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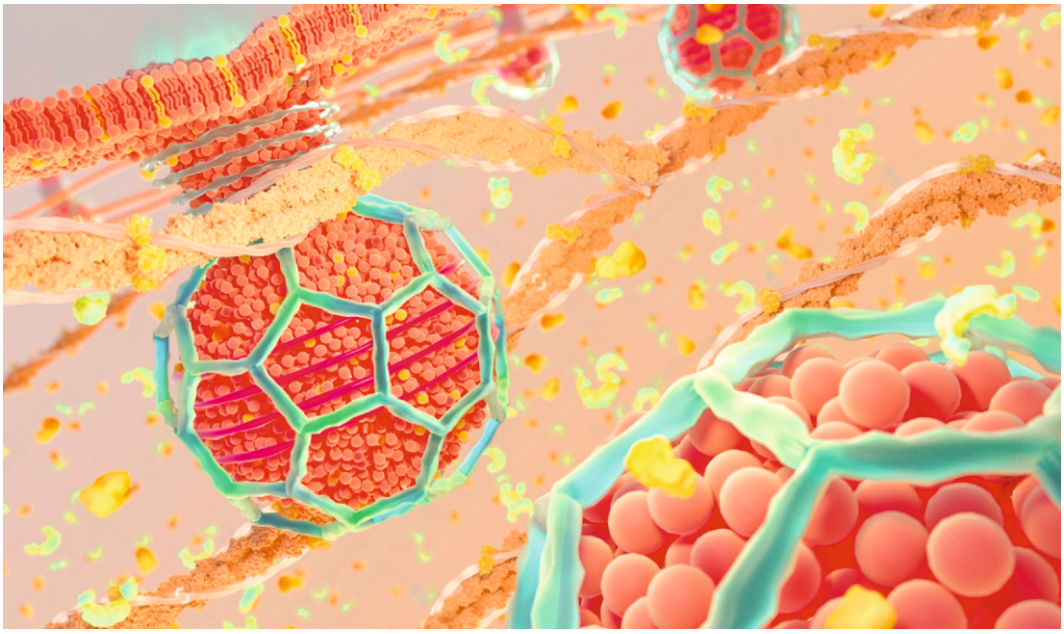

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CONTENTS

CHAPTER 1

ACTIN A CENTRAL PLAYER IN CELL SHAPE AND MOVEMENT

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ABSTRACT:

Actin is also one of the eukaryotes' most common, ubiquitous, structurally and functionally conserved proteins. The ubiquitous role of actin in cell shape and motility is due to self-assembly. Cells are mechanically supported and propelled by strands made of the protein actin. Actin is involved in many biological systems, including the cell's division and the ability to sense external pressures, internalize cellular vesicles, and move across surfaces. These intricate cellular processes rely on relationships between numerous other proteins and actin monomers and filaments. This chapter provides a summary of the most important issues in the area and makes recommendations for potential solutions. Determining the involved molecules and their molecular processes will be necessary to comprehend actin-based biological phenomena.

KEYWORDS:

Actin-filaments, ARP2/3 complex,Animal cells, Eukarytoics cells, Myosin motor.

INTRODUCTION

From a common ancestor that existed about 3 billion years ago, life on earth developed through divergent evolution. Its 400 genes included the actin ancestor gene. Actin and its bacterial equivalents polymerize into filaments that have numerous benefits for cells [1]. Actin is one of the most common proteins in the world because of its high cellular concentrations. Actin filaments provide internal mechanical support, track the movement of intracellular materials, and power to propel cell movement, making it crucial for the survival of the majority of cells[1]. Actin relatives are used by many contemporary prokaryotic species to preserve asymmetrical forms and transport DNA through the cytoplasm. Most eukaryotes have genes for myosin motor proteins, which exert pressures on actin filaments, and nearly all eukaryotes have genes for actin [2]. In mammals, actin filaments work in conjunction with intermediate filaments and microtubules, two other cytoskeletal polymers.In muscle, where they make up highly regular groups of filaments that account for more than half of the total protein, actin, and myosin were first identified in the 1940s.

An early understanding of actin assembly and function in all cells, including the process by which myosin produces force and movement from ATP hydrolysis, was established by research on muscle[3]. Actin and myosin were found in other cells 20 years later, demonstrating that muscle filaments are a specific type of cellular structure. Many proteins that control actin have since been discovered, their methods of action have been examined, and the proteins have been connected to cellular functions.Under physiological circumstances, actin monomers spontaneously polymerize to form long, stable filaments with a helical arrangement of subunits [4]. Small oligomers are very unstable, so polymerization begins slowly. However, once strands have been formed, actin polymerizes quickly and almost entirely. Actin filaments are polar because their components all face the same way. The filament develops much more quickly on one end than the other. Soon after assembling

into filaments, actin hydrolyzes the bound ATP's terminal phosphate and gently dissociates the phosphate. Actin binds an adenine nucleotide (ATP or ADP). These chemical processes cause minor alterations in the actin subunit structure that set up ADP-actin filaments for disassembly by regulatory proteins[4]. More than 100 accessory proteins are used by eukaryotic cells to keep a supply of actin monomers, start polymerization, shorten actin filaments, control their assembly and turnover, and crosslink filaments into networks or bundles. A branch can develop on the side of an existing filament, a filament can be severed to form two new ends, or a filament can be created from scratch using monomers as the starting material. Since the genes for the majority of these auxiliary proteins were already present when the top branches of the phylogenetic tree developed roughly 1 billion years ago, amoebas, fungi, and animals share many molecular mechanisms that control their actin systems. Several actin-binding proteins, including myosin, are not present in some organisms, including the intestinal parasite Giardia. These animals might have diverged before the emergence of these genes, or they might have lost these genes, similar to how plants lost more than 200 genes necessary for the cilia and flagella assembly[5].Actin filament polymerization powers the eukaryotic cells' crawling locomotion, which is a defining trait of amoebas and animal cells. To regulate the composition of the cell membrane and the cell's interface with the world, actin polymerization also helps membrane vesicles internalize. There are two kinds of movements produced by myosin motor protein interactions with actin filaments. First, myosin exerts a force between actin filaments, causing contractions that pinch dividing cells in half, pull up the rear of moving cells, and alter cellular shapes to create tissues. Muscle cells are contracted by a similar process[6]. Second, these cargos are moved over short lengths along actin filaments by myosins linked to subcellular organelles and macromolecular complexes of proteins and RNA. Actin filament tracks distribute nearly all of the organelles and secretory vesicles to progeny cells before cell division in budding yeast cells, which are small. Many other cell types use microtubules and their motors to move cargo over greater distances.To understand how the actin system or any biological system functions, it must first have a full inventory of its components. Understand how every component of the actin system communicates with each other[6].

LITERATURE REVIEW

Certainly intricate, cell locomotion requires the concerted action of the cytoskeletal, membrane, and adhesion systems. Actin filaments themselves are probably engaged in a variety of mechanisms that produce forces. We need to break down locomotion into different kinds of motility to start a molecular analysis. depicts a typical spatial/mechanical breakdown for a single cell traveling over a two-dimensional substrate[6]. A protrusion is a term used to describe the forward motion of the cell's front membrane. It has received a great deal of attention in recent years and is likely the aspect of locomotion for which we are most close to understanding the molecular foundation of force production. The protrusion cannot become moving along the substrate without adhesion[6]. Traction is the word used to describe the mechanism that causes the nucleus and cell body to advance. This type of motility, which is probably the most crucial for producing overall locomotion, is also the least understood from a mechanical or molecular perspective. Deadhesion and tail retraction are the two mechanistically separate processes that make up the final stage of the movement. Depending on the cell type, this process may or may not be highly motile. Deadhesion develops because the axon is less tightly bound to the substrate than the front of the growth cones in neurons, which lack tail retraction and spin out an axon as they travel. Strongly adhering cells, like cultured fibroblasts, typically have an extended tail that is firmly adherent and leaves a path of cytoplasmic fragments as they move. The movement rate in these cells may be slowed down by deadhesion or tail retraction. The tail is more rounded and this process is more

effective in cells with weak adhesive and rapid movement, such as amoebae and white blood cells[7]. At the leading edge of motile cells, protrusive structures with dense arrays of actin filaments are extremely dynamic. Generally speaking, these filaments are arranged so that their barbed ends (fast-growing, or plus ends) are oriented preferentially in the direction of protrusion where research has been feasible [7]. Filopodia are narrow cylinders that can protrude tens of microns from the main cortex and are the simplest protrusive structures. Long actin strands that are tightly bunched together and pointed in the protrusion direction make up filopodia. Cross-linking proteins like fimbrin keep the strands in the bundle together [8]. Although they are found in many different kinds of motile cells, filopodia have primarily been studied in neuronal growthcones. The leading margins of cultured fibroblasts and many other motile cells are dominated by lamellipodia, which are thin protruding sheets. Lamellipodia that lift off the substrate and advance give fibroblast leading edges their distinctive ruffled look. Actin filaments are organized into a web that forms lamellipodia as an orthogonal cross-weave between two groups of filaments that are oriented at roughly a 45° angle to the direction of the protrusion [8]. Lamellipodia are punctuated by rib-like microspikes that mimic short filopodia in many different cell types. In microspikes, the orthogonal strands condense into a compact bundle. Amoeboid cells frequently extend through thicker structures known as pseudopods. Due to issues with maintaining their organization during fixation, it has been challenging to investigate the organization of actin in pseudopods. Their cortex is believed to be predominated by a cross-linked mesh of actin filaments that has a less polarised organization and may resemble the organization found in thicker lamellipodia of specific kinds of growth cones[9].

However, protruding areas of both of these cell types have filaments with their barbed ends facing forward, primarily on the ventral surface of growth cones, and as far as we can tell from the information provided, thin tips protruding along the ventral surface of amoeba.The two types of proposed processes for producing protrusive force are those that rely on motor proteins to propel protrusion and those that use actin polymerization to generate force. Energy is needed for force production, and in the cytoplasm, this energy must eventually come from the chemical energy of nucleotide hydrolysis[10]. In motor-based models, a myosin-like barbed end-directed motor converts hydrolysis energy straight into force, moving the membrane tip forward. In protrusive structures, where they may serve as the motor, certain myosin I isoforms are more abundant, and kinetic analysis of filopodial protrusion has been argued to support a motor-driven paradigm[10].The polarity of actin filaments in protrusive structures is consistent with the notion that polymerization alone could propel a membrane forward, though it may be less intuitive than using a motor protein. But there is a tonne of data that supports this model. Pure actin polymerization within a lipid vesicle can distort the membrane, and other protein polymerization processes, such as tubulin polymerization can also result in membrane-deforming force[11].

The mechanical characteristics and shapes of cells, which are frequently essential to their activities, are a result of the protein polymers that make up the cytoskeleton. A ghostly meshwork of cytoskeletal polymers is left behind after the membranes of a human cell are broken down to liberate soluble components. Actin filaments, microtubules, and intermediate filaments are among the polymers, which come in different ratios and shapes. Amoeboid and animal cell movement and mechanical structure are provided by actin filaments(Figure.1A-D). All eukaryotes rely on microtubules for the long-distance movement of large particles and the division of chromosomes. In vertebrates, intermediate filaments serve as intracellular tendons and ligaments to withstand mechanical pressures[12].Although some crosslinking proteins exchange quickly and the three cytoskeletal polymers themselves flip over on time scales of seconds to minutes, interactions among the three polymers strengthen the

cytoskeleton. These characteristics endow the cytoplasm with beneficial qualities, such as the ability to be malleable when deformed slowly and stiff when deformed quickly. Despite having a cell membrane surrounding them, even the cells of plants and fungi use cytoskeletal polymers to control the shape of their compartments[12]. The cytoskeleton is also a component of a system that detects the mechanical characteristics of the environment around the cell as well as external forces acting on the cell. This system can affect a variety of cellular processes, including gene translation and differentiation [12].

In budding and fission yeast, actin filaments form at sites of plasma membrane internalization. In these "actin patches," filaments assemble from scratch, exert force on the plasma membrane to create and internalize an endocytic vesicle, and then disassemble in a process that is self-limited in time and space. Even though between 30 and 50 participating proteins have been found using sophisticated molecular and genetic tools, the parts inventory still seems to be lacking. In addition to yeast, many other cells also link actin with endocytosis, and a similar group of molecular players is involved [13]. The spontaneous assembly of membrane proteins, including clathrin and adaptor proteins, is the first step in the process of endocytosis at numerous independent locations. Proteins from the WASp family and specific class-I myosins that bind to and/or trigger the Arp 2/3 complex, which forms new filaments as branches on older filaments, are the next to be recruited. It is still unknown where the initial strands came from. Actin filaments are capped by a protein, and among other proteins, fimbrin links the edges of the filaments together.When specialized proteins interact with a membrane, they can cause curvature, even though the assembly of such a network of filaments by itself can generate force adequate to deform a membrane. As the endocytic vesicle enters the cytoplasm, the density of actin filaments quickly decreases. This process relies on the filament-severing protein cofilin and may be helped by the proteins Aip1 and coronin. Even though actin patches are one of the best-characterized actin systems, our knowledge of these reactions is still restricted, and some seemingly incongruous observations show how little we do[14].

Some bacteria that invade eukaryotic cells use cellular proteins as their own to construct a comet tail of actin filaments that allows them to move through the cytoplasm. The Arp2/3 complex is enlisted by nucleation-promoting proteins on the bacterium's surface to polymerize actin strands (Figure.1E). The expansion of those strands propels the bacterium. The entire procedure can be recreated using the bacterial nucleation-promoting protein on a bead or lipid vesicle surface in a solution with pure actin, profilin, Arp2/3 complex, a capping protein, and the severing protein cofilin , and computer-simulated[15].Animal cells are distinguished by the presence of actin fibers, which are necessary for cell movement. Immune system cells may move, for instance, to look for and eliminate pathogens or cancerous cells. Some cells in animal embryos move around the body as they grow by squeezing between adjacent cells and through the extracellular matrix. Similar processes are used by cancer cells to spread throughout the body (Figure 1F).

