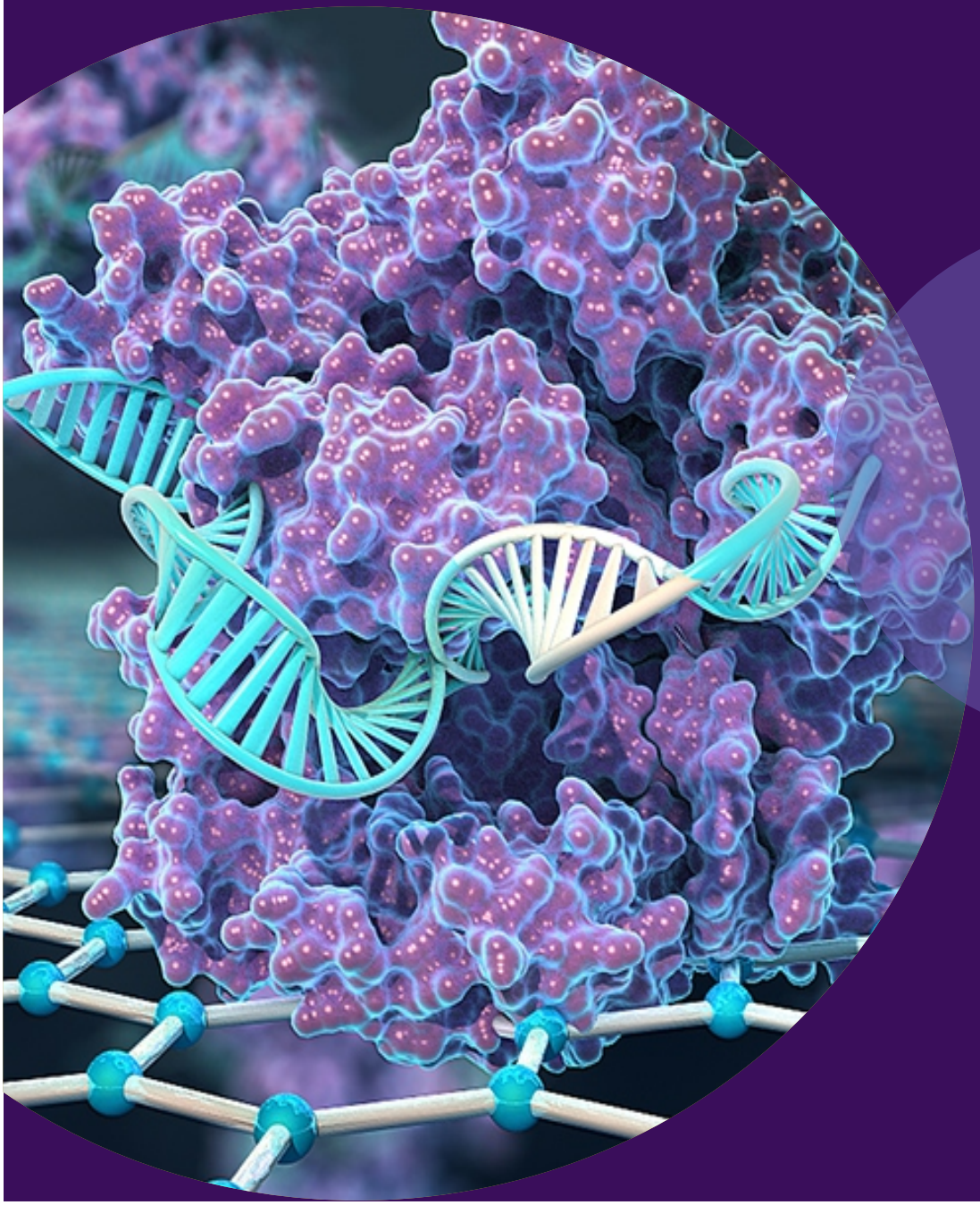
BIOTECHNOLOGY AND GENETIC ENGINEERING

Dr. Kumudini
Shreya Banerjee



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CONTENTS

CHAPTER 1

INTRODUCTION AND DEFINITION OF BIOTECHNOLOGY AND GENETIC ENGINEERING

Dr. Kumudini, Professor,
Department of Biotechnology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: kumudini.satyan@jainuniversity.ac.in

ABSTRACT:

Before the twentieth century, traditional processes like creating bread, wine, beer, and other traditional goods like cheese and curd were referred to as "biotechnology." None of them, however, could be categorised as biotechnology in the contemporary sense. Biotechnology does not include practises like plant cloning by grafting or the selective breeding of organisms to change their genetic makeup. It is possible to refer to fermentation as classical biotechnology or traditional biotechnology when it is used to prepare and manufacture goods like alcohol, beer, wine, dairy products, different forms of organic acids like vinegar and citric acid, amino acids, and vitamins. The practise of using live organisms, such yeast or bacteria, to create beneficial substances or products is known as fermentation. In that it makes use of living things, contemporary biotechnology is akin to traditional biotechnology. So, what about contemporary biotechnology is novel? Modernity is not found in the use of many living things, but rather in the methods used to do so. The face of traditional biotechnology has been permanently altered by the advent of a significant number of innovative approaches.

KEYWORDS:

Cells, Centrifugation, DNA, Macromolecule, Nucleic Acid, Technology.

INTRODUCTION

These cutting-edge methods, which are mostly used on cells and molecules, enable highly exact use of biological processes. For instance, genetic engineering has made it possible to transfer a single gene's functionality from one creature to another. So, let's first define biotechnology before delving into its specifics and the methods that make it feasible[1]–[3].

What is Biotechnology?

There are several ways to define biotechnology. It may be simply defined as the commercialization of molecular and cell biology. Biotechnology is the "controlled use of biological agents like cells or cellular components for beneficial purpose," according to the United States National Science Academy. It encompasses both traditional and contemporary biotechnology. The use of living things, cells, or cellular components in the synthesis of substances or the exact genetic modification of living things for the advantage of man is what is often referred to as biotechnology.Despite the fact that biotechnology has been used for thousands of years, the development of life sciences was revolutionised by the technological boom of the twentieth century in the fields of physics, chemistry, engineering, computer application, and information technology, which ultimately led to the development of modern biotechnology.

In addition to engineering and information technology, a variety of biochemical, biophysical, and molecular approaches have helped life scientists generate novel medications, diagnostic tools, vaccinations, foods, cosmetics, and compounds with industrial applications. Crop plants that have been genetically modified to withstand the stress of pests, diseases, and harsh environmental conditions have been created. In order to further the research on the genomes and proteomics of both humans and other creatures, new tools and methodologies have also been created. A new field of biotechnology called bioinformatics and computational biology has emerged as a result of the use of information technology and the internet in biotechnology, notably in genomics and proteomics. Like any other contemporary science, biotechnology is built on the information that has been accumulated through time. A complete understanding of biotechnology requires knowledge of its development's past.

Historical Viewpoints

While just 200 years old as a science, biotechnology is a very ancient technology. The term "biotechnology," which was first used in 1917, describes a massive fermentation process used to produce numerous kinds of industrial chemicals. Yet, the origins of biotechnology may be found in prehistoric societies like those of the Indus Valley and Egypt, where people first learnt about agriculture and animal domestication. They had learnt to use biotechnology even before they were aware that microbes existed. In order to breed plants and animals, gather and prepare herbs for medicine, make bread, wine, and beer, as well as a variety of fermented foods like yoghurt, cheese, and various soy products, primitive man had to become domesticated. He also developed septic systems to handle excretory and digestive waste, as well as vaccines to protect himself against disease. Archaeologists continue to find older instances of how man used microbes. The majority of these procedures have examples as early as 5000 BC.

For instance, the ancient Indus people created and consumed a variety of fermented foods, drinks, and remedies. As early as 4000 BC, the Sumerians and Egyptians employed yeast to manufacture wine and bake bread. Bacteria were utilised by people in Mesopotamia to turn wine into vinegar. By rotating crops in the field to boost agricultural yields, many ancient civilizations took use of the microscopic creatures that inhabit the ground. The Greeks utilised a variety of food preservation techniques, including drying, smoking, curing, salting, and crop rotation to increase crop production. All of these methods and procedures were used in the ancient India as well as the Middle East and South East Asia. The process of dehydration employing a salt solution was utilised in Egyptian mummification.

Genetic Resource Utilization

The ancient people understood the importance of natural genetic resources like plants in a region's economic development. The monarchs of ancient era would dispatch plant hunters to harvest valued exotic plant species that provided priceless spices and medicines. In similar fashion, colonial powers of the modern era undertook extensive plant collection trips throughout South America, Asia, and Africa, displaying their discoveries in botanic gardens. These early "gene banks" aided the globalisation of agricultural monocultures by the colonial powers.

Fermentation and Microorganisms

Although though people have been creating bread, beer, and cheese for generations, the scientific study of these biochemical processes is just a little over 200 years old. When Dutch experimenter

Anton Van Leeuwenhoek used his microscope to find microbes, he provided clues to understanding fermentation. Using analytical methods for the determination of carbon dioxide, he revealed the chemical underpinnings of the fermentation process. Two hundred years later, in 1857, a French scientist named Louis Pasteur wrote his first study on the fermentation-based production of lactic acid from sugar. Later in 1860, he released a thorough paper on the fermentation of alcohol. He described some of the intricate physiological processes that take place during fermentation in this article. He demonstrated that fermentation is a byproduct of anaerobic life and distinguished between three different forms of fermentation: gas generation, alcohol production, and acid production.

Eduard Buchner noticed near the end of the nineteenth century that adding cell-free yeast extract to an aqueous solution of carbohydrates resulted in the production of ethanol and carbon dioxide. He therefore demonstrated that cells are not necessary for the fermentation process and that the active ingredients are dispersed throughout the extract. He gave it the name "Zymase." In order to create glycerine for the explosive nitroglycerine during World War I, the fermentation process was altered in Germany. Similar to how new technology in the food and chemical sectors were developed by military weapons projects, which helped them win battles in the First World War. For manufacturing the explosive cordite, for instance, they employed the same bacterium that turns grain or molasses into acetone. Biotechnology not only helped troops die, but also helped them recover. The first antibiotic, penicillin, was discovered by Sir Alexander Fleming, and it was very effective in healing injured troops[4]–[6].

Evolution of Genetics

When Gregor Johann Mendel published the results of his research as the "rules of genetics" in 1906, biotechnology made significant strides. He foresaw the existence of "units of heredity"later referred to as genesthat did not alter from generation to generation but simply underwent recombination. In an effort to explain how creatures resemble their parents while yet differing from them, the study of genetics, which derives from the word "genesis," which refers to the beginning of anything, was developed. It was formerly thought that each gene had a direct correlation to a certain characteristic. Genetics began assisting plant breeders in enhancing their harvests by the 1920s. Genetics had revolutionised agriculture by the 1940s, paving the way for the Green Revolution of the 1960s.

Genetic Engineering and DNA: The Foundation of Modern Biotechnology

The discovery of DNA (deoxyribonucleic acid), which contains the hereditary information in the cells, revolutionised the field of genetics. Industrial, and environmental biotechnologyIn 1953, Francis Crick, James Watson, and Rosalind Franklin made the discovery that the DNA molecule was made of two strands that were twisted around one another like a spiral staircase and connected by bars that looked like rings. From a blade of grass to an elephant, DNA is nearly the same in terms of its makeup, structure, and function. The specific arrangement of the chemical bases in the DNA molecule is what varies and distinguishes each organism. This inspired scientists to consider how they can alter this arrangement and therefore alter life. In 1961, Marshall Nirenberg and H. Gobind Khorana succeeded in cracking the genetic code.

Within a short period of time, researchers and businessmen began attempting to change the genetic makeup of living things by transferring certain genes from one creature to another. By changing the genetic material at the molecular level, they were now able to transform various

living forms. In 1973, Walter Gilbert conducted the first recombinant DNA studies, and in 1975, he produced the first hybridomas. The first recombinant human therapeutic protein, insulin (humulin), was created in 1982, along with monoclonal antibodies for diagnostic use. The first biotech firm to create techniques for rearranging DNA was the US business Genentech, which started doing so in 1976. Interferon, insulin, and a variety of genetically engineered agricultural plants, such the high-solids-processing tomato with 20% less water, are all products of recombinant-DNA-assisted biotechnology. In order to research cancer, transgenic animals have been developed, such as the unlucky onco-mouse, which was engineered to get cancer 10 months after birth.

The 1980 decision of the U.S. Supreme Court permitting genetically altered microbes to be patented has helped and encouraged businesses in their research. This implies that almost every lifeform existing on our planet might possibly become into the property of the business or individual who 'creates' it. The monopolisation of life by a small number of very large corporations is one of the biggest dangers posed by the emerging biosciences.Across the globe, 600 pharmaceutical firms are working on developing genetically altered products. Errors are inevitable. However, a mistake with something as potent as genetic engineering might have far-reaching consequences. The whole gene revolution is on the point of being turned over to a select group of global corporations. We must set strict regulations on the genetics supply sector and endeavour to ensure that new technologies are used for the benefit of the whole world community.

The Area of Biotechnology and Its Importance

Biotechnology used to be primarily focused on producing food and medications. It also made an effort to address environmental issues. Due to the strong demand for diverse compounds including ethanol, butanol, glycerine, and acetone, among others, companies related to fermentation technology grew significantly in the nineteenth century. A brand-new field, known as "bioprocess technology," has emerged as a result of the development of the fermentation process via its interaction with chemical engineering. The use of bioprocess technology in fermentation may be used to produce proteins and enzymes on a large scale. Processes are created to economically produce huge amounts of chemicals, antibiotics, proteins, and enzymes by using the concepts of biology, chemistry, and engineering sciences.

Upstream, downstream, and media and buffer preparation are all included in bioprocess technology. The microbe receives the medium, substrate, and ideal chemical environment from upstream processes in order to carry out the necessary biochemical reactions and generate the product. The procedure of separating the pure product from the fermentation medium is known as downstream processing. Hence, biotechnologynow referred to as classical biotechnologyevolved from fermentation technology. Today, if we take a closer look at biotechnology, we can see that it has applications in a number of industries, including food, agriculture, medical, and fixing environmental issues. Because of this, biotechnology has been divided into a variety of fields, including agricultural, medicinal, pharmaceutical, industrial, and environmental biotechnology.

In addition to bioprocess technology, recombinant DNA (rDNA) and hybridoma technology are the basic foundations of contemporary biotechnology. The primary technique for producing genetically modified species, such as plants, animals, and microorganisms, as well as for

addressing key issues in the life sciences is rDNA technology. In reality, the production and sale of recombinant human insulin in the United States in 1982 marked the beginning of contemporary biotechnology. Early in the 1970s, research experts established procedures for building vectors, which they used to combine fragments of DNA to make new ones (recombinant DNA) that could be put into the bacteria E. coli. This work eventually culminated in the historic discovery (transformation). The bacteria would be able to make insulin or any other therapeutic protein or enzyme in huge numbers by using bioprocess technology if one of the new DNA's pieces has the gene for it.

Hybridoma technology is another method for producing human therapeutic proteins, vaccines, and diagnostic proteins. In 1975, the first hybridoma trials were conducted. With hybridoma technology, a myeloma cell is joined with a B-lymphocyte that secretes an antibody against a certain antigen. The resultant (a malignant B-lymphocyte) cell will continue to grow and divide forever, generating significant amounts of the antibody that can subsequently be extracted, whether it is injected into the abdomen of a mouse or cultivated in a bioreactor using bioprocess technology. Monoclonal antibodies (MAb), the resultant proteins, are most often utilised in diagnostic tests. Pregnancy tests are the most well-known MAb-containing diagnostic tool.

With the use of rDNA technology, new crop plant types with enhanced agronomic and nutritional properties may be created in agriculture. There are transgenic plants that can withstand biotic and abiotic stressors like salt, drought, and disease.It is possible to develop and employ recombinant microorganisms, plant cells, and animal cells for the mass production of crucial enzymes and chemicals for industry. Protease, amylase, lipase, glucose isomerase, invertase, and other similar enzymes are examples. The starch business employs the enzyme amylase. In order to convert glucose syrup into fructose, glucose isomerase is used. To remove stains, detergent products include lipases and proteases. Moreover, the meat and leather industries utilise protease to soften leather and eliminate hair from meat.

Several additional proteins (for human and veterinary medicines, vaccinations, and diagnostics) have been produced since the production of human insulin using recombinant e. coli started in 1982. There are several human therapeutic proteins or vaccines available today that are produced using cutting-edge biotechnology techniques, have received official approval, and are sold on the domestic market. More than 200 more human therapeutic and vaccine proteins are now undergoing clinical trials. Cancer, AIDS, heart disease, multiple sclerosis, Lyme disease, herpes, rheumatoid arthritis, and viral disorders are among the illnesses for which products are now being evaluated. Moreover, items are being created to speed up wound healing, lessen bleeding from surgical operations, and avoid organ rejection[7]–[9].The future of this fascinating new area of contemporary biotechnology is impossible to foresee. There is no question about its capacity to advance both the global economy and quality of life for people. Yet, as life sciences and biotechnology research and development expand, a number of societal, environmental, and ethical issues surface. Several groups are researching different problems and addressing the common worries.

DISCUSSION

The use of biological sciences for commercial purposes is known as biotechnology. It significantly affects a variety of applied sciences, production methods, health and medical care, agriculture, and environmental sciences. It achieved significant advancements in the health and medical fields with monitoring and diagnostic systems. Monitoring the utilisation of both

conventional and unconventional energy sources is a key function of biotechnology.Commercial biotechnology goods are already on the market and include things like updated diagnostics, recombinant therapeutic proteins, vaccinations, and vaccines, as well as biochips or DNA chips. The design and results of gene analysis in the area of molecular medicine are being revolutionised by the biochips or DNA microarrays that are now being manufactured. Using genetically modified microbes, bioremediation methods are currently used commercially to clean drinking water, freshwater ecosystems, and rivers of hazardous manufacturing effluents.

Biotechnology and the computer industry are comparable in terms of their economic potential. The consumer market is poised for a biotech sector explosion. Many new biotech goods will soon be available to consumers, such as super-nutritious meals and foods containing vaccinations, which will alter how people see agriculture.Along with the commonalities in their economic potential, there are also technological similarities. There are similarities between computer code and genetic code. Both computers and biological things arrange their crucial data in a similar ways. The binary code, a set of ones and zeros, is used to control computers. A quaternary code, which consists of four elements, is used by all living things. Instead of ones and zeros, a group of four molecules called adenine, thymine, guanine, and cytosinecommonly abbreviated A, T, G, and C by geneticistsconvey the information. These four substances are joined together to produce genes, which, like computer code, carry the instructions that tell the cells whether you should be a lemming or a linebacker-sized person.

Around the middle of the 1970s, scientists discovered that they could change these four molecules to create new genes. With the invention of recombinant DNA in 1974, even high school students have been able to cut and sew genes together. Both the advancement of this technology and the expansion of the biotech sector globally have been astounding.

A Cross-Disciplinary Problem

The fundamental sciencesphysics, chemistry, and biologyappears to be independent of one another but is really not the case. Without the participation of other scientific fields, research and advancement in one field of study are in no way conceivable. The intimate interaction between the physical, chemical, and biological sciences by the middle of the 20th century led to enormous development in every field of study. Several hybrid fields have been developed as a result of the strong interaction between these sciences.Contemporary biotechnology is really an interdisciplinary discipline that combines the basic concepts of biology with those of the other sciences, such as arithmetic, statistics, and engineering. A new subfield called bioinformatics has developed as a result of its interplay with information technology.

A Quantitative Strategy

Despite the fact that there is a gradation in complexity from one lifeform to another, all lifeforms, from a virus to a person, are very complicated in their structure and functioning. All of these lifeforms follow the basic rules of physics and chemistry in their growth and development, despite their complicated structural makeup and the hundreds of biochemical interactions they entail. They abide by the principles of thermodynamics, conservation of matter, mass action, and other related laws. An organism uses substrate or food items as a source of energy and matter while it is growing. It will either be secreted as products or digested in the body and integrated into the cells and tissues. The body will utilise the energy of the substrate or food source to build itself up or to produce the product that is a result of its metabolic processes in order to preserve

its existence. The production of products at a certain set of physical, chemical, and biological circumstances is exactly proportional to the substrate consumption, as can be shown if we concentrate primarily on the consumption of specific compounds and the products that are formed by organisms. The following equation illustrates aerobic cell development, in which only CO2 and water are produced as extracellular waste at the end of the process.The anaerobic mode of life is represented by this equation. One mole of glucose is transformed into two moles of ethyl alcohol and two moles of carbon dioxide when alcohol is fermented by yeast in the absence of oxygen. Secondly, only a limited number of these tests are carried out in labs. It is possible to standardise a laboratory-scale process in terms of the molar concentrations of the substrate used and the end products produced. It's also necessary to adjust other culture-related variables including medium composition, pH, and temperature. The experiment has to be scaled up to the industrial level after the conditions are perfected for small-scale volumes like 500 ml or 1 litre cultures. It is difficult to transform a small-scale laboratory procedure into a large-scale procedure fit for an enterprise. Throughout this scaling up process, a great deal of unexpected issues may surface, and they must be effectively resolved. Scaling up, also known as process development, is the process of converting a laboratory-scale experiment into an industrial process by using the principles of biochemical engineering.

Process Improvement or Growth

The volume of the product that the industry wishes to produce is the major factor in determining how to scale up a laboratory-size experiment or method into an industrial production process. To raise a small-scale experiment to the level of an enterprise, a predetermined set of processes must be used, depending on the nature of the microbiological or biochemical activity. To address the increasing demand for alcohol, acetone, glycerine, and butanol-based compounds during the First World War, the first large-scale fermentation method was created. Making explosives like cordite requires the use of acetone. The old technique of making acetone by distilling wood was unable to satisfy the demand. Dr. Chaim Weizman made the discovery of the bacteria Clostrudium acetobutylicum at that time (1912). David Lloyd George, the then-British minister of armaments, got in touch with Dr. Weizmann and asked him to come up with a novel way to produce acetone. In order to produce acetone, Dr. Weizmann created a microbiological technique and set up many plants in nations such as India, the United States, and Canada. In 1917, the British government issued the Balfour statement in support of a national home for Jews in Palestine as payment for Weizmann's invaluable services. Dr. Weizmann received an invitation to serve as the country of Israel's first president in 1948. As a result, Israel and microbiology are closely related.The following are the main factors to take into account while scaling up a microbial or fermentation process. In order for cells to be able to reproduce and expand unrestrictedly, there must be a sufficient amount of cell biomass and it must be distributed evenly throughout the culture medium.

1. The substrate must be delivered to the site of action within the cell;
2. The substrate molecules present in the culture media must come into contact with the cell surface.

For the process to be as efficient as possible, the concentration of substrate molecules within the cell must be high enough. Mass transfer is the term used to describe the movement of substrate and product molecules across the cell membrane.The byproducts created during reactions need to be carried out of cells and removed from the area of activity.Throughout the responding system,

there shouldn't be any localised holding up or buildup of products. Cell development and product synthesis may be inhibited as a result of this.

There should be enough oxygen in the culture medium if aeration plays a role in the development or production of the product. Mass transfer and oxygen diffusion to the bacteria are significant rate-limiting elements in many microbial systems.

The degree of sterility that is maintained in the responding system determines the process' quality and effectiveness. Unwanted microorganism contamination may affect both the product's quality and the process's effectiveness.The pH and temperature of the culture's chemical and physical surroundings must be at their ideal levels.In specifically created culture tanks known as fermentors or bioreactors, which are often employed in microbial process investigations, all these parameters may be maintained.

While the underlying concepts are the same, the technical development of using live cells for human benefit distinguishes between traditional and contemporary biotechnology. Early twentieth-century classical biotechnology was essentially a microbial-based fermentation process that had been transformed into an industrial process using the concepts of biochemical engineering. It is a combination of fermentation and biochemical engineering, to put it simply. Recombinant DNA and hybridoma technology, which allow for genetic modification of cells and organisms (including those of microorganisms, plants, and animals) and their application for various reasons, are the foundation of contemporary biotechnology.

The ownership of transgenic organisms, bioprocess technologies, and the sociopolitical, ethical, and economic repercussions that follow these trials are all significant aspects of contemporary biotechnology.Transgenic agricultural plants are employed in agriculture to increase production thanks to plant biotechnology. It is feasible to pass a characteristic from one plant to another using traditional plant breeding procedures. But, there are restrictions. It is very non-specific in that the resulting hybrid plant could not possess the desired characteristic. Only related plants may hybridise and transmit genes to one another. Yet thanks to genetic engineering, any organism's specialised gene might be transferred to a plant. Transgenic plants are widely used in industries other than agriculture. For instance, bioremediation of heavy metal-containing hazardous pollutants, such as mercury and arsenic. The genetically modified species, including both plants and animals, may also serve as bioreactors for the synthesis of medicinal proteins like hormones and vaccines. In addition to this, genetic engineering or recombinant DNA technology is a potent tool in molecular biology. In addition to genetic engineering, hybridoma technology has increased the power of contemporary biotechnology in the pharmaceutical and medical sectors. The discovery and characterisation of novel genes, proteins, and genomes have generated a vast amount of computerised data as well as several challenges that are related to it, giving rise to the fields of bioinformatics and computational biology.

A key turning point in the history of biotechnology, the completion of a "working draught" of the human genome, was announced in June 2000 during a press conference at the White House and revealed in the magazine Nature's issue from February 15, 2001. Just 32,000 genes, or around 2 to 3% of the whole genome sequence, are reported in the publication. There is no gene coding in the remaining 97% of the sequence. Even the existence of these sequences' functions is unknown. But, geneticists, molecular biologists, and pharmacologists can accomplish a number of things with these 32,000 genes. For instance, with all of these 32,000 genes on a microchip, it is conceivable to conduct a high-throughput drug screening for a particular population or tribe

and create a population- or tribe-specific designer medication. One particular gene, Her-2 Neu, which is overexpressed in certain individuals with breast cancer, is well-treated with a recently produced medication. Rapid screening of genomes and proteomes is made possible by the development of gene chips, which are complete genomes or specific DNA sequences immobilised on a microscopic silica, glass, or nylon chip. These chips are used for various purposes, including drug development and toxicological and pharmacological testing of drugs. Similar to this, protein molecules may be immobilised on tiny chips in the form of protein chips. These protein and DNA chips have altered genomic and proteomics research, and new areas like pharmacogenomics and toxicogenomics have evolved as a result[10]–[12].

CONCLUSION

In terms of biotechnology research and development, the US is in the forefront. From the beginning of biotechnology research, the United States has supported biotechnology businesses by establishing laws and regulations that maximise the advancement of biotechnology. The federal government has supported basic research at the National Institutes of Health, the leading biotechnology research organisation in the United States, and other scientific institutions by providing funding, accelerating the administrative processes for approving new medications, encouraging private-sector research investment and small business development through tax incentives, and promoting intellectual property protection and opening up international markets for biotechnology inventions and products. The United States has created online resources and public databases that enable scientists to coordinate their work and study results for quick and organised investigations

The government also supports the independence of scientific inquiry while enhancing science education. The government has established policies to safeguard patients from the improper use of new medications and private medical information. It has also given federal regulatory bodies the funding to continue conducting a thorough, scientific evaluation of and regulating biotechnology goods.

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CHAPTER 2

FUNDAMENTALS OF BIOCHEMICAL ENGINEERING: A REVIEW

Dr. Parvathi Jayasankar, Assistant Professor,
Department of Chemistry, School of Sciences, B-II, Jain (Deemed to be University), JC Road, Bangalore-560027, India
Email Id: parvathi.jaysankar@jainuniversity.ac.in

ABSTRACT:

We are all aware of the possibilities opened up by molecular biology research. Living cells and their constituent parts may be utilised to create a wide range of beneficial chemicals, including medicines and other goods. Yet molecular biology requires the help of biochemical engineering to be successful as a business venture. Biochemical engineering is a crucial field of biotechnology that deals with the industrial scale practical use of biological agents (whole cell systems and biocatalysts) and the approaches and procedures connected with it. A variety of biotechnology fields, including biochemical processes, enzyme technology, environmental biotechnology, microbial manipulations, bioseparation technology, plant and animal cell cultures, and food technology, may benefit from biochemical engineering. It entails the creation of novel process technologies, the design of bioreactors, and the creation of effective and financially viable extraction and purification techniques (downstream processing).

KEYWORDS:

Biochemical Engineering, biological agents, Plant, Bioreactors.

INTRODUCTION

Developing a bioprocess technology to fully use the potential of the biological system requires a thorough grasp of the behaviour of biological agentsliving cells or their componentsas well as skill in the principles of biochemical engineering. Hence, biochemical engineering is crucial in converting a biological laboratory experiment into an industrial process that can be sustained financially. Living cells are very delicate and need a highly particular sort of chemical and physical environment, including dietary requirements, pH, and temperature, which makes handling them in the creation of bioprocesses quite challenging. It's possible that the cell-produced item won't be released into the medium. In such circumstances, the products must be extracted by rupturing the cells. Most of the time, the product may be coupled with other cellular products that are identical to it, from which the genuine product must be cheaply isolated and purified. The following are the main issues in developing a bioprocess technology that is cost-effective: The low substrate concentration in the culture medium [1]–[3].

The reaction must be carried out at a temperature and pH that promote culture development and maximal product production. The pressure within the bioreactor increases as a result of the gas produced by the expanding cells.There are several issues that might occur when a laboratory technique is scaled up to an industrial operation. For the technology to be successful, these issues must be effectively resolved.Operational concerns for bioreactors for various cell cultures, as well as suspension and immobilised cell cultures of plants, animals, microorganisms, and genetically modified organisms, are some of the key study topics in biochemical engineering.

1. Bioreactor selection, scalability, operation, and control (fermentors).
2. Processes for recovering and purifying goods.
3. Applications of bioprocess engineering in medicine.
4. A blend of civilizations.
5. Microbial growth stoichiometry and product production.
6. Engineering of metabolic pathways.

Innovative solutions for less expensive and more sustainable energy and chemical feedstock alternatives are among the applications of biochemical engineering. There are also new biochemical engineering methods for efficient pollution management.

PH CONCEPT

The pH of cellular fluids has a significant impact on the metabolic events that are essential for life. The chemical makeup of pH, its measurement, and its biological significance are covered in this section of the chapter. The chemical environment, including the medium's acidic or basic character, has a significant impact on biomolecules like proteins in terms of both their structure and function. The pH level of the biological medium also regulates the many biochemical processes that take place in a living organism.Understanding pH and buffer requires knowledge of the chemistry of acid-base reactions as well as water dissociation.

Features of Acids and Bases in General

Acids, such as vinegar, which is a diluted solution of acetic acid, are often a family of chemicals that taste sour (but do not use this approach to identify a component). Bases, or alkaline compounds, are distinguished by their acrid flavour and slick texture. Svante Arrhenius provided the first accurate definition of an acid and a base, which is known as the Arrhenius Theory.

Mathematical Factors

In the lab, scientists measure a wide variety of things. Many observations are quantitative, even if many are qualitativewhat hue, what condition, etc.). Titrations, measuring the mass of a reactant or the volume of a liquid, and other more complex measurements call for careful value determination and must be reported with the correct unit. For other chemists who want to reproduce an experiment, the magnitude of the number and the unit must both be specified. The physical variables are the sizes of these quantifiable observations.

Important Variables

There is a unit for these variables. They are evaluated in comparison to an exact physical standard. These benchmarks are known as units. Mass, length, volume, duration, viscosity, heat, temperature, and other examples are examples of major variables.

Natural Variables

They are dimensionally-free integers or sets of numbers. They may express their measurement without the need of any units. The refractive index, particular gravity, specific viscosity, and other terms are examples of unitless variables.

Certain scientific phenomena, like the Reynolds number, do not have any units. Fluid mechanics uses the Reynolds number (Re), a dimensionless quantity, to describe the characteristics of fluid

flow via pipes and tunnels. The Reynolds number, which relies on the velocity, viscosity, and density of the fluid as well as the pipe's design, may be used to describe the transition from laminar to turbulent flow.

Measurements and Units

Fundamental quantities and derived quantities are the two types of physical variables that we employ in physics and chemistry. Fundamental amounts, dimensions, or basic quantities are a few physical variables that serve as the foundation for all measures and quantities. Base units are the units used to express them. Seven fundamental quantities or dimensions are listed along with their units. The basic quantities are multiplied and/or divided to create all other quantities. They likewise get their units of expression from basic units. These units are referred to as derived units, and the sum of their basic quantities make up their dimensions.

Physical variables are measured in accordance with predetermined benchmarks called units. The dimensions or basic quantities are expressed in base units, and the derived units are those that are derived from the base or fundamental units. The MKS, CGS, SI, and FPS units are only a few examples of the many unit systems. One system's units may be changed into another system's units. The legally recognised system, known as SI units, is extensively used. In science and engineering, metric units are divided into two groups. The CGS system is one cluster that is based on the centimetre, gramme, and second. The MKS system is the alternative, which is based on the metre, kilogramme, and second. Similar to this, the FPS system is an outdated British system that employs the fundamental units of foot, pound, and second.

The International System of Units is supported by a network of international agreements that connect all weights and measuring systems, metric and non-metric. Using the first two letters of its French name, Le Système International d'Unités, the International System is referred to as the SI. While the quantity of actual use varies greatly, it was first used in October 1960 and has since been formally recognised and embraced by almost every nation. It is based on seven fundamental units: length, mass, time, temperature, quantity of material, electric current, and luminous intensity, one in each of these seven categories. Lists the seven fundamental physical variables together with their SI-compliant units.

The International System of Units (SI) is maintained by a small organisation in Paris called the International Bureau of Weights and Measures (BIPM, or Bureau International des Poids et Mesures), and it is updated every few years by a global conference called the General Conference on Weights and Measures (CGPM, or Conférence Générale des Poids et Mesures), which is attended by representatives of all the industrialised nations and international scientific and engineering organisations.

Data and Calculation Errors

Measuring mistakes are common. Thus, this measurement error must be taken into account in all data analysis approaches. Sometimes it is impossible to prevent experimental mistakes while obtaining measurements, and precision may play a role. Think about the measurement of length, for instance. A table should have a length of 5 metres. In this case, we are really contrasting the length of the table with a 1 metre standard. There is always some doubt about the correctness of this comparison. That is based on how accurate the scale was that you used to measure the length. The measurement is not correct if the length is between 5 and 6 and there are no

subdivisions of metres noted on the scale that was used. Use a scale where the metre is split into centimetres so that the length of the table may be measured with centimeter-level precision, such as 5 metres and 3 centimetres, to get a more precise measurement.

There are always variable degrees of mistakes in numbers that are determined experimentally. Calculations regarding the trustworthiness of the conclusions made from this data must take experimental mistakes into account. In order to avoid misleading and confusing interpretations of the data, it is crucial for all disciplines to minimise mistakes by utilising precise measurement scales, estimate errors, and understand the principles of error propagation in computations.

Almost Complete Uncertainty

Uncertainty in the final results is always a result of experimental and measurement mistakes. The important figures criteria may be used to overcome this issue. Using this method, we define the range of error that each of the provided values is susceptible to. Within this margin of error, each reading will be erratic. Absolute error is the name given to this error value. Relative error is the phrase used when the same mistake is expressed as a percentage.

Types of Mistakes

1. In general, there are two types of experimental errors:
2. Random mistakes and systemic errors
3. A systemic mistake is one that has the same impact on all measures. The majority of the time, this mistake's origin is recognised, and by adding a correction factor, the error may be reduced. For instance, a watch with a five-minute inaccuracy (five minutes fast). In this instance, we may subtract five minutes from the clock's displayed time to get the right time. If the reality is known, a balance that displays a -0.5 gm mistake may be efficiently corrected.
4. A random mistake or unintentional error is one that happens for unclear causes. Repeating the studies in the exact same manner allows for the detection of this kind of inaccuracy. Random mistakes are present if different experimental values or outcomes are obtained after repeating the experiment under the same circumstances. By using statistical analytic techniques, these mistakes may be measured and reduced.
5. An experiment's findings or data should be trustworthy and repeatable. The dependability and repeatability of outcomes are referred to by the word precision. It also shows the degree to which random mistakes are absent from the data. Also, we use the word accuracy to describe the calibre of the data. We define accurate data as having a minimum of both systematic and random mistakes, and when both are virtually nil, and repeatable outcomes.

Statistical Analysis

Data is a collection of experiment-derived findings. A simple kind of information is data. Knowledge is communicated via information.Information is data that is important to solving your particular challenge.

When data can back up the information, it becomes fact. When facts are helpful in a successful explanation of a problem, occurrence, or process, they are considered to be knowledge.The methodical transformation of data into knowledge involves statistics in a significant way. You can make judgements under uncertainty using a quantifiable and quantitative scale with the aid of science. Data should be the basis for this decision-making, not opinions and beliefs held by

the individual. The study of probability rules, data collection, organisation, and display, data attributes, correlations between data, etc. are all part of statistical analysis of data.

Data Types and Measurement Levels

1. There are two forms of data. Both quantitative and qualitative data.
2. Data like colour, size, or any other characteristic of a population that cannot be calculated using mathematical relations is referred to as qualitative data. They serve as indicators of a person, a process, or the group or class to which they belong. Categorical variables are what they are termed.
3. The measurements that make up quantitative data take the shape of numerical values. This form of data is the only one for which the statistical analysis is appropriate. Discrete or continuous data may be found in quantitative data. Countable data are discrete data. For instance, the proportion of immature grapes in a box or basket of fruits. Continuous data is defined as data that has quantifiable properties and is represented on a continuous scale. Using the weight of the tissues utilised in an experiment as an example.
4. Many phases are involved in statistical analysis of data. In statistical analysis, measurement or counting comes first. The data and reality are connected by this measurement or counting. A collection of data is a depiction of reality on a numerical or quantifiable scale. Data is classified as main type if the analyst participated in its collection; otherwise, it is classified as secondary type.
5. Data may have any of the following formats, whether it is discrete or continuous: Interval, Nominal, Ordinal, and Ratio (NOIR)

The Methodology of Statistics

In order to gather, analyse, present, and evaluate data in order to draw a conclusion regarding the issue, statistical techniques are utilised. They are presently used in a broad range of professions to address several challenging experimental issues. Decision-makers, managers, and administrators in politics, business, and economics may make better and more accurate judgements concerning ambiguous situations by using statistical analysis techniques.The development of computer technology and software has considerably streamlined statistical analysis, and the economic, sociopolitical, and technological contexts of today have access to a wealth of statistical data. The analysis of statistical data has benefited greatly from recent advances in software engineering. There are software programmes that handle a lot of data extremely well. They are perfect for systematically processing a variety of data kinds, from tiny to very detailed forms. Despite the fact that computers help with statistical analysis, the analysis primarily relies on its capacity to produce accurate forecasts and conclusions.Four fundamental phases are involved in statistical analysis of a data set:

1. Problem definition (understanding); data gathering or compilation; data analysis; and final evaluation and reporting of outcomes.
2. Identifying the issue it is necessary to have a thorough understanding of the issue. In order to get the precise kind of data for analysis, the issue must be defined correctly.
3. Gathering data Data must be gathered from a particular demographic or group. As a result, it's important to specify the population from whom we are aiming to draw conclusions. The exact gathering of data requires sampling and experimental design. Even if advances in

computational statistics have made the process of data collecting simpler, designing the techniques to gather data remains a crucial component of statistical methods of data analysis.

4. The population and sample definitions are two crucial steps in statistical analysis.

A population is a collection of all the variables that make up an experiment or research.In statistics, we first extend the conclusion to the whole population from a small, well-defined population. In mathematics, this is referred to as inductive reasoning. Its major objective is to test a population-related hypothesis. The data in a sample is used to draw conclusions about the population.

Examining the information Data is categorised, sorted, and then appropriately processed to transform it into outcomes.Reporting the findings, the outcomes are then presented in an appropriate format, such as tables, graphs, or a list of percentages. The findings should include probability assertions, ranges of values, and errors to highlight the uncertainty condition because only a small collection or sample has been evaluated, not the full population.

Experimental Data Presentation

Data must be examined and transformed into a result that conveys the correct knowledge or information. The information we gather might come from samples or small groups that are representative of the total population.The information may come from pre-existing sources or from fresh observations of experiments intended to provide new information. Many things will influence the procedure in experimental investigations. To gather information on the effect of the variables, the variable of interest must first be determined, and only then can the other variables or factors be controlled. The most typical kind of observational research is a survey.

Analyzing Data

There are primarily two types of data analysis in statistics: exploratory approaches and confirmatory methods.With exploratory approaches, data are analysed using basic arithmetic computations, and the findings are summarised using simple illustrations.In order to analyse data using the confirmatory technique, a probability theory is used. Since it gives a way to measure, quantify, and analyse the uncertainties associated with future occurrences, probability is crucial to decision-making.

DISCUSSION

To study the patterns of variation among the variables, experimental data is presented in an appropriate graphical format. In other instances, it can be seen that the results are quite erratic and oscillate close to a mean value. The distribution that results from such a situation is known as a Gaussian distribution. For instance, we may get a dispersed distribution if we plot the variance in blood glucose levels as a function of time. An extremely erratic line will be produced if we attempt to draw a line across each number. When this occurs, even if there is a significant amount of point scatter, we draw a line through the centre of the dispersed values, assuming that the system is smooth and continuous and that the data values are free of experimental mistakes and would lie on the line. There will be many queries in such situations. Where would the curve pass more closely? Are certain data points obviously in the range of error, or should all the data points be included? For instances like this, unique statistical techniques have been created [4]–[6].

A biochemical reaction's overall performance in a bioprocess may also be significantly impacted by heat transfer. In a fermentor, the reaction may be either exothermic or endothermic. Hence, precise temperature control is required for the majority of fermentors. The agitation and respiration of the developing organisms, as well as the exothermic reaction, may all raise the temperature of the fermentor. The target temperature may be computed after the specifics of the heat transfer—the development of heat by fermentation and heat created owing to agitation and aeration—are understood. Heating and cooling equipment may be configured to deliver the ideal temperature required for the process's smooth functioning based on the total heat produced during fermentation. The bioreactor's temperature may be protected extremely effectively by a well-designed jacket around it and by the movement of steam, hot water, or cooled water. Before beginning the procedure, this facility may also be utilised for autoclaving and cleaning the fermentor as well as sterilising the media and fermentor [7]–[9].

CONCLUSION

When the fermentation is complete, the cells are filtered or centrifuged away from the soup. The product may be separated from the cell, free medium, or supernatant if it is extracellular, like numerous acids, alcohols, antibiotics, and vitamins. If the product is intracellular, the cells are taken out of the culture and disturbed using the appropriate technique. The product is then separated from the mixture using a variety of separation techniques as part of downstream processing. The structure and various stages of downstream processing heavily rely on the kind of product, its nature, and the media's constituent parts. Consequently, the integration of planning and operating all three crucial stepsupstream processing, bioreactor operations, and downstream processingis crucial for the success and efficiency of the bioprocess technology for the cost-effective manufacture of a biochemical.

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CHAPTER 3

SOCIETY PUBLIC PERCEPTION OF BIOTECHNOLOGY

Dr. Rekha MM, Assistant Professor,
Department of Chemistry, School of Sciences, B-II, Jain (Deemed to be University), JC Road, Bangalore-560027, India
Email Id: mm.rekha@jainuniversity.ac.in

ABSTRACT:

Science and Society, in Brief your opinions or impressions of things are often impacted by your cultural background and are not necessarily reasonable. They are a synthesis of our thinking, or cognitive dimension, our emotions, or affective dimension, and our behaviours, or behavioural dimension. What we know make up the cognitive dimension, feelings make up the emotional dimension, and conduct determines how we will act on the attitudes we develop. Our attitudes help us fit in with society. The public's perception of biotechnology is primarily shaped by its lack of trust and scientific illiteracy. There are benefits and drawbacks to ignorance. Effective debate with someone about a subject they are unfamiliar with is challenging. Because of their ignorance, many people will be concerned about the technology. Because of their innate fear of the unknown, people will be easily persuaded by arguments that claim all biotechnology is immoral if they are unable to comprehend the issues.

KEYWORDS:

Science, DNA, World Commission on Environment and Development, Cognitive Dimension, Society.

INTRODUCTION

Modern science and technology have greatly simplified and accelerated our way of life. Yet, some of these developments have given rise to serious worries about their long-term effects on the environment and human life. The UN-appointed Brundtland Commission, commonly known as the World Commission on Environment and Development (WCED), made its sustainable development recommendations in 1985. Sustainable development preserves the environment without causing any deterioration. A sustainable development is one that "meets the demands of the present without jeopardising the capacity of future generations to satisfy their own needs," according to the Commission. For many countries, there are several definitions and points of view on sustainable development. All of these perspectives have one thing in common: they all represent science and technology as having both advantages and disadvantages [1]–[3].Regarding the use of science and technology, there are two competing ideologies:

1. Integral Ideology
2. Reductionism as a Theology

Traditional techniques should be used in all spheres of life, from industry to agriculture, according to holistic worldview. According to this worldview, traditional and informal forms of knowledge are ignored in favour of formal education. This ideology claims that there are no issues with the agricultural industry. If we went back to eating some of the ancient or traditional grains, which are simple to farm, Earth could produce enough food to feed everyone. In order to

preserve the soil and grow a crop, it also suggests using chemical manures, pesticides, herbicides, and limited tillage. Whatever issue still exists in the globe is mostly a result of how unevenly produced food is distributed.

Reductionistic philosophy promotes the use of new information to enhance crop plants and agriculture. It suggests ongoing research and development projects to identify fresh approaches to challenges.These two organisations and its several subtypes have offered contrasting opinions on environmentally friendly sustainable development.

The Social Effects of Biotechnology

Biotechnology may first seem to be only a scientific and technology issue. Nonetheless, because of its potential effects on the environment and our way of life, biotechnology is both admired and feared. Biotechnology has developed into a discussion point for opposing socio-ethical points of view because of its ability to influence societal values, interpersonal relationships, and environmental issues.

The general public's perception of biotechnology varies significantly by nation, sex, age, education level, religious beliefs, and social groups. Overall, views are far from being as optimistic as the sector would want.Modern technology development is frequently the focus of contentious and divisive debates. At first glance, biotechnology might appear to be just a science and technology issue, but it has the power to alter social norms and reshape interpersonal relationships. As a result, different religious and social groups have very different perspectives on the advancements in biotechnology. Two significant social and ethical issues include, for instance:Should a man be allowed to pretend to be God? Should it be legal for man to use genetic engineering to clone humans and other animals and change their genetic makeup?

Who is the creator and owner of the technology? Who is the owner of the newly created, genetically altered organism in the case of genetic engineering? Who are the parents of the cloned organism in the case of cloning? The mother who is giving birth to the child or the woman who contributed the ovum should be regarded as the real mother, even in the case of test tube babies.The social effects of biotechnology as a science, a technology, and a rapidly growing industry should be discussed in discussions between the general public, scientists, governments, and religious organisations. We should create a programme that includes activities to harmonise and clarify biotechnology regulations, to understand consumer attitudes towards biotechnology, and to understand the social and ethical issues raised by this technology if we want to address the barriers to the successful development and commercialization of biotechnology products. In addition to discussing the competing pressures on governments to address social, ethical, and environmental issues, we should analyse and recognise the conflicting social and ethical perspectives of biotechnology.

Virtually every facet of human life will be impacted by the applications of biotechnology, which is a production method. There are socio-ethical ramifications for both the method and the applications. Whether biotechnology and its many applications will benefit or harm society is a topic of intense debate. Since there are so many different biotechnology applications, each one must be evaluated separately.There are primarily three main levels of disagreement in the discussion. First, there is disagreement regarding predictions of the potential social effects of this technology. Second, there is disagreement regarding the moral principles that should guide the outcomes—what should take place. Finally, there is disagreement regarding the process that will

be used to develop ethicswho makes the decisions about what should and won't happen? In actuality, the debate over the advancement of biotechnology is one over the future of our social structures and values.

Numerous social, ethical, and legal issues are prompted by the use of biotechnology in society. Many nations have yet to create a system or mechanism to deal with the social and ethical problems that biotechnology has brought to light. The social effects of biotechnology have instead been determined by government spending on research and development.Due to the need for products to address socio-ethical concerns in committee hearings and parliamentary hearings, the commercialization process for biotechnology products has been uncertain and drawn out. The establishment of a publicly derived socio-ethical framework to guide the development of biotechnology is necessary for industrial development.

Public support is essential if the successful commercialization of biotechnology is the ultimate goal. In turn, public support will depend on how socially beneficial biotechnology is. The ethics governing the social effects of biotechnology must reflect the general populace. The public's preferences for social and ethical rules to guide the development of biotechnology have not yet been the subject of any systematic research.The products of biotechnologyand the ensuing benefits and riskswill affect everyone, so it is crucial that the public understands it as a science and technology. The use of biotechnology to improve and maintain environmental quality, food safety, and human health has great potential. To address these issues that have an impact on both people and the environment, it can develop technologies and methods that are environmentally safe. However, the potential role that biotechnology could play in resolving issues with food production and the environment could be stymied without public understanding, acceptance, and support.

Intellectual Property Rights (Patenting)

The term "intellectual property rights" (IPR) refers to a variety of different legal rights that are granted by each nation. It can be viewed as a form of national recognition for the inventor's contribution to the creation of a new technology, method, or item. It is safeguarded from unauthorised use for industrial or commercial purposes.Science and biotechnology are not new to the idea of intellectual property rights. It is a result of industrialization. Due to the development of scientific research and the promotion of those products, it gained not only popular among scientists but also among the general public. The nature, consequences, and legality of intellectual property rights as they relate to gene technology, biodiversity, and inventions that draw on genetic resources and related traditional knowledge have been some of the concerns raised about modern biotechnology. The majority of institutions and universities now have their own teams or experts to handle their own IP issues [4]–[6].

Intellectual Property Rights

Various kinds of intellectual property rights exist. Patents, trademarks, industrial designs, geographic indications of sources, protection for plant species, and copyrights are the main classifications.The laws passed by the parliament or other national governing body in each nation safeguard these rights.

1. Patents: These provide protection for products, manufacturing techniques, and inventions. For a set amount of time, it grants its owner a monopoly right over the process or product's commercial application.
2. Industrial design: This refers to how products, like ornaments and instruments, are shaped and created.
3. Trademark: This refers to the distinguishing words or images used to identify goods, services, or businesses.
4. Plant variety protection: This category of IPR pertains to biotechnology. A plant variety right or plant breeder's right is what it is called. It is applied to new plant varieties created through hybridization processes or other genetic alterations.
5. Copyright: This IPR covers software, engineering drawings, handiwork, and literary and artistic works.

Patent

A 20-year period of legal protection for inventors and investors in their inventions from their work being used for profit without their permission is provided by this system of laws.

Discovery vs Invention

Between discovery and invention, there is a significant distinction. The main definition of discovery is the process of learning new things through experiments, research, and thought. It has an intellectual bent. The name itself denotes the discovery of a new idea, theory, or piece of information that either already exists or is hidden in the natural world. It increases our understanding of the world. Examples include Sir Isaac Newton's discovery of the gravitational force and Hargobind Khorana's deciphering of the genetic code.

DISCUSSION

Actually, invention is the creation of something new that doesn't already exist. There is the use of prior knowledge developed through discovery. For instance, an electric bulb is an invention, whereas electricity is a result of discovery. In a similar vein, the discovery of steam's potential and the invention of the steam engine. Gene cloning, PCR amplification, and other inventions are techniques. New knowledge or information is also distributed through invention. Examples include a new process that produced an old product, a known process that produced a new product, a new result, a new process, a new combination of materials to produce a known product, etc.

In general, a discovery cannot be patented, while an invention can. Existing and new materials are now unclear to one another. For instance, a synthetic product is a brand-new item that did not previously exist. Thus, it is unquestionably patentable. However, when a particular protein, gene, or other naturally occurring compound is isolated or purified, it is not a brand-new substance. But why is it patentable?

The cause is that isolated or purified forms of these proteins or natural products were not previously known. A new strain of microorganisms isolated from nature will also be eligible for patenting if it possesses a novel trait not present in the bacteria's previously recognised natural form. Through a joint statement of policy, the patent offices of the U.S., Europe, and Japan have clarified these issues.

Patents on Goods and Processes

According to the patent laws of many nations, a novel product that has been created synthetically or isolated and purified from natural sources is patentable. The issue at hand is whether or not a process is patentable. A new technique, method, or process used for the synthesis, isolation, or purification of a compound or microorganism that is already known to exist is patentable. Normal operating procedure cannot be patented. The procedure or the process is not patentable if the product is new but the method used is an established method; only the product is. Both the process and the product must be novel in order to be patentable.

Patent Management

When someone invents something, they must first file a patent application in their home country for the new product or process before moving on to other countries. A distinct title should be present on the patent application. The invention's novelty needs to be carefully examined. A patent attorney must complete all necessary steps, including the application filing.

Worldwide Patent Laws

According to a treaty or international agreement, each nation has its own unique type of patent law. However, there is a history of effective international cooperation through the use of conventions. These conventions help member states reach consensus and agreements on formal and substantive patent issues. The following describes a few of the conferences that influenced global patent laws.

Paris Conference:

In 1883, this convention took place. The International Convention for the Protection of Industrial Property was another name for it. The Paris Union, which now includes the vast majority of industrialised nations, has 151 members. Regarding the protection of industrial property, member nations must treat citizens of other union members on an even playing field with their own citizens. With regard to patent laws and patent applications, there are numerous agreements and understandings among the member states. The most recent modification to the Paris Convention's text was made in Stockholm (1979).

Geneva Convention:

This took place in 1963. "Convention on the Unification of Certain Points of Substantive Law on Patents for Invention" is what it is called. This outlines the typical criteria for an invention's patentability, such as that it must be novel, be capable of industrial application, involve an inventive step, etc. The European Patent Convention has incorporated many of this convention's features. For instance, the definition of "the state of the art," which must be used to evaluate and judge the degree of novelty and inventiveness of the subject matter of a patent application. This convention also decided to exclude both plant and animal varieties from patent protection.

Because it is run by the Geneva-based World Intellectual Property Organization (WIPO), the Patent Co-operation Treaty (PCT) is regarded as the international patent body. This agreement was actually signed in 1970, and it and the European Patent Convention became operative in 1978. It is the largest international organisation and has 100 members. Because a designated national system first formally accepts them before an international body (WIPO) processes them

in an international phase, the patent applications submitted under PCT are regarded as "international" in this context. The formal preliminary steps, a prior art search, and publication of the application are all part of the international phase.

European Patent Convention (EPC):

This was established in 1978 following a 1973 conference. Along with the European Patent Office (EPO) and Administrative Council as its supporting bodies, it created the European Patent Organization. Each regional patent office belongs to a member nation. Before the EPO, a single patent application may be submitted, and it will be taken into account for its member states. The application may be submitted to any of the national or regional patent offices, but the EPO will eventually examine it. When the patent is issued, it will be a collection of national patents from each member state rather than a single European Patent. For instance, European Patents from the UK, Germany, France, and so forth. As a result, a single patent application submitted via the EPO will eventually become a single unitary indivisible object of property protecting the entire European Economic Community.

The Budapest Treaty was signed in 1977 and came into effect at the end of 1980. It is also referred to as the "Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure." The International Depository Authorities' microbial culture collections are recognised by this treaty. For the purpose of filing a patent application in any member state, a novel strain of microorganisms may be deposited with any of the International Depositories.A microbial culture collection's primary duties are to accept, preserve, and offer microbial cultures to the scientific community as a service. They accept new strains of microorganisms isolated by scientists, and its identity will be verified and properly classified. Then it is deposited into their culture collections and is maintained as a new strain. These depositories act as a source of microorganisms for scientific and industrial purposes. The new organism is allotted an accession number and date by which the strain is identified and referred in the future and in patent applications.

Patenting In Biotechnology

Biotechnology in one form or another has been part of human development since the dawn of agriculture. Human ingenuity has led to increased production and greater diversity and quality of livestock and varieties of crops. Today’s food crops and domestic animals embody the benefits of many generations of selection and breeding.Biotechnology continues to offer considerable potential for enhancing human health and well being. Modern biotechnology, including gene technology, is finding increasing application in health care and in a host of industrial and agricultural industries. Effectively applied, modern biotechnology may contribute to economic growth, technological development, and human welfare. Yet it has also raised concerns about ethical and moral issues, equitable sharing of the benefits of biotechnology, environmental impact, the accelerated pace of change, and regulatory challenges. Intellectual property (IP) rights are not new in the biotechnology domain, but some of the concerns about modern biotechnology have focused on the nature, impact, and legitimacy of IP rights as they are applied to gene technology and to inventions that draw on genetic resources and associated traditional knowledge.

Varietal Protection

In conventional plant-breeding experiments for the production of desirable hybrids, there is the Plant Breeder's Right and Farmer's Right. The Plant Breeder's Right implies the protection of the new variety that has been created by the method of breeding procedures and selection. Farmer's Right means that farmers have the right to produce the seeds from a hybrid plant and raise the seedling for their agricultural purpose.

Ethical Issues in Biotechnology-Agriculture and Health Care

The government's promotional activities surrounding biotechnology have inspired debate. The use of public funds to further biotechnology research supports the use of a method that has social implications and around which there exists significant social disagreement. The government's decision to allocate funds to certain niche areas determines the applications, which are most likely to be commercialised. This history has established a relationship between the government and the public.

It has created trust and support among some, and distrust and opposition among others, because of the socio-ethical position implied in the policy [7]–[9].Using public resources for biotechnology's development raises objections from stakeholders who do not believe this technology will benefit society, or who believe that its current course of development poses socio-ethical risks.

The use of public resources for the development of biotechnology is supported by the biotech industry who believe that this technology offers significant health, environmental, and economic benefits to the public, and who believe that facilitating the development of this industry for those benefits is a government responsibility [10]–[12].

CONCLUSION

Proponents of biotechnology argue, correctly, that biotechnology is being held to standards that are not demanded of other advanced technologies such as computers or the information highway. There is considerable debate over whether biotechnology should be held to distinct standards because it is a power that raises special social questions. What is unquestionable is that biotechnology requires considerably more public trust than any other technology because of its potential power to transcend, quickly and intentionally, any God-given or natural limit to human activity.

Biotechnology patent law operates under the same general legal principles as other areas of patent law. However, the nature of biotechnology raises many unique patent law issues. Biotechnology law is becoming more complex in all industrialised nations of the world. Long-lasting, broad, international patent protection is vital to ensure a financial return after an invention travels the long pipeline from lab to marketplace. The complexity in biotechnology patenting involves patenting of living organisms and natural products, which are not patentable normally, in addition to the products and processes. For example, a gene that causes a fatal neurological disease and another gene that dramatically improves crop yields. Even though these genes exist naturally and are not patentable, the gene is not occurring in isolated and pure form. In this sense genes and other naturally occurring materials are patentable and can be commercialised. The recent awareness of patents and their elaborated studies is an outcome of biotechnological inventions.

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CHAPTER 4

EXPLORING THE ROLE OF BIOMOLECULES

Dr. Nayana Borah, Assistant Professor,
Department of Microbiology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: b.nayana@jainuniversity.ac.in

ABSTRACT:

Biomolecules are substances that are created by living things. These compound groupings come in a variety of forms, sizes, chemical and physical characteristics, and biological roles. Depending on their size and composition, the many kinds of chemicals that make up these biomolecules may be roughly categorised into two groups. Macromolecules are those molecules that are polymeric and larger in size, whereas biomolecules are those that are simple and smaller in size. In biological systems, there are four different kinds of macromolecules: lipids, proteins, carbohydrates, and nucleic acids. Three of these four varieties are polymers made out of monomers, or basic elements. Polymers are not lipids. An organism's live cells are chemical machines with chemical components and a chemical language. It is an amalgamation of many kinds of biomolecules with varying biological, chemical, and physical characteristics. All of these tiny and big macromolecules participate in various biochemical processes, which lead to the phenomenon of life.

KEYWORDS:

Biomolecules, DNA, Plant, Cells, Substances.

INTRODUCTION

Three chapters make up this section. We examine tiny molecules in the first chapter, including the precursors of macromolecules. Monosaccharides, also known as sugars, amino acids, nucleotides, vitamins, coenzymes, and fatty acids are included in this. Several of these molecules serve as the macromolecules' building blocks. For instance, proteins are composed of amino acids. All of these molecule's macro and microare in a state of flux or dynamic state in biological systems. That is, they undergo chemical changes continuously in order to preserve the condition of life. Basically, the metabolism of an organism is the result of all these biochemical changes together. These metabolic processes, which may be linear or cyclic, are linked together and organised into distinct metabolic pathways. In terms of both structure and functionality, biopolymers, also known as biological macromolecules, represent the majority of biomolecules. All cellular components, including cell walls, membranes, organelle membranes, microtubules, flagella, and celia, include a variety of biomolecules that are actively involved in a variety of metabolic processes.

As biopolymers, these macromolecules are also referred to. They may be divided into four main categories: lipids, proteins, carbohydrates, and nucleic acids. Little repeating units or monomers of the same kind or various sorts make up all of these biopolymers. These biopolymer building components are known as building units. Lipids are macromolecules that are classified as biopolymers even though they are not polymers. The structure and behaviour of the fundamental macromolecules that make up the biological system are covered in this chapter. These

biopolymers' monomers are distinguished by the presence of certain functional groups. Consequently, prior to delving into the specifics of these monomers, it is necessary to have a fundamental grasp of the functional groups present in biomolecules.

Polymers and Monomers

The fundamental function of a cell is the assembly of tiny molecules (monomers) into much bigger ones (polymers).

Materials of Life

A pure material with just one kind of atom makes up an element. There are 92 elements that are found naturally. The living system does not include all of the elements that exist on Earth. Similar to this, biological tissue does not reflect the number of elements in the crust of the planet. Some of the earth's key elements, like aluminium, are absent from cells, whereas others that are present in cells are rare in the crust of the planet (e.g., carbon). Over 25 different elements have been found in live cells.

Functional Biological Molecular Groups

The functional groups are special groupings of atoms and molecules that provide a new product certain chemical characteristic when they are swapped for one or more hydrogen atoms in a hydrocarbon. The functional group that is present in a molecule is what gives it its chemical characteristics or "activity." For instance, the presence of the -COOH group is a characteristic of all organic acids. These are some of the significant functional groups found in biological molecules. A list of the most frequent functional groups found in different biomolecules is also provided.

Building Carbohydrate Blocks

Carbon-based molecules called carbohydrates have a lot of hydroxyl groups in them. Often referred to as sugars, the simplest carbohydrates also either an aldehyde group or a ketone group. Polyhydroxyaldehydes and polyhydroxyketones are the names given to the sugars that comprise aldehydes and ketone, respectively. All sugars fall into one of three categories: polysaccharides, oligosaccharides, or monosaccharides. The monomers or fundamental components of carbohydrates are monosaccharides. An oligosaccharide is made up of two to ten monosaccharide molecules connected by glycosidic linkages. As they include hundreds of monosaccharide units, polysaccharides are substantially bigger. Since carbohydrates include hydroxyl groups, they may engage in hydrogen bonding both inside and between chains as well as with the aqueous environment. Carbohydrate derivatives may include nitrogen, phosphate, and sulphur molecules. In order to generate glycolipids or glycoproteins, carbohydrates may also interact with lipids.

Monosaccharides

Glucose, fructose, galactose, and other simple sugars are examples of monosaccharides. The amount of carbon atoms in the backbone structures of the different sugar kinds found in nature allows for classification. They are referred to as carbon hydrates since their fundamental molecular structure is CnH2On. Based on the kind of functional group they include, the monosaccharides are divided into two groups. Aldehyde-containing sugars are referred to as

aldoses, while keto-containing sugars are referred to as ketoses. Ketoses and aldoses, the two main monosaccharides, each have four to seven carbon atoms. As a result, monosaccharides are categorised into seven categories based on how many carbon atoms they contain. These are triose, which has three carbon atoms, tetrose, which has four, pentose, which has five, hexose, which has six, and heptulose, which has seven. Both aldoses and ketoses are included in each of these groupings.

DISCUSSION

Aldotriose and aldoketose are the names of the trioses-containing aldehyde groups. The same is true for tetrose, which may be either aldo- or ketotetrose, pentose sugar, which includes aldopentose and ketopentose, and hexose sugar, which includes aldo- and ketohexose. The seven-carbon sugar known as heptose is uncommon and absent in free form. Sedoheptulose, a keto form of heptose, is a component of plants and a step in the biosynthetic process that converts carbon dioxide into glucose for photosynthesis. A list of monosaccharide classifications may be found in [1]–[3].

Protein Building Blocks

Proteins are enormous molecules made up of mixtures of twenty distinct amino acids. A protein's function is highly dependent on the specific physical structure of the protein. Each cell in a live body depends on a wide variety of proteins to operate.Alpha amino acid polymers make up all proteins, including peptides and polypeptides. Regarding the composition of all proteins, there are 20 -amino acids. Several amino acids may be present in the body either free or mixed (i.e., not associated with peptides or proteins). Certain amino acids that are not linked to proteins carry out specific tasks. A number of the amino acids present in proteins also have uses other than in the synthesis of peptides and proteins, such as tyrosine's role in the production of thyroid hormones and glutamate's role as a neurotransmitter.

Nucleotides: Building Blocks of Nucleic Acids

Our genetic material is made up of information-carrying molecules called nucleic acids. The nucleic acids are formed of repeating units or monomers known as nucleotides, much like many of our other chemicals. The constituent parts of nucleic acids are called nucleotides. The nucleotides may be thought of as one of the most significant metabolites produced by cells. The two main nucleic acids in a cell, RNA and DNA, are composed of monomeric units called nucleotides. They are necessary for countless other significant and separate cellular processes, however.

Nucleotide functions include acting as energy carrier molecules (ATP) and taking part in energy transfer processes. Most of these processes are primarily powered by ATP or adenosine triphosphate.Coenzymes that transport energy, include coenzyme A, NAD+, NADP+, FAD, and numerous other significant coenzymes.Intra-cellular messengers, which act as middlemen in a variety of crucial cellular processes, including signal-transduction events and second messengers. Cyclic-AMP (cAMP), a cyclic derivative of AMP created from ATP, is the main second messenger.Use allosteric influences on enzyme activity to regulate a variety of enzymatic processes.Activated intermediates for a variety of biosynthetic processes. A number of sugar-coupled nucleotides involved in the synthesis of glycogen and glycoproteins as well as S-

adenosylmethionine (S-AdoMet), a component of methyl-transfer processes, are among these activated intermediates.

1. Nucleic acid components (which are long chains of nucleotides)
2. A nucleotide is made up of three parts:
3. Pentose sugar, a 5-carbon sugar
4. Phosphorus group
5. Base nitrogen

The 5-carbon sugar found in nucleic acids is called deoxyribose sugar in deoxyribose nucleic acids and ribose sugar in ribonucleic acids (RNA) (DNA). The 5-carbon of the pentose sugar is connected to the phosphoric acid through an ester bond as phosphate residue. N-glycosidic connection binds the nitrogen base to the pentose sugar's carbon-1 atom. Both of these condensation reactionsthe production of an ester connection between pentose sugar and phosphoric acid and the development of an N-glycosidic link between the sugar's C-1 and nitrogen baseslead to the removal of a water molecule. The heterocyclic, highly basic chemicals purine and pyrimidine are the precursors of the nucleotides present in living things.As they are related to the nucleotides found in DNA and RNA, the chemical basicity of nucleotides is what has given them the widespread name "bases." Cells include five main types of bases. Adenine and guanine are the names of the pyrimidine derivatives, whereas thymine, cytosine, and uracil are the names of the derivatives of purine. A, G, T, C, and U are the standard acronyms used for these five bases.

The nucleoside formed by the b-N-glycosidic bond between the anomeric carbon (C-1) of ribose and the N9 of a purine or N1 of a pyrimidine is what connects the purine and pyrimidine bases in cells to D-ribose or 2'-deoxy-D-ribose. By creating an ester bond with its 5-terminal OH group, the nucleoside condenses with phosphoric acid to produce a nucleotide. The five different kinds of nucleosides and nucleotides that correspond to the five different nitrogen base types [4]–[6].

Structure and nomenclature of nucleosides and nucleotides

The base may have two different orientations around the N-glycosidic link in nucleosides and nucleotides. The terms syn and anti are used to describe these conformations. In naturally occurring nucleotides, the anti-conformation prevails.In the cell, nucleosides are typically present in their phosphorylated state. Nucleotides are the name for these. The hydroxyl group linked to the 5-carbon of ribose serves as the most prevalent site of phosphorylation of nucleotides seen in living cells. To differentiate them from the backbone numbering in the bases, the carbon atoms of the ribose found in nucleotides are given a prime (') designation. Mono-, di-, or tri-phosphorylated nucleotides may occur.To make it simple to distinguish between nucleotides and determine their structure and phosphorylation level, each nucleotide has a unique shorthand. Adenosine (5-monophosphate), which has undergone monophosphorylation, is denoted by the symbol AMP. ADP and ATP, respectively, are used to represent the di- and tri-phosphorylated forms. These acronyms are used with the assumption that the nucleotide is in its 5-phosphorylated state. Nucleotides' di- and tri-phosphates are connected by acid anhydride bonds. The high free energy (G0') for hydrolysis of acid-anhydride bonds gives them a strong potential to transfer phosphates to other molecules. The nucleotides' participation in group-transfer processes for energy transactions in the cell is caused by this feature of the nucleotides.Thymine is nearly exclusively present in DNA, but uridine is never found there.

Neither rRNA nor mRNA contain thymine, only tRNAs do. In DNA and RNA, a few less frequent nucleotides may be identified. DNA's most important modified base is 5-methylcytosine. Numerous changed nucleotides are met outside of the context of DNA and RNA that perform crucial biological roles, including a range of modified bases that are found in the tRNAs.

Alternatives to Adenosine

The cyclic form of adenosine, 3-5-cyclic adenosine monophosphate, or cAMP, is the most prevalent adenosine derivative. The second messenger function of this substance is to transmit signal transduction events from the cell surface to interior proteins, such as cAMP-dependent protein kinase (PKA). PKA phosphorylates a variety of proteins, which may have a positive or negative impact on how active they are. By directly interacting with the channel proteins, cyclic-AMP also plays a role in the control of ion channels (e.g., in the activation of odorant receptors by odorant molecules). When receptor-coupled adenylate cyclase is activated, cAMP is produced in response. Every sort of receptor may be present (e.g., hormone receptors or odorant receptors). S-adenosylmethionine, a kind of activated methionine, is used in the production of polyamines as a source of propylamine and as a methyl donor in methylation processes.

Derivatives of Guanosine

Also present in the cells as a second messenger molecule is cyclic GMP (cGMP). Its function is often to counteract the effects of cAMP. Similar to the signals that activate adenylate cyclase, receptor-mediated signals result in the formation of cGMP. Guanylate cyclase is connected to the receptor in this instance, however.Photo-reception is the most significant cGMP linked signal-transduction cascade. In this instance, however, activation of rhodopsin (in the rods) or other opsins (in the cones) by photon absorption (via 11-cis-retinal covalently linked with rhodopsin and opsins) activates transducin, which in turn activates a cGMP specific phosphodiesterase that hydrolyzes cGMP to GMP. This causes the closing of the channels and the ensuing hyperpolarization of the cell because it decreases the effective concentration of cGMP bound to gated ion channels. The long-chain hydrocarbon acids known as fatty acids, the C18 amino alcohol or sphingosine, glycerol, and cholesterol are the chemical components or building blocks of lipids. Blocks of lipids that may be discussed include fatty acids and glycerol.Lipids are a subclass of biological molecules that are soluble in organic solvents but insoluble in aqueous solutions. Human physiologically significant lipids have four main purposes:

1. They are essential elements in the structure of biological membranes.
2. They provide stored energy, mostly in the form of triacylglycerols.
3. Lipids and lipid derivatives act as hormones and vitamins, respectively.
4. Bile acids that are lipophilic help to dissolve lipids.

Lipids' Structure

Lipids are comparable to carbohydrates in their chemical makeup in that their primary elemental constituents are carbon, hydrogen, and oxygen. Yet, since there is not much oxygen present, the majority of lipids are hydrocarbons. The building blocks of fatty acids and the alcohol glycerol form the basis of the chemical structures of fats and oils, the most prevalent lipids. Conventional language dictates the phrases fats and oils. At normal temperature, fats are "hard" or solid, while oils are liquids.

Oily Acids

Being the primary constituents of triacylglycerols, which are the principal type of stored fat, fatty acids perform two key functions in the bodies of both plants and animals.

Long-chain hydrocarbon molecules called fatty acids have a carboxylic acid component at one end. In fatty acids, the carbon of the carboxylate group is the first carbon to be counted. The carboxyl group is easily ionised at physiological pH, giving fatty acids a negative charge.

Saturated and unsaturated fatty acids are the two forms of fatty acids

Unsaturated fatty acids have double bonds, but saturated fatty acids do not; they are distinguished by the absence of double bonds. The number of carbon atoms, followed by the number of unsaturation sites, is used to represent fatty acids numerically (e.g., palmitic acid is a 16-carbon fatty acid with no unsaturation and is designated by 16:0). The symbol and the number of the first carbon of the double bond serve to identify the location of unsaturation in a fatty acid.At physiological temperature, saturated fatty acids with less than eight carbon atoms are liquid, but those with more than ten are solid. In comparison to a saturated fatty acid, fatty acids with double bonds have much lower melting points.

The bulk of fatty acids in the body are obtained via eating. Fatty acid synthase and other fatty acid modifying enzymes, nevertheless, are capable of producing all the different fatty acid structures that the body requires. The highly unsaturated fatty acids linoleic acid and linolenic acid, which include unsaturation sites beyond carbons 9 and 10, are two significant exceptions to this rule. These two fatty acids are regarded as the necessary fatty acids since the body is unable to synthesis them from their precursors. This means that they must be obtained from the food. As plants can produce linoleic and linolenic acids, people may consume a variety of plants to get these fats or they can eat the flesh of animals that have ingested these plant fats [7]–[9].

CONCLUSION

Every living thing in the world constantly engages in hundreds of different chemical interactions. The nutrients that are ingested by the cells are converted into several new biomolecules and parts that are unique to each individual cell. This method is used to create sugars, amino acids, organic acids, nucleotides, lipids, and other compounds. Cell metabolism is the collective name for these processes.

Every biological process results in the formation or dissolution of a bond. The metabolism of the cell and its energy balance must be seen as interdependent factors since this process typically consumes or produces energy. Each covalent link in a molecule has energy that, when it breaks, is released and may be put to use in different ways. For instance, it may be utilised to create a new link or changed into another kind of energy like motion, heat, light, or electrical energy. Thus, activation energy is required for a molecule to break down. The majority of these reactions are achievable in chemical labs, but only at very high temperatures and pressures and with certain inorganic or organic catalysts. For a cell that can only employ catalysts, the first two options, high temperature and pressure, are out of the question. Without fail, enzymes are biological catalysts (proteins). They often need the presence of other molecules for their function. Because enzymes are so specialised, very few useless byproducts are produced. Enzymes allow the cell to carry out many thermodynamically feasible and impractical processes concurrently.

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CHAPTER 5

BIOLOGICAL MACROMOLECULES: PROPERTIES, STRUCTURE, AND FUNCTION

Dr. Nayana Borah, Assistant Professor,
Department of Microbiology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: b.nayana@jainuniversity.ac.in

ABSTRACT:

As we covered in the last chapter, carbohydrates are made up of monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Simple sugars, often known as monosaccharides, serve as the building blocks or monomers from which other forms are assembled. Two monosaccharide residues connected by glycosidic linkages make up disaccharides. This bond develops between the OH group of the anomeric carbon of one sugar (carbon No. 1) and the OH group of any other carbon atom of another sugar, ideally in the 4th or 6th position. In the case of oligosaccharides, the number of monomers ranges from three to ten, but it is undetermined in the case of polysaccharides. The structural polysaccharides are these polysaccharides. The primary component of plant cell walls and the most prevalent biopolymer in the biosphere is cellulose, a structural polysaccharide. Other examples of structural polysaccharides include the substance that makes up the exoskeleton of insects, chitin, and the primary components of bacterial cell walls, peptidoglycan.

KEYWORDS:

Biological Macromolecules, Properties, DNA, Structure, Function.

INTRODUCTION

The polymers of sugars and their numerous derivatives, such as glucosamine and galactosamine, bound together by glycosidic linkages, are referred to as polysaccharides. Storage polysaccharides and structural polysaccharides are additional categories for polysaccharides based on how they operate in biological systems. Polysaccharides are mostly stored as starch in plants and as glycogen in mammals. They serve as both plants' and animals' primary sources of energy. Moreover, there are polysaccharides that sustain the organism as a whole as well as the cells or individual cell components mechanically. Homopolysaccharides and heteropolysaccharides are terms used to describe polysaccharides that are made up of different types of sugars or sugar derivatives, respectively. Homopolysaccharides are defined as those that include the same kind of monomers or sugars.

Peptidoglycan is a heteropolysaccharide found in bacterial cell walls that gives the cells their stiffness and form. It is a polymer made up of alternating units of a sugar or sugar derivative, such as N-acetyl glucosamine and N-acetyl muramic acid. The polymer of a disaccharide, made up of two different sugars or their derivatives, may also be used to characterise it. Like with other structural polysaccharides, -1 > 4 connections bind the monomers together. Due to the existence of -1 > 4 links, most structural polysaccharides are straight chain molecules.The precursor ingredient for the formation of polymeric carbohydrates is glucose, along with other

phosphorylated sugars and their derivatives. For instance, phosphorylated glucose is used as a precursor in the biosynthetic pathways of several polysaccharides, including those that make up the structural components of plant cells, cellulose, and storage substances like starch. Like all other beginning molecules in biosynthetic pathways, glucose must first be activated if it is not already. Either phosphorylation or the binding of a sugar residue to a nucleotide causes this to happen (like ATP). As a result, glucose-6-phosphate is created.

A crucial step in both glycolysis and photosynthesis is fructose-6-phosphate. It and glucose-6-phosphate are in equilibrium, and glucose-1-phosphate and this compound are also in equilibrium. UDP-glucose is produced by the reaction between glucose-1-phosphate and UTP (uridine triphosphate), and it again polymerizes with fructose-6-phosphate to create saccharose phosphate. Saccharose (sucrose) is created once the phosphate is broken down. Chloroplasts participate in the reaction. Several varieties of disaccharides are created in a similar manner. Glycolipids and glycoproteins may also contain UDP-glucose, but not ADP-glucose.Glucose-1-phosphate is joined with ATP in the case of starch to create ADP-glucose. ADP-glucose that re-polymerizes with Glucose-6-phosphate to create a disaccharide that then goes through further polymerization to create polysaccharide phosphate to create starch, phosphate is separated from the carbohydrate. Chloroplasts also participate in this process to some extent.

Proteins

The actions of every cell are significantly influenced by proteins or polypeptides. They function as biological catalysts (i.e., enzymes), participate in the control of cellular metabolism and cell-to-cell communication, and are necessary for the formation of certain structures. These are linear chains made up of a series of 20 amino acids arranged in various ways and joined only by peptide bonds.The main amino group of one amino acid reacts with the primary carboxyl group of another amino acid to produce peptide bonds, which are then created by the elimination of a water molecule. There is a condensation reaction as a result. The polypeptide chain develops polarity as a result of this kind of connection. The C-terminus is the end that is terminated by a free carboxyl group, whereas the N-terminus is the end that is terminated by an amino group. As protein synthesis occurs from the N- to the C-terminus, amino acid sequences are written in this manner. The nucleotide sequence of the gene, the section of the DNA strand that codes for the protein, determines the precise sequence of amino acids, commonly known as the protein's fundamental structure. The amino-acid sequence of the polypeptide directly affects both the protein's three-dimensional structure and its ability to perform its intended function [1]–[3].

The Composition of Amino Acids and Protein Sequencing

Protein Composition

The peptide is first hydrolyzed into its individual amino acids by boiling in 6 N HCl at 110°C for 24 hours in order to ascertain the makeup of amino acids. By using ion-exchange chromatography, the amino acids in the hydrolysate may be isolated, and then they can be hydrolyzed by interacting with ninhydrin. When processed in this manner, alpha amino acids produce a vivid blue hue, while amino acids like proline produce a yellow tint. The optical absorbance of a solution after being heated with ninhydrin is inversely related to the concentration of amino acids in the solution. A microgram (10 n mol or nanograms) of an amino acid may be found using this method. One may go on to sequence the amino acids for a certain protein or polypeptide if they have knowledge of the relative amounts of each amino acid and

their makeup. Large, intricate, and necessary for life, biological macromolecules are constructed from smaller organic molecules. The four main categories of biological macromolecules are lipids, proteins, carbohydrates, and nucleic acids. Each of these macromolecules has a vital function in the cell and carries out a variety of tasks. While lipids are involved in energy storage, membrane construction, and signalling, carbohydrates are employed for energy storage and as structural elements. Proteins perform a wide variety of tasks, including as regulating gene expression, supporting structural processes, and catalysing enzymatic reactions. The storage and transfer of genetic data are carried out by nucleic acids.

Basic chemistry concepts including covalent and non-covalent bonding, polarity, bond rotations and vibrations, and the hydrophobic effect all influence the structure and function of macromolecules. Understanding macromolecules is crucial for comprehending the molecular basis of life and has important ramifications for a number of disciplines, including environmental science, biotechnology, and medicine. In order to illustrate the significance of biological macromolecules for the continuation of life and the variety of roles they play in cellular activities, this article gives an overview of their characteristics, structure, and functions. Overall, biological macromolecules play a variety of vital roles in the body and are necessary for life. They support the structure, hold energy, catalyse metabolic processes, convey genetic information, control cellular functions, and shield cells and tissues from damage. For improving our understanding of the molecular foundations of life and creating innovative approaches for medicine, biotechnology, and environmental science, it is essential to comprehend the characteristics, structure, and function of biological macromolecules.

Proteins' Three-Dimensional Structure

The amino acid sequence or the protein's fundamental structure determines the molecular form that each protein exhibits. Christian Anfinsen initially proved this using the RNA-hydrolyzing enzyme pancreatic Ribonuclease A. (ribonucleic acids). He demonstrated that when exposed to potent substances like urea or heat, a pure sample of ribonuclease A lost most of its features, including catalytic activity. When the denaturing agent (high temperature or chemicals like urea) was removed, the enzyme was able to restore the majority of its properties and three-dimensional structure. This basically illustrated two points. 1) A protein's three-dimensional structure and function are inextricably intertwined. 2) The sequence of the amino acids or the protein's fundamental structure contains all the data required for the polypeptide to fold into a three-dimensional structure.

Enzymes

The biocatalysts that direct practically all biological processes are enzymes. A substance known as a catalyst is one that speeds up a chemical process without affecting its quality or quantity. Enzymes are globular proteins with distinct functions, natural conformations, electrical charge distributions, and surface geometries, all of which are dependent on the tertiary structure of the individual enzymes. The three-dimensional form is determined by the tertiary structure. They are in charge of regulating metabolism since they are in charge of a single response. A typical prokaryotic cell has roughly 700 enzymes, while eukaryotic cells have thousands of enzymes.Catalysts are chemicals that promote (speed up) processes without directly participating in the reaction, and enzymes serve in this capacity. A single enzyme molecule may mediate hundreds of reactions in a single second due to its frequent utilisation. Even simple processes,

like the solubility of carbon dioxide in water, do not occur to a significant amount on their own. Yet, we may use high pressure to make it dissolve in water at larger quantities. Drinks with carbonation include high-pressure CO2. Several CO2 bubbles will discharge when the cap is removed to alleviate the pressure. Yet, under typical circumstances, the rate of CO2 dissolution in biological systems is more than 10.6 times faster than that of uncatalyzed processes. The carbonic anhydrase enzyme, which mediates the process, makes this feasible. Similar to this, every reaction in biological systems is mediated by one or more enzymes, which causes reactions to occur more quickly.Table 1 comparing the Composition of Monomers for the Four Biological Macromolecules Practice.

Table 1: Comparing the Composition of Monomers for the Four Biological Macromolecules Practice.

Macromolecule	Monomer	Basic Chemical Formula	Example
Carbohydrates	monosaccharide	CHO	Sugar, starch, cellulose
Nucleic Acids	nucleotides	CHONP	Deoxyribonucleic Acid, Ribonucleic Acid
Proteins	Amino acids	CHON + R-group	Protein hormones, enzymes
Lipids	Fatty acid and glycerol	CHO	Cholesterol, butter

C=carbon, H=hydrogen, O=oxygen, N=nitrogen, P=phosphate

Heat causes molecules to move more quickly and increases the likelihood of collision. This requires heat, which is often improper within a cell. To improve the possibility that reactants will collide in the proper spot, enzymes may be utilised in place of heat. Reactants are brought together by enzymes, which also maintain their proper orientation with regard to one another. Atoms engage in reactions that result in changes to the electron distribution. Let's talk about the pace of a reaction before getting into the specifics of the mechanism of enzyme function.

Extremely Specific Enzymes

In comparison to substrate molecules, enzyme molecules are enormous. To create the enzyme substrate complex, the substrate molecules attach to the enzyme molecules at specified locations on their surfaces, known as substrate binding sites or active sites (ES complex). With the release of free and undamaged enzyme, the ES complex ultimately ends up in the final product. The reactions that result in the creation of the product are carried out by the enzyme's active site. Just

a very tiny section of the enzyme's molecule is its active site, which complements the substrate's molecular structure. This is the primary explanation for an enzyme's substrate specificity. Molecular collisions between the reactants or substrates are often what cause chemical reactions to take place. The substrate molecules are brought close together in enzyme-mediated reactions by being absorbed by the enzyme's active sites. The process is thus made easier by the enzyme molecule.

DISCUSSION

It is believed that enzymes work on a geometric premise. The substrate binding sites in the tertiary and quaternary structures of an enzyme have the exact same shape as the substrate molecules. When a key fits in a keyhole, this aids in the binding of the proper substrate to the active centres. The Lock and Key hypothesis is a theory that describes how enzyme substrate contact works. Induced fit is the mechanism by which certain enzymes modify the shape of the active site just enough to fit the substrate molecules. The induced fit theory is the name for this hypothesis. The resulting enzyme substrate complex decreases the activation energy by either straining an already-existing link or properly positioning two molecules to promote a reaction. Due to its geometrical and electrical structure, the enzyme keeps the substrate molecules precisely where they need to be in relation to one another to support the reaction. The distinctive functional groups of the amino acid residues located in the enzyme's active region may interact with the substrate molecule's different bonds. The reaction takes place, and the new product molecule escapes from the enzyme as a result of diffusion gradients, new electrical forces that are repulsive, or changes in the structure of the enzyme or the product. The location of fresh substrate molecules changes [4]–[6].

Chemical Acids

The fundamental building block of genetic material, nucleic acids, were discovered by Friedrich Miescher in 1869. He also showed that blood CO2 content affects how breathing is regulated.Using phosphodiester bonds, nucleic acids are polymers made of nucleotides (polynucleotides). The two kinds of nucleic acidsribonucleic acids (RNA) and deoxyribonucleic acidsdepend on the type of sugar (ribose or deoxyribose) that is present in the nucleotide (DNA). RNA participates in protein synthesis, serves as a messenger (messenger RNA), and is recognised to be the carrier of genetic information (transferRNA, ribosomalRNA).

Adenosine Triphosphate (DNA)

Ribonucleic Acids (RNA) RNA is a kind of non-genetic nucleic acid, unless it is the genetic material of some viruses like HIV. They are typically straight, single-stranded molecules and relatively tiny. Cells have RNA in four main types. These are small nuclear RNA (snRNA), ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA) (snRNA). For the goal of synthesising proteins, all of these RNA molecules are created based on the DNA's nucleotide sequence.Except for certain viruses, which contain RNA as their genetic material and are referred to as RNA viruses, practically all creatures have DNA, or deoxyribonucleic acid. The very long polymer DNA is always a double-stranded helical molecule [7]–[9].

CONCLUSION

The nitrogen bases adenine (A), thymine (T), cytosine (C), and guanine (G) are found in DNA (G). Erwin Chargaff extracted DNA samples from several creatures, examined the nitrogen-

based makeup, and discovered that A, T, C, and G are all equally prevalent. Every species has its own particular A + T / C + G ratio. The Chargaff rule applies here. No matter where they came from, all double-stranded DNA samples adhere to the Chargaff principle of base equivalency. There are certain DNA viruses with single-stranded DNA as their genetic material. Base equivalency according to the Chargaff rule is not followed by single-stranded DNA. James Watson and Francis Crick established the Watson - Crick Model for the structure of DNA based on these findings plus x-ray diffraction data Rosalind Franklin collected from DNA crystals. In 1953, they made their discoveries public in the journal Nature.

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CHAPTER 6

STRUCTURE AND FUNCTION OF MACROMOLECULE

Roopashree Rangaswamy, Assistant Professor,
Department of Bio Chemistry, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: r.roopashree@jainuniversity.ac.in

ABSTRACT:

We have discussed the structure and dynamics of smaller biomolecules, which form the building blocks of the important cellular macromolecules, in earlier chapters. These biomolecules can undergo polymerization or condensation to form specific polymers of high molecular weight known as macromolecules. These macromolecules are of four distinct groupscarbohydrates, proteins, nucleic acids, and lipids. All these macromolecules are specialized for carrying out specific cellular functions, which are very closely related to their functions. So a clear understanding of their structure is required for the proper understanding of their functions in the cell metabolism. There are two types of glycosidic linkages depending on the types of anomers. Starch is the main storage form of polysaccharides in plants and glycogen in animals. They form the main energy sources for both plants and animals. There are also polysaccharides that give mechanical support to the cells or cell components or to the organism as a whole. Such polysaccharides are the structural polysaccharides. Cellulose, a structural polysaccharide, is the main component of the plant cell walls and is the most abundant biopolymer in the biosphere. Chitin, the material of the insect exoskeleton and peptidoglycan, the main components of the bacterial cell walls, are other examples of structural polysaccharides.

KEYWORDS:

Cells, Carbohydrates, Macromolecule, biomolecules, Lipids.

INTRODUCTION

Carbohydrates, as we have discussed in the previous chapter, consist of monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides, or the simple sugars, are the building blocks or the monomers by which other forms are constructed. Disaccharides consist of two monosaccharide residues linked together by glycosidic bonds. This bond forms between the OH group of anomeric carbon (carbon No.1) of one sugar and with the OH group of any other carbon atom, preferably of 4th or 6th position of another sugar. The number of monomers varies from three to ten in the case of oligosaccharides, and it is indefinite in the case of polysaccharides. The polysaccharides are defined as the polymers of sugars and their various derivatives, such as glucosamine and galactosamine linked together by glycosidic bonds [1]–[3].If the polysaccharides are composed of the same type of monomers or sugars, they are called homopolysaccharides and if more than one type of sugar or sugar derivatives are involved in the formation of the polysaccharides, they are called heteropolysaccharides.

Cellulose:

No branchings occur in cellulose, a linear molecule. Neighboring cellulose chains may form hydrogen bonds leading to the formation of microfibrils with partially crystalline parts

(micelles). Cellulose is the most important structural component of nearly all green plants' cell walls. The glucose units are linked by -1 > 4-glycosidic linkages.

Peptidoglycan

This is a heteropolysaccharide present in the cell walls of bacteria imparting rigidity and shape to the cells. It is a polymer of alternating units of a sugar or sugar derivatives; e.g., N-acetyl muramic acid and N-acetyl glucosamine. Or it can be defined as the polymer of a disaccharide, consisting of two types of sugars or their derivatives. The monomers are linked by -1 > 4 linkages as in the case of other structural polysaccharides. Most of the structural polysaccharides are straight chain molecules because of the presence of -1 > 4 linkages.

Synthesis of Carbohydrates (polysaccharides)

The characteristic biosynthetic pathway which leads to the synthesis of polymeric carbohydrates such as starch, cellulose, etc., in plants, and glycogen in animals, occurs as a part of photosynthesis in plants and as a part of carbohydrate metabolism in animals.Glucose and other phosphorylated sugars and their derivatives are the starting compound for the synthesis of polymeric carbohydrates. For example, the biosynthetic pathways of many polysaccharides that are the structural elements of the plant cells and the cellulose and storage compounds such as starch, are synthesized using the phosphorylated glucose as the precursors. If the glucose is not already activated, like all other starting compounds of biosynthetic pathways it first has to be activated. This occurs either by phosphorylation or by binding of a sugar residue to a nucleotide (like ATP). Thus, glucose-6-phosphate is formed.

Fructose-6-phosphate is an important intermediate of both photosynthesis and glycolysis. It is at equilibrium with glucose-6-phosphate and this again is at equilibrium with glucose-1-phosphate. Glucose-1-phosphate and UTP (Uridine Triphosphate) react to UDP-glucose that again polymerizes with fructose-6- phosphate to form saccharose phosphate. Upon cleavage of the phosphate, saccharose (sucrose) is produced. The reaction takes part in chloroplasts. Similarly, other types of disaccharides are produced. UDP-glucose (but not ADP-glucose) can also be incorporated into glycolipids and glycoproteins.

In the case of starch, glucose-1-phosphate is coupled to ATP to form ADP- glucose. ADP-glucose that again polymerizes with Glucose-6-phosphate to form a disaccharide, which undergoes further polymerization to form the polysaccharide phosphate. Phosphate is cleaved off from the polysaccharide to generate the starch. This reaction also takes place in part in chloroplasts.Two enzymes are necessary for starch production: one for the start and the chain elongation (-1 > 4 glycosidic linkage) with monosaccharide units, the other for the introduction of -1 > 6 glycosidic linkages in amylopectins, as well as for linking together different chains (via alpha 1 > 4 glycosidic bonds).

Proteins

Proteins or polypeptides play an outstanding part in all cell activities. They act as biological catalysts (= enzymes), take part in the regulation of the cell's metabolism and in the interaction between cells, and are required for the generation of specific structures. They are linear chains consisting of a sequence of 20 amino acids in different combinations linked exclusively by peptide bonds. The different combinations and sequences of amino acids are responsible for the diverse nature and functions of proteins. The peptide bonds are formed by the reaction of the

primary amino group of one amino acid with primary carboxyl group of another amino acid with the elimination of a molecule of water. Thus, it is a condensation reaction. This type of linkage causes a polarity for the polypeptide chain. One end has the amino group and is called the N-terminus, while the other end is terminated by a free carboxyl group and is called the C-terminus. Amino-acid sequences are written from N- to C-terminus, the direction in which protein synthesis proceeds. The exact sequence of amino acids (also called the protein's primary structure) is determined by the nucleotide sequence of the gene, the part of the DNA strand, which codes for the protein. The three-dimensional structure and the function of the protein are very closely dependent on the amino-acid sequence of the polypeptide.

There are three types of proteins based on their complexity. Some proteins are made up of a single polypeptide chain and are known as simple proteins. Those proteins having two or more polypeptide chains are called complex proteins. In some cases, the protein molecule is associated with a non-protein component known as the prosthetic group. Such proteins are known as conjugated proteins. The non-protein component may be metallic ions such as Zn+ in the case of carbonic anhydrase, hem part of hemoglobin enclosing Fe+ ion in it, or organic molecules such as vitamin derivatives such as NAD and NADP, nucleotides such as ATP and GTP, or maybe sugars, oligosaccharides, or various types of lipids.

1. Amino Acid Composition and Protein-Sequencing
2. Amino acid Composition

To determine the amino acid composition the peptide is first hydrolyzed into its constituent amino acids by heating in 6 N HCl at 110°C for 24 hours. The amino acids in the hydrolysate can be separated by ion-exchange chromatography and hydrolyzed by reacting them with ninhydrin. Alpha amino acids treated this way give an intense blue color, whereas amino acids, such as proline, give a yellow color. The concentration of amino acids in a solution is proportional to the optical absorbance of the solution after heating it with ninhydrin. This technique can detect a microgram (10 n mol or nanograms) of an amino acid. After getting the information about the amino acid composition and relative quantity of each amino acid, one can proceed to do the sequencing of amino acids for a particular protein or polypeptide.

Amino Acid Sequencing

The amino acid sequence of a protein is very important because it is essential to know the structure and function of that protein; and also it can help in identifying and isolating the gene code for the protein. So obtaining at least a partial amino acid sequence is a critical first step in studying many proteins.The first protein sequenced was the peptide hormone insulin, which controls the glucose level in blood; its deficiency can lead to the metabolic error, diabetes. Nobel Laureate, Frederic Sanger, showed for the first time that proteins have a definite amino acid sequence and a specific three-dimensional structure that is determined by the amino acid sequence. He found out that certain reagents such as fluoro-dinitro-benzene (FDNB) known as Sanger's reagent can react specifically with the free NH_2 group of the amino acid at the N-terminal of a polypeptide. Fluoro-DNB reacts with the N-terminal amino acid and on acid hydrolysis it yields a yellow DNP derivative of the amino acid, which can be separated and identified by ion-exchange chromatography. This procedure cannot be used on the same sample of polypeptide because the peptide is totally hydrolyzed on the acid hydrolysis step. But Sanger managed to use this technique with new samples of peptide in each cycle of experiments to

sequence insulin. He used more than a gram of insulin for completing this task. Now this method is used only for identifying the N-terminal amino acid of a polypeptide and not for sequencing because of the above-mentioned demerit.

The most popular direct protein-sequencing technique in use today is the Edman degradation procedure. The Edman reaction is a series of chemical reactions, which remove one amino acid at a time from amino terminus of a protein, releasing an amino-acid derivative, phenylthiohydantoin (PTH), that may be chromatographically identified (reversed phase). The Edman reagent, Phenylthiocyanate, reacts with the NH2 group of the terminal amino acid and forms an intermediate, phenylthiocarbomyle derivative. A simplified schematic diagram of the process. Release of amino acids from the amino terminus in a sequential manner is possible because the Edman procedure consists of three chemical reactions, which proceed under different pH conditions

The whole method, including the reaction steps and the identification of PTH- amino acid derivative, is automated and the instrument that can carry out these reactions is known as a sequenator. Using this automated instrumentation, more than 100 amino acids of a polypeptide can be sequenced efficiently. Polypeptides and proteins of high molecular weight have to be fragmented into smaller polypeptides of 50 to 100 amino acids before carrying out the sequencing by a sequenator. Amino acid sequencing by the Edman degradation reaction can be carried out with very minute quantities of protein to the level of micrograms. There are a number of physical techniques like mass spectrometry, NMR (nuclear magnetic resonance spectrometry), and x-ray diffraction techniques used in protein analysis and structural elucidation. There are various types of mass spectrometric techniques for the analysis of proteins. For example, matrix assisted laser desorption ionization (MALDI), mass spectrometry, and tandem mass spectrometry (2D-MS or MS-MS) are efficient techniques developed to identify and sequence proteins rapidly.

Three-Dimensional Structure of Proteins

All proteins exhibit a specific molecular shape, which is determined by the amino acid sequence or the primary structure of the protein. This was first demonstrated by Christian Anfinsen on enzyme pancreatic Ribonuclease A, which hydrolyzes RNA (ribonucleic acids). He showed that a pure sample of Ribonuclease A lost its three-dimensional structure and most of its properties including catalytic activity when treated with strong chemicals like urea or by heating. The enzyme could regain most of its properties and three-dimensional structure when the denaturing agent (high temperature or the chemicals like urea) was removed. This demonstrated mainly two things. 1) The function of a protein is closely linked to the three-dimensional structure of the protein. 2) All the information necessary for folding the polypeptide into three-dimensional structure is present in the sequence of the amino acid or the primary structure of the protein.

Based on the molecular shape at its three-dimensional structure, proteins are classified into two categoriesglobular proteins and fibrous proteins. Globular proteins have globular three-dimensional shapes and are therefore soluble proteins. Fibrous proteins have a long, thin fiber-like structure and are insoluble proteins.The structure of protein includes three stages in its transition from amino acid sequence to the functional three-dimensional structure. In multimeric proteins where there are more than one polypeptide chains, there are four stages in the process of folding and conversion to the functional three-dimensional stage. They are primary structure, secondary structure, tertiary structure, and. The sequence of R-groups along the chain is called

the primary structure. Secondary structure refers to the local folding of the polypeptide chain. Tertiary structure is the arrangement of secondary structure elements in three-dimensions and quaternary structure describes the arrangement of a protein's sub-units.

Primary Structure of Proteins

The primary structure of a protein or polypeptide is the sequence of amino acids joined by the peptide bonds formed between the COOH groups of one amino acid with the NH2 group of the other. The primary structure of a protein can be determined by the Edman degradation reaction or it can be obtained from cloning and sequencing the gene responsible for the production of the protein. Size of the protein molecule can be determined by SDS-PAGE (sodium dodecyle sulfate polyacrylamide gel electrophoresis) or electrospray-mass spectrometry (ES-MS).The main molecular forces in the primary structure are the covalent linkages— the peptide bonds between amino acidsand the disulphide linkages between the cysteine units of the polypeptides. The peptide linkages are partial double bonds and are comparatively rigid, restricting the free rotation about the bond. All peptide bonds in protein structures are found to be almost planar.

Secondary Structure of Proteins

The peptide bond has some double-bond character (40%) due to resonance. The double bond of C=O alternates with the C-N of the peptide unit. As a consequence of this resonance, all peptide bonds in protein structures are found to be almost planar; i.e., atoms between two C□ atoms (C, O, N and H) are approximately co- planar. This rigidity of the peptide bond reduces the degrees of freedom of the polypeptide during folding. One can visualize the polypeptide chain consisting of rigid planes of peptide units interconnected by C□ atoms of each amino acid residue.

Tertiary Structure of Proteins

The regular polypeptide structures or the secondary structures are assembled, or grouped together, by specific folding of the polypeptide units such as helices and sheets grouped together to form a specific shape. It is only with the complete, compact folding into tertiary structure that the proteins attain their "native conformation" and become active proteins (as a result of the creation of active sites). Forces that contribute to tertiary folding include: hydrogen bonds, hydrophobic interactions, and ionic bonds. Sulfhydryl bonds (-S-S- bonds) mentioned earlier, are a force contributing to the tertiary structure, but now they are considered a molecular interaction contributing to the primary structure of polypeptide along with peptide bonds. These are especially important because they are covalent bonds and quite strong compared to H-bonds. It was observed that certain combinations of secondary structures like helix and sheets are conserved in certain classes of proteins. These groups of secondary structures are known as motifs. The motifs form the structural elements of proteins since they are conserved. The presence of certain conserved domains and motifs in the tertiary structure of protein can be used for predicting the function of a protein or its identification.

Quaternary Structure of Protein

Some proteins are made of more than one polypeptide chain. These proteins are referred to as multimeric proteins. The polypeptides and their tertiary structures are assembled to form the native conformation and become functional proteins. The individual polypeptides are known as the subunits, which must be assembled together after each individual polypeptide has reached its tertiary structure. Examples include hemoglobin (blood protein involved in oxygen transport),

which has four subunits. Pyruvate dehydrogenase (mitochondrial protein involved in energy metabolism) has 72 subunits. There is no involvement of covalent linkages in the assembly of these subunits. The main molecular interactions involved in the assembly of subunits and formation of quaternary structures include hydrophobic and electrostatic attractions in addition to the weak Van der Waal's attractions.

Enzymes

Enzymes are the biocatalysts that control almost all cellular reactions. A catalyst is a compound, which accelerates a chemical reaction without undergoing any change in quality or quantity. Enzymes are globular proteins, each with a specific structure (native conformation), function, distribution of electrical charges, and surface geometry whose specificity depends on their tertiary structure. The tertiary structure determines the three-dimensional shape. They are responsible for control of a single reaction and are thus responsible for control of metabolism. There are about 700 enzymes in a typical prokaryotic cell and there are thousands in a eukaryotic cell.

Enzymes function as catalysts, which are substances that facilitate (speed up) reactions without actually entering into the reaction. They are used over and over, and a single enzyme molecule may mediate thousands of reactions in a single second. Even simple reactions like dissolution of carbon dioxide in water will not take place to an appreciable extent by itself. But we can make it dissolve in water in higher concentrations under high pressure. Carbonated drinks have CO2 under high pressure. On releasing the pressure by removing the cap, lots of CO2 bubbles will release. But in biological systems the dissolution of CO2 takes place under normal conditions at a rate more than 10.6 times that of uncatalyzed reactions. This is possible because of an enzyme known as carbonic anhydrase, which mediates the reaction. Similarly, all reactions in biological systems are mediated by one or more enzymes and so reactions take place at higher speeds.Enzymes operate on reactants, which are known as substrates, and convert them into products. The reaction may require energy or it may release energy. The enzyme is unaffected by the reaction.

Rate of Enzymatic Reactions

The rate of reaction defines the speed or the velocity of the reaction. The velocity of a reaction can be expressed in two ways. It can be expressed either in terms of the product formed or the substrate consumed. The rate or velocity of a reaction can also be defined as the quantity of the product formed per unit time (per second or per minute) or the quantity of the substrate consumed per minute. The quantity can be expressed in milligrams (mg) or micrograms (□g); and it can also be expressed in moles (M), millimoles (mM), or micromoles (□M).

Rate of a reaction = quantity of product formed per minute

In the case of enzyme-mediated reactions, the rate of reaction is usually represented as enzyme activity, which is related to the quantity of enzymes. The quantity of enzymes is expressed as units. It is the international unit (IU) for expressing the quantity of enzymes.

Enzymes are Highly Specific

Enzyme molecules, compared to substrate molecules, are very large. The substrate molecules bind to the enzyme molecules at certain specific sites on the surface of the enzyme molecule,

known as substrate binding sites or actives sites, to form the enzyme substrate complex (ES complex). The ES complex finally ends up in the product with the release of free and intact enzyme. The active site of the enzyme is responsible for the reactions leading to the formation of the product. The active site in the enzyme molecule is only a very small portion and is complementary to the molecular shape of the substrate. This is the main reason for the substrate specificity of an enzyme. The chemical reactions normally occur because of the molecular collisions between the reactants or the substrates. In enzyme-mediated reactions the substrate molecules are brought in proximity by taking them into the active sites of the enzyme.

1. Mechanism of Enzyme Action
2. Energy of Activation

Reactions involve making and breaking bonds. Some bonds must be broken at the start of a reaction and this requires energy. No matter how exergonic (reactions that proceed with the release of energy) the overall reaction may be, some energy must be added initially to break the necessary bonds and get the reaction started (e.g., match to paper, spark to cylinder). This is the energy of activation (Ea). The activation energy can be defined as the energy needed by a molecule to take part in a reaction. This energy is often supplied as heat but this may not be practical in a cell.All organic molecules contain energy that could be released by the breakdown of the molecule. Such a reaction would be strongly exergonic, and it does not happen spontaneously because the activation energy must be provided to get the reaction started and this energy is usually not available. To start the reaction, it is necessary to provide the activation energy, or else reduce the amount of activation energy needed.

Enzymes accelerate reactions more likely by reducing the energy required for activation. Enzymes bring down the activation energy by holding the reactants (substrates) in exactly the right orientation to each other so that contact will result in a reaction each time.Enzymes are thought to operate on a geometric principle. The tertiary and quaternary structures of an enzyme have the substrate binding sites, which have exactly the complementary shape of the substrate molecules. This helps in the binding of the appropriate substrate to the active centers just like a key fits in the keyhole. This hypothesis that explains the mechanism of enzyme substrate interaction is known as the Lock and Key hypothesis. Some enzymes change the shape of the active center slightly to accommodate the substrate molecules, a process known as induced fit. This theory is known as the induced fit theory. The enzyme substrate complex thus formed lowers the energy of activation by either stressing an existing bond or correctly orienting two molecules to favor a reaction. The enzyme holds the substrate molecules in exactly the right position relative to each other to facilitate the reaction due to geometric and electrical configuration. The characteristic functional groups of the amino acid residues present in the active sites of the enzyme molecule can interact with the various bonds of the substrate molecule. The reaction occurs and the new product molecule leaves the enzyme due to diffusion gradients, or to new repulsive electrical forces or the shape changes in either the enzyme or the product. New substrate molecules move into position.

Enzyme Kinetics

The velocity of an enzyme-mediated reaction or the enzyme activity is closely controlled by a number of factors including the environmental (cellular environment) factors such as temperature and pH. The other factors are the substrate and enzyme concentrations.The rate of a

reaction can be measured either by monitoring the formation of the product or by the disappearance of substrate. A specific quantity of enzymes is allowed to interact with an excess amount of substrate for a specific period of time. The product formed can be measured by exploiting any of its physical or chemical properties such as color development, absorbance at a particular wavelength of light radioactivity, etc. For example, if the product formed is colored or can develop any color with some other reagent, the intensity of the color can be measured by an absorption spectrophotometer. The enzyme-mediated reaction has to be conducted under physiological conditions by providing a buffer of appropriate pH and optimum temperature usually between 20°C and 37ºC. It is also necessary to provide co-factors and metal ions essential for the specific enzymes. All these physiological factors have to be maintained in vitro to keep the protein in its specific three-dimensional conformation and active state. Change in any of the parameters, particularly factors such as temperature, pH, and ionic strength can destroy the enzyme conformation or the three-dimensional structure. This change of conformation in the three-dimensional structure of an enzyme followed by the loss of activity is called denaturation.

Effect of Substrate Concentration

Substrate concentration is one of the factors that affect the rate of an enzyme- catalyzed reaction. Velocity of an enzyme-catalyzed reaction at different substrate concentrations can be measured, and the values can be plotted on a graph with substrate concentration on the x-axis and its corresponding velocity on the y-axis. It can be observed from the graph that as the concentration of substrate increases there is a corresponding increase in the velocity of reaction. However, beyond a particular concentration of substrate concentration, the velocity remains constant without any further increase. This maximum velocity of an enzyme-catalyzed reaction under substrate saturation is called the Vmax. A reaction attains its Vmax when all the active sites of the enzyme molecules are saturated with its substrate molecules so that the rate of product formation will be at its maximum. There is a substrate concentration at which the reaction attains its Vmax. Similarly, there is a substrate concentration at which the velocity of the reaction is half of the Vmax. This substrate concentration is a constant for a particular enzyme and that is known as Km or MichaelisMenton constant. Km value of an enzyme is inversely proportional to affinity of the enzyme toward its substrate. The turnover number is the number of substrate molecules converted to product per second by one molecule of enzyme. It can be calculated from the Vmax. It indirectly indicates the catalytic power of an enzyme. Higher values of turnover number point toward better catalytic power of the enzyme.

Effect of Enzyme Concentration

The effect of enzyme concentration on the rate of reaction is more or less similar to that of substrate concentration. Under excess of substrate concentration, if you increase enzyme concentration there is a corresponding increase in the rate of reaction, but only up to a particular point. After this point there is no further increase in the rate of reaction corresponding to the increase of enzyme concentration.

Effect of Temperature and pH

Enzymes always exhibit typical temperature and pH optima that are characteristics of that enzyme and appropriate for its function. Most of the enzyme works in the physiological pH, which is 7.5, even though their optimal pH is different.

Mammalian enzymes, for example, always show temperature optima around 37°C while those of the ice fish are around 0°C. Most enzymes are denatured at 45°C or so, but not those of thermophilic organisms.

Classification of Enzymes

- Enzymes are classified into six major groups or classes based on the reactions they catalyze.
- Enzyme Inhibition
- Enzymes may be regulated in several ways: by temperature and pH as mentioned earlier or by control of synthesis or locally (i.e., after its synthesis).

We are interested in the latter here. There are certain compounds called enzyme inhibitors, which inactivate the enzyme permanently or temporarily. There are many types of inhibitors. Some are deleterious (poisons) and some are important regulatory mechanisms. Some inhibitions are deliberate control mechanisms; some are detrimental to the organism. Enzymes are often inactivated by some molecule (an inhibitor) that changes their shape or blocks the active site.

Competitive Inhibitors

These are molecules that bind reversibly or irreversibly to the active site. They compete with the substrate for space in the active site. Most naturally occurring competitive inhibitors are irreversible.

Reversible Competitor

These competitors are not the substrate molecules but molecules similar to the substrate molecules. Thus, they compete with the substrate to occupy the active center of the enzyme molecule.

If they are in high concentration, they may essentially inactivate the enzyme. These are of limited importance in natural systems.

The competition is reversible and the competitor can be overwhelmed by high concentration of substrate. For example, fumaric acid is the competitive inhibitor of the enzyme, succinic dehydrogenase. The structure of fumaric acid is similar to that of succinic acid, the actual substrate of the enzyme.

Irreversible Competitor

The competitor and substrate both compete for the active site but the competitor occupies the active site permanently thus deactivating the enzyme. Non-classical competitive inhibition is the binding of S at the active site and prevents the binding of I at a different site. The reverse is also true. Carbon monoxide is an irreversible competitive inhibitor of hemoglobin. Oxygen is the substrate

Non-Competitive Inhibition

In non-competitive inhibition the inhibitor and substrate do not compete for space in the active site. The substrate enters the active site but the inhibitor reacts with some other part of the enzyme molecule. It may be reversible or non-reversible. Reversible non-competitive inhibition is a major metabolic control mechanism.

Reversible Non-competitive

This type of inhibition involves two binding sites on the enzyme molecule: the usual active site and a second regulatory site where the inhibitor binds. The inhibitor and the substrate do not compete for the same site. If the regulatory site is occupied by the inhibitor then the shape of the active site is changed so that the substrate molecules cannot fit and react. This type of inhibition, often called allosteric control, is very important in regulating metabolic pathways in the cell.In allosteric control the final product molecule often acts as an inhibitor of one of the enzymes in the pathway, typically the first.When high concentrations of the final product accumulate, some of them react with the first enzymes at its regulatory site and render it inactive. This eventually results in a decrease in the concentration of the product. When this happens the inhibitor will drop out of the regulatory site and the enzyme will become active again. This is an example of negative feedback. This is a type of reaction that we will see over and over again in biology, at all levels from cell to ecosystem. Negative feedback prevents runaway reactions. The thermostat on ACs, furnaces, and incubators is a negative feedback mechanism. When temperature is raised over the control point the heating system of the incubator is turned off.

Nucleic Acids

In 1869, Friedrich Miescher discovered nucleic acids, the molecular substrate of the genetic material. He also demonstrated that the regulation of breathing depends on CO2 concentration in the blood.Nucleic acids are polymers consisting of nucleotides that are linked by phosphodiester bonds (polynucleotides). Depending on the type of sugar present in the nucleotide (ribose or deoxyribose), they are divided into two classes of nucleic acids: ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). DNA is known to be the carrier of genetic information, RNA functions as a messenger (messenger RNA) and takes part in protein synthesis (transferRNA, ribosomalRNA).

Ribonucleic Acids (RNA)

RNA is the non-genetical nucleic acids except in some viruses such as HIV, where it is the genetic material. They are comparatively small and present chiefly as linear, single-stranded molecules. There are four major forms of RNA in cells. They are messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and small nuclear RNA (snRNA). All these RNA molecules are synthesized based on the nucleotide sequence of the genetic material, DNA, for the purpose of protein synthesis.In contrast to DNA, RNA is normally single-stranded. But it displays also some double-stranded, helical sections that are caused by folding of the single strand. These sequences are mirror images of each other and develop palindromes.Transfer RNAs are the different types of small molecules having a length in the range of 90 to 120 nucleotides. They can also fold and form secondary structures and have a clover-leaf-like model and can again fold to form a L-shaped structure. They form about 15% of the total cellular RNA. During protein synthesis they carry the respective amino acids to the ribosomes where the translation of mRNA or the protein synthesis takes place. They contain a number of rare and modified bases that stabilize its structure.

Deoxyribonucleic Acid (DNA)

Deoxyribonucleic acid or DNA, forms the genetic material of almost all organisms except some viruses, which have RNA as the genetic material and therefore are called RNA viruses. DNA is a

very long polymer and always exists as a double- stranded helical molecule.DNA contains the nitrogen bases adenine (A), Thymine (T), Cytosine (C), and Guanine (G). Erwin Chargaff isolated DNA samples from different organisms and analyzed nitrogen-based composition and observed that A and T as well as C and G are present in equal amounts. The ratio A + T/C + G is specific for every species. This is known as the Chargaff rule. All double-stranded DNA samples irrespective of their source obey the Chargaff rule of base equivalence. There are some DNA viruses, where the genetic material is single-stranded DNA. Single-stranded DNA does not obey the Chargaff rule of base equivalence. Based on these data and x-ray diffraction data developed by Rosalind Franklin obtained from crystals of DNA, James Watson and Francis Crick proposed a model, the Watson - Crick Model for the structure of DNA. They published their findings in 1953 in the journal, Nature.

DNA's Three-Dimensional Structure

The Watson Crick Model described virtually all of the characteristics of DNA as the genetic material, including the method of its replication by employing both DNA strands as matrices (this was later confirmed by further evidence). The model's additional features include. The DNA molecule is made up of two complementary anti-parallel polynucleotide chains that are coiled around one another in a rightward orientation and held together by hydrogen bonds (H-bonds) between the bases of neighbouring strands.

By experimentally demonstrating a Watson-Crick prediction, M. Meselson and F. W. Stahl clarified the process of replication in 1958 and demonstrated that DNA replicated in a semi-conservative manner.While the arrangement of nucleotides inside a nucleic acid may first seem random, we now know that this arrangement really encodes the genetic information. Yet, this does not imply that every nucleotide sequence is information-filled. There are lengthy, repeating DNA sequences, but it is unknown what they do. Nucleotide sequencing techniques have been developed since around 1975.

Chromosome Structure

A lengthy molecule, DNA. The DNA of the bacterium E. coli is around 1.44 mm long. DNA molecules in higher forms of life may reach lengths of several centimetres. Yet it only has a 20 diameter. Chromosomes are the name for the tiny, distinctive subcellular structures that contain these long, thin molecules. Under microscopes, specialised staining methods may be used to see the chromosomes.DNA is bundled with certain kinds of proteins called histone proteins in the chromosomes. There is a typical number of chromosomes that is consistent for each type of organism. There are 23 pairs of chromosomes in human cells, compared to only one chromosome in bacteria like E. coli. The DNA of bacteria is typically circular with no free ends, but the DNA of chromosomes in higher forms of life is linear with free ends. When it comes to eukaryotic cells, these chromosomes are restricted to the nucleus. Due to their length, DNA molecules are bundled into chromosomes that are just a few microns long in a very particular way. Supercoiling is a technique used in this packing. Topoisomerases, an enzyme family that promotes and maintains supercoiling in DNA, maintaining the DNA in a highly supercoiled form. The DNA molecule is in a beaded state as a result of supercoiling and packaging with histone proteins, which is a transitional step in the construction of chromosomes called nucleosomes.

It's fascinating to see how cells have condensed the immense length of DNA into chromosomes that are just a few millimetres long. Most bacterial cells have one or more forms of tiny additional chromosomal circular DNA molecules that can replicate independently in addition to the circular chromosomal DNA. The term "plasmid" refers to these spherical DNA molecules. The plasmids are in charge of several peculiar traits of organisms, such as antibiotic resistance. In gene cloning and recombinant DNA research, plasmids are crucial. These investigations often use modified plasmids as their vectors. Vectors are plasmids used in gene cloning and recombinant experimentation.

Biological Membranes and Lipids

While they belong to the hydrophobic molecule family, lipids are extremely miscible in organic solvents as hexane, chloroform, ethyl acetate, etc. They are a class of lipids with significant biological roles that exhibit amphipathic properties. An amphipathic molecule is one that has two ends, one of which is hydrophilic (loves water) and the other of which is hydrophobic (hates water). The primary elements of biological membranes are phospholipids. Phospholipids, which are formed of two layers of phospholipids, are excellent candidates for the creation of biomembranes due to their amphipathic character. Phospholipids or any other amphipathic molecules will organise themselves in an aqueous solution such that the hydrophilic ends are in touch with water molecules and the hydrophobic ends are kept away from the water. They may form vesicles, bilayers, or single-layered structures, depending on the molecular weight, amount, and water content of the amphipathic molecules.

Phospholipids

Phospholipids are structural molecules that make up the majority of all cellular membranes. Depending on the unique function of the membranes inside the cells, many kinds of proteins are dispersed throughout the bilayer of membranes in addition to lipids. A glycerol molecule with two fatty acids esterified at locations R1 and R2, as well as phosphoric acid esterified at position X, make up the structure of phospholipid.The phosphate section of the phospholipid structure has the advantage of making the molecule extremely amphipathic, which is perfect for the structure of the cell membrane.

1. The phosphate region's hydrophilic part.
2. The hydrophobic region in the tails of fatty acids.

Lecithin is the most prevalent phospholipid. Being excellent emulsifiers, phospholipids are employed in a variety of foods and home goods. Only in animal cells can cholesterol and its derivatives make up another part of the cell membrane. The membranes of plant cells do not contain it. Various phospholipid derivatives, including cerebrosides, glycosphingolipids, sphingolipids, and sphingomyelin, are found in certain specialised cells, such as neurons.All cell types, including eukaryotes and prokaryotes, as well as the cell organelles found within eukaryotic cells, are surrounded by lipid bilayer membranes. These membranes are robust and selectively permeable, shielding the cytoplasmic content from osmotic changes and other disturbances. Lipid bilayer characteristics. As we have previously established, the lipid bilayer's extremely impermeable structure is its most significant characteristic. Simply said, impermeable implies that it prevents molecules from readily passing through it. The bilayer is readily permeable only to gases and water. Due to this characteristic, neither big nor tiny polar molecules can pass through the bilayer or cell membrane on their own.