Amazing instances of both cell migration and cell process extension can be found in nerve cells. The human brain has 1.5 million km of these cellular processes, nerve cells grow processes up to 1 meter long to locate their targets, and during development, neurons destined to control the intestine migrate over long distances as neural crest cells. Given that formin is known to produce long, unbranched actin filaments in thin projections known as filopodia, they are obvious candidates to assist in this network remodeling. It has been challenging to determine the respective contributions of formins and Arp2/3 complex to motility, in part because the Arp2/3 complex is crucial for viability and active at very low concentrations (Figure.1E). Only lately have specific pharmacologic inhibitors for the Arp2/3 complex been

made available(Figure.2B). Myosin motor proteins function as structural components with actin filaments during cell locomotion to pull the cell up from the back[1].

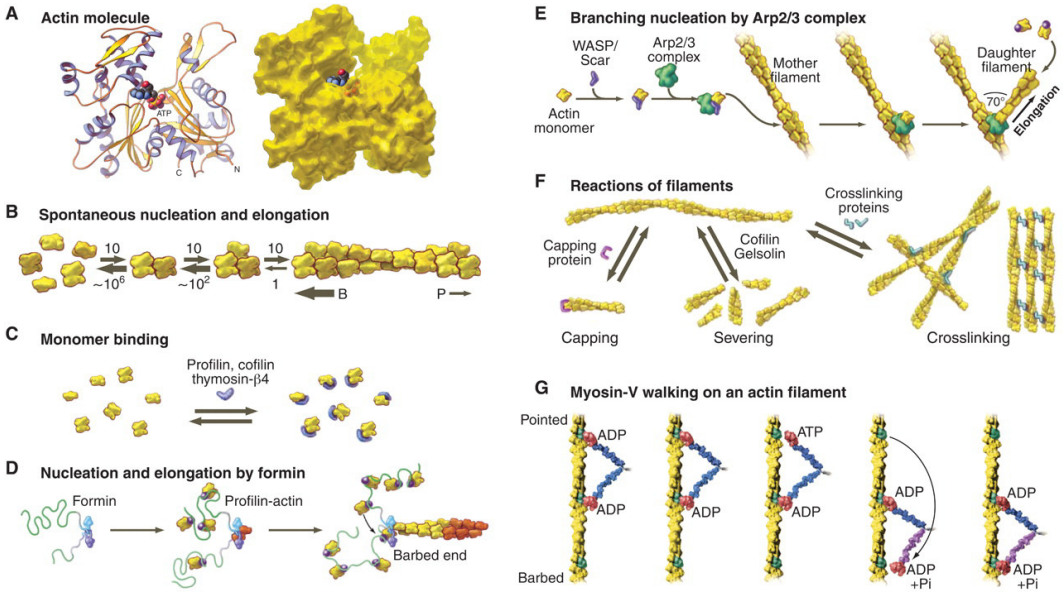

Figure 1: Structure and function of actin: Diagram showing the ribbon structure of the actin molecule. Actin dynamics regulation by the different proteins is also shown here(Science).

The final stage of the cell cycle is the physical division of two progeny cells. Animals, fungi, and amoebae can squeeze themselves in half by contracting a ring made of actin filaments and myosin-II. By drawing actin filaments together, myosin-II polymerizes to form bipolar filaments, which can cause a contraction.

The contractile ring machinery in specialized cells that gave rise to muscle was adopted by multicellular animals. Myosin-II is absent in organisms on the opposite branch of the tree, such as algae, plants, and ciliates, so cytokinesis is mainly dependent on membrane fusion in plants or unknown mechanisms.

Surprisingly, prokaryotes construct a ring of filaments that pinches these cells in half using a protein related to the microtubule subunit tubulin, much like a contractile ring but without the apparent involvement of a motor protein [1]. The correct positioning of the cleavage furrow, assembly, contraction, disassembly of the contractile ring, and fusion of the plasma membrane between the progeny cells are all necessary for successful cytokinesis (Figure.2D). The mitotic spindle, which is where the chromosomes originally congregate, contains information that is used to determine where the cleavage furrow should be located in animal cells (Figure.1E). Rho-GTPases, active signaling proteins, indicate the cleavage site near the equator. Myosin-II and the formin protein, which initiates the development of actin filaments, are accumulated in nodes. Computer models demonstrated the viability of one ring-assembly theory: myosin molecules capture actin filaments randomly growing from nearby nodes and draw the nodes together into a ring over ten minutes. Other cells' contractile ring assembly is less well-known[1]. The contractile ring contracts after the mitotic machinery divide the two daughter nuclei, forcing the cell membrane into a cleavage furrow. Surprisingly, the contractile ring's proteins spread apart as it contracts. The membranes of the two daughter cells are resolved during the membrane(Figure 2D).

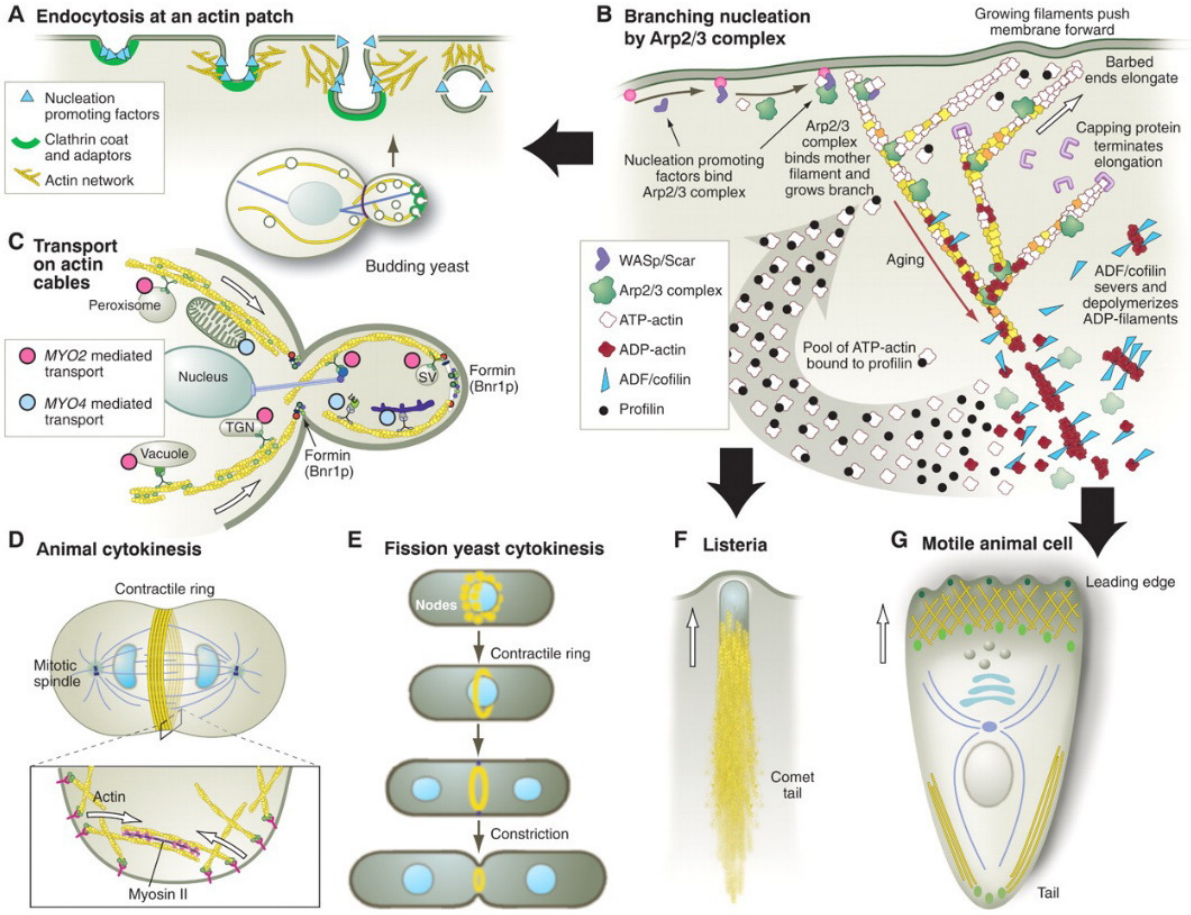

Figure 2: Role of the actin inside the cell: Actin-based movement inside the cell and the different functions of cells mediated by actin molecule (science).

Myosin motors are used by many, if not all, eukaryotic cells to move organelles along actin filaments. By controlling the secretion of cell-wall components to grow a bud from a specific spot on the plasma membrane of a mother cell, budding yeast replicate (Figure. 2A). Also play important role in the leading cell in the movement(Figure. 2G). Molecular polarity cues trigger formins to initiate actin filaments at a specified bud site. Each rapidly expanding barbed end still has formin attached to it to encourage lengthening (at 200 subunits per second) and avoid capping (Figure.2C).

Bundles made of evenly polarised filaments act as organelle movement tracks. Class-V myosin moves intracellular organelles and secretory vesicles to the bud by walking towards the barb-like extremities of these filaments (Figure.1G). Actin strands are stabilized by tropomyosin, which also has the potential to affect how the myosin motor functions. To affect the fitness and destiny of cells, myosin-V also transports specific mRNAs into the daughter along cables[1].Both plant cells and fission yeast rely on formins to construct uniformly polarised actin filament cables as tracks for the transportation of growth-supporting materials. Microtubules play a major role in long-range movements in animal cells and elongated fungal hyphae, and actin filaments don't seem to be arranged into cables of consistent polarity. However, to move organelles over small distances along the actin filaments, myosins collaborate with microtubule motors[1].

CONCLUSION

The actin system's genes share a common ancestor, so evolution should be able to help unravel the intricate processes. It should be possible to identify the underlying molecular mechanisms for each actin-based function as well as more general principles with continued emphasis on tractable model systems. The list of components is continually expanding, and novel interactions are being discovered through research using systems-level genomics methods based on genetic and physical interactions in model organisms. These developments should be essential for advancing our knowledge of these actin systems, particularly about how and where filaments are produced and put together into networks with different geometries.

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CHAPTER 2

COLLABORATION BETWEEN ACTIN AND MICROTUBULES IN CELL FUNCTION

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ABSTRACT:

Since the cytoskeleton and its constituents, actin, microtubules, and intermediate filaments, have been extensively studied, it is now known that each of these substructures plays a variety of functions within the cytoskeleton. The three cytoskeletal components do, however, participate in extensive crosstalk that is critical for fundamental biological processes, as has become clear in recent years. The establishment of neuronal and epithelial cell form and function, as well as the control of cell shape and polarity during cell migration and division, depend on actin-microtubule crosstalk. Different cytoskeletal regulators are involved in this crosstalk, which also includes diverse physical interactions like crosslinking, anchoring, and mechanical support. Therefore, the cytoskeleton should be viewed as a unified system in which subcomponents co-regulate each other to exercise their functions in a precise and highly adaptable way rather than as a collection of individual parts.

KEYWORDS:

Actin-Microtubule, Cross-Talk, Cell Function, Cell-Division, Cell Dynamics.

INTRODUCTION

The cytoskeleton, a filamentous scaffold of proteins that permeates the cytoplasm and stretches from the plasma membrane to the nucleus, is essential for a large number of cellular processes [1]. Microtubules, actin filaments, and intermediate filaments are the three filamentous components that make up the cytoskeleton in animal cells. The cytoplasm's internal structure and force-resistance stabilization are functions of all three subsystems. Microtubules and actin filaments also actively produce pressures to propel cell motility and shape changes. They are also significantly more dynamic than intermediate filaments, and these dynamic qualities play crucial roles in a variety of biochemical processes that take place within cells [2]. In the presence of GTP, tubulin dimers self-assemble into microtubules. They can switch between growing and shrinking phases, a process known as dynamic instability1, to create reasonably stiff hollow tubes made up of 13 protofilaments.

Many cells have an astral arrangement with microtubule plus end radiating outward to the cell periphery because microtubules are attached through their minus end to an organizing structure like the centrosome. Chromosome segregation during cell division, the movement and distribution of various cargoes, such as intracellular vesicles and organelles, and the preservation of polarity in migrating cells and epithelial tissues all depend on a properly functioning microtubule cytoskeleton [3].In the presence of ATP, actin monomers polymerize into double-helical threads. Microtubules are thicker and more rigid than actin strands. To carry out their role in cells, dynamic actin filaments are frequently crosslinked into bundles or

networks. The actin cytoskeleton promotes cytokinesis at the end of cell division, strengthens the membrane at the cell cortex, and is crucial for cell movement [4].

Actin and microtubule cytoskeletons' cell biology and biophysics have both been widely studied in recent decades, yielding a fairly comprehensive description of their behavior and regulation in a wide range of cellular contexts. But it is becoming more and more obvious that the two cytoskeletal systems frequently cooperate in fundamental cellular processes and that their functional and dynamic characteristics are frequently closely linked [5]. With an emphasis on the role of physical interactions mediated by associated proteins or protein complexes, we review new findings regarding the mechanisms underlying functional crosstalk between microtubules and actin in this article. Recent research demonstrates how various crosstalk mechanisms interact to regulate cell motility, shape, and polarity in neurons and epithelial cells, and cell division. Finally, we describe how additional research may fill in the knowledge gaps concerning functional actin-microtubule interactions [5].There are several distinct methods in which the actin and microtubule cytoskeletons may "talk" to one another. Although context-specific molecular actors are frequently involved, it seems that crosstalk between actin and microtubules can be reduced to a small number of (physical) mechanisms that, with minor variations, are present in a variety of distinct cellular contexts.

These mechanisms include shared regulators that affect the dynamic properties of both systems, interactions mediated by molecular components that provide direct physical crosslinks or regulate the dynamic behavior of cytoskeletal filaments, as well as more indirect mechanisms based on the mechanical effects of one cytoskeletal system on the other [5]. The functional significance of actin-microtubule crosstalk in the setting of various cellular processes is discussed in the sections that follow. In each instance, we list the particular molecular players involved along with the known or hypothesized roles of the various means of interaction discussed above. It should be emphasized that in living cells as opposed to isolated in vitro systems, direct evidence for the various means of interaction is inherently more challenging to acquire.

This discrepancy results from the challenge of co-imaging the actin cytoskeleton and microtubules at adequate spatial resolution. Additionally, numerous molecular players with a variety of different roles are frequently engaged in actin-microtubule interactions at the same time[6]. Therefore, altering them could have pleiotropic impacts on cells. Thus, the best kind of evidence in cells is sometimes dependent on the localization of substances that are known to be able to mediate actin-microtubule crosstalk [6]. Major cellular rearrangements that support morphological characteristics promoting faithful segregation of the genetic material and proper positioning of the daughter cells within the tissue support mitotic progression. From mitotic entry to cytokinesis, the actin and microtubule (MT) cytoskeleton, cell-cell adhesion, and membrane dynamics are precisely synchronized in space and time. This part covered the interplay between actin and microtubules in maintaining cellular structure.

LITERATURE REVIEW

The function of a cell is inextricably linked to its shape, which differs greatly in nature. Cellular branching processes, which can be multicellular or develop within a single cell, are essential for tissue and organ morphogenesis. This so-called single-cell or subcellular branching converts an originally relatively symmetrical unbranched cell into an elaborately branched structure through extensive cellular remodeling [5]. These branched cells can span

very large areas and perform their intended role thanks to the widespread cytoskeletal changes and cell membrane growth that cause these cellular remodeling events. Despite this obvious connection between appearance and function, little is known about the signaling processes that cause the development of these subcellular branches or what causes them to choose a specific path through the cell's cytoplasm [5]. Subcellular lumen formation by single cells involves complex cytoskeletal remodeling. We have previously shown that centrosomes are key players in the initiation of subcellular lumen formation in *Drosophila melanogaster*, but not much is known about what leads to the growth of these subcellular luminal branches or makes them progress through a particular trajectory within the cytoplasm [7]. Here, we have identified that the spectraplakin Short-stop (Shot) promotes the crosstalk between MTs and actin, which leads to the extension and guidance of the subcellular lumen within the tracheal terminal cell (TC) cytoplasm. Shot is enriched in cells undergoing the initial steps of subcellular branching as a direct response to FGF signaling. An excess of Shot induces ectopic acentrosomal luminal branching points in the embryonic and larval tracheal TC leading to cells with extra-subcellular lumina. These data provide the first evidence for the role of spectraplakins in single-cell lumen formation and branching [7]. Cytoskeletal polymers like actin filaments and microtubules are involved in a variety of crucial cell processes like movement, morphogenesis, phagocytosis, and division.

Despite the enduring dogma that actin filament and microtubule networks are different in localization, structure, and function, mounting evidence demonstrates that these components are orchestrated through complex mechanisms that are responsive to either polymer. Actin-microtubule interactions are mediated by a variety of proteins and cellular signals that have already been discovered. However, actin filament or microtubule polymers are usually used in isolation from the other system to evaluate the effects of these regulators[8]. Furthermore, novel ways and controllers of actin-microtubule interactions are still being found. Here, we look at various actin-microtubule crosstalk mechanisms with a focus on the molecular connections between the two polymer systems and their higher-order interactions[8] (Figure. 1A-F).Cells are polarised for division, shape changes, and movement through symmetry break, which is facilitated by crosstalk between the actin cytoskeleton and microtubules. These cellular processes control the metastasis of cancer cells as well as the formation of organs. Rho GTPases play a role in the production of various F-actin subtypes that affect cortical tension and rigidity and are controlled by microtubules.

For instance, the contractile ring in the cell's equatorial plane is formed when nonmuscle myosin crosslinks long, unbranched F-actin, which is produced as a result of RhoA activity stimulated by the central spindle microtubules of the mitosis spindle [9]. Compared to the poles, where short, branched F-actin is more likely to develop, this region of the cell has higher cortical tension. Growing microtubules that extend into the leading margin of migrating cells encourage Rac activation and the development of short, branched F-actin for lamellipodia formation. The idea that input from the cortex can affect microtubule stability is resonating across a wide range of disciplines. In this way, cells can dynamically react to intrinsic or extrinsic signals to make sure that their migration to create tissues or their division plane is always coupled with the segregation of DNA and cell fate determinants [9]. Actin is needed by tip-growing cells in plants for cell expansion, and microtubules are needed for cell growth orientation, but it is unclear how the two cytoskeletons are connected. It has been demonstrated that an actin cluster near the cell apex controls the direction of rapid cell

growth in tip-growing cells of the moss Physcomitrella patens [10]. The merging of microtubules close to the cell tip is necessary for the formation of this structure. found that actin is required for myosin VIII-mediated microtubule focusing and that class VIII myosin function is essential for microtubule convergence [10]. Both networks are impacted when myosin VIII functions are lost, proving that the three cytoskeletal elements are functionally connected. Findings imply that microtubules coordinate the localization of formins, and actin nucleation factors, which produce actin filaments. This positive feedback loop ensures that actin polymerization and cell expansion take place at a designated spot, producing persistent polarised growth [10].

Table 1: Actin and microtubule cytoskeletons are identified to be biologically coordinated by proteins.

Physical mechanism	coupling protein	Molecular interaction partners	cellular functions
Anchoring of microtubule minus ends by actin networks	ACF7 (Shot or Shortstop or Kakapo)	•Microtubule –TIP protein CAMSAP3 (Patronin)	•Apico-basalcell polarity in epithelial •Epithelial cell migration •Tight junction regulation in intestinal epithelia
Actin nucleationand elongation from microtubule plus ends	Complex of EB1, CLIP170 and formins (demonstrated in vitro for mDia1, mDia2, Daam1, INF1 and INF2)	•Microtubule plus ends •Actin monomers	Dendritic branching in neurons
	Navigator protein (Sickie)	•Microtubule+TIPs •Actin nucleators	Neuronal outgrowth

Microtubule development is regulated by actin-microtubule crosslinking. Proteins that crosslink microtubules to actin filaments offer one direct method of actin-microtubule communication [11]. A significant number of multi-domain proteins or protein complexes mediate this physical linkage. with microtubule and F-actin binding regions. Some of these crosslinking proteins can associate with microtubule plus-end-binding proteins (EB proteins), serving as both microtubule plus-end trackers (+TIPs) and actin-microtubule crosslinkers[11].As a consequence, these proteins can create dynamic connections between the plus ends of developing microtubules and actin bundles, which may cause microtubule growth to be redirected along actin bundles.

Another type of physical linkage happens when protein complexes connected to actin networks anchor and stabilize microtubule ends (both plus and minus ends), which is frequently seen at the cell cortex. Protein complexes that both physically capture microtubule ends and actively inhibit their dynamic properties may be involved in this kind of linkage, resulting in strong connections between actin networks and microtubule ends [5]. Alternately,

actin structures like the actin cortex and the actin in migrating protrusions may function as a strong physical barrier that keeps expanding microtubules from attempting to target the plasma membrane.

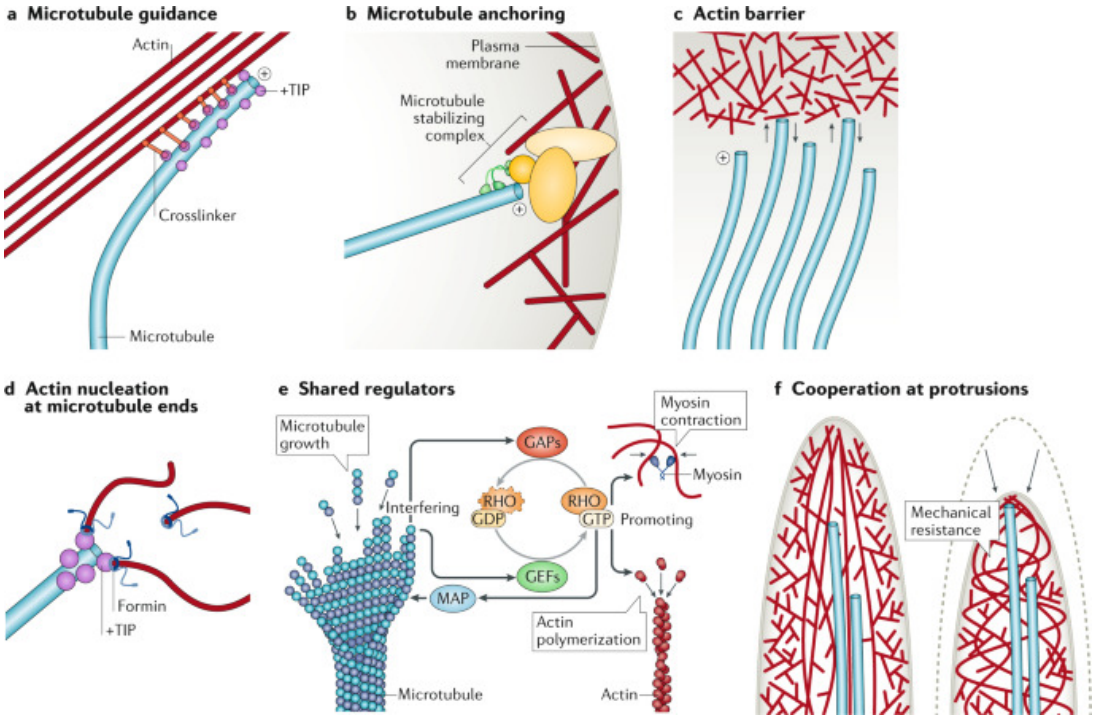

Figure 1: Acitn- microtubules crosstalk: Schematic diagram showing the different cooperation functions between the actin and microtubules (Nature).

A physical barrier prevents microtubule growth and consequently encourages the occurrence of catastrophes. Because of this, the actin cortex may prevent microtubules from passing through the membrane and interacting with membrane-bound cortical anchors or from pushing against the membrane. Evidence suggests that microtubules may directly contribute to the location of elements that encourage local actin polymerization. Formins, Ena/VASP, and Wiskott-Aldrich syndrome protein (WASP) family proteins, which may interact with microtubule ends both directly and indirectly via +TIPs mediate actin nucleation and assembly (Table 1).

Through their shared controllers, actin and microtubules also interact with one another. Members of the small GTPase RHO family are key actors in this crosstalk [5]. Through their capacity to alternate between active (GTP-bound) and dormant (GDP-bound) states, RHO GTPases function as molecular switches. The persistence length, which measures the distance over which filaments stay straight under the influence of thermal forces, reflects how much stiffer microtubules are than actin filaments. The endurance length of actin is only a few millimeters, compared to the few millimeters of microtubules. Due to their stiff polymer nature and capacity to withstand significant compressive loads, microtubules are believed to play a crucial role in providing mechanical support against membrane retraction during cell protrusion events [12]. Thus, actin-based protrusions are created by the dynamic interaction of microtubules and actin. Be aware that although actin polymerization frequently causes membrane protrusions, these protrusions can occasionally also be caused by microtubule pushing, which results from sustained polymerization or the sliding of antiparallel

microtubules by the attached motor proteins. Typically, the morphology of crawling cells is polarised, with the leading edge containing a protruding, branched F-actin network and the following edge containing an actin-myosin network that contracts[13]. Normally, microtubules stretch their plus ends towards the cell cortex at the front and back and are anchored at the centrosome or the Golgi by their minus ends. As the cell advances, nascent focal adhesions form close to the leading edge, expand in size, and ultimately release and disassemble beneath the cell body and in the back. The development of actin stress fibers and myosin-II-driven tension in the lamellar actin network is required for the maintenance and maturation of focal adhesions. The primary function of microtubules is to maintain directional migration through the mechanical stabilization of the leading edge, polarised trafficking of integrins and matrix proteases33, positioning of mitochondria to deliver ATP to fuel motility, and regulation of RHO and RAC GTPases that signal the actin cytoskeleton[14]. Microtubule bundles that have been crosslinked by tau and are positioned with their plus ends facing the axon tip are found inside axons. Instead, a mixed polarity microtubule array linked to MAP2 is present in dendrites. Additionally, a periodic arrangement of cortical actin rings supports axons Figure 2. Axons that are growing or regenerating show a growth cone at the apex of the axon that directs neuronal path-finding during neurite outgrowth. The growth cone's actin organization mimics that of a lamellipodium because it has an actin-rich leading edge that is decorated with filopodia [15].

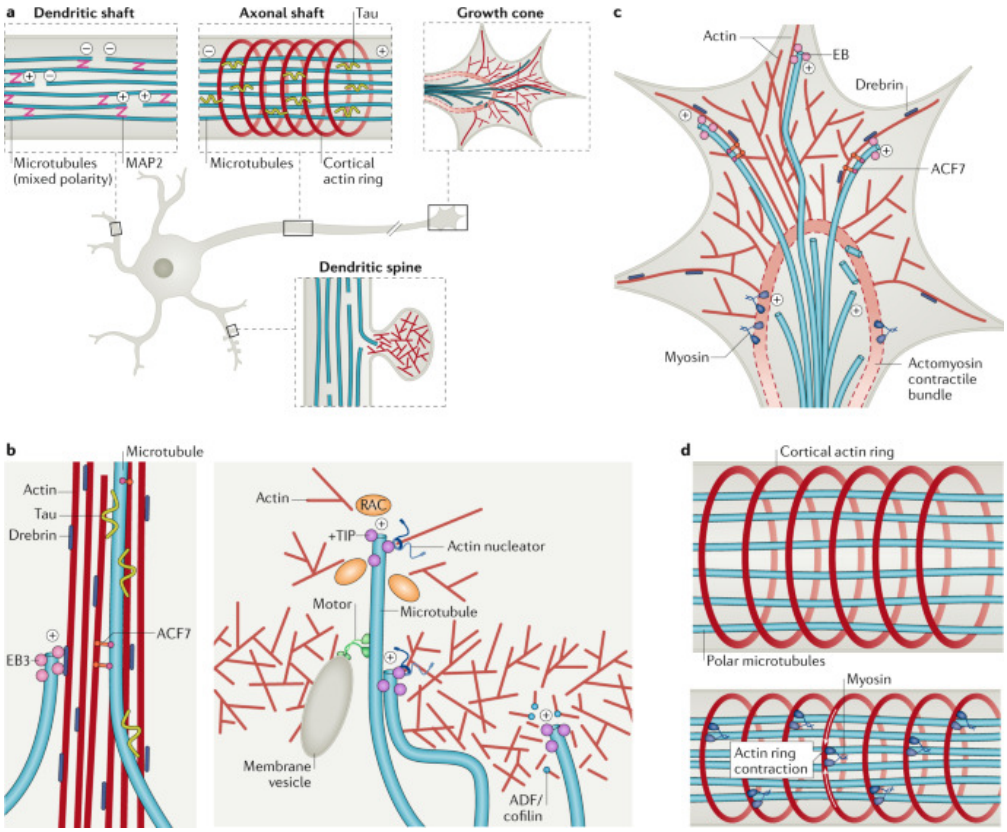

Figure 2 : Neuronal cell actin-microtubule interaction. A description of the actin- and microtubule-based cytoskeletons found in nerve cells (Nature).