DISCUSSION

The fluidity of the lipid bilayer is a significant additional characteristic. In addition to lipid molecules, the lipid bilayer also contains proteins, as we shall describe later. These structures may move about within the lipid bilayer due to the fluidity of the bilayer. This fluidity affects membrane transport, which is significant in biology. Temperature and the individual fatty acid chain structure both affect fluidity (fluidity increases at lower temperatures). The lipid bilayer is asymmetrical structurally, with varying amounts of lipid and protein in the two layers [4]–[6].

Building Blocks of Membranes

Several different types of cells' plasma and mitochondrial membranes may be partly separated and purified using subcellular fractionation methods, among other significant biological membranes. These solutions often include membranes from different organelles. Yet, since human erythrocytes lack internal membranes, it is possible to separate their plasma membranes with almost perfect purity (see CD).Proteins and phospholipids are components of every membrane. The inner mitochondrial membrane has 76% protein, whereas the myelin membrane contains just 18% protein.Myelin has a high phospholipid content, which allows it to electrically shield the nerve cell from its surroundings. The hydrophilic head of sphingomyelin is comparable to that of phosphatidylcholine because the terminal hydroxyl group of sphingosine is esterified to phosphocholine [7]–[10].

The purposes of biomembranes

1. The biomembranes serve as an excellent barrier, preventing spillage of contents or entry of extracellular substances into the cells or cell organelles.
2. Selective permeability exists in cell membranes. Sugars, organic acids, and salts like NaCl and KCl cannot readily cross lipid membranes since they are water-soluble. To make the transit of certain chemicals easier, specific protein molecules are positioned in the lipid bilayer.

CONCLUSION

The calibre of the proteins, which are dispersed throughout the membranes together with the lipids, controls how well biomembranes perform their duties. This distinguishes one biomembrane's function from another. The membranes of the cellular envelop, mitochondria, and chloroplasts, for instance, are designed specifically for carrying out a particular function that is intimately tied to the kinds of protein present in the corresponding membranes. Glycoproteins, one kind of informative molecule found in certain cell membranes, may serve as receptors for signal molecules like hormones.

Many types of membranes have various lipid compositions. A sizeable amount of phospholipids, mostly phosphoglycerides with a glycerol backbone, are present in every membrane. All phospholipids in membranes are amphipathic, meaning they include both hydrophilic and hydrophobic regions. In plasma membranes, sphingomyelin is another phospholipid that is often present.

It does not have a glycerol backbone. It comprises sphingosine, an amino alcohol with a long unsaturated hydrocarbon chain, in place of the glycerol backbone. A ceramide is created when an amide bond connects the amino group of sphingosine to a fatty acyl side chain.

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CHAPTER 7

EXPLORING THE DIFFERENT BIOCHEMICAL TECHNIQUES

Dr. Asha K, Assistant Professor,
Department of Microbiology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: k.asha@jainuniversity.ac.in

ABSTRACT:

A variety of methods based on diverse chemical and physical characteristics may be used to isolate and purify biomolecules. Molecular size and weight, interactions with electromagnetic radiations or spectroscopic qualities, solubility, molecular charge, and polarity are the primary physical and chemical characteristics that may be used to separate and characterise biomolecules. Centrifugation, gel filtration, and osmotic pressure are the methods based on the dimensions and mass of the molecules. The samples are centrifuged in cells with windows that are perpendicular to the rotor head's plane of rotation. An optical system projects the pictures of the cell (proteins) onto film or a computer while the rotor rotates. Beer's law is used to measure the concentration of the solution at different locations inside the cell by measuring the amount of light that is absorbed at each location. The degree of blackening on a photographic film or the pen deflection of the recorder of the scanning system, which is input into a computer, may both be used to measure this.

KEYWORDS:

Different Biochemical, DNA, Macromolecule, Molecules, Cell.

INTRODUCTION

Ion exchange chromatography, electrophoresis, isoelectric focusing, hydrophobic interaction, and partition chromatography are polarity- and charge-based methods. Colorimetry, UV-visible spectrophotometry, fluorescence spectroscopy, x-ray crystallography, and mass spectrometry are spectroscopy-based methods. The procedures for solubility include precipitating molecules using salts and organic solvents [1]–[3].

Based On Molecular Weight and Size Techniques

Centrifugation

A centrifuge separates particles from a solution in accordance with their sedimentation rate, which is influenced by characteristics such as size, shape, density, medium viscosity, and centrifugal force (rotor speed).

Centrifugation is the name given to the process of separating particles according to their rate of sedimentation. Cells, subcellular organelles, viruses, and big molecules like proteins and nucleic acids are the typical particles in biology. If all other variables remain constant, the rate of sedimentation will be exactly proportional to the molecular weight or size. We shall refer to all biological stuff as spherical particles in order to keep the language of mathematics simple. Centrifugation may be categorised in a variety of ways.

Centrifugation for Preparation and Analysis

The difference between the two most popular forms of centrifugationanalytical and preparativeis dependent on the latter's intended use. The physical characteristics of the sedimenting particles, such as their molecular weight or sedimentation coefficient, are measured during analytical centrifugation. Analytical ultracentrifugation employs ideal techniques. In order to see macromolecules in the solution as they move in the gravitational field, molecules are monitored using an optical system during centrifugation. The other types of centrifugations are preparative, with the goal of isolating certain, recyclable particles. Preparative centrifugation comes in a variety of forms, including rate zonal, differential, and isopycnic centrifugation.

Low-Speed Centrifugation vs Ultracentrifugation

The rate or speed at which the centrifuge is rotating is another categorization scheme. Above 30,000 rpm is the speed at which ultracentrifugation is performed. The range of high-speed centrifugation is 10,000–30,000 rpm. Centrifugation at low speeds occurs at rpms lower than 10,000. (Mostly between 3,000 to 9,000 rpm).

Zone Centrifugation Vs Moving Boundaries

The manner the samples are put into the centrifuge tube is a third approach to define centrifugation. The sample is placed in the whole tube and centrifuged in moving boundary or differential centrifugation. One separates the mixture into two parts using centrifugation: a supernatant and a pellet. However depending on its size, shape, density, and centrifugation conditions, any particle in the mixture may end up in the supernatant, the pellet, or it could be dispersed across the two fractions. The sedimented components are all mixed together in the pellet, and it is also contaminated with any unsedimented particles that were previously at the bottom of the tube. The slowest sedimenting component is the sole one that gets purified, although its yield is often quite little. By decanting the supernatant solution from the pellet, the two portions are recovered. With the production of a fresh pellet and supernatant, the supernatant may be recentrifuged at greater speeds to accomplish more purification.

The density gradient column in the isopycnic method includes all of the sample particles' densities. The gradient material is thoroughly combined with the sample. Each particle will only settle to the point in the centrifuge tube where its density is equal to the gradient density, and it will stay there. Hence, the isopycnic method divides particles into zones purely based on the variations in their buoyant densities, regardless of time. Particles from the rate zonal and isopycnic principles may participate in the final separations in several density gradient studies. For instance, the gradient's density range may be such that one component settles to the tube's density and stays there, while another component settles to the bottom of the tube. Centrifugation time for the self-generating gradient method is often quite lengthy. For instance, isopynically banding DNA requires 36 to 48 hours in a gradient of self-generating cesium chloride. It's vital to understand that raising the rotor speed won't decrease the run time; instead, it will simply change the location of the zones in the tube since the gradient material will redistribute farther down the tube due to increased centrifugal force.

Chromatography Using Gel-Filtration

Size-based separation is achieved using gel-filtration chromatography. It is also known as gel permeation chromatography or molecular exclusion chromatography. The stationary phase in gel

filtration chromatography comprises of porous beads with a well-defined range of pore diameters. If a stationary phase for gel filtration has a fractionation range, it may separate molecules with a certain molecular weight range.It is claimed that proteins are present because they can fit within all of the holes in the beads. In a gel-filtration separation, these tiny proteins elute last because they have access to both the mobile phase between the beads and the mobile phase within the beads. It is claimed that proteins are rejected if they are too big to pass through any of the pores. They elute first because they can only reach the mobile phase between the beads. Proteins of intermediate size are partly incorporated, which means that some but not all of the beads' pores can accommodate them. Then, between the big ("excluded") and tiny ("completely included") proteins, these proteins will elute.

Oxygen Pressure

Every time a molecule moves, it does so from an area of greater concentration to a region of lower concentration. In the case of a solution, it applies to molecules of both the solvent and the solute. Solvent molecules will migrate into a solution when it is isolated from a pure solvent by a membrane that only allows passage of the solvent. By exerting pressure that is equivalent to the pressure the solvent molecules use to enter the solution compartment via the membrane partition, the flow of the solvent molecules into the solution may be stopped. Osmotic pressure is the amount of force required to stop the solvent molecule from entering the solution.

Substance-Based Techniques

Ion-exchange chromatography (IEC) IEC may be used to separate practically any kind of charged molecule, from big proteins to tiny nucleotides and amino acids. It is commonly used to proteins and peptides under a variety of different circumstances. Nonetheless, standardised conditions are applied to amino acids.

The simultaneous use of IEC and GPC in protein structure research is relatively widespread.Ion-exchange chromatography is a method of separation where ions are exchanged between a mobile phase and a stationary phase according to their charges. Ions are electrostatically bound to an insoluble support matrix to form the stationary phase. The ions that need to be separated are in the mobile phase. These ions undergo reversible exchange with the immobile phase or support matrix-bound ions.

Protein molecules are separated using ion-exchange chromatography based on their electrical charge, which is affected by the distribution of amino acids on the surface and the medium's pH. Because salts have a tendency to sabotage electrostatic interactions, the molecules that need to be separated and electrostatically bound to the stationary matrix are rinsed from the column using a gradient solution of increasing ionic strength. Alternately, a pH gradient may be employed for elution since the average charge is negative when the pH is more than pI and positive when the pH is lower.

In biochemistry, ion exchangers with carboxymethyl (CM) or diethyl aminoethyl (DEAE) groups are most often utilised. Cross-linking occurs between these groups and neutral polymers like cellulose (Example: DEAE cellulose and CM cellulose) [4]–[6].Negatively charged proteins are separated using anion-exchange chromatography (anions). Positively charged groups on the inert phase of the column bind negatively charged protein sites. Anions in the solution may then swap the protein for itself. For instance, DEAE groupings.

Electrophoresis

The behaviour of charged molecules moving in a consistent electric field may provide a lot of information. Two flat metal plates are arranged parallel to one another to create a homogenous electric field with a consistent amplitude and direction throughout a certain amount of space. A consistent electric field E is created between the plates when the terminals of a power source with voltage V are connected to them. The field is not level outside of the plates and at the ends.Charged molecules, such as proteins, migrate in an electrical field during electrophoresis. Proteins are separated in an electrical field according to their size, shape, and charge. The net electrical charges of the protein molecules are influenced by the side chains of the amino acids, which are the building blocks of proteins. The isoelectric pH (IpH) of the protein and the pH of the surrounding buffer both affect how charged the protein is.

A supporting medium, such as starch, paper, polyacrylamide, or agarose, is used in the majority of electrophoretic techniques. It should be kept in mind that a 50% buffer solution makes up the real environment through which the proteins move. Moving-boundary electrophoresis is performed wholly in a liquid phase, while zone electrophoresis is electrophoresis that is performed in a supporting medium. It is possible to determine the isoelectric pH (Isoelectric focusing) and molecular weights (SDS-PAGE) of different proteins by seeing them on gels and comparing the migration lengths to standards. Protein identification and purification benefit from the use of the isoelectric pH and molecular weights.The method of electrophoresis is often used to examine both proteins and nucleic acids. DNA molecules always have negative charges, hence their rate of migration towards the anode is based on their molecular size. DNA molecules that are small and compact move more quickly than those that are big and loose. Although having the same molecular size, relaxed DNA molecules will travel more slowly than DNA molecules with compact structure (supercoiling) because the compact molecules pass through the pores of the gel matrix more quickly than the relaxed molecules, which are elongated.

The ratio of positively and negatively charged amino acids at a certain pH determines the net charge of a protein molecule. For separating charged particles, there are several kinds of matrices. For the separation of a mixture of charged amino acids and other molecules, paper electrophoresis is one method utilised. The electrophoresis matrix utilised should be stable and chemically inert. Convection should be reduced or eradicated. By adhering to the charged molecules, it shouldn't stop the molecule from moving. The polyacrylamide matrix, which satisfies all the criteria, is the best and most often used matrix.Tetramethylenediamine (TEMED) induces a polymerization process that is carried out by ammonium persulfate to create the polyacrylamide, which is a polymer matrix made of the monomers acrylamide and N and N-methylene-bis acrylamide (APS). The right amounts of acrylamide, N, N-methylene-bis-acrylamide, tetramethylenediamine (TEMED), and ammonium persulphate are combined to form the polymerization solution. By releasing free radicals after being introduced, APS will activate the molecules of acrylamide. After that, the succeeding acrylamide molecules react with the activated acrylamide molecules to form lengthy polymers. The ratio of acrylamide to bis-acrylamide controls the amount of cross-linking that occurs between these acrylamide polymer chains, resulting in a network of acrylamide chains with a certain pore size. Except from the separation of tiny nucleic acid molecules like RNA or oligonucleotides in nucleic acid sequencing, PAGE is seldom employed to analyse DNA molecules because to their huge size. Due to the enormous pore size of the matrix, agarose gels are often utilised for the electrophoresis of DNA. PAGE (polyacrylamide gel electrophoresis) comes in two flavours:

Indigenous Page

The Sds-Page (Denaturing PAGE)

In order to determine a protein's molecular weight in its active state in tertiary or quaternary structure, native PAGE is often used. The native gel experiment should be followed by a denaturing gel electrophoresis to ascertain the number of subunits, the type of the three-dimensional structure (whether containing one or more subunits), and the molecular weight of each subunit.When protein subunits have been heated under reducing conditions to denature them and bonded with the non-ionic detergent SDS, they may be separated using the electrophoretic technique known as SDS-PAGE, also known as denaturing PAGE. Protein subunits (i.e., polypeptide chains) are uniformly bound with the detergent sodium dodecyl sulphate (SDS), which has the structure CH3 (CH2)11-SO3-Na and the sodium is just a counter ion, during denaturation by boiling. All disulfide bonds in the protein are reduced with 2-mercaptoethanol (also known as beta-mercaptoethanol), and the detergent is then added to the mixture.

The detergent imparts a consistent negative charge to the polypeptide and binds in proportion to the subunit's size. As a result, every protein component in a mixture of proteins has the same charge density and will move with the same mobility in an electrical field. The PAGE gel's pores, on the other hand, are tiny enough in an SDS-PAGE system to result in molecular sieving during electrophoresis, causing the polypeptides coated with SDS to separate by size. As a result, proteins with known component sizes (or molecular masses, or MR) may be used to calibrate an SDS-PAGE gel, allowing the molecular mass of an unknown polypeptide to be ascertained. Making a plot of the log MR of the protein bands representing the standard proteins against their electrophoretic mobility relative to the dye front is the most effective way to do this (called relative mobility).

The MR (i.e., molecular size) of the unknown protein's subunit(s) may be calculated by comparing their relative mobility to the standard curve.

Using Isoelectric Focus

The pH affects how charged protein molecules are. Amphoteric molecules are those that fit this description. There is a pH where the molecule's net charge equals zero. The isoelectric point or isoelectric pH is this pH. (IpH). Isoelectric focusing may be used to separate molecules that vary in their isoelectric pH or isoelectric point (Ip). By utilising electrophoresis in a gel with a pH gradient, isoelectric focusing (IEF) is a technique for sorting proteins based on their isoelectric pH.

Ampholytes are used to create this pH gradient. These are low-molecular-weight amphoteric compounds (a combination of polyamino polycarboxilic acids) that, when mixed, can move in an electric current to their pI, creating a pH gradient.Depending on the local pH at that location in the gel, a protein put there will be either positively or negatively charged. The protein will migrate towards the anode or cathode depending on its charge when a current is applied, until it comes into contact with the region of the gel that corresponds to its pI (IpH), at which time it will have no net charge and will cease migrating.Proteins with known pI may be used to calibrate an IEF gel, and a plot of location on the gel against pI enables a calculation of the pI of unknown proteins.

Polarity-Based Practices

Hydrophobic-interaction Chromatography (HIC) (HIC)

A form of chromatography known as hydrophobic-interaction chromatography separates molecules based on how they interact hydrophobically with the stationary phase and mobile phase. The foundation of a Hydrophobic-interaction Chromatography (HIC) separation is the difference between the hydrophobic characteristics of proteins and peptides. The binding of sample molecules to a hydrophilic matrix replaced with a hydrophobic ligand is often mediated by salt solutions.

The distribution of hydrophobic or non-polar amino acid residues in protein molecules determines how hydrophobic their surfaces are on a molecular level. Protein molecules have hydrophobic residues dispersed over their surfaces, which regulate how well they interact with other hydrophobic surfaces. These hydrophobic regions of protein molecules are covered by a thin layer of water in an aqueous media, which minimises their contact with other hydrophobic surfaces. High salt concentrations may expose these hydrophobic surfaces or protein molecular clusters by disrupting the water layer that covers them, allowing them to interact with other hydrophobic molecules or surfaces. In this chromatography, the immobile phase (matrix) serves as the stationary phase. It is a matrix that has been appropriately hydrophobized, which aids in the interaction of proteins with the matrix and their binding to the matrix. The supporting matrix, such as agarose, connected with hydrophobic groups like alkyls (hexyl, octyl), or phenyls, is the most often utilised stationary phase in HIC. By using a mobile phase with variable hydrophobicity or ionic strength, which disrupts the connection between the bound proteins and the matrix, these bound proteins may be removed from the column.In preparative biochemistry, hydrophobic-interaction chromatography has developed into one of the most effective techniques. The ability to clear nucleic acids makes it a vital tool for the purification of therapeutic proteins. Its speed, resolution, and capacity rival ion-exchange chromatography. Its selectivity is complementary to ion-exchange chromatography and size exclusion chromatography.

Chromatography by Partition

Chromatography is a method that takes use of the differences in interactions between molecules that need to be separated and molecules that are immobilised on a solid support, or the stationary phase, in a solution, or the mobile phase. In a column, the mobile phase is often let to pass over the stationary phase. Different molecules will elute at various times as a result of the various interactions. The molecules that bind most strongly with the column matrix are eluted last, while those that are just faintly adsorbed are eluted first. To get rid of the molecules that are most firmly adsorbed, it is sometimes required to alter the mobile phase's (buffer's) chemical makeup throughout the elution procedure [7]–[9].

DISCUSSION

The mixture of substances to be separated in partition chromatography is divided between the stationary phase and the mobile phase (both are liquids).A liquid that is sustained atop a solid matrix by covalent cross-linking, hydrogen bonds, or other small molecular interactions is known as the stationary phase. The stationary phase is passed over by the solvent-containing mobile phase. Substances that are more attracted to the mobile phase will be eluted from the

chromatography column and removed from the stationary phase. So, the degree of the components' affinity for the liquid in the stationary phase or the mobile phase is what determines how well they separate from one another in a combination. The polarity of the solvents present in both the stationary phase and the mobile phase affects this affinity.

There are two forms of partition chromatography: reverse phase partition chromatography and normal phase partition chromatography. The stationary phase in normal phase partition chromatography is a polar solvent supported on a solid matrix. For instance, in paper chromatography and TLC, the stationary phase is water, which is supported by cellulose (in paper chromatography) and silica gel, respectively. A non-polar organic solvent or solvents with varying polarities make up the mobile phase.In reverse phase partition chromatography, a non-polar solvent serves as the stationary phase and is chemically bonded to a porous support matrix. For instance, octadecylsilane, a solvent, is chemically linked to silica, a porous support matrix. A polar solvent beginning with water, a buffer or alcohol, acetonitrile, etc. may be the mobile phase. So, during elution by the polar solvent, the molecules that are more polar will be eluted fast, and the molecules that are least polar will be eluted towards the end. This is because non-polar chemicals will interact with the stationary phase efficiently. By altering the composition of the mobile phase or eluting solvent, one may adjust the polarity, which in turn affects the retention and separation of the mixture's component molecules.

HPLC may be used to carry out any sort of liquid chromatography, including adsorption and partition chromatography (high-performance liquid chromatography). Many polar, non-polar, and ionic compounds, including proteins, sugars, oligosaccharides, vitamins, peptides and proteins, amino acids, lipids, and others, may be separated using reverse phase HPLC.

Techniques Based On Spectroscopy

Using the electromagnetic radiation that has been absorbed, emitted, or scattered by a substance in order to investigate it qualitatively or quantitatively or to examine physical processes is known as spectroscopy. Atoms, molecules, atomic or molecular ions, or solids may make up the matter. It is possible for radiation to be redirected or for atoms or molecules to shift between different energy levels as a result of radiation's interaction with matter.

In the beginning, visible light was more often used in spectroscopic investigations and procedures, especially for identifying substances and doing quantitative estimations. The use of spectroscopic methods has greatly expanded as a result of recent apparatus advancements. It is possible to examine molecules and atoms using a variety of spectroscopic methods that take use of the unique features of diverse electromagnetic spectrums, including UV, IR, x-rays, microwaves, and radio waves.

1. Absorption: As molecules take in a certain kind of electromagnetic radiation from the electromagnetic spectrum, electrons move from one energy level to another, transferring energy from the radiation to the absorber, whether it be an atom, molecule, or solid.
2. Emission: Atoms or molecules that are stimulated to high energy levels by electromagnetic radiation have the ability to decay to lower energy levels by emitting radiation (emission) in the form of heat or light. The shift from higher to lower energy levels is known as nonradiative decay if no radiation is released.

3. As light interacts with matter, it is redirected, a process known as scattering. A transfer of energy may or may not result in scattering (i.e., the scattered radiation might or might not have a slightly different wavelength compared to the light incident on the sample).

Magnetic Fields and Radiation

1. An oscillating magnetic field component, M, and an oscillating electric field component, E, make up the transverse energy wave known as electromagnetic radiation. The directions of the electric and magnetic fields are orthogonal to one another and to the wave's path of propagation.
2. We categorise electromagnetic radiation into many spectral zones for ease of discussion. Although while the radiation in each of these areas still consists of electromagnetic waves, their highly diverse intensities lead them to interact with matter in very distinct ways. For instance, the visible portion of the spectrum, which is both transmitted by the human eye's lens and absorbed by the retina's photoreceptors, is the only one that the human eye can perceive. Apart for the fact that humans can directly see 400 nm photons, the nature of electromagnetic radiation at 350 nm and 400 nm is identical. There are some ambiguous borders between some of the areas.

The visible spectrum

The electromagnetic radiation's visible spectrum spans from 400 to 750 nm. The eye's lens absorbs light at short wavelengths, while the retina's photoreceptors become less sensitive to light at longer wavelengths, which causes the long wavelength cutoff. If the light source is strong enough, light with wavelengths longer than 750 nm may be visible.

Colorimetry

The interaction of light with coloured solutions is known as colorimetry. The colorimeter, which is the predecessor of the spectrophotometer, is the tool used for this. Certain light wavelengths will be absorbed by a coloured solution when light travels through it. The absorbed wavelengths are influenced by the hue of the solution. A reddish solution will emit light that is red in hue if white light is allowed to pass through it. Except for the red hue, all other wavelengths are absorbed by the solution. If you use the complimentary hue of red, the concentration of the light-absorbing molecules (red-colored chemical) in the solution will be exactly proportional to the quantity of light absorbed by the solution. The substance may be chemically changed to generate a colour if it is not already coloured.

Using coloured filters, which can only absorb a certain range of wavelengths, colorimetry chooses the colour or wavelength of the light. The filter's constrained range is referred to as its bandwidth. The Beer-Lambert Law governs the link between a solution's ability to absorb light and the concentration of molecules that can do so. This law is a synthesis of two different laws. The first rule links the absorbance (amount of light absorbed) with the concentration of the absorbing molecules, while the second law links the amount of light absorbed with the length of the light's route or the thickness of the absorbing medium. In these studies, the colour filter should be the test solution's complimentary colour.A tungsten light source, an appropriate colour filter, a cuvette (specially manufactured clear light-insensitive tubes holding the sample), and a photosensitive detector to track transmitted light make up a colorimeter, the device used for colorimetry.

Law of Beer-Lambert

The linear connection between absorbance and concentration of an absorbing species is known as the Beer-Lambert Law (also known as Beer's Law). The route length or thickness of the absorbing medium, as well as the concentration of the absorbing species of the molecules in the solution, are directly proportional to the absorbance of a solution at a given wavelength. The Beer- Lambert law, which is often expressed as the following:

UV-Visible Spectrophotometry

Spectrophotometry is a method that is often used to estimate the quantity and quality of biomolecules such proteins, sugars, carbohydrates, amino acids, nucleic acids, vitamins, etc. This method uses a spectrophotometer as the instrument, which is likewise based on the Beer-Lambert law.An instrument called a spectrophotometer is used to quantify how much light a sample absorbs. The device measures the amount of light that reaches a detector after travelling through a sample using a light beam. The absorbance in the UV and visible portions of the spectrum is measured using a UV-visible spectrophotometer. This device is a more sophisticated kind of colorimeter since it can produce monochromatic light. The light will be divided into its individual hues by a prism or grating, which will then allow us to direct the monochromatic light of our choosing at the sample solution under investigation.

A stream of photons makes up the light beam. There is a possibility that an analyte molecule will absorb a photon when it comes into contact with the analyte (the molecule being investigated). The amount of photons in the light beam decreases as a result of this absorption, which also lowers the intensity of the light beam.The whole visible spectrum may be measured and produced using a spectrophotometer. ('photometer' is a tool for measuring light intensity, and'spectro' refers to the whole spectrum of continuous wavelengths.) The light source is adjusted to emit photons, and when the beam of light passes through the cell holding the sample solution, some of the photons are absorbed (removed). The amount of light that reaches the detector has a lower intensity than the amount of light that the light source emits. As the bandwidths of the filters in a colorimeter are so wide, monochromatic light cannot be produced. Consequently, it is challenging to analyse two substances with similar absorption patterns.

A UV-visible spectrophotometer, which can also generate the whole spectrum of UV light, may be used to study substances that can absorb ultraviolet light. There are two source lamps in a UV-visible spectrophotometer: one is a tungsten filament that produces wavelengths in the visible range, and the other is a hydrogen or deuterium lamp that produces wavelengths in the UV region.A single-beam mode of operation was typical in earlier instruments. To evaluate any background absorption, a cell containing just solvent was first put in the light beam. Thereafter, the absorbing solution was added to the cell, and the solute absorbance was calculated from the difference between the two observations. This approach needed a very steady radiation source in order to minimise mistakes brought on by source instability. The majority of contemporary spectrophotometers run in double-beam mode. Here, the source beam is divided into two halves (by a variety of methods), one of which is sent through a reference cell that just contains solvent and the other via a sample cell that also contains the absorber solution. The absorbance is determined by an electronic comparison of the two beam intensities.

A spectrophotometer may be used to scan the whole wavelength range, from the shortest UV wavelength to the longest visible wavelength, and give the compound's absorption spectra. The

graphical depiction of absorptions versus their corresponding wavelength for a certain substance is called an absorption spectrum.The absorbing solution is placed in a container known as a cell or cuvette with optically flat sides held perpendicular to the radiation beam for the extremely common situation of absorption measurements.

Fluorescence Spectroscopy (Fluorimetry)

The quantity of the material producing light in a sample may be determined by measuring the light that the emitting atoms or molecules emit. As electromagnetic radiation is absorbed, it excites atoms or molecules to high energy levels, where they may then produce radiation to decelerate to lower energies (emission or luminescence). This light emission is often referred to as atomic or optical emission for atoms excited by high-temperature energy sources, and atomic fluorescence for atoms stimulated by light. If a transition happens between electron states with the same spin and a molecule, it is referred to as fluorescence and as phosphorescence, respectively. When analytes are present in low quantities, the emission intensity of an emitting substance is directly proportional to their concentration and may be used to quantify the emitting species of molecule.By linking with a fluorescent probe termed fluor, fluorescence spectroscopy may be utilised for the detection and assessment of non-fluorescence molecules as well. Extrinsic fluorescence is the name given to this kind of fluorescence. In the case of intrinsic fluorescence, the fluorescence phenomenon is present in the native substance. Fluorescent probes are now widely employed to mark biomolecules so that their presence may be determined using DNA and amino acid sequencing. Fluorescently tagged molecules may be detected and estimated with 1,000 times more sensitivity compared with traditional techniques like absorption spectroscopy. To identify amino acids, peptides, or proteins that have been separated by chromatography or electrophoresis, for instance, fluorescent substances like dansyl chloride or o-phthalaldehyde may be utilised.

Crystallography using X-rays

A potent method for examining the three-dimensional structure of crystals, including macromolecules like proteins and nucleic acids, is X-ray crystallography.The method is sometimes referred to as the x-ray diffraction method. Many techniques may be used to research three-dimensional structure. Yet at the moment, x-ray crystallography is the most efficient of these methods. The other approaches undoubtedly complement crystallography and have a valuable position in the arsenal of instruments we use to investigate the structural makeup of molecules.The most straightforward method for figuring out an object's form is to just look at it, if you think about it. You use a microscope if they are little. Nevertheless, the smallest item that can be observed with a light microscope is limited. The "diffraction limit" states that objects substantially smaller than the wavelength of the light being used cannot be imaged. Atoms are spaced apart by lengths on the order of 0.1nm, or 1 nanometer, whereas the wavelength of visible light is measured in hundreds of nanometers. In terms of the electromagnetic spectrum, x-rays have the correct wavelength range to investigate and resolve the atomic arrangement in a molecule or crystal.

For x-ray crystallographic research, there are three fundamental prerequisites. These are the study crystal, the x-ray source, and the x-ray detector. An appropriately mounted protein crystal is exposed to a small beam of radiation of the right wavelength, which causes a diffraction pattern to appear on a photographic plate behind the crystal or is detected by a radiation counter.

The electrons in each atom of the molecule distort the x-ray as it travels through the crystal. The amount of x-ray scattering depends on how many electrons are present in the atom. Carbon will scatter x-rays with an intensity that is six times higher than a hydrogen atom. Each atoms' dispersed x-rays may either strengthen or cancel one another, creating the distinctive pattern for each kind of molecule. It is possible to identify the three-dimensional structure by comparing a number of patterns captured at various angles. With a mathematical procedure known as the Fourier Transform, which is a computer-aided software, the information included in the x-ray diffraction pattern is extracted and transformed into the picture of the three-dimensional structure. Although using computers to help in the process, there are a lot of intricate mathematical computations.Lastly, a crystallographic experiment produces a map of the distribution of electrons inside the molecule rather than an actual image of the atoms (i.e., an electron density map). The electron density map, however, offers us a very accurate representation of the molecule since the majority of the electrons are firmly clustered around the nucleus.It would be impossible to detect X-ray scattering from a single molecule above the noise level, which would also include scattering from air and water. Huge numbers of molecules are arranged in a single orientation in a crystal such that dispersed waves may combine in phase and increase the signal to detectable levels. A crystal functions almost like an amplifier.

Spectrometry by Mass

While using mass spectrometry, an object is exposed to an electron beam that is powerful enough to break apart molecules. The magnetic field is used to accelerate the positive fragments (cations and radical cations), which are then separated according to their mass-to-charge ratio in a vacuum. The value m/e is identical to the molecular weight of the fragment since the majority of the ions generated by the mass spectrometer have a unit positive charge. The reassembling of pieces in order to create the original molecule is a step in the processing of mass spectroscopy data. The mass spectrometer is seen schematically in. The ionisation chamber is kept at a very high vacuum, and only a very small amount of sample molecules are allowed in. There, they are subjected to a high-energy electron beam bombardment. The molecules break apart, and the resulting positive ions are driven through a charged array and into an analytical tube. An applied magnetic field causes the charged molecules' route to be altered. Low mass (low momentum) ions will be most strongly deflected by this field and will crash with the analyzer walls. Similar to low momentum ions, large momentum ions will not be deflected enough and will likewise strike the analyzer wall. Yet, ions with the correct mass-to-charge ratio will adhere to the analyzer's route and leave via

The chemical routes that lead to fragmentation are straightforward and predictable, and the ions that result will represent the most stable cations and radical cations that the molecule is capable of producing. The molecular ion (M+) is the largest molecular weight peak that is often seen in a spectrum and represents the parent molecule, minus one electron. Typically, owing to the natural isotopic abundance of 13C, 2H, and other molecules, minor peaks are also seen above the predicted molecular weight. A good example of a molecule that does not exhibit molecular ions is alcohol, whose maximum molecular weight peak occurs at m/e one less than the molecular ion (m-1). While the mass-to-charge ratio may be used to identify fragments, it is often more useful to identify them by the lost mass. In other words, the loss of a methyl group will result in a peak at m-15, the loss of an ethyl group at m-29, etc.

For instance, displays the mass spectrum of toluene (methyl benzene). A prominent molecular ion is seen in the spectrum at m/e = 92, along with smaller peaks at m+1 and m+2, a base peak at m/e = 91, and a variety of minor peaks at m/e = 65 and below. Once again, the molecular ion indicates the loss of one electron, and the peaks above it are caused by isotopic abundance. To generate the comparatively stable benzyl cation, a hydrogen atom is lost in toluene, which results in the base peak. Its prominent peak at m/e = 91 is a distinguishing feature of compounds containing a benzyl unit and is believed to undergo rearrangement to generate the relatively stable tropylium cation. The lesser peaks below this one result from more intricate fragmentation. The minor peak at m/e = 65 shows loss of neutral acetylene from the tropylium ion.

Mass spectrometry was first exclusively used to identify and estimate the molecular weight of very tiny organic compounds. There was relatively little application in the case of biomolecules. Nevertheless, as a result of technical development, instruments have grown more advanced, and there are now a variety of applications for the study of proteins and nucleic acids. Depending on the method of ionisation, the ion sorting method, or the analysis and detection method, there are several kinds of mass spectrometry. For instance, tandem mass spectrometry, often known as MS-MS, is a device that combines two mass spectrometric systems in succession and is typically used to sequence amino acids. MS-MS or MALDI-TOF MS may be used to sequence 50 amino acid polypeptides (Matrix assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry). Another kind of specialised mass spectrometry uses a laser to ionise the sample, and time of flight is used to sort the ions. This is another effective way for identifying bigger proteins by their molecular fingerprint and determining their molecular weight. The pattern of ionisation (MS spectrum) of a protein molecule, which is particularly unique to that molecule, is the molecular fingerprint. By contrasting the molecular fingerprint of the protein molecule with that of known samples, the protein molecule may be located. The most used ionisation technique for protein investigations is called electron spray ionisation, or ESI.

When separation methods like capillary electrophoresis and chromatographic procedures are coupled with mass spectrometry, protein investigations become easier and quicker. In such systems, the purified or fractionated protein sample may be transported directly to the mass spectrometry ionisation chamber and identified or sequenced.

The outflow of the chromatography column or capillary electrophoresis serves as the entrance of the mass spectrometer. Multidimensional Protein Identification Technology, or MuD-PIT, is the term used to describe this kind of equipment and protein identification.

To separate proteins and enzymes from other macromolecules like carbohydrates and nucleic acids, a method as straightforward and popular as this one is utilised. The amount of dissolved chemical species (such as salts 6M (NH4)2SO4)), pH of the solution (a protein's minimal solubility occurs at the pI), type of the solvent, and other dissolved chemicals are all factors that affect a protein's solubility in an aqueous medium (i.e., urea and temperature). A protein may "drop out of solution" due to a change in any one of these elements (or all of them).

Rainfall from an Organic Solvent

The precipitation of proteins and enzymes may also be accomplished using organic solvents like acetone.

Another crucial chemical for precipitating proteins and other biomolecules is the organic polymer polyethylene glycol (PGE). PGE is a polymer, hence its molecular weight may vary. The kind employed to precipitate proteins molecularly has a molecular weight of 6,000 or 20,000 [10]–[12].

CONCLUSION

Precipitation varies from salt precipitation in very minor ways. The dielectric constant of the solution is decreased in the presence of organic solvents, which increases the attraction between the oppositely charged amino acid residues on the surfaces of the protein molecules. This causes the protein to coagulate and form aggregates, which causes the precipitation. Nevertheless, a number of other parameters, like the temperature, the ionic strength of the solution, and the organic solvent used, affect the process. This process is called denaturing. By simulating similar in vivo settings, one can often restore the protein to full biological function ("the native conformation") under mild denaturing conditions (via dialysis). One of the gentlest ways to denature protein and precipitate it without harming the protein's viability is by salt precipitation. Salt disrupts the interaction of water molecules with protein molecules when it is introduced to the extract because it dissolves to produce ions that are hydrated by hydrogen bonding. This will reveal the protein's hydrophobic regions and cause interactions between protein molecules that are hydrophobic.

Finally, this causes protein molecules to group together and precipitate. The proteins that salt precipitates are not irreversibly altered. They may easily be dissolved again in a buffer to reactivate the reaction. Either gel filtration or dialysis may be used to eliminate the protein's salt content.

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CHAPTER 8

AN ANALYSIS OF CELL AND DEVELOPMENT

Upendra Sharma U S, Assistant Professor,
Department of Life Science, School of Sciences, B-II, Jain (Deemed to be University), JC Road,
Bangalore-560027, India
Email Id: upendra.sharma@jainuniversity.ac.in

ABSTRACT:

The cell is the fundamental building block of life and the basis of all creatures' structure and functionality. The third section goes through the cell's numerous components. This section consists of three chapters. We will talk about the cell and its many levels of structure in the first chapter. We'll look at how cells and their many organelles are organised into tissues and organs, as well as their structure and function. Thischapter mostly covers the numerous actions that cells engage in. The processes through which cells reproduce, divide, and cycle, as well as the ways in which they interact with one another and with their surroundings. The detailed discussion of many physiological functions of cells, including their part in motions, nourishment, internal transport, gaseous exchange, defensive systems, etc. The majority of the chapter's last section is dedicated to detailing how plants and animals reproduce and develop, as well as the natural process through which cells die.

KEYWORDS:

Cell Development, Components of Cells, Prokaryotes, Natural Process.

INTRODUCTION

We also go over different organs and organ systems, as well as how they work together to form a functioning body. A population is a collection of organisms that is influenced by both genetic and environmental variables. The evolution of populations and biodiversity is also examined, along with a number of other aspects including adaptation and natural selection. The interaction of species with one another and with the ecosystem, as well as how this may alter environmental and climatic conditions, will be covered in the chapter's last section [1]–[3].It discusses a variety of old and contemporary methods that are used for in-depth analyses of the composition and molecular processes of cells. There includes a thorough discussion of methods like microscopy, including both light microscopy and numerous other kinds including electron microscopy, fluorescence microscopy, flow cytometry, cell fractionation techniques, assessment of cell development, etc.

Structure and Components of Cells

The "sacks" are composed of two layers of phospholipids. The membrane is semi-permeable, allowing certain substances to enter or exit the cell while preventing others. With a microscope, one may enlarge tiny things like cells to see the finer intricacies of their structure. To investigate cells, both optical and electron microscopes are used. Cytology is the microscope-based study of cells. From bacterial cells to human brain cells, the majority of cell types share a few basic functions. Cell biology is the study of these fundamental biological processes and 90% of a cell's volume is fluid (cytoplasm), which includes proteins, free amino acids, glucose, and a variety of

other substances. Gene expression and regulation are influenced by the cell environment, which includes the cytoplasm and nucleus's contents as well as how the DNA is arranged. This makes the environment of the cell an essential component of heredity [4]–[6].

Viruses, Eukaryotes, And Prokaryotes

Prokaryotes and eukaryotes are two different classifications of cells based on how they are fundamentally built and how they produce energy. The demand for energy in cells determines how they are categorised as well. Autotrophs are "self-feeders" that produce their own food using either light or chemical energy. Autotrophs include, for example, plants. Heterotrophs, or "other feeders," on the other hand, get their energy from other autotrophs or heterotrophs. Heterotrophs include several types of microorganisms and animals.

Prokaryotic Cell Characteristics

Blue-green algae and bacteria are examples of prokaryotes (cyanobacteria). Prokaryotes are, to put it simply, molecules encased in a membrane and cell wall. Prokaryotic cells lack the distinctive "organelles" with a subcellular membrane that characterise eukaryotic cells, although they may have membrane systems as an extension or infoldings of the cell membrane within the cell wall. The nucleus lacks a membrane and is poorly structured.Photosynthetic pigments may be present in prokaryotic cells, such as those in cyanobacteria (sometimes known as "blue-green algae"). Some prokaryotic cells contain exterior flagella that resemble whips for movement or pili that resemble hairs for attachment. There are many different prokaryotic cell types, including cocci (round), bacilli (rods), and spirilla or spirochetes (helical cells). Eukaryotes include both single-celled and multicellular species, whereas prokaryotes are all unicellular organisms.Multicellular organisms are built from a sophisticated network of interdependent cells. For control and cell-to-cell communication, new mechanisms are required. Also, distinct pathways must exist for a single fertilised egg to grow into all the various bodily tissues. There are 1014 cells in humans, which make about 200 different types of tissues.

Fundamental Structure of Eukaryotes

1. The following are found in the fundamental eukaryotic cell:
2. Cytoskeleton, which consists of microfilaments and microtubules that suspend organelles, provide structure to the cell, and permit mobility. Plasma membrane.
3. The presence of distinctive subcellular organelles with membranes.

Characteristic Organelles and biomembranes

1. Membrane Plasma
2. A barrier-forming lipid-protein-carbohydrate complex that also houses transport and signalling mechanisms.
3. Nucleus
4. Around the nucleolus and chromosomes is a double membrane. The cytoplasm may communicate specifically with pores. The RNA that makes up the ribosome is synthesised in the nucleolus.
5. Mitochondria
6. Surrounded by a double membrane that has cristae-named folds. utilises cellular respiration and metabolism to produce energy. Contains its own DNA and is thought to have started out as a bacterium that was captured.

Chloroplasts (plastids)

A stack of thylakoid membranes enclosed by a double membrane. Responsible for capturing light energy for the synthesis of sugars during photosynthesis. Contains DNA and, like mitochondria, is thought to have started as a blue-green algae that was caught. They only exist in plants.Rough endoplasmic reticulum (RER): An internal network of channels formed by connected membranes. Ribosomes, which give the surface its "rough" appearance, are all over it and are syncing proteins for secretion or membrane localization.An internal network of channels formed by interconnected membranes is known as the smooth endoplasmic reticulum (SER). A location where lipids are synthesised and metabolised. Contains additional enzymes for the detoxification of chemicals, such as drugs and pesticides.

Complex of proteins and RNA that produces protein.

Golgi equipment

Stacks of membranes in succession. Materials are transported from the RER to the Golgi apparatus by vesicles, which are small membranes surrounded by bags. While the proteins are "processed" to a mature form, vesicles move back and forth between the stacks. Vesicles then carry newly formed membrane and secreted proteins to their final destinations including secretion or membrane localization.

Lysosymes

A membrane-bound organelle that is responsible for degrading proteins and membranes in the cell, and also helps degrade materials ingested by the cell.

Vacuoles

1. Membrane surrounded “bags” that contain water and storage materials in plants.
2. Peroxisomes or Microbodies
3. Produce and degrade hydrogen peroxide, a toxic compound that can be produced during metabolism.
4. Basic Characteristics of Viruses

Simply stated, viruses are merely genetic information surrounded by a protein coat. They may contain external structures and a membrane. Viruses are obligate intracellular parasites meaning that they require host cells to reproduce. In the viral life cycle, a virus infects a cell, allowing the viral genetic information to direct the synthesis of new virus particles by the cell. There are many kinds of viruses. Those infecting humans include polio, influenza, herpes, smallpox, chickenpox, and human immunodeficiency virus (HIV) causing AIDS.

Cell Membrane

1. Cell membranes are selective barriers that separate individual cells and cellular compartments.
2. Membranes are assemblies of carbohydrates, proteins, and lipids held together by non-covalent forces. They regulate the transport of molecules, control information flow between cells, generate signals to alter cell behaviour, contain molecules responsible for cell adhesion in the formation of tissues, and can separate charged molecules for cell signalling and energy generation.

3. Cell membranes are dynamic, constantly being formed and degraded. Membrane vesicles move between cell organelles and the cell surface. Inability to degrade membrane components can lead to lysosomal storage diseases.
4. Lipids of cell membranes include phospholipids composed of glycerol, fatty acids, phosphates, and a hydrophobic organic derivative such as choline or phosphoinositol. Cholesterol is a lipid component of cell membranes that regulates membrane fluidity and is a part of membrane signalling systems. The lipids of membranes create a hydrophobic barrier between aqueous compartments of a cell. The major structure of the lipid portion of the membrane is a lipid bilayer with hydrophobic cores made up predominately of fatty acid chains and hydrophilic surfaces.
5. Membrane proteins determine functions of cell membranes, including serving as pumps, gates, receptors, cell adhesion molecules, energy transducers, and enzymes. Peripheral membrane proteins are associated with the surfaces of membranes while integral membrane proteins are embedded in the membrane and may pass through the lipid bilayer one or more times.
6. Carbohydrates covalently linked to proteins (glycoproteins) or lipids (glycolipids) are also a part of cell membranes, and function as adhesion and address loci for cells.
7. The Fluid Mosaic Model describes membranes as a fluid lipid bilayer with floating proteins and carbohydrates (see CD) (see CD).
8. Cell junctions are a special set of proteins that anchor cells together (desmosomes), occlude water passing between cells (tight junctions), and allow cell-to-cell direct communication (gap junctions) (gap junctions).

Cell Organelles

Cells contain a variety of subcellular particles specialised for carrying out a specific function. Most of them are membrane-enclosed structures. The material that is seen within the cell enclosed by the cell membrane can be divided into a central concentrated structure known as the nucleus and the surrounding semifluid, the cytoplasm. Both the cytoplasm and nucleus together form the protoplasm. The protoplast includes the cell membrane also.

Nucleus

The nucleus is the hallmark of eukaryotic cells; the very term eukaryotic means having a “true nucleus.” It is the largest of the organelles, and is the location of the majority of different types of nucleic acids. It has got an important role in controlling the shape and features of the cell. Deoxyribonucleic acid (DNA) is the physical carrier of inheritance and with the exception of plastid DNA and mitochondrial DNA (cpDNA and mDNA), all DNA is restricted to the nucleus. Ribonucleic acid (RNA) is formed in the nucleus by coding off of the DNA bases. RNA moves out into the cytoplasm. The nucleolus is an area of the nucleus (usually two nucleoli per nucleus) where ribosomes are constructed.

The nucleus is enveloped in a pair of membranes enclosing a lumen that is continuous with the endoplasmic reticulum. However, the nuclear envelope is perforated by thousands of nuclear pore complex (NPCs) that control the passage of molecules in and out of the nucleus. The nuclear pore is formed by the fusion of outer and inner membrane. The outer membrane is the continuation of endoplasmic reticulum. The term nucleoplasm is still used to describe the contents of the nucleus.

Chromatin

The nucleus contains the chromosomes of the cell. Each chromosome consists of a single molecule of DNA complexed with an equal mass of proteins. Collectively, the DNA of the nucleus with its associated proteins is called chromatin. Most of the protein consists of multiple copies of five kinds of histones. These are basic proteins, bristling with positively charged arginine and lysine residues. (Both Arg and Lys have a free amino group on their R group, which attracts protons (H+) giving them a positive charge.) Chromatin also contains small amounts of a wide variety of non-histone proteins. Most of these are transcription factors. (e.g., the steroid receptors) and their association with the DNA is more transient.

During cell division chromatin becomes more condensed and becomes chromosomes of specific shapes. During the interphase, the chromatin becomes dispersed and becomes very light and cannot be visible very easily (except for special cases like the polytene chromosomes of drosophila and some other flies) (except for special cases like the polytene chromosomes of drosophila and some other flies). However, the density of the chromatin (that is, how tightly it is packed) varies throughout the nucleus:

1. Dense regions are called heterochromatin.
2. Less dense regions are called euchromatin.
3. Heterochromatin is found in parts of the chromosome where there are few or no genes, such as centromeres, telomeres, transposons, and other "junk." DNA is densely packed. Those genes present in heterochromatin are generally inactive; that is, not transcribed.

Euchromatin is found in parts of the chromosome that are active in gene transcription; it is loosely packed in loops of 30-nm fibres. These are separated from adjacent heterochromatin by insulators. The loops are often found near the nuclear pore complexes (which makes sense for the gene transcripts to get to the cytosol) (which makes sense for the gene transcripts to get to the cytosol).

Nucleolus

During the period between cell divisions, when the chromosomes are in their extended state, one or more of them (ten in human cells) have loops extending into a spherical mass called the nucleolus.

They are the sites of synthesis of three (of the four) kinds of rRNA (ribosomal RNA) molecules (28S, 18S, 5.8S) used in the assembly of the large and small subunits of ribosomes. The synthesis of these rRNA takes place in the denser regions of nucleolus known as the nucleolar organiser region. (The 5S rRNA molecules are synthesised at other locations in the nucleus.). 28S, 18S, and 5.8S ribosomal RNA is transcribed (by RNA polymerase I) from hundreds to thousands of tandemly arranged rDNA genes distributed (in humans) on ten different chromosomes.

The rDNA-containing regions of these ten chromosomes cluster together in the nucleolus. Once formed, the rRNA molecules associate with the dozens of different ribosomal proteins used in the assembly of the large and small subunits of the ribosome. But all proteins are synthesised in the cytosol and all the ribosomes are needed in the cytosol to do their work so there must be a mechanism for the transport of these large structures in and out of the nucleus. This is one of the functions of the nuclear pore complexes.

Nucleosomes

Two copies of each of four kinds of histones—H2A, H2B, H3, and H4 form a core of protein, the nucleosome core. Around this is wrapped 147 base pairs of DNA to form the structures called Nucleosomes on DNA chain just like beads of a chain.

The cytoplasm was defined earlier as the material between the plasma membrane (cell membrane) and the nuclear envelope. Fibrous proteins that occur in the cytoplasm, referred to as the cytoskeleton, maintain the shape of the cell as well as anchoring organelles, moving the cell and controlling internal movement of structures. Microtubules function in cell division and serve as a "temporary scaffolding" for other organelles. Actin filaments are thin threads that function in cell division and cell motility. Intermediate filaments are between the size of the microtubules and the actin filaments.

Endoplasmic Reticulum

Endoplasmic reticulum is a network of tubules, vesicles, and sacs that are interconnected. They may serve specialised functions in the cell including protein synthesis, sequestration of calcium, production of steroids, storage and production of glycogen, and insertion of membrane proteins. There are two types of endoplasmic reticulum (ER): rough endoplasmic reticulum, which gets its name from the presence of ribosomes on its surface during protein synthesis and smooth ER without any ribosomes. Rough endoplasmic reticulum may either be vesicular or tubular. Or it may consist of stacks of flattened cisternae (like sheets) that may have bridging areas connecting the individual sheets. The ribosomes sit on the outer surfaces of the sacs (or cisternae) (or cisternae). They resemble small beads sitting in rosettes or in a linear pattern.

Vacuoles and Vesicles

Vacuoles are single-membrane organelles that are essentially part of the outside that is located within the cell. The single membrane in plant cells is known as a tonoplast. Many organisms will use vacuoles as storage areas. Vesicles are much smaller than vacuoles and function in transport within and to the outside of the cell.

Ribosomes

Ribosomes are protein-synthesizing machines of the cell. They translate the information encoded in messenger RNA (mRNA) into a protein. They are not membrane-bound and thus occur in both prokaryotes and eukaryotes. Eukaryotic ribosomes are slightly larger than prokaryotic ones. The ribosomes of the prokaryotes are 80S and those found in bacteria and eukaryotic organelles like mitochondria and chloroplasts are 70S. The presence of bacterial (prokaryotic) ribosomes (70S) in chloroplasts and mitochondria shows the prokaryotic origin of these organelles.Despite these differences, the basic structure and operations of bacterial and eukaryotic ribosomes are very similar. Structurally, the ribosome consists of a small and larger subunit. These two units join together when the ribosome attaches to messenger RNA to produce a protein in the cytoplasm. Biochemically the ribosomes are ribonucleoprotein complexes consisting of ribosomal RNA (rRNA) and some 50 structural proteins. In eukaryotes, ribosomes that synthesise proteins for use within the cytosol (e.g., enzymes of glycolysis) are suspended in the cytosol. Ribosomes that synthesise proteins destined for secretion (by exocytosis), the plasma membrane (e.g., cell surface receptors), lysosomes, etc. are attached to the cytosolic face of the membranes of the endoplasmic reticulum. During protein synthesis, ribosomes form a complex with mRNA and

move along the molecule. During active periods of protein synthesis a number of ribosomes attach to the rRNA and such structures are known as polyribosomes

DISCUSSION

Golgi Complex

Golgi complex is another important membrane-bound organelle present in eukaryotic cells, both in plants and animals. It is usually present in association with or near ER. The Golgi complex consists of a pile of flattened membrane-bound sacs called cisternae, which are formed by the fusion of vesicles. The vesicles are formed from the ER. The Golgi complex has an outer convex surface and an inner concave surface. The vesicles from the ER fuse together and form cisternae on the outer or convex surface and vesicles bud off constantly from the lower or concave side. Thus, an entire stack consists of a number of cisternae, which are moving from the outer convex to the inner concave face of the Golgi complex.The nascent protein transported from ER in the form of vesicles undergoes post-synthetic modifications inside the cisternae of the Golgi complex. One of the important modifications on protein molecules is the addition of carbohydrate moiety to the proteins, a process known as glycozilation. Glycoproteins function as the receptors on the cell surfaces along with phospholipids of the membranes. Another important function of the Golgi complex is the production of lysosomes. Lysosomes are the vesicles containing hydrolytic enzymes.

Lysosomes

These are single, membrane-bound vesicles present in eukaryotic cells containing hydrolytic enzymes such as nucleases, proteases, and lipases. These enzymes are synthesised in ER and are transported to the Golgi complex through their cisternae and vesicles. From the Golgi complex these are removed as vesicles known as primary lysosomes. The lysosomes are the defence units of cells and in unicellular eukaryotic organisms such as protozoans they form the digestive enzymes for digesting the food materials engulfed by the organisms.Lysosomes fuse with the phagocytic vesicle or the phagosomes (the material engulfed by the cells) to form phagolysosomes. The enzymes of the lysosomes will digest the food material or the invading organisms present within the phagocytic vesicle. Therefore, lysosomes are abundant in those cells which are specialised for phagocytosis.

Mitochondria

Mitochondria are another very important membrane-bound organelle present in both animal and plant cells. They are short cylindrical structures. There are two membranes covering this organelle, an outer membrane and an inner membrane with a space in between. The inner membrane has a number of infoldings into the matrix known as cristae. The matrix of mitochondria contains 70S ribosomes and a circular DNA in addition to a large number of enzymes. On the surfaces of the cristae there are a large number of granular structures known as oxysomes.The main function of mitochondria is that they are the centres of power generation inside the cells. The final stage of respiration, the cellular respiration, takes place in mitochondria. Cellular respiration or the oxidative breakdown of glucose occurs in three steps – glycolysis, Kreb's cycle and electron transport system and oxidative phosphorylation (synthesis of ATP) (synthesis of ATP). The site of glycolysis is outside the mitochondria in the cytoplasm.

The other two steps take place inside the mitochondria. The site of Krebs cycle is in the matrix and that of ETS is on the inner side of the cristae.

Plastids

Plastids are special organelles present only in plants. Plastids are of different shapes covered by two membranes similar to that of mitochondria. Depending on the function, plastids are classified into three groups. They are chloroplasts, chromoplasts, and leucoplasts. Among these, chloroplasts are the most important from the functional point of view.

Chloroplasts

Chloroplasts are green plastids specialised for photosynthesis. They contain photosynthetic pigments such as chlorophylls and carotenoids. They generally have a discoid shape with a double membrane covering enclosing the matrix known as the stroma. The stroma is traversed by a system of double membranes known as lamellae. Some of these lamellae appear in the form of flattened and discoid sacs enclosing an inner space. These membrane structures are known as thylakoid membranes. Thylakoid membranes are present in piles of discs interconnected by the normal types of double membranes. These stacked thylakoids are called grana and the interconnecting membrane is called grana lamellae. There are membranes without any grana traversing the stroma and they are known as stroma lamellae. The photosynthetic pigments are present on the thylakoid membranes as pigment protein complexes. They are organised into light-harvesting systems known as pigment systems I and II (PSI and PSII). Thylakoid membranes are the centres of light reaction, where the light energy is converted into chemical energy. The components required for the light harvesting and its conversion into the reducing powers of NADPH2 and ATP are distributed on the thylakoid membrane in a much organised manner [7]–[9].

The second reaction of the photosynthesis—the dark reaction or the Calvin cycleoccurs in the matrix or the stroma of the chloroplast. The stroma is a mixture containing all the required enzymes and other compounds including the intermediates of the Calvin cycle essential for operating the absorption and reduction of carbon dioxide to carbohydrate. The stroma also contains a circular DNA molecule and 70S ribosomes needed for protein synthesis. The presence of these things indicates that chloroplasts like mitochondria are semi-autonomous organelles.The presence of a circular DNA molecule and 70S ribosomes and the absence of membrane-bound organelles and the presence of membrane infoldings postulate the prokaryotic origin of chloroplasts and mitochondria. It is believed that chloroplasts evolved from a symbiotic type of blue-green algae (cyanobacteria) and mitochondria might have evolved from a symbiotic type of bacteria.

Chromoplasts

Chromoplasts are non-photosynthetic coloured plastids. They do not have non- photosynthetic pigments. They contain red, orange, or yellow pigments and are present in the coloured parts of the plant such as flowers, fruits, etc.

Leucoplasts

These plastids do not contain any pigments and therefore are colourless. These plastids sometimes form the precursors of chloroplasts or change into structures for food storage.

Depending on the type of stored food they are further classified into amyloplasts, lipidoplasts, and proteoplasts. Amyloplasts contain starch and are also known as starch grains, which are present in the storage regions of the plants such as tubers and grains. Lipidoplasts store lipids and proteoplasts or aleuron grains store proteins. These are also present in the storage parts of plants such as endosperm of seeds and pulses.

Peroxisomes

These are small, single membrane-bound granular structures seen in eukaryotic cells. They are small lysosomes containing the enzyme catalase or peroxidase. These structures are involved in a number of metabolic reactions, which involve oxidation. Peroxidase enzymes are needed for the oxidation of peroxides formed in the cells as byproducts of other metabolic reactions.

Cytoskeleton

This is a network of fibres made up of fibrous proteins present inside the cells, which gives mechanical support and shape to the cells. In addition to this, there are some other specific functions for which it is modified. The fibres of cytoskeleton are of two types: microtubules and microfilaments. The other functions of these fibres of cytoskeleton include movements of cells, as the components of cilia and flagella, and the movements of organelles and components within the cells. For example, movements of chromosomes by spindle fibres during cell division.

Microtubules

Microtubules are present in spindle fibres, cilia, and flagella. These fibres are hollow cylindrical fibres consisting of proteins tubulin. In flagella, cilia, and spindle the microtubules undergo sliding movements. They are responsible for the movement of chromosomes attached to the spindle fibres during cell division and for the rhythmic movements of cilia and flagella. They are also responsible for the movements of other organelles such as lysosomes, Golgi complex, etc. In addition to all these they give mechanical support to the cells, particularly in the case of animal cells, which do not have a rigid cell wall.

Microfilaments

These filaments are present in bundles or sheets just below the cell surface membranes. These are very thin filaments and are composed of fibrous protein actin. They are supposed to be involved in the phagocytosis or endocytosis and exocytosis. In addition to this they are also involved in the movements of cells and cellular parts [10]–[12].

CONCLUSION

Cells are structural and functional units of life. According to the cell theory all living things are composed of one or more cells. One-celled organisms are called unicellular organisms and those with more than one cell are called multicellular organisms. Virus particles do not have any cells and therefore, are termed as acellular. No matter what type of cell we are considering, all cells have certain features in common: cell membrane, nucleic acids, cytoplasm, and ribosomes. Cells are small ‘sacks’ composed mostly of water.The fundamental processes of life, such as cell proliferation, division, differentiation, and development, are explored in the subjects of cell and developmental biology. Understanding the molecular basis of life and creating innovative approaches for health, biotechnology, and environmental research both depend on the study of cells and developmental biology. By examining recent findings on cell shape, cytoskeleton

structure, and dynamics in developing tissues, this research paper offers an understanding of cell and developmental biology. In order to comprehend the principles of how a cell functions and interacts with other cells, the paper also examines insights into cell growth and proliferation.