Dendritic spines, which are tiny protrusions along the axis of the dendrite and receive information from axons, are present (Figure. 2A-C). These spines' bases are formed by actin, which also creates dynamic patches inside the spine. Actin-microtubule crosstalk in neuronal

morphogenesis is largely understood through research on neurons grown on rigid 2D substrates. An intricate force equilibrium between the longitudinal tension in the actin-spectrin cortex and the bending resistance of the microtubule bundle controls the overall shape and mechanical resilience of the axon[15]. Axons in development also have a growth cone with an actin-rich leading edge and filopodia at the tip. Small protrusions called dendritic spines are present along the stem of dendrites. At the base of these spines, actin creates a ring and dynamic regions inside of them. Neurite outgrowth involves actin-microtubule interaction. Actin can serve as a barrier for the entrance of microtubules, but actin bundles can also direct developing microtubules into filopodia by crosslinking with tau, actin crosslinking family protein, and end-binding protein 3 (EB3) figure . Then, by facilitating the movement of vesicles and organelles into the growing protrusion, promoting actin polymerization through RAC signaling, and potentially also by recruiting actin nucleators to the microtubule plus tips, microtubules stabilize the developing neurites. Growth cones exhibit actin-microtubule interaction.

Microtubules can be physically blocked by tangential actomyosin contractile bundles at the back, but a limited number of dynamic microtubules invade the growth cone. When the neuronal microtubule bundle enters the growth cone, it spreads out. A balance between forward polymerization and rearward transport by retrograde flow of lamellipodial actin controls microtubule penetration. ACF7-EB1, drebrin-EB3, and possibly tau crosslink microtubules to filopodial actin bundles to prevent backward flow.

Axon lengthening is likely aided by microtubules, which offer mechanical resistance to membrane retraction. Crosstalk between actin and microtubules in axonal filaments. To push the microtubules closer together and facilitate their subsequent crosslinking into a stable microtubule bundle, myosin II contractility drives the inward motion of contractile actin arcs from the sides to the center of the growth cone neck during the formation of a new segment of axon shaft. Two centrosomes that migrate to the opposites of the cell rearrange the microtubules into a bipolar mitotic spindle [5].

The kinetochore microtubules attach to the chromosomes, the antiparallel central spindle microtubules interdigitate at the spindle midzone and push the spindle poles apart, and the astral microtubules extend to the cell membrane, forming the three distinct microtubule populations that make up the mitotic spindle.

After the symmetry break, the contractile actomyosin cortex develops distinct cortical regions at the cell poles and an actomyosin contractile ring close to the spindle midzone, working in tandem with chromosome segregation to first drive mitotic cell rounding by developing a uniformly high tension (Figure. 2D). Pushing and pulling pressures produced by the plus ends of the astral microtubules in contact with the cortex control where the spindle is located. Pulling forces are produced when microtubule ends are caught by membrane-bound dynein motors and by viscous drag on organelles that are moved along astral microtubules, whereas pushing forces are produced when microtubules grow against the membrane. Polarity factors that favor dynein recruitment to the cell poles regulate the orientation of the spindle. Additionally, the spindle position is sensitive to cell size and shape due to the balance of pushing and pulling pressures [5].

CONCLUSION

The cytoskeleton controls the network's organizing and mechanical characteristics, allowing actin and microtubule filaments and their supporting proteins to carry out essential cellular functions.

For crucial biological processes, such as the creation and maintenance of cell shape, cell migration and division, intracellular transport, and intercellular communication, microtubules, and the actin cytoskeleton are engaged reciprocally. As a result, the cytoskeleton is not to be viewed as a group of different components but rather as a universal system in which constituents co-regulate one another to carry out their functions in a reliable and highly adaptable way.Finally, we provided a summary of the fundamental (physical) processes in this chapter that could be used to control the dynamics and organization of the actin and microtubule cytoskeletons. Additionally, discussed a thorough explanation of how these various mechanisms, appear to collaborate in a variety of diverse situations to drive cellular function.

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CHAPTER 3

ACTIN BINDING PROTEINS

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ABSTRACT:

Wide-ranging cellular activities are carried out by the complex actin cytoskeleton. Several ABPs that control actin-driven assembly movement without the aid of motor proteins including ADF/cofilin, profilin, gelsolin, thymosin beta4, DNase I, CapZ, tropomodulin, and Arp2/3. The following cellular tasks are carried out by these ABPs by they keep the population of unassembled but assembly-ready actin monomers (profilin), they control the filament polymerization stage (ADF/cofilin, profilin), they bind to and stop the growth of actin filament ends (gelsolin), they initiate actin assembly (gelsolin, Arp2/3, cofilin), they sever actin filaments (gelsolin, ADF While some of these ABPs can create regulatory ternary complexes, others are optional. There are valid grounds for continuing to study all of the structures and functions of those that play significant roles in human disorders.

KEYWORDS:

Actin, Cytoskeleton, Capping Protein, Profilin, Formin.

INTRODUCTION

Actin microfilaments, microtubules, and intermediatefilaments make up the interior cytoskeleton of eukaryotic cells. Strong and flexible, the cytoskeleton is always prepared to adjust to the needs of the cell. The ability of actin to generate movement in the absence of motor proteins is a crucial characteristic. Actively moving cells produce ruffled membranes at the cell membrane microfilament assembly where the membrane protrudes forward [1]. By serving as the internal support rods in microvilli, preserving cell shape, and anchoring cytoskeletal proteins, microfilaments can also serve a passive structural function. Examining how actin binding proteins (ABPs) regulate these processes is the main goal of this study. Finally, we pose some intriguing and difficult issues for additional study [1].

One of the three main cytoskeletal polymers, filaments are assembled reversibly from the evolutionarily old and highly conserved actin protein. the actin molecule's structure explains its functional characteristics, such as its ability to polymerize dynamically and be controlled by a number of actin-binding proteins [2]. Other works in this compilation describe the roles of actin in numerous cellular processes, such as interactions with myosin motor proteins intracellular transport cellular structure and motility muscle contraction and cytokinesis [3][4].

Actin binding proteins (ABPs) are thought to be a part of the cytoskeleton, which provides cells structure and mobility. A variety of proteins that govern actin polymerization, severing of actin filaments, and cross-linking of actin filaments into networks, which may be used by molecular motors, control the complex dynamic properties of the actin cytoskeleton at multiple levels. The preservation of the cytoplasm's viscoelastic properties and the integrity

of macromolecules connected to the plasma membrane depend on proteins that cross-link F-actin. The majority of these F-actin cross-linking proteins contain a calponin-like actin-binding region. Some of them have also been used as platforms. Numerous study teams have discovered various proteins that interact with ABPs over time, but it is still largely unknown how these interactions affect ABPs [5]. ABPs can migrate to the nucleus in addition to organizing the cytoskeletal structure, according to new data. This fact is consistent with what has lately been discovered about actin's potential role in nuclear function. Recent information and analysis of results from published studies have also suggested that scaffold proteins like filamin A (FLNa) may be broken down through a process called proteolysis, and that the byproducts of this reaction may function as signalling molecules that link nuclear and cytosolic pathways. Here is a review of some of the pertinent data in this field [5].

Actin filament polymerization against membranes generates force for many biological processes, including organelle dynamics, endocytosis, phagocytosis, migration, and morphogenesis. As a result, abnormal actin cytoskeleton movements are related to a variety of illnesses, such as cancer, as well as immunological and neurological conditions. Many cellular, developmental, and pathological processes can be explained by understanding how actin filaments produce forces in cells, how force generation is controlled by the interaction of actin-binding proteins, and how the actin-regulatory machinery reacts to mechanical load. It has also become clear that important actin-binding proteins are mechanically regulated, and as a result, their activities are not exclusively controlled by biochemical signaling pathways. this translates into force production in endocytosis and mesenchymal cell migration using an updated view of actin dynamics as a framework [6].

Profilin, which can interact with a variety of acting binding proteins and link membrane lipids to cytoskeleton elements, are emerging as crucial regulators of actin dynamics after being initially discovered as G-actin sequestering proteins. Lately, it was discovered that profilin contains residues specifically suited for binding to microtubules in addition to its actin, poly-proline, and phosphatidylinositol binding domains [7]. By capping the barbed ends of actin filaments, capping protein (CP) is a key regulator of actin assembly kinetics. Numerous proteins and phospholipids have the ability to control the capping action of CP in a variety of ways, some direct. A 30-amino-acid region known as the capping protein interacting (CPI) motif is both required and adequate to bind to and inhibit CP. A variety of unrelated proteins, many of which are engaged in membrane interactions, contain this motif [8].All organisms contain the small actin-binding proteins known as cofilins and actin-depolymerizing factor (ADF). Actin filaments are depolymerized and severed by ADF/cofilins in vitro, promoting actin movements.

However, there is debate over whether ADF/cofilins add to actin dynamics in cells by severing "old" actin filaments or by facilitating actin filament assembly. Multiple isoforms of mammalian ADF/cofilins, which may influence actin dynamics via various processes, complicate analysis of these proteins further. It has been found mammalian ADF and cofilin-1 support cytoskeletal dynamics by depolymerizing actin filaments, and this activity is essential for a number of processes, including cytokinesis and cell motility [9]. A conserved 125 residue motif found within a 250 residue actin-binding domain is shared by a family of actin-crosslinking proteins. This domain is combined with other functional domains and spacer segments made up of varying numbers of repeated -helical or -sheet motifs to create proteins that vary in their capacity to make actin bundles or networks and associate with the

plasma membrane [10]. The first identified non-muscle F-actin-binding protein, now known as filamin A, is the most effective among numerous F-actin cross-linking molecules (FLNa) [11].

The most effective actin filament breaking protein so far discovered is gelsolin. When non-covalent bonds between actin molecules in a filament become feeble enough to split it in half, this is known as Severing. Gelsolin separates stoichiometrically and almost entirely. Gelsolin attaches to the side of an actin filament, which starts the Severing process. Gelsolin binds filaments rapidly but severs slowly the delay may reflect the time needed for structural rearrangement within gelsolin and in the filament prior to severing. Gelsolin causes the actin filament to kink and alter conformation, which suggests a mechanical explanation for severing [12].

LITERATURE REVIEW

Over view of actin binding protein: Actin behaves very differently in cells than it does when it is a pure protein in a test tube. More than 99% of purified actin would polymerize in seconds at the overall amounts found in cells (50-200 m), and subunits would switch on and off the barbed ends of the protein about once per second and more slowly at the pointed end. At concentrations between 25 and 100 m, orders of magnitude above the critical concentration, approximately half of the total actin in cells depolymerizes, in contrast to this essentially static situation. Furthermore, unlike comparatively inert actin filaments in a test tube, filaments assemble and turn over on timescales of tens of seconds. Actin-binding proteins account for these differences by regulating nearly every aspect of actin assembly [2].

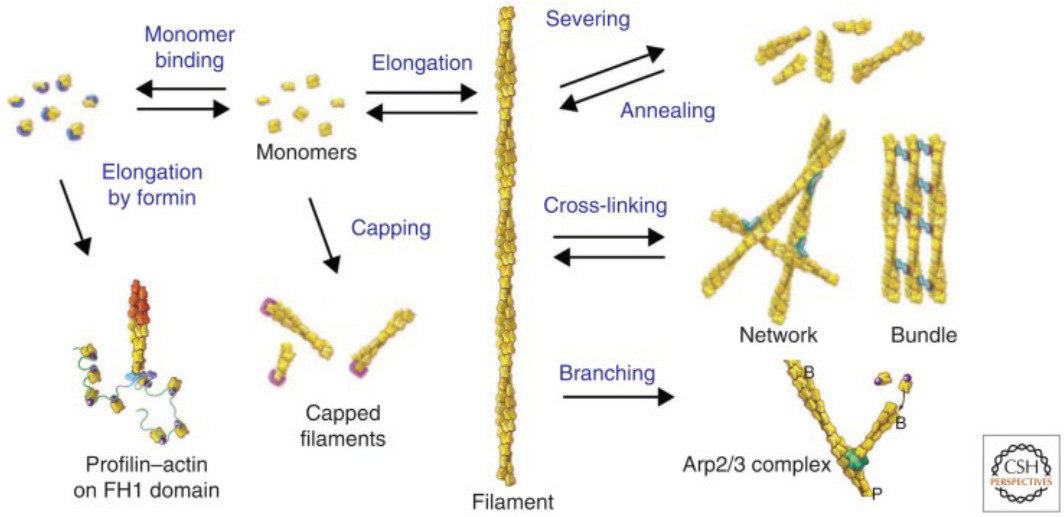

Figure 1: Actin binding proteins:Diagrame showing the actin dynamics regulated by the different actin binding protein (CHS).

Actin- monomer-binding proteins: The majority of organisms depend on the small (13–14 kDa), actin-monomer-binding protein profilin for survival. Most of the unpolymerized actin in the cytoplasm is bound to profilin due to its affinity (Kd = 0.1 m) for ATP-actin monomers and a cellular concentration in the range of 50-100 m, with the exception of mammalian cells that produce thymosin-4. The barbed end of an actin monomer, when bound to profilin, suppresses nucleation and elongation at pointed ends but not at barbed ends. Profilin dissociates quickly after a profilin-actin complex attaches, freeing the end for additional

elongation. Thymosin-4 sequestered actin monomers can engage in elongation because profilin and thymosin-4 compete for binding actin monomers) [2]. Both proteins quickly switch on and off actin monomers, enabling this shuttling mechanism. The majority of the actin monomers are bound to either profilin or thymosin-4 given the physiological concentrations of all three proteins, leaving a low (submicromolar) quantity of free actin monomers (Figure 1).

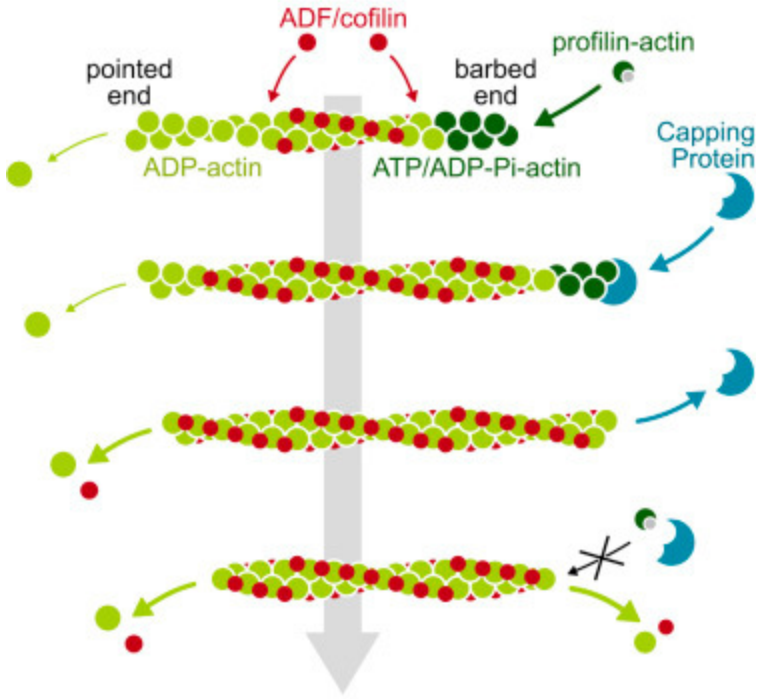

Figure 2:Actin monomer binding proteins: Actin monomer binding proteins binds with the actin monomer and inhibits their addition to the filaments (science direct).

Originally thought to be a thymosin hormone, thymosin-4 is a peptide with 43 acids that is also the most prevalent actin-monomer-binding protein in some cells, such as leukocytes and platelets. Thymosin-amino IV's terminus creates a short helix that binds in the barbed-end groove, and the remaining portion of the peptide is made up of an extended region that binds the front surface of actin and a second helix that terminates the pointed end at the top of the nucleotide-binding cleft Thymosin-4 can sequester a significant pool of actin monomers at concentrations of >100 m in the cytoplasm and a micromolar affinity for Mg-ATP-actin, stopping them from participating in any polymerization reactions due to steric interference with all of the interactions necessary for polymerization [13]. The helix that attaches to the barbed-end groove of actin is one of many proteins that share sequences with the amino-terminal half of thymosin-4. Using either a polyproline track that binds profilin or an actin-monomer-binding site related to a WH2 domain, VASP can transport either free actin monomers or profilin-actin to the barbed end of the. With a dwell period on barbed ends of only 1.5 sec, VASP is significantly less processive than formins (Figure 2). For survival, mice require at least one of their three Ena/VASP genes. These proteins are concentrated at the tips of filopodia and the leading edge of motile cells, where they help the filaments develop [14].

Actin filaments polymerase:

Actin kinetics are effectively regulated by formin proteins. The majority of eukaryotes have different formin isoforms, indicating a variety of cellular functions. Having a variety of domains and useful patterns, formins are modular proteins. The Formin homology 2 (FH2) domain links the barbed ends of the actin filaments and moves processively as they lengthen or depolymerize (Figure.3). Formins also initiate actin filaments and interact processively with the growing end of the filament to either inhibit or support the extension of the barbed end. All formins' FH2 domains were used to evaluate slow barbed-end elongation on their own. The existence of two conformations in the complex between the FH2 domain and the filament's end provides a clear reason [15]. Unlike the closed state, actin monomers can bind to the open form. Barbed ends are open anywhere from 5% to 90% of the time, depending on the shape. If the formin construct has an FH1 domain in addition to the dimer of FH2 domains, profilin can surmount this inhibition and bias polymerization towards filaments with formins (Figure 3). The FH1 domain is a flexible "tentacle" with one to 14 polyproline tracks that binds profilin-actin complexes [15].

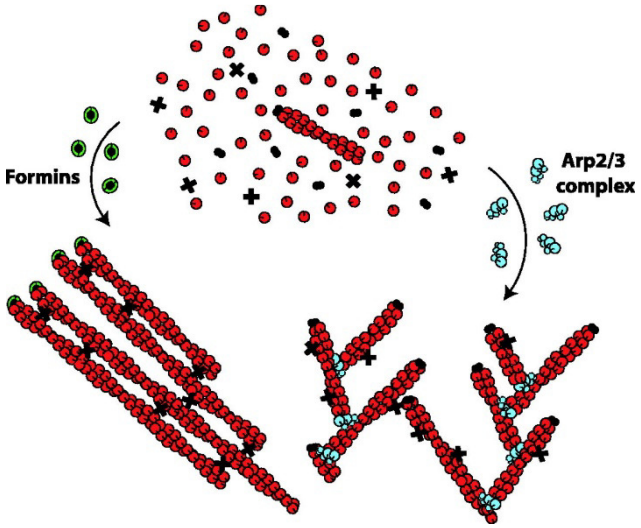

Figure 3: Polymerization by formin: Actin polymerization in presence of the formins and Arp2/3 complex (PNAS).

It is positioned amino-terminal to the FH2 domain. After rate-limiting binding of profilin-actin to multiple sites in the FH1 domain, diffusion of the FH1 domains quickly delivers profilin to the filament's end, enabling rapid extension despite the end's partial closure. Elongation can occur five times quicker than for a free barbed end in certain circumstances, such as formin mDia1, which is 90% open and has an FH1 domain with 14 potential profilin-actin-binding sites. Despite the quick elongation, all of the tested formins are surprisingly processive, successfully "stepping" onto the newly added subunit for tens of thousands of cycles. This polymerase activity causes actin filaments connected to a formin to grow rapidly and persistently in the cell while inhibiting capping by capping protein. For instance, formin mDia1 in fibroblasts develops filaments at a rate of 700 subunits/sec[15].

Cross linking proteins: Actin filament networks and bundles in microvilli, filopodia, and intracellular cables are examples of higher-order structures that are physically connected to one another by a large family of cross-linking proteins. Two actin-binding sites in the same polypeptide or in two subunits of oligomeric proteins are needed to connect two filaments.

Many of these proteins have two calponin-homology actin-binding domains (ABDs), but the spacing between the pairs of ABDs differs greatly (Figure 4). The creation of actin filament bundles is aided by the tandem ABDs of fimbrin and the two-fold symmetry of fascin, whereas the widely spaced ABDs of filamin (ABP) cross-link less organised networks of filaments, such as those at the leading edge of motile cells [2].

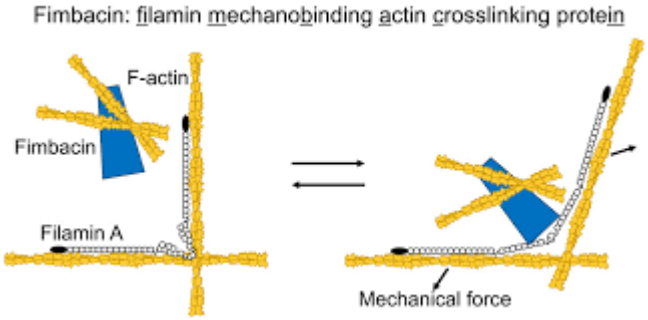

Figure 4: Crosslinking proteins: Showing the binding and function of the actin cross-linking protein (ACS PUBLICATION).

ABDs usually have a low affinity for actin filaments (Kd 10 m), so they switch actin filaments on and off in a matter of milliseconds. The mechanical properties of actin filament networks vary significantly from those of covalently cross-linked synthetic polymers due to the rapid exchange of these linking proteins. Cross-linked actin networks are stiff when deformed quickly, but the rapidly rearranging cross-links do not withstand gradual deformation. This explains why cells deformable on timeframes of tens of seconds but stiff and elastic on fast timescales[2].

CONCLUSION

Actin subunits are used by a system of proteins created by evolution to construct a wide variety of diverse structures in prokaryotes and eukaryotes. They vary from the sarcomeres of striated muscles, which are stable, to the force-producing branched networks at the leading edges of motile cells, which turn over in a matter of seconds.

This chapter's comprehension of the processes governing actin filament dynamics in all of the or another in this outline Actin, a crucial element of the cytoskeleton, is essential for eukaryotic cells. In addition to its roles in endocytosis and intracellular trafficking, contractility, motility, and cell division, the actin cytoskeleton also plays a role in the development and preservation of cell morphology and polarity. Actin-binding proteins (ABPs), which are abundant in cells, control the formation and disassembly of actin filaments as well as how they are organized into useful higher-order networks. In turn, particular signaling networks regulate the activities of these proteins.

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CHAPTER 4

ACTIN REGULATION BY FORMIN

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ABSTRACT

Numerous essential cellular functions, including cell division, migration, and adhesion, depending on the actomyosin cytoskeleton network. The cytoskeleton's ability to receive, produce, react to, and send mechanical signals throughout the cytoskeleton network within the cells and throughout the tissue via cell-extracellular matrix and cell-cell adhesions underlies all of its biological functions. Actin polymerization, which is controlled by a variety of cellular components, is essential to the cytoskeleton's operations. One of these is the formin family of proteins, which binds the F-actin, the barbed end of an actin filament, and is known to play a significant role in encouraging actin polymerization.Unbranched actin filaments and various types of actin filament bundles are elongated by formins, which also serve as a nucleus for many cellular processes. Formins form a processive association with the filaments' rapidly expanding extremities and shield them from capping. Formin-dependent actin polymerization is sensitively regulated by the force and torque applied to the F-actin.

KEYWORDS:

Actin Filaments, Cellular Process, Plant Formin, Formin Family, Formin Homology Domain.

INTRODUCTION

The biochemical procedure known as actin remodelling enables dynamic changes in cellular structure. Actin filament remodelling is a crucial component of cellular activity that takes place in a cyclic pattern on cell surfaces. Actin monomers polymerize during remodelling in reaction to signalling cascades that result from environmental stimuli [1]. The intracellular structure of the cytoskeleton is influenced by actin, which frequently has an impact on the cell membrane as a result. Actin strands disintegrate back into monomers when environmental factors are present once more, ending the cycle. During the actin remodelling process, actin-binding proteins (ABPs) support the change of actin filaments [1]. These proteins are responsible for the varied structure and morphological variations of eukaryotic cells. Despite its intricacy, actin remodelling has the potential to completely reorganise the cytoskeletal system in less than a minute [2].

Formins (formin homology proteins) are a family of proteins that interact with the fast-growing (barbed end) of actin strands during the polymerization of actin[3]. Most formins are activator proteins for the Rho-GTPase. Formins play a role in several cellular processes including cell polarity, cytokinesis, migration, and SRF transcriptional activity. They also control the actin and microtubule cytoskeleton [4]. While some formins have been given roles within the nucleus, formins are multidomain proteins that interact with a variety of signalling molecules and cytoskeletal proteins[5].The formin protein regulates the formation of straight

filaments from globular actin monomers in eukaryotes. Formin proteins are large, 1,000 amino acid residue molecules made up of diverse combinations of functional domains. The formin homology domains FH1, FH2, and FH3 are the most significant regions [5].

Actin molecules are bound by the formin homology 2 (FH2) domain, which has 400 amino acid residues. The three-dimensional (3D) structure indicates that the FH2 domain is a member of the all-alpha family of proteins [6]. It is made up of 20 alpha helices that have been put together to resemble a circle. According to, the FH2 domains group together to create homodimers, which take on the shape of a ring-like structure that encircles the elongating actin filament at its fast-growing end and encourages elongation [7]. A proline-rich region known as the FH1 domain increases the delivery of new actin monomers onto the expanding filaments by binding to profilin-actin complexes.Formin dimerization and formin localization within the cell are both regulated by the FH3 region. Two additional domains are involved in the control of formin activity. One is the GTPase-binding domain (GBD), which interacts with the molecular switch Rho-GTPase to initiate the function of formin [7].

The second is the diaphanous autoregulatory domain (DAD), which collaborates with the GBD domain to maintain formin's dormant condition. A multigene family that encodes for formins has different sizes and compositions depending on the organism. Although seven paralogous families of formins from bilateral animals have been identified, it is still unknown how these formins evolved[7]. Other eukaryotes' formins have also been characterised, but the formin multigene family's origin and mode of evolution have not been investigated. No prokaryotic formin genes have been discovered thus far. Here, we look into the origins of various formin genes and their evolutionary connections[8]. To understand the beginning and evolution of the formin gene family and to deduce the domain structure of the ancestral formin molecule, we surveyed a large collection of genomes and proteomes[8].

The actin cables that control polarised secretion and development in budding yeast are assembled under the control of the formins Bni1p and Bnr1p. it has been that four of the six yeast Rho proteins (Cdc42p and Rho1–5p) are involved in the control of formin function. it has been demonstrated that activating the formins Bni1p and Bnr1p is the crucial role of Rho3p and Rho4p, and that activated alleles of either formin can operate without these Rho proteins[9].

Rho1p, acting via protein kinase C (Pkc1p), the main effector for Rho1p signalling to the actin cytoskeleton, is required for formin activation at high temperatures via a different signalling route. A MAPK pathway is also activated by Pkc1p, but it is not involved in the stimulation of formin. Cdc42p is not necessary for formin-dependent cable assembly, but without it, the cable assembly is improperly organised during the start of bud development. These findings demonstrate that three separate, crucial Rho signalling pathways regulate the function of formin[9].