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CHAPTER 9

TISSUES AND ORGANS: THE BUILDING BLOCKS OF THE HUMAN BODY

Dr. Vandana C D, Assistant Professor,
Department of Biotechnology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: vandana.cd@jainuniversity.ac.in

ABSTRACT:

In multicellular organisms cells cooperate for the well-being of the whole body. Groups of cells differentiate and emphasize certain functions while losing others. Basic categories of differentiated cells include secretory cells in both plants and animals with highly developed powers of secretion, for example, of enzymes, hormones (in animals), honey, and mucilage (in plants); fat cells for storage of fat; muscle cells which are highly contractile; nerve cells with high irritability; and reproductive cells that produce gametes. Each kind of cell has reduced or given up some of the functions of the other kinds. There are three basic cell shapes in epithelial tissues: columnar, cubical, and squamous (scale-like). The deep columnar cells often have a secretory function, and the nucleus is pushed to the bottom by the maid and stored secretions near the surface from which they will exit (e.g., the cells lining the stomach, which secrete mucus). Cubical cells form the walls of small ducts as from salivary glands. Squamous cells are very flat, and the nucleus may form a bulge; they look something like a fried egg. The thinness permits diffusion of molecules across membranes (e.g., alveolar walls in lungs).

KEYWORDS:

Tissues, Organs, DNA, Human Body, Nucleic Acid.

INTRODUCTION

The cells with the same function and structure are arranged together to form tissues. Each tissue carries out a specific function for which the cells are specialized. Different types of tissues are organized together and form specific structures called organs, which cooperate with other similar organs to carry out specific functions of the body. In animals, different organs cooperate to form a system which carries out a specific function of the body. For example, the circulatory system, digestive system, nervous system, excretory system, etc [1]–[3].

Animal Tissues

In animals, there are four basic types of tissues: epithelial or linings, connective or supporting, muscular, and nervous. An organ of the body may have all the four types of tissues. For example, the stomach, an organ of the digestive system has all the four types of tissues. (See CD)

Epithelial Tissue

The cells are arranged in single or multilayered sheets. They basically form the covering on the external and internal surfaces of the organs and body parts. Epithelial cells are not supplied with blood vessels.

They protect the internal tissues from physical injury and infection. The free surface of the epithelial tissue may be of different types depending on its special function such as secretory, absorption, or excretory functions. Epithelial cells are basically classified according to their shapes.

Thick layers of cells (e.g., skin) prevent diffusion. In addition to the above three basic types there are some modified forms of these tissues. They are the following. Ciliated epithelium, the columnar cells with numerous cilia on their free surface, which lines the respiratory passage. Psudostratified epithelium, which forms a single layer of cells but on sectional view appears to be multilayered. The last one is the stratified epithelium, which are multilayered and form a very tough and impervious barrier [4]–[6].The secretory or glandular cells may be present individually as in the case of goblet cells or in groups forming multicellular glands. An epithelial tissue having many goblet cells that secrete mucus is called mucus membrane. If the glandular cells or glands discharge their secretion on the surface of the cells or through a duct, they are called exocrine glands. But there are glands that discharge their secretion directly into the bloodstream and do not have any ducts. They are called the ductless glands.

Connective Tissue

This includes the various types of supporting tissues in the body. Connective tissues are cells in a matrix. The matrix may be a fluid, semi-fluid, or a composite structure made up of secretory products of cells such as fibrous proteins. Blood is a connective tissue in which cells are embedded in a fluid matrix. In fibrous connective tissue cells are scattered among the collagen fibers (fibrous protein) they secrete. In bone and cartilage, cells are scattered throughout the hard or pliable matrix.

In cartilage, the cells known as chondroblasts deposit in the matrix. The cell, along with the matrix, forms the chondrocytes. The cartilage is hard but flexible because the matrix is compressible and elastic. Bone is a calcified connective tissue. The cells are embedded in a hard matrix.

The cells in the bone tissue are called osteoblasts, which are present in lacunae. Lacunae are present throughout the tissue. The main inorganic component of bone is hydroxyapatite.

Muscle Tissues

Muscle tissues are made up of highly differentiated contractile cells or fibers held together by connective tissues. Muscle tissues are of three types. Striated muscle cells are large, multinucleate, and column-shaped cells; they are chiefly attached to the skeleton and are known as skeletal muscles or voluntary muscles. Voluntary muscles are under the control of the voluntary nervous system.

They show powerful rapid contractions. They are attached to the bones in the trunk, limbs, and head. Smooth muscle cells are small and mononucleate; they are found in the walls of tubes such as blood vessels, glandular ducts, and the digestive system. They are also known as unstriated or involuntary muscles. The involuntary muscles are under the control of the autonomic nervous system and show sustained rhythmical contraction and relaxation movements. Cardiac muscle cells of the heart are small, striated, and branched. They are present only in the heart. They show rapid rhythmical contractions and relaxation movements with long refractory periods and do not show any fatigue.

Nervous Tissues

Nervous tissues consist of nerve cells, the neurons and associated neuroglial cells. Neurons are capable of generating and transmitting electrical impulses. These cells also act as supporting connective tissue in the brain and spinal cord. The neurons transmit the stimuli from receptors such as skin to the effectors such as muscles and glands that then react to the stimuli.Each neuron may have thousands of branches that connect it to other neurons. The branches are called dendrites or axons. The nerve fibers or the axons are completely ensheathed by a myelin sheath formed by Schwann cells. Such nerve fibers are called myelinated. When bundles of nerve fibers are ensheathed in connective tissue, they form the nerves. Dendrites carry messages toward the cell body; axons carry messages away from the cell body to another neuron. Axons extend for as long as four feet in humans. In some animals, axons are even longer.

In the beginning, we thought that axons and dendrites simply ran through the body continuously, like wires. Then we discovered a space between each axon and dendrite. We call this space a synaptic gap, or synapse. The synapse is the space between the axon of one neuron and the dendrites of the next neuron in a nerve pathway. That gap is extremely smallabout one-millionth of an inch.

Researchers originally thought that electrical impulses jumped these gaps, like electricity jumps across the gap in a spark plug. Now we know this is not true. Chemicals, not electrical impulses, travel across the gaps. These chemicals are neurotransmitters. Today, we know of about 50 neurotransmitters. Undoubtedly there are more things waiting to be discovered.Our bodies synthesize (make) neurotransmitters. Some of the chemical building blocks for neurotransmitters, such as amino acids, come from the foods we eat. Neurons include places to store neurotransmitters. Acetylcholine is an example of a neurotransmitter. These storage areas, called vesicles, are located close to the ending of each axon. Neurons synthesize some neurotransmitters right in the vesicle. Other neurotransmitters are synthesized in the body of the cell and shipped down to the vesicle.

Most addictive drugs change the effect of neurotransmitters on neurons. To understand how these drugs work, we need to know about neurotransmitters and how they act as chemical "messengers."Neurotransmitters are molecules-groups of atoms, joined by a chemical bond, that act as a unit. In order to be called a neurotransmitter, a molecule must meet three criteria:

1. First, the molecule must be present in the brain and distributed unevenly. That is, the molecule must be spread out among different types of neurons, and across regions of the brain that have different functions.
2. Second is a chemical criterion. The enzymes that help to create the neurotransmitter must be present in the brain. Also, these enzymes must be present in areas where the neurotransmitter is found.
3. Third is the criterion of mimicry. Suppose that we directly inject a neurotransmitter into a part of the brain known to contain certain neurons. This injection should mimic (imitate) the effects of electrically stimulating the same neurons.

Combinations of these tissues make up the organs in the human body. Organs are united into systems: digestive, circulatory, respiratory, excretory, endocrine, nervous, locomotory, and reproductive systems.

Plant Tissues

Vascular plants have distinctive cell types, all of which are surrounded by a cell wall of cellulose fibers and other molecules secreted by the cells. Just as in animals, cells are organized into tissues that perform different functions, but plants do not have organ systems like those of animals. The tissues of plants are grouped into three basic kinds: ground, vascular, and dermal. Meristem is a special embryonic tissue.Plants differ from animals in that the tips of roots and stems, called apical meristem, remain embryonic and retain the ability to form new structures (e.g., leaves, stems, flowers, and roots). Hormones secreted by meristem cells are transported elsewhere in the plant; meristem is in part analogous to the endocrine system in animals.

Ground Tissues (Simple tissues)

Ground tissues or the simple tissues include parenchyma, collenchyma, and sclerenchyma. Thin-walled parenchyma cells have a variety of functions such as photosynthesis, starch storage, and secretion; they retain the capacity to divide and are important in repair of damage. They form the large part of the bulk of various organs such as stem, root, etc. In some parts they are modified to perform some special functions. For example:

Epidermis: This is a single-layered tissue that covers the whole plant body. It protects the internal part from infection and loss of water. This layer of cells has a waxy coating on the surface, which is secreted by the cells. This waxy layer is called cuticle, which helps to reduce the water loss.

Mesophylls: These types of parenchyma cells are found in the leaves between the two epidermal layers.

These are specialized for carrying out photosynthesis. Parenchyma cells containing chlorophyll are also known as chlorenchyma. If the cells of the mesophyll tissue are tightly packed without air space, they are known as palisade parenchyma or mesophyll; if a lot of air space is present it is called spongy parenchyma. Endodermis, pericycle, and companion cells, etc. are also an example of modified parenchyma cells.

Collenchyma: These cells resemble parenchyma cells but are characterized by the presence of extra cellulose at the corners of the cells. Their walls are thickened and made strong with cellulose and pectin. Collenchyma cells help strengthen the plant parts in which they occur. Celery strings are an example.

Sclerenchyma: Sclerenchyma cells have very thick secondary walls that are commonly impregnated with lignin, which makes them quite rigid. The lignified sclerenchyma of flax plants is made into linen threads for weaving, sewing, and paper making. Wood is made of lignified xylem cells. The hardness of a coconut shell or a peach pit is caused by lignified cells. Ground tissues are analogous to the supporting connective tissue and skeletal elements in animals. Sclerenchyma cells act as supporting elements in plants. Mature sclerenchyma cells can’t elongate.

The two types of sclerenchyma cells are fibers and sclereids. Fibers are long, slender, and tapered cells that occur in bundles. Sclereids are shorter than fibers and shaped irregularly. Nutshells and seed coats are composed of sclereids. Sclereids scattered among the soft parenchyma tissue of the pear give it a gritty texture.

Complex Tissues

Complex tissues consist of more than one type of cells. Vascular tissues of plants include xylem and phloem; this is the plant's circulatory system. Xylem and phloem are the complex tissues.Xylem consists of four types of cells—trachieds, vessel elements, parenchyma, and fibers. Trachieds are single cells that are elongated and lignified. At maturity, trachieds cells are dead and form interconnected tubes throughout the plant. Vessels are long, tubular structures formed by the fusion of several cells end to end in a row. They conduct water and dissolve nutrients that the plant absorbs from the soil; their thick, sclerified walls allow them to give mechanical support to the plant. Wood is made of xylem cells. Xylem parenchyma has thin cellulose cell walls and living contents similar to the typical parenchyma cells. Xylem fibers are shorter and thinner than trachieds and have much thicker walls.Phloem consists of tubular cells modified for translocation. These tubular cells have interconnected cytoplasm and they conduct other solutes, chiefly nutrients (e.g., carbohydrates) from areas of food production such as leaves to areas of food storage such as tubers. There are five types of cells in phloem. They are sieve tube elements, companion cells, parenchyma, fibers, and schlerids. Sieve tubes are long tube-like structures formed by the end to end fusion of sieve tube elements. Adjacent to the sieve tube elements lie the companion cells with dense cytoplasm. Phloem parenchyma is similar to the ordinary parenchyma cells and the phloem fibers are like the sclerenchyma fibers.

Dermal tissues include epidermis and cuticle. The epidermis is a continuous layer of tightly packed cells. It is usually coated with a cuticle of waxes embedded in a fatty substance; this is analogous to keratinized outer layer of skin, including your own, in animals that live on land. Leaf epidermis is perforated by stomata for gas exchange between the photosynthetic mesophyll (parenchyma) and the surrounding atmosphere. Thus, leaves function in part like lungs. All these tissue types—both simple and complex tissuesare distributed all over the plant parts, but their position and orientations are different in different organs like stem, roots, leaves flowers, fruits, etc.

Organs

Various types of tissues are associated together to carry out a specific function of the body and such structures are known as organs. In animals, stomach, heart, brain, etc., are specific organs carrying out specific functions due to the interaction of various tissues. The heart is involved in the pumping of blood, which in turn is circulated to other organs and tissues by a network of arteries. Blood from various organs and tissues is brought back to the heart by another network of tubes called veins. Thus, forms an important system called the circulatory system. Kidneys are another example of organs. They are involved in the excretion of metabolic waste and other toxins produced in the body. Similarly, there are a large number of organs such as stomach, intestine, liver, pancreas muscles, reproductive organs such as testes, ovaries, external genitalia, etc., that carry out specific functions in association with other organs. There are varieties of glandular organs, both ductless glands such as pituitary, thymus, adrenal, etc., and glands with ducts such as the salivary glands that execute their function primarily by secreting specific enzymes and hormones to carry out various metabolic activities.

Evolution of Population

Evolution is a change in the gene pool of a population over time. A gene is a hereditary unit that can be passed on unaltered for many generations. The gene pool is the set of all genes in a

species or population. A population is a group of organisms of the same species usually found in a clearly defined geographical area.The English moth or the peppered moth, biston betularia, is a frequently cited example of observed evolution. In this moth there are two color morphs, light and dark. Dr. Henry Bernard Davis Kettlewell, a British lepidopterist and medical doctor, is notable for his experiments on the peppered moth, most of which were done in Manchester, England. He found that dark moths constituted less than 2% of the population prior to 1848. The frequency of the dark morph increased in the years following. By 1898, 95% of the moths in Manchester and other highly industrialized areas were of the dark type. Their frequency was less in rural areas. The moth population changed from mostly light colored moths to mostly dark colored moths. The moths' color was primarily determined by a single gene. So, the change in frequency of dark colored moths represented a change in the gene pool. This change was, by definition, evolution.

The increase in relative abundance of the dark type was due to natural selection. The late eighteenth century was the time of England's industrial revolution. Soot from factories darkened the birch trees the moths landed on. Against a sooty background, birds could see the lighter colored moths better and ate more of them. As a result, more dark moths survived until reproductive age and left offspring. The greater number of offspring left by dark moths is what caused their increase in frequency. This is an example of natural selection.Populations evolve. In order to understand evolution, it is necessary to view populations as a collection of individuals, each harboring a different set of traits. A single organism is never typical of an entire population unless there is no variation within that population. Individual organisms do not evolve; they retain the same genes throughout their life. When a population is evolving, the ratio of different genetic types is changingeach individual organism within a population does not change. For example, in the previous example, the frequency of black moths increased; the moths did not turn from light gray to dark in concert. The process of evolution can be summarized in three sentences: Genes mutate. Individuals are selected. Populations evolve.

The word evolution has a variety of meanings. The fact that all organisms are linked via descent to a common ancestor is often called evolution. The theory of how the first living organisms appeared is often called evolution. This should be called abiogenesis. And frequently, people use the word evolution when they really mean natural selectionone of the many mechanisms of evolution. Phenotype is the morphological, physiological, biochemical, behavioral, and other properties exhibited by a living organism. Genotype is the genetic makeup of an organism.

Evolution can occur without morphological change; and morphological change can occur without evolution. Humans are larger now than in the recent past, a result of better diet and medicine. Phenotypic changes like this, induced solely by changes in environment, do not count as evolution because they are not heritable; in other words, the change is not passed on to the organism's offspring. Most changes due to environment are fairly subtle, for example, size differences. Large- scale phenotypic changes are obviously due to genetic changes, and therefore are evolution.

Biodiversity

Biodiversity is the occurrence of all lifeforms in the biosphere. The phenomenon of speciation increases biodiversity. Biodiversity can be for a specific region or geographical area and similarly can be within a species. Within a species there can be varieties or sub-species, strains, and types. This variation within a species constitutes the biodiversity within a species. It is

directly linked to the stability of the ecosystem. The magnitude of the biodiversity is not completely studied. The total number of species collected, named, and classified in taxonomic groups is around 1.5 million. This number is only a small fraction, about 10% of all living organisms, in this biosphere. The remaining, more than 90%, remains to be identified and classified.Out of this 1.5 million known species, 750,000 are insects. The remaining part includes 280,000 animal species and 250,000 numbers of plant species. There are approximately 69,000 fungi, 27,000 algae, 3,000 protozoans, and about 3,000 prokaryotes including eubacteria and archaebacteria. Among these known groups, some have been studied extensively and others have been studied very poorly.

Biodiversity, which is created by speciation and evolution, has a direct impact on the stability of the ecosystem and the biosphere. Due to many man-made changes in the environment through deforestation and construction of big dams, there is disturbances in the habitat of the species, slowly leading to their mass extinction and destabilization. This loss of biodiversity is non-reversible unless we take special precautions. The phenomenon of extinction is opposite to that of speciation.Extinction is the ultimate fate of all species. The reasons for extinction are numerous. A species can be competitively excluded by a closely related species, the habitat a species lives in can disappear, and/or the organisms that the species exploits could come up with an unbeatable defense. Some species enjoy a long tenure on the planet while others are short-lived. Some biologists believe species are programed to go extinct in a manner analogous to organisms being destined to die. This is ordinary extinction. The majority, however, believe that if the environment stays fairly constant, a well-adapted species could continue to survive indefinitely.Mass extinctions shape the overall pattern of macroevolution. If you view evolution as a branching tree, it's best to picture it as one that has been severely pruned a few times in its life. The history of life on this earth includes many episodes of mass extinction in which many groups of organisms were wiped off the face of the planet. Mass extinctions are followed by periods of radiation where new species evolve to fill the empty niches left behind. It is probable that surviving a mass extinction is largely a function of luck. Thus, contingency plays a large role in patterns of macroevolution.

Adaptation

The existence of an organism in its environment or habitat is closely related to the special features of that organism or the adaptations. Adaptation is the special feature of an organism's morphology, anatomy, and physiology, which improves its interaction with its environment. Adaptations usually have the following characteristics.

1. Special features are especially suited to a specific habitat.
2. These special features are often complex.
3. These special features help organisms to live in their environment and capture food, regulate the body's physiology, reproduce, disperse, and defend against enemies.

Adaptation is one of the important factors that drives the process of evolution. Adaptations are created through mutation and natural selection. Evolution requires genetic variation. In order for continuing evolution there must be mechanisms to increase or create genetic variation and mechanisms to decrease it. Mutation is a change in a gene. These changes are the source of new genetic variation. Natural selection operates on this variation. If these variations are suited to the changed environment that organism will outperform the others, which leads to the evolution of

the population. If these new changes created through mutation are not suitable for existence in that environment, they will lead to extinction.

Natural Selection

Some types of organisms within a population leave more offspring than others. Over time, the frequency of the more prolific type will increase. The difference in reproductive capability is called natural selection. Natural selection is the only mechanism of adaptive evolution; it is defined as differential reproductive success of pre-existing classes of genetic variants in the gene pool.The most common action of natural selection is to remove unfit variants as they arise via mutation. In other words, natural selection usually prevents new alleles from increasing in frequency. This led a famous evolutionist, George Williams, to say "Evolution proceeds in spite of natural selection."

Natural selection can maintain or deplete genetic variation depending on how it acts. When selection acts to weed out deleterious alleles, or causes an allele to sweep to fixation, it depletes genetic variation. When heterozygotes are fit than either of the homozygotes, however, selection causes genetic variation to be maintained. (A heterozygote is an organism that has two different alleles at a locus; a homozygote is an organism that has two identical alleles at a locus.) This is called balancing selection. An example of this is the maintenance of sickle cell alleles in human populations subject to malaria. Variation at a single locus determines whether red blood cells are shaped normally or sickled. If a human has two alleles for sickle cell, he/she develops anemia—the shape of sickle cells precludes them from carrying normal levels of oxygen. However, heterozygotes who have one copy of the sickle cell allele coupled with one normal allele enjoy some resistance to malariathe shape of sickle cells make it harder for the plasmodia (malaria-causing agents) to enter the cell. Thus, individual homozygous for the normal allele suffer more malaria than heterozygotes. Individual homozygous for the sickle cell are anemic. Heterozygotes have the highest fitness of these three types. Heterozygotes pass on both sickle cell and normal alleles to the next generation. Thus, neither allele can be eliminated from the gene pool. The sickle cell allele is at its highest frequency in regions of Africa where malaria is most pervasive. Balancing selection involves opposing selection forces. An equilibrium results when two alleles selected in the homozygous state are retained because of the superiority of heterozygotes. Balancing selection is rare in natural population .

Organization of Life

Life on Earth is incredibly extensive and to make it easier to study, biologists have broken living systems up into generalized hierarchical levels as follows:

- ■ Molecules ■ Organisms
- ■ Organelles ■ Populations
- ■ Cells ■ Communities
- ■ Tissues ■ Ecosystems
- ■ Organs ■ Biosphere

The lowest level of the biological hierarchy begins with molecules. Examples include proteins, DNA, lipids, etc. Many such specialized molecules are organized into cells, the basic unit of life. There are single-celled organisms such as bacteria, amoeba, yeast, etc., in which the body consists of a single cell. When the body consists of more than one cell it is called multicellular.

Multicellular organisms are collection of various types of specialized cells. A group of specialized cells carrying out a specific function is called a tissue. For example, muscle tissue, nervous tissue, connective tissue, etc. When different types of tissues are organized together to perform a common function it is called an organ. Examples include, liver, stomach, heart, etc. When a number of organs function together to accomplish a specific function of the body, it forms an organ system. For example, the stomach, liver, intestine, pancreas, salivary glands, etc. work together to form the digestive system. In an organism there are a number of organ systems that work in an associated way to form the organism and its life activities. Each individual organism is a member of a large population, which exists in a habitat.

A population is a group of organisms belonging to a species. A group of different species that live and interact in a particular area or environment is known as a community. The communities, along with the environment in which they exist, are known as ecosystems. An ecosystem consists of biomes, which are large geographical areas of the world. Each biome is a part of the biosphere, which includes the entire living population on the Earth along with its physical environment.

Size and Complexity

Living organisms greatly differ in size and complexity of their body. They range from minute unicellular bacteria to very big multicellular organisms such as blue whales and redwood trees. Primitive cell forms such as bacteria and blue-green algae are very simple in organization and function. The cells and organisms are very small and cannot be seen with the naked eye. A microscope is needed for observing these microorganisms. The multicellular organisms and their cells are very complex in organization and function. The body of higher plants and animals consists of billions of various types of specialized, structurally and functionally complex cells. Therefore, their body and its function is highly complicated.

Variations in the body size affect various other body measurements differently. This is because the volume of cells and so the volume of the entire organism increases much faster than the surface area. Entire single-celled organisms and most primitive multicellular organisms use their cell surfaces to acquire nutrients and dispose of wastes. But the amount of nutrients needed, and the quantity of wastes produced, is related to cell volume. Since the surface area to volume ratio of a cell decreases as its size increases, cells have an upper limit on how much volume they can sustain with a given surface area. Large organisms have less surface area relative to mass than do small organisms. This relationship affects the efficient exchange of material between the body and the environment.

Allometric relationships describe the effect of body size on biological features. These relationships can reveal general patterns of how organisms function; for example, how much they sleep, their food requirements, and their brain size. Allometric relationships also have practical applications, as in the proper determination of drug dosages for animals of differing body sizes. For multicellular organisms, increases in overall body size are mostly due to increases in cell number, not cell size. This is also because of surface area to volume ratio limitations on cells.The evolution of complexity in multicellular organisms is driven by the specialization of cells. Multicellular complexity requires coordination among body cells. Internal communication mechanisms such as hormones and the nervous system help make this possible. Complexity also requires many body cells to give up reproduction in support of a relatively few cells that do reproduce [7]–[9].

DISCUSSION

Living organisms and the physical environment have a close relationship. They interact with each other. The biosphere is the parts of Earth inhabited by living organisms. The biosphere consists of specific geographical areas known as biomes. A biome is a collection of different types of ecosystems. The ecosystems include grasslands, rain forests, streams, lakes, sea, deserts etc., with various types of organisms starting from bacteria, fungi, algae, and various other types of plants and animals. There are millions of known species of organisms and there are many millions to be discovered. Each organism lives in a specialized regional environment within the ecosystem known as the habitat. An organism can live in a specific habitat because it is adapted to live in that habitat. Deep-sea vent, bottom of sea, arctic rivers, and river banks, etc. are examples of habitat.

Organisms living in a specific environment interact with the environment and also with themselves in very different ways. There are big trees growing along the bank of the stream. Since the trees are very big, they make half of the stream a shady area, and this may make the temperature of the water a bit lower than that in the middle region. It is because the water at the bank side is not directly heated by the sun. Similarly, there are many algae floating in the water freely, and this may reduce the penetration of sunlight. So the light intensity under the water may be decreased. All the organisms living in an environment along with that physical environment form an ecosystem. The organisms living in a fresh water pond, along with the pond, form an aquatic ecosystem. All organisms on land along with their environment form the terrestrial ecosystem.These herbivores form the food of carnivores, which are the secondary consumers. And finally the decomposers act on the dead remains of all these organisms including the producers, decompose the organic materials into inorganic materials, and thus cycle back the materials to the environment. This forms the food chain in the ecosystem. In each step of the food chain energy is also transferred. In each step a portion of the energy is lost in the form of heat. Thus, heat is flowing in one direction and is not cycled back. The energy enters the ecosystem from the sun through producers and leaves the ecosystem in all steps of the food chain in the form of metabolic heat [10]–[12].

CONCLUSION

The materials in the form of nutrients required for life are cycled between organisms and the environment. The materials are absorbed by the producers for synthesizing the nutrients and are cycled among the consumers and finally returned to the environment by the activity of saprophytes and other decomposers such as fungi and bacteria. Considering the flow of energy and nutrients in the ecosystem and in the biosphere, it can be considered a single living organism.

The energy flow in an ecosystem obeys the laws of thermodynamics. It is an open system. An open system allows the free flow or exchange of energy and matter such as water, carbon dioxide, nitrogen, food materials, and even the movement of organisms from one ecosystem to another. There are producers, consumers, and decomposers in ecosystems. The producers are the photosynthetic organisms or the autotrophs. The producers of the ecosystem take energy from sunlight and convert it into chemical energy. This energy is passed on to consumers and then to decomposers, which cycles back the materials to the environment. But the energy flows only in one direction and is not cycled back. Herbivorous animals consume the organic food synthesized by the producers, which form the primary consumers.

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CHAPTER 10

TOOLS OF GENETIC ENGINEERING: AN ANALYSIS

Dr. Vandana C D, Assistant Professor,
Department of Biotechnology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: vandana.cd@jainuniversity.ac.in

ABSTRACT:

A pile of stones is not a home, and a collection of facts is not necessarily science. Science is made up of facts, just as buildings are composed of stones. Restricting and modifying enzymes, specific techniques for amplifying DNA, and vectors for conveying this recombinant DNA molecule are all necessary components of the fundamental approach for changing genes and cloning recombinant DNA molecules. Several of the fundamental instruments needed for genetic engineering and cloning will be introduced in this chapter. The more sophisticated technologies for altering the sequence of DNA or protein molecules and observing the results of these changes are described in the following chapters. Bacteriophages use bacteria as their host cells, and every invader develops a defensive mechanism. This mechanism is the host restriction/modification system, which includes a methylating enzyme in addition to the restriction endonuclease in bacteria. As a component of this defensive mechanism, restriction endonucleases cut or cleave specific sequences on the phage DNA, rendering the phage harmless. Invading phage DNA will be digested at the recognition sites, but the host DNA itself has to be protected from this digestion in some way.

KEYWORDS:

Genetic Engineering, Centrifugation, DNA, Macromolecule.

INTRODUCTION

The processes of host restriction, whereby bacteriophages isolated from one strain of Eschericia coli will infect bacteria of that strain but not of other E. coli strains, were the focus of phage biologists' study in the 1960s (in other words, the phage would be restricted in others). By these investigations, the first restriction endonuclease, or restriction enzyme, was isolated from E. coli (Meselson and Yuan 1968). As these restriction enzymes were shown to be strain dependent and to cut DNA into smaller bits, it was hypothesised that they would also detect and cut certain target sequences. Two years later, Smith and colleagues isolated HindII, a different restriction enzyme from Haemophilus influenza strain Rd, and demonstrated that it recognises a particular sequence of 5′-GT (T/C). Cleaving the sequence on both strands exactly in the centre (where the dot is) results in (G/A) AC-3′. (Kelly and Smith 1970; Smith and Wilcox 1970). The first enzyme that was isolated from Serratia marcescens is known as SmaI, while the third enzyme that was isolated from Hemophilus influenzae, strain d, is known as HindIII. This has been acknowledged as the norm for abbreviation [1]–[3].

This is accomplished by altering the bacterial DNA by adding a methyl group to the target recognition sites (modification). In other words, an EcoRI methylase that alters the identical region in the bacterial genome is required for an E. coli EcoRI restriction enzyme to identify the sequence 5′-GAATTC-3′ on phage DNA. Restrictions endonucleases may be divided into four

main kinds according on the degree of enzyme complexity, the need for cofactors, the characteristics of the recognition sequence, and a variety of other factors.

Endonucleases of Type I

They were the first defined systems, and they include a single enzyme with various subunits for recognition, cleavage, and methylation (all-in-one). Its method of cleavage depends on the translocation of DNA until a mechanical collision takes place (often at a distance of up to 1000 bp), resulting in somewhat haphazard pieces. As a result, they are inappropriate for particular gene cloning tasks since they lack a defined cleavage point.

Endonucleases of Type II

These are the most prevalent varieties of restriction enzymes, and they make up the majority of the commercially available enzymes often employed in ordinary cloning procedures. The restriction and modification genes are two distinct genes. Nonetheless, certain Type II enzymes may attach as heterodimers and detect asymmetric sites; regardless, they are quite specific and have generally constant cut locations. Type II enzymes typically bind DNA as homodimers, recognising symmetric sequences. As is the case with the majority of 6-base-cutters, the sites might either be continuous (such as GAATTC for EcoRI),The most frequent Type II enzymes employed in research identify four to eight bases; however, the recognition motif is palindromic, meaning that the nucleotides are mirror images via an axis that passes in the centre of the sequence and that each strand reads the identical sequence from 5′ to 3′.

Endonucleases of Type II

This particular class of enzymes really detects asymmetric sequences and functions in pairs. They split up to 20 base pairs (bp) away from the recognition sequence on one side. On DNA with many motifs, they are substantially more active. By blunting the ends of digestion products, their recognition site cannot be eliminated, which may be used in certain cloning applications, such as creating deletions along a DNA molecule.

Endonucleases of Type III

The mod (modification) and res (restriction) genes encode a complex of two subunits that make up Type III enzymes. The recognition sequence consists of a pair of inversely oriented nonpalindromic sites. The enzyme then starts to break down at a certain distance (between 24 and 26 bp) from one of the copies. These enzymes are not suited for cloning since the precise cut site is not known in advance.

Endonucleases of Type IV

These enzymes are capable of identifying altered (even methylated) DNA. They cleave outside of their recognition sites and are fairly big proteins with two catalytic subunits. Enzymes that detect continuous sequences, like CTGAAG for Eco57I, cleave on only one side of the motif, while enzymes that recognise discontinuous sequences, like CGANNNNNNTGC for BcgI, cleave on both sides.

Both iso- and neo-schizomers

All of the host strains that various phages infect have evolved restriction/modification mechanisms as a means of self-preservation. Consequently, it is not unexpected that if bacteria

are infected with a phage containing the same recognition pattern, enzymes from other bacteria may identify the motif. These two enzymes are known as isoschizomers if they identify the same DNA sequence motif but cleave at precisely the same spot despite having been isolated from different bacteria (thus the distinct enzyme names). Neoschizomers are enzymes that do the opposite.

Map Restrictions

Under normal circumstances, the recognition motifs are extremely precise; as a result, a restriction enzyme will cut a particular sequence of DNA to produce a particular set of distinct fragments, but a different enzyme would produce a different set of fragments from the same DNA sequence. Moreover, several digests may be performed simultaneously or sequentially (first cut with the same enzyme, then purify the resulting fragments, and last do the second digestion) (two different restriction enzymes can be used to digest the DNA molecule at the same time, if their buffer requirements are similar). The two restriction enzymes will digest from their respective recognition sequences in any scenario, producing a collection of smaller pieces than single digests. Even if the whole DNA sequence is unknown, these single and multiple digests may be applied to any DNA sequence to produce a so-called restriction map of the sequence.

Restriction Polymorphism in Fragment Length

We have polymorphisms because our genome is significantly varied within a community. There are several ways to identify these polymorphisms, but restriction fragment length polymorphism, or RFLP, was one of the first techniques to successfully detect a subset of these polymorphisms. This approach depends, as the name suggests, on whether or not polymorphisms between people alter the restriction enzyme recognition motif or the fragment that the digesting process generates (thus it would not apply to polymorphisms, which do not create a readily detectable change in such recognition motifs or in the length of the restriction fragments).

Vectors are DNA molecules that have the ability to reproduce on their own. As a result, they may be utilised to deliver and amplify insert DNA in vivo. The kinds and uses of vectors are many. This section will discuss the most popular vectors, including plasmids, phage vectors, cosmids, bacterial artificial chromosomes, and yeast artificial chromosomes. The size of the insert each of them vectors can support as well as the application for which they may be employed vary. Plasmids, for instance, may carry inserts up to 10 kb, phage vectors can carry inserts up to 20 kb, and YAC vectors can carry inserts up to 100 kb. Another option is to pick vectors depending on the application, such as cloning, sequencing, making RNA or DNA probes, or expressing proteins, rather than the size of the insert (Hartl et al. 1988; Howe 2007; Nair 2008).

Plasmids

Plasmids are extrachromosomal DNA molecules found in prokaryotes. They serve a variety of purposes, including producing conjugation pili (F plasmids), providing antibiotic resistance, and serving as vectors.Plasmids are typically tiny, circular, double-stranded DNA molecules that can reproduce independently inside bacteria, albeit this replication is still dependent on the host. There are two types of plasmids: plasmids may be either strict, reproducing just once or twice every generation (low copy number plasmids), or loose, reproducing somewhere between 10 and

200 copies per generation (high copy number plasmids). All plasmids must include the following DNA sequences in order to be used for cloning:

1. Autonomous replication inside the host cell requires the origin of replication, or ori. A bacterial ori is needed if the host is a bacterium, and a yeast ori is utilised if the host is yeast.
2. Selec- tive markers, most often antibiotic resistance genes, are needed to select the recombinant bacteria that carry the plasmid.
3. The multiple cloning site (MCS), which will be utilised to insert the foreign DNA, is an engineered DNA sequence that has numerous restriction sites that are distinct (i.e., that are not found anywhere else in the plasmid).

A host-compatible promoter for expression vectors, RNA termination sequences, or an MCS placed into the beta-galactosidase (lacZ) gene to be employed for blue-white screening are other qualities of platforms that may be anticipated in addition to these three fundamental ones (as discussed in Section 2.4.2).These naturally existing plasmids have the drawback of not being very versatile in the unique cloning sites they have and the difficulty of choosing recom- binants. The majority of the regularly used laboratory plasmids today are based on the naturally occurring E. coli plasmid ColE1. The pBR322 plasmid, created by Paco Bolivar and Ray Rodrigues, was the first vector to become widely used in laboratories (Bolivar et al. 1977). Plasmids used the resistance gene on the plasmid to assist bacteria avoid the fatal while screening for bacteria with ColE1 [4]–[6].

DISCUSSION

Virus Vectors

In a normal molecular biology lab, plasmids used to be rather popular, especially if one wanted to analyse bigger DNA fragments like those for cDNA or genomic DNA libraries. Phage-based vectors are no longer as widely employed as they once were, nevertheless, as a result of technological advancements. But, from a historical standpoint, we shall provide a succinct summary of phage vectors [7]–[9].

Phagemids and cosmids

Collins and Bruning created cosmids, which are basically plasmid vectors that include phage cos sites (1978). They can replicate in the cell like plasmids since they are based on plasmids and have an origin of replication, but because they contain cos sites they can also be packed like phages and have a double life.

Moreover, since they may be packed like phage particles, they can have a carrying capacity of up to 45 kb inserts, which is considerably higher than that of a regular plasmid and even higher than that of a typical phage vector, which has a carrying capacity of just 25 kb. Cosmids, like phage vectors, are no longer as widely employed as they once were; instead, more sophisticated specialised vectors are now in use.Similar to phagemids, they are not as prevalent as they once were, although one can still find the classic phagemid, pBluescript, in many labs. In essence, phagemids are plasmids that contain an origin of replication for single-stranded phages (like f1); as a result, bacteria that are transformed with this plasmid and infected with a helper phage (like M13 or f1) can produce single-stranded copies of plasmids, which can then be packaged into phage heads.

Particular Vectors

Researchers have created modified vectors for their own specific uses as recombinant DNA technology has improved. Although the quantity and diversity of specialised vectors vary depending on the model organism and area of interest, we will only focus on a select subset of them in this section. In this section, we'll mostly talk about bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), expression vectors like the pGEX series or pCMV-based plasmids, and vectors that encode the enhanced green fluorescent protein (pEGFP). It should be emphasised once more that this is a very limited selection and does not in any way imply that these are the expression vectors that are most frequently used; on the contrary, a sizable number of Drosophila vectors, plant vectors, and vectors for expression in other organisms have been left out in order to make the chapter as brief as possible. These vectors just serve as examples in real life.

Artificial Bacterial Chromosomes

In addition to a selectable marker and typically phage promoters like the T7 promoter for transcription of the cloned genes, a typical BAC vector also includes ori and rep sequences to ensure replication of the vector and the copy number, par sequences for even partitioning of the DNA to daughter cells, and other necessary components. Due to its stability (the risk of the large-sized insert being rearranged is negligible), BAC vectors have been the preferred vectors for genome sequencing studies, and they are often employed to build BAC libraries. These BAC genomic

Yeast Synthetic Chromosomes

Researchers began gathering physical information about the structure of chromosomes as the study of the human genome got under way, even before the official Human Genome Project was established. However, since lambda or cosmid vectors can typically hold up to 5 or 10 kb of DNA, this presented a significant challenge.

Symbolic Vectors

Depending on which cell type from which creature will be utilised for gene expression, there are many expression vectors from which to pick if one wants to synthesis certain proteins for further analysis [10]–[12].By analysing recent studies on the background and methods of genetic engineering, as well as the enzymes employed in restriction endonuclease, electrophoresis, denaturing, transfer to filter, probing, and visual detection, this research paper analyses the tools of genetic engineering. The most popular technologies and techniques for functional genome analysis, such as the Roche/454 Life Science, Applied Biosystems SOLiD, and Illumina Genome Analyzer, are also covered in this article. The article also discusses the moral dilemmas raised by genetic engineering techniques like oncogene activation and insertional mutagenesis.

CONCLUSION

The processes of host restriction, whereby bacteriophages isolated from one strain of Eschericia coli will infect bacteria of that strain but not of other E. coli strains, were the focus of phage biologists' study in the 1960s (in other words, the phage would be restricted in others). By these investigations, the first restriction endonuclease, or restriction enzyme, was isolated from E. coli (Meselson and Yuan 1968). As these restriction enzymes were shown to be strain dependent and

to cut DNA into smaller bits, it was hypothesised that they would also detect and cut particular target sequences.Genetic engineering is a technique that modifies an organism's DNA using technology developed in labs. This procedure entails altering a single base pair, erasing a section of DNA, or including new DNA. Over time, the genetic engineering toolkit has developed from laboratory cloning for analysis to completely synthetic biology for new biomedical capabilities.

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CHAPTER 11

DNA LIBRARIES: CONSTRUCTION, SCREENING, AND APPLICATIONS

Dr Gopal Krishna Menon, Assistant Professor,
Department of Microbiology, School of Sciences, Jain (Deemed to be University), JC Road, Bangalore 560027., India
Email Id: m.gopalakrishnan@jainuniversity.ac.in

ABSTRACT:

A DNA library is simply a representation of the full DNA set (genomic or complementary), often found in bacteria, in a live creature. We will study about the two main categories of libraries in this chapter, as well as how and why they are created and screened. A genomic DNA library is the representation of an organism's whole genome, with coding and noncoding portions included. The protein-coding sections of the genome are the only ones that are represented in the cDNA library, which is created by the reverse transcription of mRNA transcripts. As a result, the two libraries are used for various reasons that will be covered in more depth. Several experimental approaches, different DNA sources, different types of vectors, and different types of libraries are employed. As was already established, the goal of the research will decide the sort of library required, and this will in turn dictate the kind of vector required to build that library. After the identification of these key locations, the genomic DNA is digested into pieces (often partial digestion, resulting in longer and overlapping fragments) and cloned into the appropriate vector with suitable sticky ends. Then bacteria get these ligation products (either through transformation or through viral packaging and infection).

KEYWORDS:

Cells, Centrifugation, DNA, Macromolecule, Nucleic acid, PAGE, Process, Technology.

INTRODUCTION

The preparation of representative DNA fragments, creation of the proper vector, cloning and transformation, selection, and screening are the fundamental components of all DNA libraries.As these techniques are largely the same for cloning any specific DNA, this chapter will only examine the fundamental ideas and possible applications since the vectors suitable for various kinds of libraries were detailed. (For specific technical information on library building, please see published publications and particular laboratory guides.) The library must include up to millions of unique bacteria, each with a unique DNA fragment, which is the key distinction from regular cloning (for sequencing, expression, and other uses). Hence, representation/coverage and trans- formation efficiency become more crucial [1]–[3].

Genetic DNA databases

The cloned DNA fragments will have several repeat motifs, introns, promoter regions, and other regulatory areas in addition to coding sequences. On. As a result, these libraries are helpful for searching for DNA's non-coding sections. It's important to note that since these are genomic DNA fragments, the specific tissue from which the DNA was obtained makes little difference as

long as the tissue is from the target organism (for example, if you want to screen for a human intron, you would have to use a human genomic DNA library regardless of the tissue). In this diagram, the cloning vector is digested and ligated with partial or complete genome digestions; the ligation results are bacteria. Every bacterial clone should, ideally, have a unique genetic segment.

A cDNA library

Complementary DNA, also known as cDNA, is DNA that has been generated to be complementary to mature mRNA and solely represents the exons or coding sections. In other words, a cDNA library is a representation of all the expressed genes in a particular cell at a given time and under a specific situation. It includes the majority, if not all, mature mRNA transcripts that have been recovered from that cell type. Consider the fact that a neuron may not include a dopamine neurotransmitter synthesising enzyme unless a demand exists for it, or that insulin would not be produced from pancreatic -islet cells unless there is a need for it. Similarly, similar to how genes expressed in healthy breast tissue vary from those expressed in benign or malignant breast cancers, genes expressed in youthful mouse muscles will differ from those expressed in aged mouse muscles. As a result, the genes that are expressed will vary within a single organism depending on the cell type, stage of development, and environmental factors. So, the source of the mRNA differs significantly from genomic DNA libraries and is critical for answering the particular issue presented.

Finding the source from which the mRNA will be obtained is the first step. Afterwards, these mRNA species will be utilised to produce DNA using the so-called reverse transcriptase enzyme, which was initially identified in retroviruses. This is required for two reasons: first, because RNA is generally highly susceptible to RNase degradation, which makes it crucial to convert RNA into a more stable DNA double helix; and second, because another double-stranded molecule that can be similarly modified must be used in order to clone into a DNA-based vector. One thing to keep in mind is that the reverse transcriptase will need a primer to start synthesising the complementary DNA, much like DNA polymerases but unlike RNA polymerases. We may leverage the unique changes of the mRNA, most notably the poly (A) tail, as the mRNA species is our target RNA in this case.

This poly(A) tail not only enables reverse transcription of most mRNA through the use of oligo(dT) primers that will anneal to this stretch of sequences, but also allows the researcher to enrich for the mature mRNA transcripts from a total RNA mixture isolated from the cells (typically using oligo(dT) columns). For the purposes of this book, it is important to keep in mind that the cDNA libraries produced in this manner will not contain any regulatory sequences, such as promoters, any introns, or any other noncodon sequences. The choice of vector, cloning, and transformation steps were already explained, so we won't go into too much detail here (suffice to say that there are a few "tricks" one will need to do to clone in the reverse

Only expressed genes, or mature mRNA species that have been transcribed, changed, and translocated, will be represented by the cDNAs. This also implies that, unlike genomic DNA, which is generally unaltered in practically all somatic cells of the same organism, cDNAs from the same organism may change from one cell type to another, from one developmental age to another, or from one circumstance to another.The same cDNA material can be used for microarray experiments as well as RT-PCR (reverse transcription-polymerase chain reaction) experiments, Northern blots, and even for directly analysing relative expression levels in

different cells or cells under different conditions (although some modifications to the procedure may be needed. The same issue is fundamentally investigated by all three assays: how the gene expression is impacted in various cells or tissues, in the same cell at various developmental stages, or in response to various treatment regimens. viewings at libraries

DISCUSSION

After the library has been acquired, it must next be screened for a specific gene of interest. Typically, this screening involves employing a homologous DNA probe that must be either radioactively or nonradioactively tagged. This probe could be the equivalent DNA fragment from a different species (such as the human gene's coding region used to screen a mouse cDNA library from the target tissue), a portion of a coding region to "hunt" for a promoter region, a portion of a gene from one tissue to search for homologues in another tissue, and so on. According to the fundamental tenet of probe-based screening, single-stranded DNA or RNA species will hybridise to libraries' complementary sequences.

There are several techniques to create a labelled probe; for instance, to create a radioactive DNA probe, one must first extract the labelling fragment (for instance, digested and purified human cDNA from a plasmid vector). Then, the labelling fragment must be denatured (the common and easiest method is by temperature). Once the labelled (or radiolabeled) DNA has been denatured, it is extended by DNA polymerase using specific primers, but one of the nucleotides will contain a radioisotope (for example, a combination of dATP, dGTP, dTTP, and 32P-dCTP). The single strands that contain the radioactivity can then be used as a probe to screen for the desired library (whether genomic or cDNA).A protein-coding gene must first be copied to an mRNA transcript in order to be expressed. Monitoring transcription is therefore the initial level of a monitoring expression. There are other methods for doing this; the first one discussed here, known as a reverse transcription polymerase chain reaction, or RT-PCR, likewise depends on the creation of cDNA. The classic Northern blotting is the second method, and the microarray, or DNA chip assay, is the third method that will be discussed in this chapter [4]–[6].

In addition to the restriction-modification system that has already been discussed, there are numerous other enzymes that alter DNA. Only a few of these that are frequently used for cloning applications will be covered in this section: ligases, which are frequently used to join two different DNA fragments and thereby for cloning, polymerases, which are frequently used to amplify the target DNA to be cloned, and alkaline phosphatases, which are used to phosphate the 5′-phosphate groups on vector DNA to be used in cloning. In subsequent chapters, we'll cover nucleases, reverse transcriptases, and other modifying enzymes.

DNA polymerases are DNA-dependent DNA polymerases, which means they synthesise complementary DNA molecules in a 5′-to-3′ direction using a DNA strand as a template. This process is referred to as the 5′-3′-polymerase activity. Since T4 DNA polymerase has a much higher exonuclease activity than the Klenow fragment, it is the preferred enzyme for breaking down 3′ overhangs to produce blunt-ended DNA molecules. T4 DNA polymerase can therefore be used in almost all reactions in which the Klenow fragment is used [1]–[3]. Since the aforementioned DNA polymerases are inactive at high temperatures, they are not very useful for applications like a polymerase chain reaction (PCR), where DNA double strands are melted at a high temperature. The discovery of a heat-stable DNA polymerase from the hot springs-dwelling bacterium Thermus aquaticus in 1976 was welcomed news by the biotechnology community almost ten years later for PCR applications (Chien, Edgar, and Trela 1976). The reaction that this

enzyme catalyses is essentially the same, with the exception that magnesium is not needed as a cofactor and that this enzyme retains catalytic activity at 75–80°C, has a half-life of about 1.5 hours at 95°C, and is largely inactive at 37°C. Since then, a number of additional variants have been reported; these can be purchased by researchers commercially: For instance, Pyrococcus furiosus polymerase has been isolated and has a significantly lower rate of replication errors.

The more popular ligase enzyme for genetic cloning is T4 DNA ligase. Although it would also work on blunt-ended fragments, this enzyme binds DNA fragments with overlapping sticky ends more effectively than it does with blunt-ended fragments (though blunt-ended ligations typically require a higher enzyme concentration and the optimal temperature of 16°C). In order to reduce the number of vector self-ligations or insert tandem repeats, ligation reactions are typically empirically optimised. 1 molecule of vector to 3 or 5 molecules of insert is the suggested ratio for a ligation reaction. Regardless of the vector-to-insert ratio used, this is still an enzymatic reaction, so it will not be 100% efficient. Thus, a variety of fragments will be ligated during the ligation reaction, including correctly ligated and incorrectly ligated fragments, self-annealing of the plasmid vector, and occasionally even tandem ligations. When two different restriction enzymes are used for cloning (RE1 on one end of the fragment and RE2 on the other end of the fragment), one of the ligation errors—ligating the insert to the vector DNA in the wrong orientation—is typically avoided. This is crucial if the insert DNA is intended to be used for protein expression (where the orientation will affect promoter-driven expression).

To prevent misunderstanding, it is important to first underline the difference between real-time PCR, also known as Q-RT-PCR, and reverse transcription polymerase chain reaction (RT-PCR), which is sometimes shortened as RT-PCR. This section's discussion on Q-RT-PCR will come later. It is more challenging to compare expression levels using the mRNA species because RNA is substantially less stable than DNA, or more susceptible to destruction by RNases, which are widely distributed.Because of this, researchers often use the cDNA produced from mRNA during reverse transcription to compare the levels of expression in various samples. A housekeeping gene transcript, such as that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH or G3PDH) enzyme or the -tubulin gene, is often employed for normalisation across several samples, as is the case with every experiment, in order to retain some degree of quantification. The cDNA mix produced from the mRNA of various samples is then subjected to PCR amplification using primers specific for the gene of interest (often the first strand is adequate for this purpose).

The main difference between quantitative real-time PCR (Q-PCR or qPCR for short) and PCR amplification is the real-time monitoring of the amplification process. Real-time means that a mechanism is needed to identify DNA throughout each cycle of amplification. SYBR Green, a non-specific fluorescent DNA dye that intercalates double-stranded DNA, may be used for the detection. As a result, with each cycle of PCR, more SYBR Green fluorescence will be linked to DNA. Alternatively, target DNA-specific probes may be employed to measure the quantity of DNA in each cycle. These are typically fluorescent probes that are quenched when unattached to DNA and only glow when bound to double-stranded DNA [7]–[9].

In the North Blotting

The Northern blotting method, created by James Alwine, George Stark, and David Kemp, is used to analyse RNA, most often mRNA, in samples. It was given that name in honour of Edwin Southern's Southern blot, which is used to detect DNA. The nucleic acids in the sample are

separated in an electrophoresis gel for Southern blotting and Northern blotting, respectively, and then blotted on a membrane before being probed for screening. The probes may be labelled RNA, DNA, or oligonucleotide in nature and can be used in both Southern and Northern blotting, while DNA probes are favoured due to their stability despite RNA probes' superior sensitivity in Northern blots. While nonradioactive probes like those that employ chemiluminescence may also be selected because to their better sensitivity and reduced biohazard as compared to radioactive probes, radioactive labelling is still the most common method used.

Basically, there are two main approaches to manufacture probes: In vitro transcription may be used to create RNA probes, while end-labeling of cDNA fragments or PCR amplification using labelled deoxynucleotides can be used to create DNA probes. You may create single-stranded or double-stranded short oligonucleotide probes. Regardless of the approach used, the probes must have a significant amount of complementarity to the target sequence and little mismatch (none, if possible).

Test for Nuclease Prevention

The classic Northern blot is less sensitive than nuclease protection assays, and in particular, the ribonuclease protection assay (RPA), which will be reviewed here, for the detection and quantification of specific RNA species in the total RNA isolate.A particular target RNA (or RNAs; several RNA species may be detected concurrently if the probes are of various lengths) is hybridised with a target-specific antisense probe to create a double-stranded hybrid (DNA/RNA or RNA/RNA), which is the basis of the nuclease protection test. Using single-stranded RNA-specific nucleases, any unhybridized probe or unhybridized RNA in the sample will be digested (or S1 nuclease when the probe is a DNA molecule). The double-stranded nucleic acid hybrids are then precipitated and subjected to gel electrophoresis analysis after nucleases have been inactivated.

Analysis Using Microarray

One has to know which gene can be expressed in order to produce probes specific for that transcript in both RT-PCR and Northern blotting. A more broad strategy for screening gene expression is needed in order to answer the more open-ended and generic issue of "which genes may be expressed differentially across samples." This may be accomplished very well with the use of microarray or DNA chip technology, which is now the norm for examining global gene expression patterns.

CONCLUSION

There are several microarray formats that are often offered for sale in the market, including oligonucleotide or cDNA arrays, cancer arrays, cell cycle arrays, toxicological arrays, and others. But, in order to meet the unique requirements of the experimenter, it is also feasible to create customised arrays. Nowadays, spot arrays, in which oligonucleotide DNA or cDNA, which is specific to each gene, is affixed to a solid surface, are the most often used format (this could be glass, nylon, silicon, or plastic). Microarrays operate on the same principle as reverse Northern blots, which are nucleic acid hybridizations with the origination reversed: the probe is attached to the microarray surface unlabeled, and the array is screened either radioactively or, more frequently these days, with fluorescently labelled cDNAs from various samples.

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CHAPTER 12

PURIFICATION OF PROTEINS AND MUTAGENESIS

Shreya Banerjee, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- shreya.maulik@atlasuniversity.edu.in

ABSTRACT:

The expressed proteins are sometimes only examined using Western blots, immunofluorescence, immunohistochemistry, or other techniques. Nevertheless, many of these patients lack access to the proteins needed for more research (i.e., they are either denatured for sodium dodecyl sulphate poplyacrylamide gel electrophoresis, or fixed for immunostaining assays). Yet, in rare circumstances, further biochemical or molecular examinations may call for a native protein. In these situations, it is necessary to remove the proteins from the cells; the level of removal depends on what we want to do with the proteins afterward. These carefully controlled post-translational modifications are really chemical changes made to a polypeptide after it has been synthesised and folded. Merely tracking a protein's expression may not always reveal the whole spectrum of these alterations, making it difficult to determine whether an expressed protein is indeed active or not. Yet, in addition to information regarding the degree of protein expression, 2D gel electrophoresis and other proteomic methods may also reveal any post-translational changes.

KEYWORDS:

Proteins, Mutagenesis, Immunohistochemistry, Polyclonal.

INTRODUCTION

We will focus on preparative or crude purifications (or rather, enrichments), based on affinity purification methods like immunoprecipitation or GST pull-down assays. Biochemical and analytical-grade purification through various chromatography methods are largely covered in a variety of biochemistry textbooks.The majority of crude purification kits or systems use on weak, noncovalent affinity contacts that are still exceedingly specific to the analyte and interacting molecule.

This technique of purification depends on the protein under analysis having tags, or short peptide labels, such the Flag tag, HA tag, or His-tag (a typical plasmid for an HA tagged clone is given. Affinity purification, which is frequently used, relies on the affinity between a tag peptide and an antibody that is specific for it, such as the anti-Flag antibody and the Flag tag (discussed in the following sections); however, in this section, we will first look at the interaction between the His-tag and nickel.

The N- or C-terminus of a protein typically contains at least 5 or 6 histidines in a polyhistidine tag (cloning of tagged or fusion proteins was explained. Hence, histidines' affinity for certain metals, such as nickel, which was mentioned in this chapter, or other materials, such as cobalt resins, may be used to purify his-tagged proteins [1]–[3].

Purification of Affinity Using Nickel Columns

The principle is similar to that of HA affinity purification in that the histidines in the tag will exhibit an affinity for the nickel in the resin or agarose beads and will be retained in the precipitate while all other proteins will be in the supernatant and may be discarded. The protein of interest may be eluted and removed (cleaved) from its tag to yield a pure protein after purification, or the column or beads may be employed directly for further identification of interaction partners in co-immunoprecipitations.

Using Monoclonal and Polyclonal Antibodies for Affinity Purification

The majority of the time, indirect detection techniques are employed to analyse or purify proteins, and these techniques sometimes depend on an antibody's affinity for the relevant antigens.The immune cells that make up the body's defensive mechanism create antibodies. B cells in particular of the adaptive immunity create antibodies particular for antigens. A polyclonal antibody is a collection of antibodies (often immunoglobulin IgG) from several B cells that circulate in the blood of an immunised animal and are each specific for a separate antigen epitope. In our situation, the protein we wish to analyse is mostly this antigen. Nevertheless, monoclonal antibodies are produced by a single B cell clone and are directed to a particular epitope.

To do this, immortalised myeloma cells and B cells from the spleen are joined using hybridoma technology, and the resulting clones from each are then purified using affinity purification. Monoclonal antibodies are especially helpful for this process. For proteins that have been fused to tags like the HA or the Flag, particular monoclonal antibody-bound agarose or sepharose beads or resins, such the Flag-agarose beads, are available for immunoprecipitation-based purification. The process of purification is thus quite similar to that described for His-tag nickel affinity. Proteins may be detected using mono- or polyclonal antibodies using a number of techniques, such as immunohistochemistry, immunofluorescence,

Monitoring Cellular Expressions

It is evident that the goal of using an expression vector to clone a gene of interest is to examine the protein that results from that gene. The degree of expression, subcellular localization, localisation and intensity in various tissues, etc., are only a few of the numerous factors that may be studied. Once again, the specificity of antigen-antibody interaction is used to track the expression of proteins.The first thing one may do is do a Western blot on cell lysates to investigate the level of expression in a semiquantitative way. As absolute quantification via antigen-antibody interaction is not attainable, samples are normalised using a housekeeping gene like actin or -tubulin, and the antibody reactivity for our target protein is then evaluated in relation to this housekeeping gene. This approach may also be changed to address other issues. For instance, subcellular fractionation of the cells, followed by lysis and Western blotting, can be used to investigate expression in a specific subcellular location.

The fundamentals of immunohistochemistry and immunofluorescence are the same, but the former is used to study protein expression in tissue slices using an enzyme-conjugated secondary antibody for colour reaction, whereas the latter relies on a fluorescent dye-conjugated secondary anti-body for fluorescent detection of proteins in cells (for technical details, please refer to Appendix B).Radioactive labelling may be utilised for quantitative measurements. Among the

most popular expression assays where radioactive labelling is easily applied are in vitro transcription and translation methods (the details of this expression system are discussed in this chapter). Fundamentally, the acquired mRNA will be utilised to translate the protein in vitro with the aid of a rabbit reticulolysate, a HeLa cell extract, or something similar following transcription from plasmid DNA utilising a viral RNA polymerase, buffer, and NTPs in a reaction tube.

Green Fluorescent Proteins for Fusion Protein Production

As with immunohistochemistry, immunofluorescence relies on the use of antibodies, necessitating the fixation and permeabilization of cells in order for the antibodies to pass through the lipid bilayer. Immunofluorescence is a useful technique for monitoring the expression of proteins in cells as well as their subcellular localizations. After this treatment, the cells are no longer alive, hence the test can only reveal the presence and/or location of the protein for a brief period of time. This image does not provide the whole picture since proteins are dynamic inside cells.Therefore, the discovery of fluorescent proteins has been crucial for the study of proteins in living cells. As a result, Osamu Shimomura, Martin Chalfie, and Roger GFP received the Nobel Prize in Chemistry in 2008 for their work. GFP was initially isolated from the jellyfish Aequorea victoria and quickly gained popularity as a molecular biology tool (Tsien 1998). Eventually, red fluorescent proteins were recovered from additional species, along with other improved forms of GFP including EGFP (enhanced GFP), BFP (blue fluorescent protein), and YFP (yellow fluorescent protein) (a map of pEGFP. As all of these proteins have a fluorophore domain that produces fluorescence when excited at a certain wavelength, fixing cells is not required, allowing for the use of live experiments.

Bicistronic vectors can monitor transfection and the expression of the target gene at the same time using GFP and other fluorescent proteins. The existence of an internal ribosome entry site (IRES) between the coding sequence of the gene to be studied and that of GFP is required for these bicistronic vectors to function. In contrast to bacteria, which may translate several coding units (cistrons) from a single transcript, eukaryotic cells typically only translate from the 5′ end of a transcript. IRES sequences, which are patterns that may entice ribosomes to translate from inside an mRNA, were initially discovered in polioviruses in 1988. By engineering a reporter gene, such as GFP, after an IRES sequence in bicistronic vectors, this characteristic is taken advantage of to ensure that the target gene is copied to the mRNA before the IRES and GFP sequences. It is hypothesised that the reporter gene causes the protein of interest to be expressed in the cells that carry it.