Actin filaments are protein polymers that support a variety of biological processes in eukaryotic cells throughout the cell cycle, including cell migration, vesicle trafficking, and polarity formation[10]. Uncertainty exists regarding the mechanisms governing the geographic and temporal control of actin assembly in vivo. Cables, which are collections of unbranched actin strands, are formed by formin proteins. To reproduce the physiological actin cable construction initiated by formins[10].

LITERATURE REVIEW

Numerous cellular processes, such as polarity establishment, morphogenesis, and motility, are driven by the close coupling between the biochemical and mechanical characteristics of the actin cytoskeleton. Actin filaments, which are semi-flexible polymers, can function as biological active springs or "dashpots" (in layman's terms, shock absorbers or fluidizers) able to exert or resist force in a cellular environment. This is made possible by the molecular motor myosin and semi-flexible actin filaments. Actin filaments can arrange themselves into a variety of architectural configurations to modulate their mechanical properties, leading to a variety of cellular organisations such as branched or crosslinked networks in the lamellipodium, parallel bundles in filopodia, and antiparallel structures in contractile fibres [11].

Formins are well-known for controlling how cells' actin cytoskeletons behave. The formin family only has a small number of members in yeasts, but there are many formin genes in both plants and mammals, many of which have similar functions. Findings collectively point to For1A and For1D as actin dynamics controllers that, despite their similarity, have evolved to serve different purposes [12]. Actin network formation can frequently be timely controlled by altering the early actin nucleation. The plant class I formin family is a significant class of actin nucleators that are found at the cell surface and react quickly to external chemical and environmental stimuli. Plant class, I formins have comparable biophysical characteristics to mammalian integrins, which are structurally integrated within the extracellular matrix-plasma membrane-actin cytoskeleton (ECM-PM-AC) continuum [13]. The molecular mechanisms that spatiotemporally underpin the mechanosensing and mechanic regulation of formin for remodelling actin, however, during various signalling transductions remain unknown. Here, the focus will be on recent advances in our understanding of how class I formin condensation controls the biochemical processes that tune actin polymerization during plant immune signalling, as well as how the CW-PM-AC continuum structural elements of plants control formin condensation at a nanometer scale [13].

The model plant Arabidopsis thaliana's fully sequenced genome shows the existence of a diverse multigene family of formin-like sequences with more than 20 isoforms. Recent discoveries from biochemical, cell biological, and reverse-genetic studies of this family of actin nucleation factors are highlighted in this overview. Major roles for plant formins during cytokinesis and cell expansion are suggested by significant advancements in our knowledge of cellular function. The need to investigate molecular mechanisms outside of mammalian and yeast systems is highlighted by biochemical research on a subset of plant formins. Notably, proof for the first non-processive formin (AtFH1) in eukaryotes is provided by a combination of timelapse, single-filament imaging with TIRFM and solution-based assays for actin dynamics [13]. Despite these developments, it is still challenging to agree on the activities of plant formins and cellular processes. The vast differences in domain structure among plant formins pose a challenge to summarising formin properties. Plant formins will be unable to produce homology-based forecasts that rely on conserved domains other than the FH1 and FH2 [13].Arabidopsis class I has no similarity with the auto-inhibitory domain (DID) or diaphanous autoregulatory domain (DAD) of mDia1, a member of the mammalian formin family, aside from the FH1 and FH2 domains. Additionally, the normal Arabidopsis class I formin has a transmembrane domain (TM) and a putative signal peptide (Sp) at the N-terminus. Sp stands for signal peptide, TM for a transmembrane domain, FH1, FH2, FH3,

diaphanous autoregulatory domain, DID, auto-inhibitory domain, and CC for the projected coiled coil. (Figure 1).

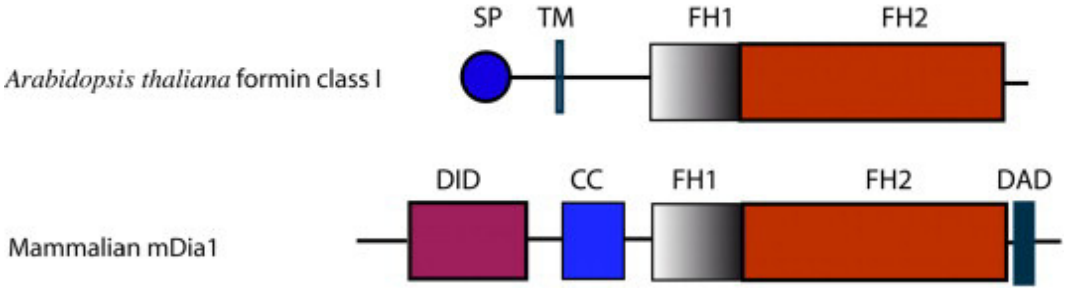

Figure1: Formin domain: Comparing the domain structure of a normal class I formin from Arabidopsis to one that resembles a diaphanous formin from a mammal (Science Direct).

Phagocytosis is a process used by neutrophils, dendritic cells, and macrophages to collect and eliminate invasive pathogens. The process is started when certain phagocytic receptors, such as immunoglobulin (FcR) and complement C3bi (CR3) receptors (integrin M2, Mac1), engage with ligands on the surface of pathogens. Rho-family small GTPases regulate the localised actin-filament assembly that drives particle engulfment. RhoA controls CR3-mediated phagocytosis through an unidentified process [14]. mDia1 is recruited around fibronectin-coated beads in a RhoA-dependent way in fibroblasts, and mammalian Diaphanous-related (mDia) formins contribute to the generation of a diverse set of actin-remodeling events downstream of RhoA. It has been demonstrated that the CR3-mediated phagocytosis recruits the RhoA effector mDia1 early and that it colocalizes with polymerized actin in the phagocytic cup. While inhibiting mDia activity has no impact on FcR-mediated phagocytosis, it suppresses CR3-mediated phagocytosis. These findings point to a novel role for mDia proteins in the control of actin polymerization during phagocytosis mediated by CR3 [14].

All organisms contain a family of conserved proteins called formins, which control the dynamics of actin. There are 15 different formin alleles in mammals. Surprising variation between these isoforms has been found through research so far (Figure 2A). All of the investigated proteins similarly affect actin dynamics by accelerating the rate of nucleation, changing the elongation/depolymerization rates of the filament's barbed ends, and opposing the capping protein. The strength of each impact can, however, differ significantly between formins. A portion of the filaments also firmly bind to the sides of the filaments and bundle them [15].

Actin cytoskeletal dynamics must be tightly regulated for cellular survival. The quick assembly of filament nuclei that elongate and are integrated into various and specialised actin-based structures is made possible by various families of nucleation-promoting factors. The formin protein family also controls the lengthening of unbranched actin filaments in addition to encouraging filament formation. Processive association of formins with growing filament ends is accomplished by the highly conserved, dimeric formin homology (FH) 2 region continuously binding to barbed filament ends. Actin subunit addition is mediated by FH2 dimers in conjunction with the FH1 domain and C-terminal tail region at rates that can significantly surpass the rate of spontaneous assembly(Figure 2B,2C).

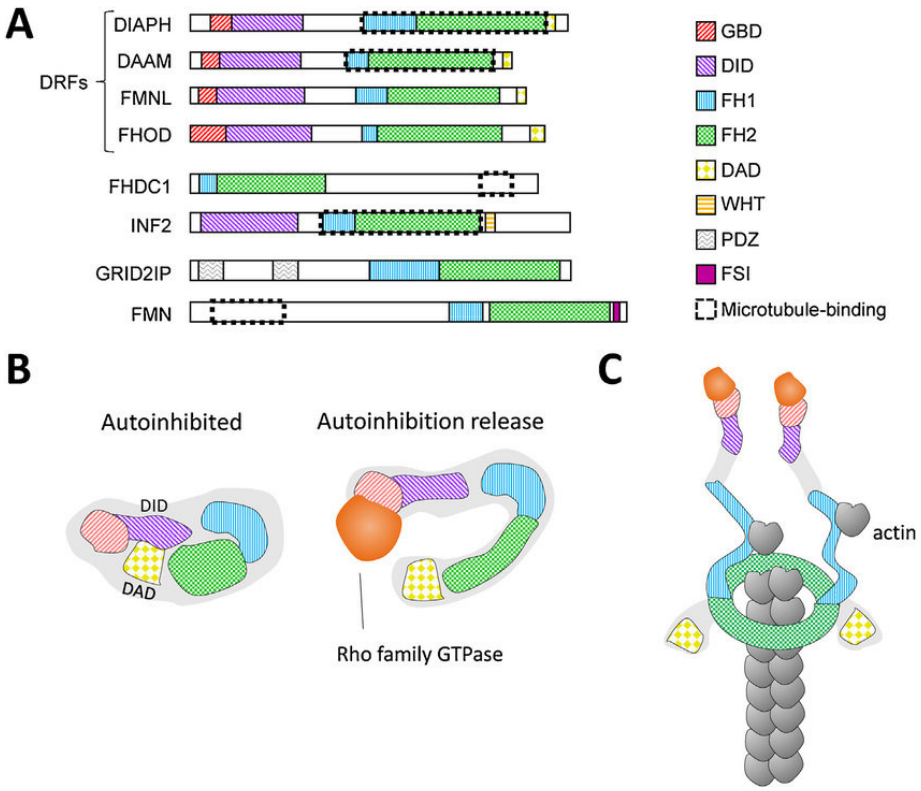

Figure 2 : Domain organization of mammalian formins subfamily (A) Domain organization of the different formin protein. (B) Showin the autoinhibition and relaeased of theautoinhibition by formin.(C) Lengthin gof actin filaments by the formin(Research gate).

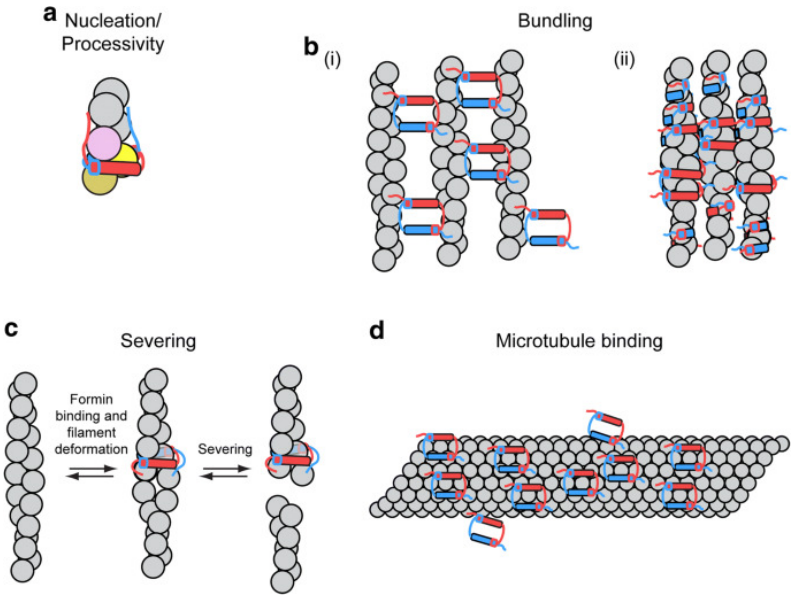

Figure 3: Function of the formins: Different actin dynamics and assembly controlled by formins (Spring link).

Yeast formin Bni1p's FH2 domain's structure has Five subdomains known as a lasso, linkage, knob, coiled-coil, and post make up the FH2 domain. Based on the crystalline symmetry, it

was determined that Bni1p FH2 had a dimer-like structure. The partner FH2's "lasso" and "post," which show a closed ring structure, interact specifically to cause dimer formation. FH2 lacking the ability to form dimers lost the ability to polymerize actin, demonstrating that the ability to make dimers is necessary for the activity [7]. Formin performed different function like the actin nucleation,bundling and some time role in the filaments severing(Figure.3A- 3C). Recently, the intricate structure of the actin and Bni1p FH2 was discovered. The structure demonstrated that the interaction with the actin monomer occurs at the post/lasso and knob regions. A bridge is a structural component with these two contact sites; each bridge engages with one actin monomer [7]. The actin monomers in the crystal are connected by 21 helical symmetry, and Bni1p FH2 formed a helical oligomer around the actin molecules. By swapping the alternate lasso-post connection, Research also uncovered the specifics of the "stair-stepping" model for the actin polymerization: the "stair-stepping" of each bridge on the barbed end maintains a space between the actin filament and FH2 to accommodate the next incoming actin monomer, and this supports the process However, the helical strain is inherently brought on by the actin filament's stair-stepping (Figure.3D). Therefore, it has also been freely suggested to use the screw model to reduce the helical tension [7].

CONCLUSION

The chapter's findings have been used to demonstrate how formin regulates actin. Formins, proteins with an FH2 domain and the capacity to generate straight F-actin from scratch, are essential for controlling the cytoskeleton. Initially believed to control actin primarily, new research has revealed a role for formins in controlling microtubule dynamics. Most recently, it was discovered that some formins can coordinate the arrangement of the actin and microtubule cytoskeletons. These functional characteristics are relevant to biological processes in a developmental or organismal context, although biochemical analyses of this family of proteins have revealed many insights into how formins regulate various cytoskeletal reorganisations. Studies on the genetics of development in fungi, Dictyostelium, vertebrates, plants, and other model animals have shown that formins play conserved functions in the assembly of actin cables, cell polarity, and cytokinesis. However, formins unique to individual organisms have also been found to have roles. As a result, formins carry out both general and specialised tasks, some of which are consistent with earlier biochemical evidence and others of which reveal formins' novel properties. The studies to date have shown the importance of the flexibility within the formin family to control a wide range of diverse cytoskeletal processes during development, even though not all family members have been studied across all organisms.