Protein Post-Translational Modifications

An expressed protein-coding gene initially produces mRNA before being translated into a polypeptide. A variety of post-translational changes, such as glycosylation, may be required to make the protein functional or to transport it through the Golgi network to the right subcellular location. Nonetheless, this polypeptide is not always functional upon synthesis and folding. Alternately, a variety of changes, including phosphorylation of an enzyme, sumoylation of a transcription factor, acetylation, or methylation of histone proteins, might alter a protein's function. The protein may also be misfolded or no longer required, in which case ubiquitination labels it for proteolytic destruction (in fact, there are many different formats of ubiquitination, each with a different meaning). Alternatively, a protein could need to be broken down in order to be activated (as when a procaspase is converted into an active caspase), or disulfide bridges might need to be generated for functioning secreted proteins like insulin.In tests like Western

blots or immunohistochemistry, phosphor-specific or sumoylation-specific antibodies may be utilised as a potential solution to the issue. To learn more about the chemical change of a specific amino acid and how crucial that residue is for the functioning of the protein, site-directed mutagenesis may be employed in conjunction with other methods [4]–[6].

DISCUSSION

The easiest approach to comprehend a gene's role is to look at what occurs when it is absent. Using patients who exhibit certain symptoms as a starting point, geneticists look for the deletion or deficiency of whatever gene(s) may be the cause of these symptoms. Instead, molecular scientists may choose for a more direct method and alter or remove a gene entirely or partially in a model system (which might be bacteria, cells, animals, or plants) and then watch what happens to the organism's physiology or behavior [7]–[9]. Apart from improving or changing the features of the protein encoded by the gene, mutagenesis may also be used to investigate the function of certain amino acids or areas in proteins for both academic and industrial objectives, such as medication development.

Studies on Deletion

Deletion studies, as the name suggests, rely on the genetic manipulation of substantial portions of the gene sequence. The easiest way to make these deletions (which may then be utilised for cloning purposes) is via PCR using primers that specify the deletion's borders. This method is often used to identify the function of various protein domains, including nuclear localization sequences (NLS), DNA-binding domains (DBD), activation domains (AD), and others, for protein coding sequences. One may then track which section of the protein is accountable for that specific function by deleting various parts of the protein and analysing the changes in the protein's function.

The deletion of portions of various sizes from both the N- and C- termini is a common procedure; nevertheless, intelligent design of the studies often requires previous research on the gene sequence and its translation, as well as bioinformatic analysis of potential domains, if relevant. In that the same restriction sites as the full-length coding sequence may be chosen and cloned to the vector in the same way, the cloning of N- and C-terminal deletions is comparatively simple. Therefore, a new approach must be developed when internal domains inside the coding sequence need to be eliminated. The method described here is one of the easiest methods to create internal deletions, however it may not work for all coding sequences since site-directed mutagenesis uses restriction enzymes. As the name suggests, site-directed mutagenesis refers to the creation of mutations at specific locations along the gene sequence for a particular reason (such as changing a phos- phorylation site, catalytic activity, ligand binding property, etc.). There are other ways to produce these site-directed mutations, however because to space limitations, we will only focus on a straightforward PCR-based approach.

The sequence is then amplified in two distinct locations using each primer in conjunction with the forward or reverse primer, inducing the desired mutation at the target site (hence, site directed). When the double-stranded PCR products are denatured and annealed in these first few cycles, each single strand may cross-anneal to the single strand from the other PCR product, and only one of these cross-annealed pairs will be productive. The products of this first step of PCR are then used in a second PCR, initially as primers for each other briefly. The forward and

reverse primers will be added to this second PCR phase to complete the process and produce the whole altered sequence.

There are further site-directed mutagenesis techniques that amplify the complete genome using PCR and two primers that introduce mutations. When the freshly manufactured copy of the plasmid DNA is altered as above but the parental plasmid DNA is wild-type in certain whole-plasmid sequencing procedures, a different strategy is used to identify the wild-type parental strand from the mutated daughter strand (otherwise, only half of the transformed bacteria will have the mutant plasmid). The success of this tactic depends on the fact that the restriction enzyme DpnI will only cleave the pattern GATC if the adenine is methylated (i.e., GmATC), and won't cleave the unmethylated sequence. The plasmid is first multiplied in a bacterial strain called dam+, which has a DNA methylase called dam that adds a methyl group to the adenine in the same motif (i.e., GATC). The freshly synthesised DNA created by PCR amplification (in vitro) will not have this methylation, which may then be utilised to have the parental plasmid digested by DpnI, leaving only the newly created plasmids for transformation. The parental plasmid will thus have methylated GATC.

Random Mutation

Genes have historically undergone mutations by chemical or irradiation mutagenesis (as in Morgan's Drosophila studies, for example). Although these chemical or physical mutagenesis techniques are exceedingly effective at changing genes, they are also quite dangerous and hence not entirely desirable.

Today, a variety of other techniques are being used to produce random mutations in genes, including error-prone PCR, degenerate oligonucleotide primers, or mutant bacterial strains (such as the Eschericia coli strain XL1red, whose DNA repair proteins are defective, resulting in a mutation rate that is roughly 5000-fold higher than the wild-type strain). Simple error-prone PCR depends on the ability of Taq DNA polymerase or related polymerases to incorporate incorrect nucleotides in the presence of high Mg2+ and Mn2+ concentrations. On the other hand, degenerate primers would result in site-directed random mutations. A degenerate sequence is created in specific residues (N, for any nucleotide) to introduce relatively random mutations at particular residues.

This method is fundamentally based on the same principle as site-directed mutagenesis. The only significant difference is that instead of changing a specific nucleotide (or set of nucleotides) into other known nucleotides (such as A > G in, introducing a specific. In order to examine the relationships between the structure and function of proteins or enzymes, enhance the catalytic characteristics of enzymes, or create proteins with desired qualities, these random mutagenesis products may be utilised to create a mutant library. High-throughput screening techniques must be developed, nevertheless, in order to select for these desirable features since a large array of mutants is created.

Protein engineering, directed evolution, and enzyme engineering

A protein or enzyme that has been artificially evolved to have the required qualities is the objective of a directed evolution experiment (i.e., directed towards a certain goal). There are several ways to do this, but the most popular ones involve random mutagenesis, primarily error-prone PCR, or the use of a strain that is highly mutagenic [10]–[12].

Alkaline phosphotransferases

Alkaline phosphatases (AP) remove the 5′ phosphate groups from nucleic acids to modify them, and they are primarily active at alkaline pHs. The cold water shrimp alkaline phospha- tase and calf intestinal alkaline phosphatase are the two alkaline phospha- tases that are most frequently used in cloning laboratories.

Recombinases

As a long-term method of subcloning DNA sequences from one vector to numerous others, site-specific recombination can also be used for cloning. The typical exam- ple is the GATEWAYTM series of vectors, which is based on the bacteriophage lambda recombination mechanism, consisting of the att sites (attB site on E. coli and the attP site on the phage) and the recombinase enzyme (see phage vectors). The system also includes an entry plasmid and a donor plasmid, which contain modified att sites for improved efficiency. The initial cloning to the entry plasmid is generally carried out using restriction enzyme fragments or PCR prod- ucts cloned through the incorporation of attB sites through the appropriate design of PCR primers, as can be seen [4]–[6].

Cloning means copying, that is, making identical copies of what one has in the case of genetic engineering, what one tries to clone is usually the recombinant DNA, or plasmid, that one has engineered (Hartl et al. 1988; Howe 2007; Nair 2008; Primrose et al. 2006). (Hartl et al. 1988; Howe 2007; Nair 2008; Primrose et al. 2006). For relatively short pieces of DNA, up to several kilo bases long, high-efficiency DNA polymerases that are commercially available can be used to amplify and copy this recombinant DNA. However, for much longer sizes, PCR ampli- fication becomes rather impractical. Therefore, a more practical method to make genetic replicas of the recombined DNA frag- ments became crucial, and bacteria came to the rescue!

Bacterial Transformation

Once the recombinant plasmid is prepared by ligating the DNA of interest to complementary restriction sites on a rel- evant plasmid, one needs to efficiently transfer this recom- binant plasmid into bacteria. There are many different ways by which one can achieve this—historically one of the first effective methods that researchers came up with was to trans- fer this DNA with phage vectors, and later this method was largely replaced by treating bacteria with calcium chloride

Since transformation efficiency, no matter how close, is hardly ever 100%, the bacterial mixture will inevitably contain both nontransformants and transformants, and among the transfor- mant bacteria some will inevitably contain nonrecombinant plasmids as well as recombinants [7]–[9].

Screening for Recombinants

There are many different ways to screen for recombinants, depending on which vector one uses for cloning. The most common method used for the screening of transformants, for instance, is antibiotic screening, although other methods such as blue-white screening and colony PCR are also available. If cloning into a phage vector, however, then a different method for choosing the recombinant phages would be needed. This section will summarise some of the basic methods of screen- ing used for plasmid cloning [10]–[12]. For plasmid clonings, antibiotic screening is the sim- plest and fastest way to choose transformants. In this method, one plates the transformation

mixture onto an agar plate that includes the relevant antibiotic (whichever resistance gene is present on the plasmid as a selection pressure) (whichever resistance gene is present on the plasmid as a selection pressure). The nontransformants do not carry the plasmid, and therefore will not survive on the antibiotic-containing plate, whereas all the transformants (recombinant or nonrecombinant) will produce colonies due to the presence of the resistance gene.

CONCLUSION

In order to select the desired characteristic under selection pressure, such as resistance to heavy metals, salt tolerance, better affinity to substrate, etc., the gene of interest is altered randomly, producing a vast mutant library. The mutants are then amplified and their DNA is extracted once the positives have been chosen so that the mutation causing the desired attribute can be sequenced and identified.

A sample of blood serum containing immunoglobulins from multiple B cell clones against many distinct epitopes of the protein of interest is taken after an animal, commonly a rabbit or sometimes a goat, is immunised with the protein of interest. This procedure produces polyclonal antibodies.

This approach is very simple and affordable to use, but it has the drawback that when IgGs are circulating in the blood against a variety of distinct epitopes, some cross-reactivity with related epitopes on other proteins may occur. Contrarily, as monoclonal antibodies are IgGs produced by a single B cell clone against a single epitope, they are often more specific and less prone to cause cross-reactivity. Yet, as the name suggests, the production of monoclonal antibodies necessitates the propagation of several B cell clones in the laboratory.

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CHAPTER 13

CELL CULTURE: TECHNIQUES, APPLICATIONS, AND ADVANCEMENTS

Aditya Gupte, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- aditya.gupte@atlasuniversity.edu.in

ABSTRACT:

Being warm-blooded animals, frogs were often used in early cell culture studies because their cells did not need incubation. As research accelerated and got more complex, a model system that was more like human systems became essential. As they produced continuous cell lines and made creating transgenics easier, rodents were first employed. And growing human cells proved more difficult. This problem was first resolved largely because of Henrietta Lacks, a freed slave who resided on the plantation of her family.Cell culture is a laboratory technique that allows for the controlled growth of eukaryotic or prokaryotic cells away from their natural environment. In cellular and molecular biology, cell culture has become a crucial tool that offers great model systems for understanding cell behaviour, how medications and other substances interact with cells, how to make proteins and vaccines, and more. This study reviews current research on the methods, uses, and developments of cell culture to provide an analysis of this laboratory technique.

KEYWORDS:

Cell Culture, animals, DNA, Vaccines.

INTRODUCTION

Henrietta had a severe tumour all over her body. Her cervical cancer samples yielded cells that were removed, known as HeLa cells that grew well in culture systems and are still being used today, more than 60 years later. Organ culture is the three-dimensional cultivation of undisintegrated tissue (thereby preserving some of the features of the organ although it is detached from the body). Organotypic culture, on the other hand, describes the recombination or coculture of cells from many lineages in vitro in order to imitate some of the properties of the organ. Insect, plant, or animal cells may, of course, be described using any of the aforementioned terms [1]–[3].A primary cell culture, which is created directly from tissue, is the most accurate representation of the target tissue; nevertheless, these cells have a brief ex vivo life cycle (i.e., outside the organism). It is challenging to establish a primary cell culture since each tissue is not homogeneous and is composed of several cell types. As well as cell-to-cell contacts, the tissue is typically coated with extracellular matrix proteins, to which the cells are strongly linked. As a result, the matrix proteins must first be disassembled, the cells must then be mechanically or enzymatically disaggregated (blood cells are already separated, though), viable cells of interest must then be separated, and the cells must then be cultured with the aid of essential growth factors and supplements, all the while avoiding contamination. Cell strains may be created in the lab by multiplying primary cells over time, but they must first go through a rigorous validation process before being labelled as cell lines.

Cell lines are just modified or immortalised cells that can be kept in vitro for a long time with little to no physiologic change. These cells might be original cells that have been altered to express viral onco-genes and exhibit limitless growth potential, or they could be derivation of immortalised tumour cells.

Depending on the type of culture that one wants to use in their research, important decisions must be made regarding the source of cells, dispersion techniques (not all tissues can be dispersed in the same way due to differences in matrix material), defined media (i.e., antibiotics, nutritional supplements, growth factors, hormones, etc.), temperature, pH, incubators, and method of cell propagation over time.Cells may be grown as adherent or suspension cultures depending on their origin or source. For instance, since they must cling firmly to an extracellular matrix, fibroblast or epidermal cells must be cultivated as adherent cultures. On the other hand, leukocytes, or white blood cells, are very mobile cells that often travel in the circulation without any significant connection. They may thus be grown as suspension cells. The majority of cell lines have been immortalised by oncogenes, therefore they are unable to be stopped by contact, even if adherent cells are normally kept as monolayer cultures (i.e., cultured in a single layer, and growth is afterwards prevented by touch, or cells are passaged beforehand). Although cells may grow larger after they reach confluency, they must be routinely passaged (or diluted) before that point. Suspension cells must also be passaged for the obvious reason that, in a densely packed population of cells, the medium's nutrients will eventually deplete and must be supplied [4]–[6].

Tissue culture has the advantage of allowing control of the physiochemical environment due to the use of semidefined media, where nutrient concentrations can be determined but the content and amount of growth factors and other macromolecules within sera, such as foetal calf serum or horse serum, cannot be determined and there are variations between batches. Moreover, predefined incubation circumstances might be applied (such as temperature, CO2 concentration, etc.). If cells are stored in liquid nitrogen, they may be used almost indefinitely, especially cell lines. Cell lines and even primary tissue cultures are very homogeneous and become more uniform after a few rounds (mainly owing to selection pressure or culture conditions). The fact that the tissues of the organism get the chemicals or reagents through blood after they have been digested may be considered a disadvantage of studies in which the cells in culture are often exposed to the chemicals or reagents under consideration right away.

Nevertheless, there are also disadvantages, such as the need for specialised expertise and very aseptic surroundings, which are not always available. Animal cells develop slowly, even in the absence of a considerable risk of microbial, yeast, mould, or fungal infection. Moreover, when left in culture for too long, cells may change their characteristics, such as losing their differentiated condition, being more aggressive, or displaying signs of senescence. In these circumstances, only a small number of cells may be created.

Cellular Genetic Engineering

The method used to alter the genetic composition of a cell largely relies on the kind of cell being worked on, albeit these methods may be categorised into five major groups:

1. Electrical, as in the case of electroporation
2. Mechanical, such as a microinjection or gene cannon
3. Chemical, such as liposomes or calcium phosphate
4. Viral, such as when utilising lentiviral, retroviral, or baculoviral vectors

5. Laser, utilised for phototransfections and other things.

No matter how foreign DNA is inserted into the target cell, not all cells will accept it. Defined as the percentage of effectively completely transformed cells in the entire population of cells, "transfection efficiency" is a term we must use. In order to enhance the transfection, a reporter like GFP is often used to estimate the transfection efficacy obtained under different conditions. Each cell type will have a varied transfection efficacy based on the method utilised, which will be influenced among other things by the amount of DNA or reagent used, hence optimising the transfection parameters for each cell type is required before doing an experiment. Confluence of the cell is also essential for optimal transfection; usually, populations of cells that are between 40% and 70% confluent have higher transfection efficiencies than those that are either too dense or too sparse.

Electrical Techniques

Similar to how bacteria were electroporated, the underlying concept behind electroporating animal cells is the same. When an electrical field is applied to cells suspended in a suitable solution, the cell membrane briefly opens, allowing DNA from the solution to enter the cell. Nevertheless, this is a risky technique, and the intensity of the electrical field and the time must be carefully weighed in order to prevent harming the cell membrane.

Mechanical Techniques

In order to directly affect the membrane's integrity, this DNA transfer approach employs mechanical devices like a gene gun and a microscale needle, or microinjection. They are especially useful when just a small number of cells need to be transfected or when the target cell is difficult to physically enter, such as a plant cell with a cell wall.In the microinjection process, DNA is delivered to cells using a glass micropipette that may breach cell walls, membranes, or even nuclear envelopes. This procedure is carried out using a specialised instrument known as a micromanipulator under an optical microscope. A blunt pipette is used to anchor the target cell while a glass micropipette or a microinjection needle is used to deliver the DNA. This procedure may be used to insert DNA into a fertilised eggs male pronucleus, even for the creation of transgenic animals.

A gene gun, often referred to as a particle gun or a biolistic device, was developed first to effectively transfer DNA into plant cells since chemical approaches had trouble penetrating their cell membranes. In essence, the DNA is supplied into the cell together with the heavy metal, such as gold or tungsten that has been coated with the DNA to be transferred. The metal is accelerated under helium or a similar propellant. Nevertheless, the survival of the cells after the technique (and hence the efficacy of the transformation) may be inadequate if membrane integrity is substantially compromised.

Chemical Methods

DNA is precipitated utilising a variety of substances during chemical processes, and the cell then absorbs it. The method most often used makes use of calcium phosphate cotransfection. Calcium phosphate is a fairly affordable method of transfection. In essence, the plasmid DNA is permitted to precipitate as a result of the calcium ions (calcium chloride) in the solution. This precipitate is then added to a phosphate buffer, which is intended to speed up the cell's ability to endocytose or

phagocytose the precipitated DNA. One issue with this approach is the relatively high amount of variability among parallel testing.

Liposome-mediated transfection may also be categorised as a chemical procedure where DNA is encased by lipid molecules with hydrophilic modifications (for simple dissolution and dispersion) and the lipid component promotes easy integration with the cell membrane. The positively charged head groups will interact with the negatively charged DNA molecules more readily if the liposome is formed of cationic lipids. Since liposomes are essentially a lipid bilayer, they may entrap DNA molecules (or even medicines, depending on the application). Since their lipid bilayer is compatible with the phospholipid bilayer of the cell membrane, liposomes often have a high transfection efficiency for animal cells, permitting either the fusion of the liposome with the membrane or endocytosis. The DNA that was thus transferred to the cell first exits endosomes, then the cell nucleus. Yet, due to the cell walls of bacteria and plants, it is difficult for liposomes to deliver DNA to these organisms.

Viral Methods

In vivo delivery, integration into the genome, and stable transfections are three common uses for viral vectors. Host specificity, or the fact that each virus has a certain cell type that it can normally infect, is a property that all viruses that have been exploited for their ability to transfer DNA into target cells have. Hence, a different sort of viral vector may be required depending on the kind of cell. The goal of using viral vectors to transport our desired DNA to the target cell is that the DNA stays in the target cell even if viruses are infectious; in other words, the viral vectors shouldn't cause the target cell to lyse once they have successfully done so. This results in the viral genome often only containing the infectious genes required for a single cycle of infection, and this is another characteristic shared by all viral vectors. There is another another issue (or difficulty) with creating and using viral vectors.

As all viral vectors are designed to be non-infectious, after they have delivered the DNA payload to the target cell, they are unable to produce further viral particles. Nevertheless, this biosafety measure also raises a practical problem: how to first encapsulate the payload DNA into viral particles. A "packing cell line," which has some of the crucial viral genes stably integrated into its genome, is often utilised to do this. As an additional safety measure (so that the viral particles can only be packed if three or more components are present at the same time), some of these genes are now further divided between packaging cell lines and helper vectors in more recent viral vectors.

To adequately infect target cells with the required viral titer, which is a functional indication of viral infectivity, it is important to measure the amount of the virus after the packing cells have created the viral particles. The most popular method for calculating the viral titer uses plaque assays, which enable the computation of plaque-forming units (PFUs). Confluent cells are grown on multiwell plates and then exposed to progressively lower concentrations of the viral material.

Adenoviruses have a double-stranded linear DNA genome but no viral envelope. As they can infect a number of vertebrates, including humans, they provide a valuable resource as gene therapy vectors for therapeutic application. Adenovirus-based vectors can therefore infect human cells in addition to many other mammalian model cell lines (mouse, rat, etc.) and be used, for instance, in gene therapy for Parkinson's disease. They can accommodate rather large DNA inserts and transfer them into both dividing and nondividing cells. Nonetheless, the packing

capacity is still lower when compared to other viral vectors as retro- or lentiviral vectors. Adenoviral vectors often include a partial genomic sequence that is replication-deficient, therefore it may be essential to use a helper vector that incorporates portion of the genome or engages in homologous recombination with the recombinant vector.

Baculoviruses, rod-shaped viruses with circular double-stranded DNA genomes, often infect invertebrates, notably insects like moths and mosquitoes. As a result, DNA is transferred into insect cells like Sf9 cells using baculoviral vectors, generally with the intention of creating a large amount of proteins for future research. To put it simply, baculoviruses are preferred over other viruses if mass protein synthesis is all that is required. This is due to the nonpathogenic nature of baculoviruses, which prevents them from infecting either plants or animals. By cloning the protein's coding sequence under the polyhedrin promoter, which normally produces significant amounts of the baculoviral polyhedrin protein close to the end of the infection, it is possible to produce proteins on a big scale and at a high level. All of the many baculoviral expression techniques, such as the bac-to-bac system (Invitrogen) and the flash-bac system (Oxford Expression Technologies), are supported by the strong expression of the gene of interest (GOI) from the viral polyhedrin promoter.

The reverse transcriptase enzyme is used by retroviruses, which are RNA viruses that generate DNA from their RNA genomes for integration into the host chromosomes.Since they are replication competent, retroviral vectors have the limitation that they can only infect dividing cells. In addition, they could activate any dormant illnesses or endogenous retroviruses in the target cells. Therefore, using the generated recombinant retroviral particles with dormant primary cells won't be useful (such a myocyte). For these cell types, lentiviral vectors are often the best choice. The preintegration complex or the viral shell may pierce the nuclear membrane when they infect nondividing quiescent cells (in G0, such as neurons or muscle), allowing for stable integration of the insert DNA into the genome. Lentiviruses, a subtype of retroviruses, are the foundation of lentiviral vectors.

Laser Methods

Based on the hypothesis that a temporary hole may be made in the cell membrane when a laser is directed to a particular spot on it, enabling nucleic acids like DNA or RNA to enter the cell. This is very useful when analysing a few primary cells (such as a single cortical neuron), but it is less useful when analysing a large number of cells. Yet, it has the advantage of enabling local translation, which is not achievable with the bulk of the aforementioned techniques, by delivering mRNAs to particular cellular locations.

Reporter Genes

While expression plasmids have already been discussed, we won't go into great detail concerning those vectors here. We merely want to remind readers that the plasmids used for transfection and analysis in (animal) cells may be broadly categorised into four types based on the promoters utilised:

1. Enhancer elements or activating regulatory motifs are generally studied using plasmids with minimal promoters, such as the HSV tk (herpes simplex virus thymidine kinase) promoter.

2. (Again, it should be emphasised that the plasmids mentioned above are mammalian expression plasmids; if one is working with, for example, Drosophila or plant cells, one will need to refer to the equivalents of minimum and constitutive promoters for the respective organism) (Again, it should be noted that the plasmids given above are mammalian expression plasmids; if one is dealing with, for example, Drosophila or plant cells, one will need to refer to the equivalents of minimal and constitutive promoters for the specific organism.)
3. Plasmids with promoters that are unique to a certain kind of cell.
4. Plasmids with regulable promoters.

We will instead focus on a specific group of vectors that are used as reporters in this chapter. A reporter gene is often included in these vectors, and it is used to monitor the expression of plasmids transfected into target cells. They may be used to monitor a promoter's activity in a cell culture system or as positive checks to make sure that target cells are successfully transfected. Traditional reporters include fluorescent proteins like green fluorescent protein (GFP) or its derivatives, luciferase, or enzymes like -galactosidase (lacZ).

The typical molecule for many reporter assays is -galactosidase (lacZ), due to its extensive research. This enzyme's role in the lac operon of bacteria, where it catalyses the breakdown of lactose into its monomers of glucose and galactose, has been the subject of much investigation. The enzyme may also hydrolyze other comparable substrates, most notably the colourless X-gal molecule (5-bromo-4-chloro-indolyl—D-galactopyranoside), which is hydrolyzed into galactose and 5-bromo-4-chloro-3-hydroxyindole, which after aggregation and oxidation produces an insoluble blue-colored complex. Due to its colour response, LacZ is a strong reporter in many applications. LacZ enzyme use in complementation-based

The enzyme luciferase was first discovered in a particular type of firefly. It may catalyse the oxidation of the substrate luciferin in the presence of ATP, producing oxy-luciferin and bioluminescence. Since the amount of bioluminescence generated can be detected directly with the use of a luminometer and is closely connected with the amount of enzyme synthesised in the cell, this approach of measuring promoter activity has grown in favour (hence, the strength of promoter driving the expression of this enzyme).

Forms of Transfection

The introduction of foreign DNA into cells is often referred to as "transfection," albeit this DNA may remain extrachromosomal and not always be integrated into the genome. According to whether there is stable integration or not, transfections may be loosely separated into transient and stable transfections.

A transient infection

Transient transfection refers to scenarios in which the gene trans- ferred to the cell spreads to the daughter cells at each cell division and in the absence of selection pressure. As a consequence, the high level of expression of a transfected gene only lasts for a few period of time after transfection before it starts to decline as DNA is lost. High-copy number vectors with potent promoters are often used for the high-level synthesis of proteins, markers, or reporters, as well as the proper analysis. While the study is quite speedy, it has the disadvantage that each cell has a significant amount of plasmid DNA, which is inconsistent with the remainder of the genome,

which is diploid. Moreover, it is not integrated, which causes it to deteriorate with time. The results of transient transfections must thus be carefully analysed since protein overexpression may very well be the cause of the data [7]–[9].

Trustworthy Transmission

Stable transfection, in contrast to transient transfection, leads to the eventual integration of transfected DNA into the genome, although with varying probabilities (the efficiency of integration depends on cell type, method used, or selection pressure applied). While there is eventually only one copy of the transfected gene, as opposed to tens or hundreds, and this copy is more physiological, the results of stable transfections are more reliable. Moreover, the stable integration guarantees transmission across many generations to daughter cells. Viral vectors are often used for the creation of stable cell lines because of their capacity to integrate into the genomes of their target cells.

Recombination and Genome Incorporation Recombination is the splitting and rejoining of DNA from a variety of sources, including other species, cells, or chromosomes. Examples of naturally occurring recombination processes include homologous recombination, such as cross-over, site-specific recombination, like integration of the viral genome, and nonhomologous end-joining, like a DNA double-strand break repair pathway. The Rec recombinase enzymes, among other crucial enzymes required for recombination processes, may all belong to the same family. We'll examine the basics of homologous and site-specific recombination processes fast since many genetic techniques for modifying animals or plants leverage these ideas (Watson et al. 2008).

Customized Recombination

DNA molecules that are generally similar but not identical interchange complementary sequence regions during homologous recombination. It is mostly studied in relation to post-replicative recombination repair and meiotic crossing over. Homologous recombination does not begin at any particular location; rather, it can occur between any two of the recombination hot spots, which are scattered almost at random along the sequence of each chromosome.

The strand exchange in one of the chromosomes is initiated by removing a few nucleotides at these double-strand break sites, which results in short sections of 3′ overhangs. To attach to the single-strand binding protein RPA, these 3′ overhangs, which are now single strands, invade comparable locations in the other DNA molecule (hence homolo- gous recombination). A four-way Holliday junction forms at the exchange (this Holliday junction structure is also observed in site-specific recombination, although the context is slightly different there). DNA molecules with switched strands are produced as a result of resolution of the Holliday junction, which is a very difficult process in and of itself and is not the subject of this discussion (Watson et al. 2008).When DNA damage cannot be repaired, it is omitted from the replication process, leaving the initial damage unrepaired. The post-replicative recombination repair is what is happening here. Moreover, it is used to fix double-strand breaks.

Site-Specific Recombination

Nevertheless, recombination only takes place in a certain region of between 30 and 200 bp, outside of which little or no homology is required. Theoretically, homologous and site-specific recombination employ very similar methods. Because of the asymmetry of these sites, the recombinase enzyme may recognise both the left and right sections of this motif.

The two recombinases that are most often used in genetic modification are Cre and Flp (which will be extensively covered in the following chapters). At Holliday Junctions, strand switch happens once more (Watson et al. 2008).The outcomes of strand exchange may include integration, deletion, or inversion, depending on the source or strands to be exchanged (same or different DNAs), and the direction of the recombination.

Volume of Expression

The degree of exoge- nous gene expression in the cells, which is directly connected with the promoter present in the vector, is the second factor to consider. The target gene is expressed using the same mechanisms as genes that are expressed in live cells: a strong promoter will increase the quantity of gene expression, while a weaker promoter will cause the target gene to create fewer transcripts. Despite the fact that certain promoters are active in all cell types and conditions, others are either tissue- or developmental-time-specific or only expressed in specific situations when inducing chemicals are present. As a result, these same methods will be used to modify cells' genetic makeup.

Partially Expressed

A gene that has been inserted into cells will always be expressed, regardless of the context, under scenarios known as constitutive expression. Many mammalian expression vectors, including the pCMV family, use strong, constantly active promoters. This is particularly advised if high amounts of protein expression are required for investigative purposes.

Simple Induction of Expression

One may cause expression in cells by selecting a promoter that is responsive to a variety of stimuli, which in turn regulates the activity of transcription factors. Certain promoters may be activated by thermal stimuli (such as heat shock promoters), others by chemicals (such as lactose, lactose analogue IPTG, or tetracycline-inducible promoters), and still others can be controlled by oxygen levels (such as hypoxia-inducible promoters) [10]–[12].

CONCLUSION

Let's start with some definitions as there are several different types of cultures. Tissue culture is the in vitro cultivation of a whole tissue, such as the skin's epidermis. It stands to reason that certain tissues are easier to establish in vitro than others due to the cells' intrinsic properties, such as their potential for cell division. The in vitro growing of sporadic cells derived from primary tissue, a cell line, or a cell strain is known as cell culture. Also known as primary cell culture, this procedure (we will discuss the dif- ference of these latter two terms later).The study examines the numerous cell culture types, including microbial, plant, and animal cell cultures, as well as their uses in a range of disciplines, including biotechnology, medicine, and environmental science. The report also emphasises the need of comprehending the circumstances necessary for developing a successful cell culture, such as the usage of suitable culture media, temperature, and pH. The development of three-dimensional (3D) cell culture models and how they are used in oncology research are also covered in the study. According to the research paper's conclusions, cell culture is an essential tool for expanding our understanding of cellular and molecular biology and creating fresh approaches to biotechnology, medicine, and environmental science.

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CHAPTER 14

GENETIC MANIPULATION OF STEM CELLS AND ANIMALS

Aditya Gupte, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- aditya.gupte@atlasuniversity.edu.in

ABSTRACT:

Self-renewal and differentiation are characteristics of stem cells. Both embryonic and adult bodily organs may be used to harvest stem cells. Moreover, they are capable of differentiating (potency) and assuming any cell type in the body's features (Plasticity). They may be utilised to increase the production of livestock due to their effective traits, which include chimaera generation, gene targeting, nuclear transfer, animal cloning, gene transfer, and genetic engineering. In addition, stem cells may be utilised to treat a number of clinical diseases, such as heart abnormalities, spinal cord injuries, tendon, ligament, and cartilage repairs, as well as to speed up the healing of wounds. This overview describes the characteristics, historical background, categorization, and adaptability of stem cells. Stem cell uses in many productive and therapeutic domains are also covered.

KEYWORDS:

Genetic Manipulation, Stem Cells, DNA, Macromolecule, Genetic Engineering.

INTRODUCTION

Nowadays, stem cells are used extensively in research across many disciplines, including veterinary, agricultural, and human. Genetic engineering and medicine. These cells are known as stem cells because they are capable of both self-renewal and differentiation (Anand et al., 2011). According to self-renewal, stem cells have the capacity for unrestricted, ongoing cell division while yet retaining their undifferentiated status. Under the right experimental, physiological, and cultural circumstances, stem cells have the capacity to develop into one or more other (different) cell types [1]–[3].As stem cells split, the new daughter cell has two options: it may either stay a stem cell or it can develop into a specialised cell (such as a muscle, blood, or nerve cell) with a specific purpose. For stem cells to proliferate and activate the genes necessary for cell type differentiation, a signal is required.

Origin and categorization of stem cells

Embryonic stem cells and adult stem cells are the two main divisions of stem cells. Adult stem cells are produced from adult body organs, while embryonic stem cells are separated from the inner cell mass (ICM) of the embryo. The ability to self-renew and differentiate is a trait of stem cells. Body organs from both embryos and adults may be used to harvest stem cells. Moreover, they possess the capacity for differentiation and the capacity to adopt the features of any kind of body cell (Plasticity).These productive traits allow for the use of chimaera formation, gene targeting, nuclear transfer, animal cloning, gene transfer, and genetic engineering to increase the output of cattle. Moreover, stem cells may be employed to treat a number of clinical problems, including heart abnormalities, spinal injuries, tendon, ligament, and cartilage repairs, as well as wound healing. The characteristics, history, categorization, and flexibility of stem cells are

described in this review. Furthermore included are stem cell uses in different productive and therapeutic domains.

The Inner Cell Mass (ICM) and the Trophoblast are formed by the cells of the embryo (blastocyst). Ectoderm, Mesoderm, and Endoderm, which differentiate into the animal's internal organs, are formed from embryonic stem cells (also known as ICM). The placenta is created by the trophoblast. Nervous system, teeth, hair, exocrine glands, and mammary glands are among the organs that arise from ectoderm.Endoderm is the respiratory and digestive systems' epithelial lining. Liver, pancreas, thyroid gland follicular lining, thymic cells, auditory tube and tympanic membrane epithelium, urinary bladder, and a portion of the urethra.Animals that are young or adults include adult stem cells, also known as somatic stem cells. The many kinds include

Blood-forming stem cells

Stem cells have the capacity to develop all immunological and blood cells. By the daily creation of billions of new blood cells, these cells are in charge of ensuring that the blood is constantly renewed.

These are the only adult tissues with the greatest capacity for self-renewal found in umbilical cord blood and bone marrow.

Mechanized stem cells

They are quite helpful in treating a variety of chronic and crippling clinical diseases in dogs, horses, and caprines. They may be a useful tool for cell treatment in regenerative medicine. They come from stromal cells. They are separated from the placenta, bone marrow, lung, adipose tissue, blood, teeth (periodontal ligament), and other tissues.

1. A central nervous system cell called a neural stem cell is one that is mainly undifferentiated.
2. The ability of neural stem cells (NSCs) to produce progeny cells that develop and differentiate into neurons and glial cells.
3. The discovery of stem cells in the adult monkey brain was initially made public by Lewis in 1968. In mature rats, neurogenesis persists, as Altman and Das (1965) found. Adult neurogenesis is limited to two regions of the brain: the dentate gyrus of the hippocampus formation and the subventricular zone, which borders the lateral ventricles (Alvarez-Buylla et al., 2002).

Dermal Stem Cells

During adulthood, the epidermis continually regenerates, and the hair follicle goes through an ongoing cycle of development and degeneration.Adult skin homeostasis and hair regeneration are maintained by stem cells (SCs) found in the epidermis and hair follicle.

Stem Cells in the Retina:

By transmitting electrochemical signals to the secondary neurons and visual brain, the retina aids in the perception of light. Endogenous retinal stem cells, such as neural stem cells, Muller cells, and retinal stem cells from the ciliary marginal zone, are sources of stem cells for retinal regeneration.

Embryonic Stem Cells

They serve as a source of cells for the mammary gland development throughout puberty and pregnancy. According to Liu et al. (2005), they are crucial in the growth of myoepithelial cells in the mammary gland and the development of breast cancer.

Stem Cell Adaptability

The ability of stem cells to adopt the traits of cells from different parts of the body is referred to as plasticity (Wagers and Weissman, 2004). For instance, bone marrow stem cells may seize control and transform into liver or lung cells.

The capacity of stem cells

Each kind of cell in the body may develop from totipotent stem cells. The term "Toti" is derived from a Latin word that signifies full, whole, or total. An egg divides many times after fertilisation to produce an embryo, which then develops into a foetus. Totipotent cells are those that are created during the first few divisions.

Pluripotent (Latin: pluripotentia; English: having the capacity for a wide range of functions)

It is possible for pluripotent stem cells to develop into practically any kind of body cell. After going through their first few divisions, totipotent stem cells become pluripotent stem cells. Pluripotent cells include foetal stem cells and embryonic stem cells (Inner Cell Mass) during the blastocyst stage. Since totipotent and pluripotent cells are necessary for the growth of new organisms, they may be detected in the embryo's early stages of development [4]–[6].

DISCUSSION

These stem cells are multipotent, meaning they have the capacity to develop into several cell types. Unfortunately, it is only applicable to a family of cells that are closely connected. They mostly affect blood, heart, muscle, and nerve cells. These cells serve as the body's mechanism for repairing damaged tissues. Adult stem cells serve as an illustration for this group [7]–[9].

- Oligopotent (capable of differentiating into a small number of cell types)
- They may change into a few distinct kinds of cells, including lymphoid and myeloid cells.
- Unipotent (capable of differentiating into only one kind of cell)
- These stem cells have the ability to self-renew and can only create one kind of cell.
- This sets it apart from progenitor cells, which are unable to replenish themselves.

Source of the transplant is the patient's own stem cells when done through the autologous approach (AUTO transplant) (autologous). Either the patient's own body cells or the cord blood of the patient make up these cells.Currently, instead of bone marrow, doctors often get stem cells for autologous transplants from peripheral blood.The allogeneic transplantation route (ALLO transplant). Another donor is the source of the stem cells (allogeneic). They may either be a) Familial allogeneic (mostly related people, such as a parent, kid, sister, or brother) or b) Unrelated allogeneic (completely unrelated donors). In this case, the stem cells are taken from the donor's body or cord blood (Umbilical cord transplant). According to Slavin et al. (1998), myeloablative conditioning, which is linked to risky early and late consequences, is regarded as a

prerequisite for allogeneic blood or marrow transplantation (allogeneic BMT), which is used to treat malignant hematologic illnesses and genetic diseases. Immunemediated graft-versus-leukaemia (GVL) effects are the principal advantage of \sallogeneic BMT. In order to create host-versus-graft tolerance for the engraftment of donor immune hematopoietic cells for the production of GVL effects to displace remaining malignant or genetically aberrant host cells, they introduced the use of relatively non-myeloablative conditioning prior to allogeneic BMT.

The main restrictions, however, are a lifetime of immunosuppression and the possibility of rejection. Undifferentiated cardiomyocytes generated from mouse embryonic stem cells survived in a normal heart following transplantation, according to Naito et al. (2004). They said that this was a desirable approach to treating cardiac problems.Human mesenchymal stem cell transplantation was examined by Neimeyer et al. in 2010.

Uses of stem cells in the development of livestock

The effective use of stem cells has established itself as a key strategy for improving livestock reproduction. Stem cells are utilised in a variety of fields, including gene targeting. Zwaka and Thomson (2003) reported using an electroporation technique based on the physical properties of human ES cells to successfully target the genes HPRT1 (hypoxin phosphoribosyltransferase-1) and POU5F1 (octamer-binding transcription factor 4; also known as POU domain, class 5, transcription factor 1) using stem cells (POU5F1)

Animal cloning (Stice and Keefer, 1993), nuclear transfer (Harrison et al., 2002), chimaera animal production (Cibelli et al., 1998), transgenic animal production (Saito et al., 2001), and genetic engineering all include the effective development of bovine nuclear transfer embryos (Soto and Ross, 2016) - The development of transgenic animals may improve their genetic improvement due to the skillful utilisation of stem cells (Wheeler, 2007). Stem cells may be genetically modified to carry out tasks for which they are not ordinarily intended. Chemotherapeutic chemicals may be administered using this method to treat cancer and tumours. According to Hu and Fu (2012), knowing the fundamental traits of cancer stem cells can help researchers create new treatments that target the cancer stem cell that causes the disease in the first place. Tissue loss after spinal injuries includes tracts of myelinated fibres that convey nerve signals.

The ability of nervous tissue to regenerate is constrained. Recovery from spinal injuries is aided by the transplantation of stem cells that may develop into neurons and supporting cells, according to Int.J.Curr.Microbiol.App.Sci (2018) 7(3): 67-77 74. According to Teng et al. (2002), traumatic spinal cord injuries in animals might be treated using a special polymer scaffold seeded with neural stem cells.Embryonic stem cells aid in the regeneration of the severely damaged heart and provide further proof of their immunological privilege (Zhu et al., 2017). Myocardial healing was accomplished using the engraftement of cardiac cells produced from pluripotent stem cells in a pig model of myocardial ischemia-reperfusion damage. Adult animals' difficult-to-heal cartilaginous tissue is repaired using mesenchymal stem cells. It is used to cure cartilage abnormalities and differentiates into chondrogenic lineage. Furthermore capable of osteogenic development are mesenchymal stem cells.The possibility of enhancing bone repair and regeneration using autologous stem cell treatment was investigated by Bruder et al. in 1998. Cheng et al. (2015) investigated the therapeutic potential of Wharton's jelly tissue transplantation by reducing the symptoms of traumatic brain damage [10]–[12].

CONCLUSION

In conclusion, stem cells offer a wide range of uses in both the treatment and production of cattle because of their comprehensive features. In terms of cell-based therapeutics, stem cells are seen to be the most promising component. Stem cells are mostly employed to treat dogs and horses in the area of veterinary medicine. Unfortunately, the utilisation of stem cell therapy is limited by the high cost of care. As stem cell manufacturing is greatly boosted, the cost of stem cell treatment will potentially decrease. Therapeutic usage of stem cells requires several studies to standardise desired therapy regimens, delivery methods, and dose. Stem cells may also be employed to treat a variety of chronic diseases that have little chance of recovery.

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CHAPTER 15

GENETIC MANIPULATION OF PLANTS: AN ANALYSIS

Harsh Mohanty, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- harsh.mohanty@atlasuniversity.edu.in

ABSTRACT:

Over 10 million farmers in 25 countries planted transgenic plants on an area that increased from 44 million hectares in 2000 to 125 million hectares in 2008. (Marshall 2009). The majority of these crops (58.6%) are herbicide-resistant; the rest are either insect- or stack-trait resistant. Notwithstanding the continuous discussion about the ethics of environmental safety, economics, and gene diversity-related concerns, many people still consider transgenic crops as a source of oral vaccinations, biofuels, or improved/high-quality food items. As a consequence, genetic engineering is either employed to modify plants to produce new goods or to give them new roles, such increasing crop quality or quantity.

There are a number of moral issues with genetically modified agricultural plants (a subset of genetically modified organisms, or GMOs). As it is well-known for its Bt cotton, a trade-mark genetically modified cotton that produces an insecticide as a consequence of the expression of a bacterial toxin-producing cry gene inside the cotton, one of the original companies, Monsanto (St. Louis, Missouri), is the primary target.

KEYWORDS:

Cells, Genetic Manipulation, DNA, Plants.

INTRODUCTION

The cry genes in Bacillus thuringiensis (Bt) create cry (crystal) proteins, endotoxins that are activated in the acidic pH of an insect's stomach. As a result, the insect perishes. The Bt potato was really developed by Monsanto, which also obtained approval from the EPA in 1995. The Bt soybean, Bt maize, and other plants were later produced by the business using the same genes. Although the use of conventional pesticides in the field was initially drastically reduced by these insect-resistant GM crops, known as Bt plants, creating a tremendous amount of hype for the biotech industry, pesticide consumption has been estimated to reach over 1 billion pounds annually in the United States alone (Alavanja 2009). Yet, a Bt cotton test in India reveals no discernible gains in output or discernible reductions in the use of pesticides in areas where Bt cotton is grown [1]–[3].

Disinformation, mistreatment of farmers, or Bt cotton itself may or may not be to blame for this. Globally, pesticide sales were actually growing, maybe with the probable exception of North America, where Bt cotton has significantly reduced the need of pesticides. Yet, China uses almost five times as many pesticides per acre than the US does, for instance (Plumer 2013). The reader may do their own study on the financial and economic ties, as well as the ethical issues, and narrow their attention on the procedures used to make transgenic plants.

Dicotyledons, Monocotyledons, and Commercial Crops

Flowering plants are traditionally split into two groups: monocots and dicots. Despite the fact that certain species are difficult to categorise, many model organisms employed in plant laboratories may be readily distinguished by a number of features.

Monocots might be considered the most important crops in terms of industry since they are the main source of the world's nourishment. Consequently, much research on the geneticIn 1992, the FDA approved the Flavr Savr tomato as the first genetically modified food product that was commercially marketed. A transgenic antisense gene that suppresses the production of a gene called polyga-lactouronase, which prevents the breakdown of pectin in the cell wall and decreases softening, was inserted into this tomato, which was developed by the firm Calgene LLC (Davis, California; now a Monsanto subsidiary). Flavr Savr tomatoes didn't really provide any observable benefits, and they didn't stay on the market for very long since they didn't hold firm and needed to be harvested in the same way as standard wild-type varieties [4]–[6].

A research team from the Swiss Federal Institute of Technology and University of Freiburg has genetically altered Golden Rice to produce beta-carotene in rice in order to improve the nutritional value of this staple crop for the vast majority of the population of the world who consume rice but are vitamin A deficient.Another Monsanto product, the Roundup Ready Soybean, has been genetically altered to have improved resistance to the company's well-known herbicide, glyphosate, which harms both crops and plants because it prevents the formation of key amino acids. This substance inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyses an important stage in the production of these essential amino acids. The commercial crop is protected from the herbicide by the EPSPS variant that is produced by the genetically engineered Roundup Ready type. The main problem with the use of Roundup Ready has been that, while the system initially worked well and allowed for the effective removal of herbs with only a small amount of glyphosate, over time, herbs have developed resistance to this herbicide (much like bacteria and antibiotics do), and as a result, the use of herbicide has gradually increased, effectively outweighing the advantages of genetic modification (i.e., less use of herbicidesnot more).

A French team presented two-year research on rats given a regular diet vs a diet containing Roundup herbicide and/or Roundup Ready maize, claiming that this exposure led to a high cancer incidence over time (along with several media events previous to the article publication) (Seralini et al. 2012). Obviously, advocates against GMO goods and regulations were outraged by this, causing a global controversy (Arjo et al. 2013). Yet a comprehensive examination of the data made it evident that the results did not support the interpretations. One difference between the GM-fed male rats and the control group was that fewer male GM-fed rats (3 out of 10; 30% vs. 7 of 30) died from tumours. Also, there was no dosage dependency. In comparison to rats given a diet containing 22% GM, those fed a diet containing 11% GM died more often (Arjo et al. 2013; Seralini et al. 2012). As a consequence, scientists from all around the globe began to closely examine the piece right once (Arjo et al. 2013; Ollivier 2013; Sanders et al. 2013). The publication was finally withdrawn despite the authors' thorough replies to several of these complaints (Seralini et al. 2013).

Several more transgenic crop experiments have been made, some of which are presently in the commercialization stage. For instance, a Japanese team effectively and sustainably suppressed the expression of N-methyltransferase enzymes involved in caffeine production to develop a

transgenically decaffeinated plant (Ogita et al. 2003). This chapter will briefly examine some of the most well-known plant expression vectors, plant cell or tissue culture methods, modification strategies, and associated issues that are employed in some of these industrially relevant plants.

1. Techniques for Manipulating Plants
2. Plant Cell and Tissue Culture

Plant cells are totipotent, which implies that any section of the plant might potentially develop vegetatively and generate a full plant. This is how plant cells vary from animal cells. Because of this characteristic, controlling plant cells and tissues in cell and tissue culture is simpler than manipulating mammalian cells. Among many other plant components, the plant may include embryonic cells, specialised organs, callus, or cultivated scattered cells. If a plant is cut or damaged, a callusa mass of rapidly proliferating cells—forms, just as how animal wound sites form calluses of rapidly proliferating fibroblasts or progenitor cells. Thus, a callus may develop into root, shoot, and stem structures in tissue culture (Dodds and Roberts 1985).

The same aseptic culture conditions apply to plant cell culture as they do to mammalian cell culture. Even though the media should be customised for each plant cell, culture media are based on the nutritional requirements of plant cells and follow a few basic rules. Plant growth hormones like auxins and cytokinins, vitamins like vitamin C, biotin, and riboflavin, sugar (mainly sucrose of D-glucose), amino acids, nicotinic acid, glycine, and pyridoxine, and inorganic ions like nitrogen, magnesium, and phosphorus are often added to the culture media (Dodds and Roberts 1985). The lab should be optimised using a precise methodology. Monocots and dicots may need a distinct growth media, which may either be aqueous or matrix-based (containing the starch of sucrose polymers).Plant cells may be genetically modified using polycationic substances like calcium, liposomes, electroporation, and other tools. Several of the aforementioned gene delivery strategies are substantially less successful when attempting to genetically change plant cells due to the hard construction of the plant cell wall. As a consequence, a variety of strategies have been created to penetrate the cell wall, improving transformation efficiency.

Genetic Pilot

Nucleic acids may be directly delivered into organisms that are typically difficult to transfer, such as bacteria, yeast, or plant cells, using a gene cannon. It is also known as a particle bombardment technique or a biolistic particle delivery system. Helium or another source is often used to accelerate gold or tungsten particles that have been coated with nucleic acids, enabling them to penetrate both the cell wall and the cell membrane in such animals. After the nucleic acid has been released from the particle, one of two outcomes will occur: either a transitory expression or a steady expression (if the host chromosome is integrated, which has a very low probability of happening).

Protoplasts

Since the highly structured and inflexible cell walls of plants are a severe impediment to DNA delivery, several solutions include stripping the cell of its cell wall, leaving just its plasma membrane. This is known as a protoplast. The first protoplasts were cultivated from onion bulb scales by Klercker in 1892, but the technique wasn't perfected for sterile tissue culture applications until the 1960s (Compton et al. 1996). The two primary techniques for obtaining a

protoplast are (a) mechanically chopping or slicing plant tissues, which would damage many of the cell walls (the original technique also results in the loss of many cells, which is why it is not very popular), or (b) a milder technique that uses hydrolytic enzymes to remove the cell wall while preserving the cell membrane (the preferred and more com- mon version). Similar to other plant cultures, ammonium nitrate, calcium, organic compounds, sugar, plant growth hormones, and other nutrients are usually needed to promote cell division; however, the culture conditions must be specific for protoplasts. As protoplasts are often light-sensitive, they should be maintained in dark surroundings until they synthesise a new cell wall (Compton et al. 1996).

A protoplast may be genetically altered in the same ways as a conventional mammalian cell, such as via electroporation, the use of polycationic chemicals, liposomes, etc., after its cell wall has been removed and all that is left is its cell membrane. Agrobacterium tomafeciens and protoplasts may also co-produce (Compton et al. 1996).

Agrobacterium

The plant pathogen tumfaciens causes crown gall disease, a kind of plant tumour. These bacteria transfer a tumor-inducing (Ti) plasmid to several monocot or dicot plants. In this Ti plasmid, genes for nopaline synthesis and tumour induction are found in a structure known as T-DNA (transferred DNA). Certain Ti plasmids may have several T regions, and these T regions, which are determined by the left and right T-DNA boundaries, are generally 10 to 30 kbp in size (Gelvin 2003). The VirD2 protein is subsequently used to coat the single-stranded T strand, which is then transported to the plant with the aid of a number of additional virulence proteins. The T region is released when the virulence endonucleases recognise and cleave these border sequences.

For Agrobacterium-mediated transformation, protoplasts are normally separated for a few days previous to the transformation day and then co-cultivated with the genetically modified Agrobacterium (usually at a 1 plant cell: 100 bacteria ratio). Antibiotics are then added to the culture mix to get rid of the bacteria after co-cultivation, and transformed plant cells are chosen using a selection marker (Gelvin 2003).

The earliest vector systems utilised historically were cointegrate Ti plasmids (or hybrid Ti plasmids), however they are no longer often employed since easier manipulation tools have been created. Engineered T-DNA binary vectors are more frequently used in Agrobacterium-mediated transfection, which employs two distinct vectorsone with a helper Ti plasmid that lacks the T-DNA but contains virulence genes and another with the T-DNA present on a smaller binary vector, where the transgene is cloned between the left and right borders but there is no virulence region (sometimes also referred to as the mini-Ti). This mini-Ti can also express itself in plants and reporter vectors.

DISCUSSION

The report also emphasises how critical it is to comprehend the moral issues raised by plant genetic engineering, such as the possibility of unintended consequences and the effect on biodiversity. The paper also highlights developments in plant bioreactor culture and their industrial applications. The results of this study indicate that plant genetic engineering is a vital tool for expanding our understanding of plant biology and creating fresh approaches to agriculture, biotechnology, and environmental science. Sometimes the potential expression

plasmids are also determined by the sort of DNA delivery technology (Liu et al. 2013). All of these plant expression systems, however, nevertheless have the same characteristic:

1. A plant cell promoter with exceptional potency. Several plant expression plasmids employ the promoters of the genes of the cauliflower mosaic virus (CaMV).
2. One may also utilise tissue-specific promoters, such as a fruit-specific, leaf-specific, or seed-specific promoter, depending on where the transgenic expression is necessary.
   Among the selectable markers are nptII, which codes for kanamycin resistance, hptII, which codes for hygromycin resistance, and pat, which codes for phosphinothricin N-acetyltransferase, which detoxifies the plant growth inhibitor phosphinothricin (ppt). Selectable markers are frequently eliminated from food crops prior to their release on the market due to biosafety concerns (typically through Cre-LoxP, Flp-FRT, or similar site-specific recombinase-mediated cleavage of the marker gene and/or the transgene), but doing so also enables the producer to avoid labelling their product as "GMO."
3. Reporter genes such as smgfp (a soluble version of the so-called soluble-modified GFP, which is a codon-modified green fluorescent protein), luc, and gusA (-glucuronidase) would cleave the colourless X-gluc, 5-bromo-4-chloro-3-indolyl glucuronide, into 5, 5′-dibromo-4, 4′- dich (encoding the enzyme luciferase).

The bamboo mosaic virus (BaMV) and the tobacco rattle virus (TRV), among other plant viruses, are used as gene silencing vectors (Liou et al. 2014). Using virus-induced gene silencing (VIGS), a method of reducing gene expression from plant genes, to benefit from the body's built-in antiviral defences (Purkayashta and Dasgupta 2009).Figure 1 genetic modification.

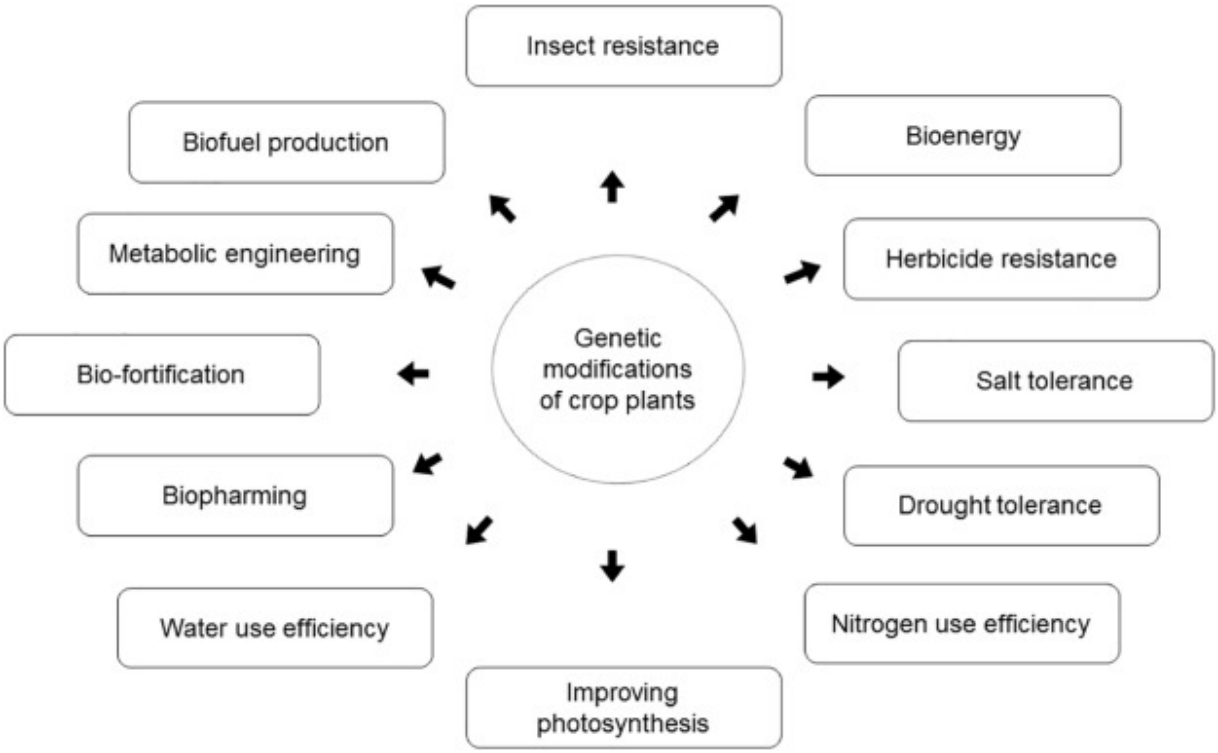

Figure 1: Genetic Modification

Remember that the nuclear, mitochondrial, and plastid genomes are all distinct from one another in plant cells (mainly chlo- roplast). The manipulation of the chloroplast genome has been realised in a small number of plant species, starting with tobacco, due to its many advantages, including exclusively homologous recombination-mediated integration, a high number of chloroplasts (hence the high levels of stable expression in green tissues, though there are issues with expression in nongreen tissues, such as roots or seeds), a lack of gene silencing mechanisms, and maternal inheritance (Bock 2014). The gene gun is the most popular strategy since the transgene must pass through numerous membrane layers; nevertheless, plastid transformation techniques are still in their infancy (Bock 2014).

Codon optimization is suggested for the transgene to be translated as effectively as feasible in plants since codon use differs across bacteria, fungus, humans, and plants. If, for example, a human gene is to be expressed in plants, one must look at the codon frequencies for the same amino acid in both people and plants. Consider that a protein containing the amino acid glycine is encoded by the GGU codon in humans, however plants only have a 2% codon frequency. This would limit the efficacy of translation. A codon called GGC, which also codes for glycine, is present in 30% of plants. The efficacy of translation in plants would thus be considerably increased by replacing GGU with GGC. This is especially true for organelle genomes, which often exhibit more variation in codon frequency than nuclear genomes.It should go without saying that the methods of plant genetic alteration we have covered here are merely the beginning. Nonetheless, we do hope that the chapter provides the reader with a broad understanding of the variety of things that may be done with plants, from biopharmaceuticals to biofuels [7]–[9].

The Food and Agriculture Organization said that by 2010, genetically modified agricultural plants have been planted on around 150 million hectares of land worldwide. They included transgenic plants developed for phytoremediation or as pharmaceutical bioreactors, such as bananas that generate vaccines, as well as crops that were nutrient-fortified, like the previously noted Golden Rice. In general, it appears that the United States, Canada, and Argentina are the top producers and exporters of transgenic or GM crops; in fact, despite not being widely commercialised, the value of GM rice is estimated to be around 64 million USD annually, indicating that it is an important staple crop (Demont and Stein 2013).

CONCLUSION

In addition to animals and plants, zinc finger nucleases (ZFNs) and other new genome editing tools have made gene targeting significantly simpler (Puchta and Fauser 2013), which may open up new possibilities for the development of transgenic ras when combined with synthetic biology techniques.

About 25 years after the introduction of the first genetically modified food crop, Flavr Savr, gene targeting in plants is continuously being enhanced, updated, and field-tested. Even now, and maybe even now, the green revolution is in progress.Plants can have their DNA altered through genetic engineering to create desired features like disease resistance, increased yield, and higher nutritional value. From basic selection to contemporary biotechnology technologies like genetic engineering and gene editing, this approach has been employed for decades. By examining recent studies on the methods, developments, and applications of this procedure, this research paper analyses plant genetic manipulation. The many approaches for genetically altering plants

are examined in the research, including conventional breeding methods and contemporary biotechnology technologies like genetic engineering and gene editing.