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CHAPTER 5

ACTIN'S ROLE IN CANCER

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ABSTRACT

The cytoskeleton plays a key role in many of the characteristics of malignancy. Actin regulatory proteins have been linked to cancer in recent years, and data suggests that their dysregulation indicates a poor clinical outlook. The expression of several inducers of actin polymerization is noticeably decreased in some cancers, although the increased cytoskeletal activity is frequently linked to cancer development. It is unclear how these changes affect tumorigenesis and dissemination. Consequently, the complexity of cytoskeletal induction of cancer progression presents significant challenges for therapeutic intervention; it is not always clear which cytoskeletal regulator should be targeted to obstruct cancer progression and whether this targeting may unintentionally enhance alternative invasive pathways that can worsen tumour growth. It would be impossible to cover the complete array of cytoskeletal machinery in eukaryotic cells in a single overview because they are so numerous and complicated. The system is made up of and controlled by hundreds of proteins. As a result, we will concentrate on the actin cytoskeleton in this section, which includes the biological machinery responsible for the majority of the crucial cellular functions that are changed in cancer, with a concentration on actin nucleating factors and nucleation-promoting factors. We conclude by talking about contemporary cancer treatment approaches that focus on the cytoskeleton.

KEYWORDS

Actin cytoskeleton, Cancer cells, Cell movement, cell migration, Cancer progression.

INTRODUCTION

One of the most prevalent proteins in eukaryotic cells, actin is essential for a variety of biological processes, including cell motility, muscle contraction, adhesion, cell division, junction formation, cellular membrane protrusion, vesicle trafficking, chromatin remodelling, and cell integrity maintenance [1]. Actin was discovered to have six distinct isoforms in mammals, each of which is transcribed by a different gene. While cyto-actin and cyto-actin are the actin variants found primarily in non-muscle cells, skeletal-actin, cardiac-actin, smooth-actin, and smooth-actin isoforms are found largely in muscles[2]. These various isoforms, which only vary from one another slightly in the N-terminal region of a few amino acids, have shared and distinct cellular roles [2]. Monomeric globular actin (G-actin), which polymerizes into a polymeric thread known as filamentous actin, makes up the actin cytoskeleton (F-actin). F-elongation actin's phase is energetically advantageous, but the process's initial, or "nucleation" step, is energetically unfavourable and therefore highly controlled[3]. Actin polymerization, also known as actin treadmilling, is the process of adding new G-actin monomers bound to ATP molecules to the filament's expanding "barbed" or "+" end while depolymerizing G-actin monomers bound to ADP at the filament's "pointed.

The actin-related protein 2/3 (Arp2/3) complex, the Formin protein family, or the more newly identified proteins Spire, Cordon-bleu, and Leiomodin can all break through the kinetic barrier of the nucleation phase. Actin dynamics are further controlled by proteins involved in severing, depolymerizing, capping, and motor activities after the nucleation phase [4].

The disruption of proteins involved in the treadmilling procedures can alter the activity of cancer cells, which may then have an impact on the development of the disease. Serial mutations in cancer cells lead to the development of increased cellular skills known as "Hallmarks of cancer," which heavily rely on the cytoskeleton. Anomalous signalling cues are the cause of the multistep processes of invasion and metastatic dissemination, which also require the capacity of the cancer cells to migrate. The cytoskeleton controls and carries out all of these abnormal signalling cues[5]. The cytoskeletal-regulating proteins are elevated or overexpressed in a variety of cancer types, which promotes spread and metastases. However, it has also been demonstrated that suppressing some cytoskeletal regulatory complexes accelerates the development and metastatic dissemination of malignancy [6]. These events are not unexpected given that numerous studies on the cytoskeletal dynamics of migrating cells have shown that, when a specific actin-based architecture is blocked, a different structure can compensate to encourage migration and invasion .

The exact equilibrium of the cytoskeletal architecture can therefore be changed to favour a virulent phenotype by upregulating or downregulating actin regulatory networks. However, since our understanding of these processes is still limited, it is challenging to develop treatments that specifically target these networks. The most current research on actin cytoskeletal elements and their dysfunction in cancer cells will be covered in-depth in the parts that follow. We will pay close attention to actin nucleating factors, as well as factors that promote type I and type II actin nucleation, and how these interactions affect the pathogenesis of illnesses. Lastly, we will go over present therapeutic approaches used to specifically target the cytoskeleton of cancer cells as well as the promise of actin-inhibitory drugs in the future. Therefore, movement and the spread of metastatic disease depend on conventional actin cytoskeletal patterns in cancer cells.

Cancer cells also use specialised actin structures called invadopodia to enter nearby or distant tissue and flee the main tumour. Both Arp2/3 and Formin activity help these protrusions develop, which are made up of both branched and unbranched actin filaments [7]. Neural Wiskott-Aldrich syndrome protein (N-WASp), the actin-binding protein, Fascin, and Ena/VASP proteins are among the regulating proteins involved in their development. Growth factors, cell-cell contacts, and cues from the ECM all play a role in the generation of invasions at various phases. To aid penetration, they degrade the ECM using matrix metalloproteinases (MMPs). They therefore stand in for important actin structures that support the development of malignancy[8].

LITERATURE REVIEW

In mammalian cells, the actin microfilament network is crucial for preserving cell structure and function. In cellular processes like cell adhesion, motility, cellular signalling, intracellular transport, and cytokinesis, it plays a variety of functions. The shape, motility, and adhesiveness of transformed cancer cells change, along with alterations in the way the cytoskeleton is organised. Because of this, cytoskeletal microfilaments are now potential targets for chemotherapy. Since actin-targeting medicines damage actin microfilaments in

both normal and malignant cells, they have not yet been tested in clinical studies. Actin filament communities need to be addressed more precisely to get around this issue. Actin filaments vary from one another, and there is mounting evidence that diverse groups of actin filaments exist within a cell and are physically organised into various cellular compartments, each of which serves a specific purpose. The related actin binding proteins play a major role in controlling the shape and operation of the actin cytoskeleton. Over 40 variants of the natural actin filament component tropomyosin (Tm) have been found in non-muscle cells. Tm variants are geographically distinct, and recent research indicates that they may be able to regulate the actin microfilament's functional potential. Therefore, the makeup of these functionally different actin filaments may play a significant role in deciding a cancer cell's capacity to survive. If the tropomyosin makeup of actin filament populations can be used to distinguish them from those of heart and skeletal muscle, this becomes a potent anticancer therapeutic strategy [9].

Invasion into the neighbouring tissue, intravasation, passage through the blood or lymph, extravasation, and development at a new location are all stages of the multi-stage process known as cancer cell metastasis. Cell movement, which is fueled by rounds of actin polymerization, cell adhesion, and actomyosin contraction, is necessary for many of these processes. Long-term in vitro research on these processes in cancer cells has frequently produced findings that appear to be at odds with one another. Understanding how the vast array of in vitro data links to the movement of cancer cells in living tumour tissue is now the task. Actin protrusion and acto-myosin contracture will be the main topics covered in this overview. We'll start by outlining some overarching concepts that summarise the generally recognised processes for the coordinated control of actin polymerization and contraction. Then, we'll go over more recent studies that look at how manipulating actin dynamics experimentally impacts cancer cell invasion in vivo and complicated settings [10]. 90% of cancer patients die from metastasis, making it the most important problem related to the illness. It follows that it is not surprising that many scientists are working to create medications that either target or avoid them. The development of additional tumour sites is closely correlated with processes such as the epithelial-to-mesenchymal transition and its reversal, the mesenchymal-to-epithelial transition.

The cells' ability to migrate is acquired during the transition of their trait to mesenchymal. The growth of invasive structures like invadopodia, lamellipodia, and filopodia makes it feasible for cancer cells to migrate. The actin cytoskeleton's rearrangement is necessary for these modifications. Actin-binding proteins in turn regulate the polymerization and depolymerization of actin. The accumulation of actin and actin-associated proteins in the nucleus of many tumour cells raises the possibility that actin may also influence the development of cancer by controlling gene expression. Once the cancer cell enters a new environment, it once more develops epithelium characteristics and begins to proliferate. A possible approach to the metastasis issue is to target epithelial-to-mesenchymal or/and mesenchymal-to-epithelial changes by regulating the expression of their key constituents [11]. lethal natural killer (NK) cell tolerance in breast cancer cells is mediated by the actin cytoskeleton.

We called this response, which occurred when a substantial portion of breast cancer cells reacted to NK-cell assault by accumulating a lot of F-actin nearby the immunologic synapse, "actin response." Direct proof that the actin response is linked to tumour cell resilience to

NK-cell-mediated cell death was given by live-cell imaging. When compared to susceptible cell lines, high-throughput imaging flow cytometry studies revealed that actin response-competent cells were considerably more concentrated in breast cancer cell lines that were extremely resistant to NK cells. The actin reaction was linked with decreased intracellular levels of the cytotoxic protease granzyme B and a lower rate of apoptosis in target cells but not with a deficiency in NK-cell activation. Granzyme B levels significantly increased in target cells after the actin response was inhibited by CDC42 or N-WASP knockdown, which was adequate to transform refractory breast cancer cell lines into an extremely susceptible phenotype. Using primary NK cells taken from donors as effector cells, the actin reaction and its protective effects were completely recapitulated [12].

There is a lot of debate right now regarding the role of autophagy in the development of cancer. It has to do with the potential of changing this process' character based on the therapy from cytotoxic to cytoprotective, and vice versa. While this is going on, cytoprotective autophagy may also play a role in antibiotic resistance, as it becomes more pronounced in patients who have a worse outlook.

Although the precise mechanism of this connection is not yet completely known, it has been proposed that a cytoskeleton may be one of the pieces of the puzzle. Actin's role in autophagy is receiving more and more focus in the most recent literature studies. This protein plays a crucial part in the development of autophagosomes, which are essential to the process. However, given that altering the actin pool is successful, it appears to be a viable option for overcoming cancer's autophagy-dependent multidrug resistance[13]. Regulating cell migration will result in effective cancer treatments because it is a crucial stage in tumour invasion and metastasis. Depending on the sort of cell and level of differentiation, cancer cells migrate in distinct ways. Different processes control the various subtypes of cell movement. The main process of cell movement and a prerequisite for the majority of cell migration types is reorganisation of the actin cytoskeleton.

Rho, Rac, and Cdc42 are examples of Rho family small GTPases that control actin rearrangement. To downstream effectors, these tiny GTPases deliver external chemotactic signals. Wiskott-Aldrich syndrome protein (WASP) family members are important cell migration factors among these downstream effectors. Protrusive membrane structures involved in cell movement and the breakdown of the extracellular matrix are created by activated WASP family proteins. Cancer cells' motility and infiltration are suppressed when Rho family small GTPase signalling is inhibited. Thus, it is possible to regulate cancer cell invasion and metastasis by modulating cell movement via the actin cytoskeleton[14] The actin cytoskeleton can be arranged in various ways to produce a variety of distinctive structures that affect the shape and movement of cells (Figure 1).

Cell movement and invasion are facilitated by actin frameworks(Figure.1). Changes in the actin cytoskeleton's control cause cancer cells (in blue) to migrate more easily, which promotes the spread of invasive diseases. At the leading edge, lamellipodia and filopodia stimulate forces that guide the cell in a specific path. Blebbing brought on by cell cortical reorganisation can promote amoeboid-like movement. Invadopodia enable invasion through the epithelium (green) and basal membrane in response to environmental stimuli (such as growth hormones, cell-cell contacts, and ECM signals).

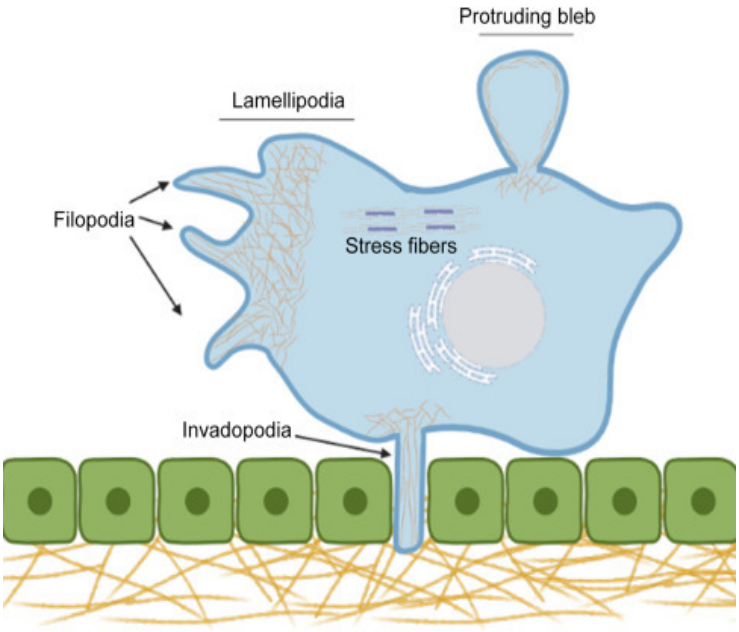

Figure 1: Cell migration and invasion in the presence of actin. Diagrame showingthe different role of the actin in the cell migration and cell invasion (Sciecne direct).

Actin bundles, crosslinked networks, and branched actin networks are three general categories for them. We won't go into detail about the various actin configurations or the molecules involved in their creation here because they have already been covered in-depth elsewhere. Migrating cells' lamellipodia and filopodia, both sustained by actin, make up their leading margin. Actin strands that have been branched together make up the majority of the lamellipodial network, which is produced at the leading edge of migrating cells and mimics a two-dimensional sheet. Arp2/3 activity, which is aided by nucleation-promoting agents like WASp family verprolin-homologous proteins (WAVE), and Formin activity (covered in the following parts) are both involved in the assembly of the lamellipodia [15]. Stress/contractile fibres, which are also composed of unbranched bundles of actin filaments and can be broadly divided into ventral stress fibres and transverse arcs, are additional actin-containing structures that are missing from the lamellipodia/filopodia areas.