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CHAPTER 16

EXPRESSION OF NON-NATIVE GENES IN A SURROGATE HOST ORGANISM

Harsh Mohanty, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- harsh.mohanty@atlasuniversity.edu.in

ABSTRACT:

An organism of interest may benefit from genetic engineering by having better functioning metabolic and functional systems. Nonetheless, it often happens that one wants to add new phenotypic traits and/or capabilities to a particular host organism. Under these conditions, non-native genes may be expressed inside the host organism using the concepts of genetic engineering, resulting in the creation of hitherto unobtainable protein products. The most important things to take into account stem mostly from the variations in how various species have evolved to efficiently duplicate, repair, and express their native genetic structures. So, the capacity of the replication and expression machinery to detect and interact with the target gene must be taken into account when determining the correct expression of foreign genes in a surrogate host. The major focus of this chapter will be on how prokaryotic and eukaryotic organisms vary in their gene expression machinery and methods.

KEYWORDS:

Cells, Non-Native Genes, DNA, Macromolecule.

INTRODUCTION

During the last 50 years, this approach has been tremendously beneficial for the advancement of biotechnology and fundamental scientific research, but it has also become evident that there are many parameters that need to be taken into account in order to correctly express exogenous genetic constructs.How these discrepancies can be resolved when expressing a prokaryotic gene in a eukaryotic organism, or vice versa, will be discussed. A number of factors will be taken into consideration, including the existence or absence of exons, the functionality of polycistronic expression systems, and variations in ribosomal interaction with the gene sequence [1], [2].

Regardless of how closely related the surrogate host is to the original organism, there are, of course, extra issues to be aware of. Concerns like differences in the codon usage bias of the surrogate versus the native host, as well as how differences in the overall GC content of each organism can affect the efficiency of gene expression and long-term maintenance of the construct, will be taken into consideration in light of the mechanisms employed by the host to recognise and remove foreign DNA, in order to properly prepare investigators for the expression of genes in a wide variety of non-native organisms. This will provide a fundamental grasp of how non-native entities may be expressed using the biochemical processes responsible for genetic replication and expression.

The primary regulatory signals that regulate gene expression will also be described in depth, with an emphasis on how they must be altered before external expression. This section will

specifically concentrate on the existence, placement, and composition of common promoter elements, the role of restriction and other regulatory sites in relation to expression across broad host categories, and the function and location of the Kozak sequence. Aspects of the exogenous gene expression's potential phenotypic impacts will also be taken into account, particularly in light of the possibility of interacting with host metabolism or controlling potential protein aggregation inside the surrogate host. This will provide readers a fundamental grasp of how common sequences may be used to either boost or moderate a gene's production inside a surrogate host to ensure effective expression.

Lastly, the production of the whole bacterial luciferase gene cassette in a human kidney cell host will be discussed as a case study to demonstrate how these procedures must be used in unison in order to express non-native genes in a surrogate host organism. In this particular instance, a number of different factors were taken into account at the same time in order to express a set of six genes that were thought to be exclusively functional in prokaryotic organisms in a eukaryotic surrogate. The culmination of more than 20 years of research by numerous groups is the full expression of the bacterial luciferase gene cassette, which nicely illustrates how each of the major topics discussed in this chapter was necessary to successfully produce autonomous bioluminescence from a highly varied surrogate host. It will restate the previously discussed points and provide the reader a concise rundown of how these ideas might be put into practise in the laboratory to accomplish a certain objective.

Gene expression mechanisms

Prior to exogenously expressing a gene in a different host organism, it is critical to comprehend the fundamentals of gene expression and maintenance. Our comprehension of innate genetic operation paves the way for a deeper comprehension of the alterations that improve the expression of non-native genes. Fortunately, replication, transcription, and translation are the same fundamental mechanisms that apply to all genes regardless of whether they originate from prokaryotes or eukaryotes. The related proteins engaged in each of these steps are what distinguish eukaryotic from prokaryotic gene expression the most. Yet ultimately, the goal remains the same: to convert mRNA from messenger RNA (mRNA) into a protein that will perform a specific function.

Replication

Whether an organism is eukaryotic or prokaryotic, the process of replication always has the same ultimate objective in mind: preserving genetic material for the next generation. In addition to providing a mechanism for transmitting genetic information, replication is a crucial step in the gene expression process since any mistakes made during this time might change the genetic code and be passed on to next generations. The site of replication and the structure of the genome itself account for the main variations in replication between prokaryotes and eukaryotes. Prokaryotic organisms normally store their DNA as circular chromosomes in the cell's uncompartmentalized cytoplasm. Yet, in eukaryotic species, the DNA is kept in the cell's nucleus in the form of linear chromosomes. Yet in both prokaryotes and eukaryotes, the process of DNA replication is the same. When DNA helicase binds and causes the DNA to unwind, that location is known as the genesis of replication.

Transcription

Beginning of transcription

Initiation, elongation, and termination are the three fundamental stages of transcription, which produces an mRNA message from a DNA template in both eukaryotic and prokaryotic species. Prokaryotes only have one coding area for genetic information, but eukaryotes have both coding and non-coding sections, known as exons and introns, respectively. This is a significant distinction. Exons contain the genetic material that has to be translated and transcribed, while introns divide up exon sequences with non-coding genetic material (Watson et al., 2008).

The accuracy of protein translation, the efficiency of transcription and translation, and even the process of protein folding can all be directly impacted by codon usage patterns, contrary to what was previously thought. Despite this, more recent research has disproved the notion that synonymous codon substitutions are simply examples of fortuitous silent mutations (Angov, 2011; Zhang et al., 2009). So, it is plausible that an organism's unique pattern of codon use has coevolved with other biological components in order to guarantee the best possible levels of gene expression and protein function for the host genes in their environment (Grantham et al., 1981). For instance, in prokaryotes, the intracellular quantity of the matching tRNA positively correlates with the frequency of a codon being utilised (Bulmer, 1987; Dong et al., 1996). So, it follows that the presence of difference in their respective codon use pattern compared to the host organism hinders the production of non-native genes. The lengthy history of foreign gene expression has validated this theory, showing that same DNA sequences are often expressed with varying efficiency in other species [3]–[5].

This is because the foreign DNA sequence contains codons that are rarely used in the host, which results in low levels of translational efficiency and protein expression (Kane, 1995; Kim & Lee, 2006; Rosano & Ceccarelli, 2009). This is because there is an imbalance between the codons used in the target gene sequence and the pool of charged tRNA that is available in the host, which lowers the translation elongation rate. Any conflict between the host translation machinery and the mRNA secondary structure resulting from variations in GC content from alternative codon use patterns subsequently exacerbates these expression issues (Kim & Lee, 2006; Wu et al., 2004).

Codon optimization is a popular technique for resolving these issues and improving the expression of non-native genes in a surrogate host. In order to precisely match the host codon use bias and maintain 100% similarity to the original amino acid sequence, unusual codons within the DNA sequence must be replaced. The simultaneous adjustment of projected mRNA secondary structures that can emerge from changes in the GC concentration is also made possible by this codon optimization method. This method is particularly useful for getting rid of structures near the 5' end of coding areas, where they are more likely to interfere with the production of subsequent proteins (Wu et al., 2004) In order to improve expression, cis-acting negative regulatory regions are also removed from the coding sequence. This lowers the likelihood that the gene will be repressed (Graf et al., 2000). Both resynthesising the target gene from scratch and site-directed mutagenesis in many stages on directly cloned DNA may be used experimentally to accomplish codon optimization. The former approach could be chosen if just a few codons need to be modified, but as gene synthesis techniques have advanced, it has become less expensive and quicker to produce synthetic DNA sequences, making the latter approach more and more realistic. When produced in an E. coli host, it has been shown that the codon

optimization procedure increases the expression of a normal mammalian gene by five to fifteen times (Burgess-Brown et al., 2008; Gustafsson et al., 2004). Similarly, this approach may also dramatically enhance the expression of bacterial genes in eukaryotic cells (Patterson et al., 2005; Zolotukhin et al., 1996; Zur Megede et al., 2000).

Exogenous gene removal and silencing mechanisms

The foreign DNA must be physically introduced into the host cell and then correctly incorporated into the host's gene expression and regulatory network in order for an exogenous gene to be produced in a non-native host. The introduction of genetic material into prokaryotic and eukaryotic hosts has been made possible by decades of research in the fields of molecular and cellular biotechnology. However, once the gene has been introduced into the host cell, it must first be recognised and processed by the replication, transcription, and translation machinery of the host cell before it can be expressed as a functional protein. Yet, many species have developed defensive mechanisms that remove or quiet foreign DNA in order to protect themselves from this potentially harmful process as expression of a foreign gene is often hazardous to host survival under wild-type circumstances. In bacteria, for instance, restriction endonucleases that detect certain, non-self-nucleotide sequences may cut the invasive foreign DNA, a process known as restriction. The native genetic material is often methylated at specific locations throughout this process by methylase enzymes, enabling the preservation and expression of native DNA sequences and preventing identification and breakdown by restriction endonucleases. Since its initial discovery in the 1960s, the restriction modification system has been shown to be widespread in many bacterial species (Wilson & Murray, 1991). However other defensive mechanisms have also been created to shield the host from the expression of foreign genetic material, in addition to the restriction system. It has been shown that the interaction of Gram-negative bacteria with the histone-like nucleoid structuring (H-NS) protein allows for the selective repression of horizontally acquired genes. The term "xenogeneic silencing" was initially used to describe this phenomena by Navarre, Lucchini, Oshim, and associates in 2006. (Lucchini et al., 2006; Navarre et al., 2006; Oshima et al., 2006). The family of nucleoid-associated proteins that bind to AT-rich DNA sequences with a low degree of sequence specificity includes the H-NS protein, which is responsible for xenogeneic silencing. H-NS protein targets the laterally acquired sequence in xenogeneic silencing because it has a lower GC content than the host genome, which enables it to preferentially suppress the expression of alien DNA.

There is currently no mechanism for the direct removal of foreign genetic material from eukaryotic organisms, in contrast to prokaryotic methods for silencing exogenous DNA sequences. Yet, epigenetic alteration often results in poor effectiveness in the expression of external DNA in plants and mammalian cells. These alterations cause the transgene's expression to be erratic and, in severe circumstances, eventually silence it. By modifications to the sequence's methylation status, histone modification, or RNA interference, silencing may take place at either the transcriptional or post-transcriptional level (Pal-Bhadra et al., 2002; Pikaart et al., 1998; Riu et al., 2007). No matter what safeguards are put in place, the host uses each of these systems to control the expression of foreign genes and shield itself from negative consequences. The expression of the transgenic may be significantly influenced by the place of insertion due to the random integration that occurs when a foreign gene is introduced into the host genome, which is a last worry that is now unavoidable. The expression level varies greatly across individual expression efforts depending on the region of integration, different position

effects, and epigenetic processes. Although it is often impossible to effectively manage the genomic insertion place of foreign genes, a number of components have been suggested that may be used to mitigate the impact of position on transgenic expression and achieve sustained transgene expression. Section 4.4 discusses these components. Regulatory sequences that need to be taken into account for the best expression

Researchers can start to develop theoretical guidelines for the rational design of DNA sequences that are best tuned for heterologous expression in their target organism by developing a thorough understanding of the mechanisms underlying gene expression and understanding how variables like GC content and codon usage bias influence protein expression in non-native hosts. This strategy is particularly appealing since it allows for the de novo generation of full genes and even entire expression cassettes, which makes it feasible to quickly design a gene sequence and get to work. Nevertheless, before an exogenous gene sequence is successfully expressed, there are still certain other issues that need to be resolved. To ensure optimum expression, regulatory sequences that are not translated or transcribed should also be taken into account in addition to the coding region's optimization. While these components are not expressed in the finished protein product, they are crucial for the transcription, translation, and long-term maintenance of the target genes in the surrogate host, making it as critical to optimise them as the coding sequence itself.

Regulating Components in Transcription

Transcription by RNA polymerase is the first step in the process that transforms a gene into a useful protein. As a result, transcription initiation often serves as a crucial point of control for the production of external proteins. The promoter sequence necessary to entice the host's transcriptional machinery is what drives the binding and recruitment of the polymerase that will convert the DNA to mRNA. The effectiveness of the design approach is greatly influenced by selecting a promoter that can be processed by the host's machinery, even while the promoter itself is not transcribed or translated. For high level expression of external genes, powerful constitutive promoters that are often utilised to drive the expression of endogenous housekeeping genes in the expression host are typically selected. For instance, the T7, alcohol dehydrogenase 1 (ADH1), and human elongation factor 1 (EF1) promoters are often used in E. coli, S. cerevisiae, and mammalian cells, respectively, for the production of heterologous proteins. In mammalian cells, transgenic expression is also regulated by viral promoters like the Simian virus 40 (SV40) regulatory sequence and the cytomegalovirus immediate early (CMV IE) promoter. While the intensity of the utilised promoter might at least in part affect the degree of transgenic expression, it is vital to remember that different promoters can have varied rates of transcription across various cell lines. Because of this, choosing an acceptable promoter should be done after carefully considering each individual circumstance. Recent research (Norrman et al., 2010; Qin et al., 2010) have comprehensively examined several of the frequently used promoters in a number of cell types. These kinds of references are a great source of knowledge for creating structures with particular expression requirements.

Also, it's important to keep in mind that, like gene sequences, promoter sequences may be created from scratch. If no native alternative promoter sequences are available, creating a specialised primer upstream of a gene construct may be advantageous. Many prokaryotic and eukaryotic promoters have been examined, and it was shown that many of them have a conserved core sequence that is necessary for RNA polymerase and its cofactors to recognise and

bind to the promoter. It may be feasible to explicitly create a promoter sequence by using these conserved elements, enabling one to customise the expression of their genetic material.

The Regulatory Components of Translation

The existence of certain, conserved sequences at the 5' untranslated region of mRNA sequences is necessary for the start of translation, much as a core promoter sequence is required for the start of transcription. This purpose is served in bacterial organisms by the Shine-Dalgarno sequence, which acts as the ribosome binding site (RBS). The six nucleotides in this consensus sequence, AGGAGG, complement the anti-Shine-Dalgarno motif found at the 3' end of the

The Kozak sequence serves as the primary initiator for translation in eukaryotes, and the 40S ribosomal subunit contributes to this function by connecting to initiation proteins that aid in the process of scanning the mRNA (Kozak, 1986, 1987). While it is not always the case, this translational process often starts at the AUG codon that is closest to the 5' end of the mRNA. According to research by Kozak et al., the distance from the 5' end, the sequence surrounding the first AUG codon, and its steric connection to the 40S ribosomal subunit all play a role in defining the precise position of the initiation site. However it has repeatedly been shown that putting the promoter and the Kozak region ahead of the starting codon helps to boost the production of the target gene sequences (Morita et al., 2000).

When expressing prokaryotic genes in eukaryotic hosts or vice versa, extra considerations must be made in addition to optimising the codon use pattern in the coding area. Due to the existence of intervening, non-coding sections in the sequence, genes that have been cloned straight from the genomic library of a eukaryotic organism often cannot be produced properly in a prokaryotic host. Prokaryotes lack the RNA splicing processes needed to cut out these intron regions and create a mature mRNA, in contrast to eukaryotes. Hence, before inserting the expression construct into the prokaryotic host, any introns that are present must be removed.

Elements allowing numerous genes to be expressed at once in eukaryotes

Yet, the inability of the host to produce proteins polycistronically from a single mRNA is a substantial barrier to the expression of genomically cloned bacterial genes in a eukaryotic host. Translation in eukaryotic cells typically requires the presence of a methyl-7-G(5')pppN cap at the 5' end of the mRNA before being recognised by the translation initiation complex at the start of peptide synthesis, in contrast to prokaryotes where translation of multiple adjacent genes from one promoter is common (Pestova et al., 2001). Yet, there exist methods that enable the simultaneous expression of two or more genes in eukaryotic cells. At the most fundamental level, it is feasible to introduce numerous vectors or a single vector carrying multiple promoters in order to express each gene independently from its own promoter.

Components for sustained expression and maintenance

For transgenic expression to persist in mammalian cells, foreign DNA sequences must often be integrated into the host chromosome. The surrounding sequences and chromatin structure may have a significant influence on the integrated gene's degree of expression since the insertion event that precedes expression is mainly random. The two main problems with transgenic expression as a result are unstable expression and considerable diversity between individual clones. Also, the host's health or capacity to survive may suffer if the foreign genes are inserted into or near to a necessary host gene. Many DNA elements have been identified that may avoid

these kinds of position effects and stabilise transgenic expression to help regulate for this kind of negative regulation. A case study in exogenous expression, the bacterial luciferase gene cassette is expressed in mammals.There have been many instances of exogenously expressed genes throughout time. The recent adaptation of the bacterial luciferase gene cassette to operate on its own in a human cell line serves as an illustration of many of the issues raised here. Prior to its initial effective expression in a eukaryotic cell, the bacterial luciferase gene cassette, also known as the lux cassette, had been used in prokaryotic systems for about 20 years. Even then, it took almost another decade before it was successfully produced in a human cell line. It is feasible to examine the genetic alterations necessary for exogenous gene expression as well as the reasoning behind these adjustments by tracking the evolution of the lux system from a solely bacterial genetic system to its maturation into a eukaryotic reporter cassette.

Background of bacterial luciferase

Five genes make up the bacterial luciferase (lux) gene cassette, and their combined protein products result in a luminescent signal at 490 nm in the visible spectrum (Close et al., 2009). The heterodimeric luciferase protein is made up of two of the five genes (luxA and luxB), while the other three genes (luxC, luxD, and luxE) produce the long chain aliphatic aldehyde co-substrate that the luciferase protein reacts with (Meighen, 1991). The remaining co-substrates, $FMNH_2$ and O_2, may be directly scavenged by the enzyme since they are naturally present in the host. The substrate complex oxidises and releases a photon at 490 nm upon attachment to the luciferase dimer. This reaction has a very sluggish turnover, with the process lasting up to 20 seconds at 20 °C (Hastings & Nealson, 1977).

The bioluminescent process that the luciferase gene cassette in bacteria catalyses. Reproduced from with thanks to the bioluminescent system that these genes encode for is considerably different from those often seen in eukaryotes, such as the firefly or Renilla luciferase systems, despite the fact that these genes are extensively dispersed in prokaryotic species. The lux system is structured as a single operon, with all of the genes necessary for bioluminescent output being driven by a single promoter, in contrast to these eukaryotic bioluminescence systems. Moreover, because of its prokaryotic origin, it is designed to operate in cellular environments without substantial compartmentalization. So, it is not unexpected that significant genetic alterations were necessary before effective expression in the cellular backdrop of a distantly related human was possible. These changes provide an intriguing case study of the factors to be taken into account when any gene is expressed exogenously in an organism that is not its original host.

First exogenous expression efforts

In the 1980s, efforts were made for the first time to express the lux system outside of bacteria. There has been an increasing interest in evolving this system to function in a wider variety of organisms after realising the advantages offered by the fully autonomous expression of light as a bioluminescent reporter system in bacterial species. This is so that it can be utilised in an increasingly broad range of situations. As opposed to complete cassette expression, these early experiments concentrated on the expression of just the luxA and luxB genes in an effort to first figure out how to make the luciferase operate before applying what was learnt to the production of the other lux genes.

The first change made to the expression of the luxA and luxb genes was to provide each of them separate promoters since eukaryotic organisms cannot express polycistronic genes (Koncz et al.,

1987). This approach made it possible for the independent transcription of each mRNA sequence. Yet, since they were all positioned on the same plasmid, their physical sites of expression in the host ought to be close to one another. This method of production minimises the possibility that the luxA and luxB protein products would interact in vivo to form a functional heterodimer while avoiding the need for polycistronic expression. Cell extracts from plants that had this system expressed were able to generate light when exposed to an aldehyde substrate. Even while this showed that at least a piece of the lux cassette could be expressed exogenously, autonomous bioluminescent expression was still far from feasible.

After studying the dual promoter system in plants, various research teams tried expressing the luxA and luxb genes as fusion products in yeast, Drosophila, and even murine cell lines (Boylan et al., 1989; Kirchner et al., 1989). (Pazzagli et al., 1992). The findings of these studies were largely comparable regardless of the host origin. Upon treatment with the aldehyde substrate in yeast cells, the bioluminescent expression was noticeable above background but less common than bioluminescence from other prokaryotic systems examined under the same circumstances (Boylan et al., 1989). An intriguing issue was discovered when expression using this approach was tried using higher eukaryotic hosts like Drosophila and mouse cell lines; bioluminescence was detected but was found to be very temperature sensitive.

The murine Ltk- cell line needs higher temperatures to develop, therefore the lux luciferase proteins were unable to sustain high levels of stability after gene expression. This caused Ltk- cells transfected with the luxA and luxB genes to produce bioluminescence at very low levels when cultured at their preferred temperature of 37°C. Bioluminescent detection rose tenfold when the growth temperature was lowered to 30°C, an acceptable but not optimal temperature (Pazzagli et al., 1992). Further research in E. coli revealed that hosts expressing LuxA-LuxB fusion proteins were capable of producing a greater than 50,000-fold increase in bioluminescent production when grown at 23°C as opposed to growth at 37°C, further confirming the temperature-dependent nature of this bioluminescent decrease (Escher et al., 1989). This emphasises the need to assess both the physiological constraints limiting production of the protein encoded by a target gene as well as any possible genetic barriers to exogenous expression of that gene. It would take another ten years until this restriction was removed, which enabled the production of the whole lux cassette in a yeast cell type. This restriction proved to be a substantial obstacle in the development of routine eukaryotic expression of these genes [6], [7].

DISCUSSION

Work on the expression of the whole lux cassette in a eukaryotic host continues using the knowledge gained from the dual-promoter and fusion-based expression of the luxA and luxB genes described above. The choice to produce lux genes from the bacteria Photorhabdus luminescens rather than the traditional lux model organism, Vibrio harveyi, led to the first significant advancement (Gupta et al., 2003). P. luminescens is a terrestrial rather than a marine bacterium, in contrast to the V. harveyi template organism utilised in the earlier efforts. As a result, although performing the same function in vivo as those encoded by V. harveyi, it has a higher natural growth temperature, which results in the stability of its protein products at a higher temperature. How crucial the selection procedure might be when expressing genes in a foreign host is shown by this simple modification in the selection for the source of the external genes. No combination of genetic alterations would have been able to induce high-level expression in a

eukaryotic host at its optimum growing temperature without the intrinsic structural stability provided by the P. luminescens proteins.

After the inherent issues with gene expression at the normal yeast growth temperature had been resolved, other genetic alterations would need to be taken into account before the whole lux cassette could be produced autonomously. The first crucial factor to take into account was how to encourage the genes' own constitutive, robust expression. This was done by including yeast-specific promoter sequences, which have been shown in the past to generate high-level expression under most growth circumstances. The original upstream regions from the wild-type bacterial species that either include an inducer binding site or an AT rich region were replaced with these promoters, the glyceraldehyde 3' phosphate dehydrogenase (GPD) and alcohol dehydrogenase 1 (ADH1) promoters (Meighen, 1991). When the genes were expressed in the yeast surrogate, there would be high levels of transcription thanks to the substitution of this AT rich promoter region with known, host-expressible promoters [8], [9].

Despite the fact that these IRES components are known to be present in many other species, the researchers utilised an IRES sequence that was native to S. cerevisiae to make sure it would work well in this system. The sheer number of genes that need to be expressed for autonomous light generation utilising the lux cassette still posed a considerable barrier for exogenous expression, even with the inclusion of these IRES linker sections and several promoters. To solve this issue, it was found that splitting up the expression of the lux cassette across two distinct expression vectors was the most effective expression method [10]–[12].

CONCLUSION

Since the integration location of the gene sequences cannot be controlled by this process, a dual vector expression strategy could potentially result in distal integration of the gene sequences and raise the likelihood that expression of the various gene groups would take place with various efficiencies despite using the same promoter sequences. The next step was for the researchers to devise a strategy for the simultaneous expression of the five lux cassette genes inside the adoptive host. As S. cerevisiae is a eukaryote, it is unable to express the cassette in a polycistronic manner naturally, as would happen in a prokaryotic host under wild-type circumstances. IRES sites were included into the polycistronic expression scheme to get around this obstacle. When numerous ORFs are expressed, they may be transcribed to a single piece of mRNA and then translated separately by cap-independent ribosome recruitment during translation thanks to these IRES sites, which serve as linker sequences between the various lux genes.

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CHAPTER 17

EXPLORING THE DIFFERENT GENETIC TECHNIQUES

Sukanya Dikshit, Assistant Professor
ISME - School of Management & Entrepreneurship, ATLAS SkillTech University, Mumbai, India
Email Id- sukanya.dikshit@atlasuniversity.edu.in

ABSTRACT:

The major focus of this chapter is on the numerous methods often used in molecular biology and genetics. Several approaches have been developed to investigate how chromosomes behave during mitosis and meiosis. There has been a highly active endeavour to learn more about chromosomes since it was discovered that they are the locations of genetic factors. As a result, several histological and cytological methods have been developed to see and analyse the movement and structure of chromosomes. In addition, cytological studies of chromosomes and their function have been made easier thanks to microscopy. Lymphocytes may be used to create human mitotic chromosomes by utilising the drug colchicine to stop cell division during the metaphase. After that, the cells may be treated with a hypotonic solution to release the chromosomes. Seeing the chromosomes may be done using a variety of staining methods. The location of the centromere and the length of the chromosomes were first used for chromosomal identification.

KEYWORDS:

Cells, Genetic Techniques, DNA, Macromolecule, Cytological Methods.

INTRODUCTION

Recombination has been employed in other creatures as well as bacteria to assist understand the underlying principle. The indirect result of these procedures is site-directed mutagenesis and gene therapy. The methods used in plant breeding and innovative approaches in that field have contributed to the Green Revolution. This chapter covers a number of fundamental methods for studying chromosomes and their mutations, bacterial recombination, certain elements of plant breeding, human pedigree analysis, and DNA separation and purification [1]–[3].

Chromosomal methods

Beginning in 1956, when Joe Hin Tjio and Albert Levan employed a hypotonic solution to split apart the cell and liberate the chromosomes, cytological investigations of chromosomes were first conducted. The fact that the human chromosome number is 46 (2n = 46) was shown for the first time. The chromosomal basis of heredity and the connection between genetic disorders and chromosomal abnormalities were further studied using chromosomal methods. It was established in 1959 that Down's syndrome is caused by an extra chromosome (2n + 1 = 47). It was later determined to be chromosome 21. To create widely used preparations of human chromosomes, new procedures and staining techniques have been devised.

The labelling of the chromosomes with fluorescent dyes or radiolabeled substances is one of a variety of additional selective staining and banding methods used today to detect particular

specific areas of the chromosome. The in situ hybridization of certain chromosomes with radiolabeled or fluorescently labelled nucleic acid probes to identify the location of particular genes is another potent recent approach. Some of the commonly utilised chromosomal procedures are listed below. In order to examine chromosome activity during mitosis and meiosis, as well as polyploids and other sorts of chromosomal abnormalities, the same types of procedures were also used to plant cells, namely root tips and flower buds.

Methods for Staining Nucleic Acids

Some nucleic acid-staining methods may be used to identify chromosomes and their particular regions since nucleic acids are a necessary component of chromosomes. For the purpose of seeing chromosomes, three staining methods are often utilised.

a) Histochemical stains: Depending on their chemical makeup, these stains selectively attach to certain cellular sections or components. a some of the most significant dyes and their uses
b) Antibody-based stains: Antibodies-based stains are very specific in the substances they attach to. They can very precisely bind certain gene sequences. If the antibodies are fluorescently tagged, the nucleic acid or chromosomal fragments may be seen. Immunostaining is the name for this kind of staining.
c) Radiolabeled stains: These stains allow for the visualisation of the nucleic acids contained inside the nucleus. Here, radiolabeled nucleotides are employed (labeled with 3H; for example, 3H labelled uridine, which may be used specifically to detect and quantify RNA content). This is another another in vivo labelling method. Autoradiography must be used in conjunction with radiolabeling for visualisation or detection.

Patterns of Chromosomal Banding

The majority of chromosomes exhibit a banding pattern during prophase and metaphase. Yet in the case of bigger chromosomes, such as the polytene chromosomes of drosophila melanogaster, or the fruit fly, this banding pattern is more obvious and distinct. The areas with banding patterns are those with high levels of heterochromatin and higher levels of histone-DNA interaction. The common nuclear dyes or chromosomal dyes like orcein may readily stain these complexes. Although there are more genes present in the areas between the bands, which are truly the active portions of chromatin, there is a relatively low concentration of DNA and histone proteins in these areas. They seem unstained or faintly tinted as a result. Using fluorescently labelled antibodies against DNA-dependent RNA polymerase, which are often found in euchromatin areas needed for transcription, one may use immunostaining to identify these interband regions.

It is now possible to distinguish or accurately identify certain chromosomal homologs, chromosome sections, and/or chromosome bands thanks to specialised staining methods. The improvements in genetic mapping methods using fluorescence in situ hybridization, or FISH, have sparked a resurgence of interest in the chromosomal or cytogenetic state of many species. Several banding patterns may exist depending on the kind of dye, fluorochrome, or chromosomal preparation used. Banding patterns like G-banding, Q-banding, C-banding, and R-banding are among them. For karyotypic analysis, the information produced by several chromosomal banding procedures may be employed.

Q-banding: This banding pattern is produced by fluorescein or the fluorescent dye quinacrin treatment. A yellow fluorescence of varying intensity, as shown in the graphic on the CD, may be used to identify them. Heterochromatin makes up the vast majority of the stained DNA. Quinacrin binds to A-T and G-C-rich areas, but only A-T-quinacrin regions glow. In contrast to euchromatin, heterochromatin has more A-T areas. Thus, heterochromatin areas are preferentially designated using this banding approach. The characteristics of the banding areas and the specificity of the fluorochrome rely on the distribution of A-T and its connection with other molecules, such as histone proteins, rather than only on their attraction to regions rich in A-T.

G-banding: This pretreatment method is not reliant on fluorochromes. Animal cells can use it effectively. Without pretreatment, it is comparable to the C-banding method. The 23 pairs of human chromosomes condense during mitosis and may be seen under a light microscope. In order to perform a karyotype study, cells in mitosis are typically blocked and the condensed chromosomes are stained with Giemsa dye. Chromosome areas rich in the base pairs Adenine (A) and Thymine (T) are stained by the dye, creating a black band. It's a frequent misperception that bands represent individual genes, yet even the smallest bands may include hundreds of genes and over a million base pairs. For instance, the size of one tiny band is about equivalent to the whole of one bacterium's genetic code.

Centromeric or constitutive heterochromatin is the source of the term C-banding. As compared to other areas, the centromere appears as a stained ribbon. Prior to staining, the method calls for an alkali preparation. The DNA is completely depurinated as a result of the alkaline processing. Once again renatured, the leftover DNA is dyed using a Giemsa solution made up of methylene blue, methylene violet, methylene azure, and eosin. With this staining, the heterochromatin absorbs most of the dye while the other chromosomes absorb very little. The characterisation of plant chromosomes is ideally suited for this banding approach.

R-banding: This is a method of reverse banding. The G-C-rich regions that are characteristic of euchromatins are stained as a consequence of this approach. There are no G-, Q-, or R-bandings seen in plant chromosomes.

Hy-banding: This method is often used to plant cells. A pretreatment step in the procedure entails warming the cells while HCl is present before staining them with acetocarmine. C-bands have a distinct pattern than the Hy-band. The capacity of acetocarmine to bind to DNA and the development of bands are influenced by the histone protein's interaction with DNA and full extraction of that protein.

Further tweaks to the pre-treatment process's selection of dyes and fluorochromes improved the banding methods' resolution even more. Although many of the strategies work well with animal chromosomes, plant chromosomes present significant challenges. It is unclear why this is happening.

Any of these methods will never result in a plant chromosomal banding pattern as extreme as animal chromosome banding patterns. In many species with an intraspecific variable karyotype, the constitutive heterochromatin and the residual chromatin have identical constant banding patterns.

Karyotyping

In order to ascertain the chromosomal complement in somatic or cultured cells, karyotyping is a useful research technique. It's critical to remember that karyotypes change as organisms do. The karyotype of a particular subline must be identified in order to interpret biochemical or other evidence in light of this history. The alteration in the karyotype is responsible for a number of morphological and physiological issues. There have been several technological processes that result in banding patterns on metaphase chromosomes. An area of a chromosome that may be easily distinguished from its neighbouring segments by looking darker or lighter is called a band. A continuous array of bright and dark bars is used to represent the chromosomes. A Giemsa dye combination or a Leishman dye mixture is used as the staining agent in a G-staining technique that yields G-bands. The process of putting together a karyotype is briefly described in the paragraphs that follow.

Cells with easily distinguishable, countable, and measurable chromosomes are often used to create kyotypes. The finest chromosomes for creating and analysing karyotypes are those during the mitotic metaphase, meiotic metaphase II, and pachytene of meiosis. By removing the chromosomes from the microphotograph of the whole set of chromosomes and placing them in paired configurations, a photograph karyotype may be created. An idiogram is a diagrammatic depiction of a karyotype. Measurements and a graphic of the chromosomes with all of their relative differences may be used to construct it. The diploid complement of chromosomes is represented by an idiogram.

Chromosome Illustration

A useful method for chromosomal study is chromosome painting. Chromosome painting is the process of marking chromosomes with various coloured dyes. Fluorescent in situ hybridization, often known as FISH, is used to accomplish this.Using probes that are marked with certain fluorochromes, FISH has been utilised to locate specific genetic targets. The method enables the identification of both simple and intricate chromosomal rearrangements. Moreover, complicated chromosomal abnormalities that are undetectable by standard cytogenetic banding methods may be found (see CD).

Schrock et al. described the creation of a comparable method that enables the multi-color identification of human chromosomes in the July 26, 1996 edition of Science. Multiplex-fluorescence In Situ Hybridization is the name of the procedure (M-FISH). The method was developed by enabling 24 chromosome-painting probes that had been combinatorially tagged to hybridise with human chromosomes. Finally, using computer spectral separation (classification), the emitted spectrally overlapping chromosome-specific DNA probes are resolved. It is possible to find chromosomal abnormalities with this method. The amount of the modification may be assessed based on where the probes were employed. Moreover, the new method offers details that supplement traditional banding analysis. The aneuploid breast cancer cell line SKBR3 may easily be used to detect the existence of multiple chromosomal translocations and clearly identify structural changes, including a gigantic marker chromosome (mar1). The use of these approaches should make it easier to analyse genetic and chromosomal anomalies in cancer and other human disorders. These novel methods will definitely have several therapeutic uses, and the characterisation of complicated karyotypes in particular will enhance conventional cytogenetic research [4]–[6].The fundamental stages in the chromosomal painting approach are:

1. A grouping of nucleic acid sequences that are unique to each chromosome. Other chromosomes shouldn't include these sequences.
2. By tagging them with fluorescent dyes, the chromosome-specific sequences are transformed into probes. Each chromosome's probes should be marked with a unique fluorescent dye.
3. The in situ hybridization of every probe with the cells' target chromosomes. When all probe sets are simultaneously hybridised, a chromosomal spread preparation is produced, in which each homologous pair of chromosomes appears to have a distinct hue under a fluorescent microscope.

The test will reveal if a fluorescent dye-labeled gene-specific probe has bound to the relevant chromosome at the precise location where the gene is situated.

Chromosomes that have undergone translocation will have two segments, making their presence detectable. Depending on the number of translocations, it will accept various probes and appear in two colours or multicoloured when it is exposed to the chromosomal painting procedure.

FISH has increased the effectiveness of screening cells for chromosomal abnormalities in mutagenic investigations and for determining the mutagenic potential of pesticides and other powerful environmental mutagens. Also, it has increased the ability to find chromosomal rearrangements and aberrations linked to cancer and tumours.

To ascertain how closely related chromosomes are in divergent species: When the same chromosomal paint is applied to the chromosomes of several species, it is possible to determine how much chromosome rearrangement has occurred since the species' divergence. These studies show that very diverse species have a lot of synteny.

Applications in the clinic: This method makes it simple to spot the existence of several chromosomal translocations and clearly distinguish structural changes in cancer cell lines (for instance, a large marker chromosome (mar1) in the aneuploid breast cancer cell line, SKBR3). The use of these approaches should make it easier to analyse genetic and chromosomal anomalies in cancer and other human disorders.

Motagenic Practices

Mutations are inherited alterations to the DNA or genome. A tiny portion of mutations are helpful in the process of evolution, but often these alterations are damaging to the organism. To develop better attributes in microbial systems and plants for enhanced agricultural types, mutations may be induced using a variety of mutagenic agents and chemical or physical means. For instance, enhanced agronomic traits like grain size, disease resistance, salt tolerance, early blooming, insect resistance, and so forth.

Mutagenesis in bacteria

Radiation, especially UV light, is the finest tool for causing mutation in microorganisms like bacteria. An attempt to use UV light to cause bacterial mutation is as follows:

1. To create a bacterial culture, inoculate an E. coli colony into a tiny amount of liquid medium (LB media or the minimum medium), and let the culture develop overnight at 37°C in an incubator shaker.

2. The next day, use a bent "L"-shaped glass rod (spreader) that has been flamed after being soaked in alcohol to spread 0.1 ml of the overnight culture onto LB agar plates. Before beginning the experiment, each plate has to be correctly labelled to prevent misunderstanding.
3. Now, in a laminar flow chamber or hood, place the agar plate containing the bacteria to be mutagenized beneath the UV light. Close the hood door after removing the plate cover. Put on the UV lamp and record how long the bacteria were exposed to the light. (Time the exposure in seconds. Repeating the experiment and comparing the results between 5 and 240 seconds will provide the ideal exposure period.)
4. The UV light is quite powerful. Avoid exposing your skin to Ultraviolet rays. Never use the UV light when the hood is open. Your eyes might suffer substantial, long-lasting damage from UV radiation. The UV radiation will be absorbed by the glass in the hood door. While the UV light is on, never stare at it without safety glasses. Turn off the light when the time is up.
5. Cover the plate once again, take it out of the UV box, and put it in a 37°C incubator. Incubation of plates must be done upside down. This is crucial to avoid moisture condensation building up on the agar surface.
6. After 24 hours, inspect plates to count or estimate the number of colonies present and search for the desired mutant type.

Prepare a different set of agar media plates to develop and pick the mutants based on the kind of mutant you are searching for. You construct agar media plates with minimum media, which only includes the necessary ingredients in the form of salts and elements and no organic components other than the carbon source in the form of glucose, if your goal is to get an auxotrophic mutant for arginine (Arg-). Now, replicate plate the colonies from the master plates onto the selection plate. Gently maintain the colonies on the master plate covered with a circular filter paper that fits inside the petri dish. Take the filter paper slowly, add it to the growth medium, and let it develop overnight in the incubator. You can identify the colonies on the master plate that cannot develop on the minimum media by comparing the colonies on the selection media with those of the master plate. This may be verified by observing the colonies' expansion once again on minimum media that has been supplemented with arginine (arg + plates).

Mutagenesis in seeds

The portions of agricultural plants that may be utilised to cause mutations are the seeds. Mutations may be produced using both chemical and physical mutagens. In this experiment, we may cause mutations in wheat or other experimental plants like arabidopsis by using a mutagenic chemical called EMS (ethyl methanesulphonate). A certain quantity of nutritious seeds should be soaked in water overnight. The water is removed the next day using tissue paper or filter paper. The seeds should be incubated in an appropriate concentration of aqueous EMS solution for approximately two hours at room temperature. Use some seeds as the experiment's control by incubating them in water with comparable environmental factors. Take note of the experiment's concentration and treatment duration. Following exposure, remove the seeds and thoroughly rinse them under water to get rid of any remaining mutagen. The treated seeds must be planted separately from the control in a controlled setting. The seedlings from the treated seeds may be compared to those from the control after germination to determine if the intended mutations have occurred. The experiment may be carried out again with varied mutagen concentrations and mutagen intensities.

Relationship in Bacteria

Transferring DNA from one creature to another is referred to as genetic recombination. The recipient's nucleoid may then undergo a variety of procedures to incorporate the transferred donor DNA.Gene recombination processes include:

1. Transformation: DNA fragments from a dead, degraded bacteria attach to DNA-binding proteins on the surface of a capable receiving bacterium (typically around 20 genes long). The attached DNA was subsequently divided into pieces by nuclease enzymes. The recipient bacteria gets penetrated while the other strand is destroyed. Rec a proteins then swap this donor-provided DNA fragment for a recipient-provided DNA fragment.
2. Transduction: When a bacteriophage moves DNA pieces from one bacteria to another bacterium.

a) Generalized transduction: Sometimes, when a lytic phage replicates, the capsid will form around a little piece of bacterial DNA. The donor bacterial DNA fragment is injected into the recipient bacterium by this phage when it infects another bacterium so that it may trade it for a bit of the recipient bacterium's DNA. Similar methods may be used to transport plasmids, such as the penicillinase plasmid of Staphylococcus aureus.

b) Specialized transduction: A temperate bacteriophage's lysogenic life cycle may rarely include this. Sometimes, during spontaneous induction, a little fragment of bacterial DNA is swapped for a fragment of the phage genome (that remains in the nucleoid). Each phage capsid contains a copy of this bacterial DNA, which replicates as a component of the phage genome. The phages are released, absorbed by recipient bacteria, and then injected into the donor bacterial DNA/phage DNA complex and into the recipient bacterial nucleoid.

3. Bacterial conjugation: DNA exchange between two bacteria that are still alive. A sex pilus created by the donor bacterium bonds to the receiver in gram-negative bacteria. The two bacteria then come into contact when the sex pilus retracts. Gram-positive bacteria create sticky surface molecules that bind two bacteria together. The receiver then receives DNA from the donor.

a) F+ conjugation: This occurs when a male donor bacterium transfers a F+ plasmid (which solely codes for a sex pilus) to a female recipient bacteria, but no chromosomal DNA. Although one plasmid strand stays in the donor, the other penetrates the recipient bacteria. After then, every strand creates a complimentary replica. The receiver then develops into a F+ guy and is capable of having intercourse. This procedure may also transfer other plasmids that are already in the bacterial cytoplasm, such as those that code for antibiotic resistance.

b) Conjugation using high-frequency recombinant protein: To create a Hfr male, a F+ plasmid integrates or inserts into the nucleoid. The nucleoid of the inserted F+ plasmid then splits in the middle, and one DNA strand starts to enter the recipient bacteria. The rest of the F+ plasmid seldom penetrates the recipient because the bacterial attachment often breaks before the transfer of the full chromosome is finished. As a consequence, some chromosomal DNA is transferred, maybe in exchange for a portion of the recipient's DNA, but not maleness.

c) Resistant plasmid conjugation: In this process, an R-plasmid is transferred from a donor bacteria to a recipient bacterium. Although one plasmid strand stays in the donor, the other penetrates the recipient bacteria. After then, every strand creates a complimentary

replica. The R-plasmid contains genes that are coded for sex-pilus development and various antibiotic resistance. The receiver develops masculine characteristics, becomes resistant to antibiotics, and may now spread R-plasmids to other bacteria.

This is a significant issue when treating opportunistic gram-negative infections caused by organisms like E. coli, proteus, klebsiella, enterobacter, serratia, and pseudomonas, as well as intestinal infections caused by salmonella and shigella (urinary tract infections, wound infections, pneumonia, and septicemia).Recombinant DNA technology also allows for the synthetic alteration of bacterial gene expression. Enzymes called endonucleases and ligases are often used in recombinant DNA technology. In bacteria, restriction endonuclease enzymes break down invading viral DNA without affecting the bacterium's own DNA. This helps defend bacteria against viral assaults. Restriction endonuclease enzymes identify certain palindromic deoxyribonucleotide base sequences (base sequences that read the same forward and backward on the complementary DNA strands), and then divide each DNA strand at a specified location within that sequence. For example, escherichia coli develops a restriction endonuclease termed eco R1 that detects the deoxyribonucleotide base sequence G-A-A-T-T-C and cuts the DNA strand between the G and the A. As the complementary strand bears the sequence CTTAAG, it is likewise cut between the G and the A. This leaves short, complementary, single-stranded sticky ends capable of hydrogen bonding with the complementary sticky ends of DNA fragments cut by the same enzyme.

Experiment to Carry Out Metamorphosis

Bacterial transformation is regular activity in all molecular biology labs as part of recombinant DNA experiment or gene cloning. In rDNA studies or gene cloning, we create recombinant DNA or the gene or plasmid to be cloned, which needs to be delivered to a host cell so that the DNA will proliferate within the bacterial cell. Transfer of the plasmid or the rDNA is carried out through bacterial transformation.

The first step is to pick a good host cell such as a suitable strain of e.coli like DH5 □, a typical strain accessible in many molecular biology labs, which can accept foreign DNA readily. For this we have to treat the developed bacterial cultures during their log phase of development, using CaCl2. Centrifuge the cells growing at the log phase under low rpm (3,000–5,000 for 10 minutes) at 4°C and collect the cells. Suspend the cells in chilled CaCl2 of 0.1 M. The cells in calcium chloride are able to accept the small DNA molecules. These cells in CaCl2 can be stored for a long time under low temperatures such as –20 or –70°C.Sudden exposure of this cell to the room temperature or higher can force the cell to take the DNA from outside. Take the stored competent cells, which are in the frozen condition and add the DNA sample to these cells and expose them to a higher temperature, at 42°C for two to three minutes. Some of these cells take the DNA from outside and will be transformed by intercalating with its genome. These cultures can be plated on a selection agar plate and the transformed colonies can be selected against the untransformed ones.

This transformation is extensively used in genetic engineering experiments. Any gene or DNA, before transferring into an organism, can be tested in a selected host by this transformation method. New promoters can be checked for their strength of expression. Commercially-useful enzymes and therapeutic proteins can be prepared in industrial scales. In short, any genetic engineering or gene cloning cannot be accomplished without bacterial transformation.

An Experiment of Conjugation

Take two strains of bacterial cultures of e.coli. One is the male strain or the F+, which is auxotrophic for biotin and methionine (Bio–, Met–) (Bio–, Met–). This bacterium can grow in the minimal medium, only if these two components are supplemented. Similarly, the female bacteria or the F– strain is able to produce both biotin and methionine, but are auxotrophic for threonine and leucine (Thr–, Leu–) (Thr–, Leu–). These bacteria cannot grow in the minimal medium unless the respective nutrients are supplemented.

But when these two populations are mixed and grown in media with only the salts and the carbon source (minimal media) some of the cells could grow without the supplementation of the additional amino acids and vitamin, biotin. This indicates that when grown together some female cells receive the functional genes of Thr and Leu from the male strains by conjugation. Similarly, some of the male strains receive the functional genes for biotin and methionine from the female strain. These new genetically modified strains don't need any extra nutrient supplementation in order to flourish in the minimum media.This is a typical process of natural recombination in bacteria that produces variations. Since conjugation may result in medication resistance among harmful bacteria, it is crucial in this context. Consequently, it is essential to comprehend the process of conjugation and to be aware that bacterial conjugations and genetic recombination might result in new strains with novel weapons in heterogonous cultures. Bacterial conjugations thrive in contaminated laboratory cultures, organic industry effluents, and sewage water.

Determining the Value of Genetic Recombination in Bacteria

All of the recombination processes used by bacteria were unintentionally found in labs. These genetic changes have a significant impact on human life and the environment since microbial populations and human populations interact closely, especially in the age of genetic manipulation. These processes result in the great degree of variety found among certain pathogenic organisms, such as Mycobacterium, as well as the development of drug and antibiotic resistance. Restriction endonuclease, an enzyme specialised in eradicating foreign DNA that enters its cytoplasm, is present. If these genetic recombination processes didn't exist, these enzyme systems wouldn't be necessary. The genome sequencing initiatives have shown that there is significant commonality across the gene sequences of unrelated creatures, which shows that genes have historically been transferred between species by any of the aforementioned natural processes, or natural genetic engineering techniques.

DISCUSSION

Plant Breeding Methods

The pastime of breeding plants to develop new types and enhance existing ones is one that almost anybody may take up.

Breeders may experiment with a wide variety of plants because to the simple learning curve of the crossover processes. Amateur plant breeders often experiment on qualities that are very simple to alter, including flower colour, fruit form, or plant size. Even if trials could seem straightforward, it is nevertheless possible to grow rare or stunning plants. Understanding the fundamentals of plant reproduction—discussed in previous chapters—is crucial for effectively breeding plants. This article's goal is to illustrate the straightforward procedures that may be utilised to create new plant kinds or strains.

Selection of plants

The selection of parent plants with the required traits is the first stage in the plant hybridization process. Over many generations, a process called selection may alter a plant's features. Natural and artificial selection are the two kinds. Natural selection is the mechanism by which robust and well-adapted plants persist whereas feeble and inadequately suited plants finally go extinct in the wild. From the beginning of life on Earth, this process has been ongoing, and it continues now in nature. Humans utilise artificial selection to produce more aesthetically pleasing plant varieties. People discovered many thousands of years ago that storing seed from a plant type they want to continue cultivating would enhance the likelihood of receiving a plant that was identical to the original. Yet neither our predecessors nor the procedures by which features were altered or preserved were aware of their possibilities of success. Humans didn't start learning about heredity's rules and plant reproductive mechanisms until the eighteenth and nineteenth centuries. These foundational concepts are still not fully grasped today. But, we now have enough knowledge to pick plants for breeding with a far higher degree of success guarantee than our predecessors did.

We must choose the parents for our experiment's hybridization procedure. The plants that are chosen for breeding ought to be robust and healthy. Usually, after a few of the plant's blooms have blossomed, it is simpler to identify which ones are healthy. Certain plants naturally resist self- or cross-pollination. Before breeding, it is essential to check for this since, despite obstacles being surmounted, certain plants cannot be artificially pollinated. One obstacle that cannot be overcome is the self-pollination prohibitor of certain orchids; these orchids create a chemical in their stigmas that destroys the pollen from their own blossoms. Without killing the pistil, it is impossible to remove the mechanism that does this. Choose a pollen parent (male parent) with a thick coating of yellow powder on the anther. The pollen is this powder. Examine the stigma before selecting a seed parent. It should either have a shiny, touchably sticky material on it, or a surface that is "hairy." This material or surface holds onto the pollen, allowing for fertilisation. You are prepared to start pollinating after the seed parents and pollen have been chosen.

Hybridization

The tools needed for plant breeding are user-friendly and reasonably priced. These objects are all helpful:

Tweezers, a 10- or 15-power magnifying lens, small, sharp-pointed scissors, and

Camel-hair brush little jugs or vials

Alcohol, soft wire or rubber bands, paper bags, or cellophane bags.

Paper clips, tags, a notebook, and

Marking the flowers that will act as pollen parents and seed parents is the first stage in the process. Colored thread may be used for this, one colour for the male and another for the female. Alternatively you may use paper labels that have been varnished to keep them weatherproof.

Emasculation

The next action is to shield the plant from unwelcome pollen. To avoid the chance of self-pollination or selfing, the stamens must be removed if the plant is to be cross-pollinated. The act

of removing the stamen is known as emasculation. It should be carried out prior to the anthers rupturing and releasing pollen. It could be necessary to manually open the flower before it is prepared to bloom. The following methods may be used to emasculate a plant:

1. Using tweezers to pinch off the stamens or anthers;
2. Using sharp-pointed scissors to cut off the stamens or anthers; or
3. Removing the petals to which the stamens are sometimes attached. In emasculation, a magnifying lens is quite helpful.

Defending the Plant from Outside Interference

It is important to keep foreign pollen away from the seed and pollen parents. The following techniques may be used to accomplish this: flower's closure. Using a piece of flexible wire, twine, or rubber band, the petals of many flowers, including morning glories, petunias, and lilies, may be tied shut around the floral organs. Avoid tearing the petals at all costs.

The blossom being covered

Certain flowers can't be closed, like composite flowers. You might cover the blossom with a paper bag to shield it from unwelcome pollen. You might even cover the bloom with a cellophane bag if you want to keep an eye on it at all times. A paperclip or piece of twine should be used to firmly fasten the bag.

Pollination Crossing

The process of cross-pollinating flowers may be accomplished in a number of ways. Below are the most popular techniques.Put the stamens in a little jar. Take the seed parent's protection off. With the tweezers still in your hand, carefully rub the anther along the stigma. Replace the shield.Cross-pollination may be accomplished by using a brush to transfer pollen from the anthers into a container before brushing the pollen on to the stigma if the stamens are too tiny or challenging to hold.Shake the pollen parent that is being bagged so that the pollen is gathered in the bag that is protecting it. Be cautious not to spill any pollen when you remove the bag from the pollen parent. Take the protector from the seed parent, cover it with the pollen bag, and shake it vigorously so that the pollen settles on the stigmatic surfaces. On corn, this is often done.Always wash the camel-hair brush, tweezers, and any other object that could have touched any pollen with alcohol before using a new kind of pollen. This action is crucial to avoid accidentally pollinating the seed parent with pollen that has stuck to the machinery. When using the instruments again after cleaning them, make sure they are completely dry.

Selfing

Depending on the kind of bloom, certain procedures will apply to self-pollinating flowers. Even though shaking the flower once a day for many days after the pollen develops might assist the pollen settle on the stigma, your work is over after you have closed or covered the bloom for flawless blossoms. Self-pollination is only possible in composite blooms that include both disc and ray florets. These may be selfed in the same manner as perfect flowers since they have both pistils and stamens. Self just those flowers that are on the same plant when the blooms are flawed. Pollen from the staminate flower must be transported to the stigma of the pistillate bloom on the same plant in order to self-pollinate imperfect flowers. Use any of the cross-pollination techniques listed above to accomplish this.

Labeling

The bloom should be closed or covered once again right once after pollination. Labeling the seed parent is the next step. The following is the conventional technique of labelling:The next step is to note the cross or self in a notebook once the seed parent has been designated. It is crucial to maintain thorough and accurate records on breeding activities. The written material should include all pertinent details about the cross or yourself so that you may return to it later and, if required, start the process again from scratch. For each cross or self, a different form or page should be utilised. Assigning successive numbers to each generation arising from each crossing or selfing makes it simple to keep track of the children.

A group of seeds

If the seeds are extremely little, the protector or bag cover used to keep out undesirable pollens might be utilised to gather the seeds.

Screening

By producing seedlings, collected seeds are examined for the required features. The next stages of the experiment may employ these plants.

Reverse Crossing

That is a crucial step. Once the required trait existing in one of the parents was attained, the new offspring were crossed with the parental variety.

Checking and Establishing Uniformity

This is the last phase, when the hybrid plant is tested for that particular trait using appropriate techniques like selfing, establishing the trait's stability by self-pollination, or vegetative propagation to protect the trait segregation during gamete creation. To assess the plant for stability of the desired trait, genetic, morphological, and biochemical approaches might be used.

Hybrid Power

The offspring born from mating two well-known, but dissimilar species of parents will perform better than both parents. Heterosis, sometimes referred to as hybrid vigour, is the phenomena that the hybrid organisms exhibit. Several horticultural and agriculture species, including garden plants, are taking advantage of this hybrid vigour phenomena [7]–[9].It has been discovered that human genetics may be deduced easily in big families as long as accurate records have been preserved. Pedigree analysis refers to this formal inference process. This section will cover a variety of areas of human genetics, with a focus on pedigree analysis techniques that aim to infer the genetics of human problems from information on marriage (mating) and ailment in big extended families [10]–[12].

CONCLUSION

The numerous staining techniques as well as histological and cytological procedures have led to the development of chromosomal techniques including chromosome banding and painting. Several of these methods have advanced to the point that they are now regularly used in many labs for the identification and diagnosis of certain illnesses. In order to identify numerous syndromes and chromosomal abnormalities in people, clinical labs and hospitals provide

karyotyping and pedigree analysis. The development of several mutant lines aided in the rapid discovery of a number of crucial genes. In many respects, humans are different from other species. Nonetheless, one factor that geneticists value highly is the fact that humans are immune to genetic experiments.

In reality, many long-lived creatures that postpone initial births share this trait with humans. In other words, waiting 15 years between generations makes it difficult to execute experimental crosses.

But, one must also note that such experiments on people are specifically prohibited by our morality system. This is a sad state of things since there is no other creature whose genetics would be more practical to understand, particularly when it comes to the genetics of heritable disorders.

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CHAPTER 18

PLANT AND ANIMAL DEVELOPMENT: AN ASSESSMENT

Sukanya Dikshit, Assistant Professor
ISME - School of Management & Entrepreneurship, ATLAS SkillTech University, Mumbai, India
Email Id- sukanya.dikshit@atlasuniversity.edu.in

ABSTRACT:

At least in the early stages, the pattern of development of a fertilised egg into an embryo is almost comparable in all animal species. After fertilisation, the zygote goes through a sequence of cell divisions that transforms it from a single cell to a group of cells arranged into a hollow spherical, or blastula. As the size of the cells shrinks, the number of cells multiplies quickly. Some of the blastula cells start to develop into endoderm, mesoderm, and ectoderm at this time. The endoderm typically develops into the epithelial lining of the intestine, the mesoderm creates the muscles and internal skeleton, and the ectoderm produces the animal's skin and nerves. The cellular layers are repositioned at this stage by a process called as gastrulation. During gastrulation, the endoderm replaces the mesoderm as the innermost layer of cells, while the ectoderm serves as the outermost layer.

KEYWORDS:

Cells, Centrifugation, DNA, Animal Development, Nucleic Acid.

INTRODUCTION

Endoderm may differentiate into other cell types such as liver, pancreas, and lung, among many others, but it cannot change into ectoderm or mesoderm. Gastrula is the name given to this embryonic stage. The zygote and gastrula do not significantly expand in size. Despite their significant anatomical and physiological variances, all vertebrates adhere to this developmental pattern because all of these phases are so crucial [1]–[3].When the various layers have been rearranged during gastrulation, cell differentiation begins quickly. The three layers of cells specialise and grow into the different organs required to create a fully functioning person. Human embryos begin to grow a heart only three weeks after conception, and by the eighth week of development, an embryo measuring 2.5 cm in length has a fully developed head. During this phase, almost all sorts of tissues and organs have begun to form. It develops externally discernible components during the twelfth week of development, including sex organs, fingers, nails, and toes. At this time, the endoderm also gives rise to the gut.

How can a single cell become an animal? The asymmetry in the egg cell and the instructions in the growing animal's DNA provide the basis for the solution to this basic issue in embryology and developmental biology.

The Genetic instructions that are the same in every cell and environmental signals that inform the cell of its location and intended cell type guide the formation of structures in the growing embryo. Signals may come from interactions between neighbours as well as from protein or small molecule morphogen gradients.

Growth of the Plant

In the ovule's embryo sac, the consequence of double fertilisation is the development of a zygote and an endosperm cell. The ovary contains the ovule. The embryo develops from the zygote via a sequence of mitotic cell divisions into a tiny plant. Endosperm or cotyledons develop from the endosperm cell. The ovary produces the fruit, and the ovules grow into the seed.The endosperm tissue, which is also developing, surrounds the zygote within the embryo sac after it passes through a number of repeated mitotic divisions. The proembryo is the name given to this entity. The cells of the proembryo are organised in three layers:

- Protoderm, which creates the epidermis and other surface tissues.
- Procambium, the tissue that creates the vascular tissues.
- Ground meristem, from which ground tissues are derived.

The embryo at this stage resembles an axis with meristems on each end. These meristems, which will eventually give rise to the shoot system and root system structures, are the apical shoot meristem and the apical root meristem. Also, two lumps may be seen close to the front; these are the two cotyledons, which are unique to dicot embryos. With relation to the cotyledons, the embryo is separated into areas as the cotyledons quickly lengthen. The apical shoot meristem is found in the epicotyl, which is located above where the cotyledons connect. The hypocotyl, which terminates in the radicle and houses the apical root meristem, is the area below the attachment of the cotyledons. To fit within the embryo sac, the embryonic axis often has to fold. The cotyledons may or may not absorb endosperm. It could be totally eaten throughout the embryo's development, or some might be left behind for germination. The fact that plant developing ends, or meristems, are extremely tiny yet repeatedly found above the earth as the terminal sections of shoot systems, is one of the key distinctions between the growth and development of plant systems and those of animal tissues. These meristems never cease being embryonic and are always active. As a result, they keep making new tissues and cells all throughout their lives.

Human and Animal Immune Reactions

Pathogenic organisms, such as bacteria, fungus, viruses, and various kinds of parasitic protozoans, are always a threat to all living things, whether they be plants, animals, or microorganisms. In order to combat these parasitic and deadly organisms, both humans and animals have well-developed defensive mechanisms. The immune system is a defensive mechanism found in both humans and animals, and the immunological response is the body's defence against an invader. The body is constantly protected by the immune system against the different germs and parasites prevalent in the environment. Even a simple illness might be devastating if the immune system is not functioning correctly.

System of Defense

Mammals and other higher vertebrate species have very complicated immune systems that are well-developed. The immune system becomes less sophisticated as we go backward in the evolutionary tree. Several organisms, including fish, birds, reptiles, amphibians, and others, have very rudimentary immune systems. Invertebrates, such as starfish, hydras, earthworms, insects, and so on, lack an immune system. Immune cells and lymphoid organs are the two types of specialised cells and organs that make up the immune system. The bone marrow and thymus are

examples of primary lymphoid organs, which are the lymphoid organs where immune cells develop and begin to function. Upon maturation, they go to secondary lymphoid organs where they establish themselves and carry out their functions. The lymph nodes and spleen are two examples of these organs.

The immune system has cells all across the body. Some of them are found in tissues, while others travel throughout the body in bodily fluids like blood and lymph. Phagocytic and natural killer cells are among the immune system's executioner cells (NK). The white blood cells, or lymphocytes, and macrophages are examples of phagocytic cells. The invasive organisms are engulfed by the macrophages. The primary immune cells are lymphocytes, which are further broken down into many kinds. All lymphocytes have the same morphology and cannot be differentiated. They may be categorised based on the presence of certain molecules on the cell membrane's surface and the job those molecules do. T- and B-lymphocytes, sometimes known as T-cells, are the two most significant lymphocyte subgroups.

Several phagocytic cell types make up macrophages. These cells are basophyls, eosinophyls, and neutrophils. Due to the cytoplasmic granules and the multi-lobed nucleus of these phagocytic cells, they are also known as granulocytes. There is another cell with no lobes in the nucleus and no cytoplasmic granules. Monocytes are the name for these cells. The tissue-found macrophages are descended from monocytes. From the circulation, the monocytes go into the tissues and develop into macrophages. Large cells with a lot of cytoplasm and several vacuoles make up macrophages. Histiocytes are the generic name for tissue macrophages. The macrophages found in the peritoneal cavity are known as peritoneal macrophages, whereas those found in the liver tissues are known as kupfer cells, alveolar, and lining macrophages.

The Immune System

The immunological response is the defence mechanism the immune cells display against the invasive pathogen. The body's fluid, lymph, transports foreign cells to the lymph nodes as they enter the body. The organs that resemble filters and are dispersed throughout the body are called lymph nodes. All of the immune system's cell elements, including B-cells, T-cells, and macrophages, are found in the lymph nodes. The pathogen is swallowed by the macrophages and degraded by the enzymes in the macrophages' lysosomes. The lymphocytes are exposed to these pathogen components that have been digested. The immune response, which is dependent on the type of the antigen, is followed by a number of cellular processes and the secretion of a variety of chemicals (the degraded product of the pathogen). The humoral or circulating antibody system (B-cell immunity) and cell-mediated immunity are the two main forms of immune response, respectively (T-cells immunity).

Both immune responses function by recognising antigens (foreign proteins or polysaccharides), which may be either an integral component of a virus or bacteria or a partly degraded consequence. Both systems may detect human antigens that are not produced by the person, which can lead to graft rejection.Secreted antibodies (proteins) produced by the humoral antibody system (B-cell response) attach to antigens and recognise the antigen complex for eradication. Antigens in the serum and lymph are affected by antibodies. Antibodies produced by B cells may either be free in the serum and lymph or bound to B cell membranes. The cell-mediated system responds to antigens that are present on the cell surface. T-cells create T-cell receptors, which are attached to the antigen-presenting structures on the surface of the presenting cell and identify certain antigens.

B-cells are part of the cellular immune system

Each B-cell, or B-lymphocyte, creates a unique antibody molecule (immunoglobulin or Ig). Each person produces about a million distinct B-lymphocytes. So, each person is capable of distinguishing over a million distinct antigens. Each of the naturally occurring antibody molecules, known as glycoproteins, contains two copies of two distinct proteins. There are two copies of each chain: a heavy chain that is over 400 amino acids long and a light chain that is over 200 amino acids long. Antibodies come in five main varieties. IgM, IgA, IgD, and IgE are among them. A single antibody molecule may attach to two viruses because it can bind to two antigens at once, which causes clumping. A B-cell that is already producing an antibody to the new antigen attaches to it when it enters the body. The B-cell absorbs and partly digests the antigen-antibody combination. To be recognised by helper T-cells, the antigen is presented on the cell surface by a unique receptor protein (MHC II). The helper T-cell stimulates the B-cell to divide and release antibodies that go through the blood and lymphatic system. Certain B-cells develop into memory cells such that they can generate antibodies at a low rate for an extended period of time (long-term immunity) and react fast when the antigen is re-exposed. Suppressor T-cells, a subclass of T cells, control the response. (See image on CD)

System Cell-Mediated: T-cells

It is termed a T-cell because it develops in the thymus, where T-cells do. There are many distinct types of T-cells in the system, each of which makes a unique receptor for the cell membrane. One molecule of each of two distinct proteins makes up a receptor. Each receptor only has one binding site, yet they each bind a particular antigen. Only antigens that are "presented" to a receptor by another membrane protein of the MHC type are recognised by receptors (major histocompatibility complex). Antigens given by B-cells, macrophages, or any other kind of cell are recognised by T-cell receptors. All other cells employ MCH-I receptors, while T-cells, B-cells, and macrophages use MHC-II receptors for presentation (responsible for most of tissue graft rejection). When a T-cell is exposed to an antigen, the antigen binds to the T-receptor, cell's which causes it to divide and produce helper T-cells that activate B cells that have bound antigen, suppressor T-cells that control the overall response, and cytotoxic "killer" T-cells that kill cells that have antigen bound in MHC-I.

Apoptosis

Apoptosis is characterised as exceedingly organised, planned cell death. Natural cell death may happen in two different ways. One is accomplished by this apoptosis, and the other is done through necrosis, which happens when there are pathogens or shortages. It's a really well-ordered process, apoptosis. The cells are meticulously destroyed during apoptosis. They separate from the surrounding tissue cells and their protoplasm condenses. Organelles that are membrane-bound, like mitochondria, dissolve and release their contents into the cytoplasm. Endonucleases are the enzymes that act on the components of chromatin to fragment DNA. The cell membrane begins to develop blebs towards the end stage, and the cell splits into apoptotic bodies. This kind of cell death is a natural physiological process that always happens as an organ is developing. In contrast to apoptosis, necrosis happens disorderly and is brought on by the infections' poisons acting on the cell.

Plant Defense Mechanisms Like mammals, plants are subject to a range of hostile creatures that might harm them. Insect pests, nematodes, harmful fungus, bacteria, viruses, and many more

species are among them. Abiotic stressors are a broad category of environmental pressures that affect plants in many ways. Biologic stress is the term for stress experienced by living things. Both the quantity and quality of the crops may be significantly reduced as a result of biotic stress. They continue to be healthy despite being attacked by harmful microbes and other animals. This is due to the fact that plants have a defensive mechanism to fend off invasive species. Research on plant defence systems are crucial because, if necessary, they may be utilised to genetically modify other agricultural plants by isolating and identifying any defensive response-related genes. Based on the defensive reaction, the defence system may be divided into two groups: passive or constitutive if the method of response is an already-formed type, and active or inducible if the method of response is a new type generated after the infection or assault by the pathogen [4]–[6].

Passive Protection

This form of defensive reaction is brought on by the presence of certain structural elements or metabolites in the plant's body. The outer layer of the plant surface may be a unique kind, like cuticle or wax, which the fungus or bacteria that are infecting it cannot attack or digest. Strong substances like lignin, thick bark, cuticle, etc. may effectively stop the organisms from reaching the surface of the plant. There are several secondary metabolites that are poisonous to pests and pathogens, including alkaloids, tannins, phenols, resins, and others. Some of these substances could be antibacterial, antimicrobial, or insecticidal. There are several proteins or peptides that have antibacterial activities in addition to the secondary metabolites. Examples include antifungal peptides that guard seeds against fungal infection, hydrolytic enzymes that may lyse bacteria and fungi, and proteins that render viral particles inactive by breaking down their coat proteins and nucleic acids.

Involved Defense

The term "active defence" refers to the defensive reaction that is developed fresh and was not previously existing in the cell or organism. One of the places where the alteration brought on by the defensive response may be seen is the plant cell wall. Wall apposition refers to any alterations to the cell wall brought on by an infection. When a fungus or bacterium begins to infect the plant body via the surface, the cell wall thickness at that location instantly increases to prevent penetration. The addition of additional wall components to the cell wall, notably in the infection region, is what causes the shift in thickness. The hypersensitive response is yet another intriguing mechanism or reaction (HR). The cells close to the infection site get necrotic as a result of this reaction. These cells' metabolic processes also alter. Their breathing becomes very sluggish or stops altogether. They start to build up dangerous substances. As a result, the pathogen's ability to proliferate and disseminate further from the infection site is inhibited or placed in an unfavourable situation. In reaction to the infection, the plant system or those cells (cells around the infection site) also manufacture specific new compounds known as phytoalexins. Small molecules with a low molecular weight known as phytoalexins are not present in healthy tissues and are only created in response to microbial assault or stress.

According to experimental findings, if the synthesis of phytoalexins by an infected tissue is stopped or suppressed using specific selective inhibitors, the plant's resistance to the infection is significantly weakened. It has also been shown that infections with the ability to manufacture the enzyme needed to break down phytoalexins have far higher pathogenisities than those without this ability.Abiotic stress refers to a broad range of harmful environmental conditions that are

operating on the plant body in addition to biotic stress. Plants have extremely precise responses to environmental conditions including salt, extreme heat or cold, heavy metal toxicity, drought, mineral shortage, etc. For the purpose of coping with such climatic conditions, plants have evolved a variety of physical and physiological adaptations. These changes might be made to the relevant plant components, such as the leaf, root, or stem, or they could take the shape of a physiological or biochemical shift that offsets the impact of an environment change on the plant system. For instance, abiotic stressors including drought, extreme heat, and excessive soil salinity or salinity eventually result in water loss from the cells or cellular dehydration. The plant system successfully combats it by preserving water content inside the cells by collecting a variety of non-toxic and inert metabolites and solutes (osmolytes) (known as compatible solutes). Many sugars, such as sucrose, fructose, and trehalose, as well as other sugar alcohols, including mannitol and inositol, are among the compatible solutes (myo inositol). Moreover, it has been shown that a number of novel genes are activated and expressed in response to certain abiotic stress situations. These gene products (proteins) could play a significant part in building stress tolerance. For instance, LEA proteins (late embryogenesis abundant protein) are produced in seeds that have been dried out and in the vegetative sections of plants that are submerged in water. Dehydrins are also expressed when a body is dehydrated.

Many signal transduction pathways are involved in the development of stress proteins, according to in-depth research on plant-pathogen interactions and abiotic stressors. In order to exploit the relevant genes for genetically engineering agricultural plants for varied stress tolerance, much study has been done to discover the genes linked with abiotic stressors. By transferring the appropriate gene or genes, transgenic rice and other crops have previously been created for improved salt tolerance, drought resilience, and other abiotic stressors.

Interaction between pathogens and plants

When an insect or microbe infects a plant, a series of events occur that result in the creation of certain chemicals that may defend the plant against the invaders. Elicitation is the term used to describe this process, and phytoalexins are the substances produced in response to an insect or microbial assault. In order to properly take measures to reduce crop loss in agriculture, studies on plant-pathogen interactions are crucial for understanding the molecular mechanisms of insect resistance or disease resistance in plants. In the example of the tobacco plant when it was attacked by the tobacco mosaic virus, it was shown that plants may synthesis specific polypeptides in response to a microbial assault. Pathogen-related proteins, or PR proteins, are the name given to these proteins. A variety of additional defense-related reactions, including the creation of enzymes necessary for the expression of genes associated to the manufacture of phytoalexins, wall-bound phenolics, hydrolyzing enzymes, and hydroxy-proline rich glycoproteins, were also found. These metabolites were created and gathered at the infection site to block the entrance of the invasive bacterium. These details will aid in the development of techniques, such as genetic engineering, to shield agricultural plants from disease and insect assault and so lessen crop loss.

Successive Metabolism

A variety of macromolecules, including proteins, nucleic acids, starch, and lipids like triglycerides, steroids, etc., are synthesised using biomolecules like amino acids, lipids, and carbohydrates like glucose, fatty acids, etc. These kinds of molecules, along with other closely linked processes like the production of energy and immune responses, are necessary for the

existence of life. These processes are referred to as primary metabolism, and the metabolites they produce are known as primary metabolites. Yet, as an organism reaches adulthood or when its cells reach the lag phase of development, they use extra metabolic pathways to create substances that are not necessary for performing daily tasks. Such substances are classified as secondary metabolites, and secondary metabolism is the biochemical process that produces them. Their biosynthesis is initiated by a primary metabolite or by primary metabolic intermediates. Little amounts of these secondary metabolites are created, and it is thought that they serve no purpose in the body. Nonetheless, it has been noted that sometimes they play a part in the defence against bacteria, insects, and other pests. Certain chemicals lend unique scents to body parts such as flowers or leaves, which attracts or deters insects and predators, while others are created in reaction to the assault of particular bacteria or insects. There are many different kinds of secondary metabolites found in plants, including alkaloids, steroids, terpenes, latex, tannins, and resins, which are typically synthesised and stored in specialised cells.

These secondary metabolites are created during the stationary phase of growth in the case of bacteria and fungi. The secondary metabolites amassed by bacterial and fungal cultures throughout their stationary growth phases include substances like antibiotics. The environment and stage of development affect how secondary metabolism is biosynthesized. In the case of bacteria and fungi, changing the growth circumstances and medium compositions might change the order of secondary metabolism. In microbial cultures, even a little variation in pH may shift the path of secondary metabolism.Alkaloids, latex, and antibiotics are examples of secondary metabolites with significant economic value to man. Hence, by modifying the culture conditions or by include the essential precursors in the media of the cultures, these chemicals may be produced in huge amounts.

DISCUSSION

Once upon a time, it was thought that lesser creatures lacked defensive systems. Yet, this is untrue. Although though only vertebrates have highly evolved defence mechanisms, smaller types of creatures may nonetheless have basic immune systems and defensive mechanisms. The immune system gets less complex in vertebrates as we descend from mammals to birds, reptiles, amphibians, and fish.

Lower species lack the well-developed immune system and defensive mechanisms that we have examined in the case of vertebrates, but they may nevertheless defend themselves against predators and rivals using different defence systems, some of which are unique to a given organism [7]–[9].In order to defend themselves against predators and harmful circumstances, bacteria and other microbes have a variety of structural, physiological, and biochemical mechanisms.

Many bacteria and fungi create harmful substances including antibiotics and antibacterial compounds, as well as digesting enzymes. These substances have the ability to destroy or harm an organism's cells when they come into touch with them.

Many bacteria and other microbes have the ability to make capsules. The bacterial cells that are enclosed cannot be ingested and eliminated by the phagocytic cells. Phagocytes like neutrophils and macrophages may quickly absorb and kill non-capsulated bacterial cells.A high percentage of bacteria may transform into endospores under poor circumstances. At the centre of the cell, the cytoplasm separates from the cell wall and transforms into a spore. The spore has a very thick

shell and is known as an endospore because it forms within the cell. Endospores have a high level of heat, UV, chemical, and antibiotic resistance. Although if the development of endospores is a strategy for overcoming adverse circumstances, it may also be seen as a type of protection [10]–[12].

CONCLUSION

Insects have a variety of defensive systems, which vary depending on the kind of bug. The creation of antimicrobial peptides is one of the key processes. These poisonous substances have the power to eradicate microorganisms. Several other insects known as cecropins also manufacture these poisonous antibacterial peptides. Melittin, a kind of toxin that is produced by bees and found in their venom, has hemolytic properties (lysing the RBC). The reproductive organs of insects like the fruit fly (Drosophila) create andropins, which are antibacterial peptides. The hemolymph of several other insects contains antimicrobial peptides. In response to an insect's bacterial illness, several of these antimicrobial peptides are created. They furthermore have phagocytic cells in addition to these defensive systems. These cells may assault and eliminate the diseases and germs that are encroaching.

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CHAPTER 19

EXPLORING THE ROLES OF CELLULAR TECHNIQUES

Shreya Banerjee, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- shreya.maulik@atlasuniversity.edu.in

ABSTRACT:

The microscope is a crucial instrument for research and instruction today. It is used in a variety of scientific disciplines, and improvements in microscopy are essential to important discoveries in biology, medicine, and materials science. Several approaches have developed from the basic light microscope with the goal of enabling the observation of specific objects or processes. In order to get very high resolution, to create three-dimensional photographs of surfaces or biological molecules, or to distinguish between different substances, scientists utilise electron microscopes. The numerous cellular and histological methods used to research cell structure and function are described in this chapter. The atom absorbs energy and gets excited, which is the basic idea underlying excitation and fluorescence. A higher energy level is reached by the electron. Immediately after, the electron returns to its ground state and releases a photon (or light packet), which causes that molecule or atom to glow.

KEYWORDS:

Cells, Cellular Techniques, DNA, Energy Level.

INTRODUCTION

The fine structure and operation of cells, as well as the molecular underpinnings of cell activity, are the core topics of cell biology. Since the cell is so tiny, it is quite challenging to analyse its intricate structure and see how the various parts function. The knowledge of cell biology has greatly benefited by technological advancements in the apparatus and procedures used to investigate cells and their metabolic processes at the molecular level [1]–[3].In order to create a picture, the fluorescence microscope must first excite the material by lighting it with radiation. This is followed by sorting the image from the considerably weaker radiation that was produced at the visible range. Initially, the microscope features a filter that only allows light with the required wavelength, which corresponds to the fluorescing substance, to pass through. As a result of the radiation's collision with the specimen's atoms, electrons are stimulated to a higher energy level. They produce light when they are less relaxed. The produced light must first pass through a second filter to be isolated from the considerably brighter excitation light before it can be seen. On the microscope, the fluorescing regions may be seen to stand out against a black backdrop with excellent contrast.

If a substance absorbs light at one wavelength and then emits light at another, longer wavelength, it is said to be fluorescent. Many fluorescent staining dyes are available, including fluorescein, which produces green light, texas red, which generates red light, and rhodamine. Several proteins may be seen in a cell by staining with different dyes (staining at various wavelengths). To analyse the signal changes in cell metabolism, fluorescent dyes like fura-2 are employed to

assess the quantity of free calcium ions in the cytoplasm. In genetic engineering investigations, fluorescent proteins like green fluorescent protein (GFP) may be utilised as markers. The jellyfish aequorea victoria has the fluorescent protein known as GFP, which occurs naturally.

Phase-contrast imaging

The phase-contrast microscope is often used to examine biological tissues and other items without staining them. It is a sort of light microscopy that alters the optical path of light to improve contrasts of transparent and colourless objects. Components in a cell or bacterium may be seen using a phase-contrast microscope, which is much easier than using a regular light microscope.A phase-contrast microscope makes advantage of the fact that light moving through a transparent area of the material moves more slowly and, as a result, has a different phase than unaffected light. The human eye cannot see this phase change. However, a clear phase-plate in the microscope may raise the phase change to half a wavelength, which results in a difference in brightness. As a result, in contrast to its surroundings, the transparent item stands out and shines.

In biological and medical research, the phase-contrast microscope is an essential tool. Dyeing is an option but also halts all operations in a cell when dealing with transparent and colourless components. Studying live cells is now feasible thanks to the phase-contrast microscope, and cell division is one example of a process that has been thoroughly explored with it. The 1953 Nobel Prize in Physics was given to the phase-contrast microscope.

Microscopes with a dark field

Instead of a standard condenser, these microscopes feature a dark-field condenser. Only the light that has been deflected by the object reaches the target after being focused obliquely on it by the dark-field condenser. Just the item is brilliant against the dark backdrop, making the whole background field seem to be black. Even the tiniest items, such bacteria and virus particles, may be seen because to the contrast between the specimen and the backdrop.

Atomic microscope

The resolving power of a light microscope has limitations in microscopy. A particle with a size smaller than the wavelength of visible light cannot be seen. This issue was resolved with the development of the electron microscope since electron beams have a shorter wavelength than lightwaves. An electron microscope may be used to conduct in-depth research on the subcellular architecture, organelles, etc. of viral particles. Transmission Electron Microscope (TEM) and Scanning Electron Microscope are the two primary varieties of electron microscopes (SEM).

Electron microscope transmission

The transmission electron microscope (TEM), which use electrons rather than light to function, is based on the same fundamental concepts as the light microscope. The wavelength of light puts a limit on what you can see using a light microscope. As electrons are the "light source" in TEMs, their significantly shorter wavelength enables them to achieve a thousand times higher resolution than a light microscope. You can see things that are a few angstrom in size (10-10 m). For instance, you may investigate minute characteristics in a cell or various materials at almost atomic levels. The TEM has become an important tool in medical, biological, and materials research because to the potential for high magnifications [4], [5].

The electrons that go through the vacuum in the microscope's column are emitted from a "electron source" at the top of the instrument. In contrast to the light microscope, which utilises glass lenses to focus light, the TEM uses electromagnetic lenses to concentrate electrons into a razor-thin beam. The investigated specimen is then traversed by the electron beam. Some of the electrons scatter and leave the beam, depending on the density of the substance there. Unscattered electrons strike a fluorescent screen at the bottom of the microscope, creating a "shadow picture" of the specimen with its various components shown in varying degrees of darkness based on their densities. The operator may look at the picture directly or take a snapshot of it using a camera. If specimens are frozen in hydrated condition, the electron microscope may see unstained and fixed specimens. It is referred to as cryoelectron microscopy.

Electron microscopy for scanning

An electron microscope that displays surface pictures of a material is called a scanning electron microscope (SEM). In the SEM, a stylus that scans the surface from a set distance away is used to examine a surface's structure. Surface analysis is a crucial component of physics, having applications in semiconductor physics and microelectronics in particular. Surface reactions are also crucial in chemistry, such as in catalysis. While organic molecules may also be fixed to a surface so that their structures can be studied, conducting materials function best with the SEM. This method, for instance, has been used to the investigation of DNA molecules.

Sorting cells

A technique called cell sorting allows one group of cells in a tissue to be distinguished from other cell types. It takes a variety of approaches to distinguish one kind of cell from others, which makes it a challenging technique. By treating the cells with certain proteolytic enzymes or with substances that chelate C++, the extracellular matrix and intercellular junctions holding the cells together are broken down in the first phase of cell sorting. Cell-to-cell adhesion is regulated by calcium ions. Now, the tissue may be macerated into single cells with a little shake.

By analysing the light that different cell types scatter or the fluorescence that they generate as they move through a laser beam, flow cytometry may segregate various cell types and identify various cell types. Antibodies and a fluorescent dye may be used to mark specific cells. With an electronic Fluorescence Activated Cell Sorter, the labelled cells may be distinguished from the unlabeled cells (FACS).Cells and microparticles may be identified and counted using flow cytometry. A thin stream of suspended cells is moving through a cuvette. The area where a sample stream is intercepted by a focused light beam (such as a laser or an arc lamp) or electrical field is known as the sensitive interaction zone. Each particle successively produces optical (scatter, fluorescence) or electrical signals. For the purpose of analysing cell dispersion, signals are found and shown.

The most significant aspect of flow cytometry analysis is the rapid examination of enormous numbers of particles, such as 100,000 or more (practically infinite), one after the other, usually in less than a minute. As little as 100 fluorescent molecules per cell may be detected. Small bacteria and phytoplankton are well-resolved sub-micron particles.Microscopical analysis, in contrast, is based on a very small number of cells viewed on a slide (1 to 100). It is also challenging to see structures and particles at sub-micron sizes under a microscope. Hence, a flow cytometer offers helpful data on a variety of cells and their activities.After the removal of a single sample from a test tube, fluorescence analysis enables the quantification of fluorescence from single cells all the

way up to millions of cells. The reliability of statistical information like mean fluorescence intensity (and its temporal variations or dependency on cell function) has been shown.

Fractionation of Cells

Cell biology entails separating tissues and cells into their constituent parts by fractionation in order to conduct in-depth biochemical and physiological analyses of the cell and its organelles.Breaking the cell wall (if existent) and cell membrane releases the cellular components as the initial step in isolating any subcellular particle. The two steps of cell fractionation techniques follow one another:

1. Homogenization (disrupts the tissue and releases cellular components) (disrupts the tissue and releases cellular components).
2. Centrifugation (separates the constituent components according to density, size, and form) (separates the individual components according to density, size, and shape).

Rigid cells and tissues may mechanically be homogenised using a tissue homogenizer. To provide strong shearing pressures for plant tissues, a solid abrasive may be added, such as sand, silica, alumina, etc. Soft tissues may be homogenised using liquid shear procedures, which produce moderate shearing pressures between the tissue and liquid media (homogenization buffer). Microhomogenizers are designed to cause little tissue/cell disruption by producing negligible shearing forces. By applying an osmotic shock and maintaining the cells in a hypotonic solution, soft tissues and cells similar to those of animals may be burst. Another straightforward way for rupturing cells is the freezing and thawing procedure, which rips the cells apart using ice crystals. In a sonicator, ultrasonic waves may efficiently homogenise single-celled organisms like bacteria and yeast. For cell fractionation, non-physical techniques such as enzymatic techniques may also be used. For instance, in the case of plant tissue, the enzymatic disintegration of the cell wall yields protoplasts, which are then exposed to one of the earlier techniques for disrupting cells and protoplasts. The creation of protoplasts requires a variety of enzymes, including chitinases, pectinases, lipases, proteases, and cellulases.Following tasks must be carried out throughout the cell fractionation procedure. To reduce protease activity, cell fractionation must be carried out at 4°C throughout. All media and equipment need to be precooled and kept at 4 °C. The organelles essential for isolation may be harmed by the shearing pressures required to rupture the cell membranes. If the homogenate in the high-speed blender foams, enzyme activity can be lost.

Centrifugation

Tissue that has undergone cell rupture or homogenization produces a variety of suspended cellular parts, such as different organelles, nuclei, cell membranes, etc. A appropriate centrifugation technique is used to separate the required subcellular fraction. We covered the basics and uses of centrifugation in one of the previous chapters. Particles (organelles) sediment at varying rates in a centrifugal field depending on their density, size, and shape, which affects how they separate. When the particle's density and the density of the centrifugation medium are the same, the particle stays stationary.

Centrifugation Methods

1. Varying the centrifugal force
2. Centrifugation that is rate-zonal

3. Centrifugation that is isopycnic

According to their size and density, particles are separated using differential centrifugation. Larger cell components, such as nuclei and undamaged cells, sediment during differential centrifugation at relatively modest speeds to produce pellets at the bottom of the centrifuge tube. A pellet of mitochondria and chloroplasts is created at somewhat greater speeds. Closed vesicles will first produce a pellet at lower rates of centrifugation, followed by ribosomes at higher speeds and for longer durations.Certain components may be separated as layers using density gradient centrifugation or rate-zonal centrifugation. Density gradient centrifugation is the term used to describe a centrifugation medium that exhibits a positive increase in density. A small band of the sample is put on top of the density gradient. According to the centrifugal field, the particle's size, shape, and the density differential between it and the suspending liquid, particles divide into a number of bands. The sedimentation coefficient of each component may be used to represent the rate of sedimentation as an S value.

Growth is an orderly rise in the number of components that make up cells. It relies on the cell's capacity to create fresh protoplasm from nutrients found in the surrounding environment. Growth in most organisms, including bacteria, includes an increase in cell size and the number of cell organelles (in bacteria, this is the ribosome), duplication of the genetic material or chromosomes, synthesis of new cell walls and plasma membranes, partitioning of the doubled chromosomes, the formation of septa, and finally, cytoplasmic division. This is how eukaryotic cells develop, while in unicellular creatures like bacteria, cell division is how asexual reproduction takes place. Binary fission is the name given to this kind of asexual reproduction. Similar to unicellular organisms, plant and animal cells may also be cultivated as suspension cultures in liquid medium.

There are many methods for determining how quickly cells are growing in a culture media. Changes in cell mass and changes in cell number are two separate measures that may be used to quantify the cell proliferation of organisms in a culture. The proliferation of plant and animal cells is similar to that of microbes since they may divide singly or in tiny aggregates in culture.

Techniques for Calculating Cell Mass

Both direct and indirect approaches are used in cell mass measuring procedures.

1. After centrifugation, the dry weight, wet weight, or volume of the cells may be directly measured physically.
2. Direct chemical measurement of a chemical present in the cells, such as the total amount of nitrogen, protein, or DNA.
3. Indirect measurement of chemical activity, such as rate of oxygen generation or consumption, rate of carbon dioxide production or consumption, etc.
4. The quantity of light scattered by a suspension of cells is measured by turbidity using a number of tools. Bacteria, for example, scatter light in proportion to their population. After the design and calibration of a standard curve, the turbidity or optical density of a suspension of cells is directly proportional to cell mass or cell number. While the procedure is straightforward and non-destructive, the sensitivity is only approximately 107 cells per ml for the majority of bacteria.

Techniques for Cell Number Measuring

1. Direct microscopic counts: These measurement methods use indirect viable cell counts in addition to direct counts that may be performed either visually or numerically. Counting chambers are customised slides that enable direct microscopic counts. Living cells and dead cells cannot be separated. Samples may be concentrated by centrifugation or filtration to boost sensitivity, but only thick suspensions (>107 cells per ml) can be counted. Bacterial growth in natural habitats has been monitored and measured using a variant of the direct microscopic count. T. used a variety of techniques to find and demonstrate the presence of thermophilic bacteria in boiling hot springs. D. Brock submerged microscope slides in the springs and occasionally removed them to see microscopic details. The bacteria spontaneously adhered to the glass slides in the boiling water and developed into microcolonies on the surface.
2. Electrical counting chambers: These devices count objects and gauge the size variation of cells. Bacterial cell size requires an extremely clean suspension medium. Eukaryotic cells, including blood cells, are more often counted using such electronic instruments. Placing (or spreading) a sample of a culture on a surface made of nutrient agar is how indirect viable cell counts, also known as plate counts, are conducted. Before plating, the sample or cell suspension may be diluted with a harmless diluent (for example, water or saline). Each viable unit multiplies and creates a colony when plated on an appropriate media. A colony-forming unit (cfu) is a countable colony, and the quantity of cfus is correlated with the number of viable bacteria present in the sample.

- The approach has the advantages of being sensitive (it is potentially possible to detect a single cell), allowing for visual examination, and positively identifying the organism being counted. Cons: Clumps or chains of cells grow into a single colony;
- Only live cells create colonies that are counted;
- Only organisms for which the cultural circumstances are favourable give rise to colonies.

Are appropriate for growth. Because of the latter, the approach is essentially ineffective for characterising or counting all of the bacteria present in complex microbial ecosystems like soil or the rumen or gastrointestinal tract of animals. Prokaryotes may be studied using genetic probes to show their variety and relative abundance in such a setting, however many of the species discovered by genetic methods have not yet been shown to be cultivable [6]–[8].

DISCUSSION

A viable cell may reproduce and cause a noticeable change in the medium in which it is developing if the proper nutritional and environmental circumstances are present. This might happen via the growth of a colony on an agar surface or the development of turbidity in a liquid media. The spread or pour plate technique uses a little amount (about 50 to 100ml) that is distributed evenly on an agar petriplate using a 'L'-shaped glass tube. It is then incubated under the proper circumstances. The number of organisms present in the amount of liquid dispersed on the agar plates may be connected to the number of bacterial colonies that grew in the agar plates [9], [10].

Growth is determined by turbidity

This is another another useful method for measuring growth in liquid culture environments, not only for bacterial cultures but virtually all cell types. By creating a growth curve, this technique

may be used to analyse the growth pattern of cells growing in cultures. The rise in the medium's turbidity, which may be seen using a spectrophotometer at an appropriate wavelength, can be used to quantify the growth.

Curve of Bacterial Growth

A bacterial colony that is expanding in a laboratory setting regularly doubles in size under ideal circumstances. The actual pattern of bacterial development in nature is not exponential growth; rather, it is merely a portion of the bacterial life cycle.The growth cycle comprises four distinct stages that may be identified.

1. Phase of Lag. The population doesn't alter for a while after the cells are infused into brand-new medium. Despite the absence of visible cell division, the cells may be expanding in size or mass, producing more enzymes, proteins, RNA, and other molecules, as well as engaging in increased metabolic activity. The size of the inoculum, the amount of time needed to recover from physical trauma or shock during the transfer, the amount of time needed for the synthesis of vital coenzymes or division factors, and the amount of time needed for the synthesis of new (inducible) enzymes that are required to metabolise the substrates present in the medium are all factors that appear to affect how long the lag phase lasts.
2. Period of exponential growth. The exponential phase of growth is a balanced development pattern in which all the cells regularly divide by binary fission and increase in size by geometric progression. Depending on the make-up of the growth medium and the incubation circumstances, the cells divide at a consistent pace.
3. Stage of Stability. In a batch culture, exponential growth cannot continue indefinitely (e.g., a closed system such as a test tube or flask). One of three things is limiting population growth
4. Exhaustion of the food supply
5. Accumulation of end products or inhibitory metabolites;
6. While counting viable cells during the stationary phase,

It is impossible to tell whether some cells are dying and an equal number are dividing, or if the population of cells has simply stopped growing and dividing. This exhaustion of space is referred to as a shortage of "biological space." The stationary phase, including the lag phase, is not always a calm time. Secondary metabolites, such as antibiotics, are created by bacteria during the stationary phase of their development cycle; (Secondary metabolites are defined as metabolites produced after the active stage of growth). Spore-forming bacteria must activate or uncover hundreds of genes that may be involved in the sporulation process during the stationary phase [11]–[13].

CONCLUSION

Only because of the invention and advancement of many kinds of microscopes were the cell's structural features discovered and further developed. Robert Hooke used a microscope to study numerous objects in 1667 and then wrote about the findings in a book called "Micrographia," which included a description of a cork and its capacity to float. In 1675, Anton van Leeuwenhoek examined items such as blood, insects, and other materials using a simple microscope with only one lens. The cells and germs he saw via his little microscope were the first to be described. A number of technological advancements in the eighteenth century

improved and made simpler to use microscopes, which increased the popularity of microscopy among scientists. The wide range of cellular procedures, such as cell culture, developmental cell biology, and cell separation techniques, is reflected in these titles.

The titles additionally stress the significance of comprehending the fundamentals and uses of cell culture in biomedical research, as well as the function that cell culture plays in the sector and the best ways to operate with cell lines.

The titles also emphasise the developments in bioreactor culture as well as the value of aseptic procedure, microbial control, and cell culture.

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CHAPTER 20

INVESTIGATING THE DIFFERENT APPLICATIONS OF BIOTECHNOLOGY

Shreya Banerjee, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- shreya.maulik@atlasuniversity.edu.in

ABSTRACT:

We are gradually running out of energy from fossil fuels, necessitating the search for new energy sources. They have so far included the use of nuclear power, solar and geothermal energy, as well as the exploitation of hydro, tidal, wave, and wind energy. An increasing understanding of biological solar energy systems exists now, and biotechnology developments in this field may soon make certain processes economically viable. Converting organic leftovers to liquid fuels will become a more economically appealing option when fossil fuel supplies are exhausted and grow more costly. To get biomass resources, one might go in one of three primary directions: Energy crops are grown, natural vegetation is harvested, and agricultural and other organic wastes are put to use. The process of converting the resultant biomass into useful fuels might be carried out chemically, biologically, or via a mix of both. Methane and ethanol are the two major end products that will be produced, while additional products, such as solid fuels, hydrogen, low-energy gases, methanol, and longer-chain hydrocarbons, may develop depending on the original biomass and the procedures used.

KEYWORDS:

Biotechnology, Fossil Fuels, DNA, Biological Solar Energy, Technology

INTRODUCTION

Although while biomass may eventually only be able to provide a tiny portion of the world's energy needs, it will still be very valuable in the long run. Biomass will undoubtedly become more widely exploited and used in certain regions of the globe, such as Brazil and nations with comparable climate circumstances. As compared to coal or oil, there may still be some drawbacks, but the sheer fact that it is renewable while others are not should encourage more study. Biomass will eventually be considerably more widely accessible and economically practical as a source of energy for people.

Unicell Protein

Population expansion, particularly in emerging countries, is one of the largest issues the world is now experiencing. It's possible that conventional agriculture won't be able to offer enough food, and especially protein. New agricultural techniques are widely used nowadays, high-protein cereals have been created, soybean and groundnut farming is constantly growing, and so on. Wide-spread experimental success has also been achieved with the utilisation of microorganisms as protein makers. As most microbes utilised as producers develop as single or filamentous individuals rather than as intricate multicellular creatures like plants or animals, this area of

research has come to be known as single-cell protein production (SCP).Microbes are the top possibility for producing SCPs for a variety of reasons. Some of the factors are as follows:

i. Microorganisms can grow at remarkably rapid rates under ideal conditions (some can double their mass every 0.5 to 1 hour);
ii. Microorganisms are more amenable to genetic modification than plants and animals (they can more easily be subjected to large-scale screening programmes to select for higher growth rate, improved amino acid content, etc.);
iii. Microorganisms have relatively high levels of genetic diversity; and
iv. SCP's acceptance as food for humans is influenced by a number of variables in addition to its nutritional content and safety. Most people do not like the thought of consuming food that was produced by bacteria. There are rules about what you may and cannot eat in many cultures. While dealing with people's wishes, it's also important to take into account things like odour, colour, taste, and texture. Thus, the food technologist's talents will be put to the test if SCP is intended to be consumed directly by people.

Treatment of sewerage

Any material or energy source that cannot be economically recovered or recycled at a certain time and location is considered waste. Expansion in human populations has often been accompanied by increased production of a larger variety of waste products, many of which, if allowed to build up in the ecosystem, seriously pollute the environment. Recycling organic waste from people, animals, and plants has long been a tradition in rural areas; it often results in high-quality fuel or fertiliser. Since it is impossible to dump large quantities of garbage into natural land and rivers, effective waste collection and particular treatment techniques have been developed in metropolitan settings where the majority of harmful wastes accumulate. One of the primary causes of the remarkable increase in human health and well-being over the last century was the development of these practices [1]–[3].

Several biological treatment technologies, including cesspits, septic tanks, and sewage farms, as well as gravel beds, percolating filters, and activated sludge processes combined with anaerobic digestion, have been created, mostly by empirical means. All of these systems or biotreaters have as their main goals the reduction of health risks and the quantity of oxidizable organic compounds in order to create a final effluent or outflow that can be released into the environment without causing any harm. The adaptability of diverse microbial populations' metabolisms is essential to the effectiveness of bioreactors. The essential characteristic of biotreaters is that they must include a variety of microorganisms with the combined metabolic ability to break down any substance introduced into the system. In industrialised societies, water-borne illnesses like typhoid, cholera, and dysentery have all but disappeared thanks to the careful use of microbes.

Biotechnology of the Environment

Concern about how human activity affects the environment and the legacy we leave for future generations is growing among the general people in developed nations. In attempting to preserve our current way of life, attention is being paid to minimising environmental harm and cleaning up previous environmental damage.

For instance, soil-dwelling nematodes that harm agricultural plants yearly inflict $100 billion worth of damage on US crops. The sole choice for crop protection at the moment is chemical

nematicides, yet they are among of the most dangerous and ecologically harmful pesticides now in use.It is certainly preferable to avoid environmental pollution altogether rather than having to come up with creative solutions to clean up the environment. Because of this, several agricultural companies are looking at how plants and microbes may produce processes and goods that are cleaner.The advantages of this study include: An increase in agricultural yield without a rise in the use of agrochemicals that harm the environment.Less demand to exploit the few remaining uncultivated areas due to greater production.A decrease in energy inputs as a consequence of higher productivity (mostly from reduced agrochemical manufacture).The development of alternate, renewable energy sources (e.g., biodiesel).A decrease in the number of agrochemicals (such as pesticides and herbicides) released into the environment as a consequence of the development of genetically modified crops (if appropriately handled), for example, biodegradable polymers made from plant starches or high-value specialty chemicals.

Environment-Related Issues

- The use of genetically modified crops raises worries among many environmental organisations and several biotechnologists on the impact these crops will have on the environment. They caution that the effects of its usage on the environment may not be seen until after their extensive commercial use. These worries consist of:
- Usage of herbicides Herbicide usage might go up if herbicide-resistant crops are used instead of down.

Genetic weeds and pollution. Genes that have been introduced into another species after being duplicated from a different species may "escape" and spread to other creatures, resulting in "genetic pollution." For instance, weeds that are linked to crops that are herbicide-resistant may breed to create'superweeds' that are herbicide-resistant. Medication resistance Sometimes, in addition to the "useful" gene, genes that code for antibiotic resistance are introduced into plant cells. These "marker" genes allow researchers to identify the cells that have undergone effective modification. Such antibiotic-resistance indicators in crops might spread to people or other animals, making it impossible to utilise the antibiotic for veterinary or medicinal purposes.

Surprising outcomes since it is difficult to anticipate where the genes will insert in the plant genome, certain genes introduced into genetically modified plants may be unstable (there is a high possibility that they will be lost from the plant cells) or may exhibit unexpected consequences. Resistance to pests Crops that have been genetically engineered to resist a certain pest may unintentionally (and unpredictably) affect helpful or harmless creatures (e.g., ladybirds and bees). weediness and perseverance. Intentionally or accidentally, plants that have undergone genetic modification may be more robust than their unaltered counterparts. They might essentially turn into weeds. Plants that are more "persistent" (e.g., have superior winter survival rates) may quickly supplant other plants in ecosystems. They could also invade new ecosystems if they exhibit "weediness" traits.Damage done to biodiversity and animals. Pest-resistant agricultural monocultures may potentially affect beneficial insects and unintended creatures, with repercussions for the food chain.

Taking Care of Environmental Issues

- Precautionary environmental law is in place in many nations since it is difficult to evaluate the dangers linked to the possible environmental effect of utilising genetically modified crops. Most nations use risk-assessment processes based on evaluating the

effect and interactions of genetically modified crops and their environment before permitting experimental or commercial release of genetically modified organisms. Other points of view include the following:

- Plant Genetic Engineering Achieves Almost the Same Results as Traditional Plant Breeding
- The capacity to transfer individual genes or groups of genes from one species to another enables rapid "step changes" in genetic makeup. But, in other instances, the final result is almost the same as what traditional breeding would finally produce.
- Wide-crossing
- Wide-crossing, which is considered a traditional breeding method, is used to produce novel crop types across plants that wouldn't otherwise interbreed. For instance, certain wheat types used to create bread include rye-derived genes. This is the outcome of a plant breeding experiment where the rye genes that confer disease resistance on wheat were added.
- In the natural world, different species exchange genes.
- Gene exchange between unrelated bacteria happens relatively often (this is one mechanism for antibiotic-resistance spreading). As "nature's own genetic engineer," the bacterium Agrobacterium tumefaciens frequently inserts some of its genes into plant cells.

Instability of Genes in Traditional Crops

The genes in novel crop varieties created by traditional breeding may also be unstable, especially when they come from wide-crosses. Selective breeding and variety assessment "weed out" unwanted gene combinations, much as with GM crops.

Breeding by convention is not "natural" Either

Plant breeders have long employed methods to provide more variety than what nature offers. A plant's genes will randomly mutate as a result of chemical or radiation bombardment. Tens of thousands of mutations, maybe including ones that plant breeders seek, may be found in the plants that survive.

Crops' Genes Are Transferred to Their 'Wild' Cousins

The transmission of genes from any crop to the environment is not well understood. The closest relatives of the crops are more likely to get "escaped" genes. Several cropsbut not allare produced in areas devoid of their "wild" cousins. For instance, potatoes cultivated in South American nations, where the potato has wild cousins, have a considerably lower likelihood of transferring genes than potatoes grown in Europe. There is no proof that the genes in plants that have undergone genetic modification are any more likely to "escape" than those in unmodified plants. There are currently conventionally bred crops that are herbicide-tolerant, as well as the potential for gene transfer and "superweeds." This has not presented an issue so far.

New, Sustainable Agricultural Techniques Are Required

There are worries that pest-resistant plants would eliminate crucial wildlife food supplies and have an impact on creatures that are not the intended targets. It is important to assess the ecological effect of such crops, especially before using them extensively. Yet we must also keep

in mind that many pesticides now in use are not very pest-selective, killing creatures that are not their intended targets. We must explore for alternative solutions since agriculture, as it is now done, seems to be the source of some severe consequences on wildlife biodiversity.

Since isolated plant tissues are very vulnerable to microbial assault by the inborne microbial population that competes with the plant tissue in the culture media, without any defence from the plant tissue, the majority of the early tissue culture studies failed, at least in part. In the comparatively nutrient-rich environment of a culture flask, bacteria, fungus, and other species that can be somewhat resisted by a full plant may readily outcompete an isolated segment of plant tissue. As a result, the culture must be isolated under aseptic conditions, often in a tiny flask or test tube, and competitors must be eliminated. This is often accomplished by chemically sterilising the explant's surface with a substance like bleach at a quantity and for a period that will kill or eliminate pathogens without irreparably harming the plant cells. Moreover, the culture flasks and media must be sterile.

The isolated plant cell was initially attempted to be cultured on a synthetic nutritional medium by G. Haberlandt in 1902. Despite his failure to develop and differentiate the cells, he was successful in keeping them alive for a very long period. He is thus regarded as the founder of tissue culture. After that, efforts were attempted to cultivate separate plant tissues and organs, such as the root, shoot, apex, and embryos, known as organ culture, between 1902 and 1930. Around this time, several nutritional media were assessed for their ability to cultivate and differentiate organs and tissues. For many plant species, suitable nutritional media were created for cultivating isolated embryos, anther, pollen, cells, and protoplasts as well as regenerating whole plants from the cultivated cells, tissues, and organs. Many studies were conducted between 1940 and 1970 to standardise culture conditions, produce appropriate nutrient media, and study the effects of diverse plant-hormone combinations on in vitro morphogenesis. It became feasible to insert particular genes from other creatures, such as bacteria and animals, into plant cells and protoplasts and regenerate the whole plant—the transgenic plants—with the development of genetic engineering techniques in the 1980s. The second green revolution may have been brought about by the emergence of transgenic plants or genetically modified (GM) agricultural plants with superior agronomic properties. In addition to its use in agriculture, genetic engineering of plants has contributed to our understanding of the fundamental process of gene expression and how it affects processes like morphogenesis and differentiation.

Techniques for Cell- And Tissue-Culture

Simple Technique

For the mass reproduction of unusual plants, a method called plant-tissue culture (micropropagation) may be utilised. Using very little space, resources, and time, plant-tissue culture is a method that is widely used in the nursery industry and in plant biotechnology to produce several genetically identical plants quickly.In essence, the method is taking a portion of a plant (such as a stem tip, node, meristem, embryo, or even a seed) and putting it in a sterile, nutritional media (often based on gel). Depending on whether one is attempting to make somatic undifferentiated callus tissue, increase the quantity of plantlets, develop roots, or increase the number of embryos for "manufactured seed," the growth medium's composition will alter. The main phases in plant-tissue cultivation are as follows:

1. Choose the explant or tissue.

2. Surface sterilisation of the explant using disinfectants such sodium hypochlorite or mercuric chloride to eradicate the microflora that is present on the surface, followed by repeated washings with sterile distilled water.
3. Under aseptic circumstances, insert the surface-sterilized explant of the proper size into the culture medium within a laminar air flow chamber.
4. The culture medium should include growth regulators and appropriate nutritional components in the proper proportions.
5. Inoculated cultures are moved to growth chambers or tissue-culture rooms with the proper environmental conditions, including the correct temperature (26 to 28°C), relative humidity (50 to 60%), and fluorescent lighting (16 h photoperiod).
6. Plantlets are regenerated using tissues and cells that have been cultivated.
7. After adequate acclimatisation, fully recovered plants (with shoot and root) are moved from the greenhouse to the fields.

This is a typical procedure or technique for micropropagating a certain plant species. Yet each and every plant species must have a uniform set of these criteria. Micropropagation enables the rapid generation of a large number of genetically identical plants from tiny fragments of the source plant. The initial tissue piece may have been obtained from a shoot tip, leaf, lateral bud, stem, or root tissue, depending on the species in question. The number of branches that are accessible for roots increases dramatically when the plant is put in tissue culture due to the multiplication of lateral buds and adventitious shoots or the differentiation of shoots directly from callus (an disorganised mass of cells). Several species of rooted plantlets have been successfully cultivated in containers or as field plantings after being established in production settings. The two most crucial things to keep in mind are that this technology is a way to speed asexual reproduction and that plants grown using these methods react similarly to any plant that was propagated vegetatively.

There are a number of clear benefits to micropropagation that are not available with traditional propagation methods. In a relatively short amount of time, one explant may multiply into thousands of plants. In the majority of species, the parent plant is not destroyed when the initial tissue explant is removed. Actively dividing cultures that have already been established provide a constant supply of microcuttings that may produce plants under greenhouse conditions without seasonal interruption. The nursery can quickly introduce chosen superior clones of attractive plants in sufficient numbers to make an influence on the landscape plant market using micropropagation techniques.

Biological Media

A plant's hormones and nutrients must be supplied to an isolated section of the plant in order for it to flourish in a culture dish. The culture must moreover have the capacity to expel the byproducts of cell metabolism. By cultivating on or in a predetermined culture media, this is done. The medium has to be refilled on sometimes. Cell development in vitro requires a regulated environment. Tissue cultures need a consistent and suited environment to be maintained by the nutritive medium while enclosed in a protective vessel. As a result, light and temperature control must be more meticulous than in the case of the whole plant.

All the macro- and micronutrients are present in a standard plant-tissue culture medium, together with a carbon source, vitamins, and growth promoters. The macro- and micronutrient supplements are often inorganic salts. The typical carbon source in tissue culture medium is

sucrose, while nicotinic acid, thiamine, and pyridoxine are added as significant vitamins. Amino acids like glycine and arginine are also added to certain media. The basic medium is made up of these components. According to the needs of the culture conditions and the objectives of the experiments, growth regulators are added to the basal medium. Auxins like indole acetic acid (IAA), indole butyric acid (IBA), and 2, 4-D, cytokinins like kinetin, and gibberellins like gibberellic acid are crucial growth regulators added to tissue-culture medium. Alone or in combination, these growth regulators are given at the proper dosages. Growth regulator concentrations and mixtures play important roles in the development and differentiation of cultured cells and tissues. Myo-inositol is another significant chemical component found in all tissue culture mediums. Tissue extracts from foods such coconut milk, tomato juice, yeast, casein hydrolysate, or malt may sometimes be added to the medium to satisfy specific needs. Trial and error procedures are used to identify the media compositions, special supplements, and hormonal content for a certain plant species or tissue type. For plant-tissue cultures, a variety of basal-media compositions that are tailored for various plant species are available. The Murashige and Skoog (MS) medium, created by Murashige and Skoog in 1962, is the basal media that is employed the most often.

In the past, stock solutions of mineral-salt mixes that were 10 to 100 times the final concentrations utilised in the medium were made. Two solutions, one having all the macronutrients and the other all the micronutrients, may be made as stock solutions. The calcium and magnesium phosphates and sulphates must be prevented from precipitating by maintaining these solutions at a very low concentration (10 to 20X). Iron sulphate and EDTA are combined to create iron-EDTA chelate.

Media Pouring and Storage

If no filter-sterilized components need to be added, the medium may be prepared, the pH adjusted, and the gelling agent dissolved before being poured into culture containers such tubes, baby food jars, or pink boxes and autoclaved. The solid medium's support or gelling agent is agar. It is a polysaccharide made from Gelidium amansii, a red alga. It's best to keep media in the fridge or at least somewhere dark (light causes some reagents to break down). Most types of media can be kept for at least a month under ideal storage circumstances.

Different Cultures

Culturing of organs. Organ culture is the controlled cultivation of separate organs or tissues like roots, stems, or leaves in artificial medium. Organ cultures are given names based on the organs or tissues that were utilised to create them. The different forms of organ cultivation and their distinct purposes are as follows:

Seed cultivation the creation of clean seedlings for explants or meristem culture, accelerating the germination of seeds that are challenging to germinate in vivo, and precocious germination by the use of plant growth stimulants.Culture of embryos. Removing obstacles that cause embryo abortion owing to incompatibility, removing obstacles that cause seeds to shrivel and become self-sterile, rescuing embryos during distant (interspecific or intergeneric) hybridization when endosperm development is subpar, reducing breeding cycle length, etc.

Ovule or ovary culture a typical explant is used to start somatic embryogenic cultures, to make haploid plants, to prevent wide hybrid embryos from aborting at very early stages of

development due to incompatibility barriers, and to avoid style and stigmatic incompatibility during in vitro fertilisation to create distant hybrids without impairing pollen germination and pollen tube growth.

Microspore and another culture Creating haploid plants, creating homozygous diploid lines by doubling the number of chromosomes, which shortens the time needed to create inbred lines, and identifying mutations or recessive characteristics.

Embryonic Culture

Tissue culture is really explant culture. Explant culture refers to the process of growing any excised plant tissue or portion, such as leaf tissue, stem parts, cotyledons, hypocotyls, root parts, etc. Explant culture is mostly used to stimulate callus cultures or to directly regenerate entire plantlets from it without callus development. For instance, shoot apical meristem cultivation has the following significant applications: The main goals of meristem or shoot apex culture include the production of virus-free germplasm or plantlets, mass production of desirable genotypes, facilitation of exchange between locations (production of clean material), cryopreservation (cold storage) or in vitro conservation of germplasm, among other things.

Callus Culture a callus is an undifferentiated or disorganised clump of cells. They often divide and are typically made up of parenchymatous cells. An explant begins to produce masses of cells from its surface when it is grown in a medium enriched with enough auxins. Depending on the physiological condition of the explant tissue, different auxin concentrations will be needed for various types of explants. By periodically sub-culturing to a new media, callus cultures may be kept alive for a very long time. By altering the hormone concentrations in the medium, the callus cultures may be controlled for a variety of reasons. Callus cultures may be used to prepare single cells, suspension cultures, or protoplasts, as well as to regenerate plantlets. Moreover, callus cultures may be utilised to study genetic change. In certain cases, a callus phase must occur before regeneration by somatic embryogenesis or organogenesis may take place. Callus cultures may be utilised for in vitro selection of cell and tissue variations and for the creation of beneficial somaclonal variants (genetic or epigenetic).

Suspended Cultures of Cells

A portion of callus tissue may be transferred into liquid media and continuously shaken to create single-cell cultures and suspension cultures from callus cultures. In general, the growth rate of cells in suspension culture is faster than that of solid culture. The former is preferable, especially for the mass generation of valuable metabolites. In a container like an Erlenmeyer flask, a portion of the callus is transferred to a liquid medium, and the container is then put on a rotary or reciprocal shaker.

Depending on the plant species and other variables, the culture conditions vary, but in general, cells are grown at 25°C and 100 rpm on a rotary shaker. A fine cell suspension culture with small-cell aggregates and single cells is created by subculturing over multiple generations. The tissue of the plant species and the make-up of the medium have a big impact on how long it takes to develop the cell-suspension culture. Moreover, the cells in suspension are employed for a large-scale cultivation in tanks and jar-fermentors. For the purpose of generating phytochemicals, the suspension cultures may be cultured either as batch cultures or as continuous cultures.

You may also use enzyme-based techniques to create a fine cell suspension culture. This is based on the employment of specific pectin-digesting enzymes, such pectinase or macerozyme, in the culture medium. These enzymes work on the pectin that connects neighbouring cells in plant tissues, allowing the cells to separate and develop normally as single cells. Similar to microbial cultures, cell suspension cultures may be used to induce somatic embryogenesis, create fake seeds, and cause somatic mutations, and select mutants by screening the cells. Plant cell suspension cultures' major purpose is for the bioproduction of certain essential phytochemicals or secondary metabolites using the biochemical engineering approach.

Cultivation of protoplasts

Plant protoplasts are cells that don't have cell walls. With the help of enzymes, the cell wall may be eliminated. The cells may come from leaf tissue, from another section of the plant, from suspension cultures, or from any other source. Cellulase, hemicellulase, and pectinase are mixed together and incubated with these cells for a certain amount of time. The underlying cell membrane is made visible once the enzyme combination fully digests the cell wall. This protoplast can regenerate into a whole plant after being cultured in the right media, where it will grow a new cell wall and turn into a regular cell. The somatic cell fusion that results in somatic hybrids may be carried out using plant protoplasts, which can also be employed for a variety of biochemical and metabolic experiments. Cybrids are a unique subclass of somatic hybrids that are formed when aenucleated and nucleated protoplasts fuse together instead of merging their nuclei. Moreover, protoplasts may be employed for genetic modification research employing biolistic procedures, electroporation methods, PEG-mediated DNA transfer, or direct DNA injection into the protoplast's nucleus using microsyringes.

DISCUSSION

Micropropagation

This tissue-culture technique was created for the demand-driven bulk multiplication and propagation of horticultural and agricultural plants. Apical meristems, auxiliary buds, or seedlings produced by in vitro germination of seeds are the plant components often employed for this form of propagation.It has been used commercially to mass multiply agricultural plants including cardamom, apples, eucalyptus, bananas, and many other ornamental and horticultural plants because it is quick and simple for producing a huge number of genetically identical plantlets. The major phases in the micropropagation process are as follows:You may trim healthy plants' leaves, stalks, or any other acceptable plant component. Cut the explants into appropriate sizes. Avoid using worn-out tissue components. With distilled water that has been surface sterilised, wash the dust off the tissue [4]–[6].

Surface Sterilization and Explant Preparation

i. This step of the operation should be completed aseptically or in a clean working environment. A sterile petri dish should be used after the leaf or any other tissue has been submerged in 70% ethanol for 30 seconds. The tissues are then sliced into tiny, appropriate sizes for explants using sterile scissors and forceps. The explants are put into either a 10% hypochlorite bleach solution or mercuric chloride for five minutes, gently stirring once or twice. They are then carefully cleaned by submerging them for two to three minutes in each of four beakers of sterile distilled water.

ii. Culture Initiation The culture is started using an appropriate explant, such as a terminal meristem or an auxiliary. The surface-sterilized explant is inserted into an appropriate nutritional solution containing the right growth hormones at the right quantities. The explant either starts developing callus or immediately produces shoot buds and roots to form a large number of plantlets, depending on the nutrient conditions and hormone combination. If a callus develops, it is transferred to a different medium containing a hormone combination that encourages the formation of multiple shoots. The subsequent transfer of the shoots to a different media encourages the initiation of the root. The rooted shoots are now prepared to be moved to the pots for greenhouse hardening.

iii. Shoot arrangement. If the cultures sprout more than one shoot, they are cut off and moved to a rooting medium.

iv. Creation of roots. After being moved to the rooting media, shoots form roots and are prepared to be sent to the greenhouse for hardening.

Hardening and transplantation

In order to harden the plantlets under greenhouse conditions, they may be transplanted into special pots containing sterile sand. The in vitro-created plants are acclimated to the greenhouse environment via a procedure called hardening. They may then be moved to the field.To improve the effectiveness and speed of the ways of micropropagating horticultural and agricultural plants, mass plant multiplication by micropropagation has recently been mechanised and robotized. There are currently many valuable plants, such as forest trees and various medicinal herbs that may be propagated via micropropagation techniques.

Plants Free of Virus

Producing virus-free plants is one of the fundamental goals of tissue culture plant propagation. In order to improve production and quality, this is crucial. As meristems like the apical meristem and auxiliary meristem are virus-free, these tissues may be employed as explants in mass multiplication to produce a lot of virus-free plants. Bananas, sugarcane, potatoes, tapioca, apples, and other agricultural plants have a significant risk of harm from viral infections.Meristem culture is often used to get rid of viruses and create plants that are free of them. The plantlets formed during meristem tissue culture are known as "mericlones," and the process is frequently referred to as "meristem culture" or "meristemming."Artificial seeds are somatic embryos that have been enclosed in inert polymeric substances like alginate. Also, they play a significant role in the mass production of agricultural and hybrid types [7]–[10].

CONCLUSION

By embryo rescue and in vitro fertilisation, tissue culture also offers the tools to get beyond the reproductive isolation barriers that exist between closely related wild relatives of crops. Plant hybridization by interspecific and intergeneric crosses is exceedingly challenging. Such hybridizations may produce aberrant endosperm, which causes embryos to develop too quickly and eventually die, producing sterile seeds. It may not be feasible to create new hybrids via other techniques, but it is conceivable to remove the embryo from such hybrid plants and cultivate it on a suitable nutrient to do so. This books cover a wide range of cellular techniques, such as biotechnology, genetic engineering, cell culture, and its uses in a variety of disciplines, including biology, medicine, and environmental science. The titles also stress how crucial it is to comprehend the moral and security concerns related to biotechnology applications and goods.

The titles also highlight the prospective uses of biotechnology in the manufacturing of pharmaceuticals, diagnostics, and treatments, as well as in the prevention of pollution and the development of industrial and marine biotechnology.

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CHAPTER 21

EXPLORING THE MEDICAL AND AGRICULTURE BIOTECHNOLOGY

Aditya Gupte, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- aditya.gupte@atlasuniversity.edu.in

ABSTRACT:

The use of biotechnology in medicine is expanding quickly, giving us the chance to create new, more potent medications and other therapies. Understanding what occurs when genes go awry in hereditary illnesses or malignancies and starting to create novel therapeutics that tackle the genetic cause, not the symptoms, are made possible by studying human genetics. We can better understand how viruses, fungi, and bacteria cause illness and create medications and medicines that particularly target them by analysing their genetic makeup. Traditionally, a lot of medications were made from plants, bacteria, or animals. To treat a large number of individuals, however, it is sometimes impossible to manufacture the medications in sufficient quantities, safely, or affordably. Important medicines may only be derived from threatened plant species (like the anti-cancer medication tactual from the Pacific yew), or they might be generated naturally in such meagre quantities that their extraction would be prohibitively costly. By employing "cell culture," genetic manipulation, or other methods to enhance production levels or create wholly new medications, biotechnology provides options to generate useful medicines in greater quantities.

KEYWORDS:

Cells, Medical, DNA, Biotechnology, Agriculture.

INTRODUCTION

Drugs Made from "Cultured" Cells. Like with yeasts or other microorganisms, many different kinds of plant, animal, and human cells may be grown "in culture," that is, as a suspension of cells in a large fermentor vessel of liquid that includes all of the nutrients they require. Antibiotics may be produced by microorganisms that are cultivated in this manner. These cells can be handled in a variety of ways to get them to generate vast quantities of useful chemicals [1]–[3].Vinblastine and vincristine, two naturally occurring anti-leukemia medications made from plant cells cultured in this manner, are pink periwinkle compounds. Furthermore, sedatives and cardiac medications, such as digoxin from foxglove, have been made in plant culture.

Genetic Engineering in the Creation of Medicine

It is possible to genetically modify organisms to either create more medicines, such as antibiotics, or to develop totally new types of medications. Genetically modified microorganisms that contain the human insulin gene produce a safe and plentiful supply of insulin for diabetics, and genetically modified plants are beginning to be used to produce vaccines for a variety of human and animal diseases. Both microorganisms and plants are being used to produce large quantities of medicines safely and affordably.Since the discovery of penicillin almost 50 years ago, there has probably never been a more pressing need for research into the development of new antibiotics. Why? When disease-causing microbes gain resistance, some antibiotics that

were formerly quite powerful are now proving to be worthless. It is being attempted to create wholly new antibiotics using a novel method. Streptomyces, a soil bacteria, is a source of natural herbicides, immunosuppressants, antibiotics, and anti-cancer and anti-parasitic medications. The bacteria constructs many of these priceless goods from smaller constituent parts in accordance with a "blueprint" dictated by the bacterium's DNA. By altering the blueprint—moving some of the genes and therefore some of the units—new molecules may be created. With this method, a wide variety of novel chemicals may be created, perhaps producing new antibiotics. With the growing number of illnesses brought on by antibiotic-resistant microbes, this field of study is crucial.

Biotechnology for Forests and Agriculture

Plants, animals, and microbes' genetic makeup may be changed by genetic engineering. Every day, there are more and more genes that have been separated and are transferable. While there are some additional potential uses, the technique is now mostly employed to alter crops.Before they are ready for commercial distribution, genetically modified items, like other products, go through a development and research phase. Many goods in the pipeline of research and development never make it to market. While this is true for practically all technologies, early proponents underestimated how difficult and costly genetic engineering would be. Despite the fact that in the early 1980s, biotechnology was hailed as a miraculous invention that would usher the first range of goods has shown to be modest in the new age of agricultural plenty with little impact to the environment. Some of the most significant commercial uses for biotechnology include: Herbicide- and pesticide-resistant crops that will allow for less frequent or more targeted use of agrochemicals.

1. Crops that can withstand adverse climatic circumstances like drought or cold better.
2. Innovative and more effective crop breeding techniques that enable the creation of hybrids that are impossible to create via traditional plant breeding.
3. Fresh crop types that vary qualitatively (e.g., plants that produce seeds or tubers that have an altered starch composition, and thus represent new valuable products).
4. Novel crop types designed to produce more by having varied growth traits (such a variable blooming period or growth rate).
5. These instruments may be divided into two main groups: those that boost crop performance in the field and those that create new goods with improved values.
6. Enhancing Genetic Diversity via Conventional Crop Breeding
7. As seeds from the most productive plants of one season were utilised to establish the crop the next year, food crop cultivation and improvement had their start millennia ago.

Few modern crops resemble their wild predecessors because improved crop types have been created via selection and breeding. By using these traditional plant breeding techniques, certain crops have been created that, for example, are more disease resistant or grow more quickly; other crops have bigger or tastier edible components. Plant breeders are only able to transmit genes across crops that naturally cross-fertilize due to the slowness of conventional procedures for creating new crop types.

Animal Genetics

In the recent years, researchers have been able to pinpoint the specific genes responsible for a plant's unique traits. The fact that we can now transfer these genes across plants is equally

significant since it allows us to give crop species new traits. Although it is unlikely that biotechnology will completely solve all agronomic issues, it can give farmers new choices.

Physiology and Biochemistry of Plants

We now know how plants detect their surroundings and react by germination, growth, blooming, or setting seed thanks to research on plant physiology and biochemistry. Their genes control each of these activities. It is now well known how they produce proteins, lipids, and carbs via photosynthetic processes. It is also much better known how they identify insect pests or disease-causing fungus, bacteria, or viruses (pathogens) and develop their own defensive responses.

Crop plant genetic modification

It was made possible to introduce new genes and desired features into agricultural plants thanks to the finding that genes are naturally transmitted from the bacterial pathogen agrobacterium tumefaciens when it infects plants. It is now quite simple to introduce a gene, or group of genes, into most of the main crops used throughout the globe thanks to the following development of additional genetic modification techniques.Genes introduced via genetic modification may often be transferred into "elite variety" (extremely prolific and commercially successful variations) within a financially feasible timescale thanks to advanced breeding programmes. The implanted genes may now be managed in rather complex ways thanks to a greater knowledge of how genes function, for as by turning them on or off at certain points throughout plant growth or in response to environmental cues. For instance, efforts are being made to get plants to stop spending their energy resources to "grow tall" and instead store energy as starch and sugars. Other research aims to activate the genes for defence systems in plants in response to pest or disease assault, or during growth phases when they are most likely to be vulnerable to attack.

Crop engineering

Engineered crops are by far the most common use of genetic engineering in agriculture. Almost a dozen of these devices have been certified for commercial usage after thousands of them underwent field testing. Herbicide tolerance, insect tolerance, and virus tolerance are the qualities that are most often bred into crops.

Tolerance to herbicides

Herbicide tolerance enables crops to tolerate otherwise fatal concentrations of these plant-killing toxins. Certain herbicides are ineffective on crops and destroy almost all plants. Chemical corporations may increase the market for their goods by providing herbicide-tolerant crops. And it's true that corporations having herbicides to sell are the main producers of herbicide-tolerant plants. The three herbicides with the most commercially available herbicide-tolerant crops are based on the active chemicals glufosinate, glyphosate, and bromoxynil.

Bug Tolerance

A form of the naturally occurring toxin bacillus thuringiensis (Bt), which is present in soil bacteria, is present in every one of the commercially produced insect-tolerant plants. While Bt toxins are not poisonous to humans or the majority of other non-target creatures, they are quite efficient against numerous nuisance organisms, including beetles and moth larva. Farmers and environmentalists are very concerned that the widespread use of Bt crops may result in the quick development of toxin resistance (perhaps in as little as three to five years). The Bt toxin will be

as worthless as a pesticide if resistance develops. The product's environmental advantages in this situation won't last long.

In addition to many other farmers who use Bt in its natural bacterial form, often as a spray, those who now utilise transgenic Bt crops would be impacted by the loss of Bt. These additional farmers include those that use integrated pest control and raise food organically (IPM). For these farmers, natural Bt treatments are an effective form of pest management. Organic farmers and other Bt users wonder whether the Bt crop distributors have the right to exhaust this resource based only on financial considerations.

Tolerance to viruses

Viral tolerance is the third important way that biotechnology is used in agriculture. These plants have a gene that was obtained from a virus. Plants that synthesise certain viral proteins are able to protect themselves against infections by the viruses that the proteins were derived from via an unclear method. Papaya and squash are two crops that are virus-tolerant and are permitted for commercial usage. The squash, which is immune to two viruses, is no longer available. It might be difficult to find out why things are not on the market, but it's likely that the squash didn't do well enough on the market to get traction.

Additional Engineered Goods

There have been several more genetically altered items proposed, but thus yet only a small number have been commercialised.Many tomatoes with ripening-delaying genetic engineering have received commercial approval. Delay in ripening may sometimes just increase shelf life. In contrast, the Flavr SavrTM was developed with the intention of developing a transportable, delicious winter tomato by extending its stay on the vine without softening. The Flavr SavrTM is no longer available on the market after a highly publicised launch. Transportability seems to have been the issue rather than flavour.

On the animal side, a medication called Bovine Growth Hormone (BGH) or Bovine Somatotropin (BST) has been created for dairy cows by genetically modifying a bacteria to include the hormone's gene. Despite the ongoing glut of milk in the US, the medication is given to cows to improve milk output. BGH, a substance that caused much controversy when it was originally launched, is now used on 10% of dairy herds in the United States.A rabies vaccination for wild raccoons is an intriguing product unrelated to agriculture. In this instance, a component of the rabies virus was transferred into an unrelated "carrier" virus by genetic engineering to create a "hybrid" virus. The resultant virus offers protection against rabies but doesn't carry a risk of spreading the illness. To prevent rabies in wild raccoon populations, baits laced with the vaccination have been distributed in several areas of the eastern United States. Despite concerns that the vaccination has proven ineffective or just slightly so, the USDA has authorised it. Early effectiveness tests on the medication fell short of proving that it might prevent rabies in populations of wild raccoons. The public is not being given access to data from more recent research because it is commercially sensitive information [4]–[6].

Programs for Agricultural Biotechnology Research

The list of some of the organisms that agricultural researchers are modifying follows, along with information on which products are now on the market and which are not.

Leaner meat produced by livestock and poultry that have undergone genetic engineering

Currently, no cattle with leaner meat is even close to commercialization. Early 1980s experiments to genetically modify slimmer pigs failed due to unfavourable side effects such poor fertility, rheumatoid arthritis, and weakened immune systems. There may still be some little scientific work going on.Status of Animals Designed as Drug-Production Facilities: Engineered sheep and goats may produce beneficial compounds into their milk, urine, or blood. Commercial ventures based on these animals are now being developed by businesses. None of the medications have been sold yet. It's possible that once the animals are no longer useful for making drugs, the manufacturers will wish to butcher them for food.

Animals Created to Serve as Transplant Organ Sources

Now, pigs are being genetically modified by commercial corporations so that human transplant recipients won't reject their organs. The organs are not currently offered for sale. Producers will probably wish to utilise the donor pigs' corpses as food.Animals Designed to Fight Disease: Chickens and turkeys have been created with the ability to fend against avian infections. None have been made available for sale.Fish and shellfish that have undergone genetic engineering have been created to modify hormones that speed up development in a number of labs. None have so far achieved commercial success in the US.

Many plants, including tomatoes, squash, and commodity crops like maize and soybeans, have been marketed. Genetically modified plants are consumed whole as food. Most have been genetically modified for either viral tolerance, insect resistance, or herbicide tolerance.Status of genetically modified fibre plants: Commercial use of altered insects has not been authorised. In Florida, a predatory mite that was developed has undergone field testing. Engineered honeybees and other helpful insects have been created by researchers.Many bacteria that have been genetically altered to improve their capacity to kill or deter pests have been authorised for use in commercial pesticides. These goods are used as insecticides in gardens and agricultural areas.Food Processing Aids Produced from Genetically Modified Bacteria Status: Rennet, a crucial enzyme in the production of cheese, is produced by bacteria that have undergone genetic engineering. American cheese producers often utilise genetically modified rennet (chymosin), which has received commercial approval.Animal Medicines Produced by Engineered Bacteria Status: Commercially available bovine growth hormone (BGH) is used to increase milk output in cows. Around 10% of the dairy cows in the country now utilise this hormone.

1. Biotechnology of Food And Beverages
2. Modern Biotechnology's Use in the Food Industry

Modern biotechnology approaches are playing a bigger role in the overall endeavour to enhance food production processes and broaden the range and quality of meals available to consumers. The manufacturing and processing of food, as well as the guarantee of food quality and safety, all have potential uses for modern biotechnology.Few genetically modified food crops have so far made it to the supermarket shelf, despite the fact that several are under development. In the USA, the Flavr SavrTM tomato was the first GM whole food to hit the shelves, whereas tomato puree was the first GM food available in the UK. Soybeans that have recently been introduced are genetically engineered, which has generated debate. The amount of soy or soy derivatives, such as starch, protein, or oils, in processed meals may reach up to 60%.Additionally, microorganisms, processing aids (like enzymes), or ingredients used in food production and

processing are being improved through the use of contemporary biotechnology. An illustration of this kind of genetically modified product is the enzyme chymosin, which is used in the production of "vegetarian cheese."

Biotechnology Safety

Governments in each nation create regulations based on international standards to assure the safety of the product as well as those who carry out genetic engineering studies. When the first rDNA tests were conducted, scientists themselves put a ban on more genetic testing until appropriate regulations were established. The Asilomar Conference created certain recommendations in 1977, including the following key points:The host organism used to carry out gene cloning or the cloning of rDNA should be specifically created for such research. Recombinant DNA experiments should be carried out in laminar flow-chamber housed in a clean environment, so that the recombinant organisms do not escape. The recombinant DNA molecule will be lost even if the creature manages to leave the lab. For rDNA investigations, there are certain risk-free vectors that may be utilised without concern. Furthermore, non-pathogenic E. coli has been created and used as host cells in rDNA research [7]–[9].

DISCUSSION

The majority of the time, therapeutic proteins like vaccinations, hormones, and enzymes will be the end results of contemporary biotechnology. The safety standards and laws established by the regulatory authorities should be met by the proteins intended for use as medications. Regarding the data for investigations using animal models, patient types (terminal and non-terminal), and independent clinical trials by different authorities, they are quite stringent. When a transgenic organism is introduced into the environment, further safety precautions should be taken to monitor its effects on the ecosystem. Drugs made using rDNA techniques must undergo at least three steps of clinical testing [10]–[12].

i. Selected patients are subjected to a comprehensive examination of the side effects and patient dosage tolerance during this initial stage of the trial.
ii. The second phase optimises the usage of the medicine by gathering information from pharmacological, pharmacokinetic, metabolic, and toxicological investigations on a chosen number of patients.
iii. The investigations concentrate on the drug's safety features in the final stage. These trials track not just the drug's efficacy but also any negative side effects that may occur. The interactions with other pharmaceuticals are also looked into in terms of dose range.

CONCLUSION

A novel biotechnology product, whether it be a medication or food ingredient, must undergo trial tests before the regulatory bodies would authorise or issue a licence for its commercialization and mass manufacturing.The use of living cells or chemicals produced from them for various applications is part of the broad topic of biotechnology. With the development of new tools and methods for enhancing human health, crop yields, and livestock production, biotechnology has completely transformed medicine and agriculture. By examining recent studies on the methods, developments, and applications of this discipline, this research study analyses how biotechnology is used in medicine and agriculture. The study examines the various forms of biotechnology, including genetic engineering, bioremediation, industrial biotechnology, and

marine biotechnology, as well as their uses in a range of disciplines, including environmental science, agriculture, and medicine.

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CHAPTER 22

GENE THERAPY AND GENETIC ENGINEERING: ALTERING THE GENETIC MATERIAL OF ORGANISMS

Aditya Gupte, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- aditya.gupte@atlasuniversity.edu.in

ABSTRACT:

It is difficult to imagine that the concepts of heredity were just discovered in the 1860s since they are so important to our knowledge of the biological sciences (and their importance was realised less than a century ago). Yet the understanding of the hereditary process's mechanism emerged far later than actual awareness of it. Archaeologists have revealed that farmers in China and south Asia were enhancing crops by sowing hybrid seeds that had evolved desired traits as far back as 7,000 years ago. The Chinese discovered how to create better rice strains over 6,000 years ago. Farmers and breeders have been selectively breeding their plants and animals for thousands of years in an effort to create hybrids that are more productive. Due to the fact that the exact mechanics determining inheritance were unclear, it was a bit of a hit or miss operation. Careful laboratory breeding experiments carried out over the last 150 years led to the discovery of these genetic pathways.

KEYWORDS:

Cells, Material of Organisms, Gene Therapy, Genetic Engineering.

INTRODUCTION

A five-generation pedigree of a family of horses is shown on an ancient Babylonian tablet, along with specifics on height, mane length, and other characteristics, suggesting that the people of that time were aware that certain features were passed down. This kind of selective breeding in plants and animals has continued to be used by farmers and gardeners. Every time a certain plant or animal showed a desirable feature, it was bred once again to generate more with the same qualities. For instance, farmers would choose the heads of wheat with the most or biggest kernels after harvest and keep them to use as seed the following year [1]–[3].

By the 1890s, improved microscopes had been developed, enabling scientists to learn the fundamentals of sexual reproduction and cell division. The goal of genetics study subsequently changed to figuring out exactly how features are passed down from parents to offspring. Several theories were put out to explain heredity, but only Gregor Mendel, a little-known Central European monk, came close to being correct. While his views were published in the 1860s, they weren't fully acknowledged until after his death. He lived a rather quiet life in Brno, where he taught biology and physics to high school students (now in the Czech Republic). He abandoned his scholarly endeavours in his senior years to take on the role of abbot at his monastery. Since the mechanics of inheritance are fundamentally the same for all complex lifeforms, even though Mendel's study focused on plants, the fundamental underlying principles of heredity that he uncovered also apply to humans and other animals. The rediscovery of the inheritance principles, first proposed by Gregor Johann Mendel in 1900, marked the beginning of the modern science of

genetics. The fundamentals of genetics were separately established by three scientists: Hugo de Vries, Tshermark, and Carl Correns. Sutton and Boveri noted the parallelism between the chromosomes and Mendelian "factors" during meiosis. The chromosomal foundation of inheritance was established. Bateson suggested the name "genetics" in 1906 to refer to all the fields that deal with heredity and variation. He described it as the study of variation and heredity. Seeing the variability in Mendelian inheritance, Bateson and Punnet described the linkage phenomenon another scientist, W. Johannsen, used the word "gene" in 1910 to refer to the elements or components that are in charge of an organism's ability to pass on traits to subsequent generations. It is now established by genetic and biochemical evidence that a gene is a segment of DNA that codes for a particular polypeptide [4]–[6].

Molecular genetics

Mendel demonstrated that certain qualities appear in progeny plants without any mixing of parent features via the selective growth of common pea plants (pisum sativum) over many generations. For instance, pea blooms only come in two colours: purple or white; cross-pollinated pea plants do not produce offspring with intermediate hues. Mendel identified seven features that are instantly recognisable and seem to only exist in two forms:

1. Purple or white flowers
1. 5. Yellow or green seeds are available.
2. The axil or terminal of the flower
3. 6. Inflation of the pod form or
4. The stem is either long or short and restricted.
5. A round or wrinkled seed form
6. Yellow or green pods are available.

The dominant biological idea at the time was that inherited qualities blend from generation to generation, therefore the discovery that some features do not manifest in offspring plants with intermediate forms was very significant.This "blending idea" was embraced by the majority of the top scientists in the 19th century. Another false notion put out by Charles Darwin was "pangenesis." According to this theory, the activities we do throughout our lives have an impact on the genetic "particles" in our bodies. The following generation could inherit these changed particles if they travel via the blood to the reproductive cells. The "inheritance of acquired qualities," which Lamarck incorrectly believed to be true, was basically a variant of this.

Mendel chose the common garden pea plant as the subject of his study because it is simple to cultivate them in big quantities and because their reproduction can be controlled. Male and female reproductive organs are present in pea plants. They may thus either cross-pollinate with other plants or self-pollinate. Mendel was able to cross-pollinate purebred plants in his trials with certain features and see the results over many generations. His findings regarding the nature of genetic heredity were based on this.Mendel discovered that the first progeny generation (f1) always contains yellow peas in cross-pollinated plants that only produce green or yellow peas. The next generation (f2), however, regularly has a yellow to green ratio of 3:1.

Mendel's findings from these tests may be boiled down to three basic ideas:

1. Rule of Dominance
2. The Segregation Act

3. Independent Assortment Law

According to the rule of dominance, each gene has at least two alleles, one of which may be dominant and the other can be recessive. If there is a dominant allele, it will always decide the characteristic. For instance, towering plants dominate diminutive ones in pea plants.The rule of segregation states that for each given characteristic, each parent's pair of alleles separates, and only one allele from each parent is passed on to the child. Chance determines which of a parent's two alleles will be inherited. We now understand that the process of sex-cell development causes this genetic segregation (i.e., meiosis).Different pairings of alleles are separately handed on to progeny, in accordance with the rule of independent assortment. As a consequence, novel gene combinations that come from either parent are feasible. For instance, it is not more probable that a pea plant would acquire the capacity to produce yellow peas as opposed to green ones just because it inherited the capacity to create purple blooms rather than white ones. The idea of independent assortment also explains why having six fingers on each hand isn't more or less likely in humans depending on their inherited eye colour. We now understand that this is caused by the fact that the genes for several independently assorted features are dispersed across various chromosomes.

These two inheritance tenets, combined with knowledge of the unit of inheritance and dominance, served as the foundation for our current genetics discipline. Mendel was unaware that these laws might be broken, however.Chromosomes are formed of chromatin, which is a combination of DNA and a particular family of proteins called histones, as has been previously stated. Chromosomes become severely compressed during cell division and may be seen under a light microscope. Chromosomes are largely decondensed and are not visible under a microscope during interphase.Chromosomes are not all the same, however. Certain chromosomal areas have chromatin that is consistently thick, even during interphase. They are referred to as heterochromatin, or "different" chromatin. The other areas are referred to be euchromatin (also known as "excellent" or "real" chromatin), and they are uncoiled during interphase and intensely compacted during cell division. The areas of euchromatin and heterochromatin alternate on each chromosome of a cell. When mitotic chromosomes are stained with different dyes, these different regions show up as bright and dark bands. Chromosomes may be readily recognised when dyed in this manner because each one has a distinctive banding pattern. Karyotypes are images of stained mitotic chromosomes taken for chromosomal identification.

In one instance, a somatic cell may not normally have two copies of a given chromosome. The sex chromosomes are involved in this. We'll see that these chromosomes play a role in defining a person's gender. Our discussion of sex chromosomes will concentrate on the drosophila and human sex chromosomes.Fruit flies and humans both have two distinct sex chromosomes, called X and Y. Males have one X and one Y chromosome, whereas females have two X chromosomes in each somatic cell. Males have two chromosomes that do not naturally couple up because the X and Y chromosomes are quite dissimilar. The centromere of the Y chromosome is located nearer to one end than that of the X chromosome. The DNA sequences identified on the two chromosomes likewise show minimal resemblance. The X and Y do, however, act like members of a homologous pair. The same as other homologues, they couple up during meiosis. The two kinds of sperm they produce during meiosis I are those with an X chromosome (female-producing sperm) and those with a Y chromosome (male-producing sperm). The sex chromosomes are distinguished from the other chromosomes because of some of their distinctive characteristics.

The Heredity by Chromosomes Theory

What does the segregation of chromosomes during cell division have to do with the transmission of physical characteristics (i.e., genes)? Many scientists assumed that genes were located on chromosomes fairly quickly after Mendel's work was rediscovered, but this concept needed evidence. Proof comes from a T. H. Morgan experiment on a drosophila eye colour mutation (white eyes). Morgan proved that the white eye mutation was inherited together with the X chromosome by contrasting the karyotypes of certain flies with their phenotypes. Further genes that were transferred with the X chromosome and genes that were transmitted with certain autosomes were discovered via further research. There seemed to be a specific collection of genes on each chromosome. Studies on chromosomal mapping revealed that each gene was located at a specific locus, proving that chromosomes are linear arrays of genes.Mendel's postulates might be explained in terms of chromosomal behaviour during meiosis thanks to the discovery that chromosomes are linear arrays of genes.

The Segregationist Legislation

Let's assume a premeiotic cell from a person who is heterozygous for a certain gene to clarify Mendel's rule of segregation (Aa). The homologous pair of chromosomes will each have a unique allele of that gene. Each chromosome in a homologous pair has two sister chromatids after replication. The homologous pair's two members divide into different cells during meiosis I.Two of the gametes have chromosomes with the dominant allele after meiosis II, while two of the gametes have chromosomes with the recessive allele. There is an equal likelihood that each of these gametes will take part in fertilisation. As a result, when two heterozygotes mate, the progeny will have an equal probability of acquiring a dominant or recessive gene from the male parent and a dominant or recessive allele from the female parent. As a result, there is a similar likelihood of having the genotypes AA, Aa, aA, and aa.

Independent Assortment Law

Consider a premeiotic cell from a person who is heterozygous at two gene loci to demonstrate Mendel's law of independent assortment (Aa, Bb). With the A gene, each of the bigger chromosomes has a unique allele, and for the B gene, each of the smaller chromosomes has a unique allele.Each chromosome is made up of two sister chromatids that are connected at the centromere after replication.

Due to the separate assorting of the big and small chromosomes, the initial meiotic division results in two potential chromosomal configurations in the daughter cells. The big chromosome with the dominant allele of the A gene and the little chromosome with the dominant allele of the B gene have segregated together in one of the potential combinations (let's call it "combination 1") depicted to the right. The recessive alleles of each gene have also clustered on the same chromosomes.Each gene in a diploid (2n) organism must exist in pairs; these pairings are referred to as alleles or allelic pairs. During fertilisation, the male gamete (n) provides one allele, while the female gamete (n) provides the second allele. These alleles are located at certain locations known as loci in homologous pairs of chromosomes. Some genes may undergo modifications throughout time as a consequence of random mutations, leading to altered versions of the gene. Heterozygous refers to the presence of two changed variants of a gene in a diploid cell or in an allelic pair. Homozygous refers to an allelic pair whose members share the same type of gene. But, there are many copies of a single gene present at any one moment when we

look at a population of a species. In a population, a gene may randomly change into several forms. As a result, a gene may spontaneously appear in several alternative forms. Many alleles refer to a gene that exists in more than one form. Just two of these, nevertheless, are heterozygous alleles on the homologous chromosomes in a diploid organism. For instance, the gene for flower colour may be found as one for red, yellow, or white. Yet only two of themthe heterozygous alleleare present in a person.

We often use scenarios with only two potential alleles for simplicity's sake (A and a). Yet, there are several potential alleles of a single gene. For instance, a single gene with a number of alleles that each produce a distinct colour controls the colour of the hair in mice. There are alleles for many races, including albino, black, brown, and agouti. The twist in this situation is that, depending on the situation, the same gene may be dominant or recessive. Agouti > Black > Albino is a common way to write an allelic sequence. This indicates that albino is dominant over agouti and vice versa. (And agouti must also predominate over albino.) Due to the fact that black is recessive to agouti, a mouse will be agouti if the agouti gene is present. The mouse will be black if the same black allele is combined with an albino allele since black is dominant to albino. Similar to this, the features of the human blood types A, B, and O result from interactions between three alleles (named IA, IB, and I that are located at the same locus on homologous chromosomes. IA and/or IB, but not I are required for the production of cellular antigens. These are the different allele combinations that lead to the four blood types:Be aware that people with type AB blood exhibit both of the co-dominant alleles A and B.

Connection and Crossing Over

It is often discovered that the number of offspring produced for each trait is considerably different from a 9:3:3:1 ratio, the dihybrid ratio, causing the expectations (i.e., for dihybrid crossings) to seem to be broken. The two parental phenotypes have lower numbers than expected whereas the recombinant phenotypes have larger numbers than expected. When this happens, it often happens because the genes encoding the feature under investigation are linked to one another on the same set of chromosomes, which has an impact on meiosis during prophase I. The distribution of genes after meiosis is considered to be linked when it depends on whether or not crossing over has taken place.We had previously believed that each gene was inherited separately. We have previously said that chromosomes, which are basically very long DNA strands found in the cell nucleus, are where genes are organised. It is now possible for two genes that are unrelated to one another to share a chromosome. Does these genes' independent inheritance hold true?

The formation of gametes (egg and sperm cells, each with just one copy of each chromosome, or haploid cells) from normal cells or diploid cells with two copies of each chromosome, one obtained from each parent, is a very complicated process that we must first take into consideration. The maternally derived chromosome aligns with the equivalent paternally derived chromosome at one point in this process (please review the intricacies of meiosis), and only one of the two travels to a particular gamete. A chromosome is really a collection of genes organised in lines that travel as a single unit rather than a single gene. A dog with 39 chromosomes does not merely have 39 genes; rather, each chromosome has many genes that function as one cohesive unit. As a result, the independent orientation of genes found on various chromosomes is the foundation of Mendel's law of independent assortment. Fortunately, all seven of the traits Mendel examined in the pea plant were distributed across several chromosomes, which allowed

him to get the anticipated 9:3:3:1 ratio, or the dihybrid ratio, in the F2 generation. Nevertheless, it was shown that not all genes adhere to the rule of independent assortment, leading to altered dihybrid ratios. It provides monohybrid ratios for dihybrid crossings because those genes, which are shared by the same chromosomes, are unable to move independently but sometimes act as a single gene. Yet, dihybrid crossings also result in the production of different dihybrid ratios in addition to the formation of monohybrid ratios. This demonstrates that the situation is a bit more difficult since, even though the paternal and maternal chromosomes are lined up, they can and do interchange portions. As a result, when the two chromosomes finally split, they will almost certainly each have DNA from both parents.

DISCUSSION

At this stage, a few words require definitions. When two genes on the same chromosome are near to one another, they are linked and often inherit together. These genes are referred to as linkage groups or related genes. Yet, if those genes on the same chromosome are spread apart, there is a possibility that they will swap places on the homologous chromosomes. Crossover refers to this exchange of genes between homologous chromosomes. It is strongly connected to the physical separation between the linked genes in a chromosome that the process of crossing over results in some degree or percentage of independent assortment. There is always a chance that linked genes might overlap in genuine linkage. When two homologous chromosomes cross across, DNA segments are broken and rejoined (exchange of genes). So, when genes cross across, the initial connection between them on a chromosome is broken. Genes on lengthy chromosomes seem to be inherited separately because this occurs often enough, but if genes are near together, a break is considerably less likely to develop between them than at another location on the linked chromosomes. Genetic recombination of paternal and maternal characteristics occurs as a consequence of crossing over, and the degree of this crossing over is influenced by the distance between the genes. This physical distance affects dihybrid ratio modifications, and the frequency of recombination between two genes may be used to calculate their relative distance on the chromosomes. The phenotypes of the parental and new recombinants created in the offspring of heterozygous individuals may be used to determine the recombination frequency [7]–[9].

Any exchange of genetic material between two chromosomes is referred to as genetic recombination. Proper meiosis involves the exchange of DNA segments between pairs of homologous chromosomes, which increases the possibility of genetic recombination. This results in novel gene combinations. The crossing over takes place during this step during meiosis. It permits the blending of features from the mother and father that are found on the same chromosome. The development of variety among descendants and in the population of sexually reproducing organisms depends heavily on the process of genetic recombination. Variations are also produced via mutation, however there is a distinction. Different combinations of the existing genes and their alleles result through recombination. Sexual reproduction has the potential to generate countless gene combinations, on which natural selection has been acting for millions of years to build adapted phenotypes and genotypes. Only the meiotic process allows for genetic recombination; mitosis does not allow for it. Under diploid settings, mitosis results in an equal division of cells with an equal distribution of genetic material. Chromosome pairing (synapsis) and associated crossing over do not occur during mitosis. Cell division using this strategy is necessary for the body's expansion and repair. Consequently, during the development of gametes, meiosis takes place in specialised cells of reproductive organs whereas mitosis occurs

in somatic cells. Meiotic cells vary from their mother cells both numerically and qualitatively, and the daughter cells have various qualities.

Biological Mapping

Any two genes on a chromosome have the potential to recombine. The proximity of the genes on the chromosome determines how much crossing over occurs. Crossover and non-crossover events will happen equally often if two genes are far apart, such as at the opposing ends of a chromosome. Less gene crossing over occurs when genes are near together, and non-crossover gametes will outnumber crossover gametes [10]–[12].

CONCLUSION

Seldom will two genes on the same chromosome that are near to one another cross across. When we follow genes on the same chromosomes, we may create two different kinds of gametes. In the absence of crossing over, the end products are parental gametes. Recombinant gametes are produced when crossing over occurs. Depending on whether the original cross-involved genes are in the coupling or repulsion phase, the allelic makeup of parental and recombinant gametes will differ.

Two technologies that entail modifying an organism's genetic makeup are gene therapy and genetic engineering. While genetic engineering strives to modify genes to increase an organism's capabilities beyond what is natural, gene therapy aims to rectify genetic flaws and prevent or cure genetic disorders.

By analysing recent studies on the methods, uses, and ethical issues of these technologies, this research study analyses gene therapy and genetic engineering. The study examines the numerous approaches of gene therapy and genetic engineering, such as recombinant DNA technology and gene transfer therapy, as well as its uses in a range of disciplines, including medicine, agriculture, and environmental science.

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CHAPTER 23

A REVIEW STUDY OF BREEDING OF ANIMALS AND PLANTS

Harsh Mohanty, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- harsh.mohanty@atlasuniversity.edu.in

ABSTRACT:

Three issuespopulation, food security, and the environmentcan be used to encapsulate the most urgent issues confronting the human species. By 2030, it is anticipated that there will be 8.7 billion people on the planet. The amount of arable land per person is also diminishing as a result of both the growing population (today, one million hectares of arable land are lost year) and land loss brought on by human activities. Increases in agricultural output (the total quantity of product) and productivity (the output per unit of input) must be made, and production systems' sustainability must also be increased, in order to feed more people on less land. Almost 7,000 botanical species are believed to represent the entire number of plant species that are grown as agricultural or horticultural crops. Nonetheless, since just a small number of species produce the majority of the world's crops, it is sometimes said that only 30 species "feed the world." The latter is also the main cause of the fact that just a small fraction of the total number of species that contribute to food security—roughly six million accessions collected and preserved in gene banks—belong to those species. A little more than a third of all accessions kept in collections ex situ are ancient cultivars or land races, while 15% of them are wild relatives of crop species, weedy plants, or wild plants.

KEYWORDS:

Cells, Breeding, DNA, Animals, Plant.

INTRODUCTION

The introduction of a better genotype is the first step in boosting output. By providing it with appropriate agricultural methods, such as correct irrigation, nutrition, pest control, etc., the second step is to help it reach its maximum potential. Superior genotypes may be produced using a variety of breeding procedures. For the development of new crop varieties, traditional plant breeders employed hybridization and selection techniques. Producing transgenic plants and animals using biotechnology offers whole new ways to enhance the genetic quality of agricultural plants. As compared to conventional approaches, the accuracy and speed are quite good. Of all accessions kept in collections ex situ, around half are advanced cultivars or breeders' lines. All accessions together are just partially described. The collections of minor crops and underused species, particularly landraces and wild crop relatives that are underrepresented in gene banks, clearly have a need [1]–[3].

Hence, the operations of gene banks should prioritise further investigation of small and underused species, the collecting of these genetic resources, and the evaluation of genetic diversity within and across landraces. At the same time, it's important to enhance techniques for characterising and evaluating germplasm collections, strategies for collecting and conserving germplasm, and the use of plant genetic resources overall in order to boost production.The

systematic selection and hybridization of agricultural plants for genetic improvement is known as plant breeding. From the moment he first chose a plant seed for cultivation, man began the discipline of plant breeding. Selection is thus the first technique in plant breeding. Selection plays a significant role in modern breeding methods. The notion of heredity and variability is the cornerstone upon which modern plant breeding is founded. Agronomy, plant pathology, physiology, genetics, biochemistry, molecular biology, and statistics are other subjects that plant breeders need to be knowledgeable with. A plant breeder's objective is to create a new genotype of plant with superior traits including productivity, increased resistance to biotic and abiotic stress, and enhanced agricultural and food product attributes.

The kind of plant reproduction is intimately tied to plant breeding practises (i.e., self-pollinated or cross-pollinated). The strategy will change as a result. The selection of the appropriate parents with the required traits is the first stage in the breeding process. Plants might be homozygous or heterozygous depending on the goal of the breeding. In a population that reproduces itself, all members will be homozygous for a certain characteristic. Take the hue of a flower, for instance. There are both red and white variations in the population. The genotype will be "RR" for red colour and "rr" for white colour since the plant naturally self-pollinates, and these types can only generate one kind of gamete with either "R" or "r". The population of that variety will consist mostly of heterozygous plants for flower colour and a lesser proportion of homozygous individuals if the plant is a natural cross-breeding type. A plant with red flowers will have the heterozygous state "Rr" (hybrid), in which the trait "R" is dominant over "r."

Half of the gametes produced by these plants will have the "R" gene, while the other half will carry the "r" gene. With naturally cross-pollinating plants, you may create homozygous plants for a certain characteristic by using artificial self-pollination techniques over a number of generations. Yet, it is not feasible to create a plant that is homozygous for all of a plant's traits. After five to six generations of self-pollination, a usable level of homozygosity may be reached. A population of self-pollinated plants that is heterozygous for several genes is really a population of homozygous individuals. For a specific gene, these groups are referred to be heterogeneous but homozygous. Depending on the degree of self-pollination, certain crops, including sorghum and cotton, experience varying amounts of both cross-pollination and self-pollination. These crops' populations will be a combination of both. More homozygosity for that gene will remain in the population when self-pollination rates are high. After choosing the parents, the plants may self-pollinate to create the hybrids. The resulting hybrids may be assessed, and new selection and hybridization methods can be used [4]–[6].

The introduction of new kinds to a new region of agriculture is a crucial breeding method. Some significant wheat cultivars that contributed to the. Sonsora 64 and Lerma Rojo, two well-known Green Revolution cultivars, came from Mexico. They were really created in Mexico, brought to India, and crossed with our indigenous kinds. Selecting and gathering many potential variations, sometimes known as collections, is the first stage in introducing a new variety. They may be introduced as a new variety on the basis of brief field tests, performance evaluations, selection of regionally appropriate types, and restricted field trials.All crop advances are based on selection, one of the oldest breeding strategies. Selection is the process of sorting out certain plants or groups of plants from a mixed population. Hybridization and hybrid assessment procedures are accompanied by mass selection and pure line selection techniques. Many of the new varieties are the result of artificial hybridization, which is then followed by selection for traits like disease resistance, high yield and quality, etc. The pedigree method, which keeps a thorough record of

parents and the selection criteria used in each generation, is a well-known technique. For crops that are cross-pollinated, mass selection, recurrent selection, and techniques for developing hybrid varieties and composite varieties are the most often employed breeding techniques. These techniques take use of the cross-pollinated crops' typical high heterozygosity levels. The two most significant crops that are cross-pollinated are sorghum and maize, and a variety of hybrid types have been made available.

The accessibility of plant genetic resources is crucial for developing novel selection and hybridization techniques. For each of the four plant categories under consideration (crops, their wild relatives, weeds, and wild plants), different techniques for conserving variety are most successful. In certain circumstances, combining many distinct tactics will work well. The techniques include in situ conservation, conservation and management on farms (monitoring and protecting agro-ecosystems), and ex situ conservation (administration of gene banks) (monitoring and protection of natural ecosystems). Combining morphological, agronomic, and molecular characterisation of genetic diversity is the most efficient strategy to evaluate genetic variety within a particular taxon.The demand for animal goods is rising, especially for export, and is expected to surpass that for plant products. There will be even greater pressure on the livestock industry to raise productivity since there is an increasing demand for milk and milk products. In addition to directly expanding animal production, meeting these needs would require efficient and comprehensive action in other domains (such as political, social, economic, and trade).

The existing global animal genetic resources (AnGR) are what we have to provide the current human population's need for animal products. They must be used as effectively as feasible. Any reduction in these resources will limit our potential for improving cattle in the now and the future.Hypertension and other phenotypic traits are not caused by a single "blood pressure" gene with several alleles (such as a 120/80 allele, a 100/70 allele, a 170/95 allele, etc.). A person's weight (one or more obesity genes), cholesterol level (one or more metabolism-controlling genes), renal function (salt transporter genes), smoking (a propensity for addiction), and possibly a lot more factors combine to produce this phenotype. Moreover, numerous alleles may exist for each of the contributing genes. Similar to this, the hue of a man's eyes results from the intricate interactions of two genes, each of which has a pair of incompletely dominant alleles. At least three separate genes with two or more alleles interact to determine skin colour.

Additive gene interactions and non-additive gene interactions, also known as epistasis, are the two categories into which these numerous gene interactions fall. An additive gene interaction is one in which each independent gene that influences a phenotypic equally contributes to the development of the characteristic. For instance, in wheat, the colour of the seed coat is controlled by two separate genes, each of which has its own allele.The ratio of the phenotypes obtained reveals the sort of gene interaction, or epistasis that has taken place. Epistasis is a non-additive gene interaction in which a gene effects another gene in various ways. Epistasis may refer to any kind of inhibition of a gene's action by a different, non-allelic gene. The following ratios for epistasis are some of the most typical ones.

Two genes work together to produce the trait 9:7.

One gene suppresses the expression of another gene 13:3, another gene enhances the impact of another gene 9:3:4, and a third gene masks the impact of another gene. 12:3:1.

Sex-Related Heritage

Famous scientist Thomas Hunt Morgan made it very evident that the sex chromosome was connected to a particular attribute, particularly the colour of one's eyes, which is white. Males' lack of an eye colour gene on the Y chromosome caused a skewed segregation in the progeny. As a result, a female with white eyes will have male offspring with white eyes, but one may anticipate 50% of female offspring with white eyes and 50% with red eyes. It is now widely accepted that the skewed segregation results from the close genetic relationships between the genes governing eye colour and sex determination on the X chromosome. This was the first study to demonstrate linked genes exist.

Human sex is determined by the activity of a pair of chromosomes called the sex chromosomes (the other 22 pairs are called autosomes). The phrase "sex chromosomes" or "X chromosomes" refers to those chromosomes that determine the sex of an organism. The cell's remaining chromosomes are all autosomes. X and Y chromosomes are the two different kinds of sex chromosomes. Femaleness is determined by homozygous X chromosomes, while maleness is caused by the combination of X and Y chromosomes. The female XX and the male XY are designated. We can anticipate that there is an equal chance that men and females will conceive by knowing the Mendelian principles.

There are a substantial number of genes on the X chromosome. The Y chromosome, however, has relatively few. As a result, genes on the X chromosomeoften referred to as "X-linked" genes—are in a special position. With autosomal genes (there are 22 pairs of autosomes in humans), females have two copies of each gene, as is typical. Men, on the other hand, only have one copy of each X-linked gene since they have one X chromosome. Males are thus referred to be hemizygous since they are unable to be homozygous or heterozygous (The condition of having only one allele of a pair.) As a result, males automatically express recessive alleles that are present in females (because there is no second allele to overshadow the recessive one). Males are exempt from the usual standards of dominance in this situation. Because of this, men are more likely than females to have X-linked recessive allele-related issues including haemophilia (inability of the blood to clot) and colour blindness.

A Different Nuclear Heritage

According to conventional wisdom, the nucleus of a eukaryotic cell contains the genome.The chromosomes in the cell's nucleus are the site of every genetic locus that has been mentioned up to this point. Yet, certain genes may also be found in the cytoplasm of organelles like mitochondria and chloroplasts. A circular DNA molecule found in these organelles resembles that seen in eukaryotic systems. Extra chromosomal inheritance or cytoplasmic inheritance refers to the traits they acquired. We have researched how fertilisation works. Just the nucleus of the male gamete penetrates the egg during fertilisation, leaving the cytoplasm outside. As a result, only the egg and not the male gamete contribute to the zygote's cytoplasm or cytoplasmic genes. As a result, maternal inheritance is another name for the additional chromosomal inheritance. There are now three recognised kinds of cytoplasmic inheritance:

1. The maternal role (egg cytoplasm influences the phenotype of the offspring)
2. Organelle heredity is. (mitochondria and chloroplasts)
3. Contaminated genetics (an infectious particle is transmitted during conjugation)

Cytoplasmic inheritance displays a few unique characteristics.

1. The ratios and lack of Mendelian segregation are common.
2. The persistence of traits through many generations.
3. Regulated by DNA in the mitochondria and chloroplasts.
4. Illustrates maternal inheritance since only female gametes may transfer these traits.

Alleles are various variations of a single gene. It indicates that genes located in the same region of the chromosome have slightly different base sequences. There are several genes involved in polygenic traits, etc., and each of these genes may have multiple alleles that influence a particular trait. This is far more complicated and may include a lot of chromosomes [7], [8].

DISCUSSION

Agriculture cattle breeding contributes significantly to feeding the world's population since it aims to produce food efficiently. Artificial insemination and embryo transfer, among other biotechnological techniques, have long been a crucial component of contemporary animal husbandry and have contributed to the widely acknowledged enhancements in the productivity of agricultural animals. Nevertheless, certain drawbacks could not be overcome by these methods, including the yearly rate of genetic development being between 1 and 3 percent, the inability to breed out unwanted features, and the inability to transmit genetic information across species [9], [10].It will be feasible to get over these breeding restrictions thanks to new technologies and innovative molecular-genetic tools, some of which are now accessible and others of which are in development.

The term "biotechnology in farm animals" nowadays essentially refers to reproductive and molecular biology methods designed to improve animal health, performance, and efficiency for sustainable animal production. The full sequencing of the genomes of significant domestic animals will soon allow for the differentiation of molecular phenotypes, improving the utilisation of genetic resources. Biotechnology and breakthrough genetic-molecular tools will be crucial resources for making animal production more effective, ecologically responsible, and commercially viable in light of the world's finite resources and growing population [11], [12].

CONCLUSION

Cloning and transgenic technology will pave the way for numerous new uses in agriculture, notably in the field of product diversification, as well as medical. Interdisciplinary research that promotes more reasoned and truthful social and ethical debate must go hand in hand with the development and use of biotechnology and genetic technologies in animal breeding.Since ancient times, breeding has been a fundamental technique used to improve desirable features and boost output in both animals and plants. This research article reviews recent studies on conventional and cutting-edge techniques, technological developments, and ethical issues in the breeding of animals and plants.

The numerous breeding techniques, such as inbreeding, outbreeding, and marker-assisted selection, are examined in the study, along with their uses in a variety of industries, including agriculture, medicine, and environmental science. The report also emphasises the significance of comprehending the safety and ethical concerns related to breeding, such as the possibility of unforeseen effects and the effect on biodiversity.

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CHAPTER 24

EXPLORING THE FUTURE OF GENETIC ENGINEERING

Harsh Mohanty, Assistant Professor
ISDI - School of Design & Innovation, ATLAS SkillTech University, Mumbai, India
Email Id- harsh.mohanty@atlasuniversity.edu.in

ABSTRACT:

In an endeavour to understand nature, physics and chemistry were combined to create the area of molecular biology. This study gained popularity in the 1940s postwar period. Studying the functions and mechanisms of biological macromolecules became easier with the development of nuclear physics, radioactivity, quantum mechanics, and X-ray crystallography. After the structure of DNA was discovered in 1953, efforts in molecular biology accelerated even more.The use of new tools and methods for enhancing crop yields, livestock output, and human health has revolutionised the discipline of biotechnology. By reviewing recent studies on the developments, uses, and ethical issues of this discipline, this research study evaluates the potential of genetic engineering. The numerous genetic engineering techniques, such as gene editing and synthetic biology, are examined in the study, as well as their uses in a variety of industries, including agriculture, medicine, and environmental science.

KEYWORDS:

Cells, Genetic Engineering, Crystallography, Industries, Technology.

INTRODUCTION

Another discipline emerged in the postwar period as a result of enhanced computing efforts brought on by the huge cryptography race that took place during the conflict. German engineers created Enigma during the close of World War I, which was used to encrypt German military documents. In truth, the father of modern computers is Alan Turing, a cryptanalyst who worked for the British code-breaking organisation during World War II. Turing's Turing machine, developed in 1936, is regarded as the first computer [1]–[3].So, it is conceivable to conclude that the two sister fields, which emerged during the same postwar period, worked together to grow and support one another.

The era of omics and bioinformatics

The foundation for a genome sequencing craze was already in place when Frederick Sanger created his DNA sequencing technique (see Appendix A). With the novel computational techniques created at this institution, The Institute for Genetic Research (TIGR) established the first genome sequence of an organism, Haemophilus influenza. A European collaboration was established in 1989 to sequence the yeast genome, while the Human Genome Project was introduced in 1990. J.C. Venter, a businessman and scientist, deserves attention since his team at TIGR and Celera Genomics, which he subsequently formed, created the shot-gun sequencing technology that significantly increased the pace of these genome sequencing initiatives [4]–[6].

The issue with genome sequencing projects was that it required a global effort involving thousands of labs and researchers to even attempt the sequencing of the human genome due to

the extremely slow DNA sequencing technology (especially prior to shotgun sequencing and automated sequencing approaches). The second issue, however, was primarily about data storage, analysis, and manipulation, as well as global access, in addition to data collecting.

To archive protein X-ray crystallography data, the Protein Data Bank (PDB) was established in 1972; in contrast, it took more than ten years to construct the Swiss-Prot protein sequence database. Both the Human Genome Organization (HUGO) and the National Center for Biotechnology Information (NCBI) were established in 1988. The European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Data Library, which was founded in 1980, served as the inspiration for the parallel European initiative for a nucleotide sequence database, the so-called European Bioinformatics Institute (EBI).

Model organisms were sequentially sequenced, ranging from Saccharomyces to Arabidopsis, from rice to mice, and the genome sequencing techniques, today known as genomics, quickly grew enormously. Sequence alignments, comparisons, analysis, the creation of tens of thousands of unique online tools, as well as the development of new and more specialised avenues like comparative genomics, functional genomics, structural genomics, pharmacogenomics, metagenomics, and so forth, were all part of the enormous task that was the analysis of this data. Whole transcriptomes quickly gained popularity as a result of advancements in microarray technology that enabled worldwide gene expression analysis. Indeed, genes were crucial, but they were not always expressed or not always at the same level in all cell types. Transcriptomics became the hottest trend in molecular biology as a result. It quickly became evident that just because a gene is transcribed, it does not always indicate that the protein product is created; mRNAs may be destroyed before being translated or translation may be slowed down in some other way. Proteomics, also known as whole proteome profiling, gained popularity as a result. All these omics also resulted in an exponential rise in the volume of data produced, which increased the amount of bioinformatics data and necessitated the development of new analytic tools. The cycle of research life went like that. Nowadays, additional omics are also popular, creating a mountain of data that is always rising, ranging from lipidomics to metabolomics, all of which should be merged in some way to provide valuable and educational information about the molecular processes of cells.

The San Diego Supercomputer Center (SDSC) maintains the Biology Workbench analysis environment, which is an all-in-one and simple-to-use platform for nucleotide and protein sequence data analysis. It includes restriction enzyme analysis, protein structure predictions, in silico translations, sequence alignments, and much more (Subramaniam 1998; Biology Workbench at http://workbenc.org). However, owing to an apparent lack of financing, it operates quite slowly and new, better versions are not presently being issued. Access is free, and only registration is needed for data upkeep. A comprehensive analytic tool for beginning students, Biology Workbench continues to include a wide range of sequence analyses. Yet, more user-friendly and visually appealing analytic tools are continually being created, particularly with the gathering of the vast amounts of data from high-throughput investigations (Gehlenborg et al. 2010). In order to provide biologically relevant information, these technologies primarily evaluate microarray, transcriptomics, and proteomics data and produce protein interaction networks, pathway studies, and other techniques. Systems biology is an inter- or rather cross-disciplinary field that integrates data from all other omics approaches to present an integrated and interacting network of molecules, genes, proteins, and pathways. It also studies system dynamics and the system as a whole, rather than focusing on a few molecules through a keyhole.

With this goal in mind, the Institute for Systems Biology (ISB, http://www.systemsbiology.org) was established in 2000. Over 15 years later, this organisation has had a significant effect via research on a variety of topics, including the environment, the brain, and customised media. The Systems Biology Institute of Japan, formed in 2000 (http://sbi.jp), is the organisation that funds http://systems-biology.org, a Web site for systems biology researchers. There are live systems but no such thing as living "stuff." No substance, not even a single isolated molecule from a living thing, possesses the aforementioned paradoxical characteristics on its own. They only exist in living systems, which means they don't exist below the level of a cell [7]–[9].

DISCUSSION

Artificial biology and synthetic amino acids

The synthetic biology community site defines synthetic biology as (A) the design and fabrication of novel biological components, devices, and systems, and (B) the redesign of existing, natural biological systems for practical uses. In order to use biological systems as, in a sense, a "chassis" and molecules as "Lego pieces," rearranging these pieces to come up with new uses that will (hopefully) benefit society, synthetic biology is a relatively new interdisciplinary and transdisciplinary field that combines biology, physics, bioinformatics, engineering, and many other fields. New biological system designs compete each year in the International Genetically Engineered Machine (iGEM) competition, which was founded by MIT researchers in 2003 and can be found at http://igem.org (especially noteworthy is the iGEM high school jambo- ree). The Registry of Standard Biological Parts, originally the BioBrick, is a standardised platform of parts, devices, and systems for simple assembly of synthetic systems. It was developed by the same MIT team and is freely available (for example, the BioBrick® Assembly Kit at http://www.neb.com/products/e0546-biobrick-assembly- kit). It should be mentioned that the previously described CRISPR/Cas technology for genome editing was also created by the MIT Center for Integrative Synthetic Biology.

Using synthetic biology, novel protein designs, synthetic biosensor systems, synthetic metabolic or genetic circuits, and several more applications have been created. Using such a synthetic biology method, a recombinase-based platform for logic and memory functions was developed for the long-term and stable preservation of cell memory via an integrated circuit comprising AND and OR gates (Siuti et al. 2014). The development of a synthetic biology toolbox for yeast as a biofactory (Redden, Morse, and Alper 2014) could be expected to aid in the creation of new synthetic circuits for the production of highly valuable yeast metabolites that are otherwise difficult to obtain in large quantities biochemically. In order to produce therapeutic proteins or economically significant metabolites, higher eukaryotic systems, such as plants and animals, may also be engineered via synthetic biology (Wilson, Cummings, and Roberts 2014; Ye and Fussenegger 2014).

The extension of the genetic code using synthetic amino acids, however, was perhaps one of the most fascinating advances in synthetic biology (where the author admits to being personally a little subjective here). By creating bacteria, yeasts, and mammalian neurons with enlarged genetic codes, Schultz Laboratories has been a leader in the area. However, some organisms, such as bacteria, archaea, or yeast, may occasionally use one of the stop codons to code for a 21st (or occasionally a 22nd) "unnatural" amino acid, such as a selenocysteine (Sec) or a pyrrolysine (Pyl), using either an SECIS (Sec insertion sequence) or PYLIS (Pyl insertion sequence) element. The Standard Genetic Code (Yuan et al. 2010; Zhang et al. 2005). There are

only 25 selenoproteins in humans. The Schultz lab began with this, and they originally modified the bacterial translation machinery such that one of the stop codons is reprogrammed to include additional synthetic amino acids (Wang and Schultz 2002). The Schultz group subsequently worked on or participated in a variety of synthetic biology initiatives employing artificial amino acids, such as protein evolution, where they demonstrated how synthetic codes may give antibody proteins a selection advantage (Liu et al. 2008). Also, 138 synthetic amino acids were examined, and it was shown that the majority of glutamine and glutamic acid analogues were taken up by yeast, resulting in the extension of the genetic code in this eukaryotic model organism (Liu and Schultz 1999). Five non-natural amino acids were effectively inserted into the yeast TAG codon, allowing photocross-linking for investigations of protein interaction due to the keto group on these non-natural amino acids.Moreover, human growth hormone (hGH) was engineered synthetically to include the artificial amino acid p-acetylphenylalanine (pAcF), with clinical performance similar to wild-type (Cho et al. 2011).

Optogenetics

The genetic circuit design horizons have been broadened by synthetic biology, which has also increased the toolset of amino acids and nucleotides (Bacchus et al. 2013). As a result, the scientific environment has been favourable for the emergence and development of the optogenetics discipline.The Deisseroth Laboratory was the original home of optogenetics, which was selected as the 2010 Technique of the Year. You may access the Karl Deisseroth Laboratory's official resource.

In actuality, this technology is a stunning and graceful illustration of how huge ideas truly don't need an exceptional brain per se, but rather the ability to recognise the previously unobserved. Francis Crick first suggested in the late 1970s that light may be used to precisely regulate neural activity in certain kinds of brain cells, but it wasn't until 2002 that rhodopsins were used to genetically target particular neurons in Drosophila to produce light-sensitive responses (Zemelman et al. 2002; Zemelman et al. 2003). This same group, whose neurons were genetically engineered to contain photosensitive channels, reported the first observation of light-induced modulation of Drosophila behaviour, including wing beating, flying, and other behaviours. When used with additional model species like Caenorhabditis elegans and mice, the system was swiftly modified. The primary light-sensitive proteins of microorganisms used in optogenetics are channelrhodopsin, halorhodopsin, and archaerhodopsin. Halorhodopsin was found in the late 1970s, while bacteriorhodopsins have been known since the 1970s. Yet the discovery of channelrhodopsin in the early 2000s was what really made optogenetics possible (Deisseroth 2011). Depending on the modified type being utilised, these light-sensitive channels may be made to open or shut in response to various wavelengths, which causes electrical stimulation or inhibition of the relevant neuron.

The only other elements of optogenetics required are the optical stimulation (through integrated fiberoptic and solid-state light sources for application in freely moving animals, typically directly mounted to the animal's skull) and typically another genetically knocked-in gene (Deisseroth 2011; Gautier et al. 2014; Knopfel et al. 2010).A wide variety of optogenetic reporters have been developed to study live-cell imaging of signalling and metabolism. Whereas the first generation of optogenetic reporters included calcium reporters and neural activators, the second generation has evolved to include voltage report- ers, as discussed previously, neural silencers, and many other reporters (Alford et al. 2013; Knopfel et al. 2010). Optogenetics has also been applied to

tetracycline-inducible systems, improving the amount of control over which neurons are triggered and when (Tanaka et al. 2012). In behavioural and molecular brain research, especially in the fields of learning and memory, optogenetics has therefore greatly increased in popularity (rev. in Gautier et al. 2014 and Goshen 2014). For individuals who need vision restoration, optogenetic methods for retinal prostheses have been investigated (Barrett et al. 2014). They have also been developed for use in primates, indicating potential therapeutic uses for humans (Gerits and Vanduffel 2013).

While it occupies a sizable niche in fundamental neuroscience, optogenetics is not exclusive to this area. Heart optogenetics attempts to effectively cure rhythm abnormalities and optically manipulate bioelectricity (Boyle, Entcheva, and Trayanova 2014). Though electrically nonexcitable cells are also being investigated as potential new optogenetic targets, similar applications are also possible in glial cells and skeletal muscle cells. All of the aforementioned cells share the trait of electrical excitability, making the use of light-sensitive ion channels suitable for precise and cell-specific regulation of function (Tanaka et al. 2012). Additionally, click chemistry-based novel post-translational modifications are made possible by the integration of optogenetics with unnatural amino acid incorporation and phenyl azide chemistry (Reddington et al. 2013), and one might anticipate hearing more from such integrated approaches in the future.What is ahead for genetic engineering is impossible to predict. But, it is becoming more and more obvious that new technologies will evolve far more quickly than one can interpret the data produced by such technologies, and that the only limit to research is human creativity [10]–[12].

CONCLUSION

Nonetheless, it is clear that nature's many marvels, best demonstrated by optogenetics, continue to fascinate scientists. The most recent advancements in neuroscience's connectomics field seem to be the next major development. Therefore, as a conclusion, undergraduate readers of this book should not only concentrate on their current projects, but also keep reading and looking for uncharted territory, possibly neglecting or studying previously unexplored avenues, and finding a way to incorporate them into modern technologies. The report also emphasises the significance of comprehending the moral and security concerns related to genetic engineering, such as the risk of unintended consequences and the effect on biodiversity. The impact of genetic engineering on society and its potential to improve organisms' capabilities are also covered in this essay. The results of this study indicate that genetic engineering is an important tool for expanding our understanding of cellular and molecular biology and creating new approaches for medicine, biotechnology, and environmental science, but it must be handled responsibly and ethically. The future of genetic engineering and the possibility for fresh developments and uses in the field are covered in the paper's conclusion.

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