Transverse arcs require Arp2/3, Formins, and myosin motor proteins, whereas ventral stress fibres require the action of Ena/VASP, Formins, and Arp2/3 complexes. Stress fibres typically work with focal adhesion complexes to help cells adhere to their surroundings, but their uncontrolled activity may also hasten the development of cancer. Recently, it was discovered that the increase of stress fibres encouraged breast tumour cell growth and stiffening before they developed more migratory traits. It's significant to note that disruption of normal actin dynamics in cancer cells can "sway" actin assembly in favour of a specific design (for example, from branched networks to straight protrusion, or vice versa), encouraging infiltration and metastases. It's conceivable that as an illness progresses, an actin regulator that is dominantly expressed receives the turnover of G-actin, which is typically split between a number of actin regulators.

Future therapeutic approaches must take into account the dynamic changes in cytoskeletal structures that occur during cancer cell migration because stopping just one pathway might

not be sufficient due to duplication and cancer cells' capacity to use other pathways. As a result, therapy intended to change one actin pathway may unintentionally increase invasiveness because G-actin concentrations are transferred to another main migratory pathway[1]. Both regulating the production of linear actin filaments and stabilising microtubules are shared functions of the Formins. The majority of the actin architectures described above that facilitate cell movement (such as lamellipodia, filopodia, cell cortex, and invadopodia) are created by Formin activity, so their dysregulation can result in increased cancer cell motility and invasiveness. As a result, these proteins act as key regulators in all cell types and are particularly important for cancer cell function (Table 1).

However, information on specific Formin family members raises the intriguing possibility that they may act as inhibitors of metastatic spread, potentially by preventing the cell's shift to the amoeboid movement that is promoted by cell cortex dynamics. The following part goes over the functions of members of the Formin family in the development of cancer. Numerous cancer kinds, including those of the kidney, prostate, endometrium, thyroid, and breast, have been discovered to have increased mDia1 mRNA. Therefore, it is probable that abnormal mDia1 transcript levels and/or activity give various cancers traits that are helpful for malignancy. It's possible that various FHOD isoforms control distinct elements of cell invasion and tumorigenesis. Furthermore, various cancer cell types might have varying needs for FHOD functionality to allow invasion into nearby tissues. Additionally, FMNL2 functions in CRC cells as a pro-motility and pro-metastasis regulator.

Table 1: Formin and its role in tumour progression and metastasis.

Protein name	Expression and activation during malignancy
Diaphanous (Dia)	Kidney, prostate, colon, HCC, LSCC, glioma, EOC, and breast cancer
Formin homology domain-containing protein (FHOD	Malignant cutaneous melanoma
Formin-like (FMNL)	Nasopharyngeal carcinoma, leukaemia, CRC
Dishevelled-associated activator of morphogenesis (DAAM)	Breast cancer
Spire-1, Spire-2	Breast cancer
Cordon-bleu (Cobl)	CLL, CML

It was discovered that type IV collagen-induced haptotaxis, or directional cell migration, of breast cancer cells, needed the association of Daam1 with the v3 integrin. Type IV collagen binding to integrin v3 triggers Daam1 supports the extension of invadopodia and causes haptotaxis. Malignant cells primarily spread to the lymph glands, bones, liver, lungs, and brain in breast cancer patients. In lymph node metastatic tumour cells, Daam1 is strongly expressed and triggered, according to clinical research in breast cancer patients.

CONCLUSION

Here, we discuss current studies on some of the actin cytoskeletal regulators involved in the initiation and progression of cancer, with an emphasis on nucleation factors and nucleation-promoting factors. In addition to being woven into an incredibly intricate cytoskeletal

network, a much broader collection of factors, including signaling/adaptor proteins and motor proteins, are clearly dysregulated and significantly contribute to the development of disease. Given the significance of the cytoskeleton's input to the pathophysiology of illness, targeting elements of the cytoskeleton as therapeutic methods for the prevention of cancer is an appealing idea. However, there are still significant challenges due to the complexity of cytoskeletal regulation and the duplication of pathways, as emphasised for some actin regulatory proteins in this study. It is anticipated that a better grasp of the processes by which different cytoskeletal proteins are dysregulated in cancer cells compared to healthy tissue will lead to the identification of promising targets for potential therapies. It is also intriguing to think about how expanding patient-specific data bases will aid in understanding how cytoskeletal regulatory circuits are disrupted during disease and how these findings might lead to the development of novel therapeutic approaches that are efficient without triggering alternative migration/invasion pathways. Additionally, the development of drug combinations that can concurrently block potential alternative routes used by cancer cells may advance cytoskeletal-targeting treatments.

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CHAPTER 6

BIOLOGICAL FEATURES OF THE INTERMEDIATE FILAMENTS

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ABSTRACT:

Intermediate filaments (IFs) are a diverse, essential, and ubiquitous component of the metazoan nuclear and cytoplasmic cytoskeleton. More than 70 genes, divided into six major classes, encode IF-forming proteins and are tissue-specific and differentiation-dependently regulated. A defining element shared by all IF-forming proteins is a central alpha-helical domain with long-range heptad repeats of hydrophobic residues, which promotes the formation of coiled-coil dimers, the most basic IF assembly subunit. Through interactions with various cellular proteins, IFs perform two major functions: structural support, without which incident physical trauma exposes an inherent fragility and leads to cell integrity loss, and regulation of several fundamental cellular processes such as growth, proliferation, and apoptosis.

KEYWORDS:

Alpha Helical, Domain, Desmosome, Intermediate Filament, Keratin.

Introduction

It took more than a century to discover that the proteins that form intermediate filaments (IFs) are members of a unified protein family, with members found in virtually all differentiated cells and both the cytoplasm and the nucleus [1]. Intermediate filaments (IFs) are cytoskeletal structural components of vertebrates and many invertebrate cells [2]. Branchiostoma, a cephalochordate, has been found to have homologs of the IF protein. Intermediate filaments (IFs) are made up of fibrous proteins with a central -helical rod domain that has a conserved substructure. This rod domain makes it easier to form dimeric coiled-coil complexes [3].In physiological salt solutions, intermediate filament assembly occurs spontaneously and does not require nucleotide triphosphates or proteinaceous cofactors. Intermediate filaments grow by incorporating subunits along their entire length as well as elongating by adding subunits to either end [4]. Subunits can consistently dissociate from intact filaments. The process is known as 'dynamic subunit exchange,' and it causes significant filament width inhomogeneity. Intermediate filament networks are extremely stable and retain their configuration even when cells are exposed to highly concentrated salt solutions and nonionic detergents [4].

The intermediate filaments are the most important component of the cytoskeleton, and they are made up of five major subgroups: vimentin, keratins, desmin, neurofilaments, and glial fibrillary acidic protein (GFAP), as well as a few minor subgroups (e.g., nestin, peripherin). The intermediate filaments appear ultrastructural as wavy unbranched filaments that frequently occupy a perinuclear location in the cell [5]. The original belief that intermediate filament expression was restricted to specific cell types (e.g., keratins in carcinomas, and

vimentin in sarcomas) has now been disproven [5]. The sections on intermediate filaments that follow focus not only on the normal pattern of expression of these proteins but also on situations in which intermediate filaments exhibit "anomalous expression." All mesenchymal cells express vimentin, a 57-kilodalton (kDa) intermediate filament protein. During early embryogenesis, vimentin is ubiquitously expressed in all cells and is gradually replaced in many cells by type-specific intermediate filaments [5]. Vimentin is commonly co-expressed with cell type-specific intermediate filaments in some mesenchymal tissues (e.g., desmin and vimentin coexpression in muscle cells, vimentin, and GFAP in some Schwann cells).

Vimentin is expressed in almost all mesenchymal tumors and thus has little utility in identifying specific tumors. Because vimentin and keratin are frequently coexpressed in carcinomas, vimentin expression has little value in distinguishing carcinomas from sarcomas immunohistochemically. Vimentin immunoreactivity has been promoted as a reliable indicator of tissue preservation[4]. However, vimentin expression, like that of all intermediate filaments, is fairly robust and may persist in tissues where all other immunoreactivity has been lost. 10 Vimentin-negative mesenchymal tumors, such as alveolar soft part sarcoma and perivascular epithelioid cell neoplasms, may occasionally be diagnosed by the absence of vimentin expression. In general, performing vimentin immunostains on any spindle cell neoplasm is pointless. Keratins, also known as cytokeratins, are the most complex intermediate filament protein family members, consisting of more than 20 proteins [4].

Keratins can be classified into acidic and basic subfamilies based on their molecular weights (40-67 kDa) or by their usual pattern of expression in the simple or complex epithelium Figure 1. Keratins are most commonly seen in practice as low-molecular-weight (LMW) keratins and high-molecular-weight (HMW) keratins. Keratins are highly sensitive markers for identifying carcinomas and are commonly used to differentiate epithelial/mesothelial tumors from nonepithelial tumors (e.g., lymphomas, sarcomas, melanomas)[6].

LITERATURE SURVEY

Intermediary filaments (IF, or "intermediate-sized" filaments), along with actin microfilaments, and microtubules (MT), are two of the three main types of protein filaments that make up the cytoskeletal networks of almost all eukaryotic cells. However, IF proteins make up a strikingly varied family with molecular weights ranging from 44 k to 120 k, unlike actin and tubulin, which are highly conserved molecules[7]. Long-known helical packing patterns of protein subunits in actin filaments and MT have been discovered, and their quaternary structures are immune to evolutionary divergence (like their primary sequences). However, despite significant advancements, a definitive determination of the molecular packing in any type of IF has yet to be established.

Therefore, the degree to which various IF are structurally related has not been clear aside from the fact that they have approximately comparable diameters[7]. In metazoans, intermediate filaments (IFs) are a varied, essential, and pervasive part of the nuclear and cytoplasmic cytoskeleton. More than 70 genes, divided into six main groups, code for IF-forming proteins, and their regulation depends on tissue type and differentiation IF (Figure.1)[4]. All IF-forming proteins share a central alpha-helical domain with long-range heptad repeats of hydrophobic residues, which promotes the creation of coiled-coil dimers, the most fundamental IF assembly subunit (Figure 2). By interacting with different cellular proteins, IFs regulate some basic cellular processes like growth, proliferation, and apoptosis.

Without structural support, incident physical trauma reveals an inherent fragility and results in loss of cell integrity[4].

The cytoskeleton proper in animal cells is made up of proteins from the intermediate filament (IF) supergene family, which are widespread structural elements. Since two-stranded coiled coils are the fundamental building elements of these incredibly flexible, stress-resistant cytoskeletal filaments, all IF proteins exhibit a distinctively organized, extended-helical conformation that is prone to forming them. Since IF proteins are strongly charged, they are polyampholytes that can serve a variety of purposes [5]. We provide a summary of the molecular and structural characteristics of vimentin, keratins, and nuclear lamins as our main examples. These in turn provide evidence for IF proteins' capacity to assemble into distinctive, highly diverse supramolecular assemblies and biomaterials, which can be found, for instance, at the inner nuclear membrane, throughout the cytoplasm, and in extremely complex extracellular appendages like hair and nails in vertebrate organisms [5]. Our ultimate goal is to pave the way for a more logical comprehension of the immediate effects that missense mutations in IF genes have on cellular functions and their significant influence on the emergence of the various IF diseases that are caused by them [5].

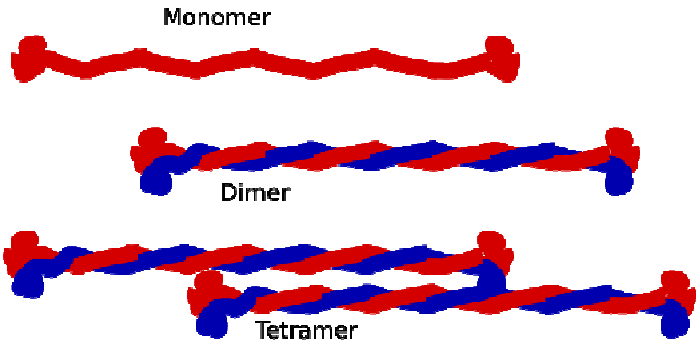

Figure 1: Intermediated filaments: Diagram showing the organization of the different forms of the intermediated filaments (Wikipedia).

The amino acid sequence of human epidermal keratin derived from cloned cDNAs was first used to predict the structure of proteins that make intermediate filaments (IF) [8]. The two types of keratins only have about 30% of their amino acid sequences in common, but they do have comparable secondary structure domain patterns, according to the analysis of a second keratin sequence. According to the first hypothesis, the central alpha-helical rod domain of all IF proteins is made up of four alpha helices (designated 1A, 1B, 2A, and 2B) that are spaced apart by three linker regions [9]. A coiled-coil structure, which is a pair of two intertwined proteins, serves as the fundamental building component of an intermediate filament. This term refers to the fact that both the intertwined pair and each protein's helical structure are helical. The two proteins that make up a coiled-coil bond together through hydrophobic interactions, according to a structural analysis of a pair of keratins [10]. The central domain's charged residues play a relatively minor part in the binding of the pair there [10]. Cytoplasmic IFs form non-polar unit-length strands after assembling (ULFs). Similar ULFs couple up laterally to form protofilaments, which pair up head-to-tail to form protofibrils, which pair up laterally to form staggered, antiparallel, soluble tetramers, four of which wind together to form an intermediate filament. Compaction, where ULF tightens and assumes a smaller diameter, is a stage in the assembly process [11]. The causes of this compaction are unclear, and it is frequently noted that IF has sizes between 6 and 12 nm [11].

Non-alpha helical regions, the N- and C-termini of IF proteins differ greatly among IF families in terms of length and structure. DNA is bound by the N-terminal "head region [12]."Vimentin heads can change nuclear architecture and chromatin distribution, and the liberation of heads by the HIV-1 protease may be crucial for the cytopathogenesis and carcinogenesis linked with HIV-1. The head area can be phosphorylated, which may impact filament stability. It has been demonstrated that the head and the rod domain of the same protein communicate [12]. The C-terminal "tail region" of various IF proteins varies greatly in length. Tetramers' anti-parallel orientation prevents them from acting as a foundation for cell motility and intracellular transport, unlike microtubules and microfilaments, which have a plus end and a minus end. Additionally, intermediate filaments lack a nucleoside triphosphate binding region, in contrast to actin or tubulin. Cytoplasmic IFs are active as opposed to microtubules and actin fibers, which treadmill [12].

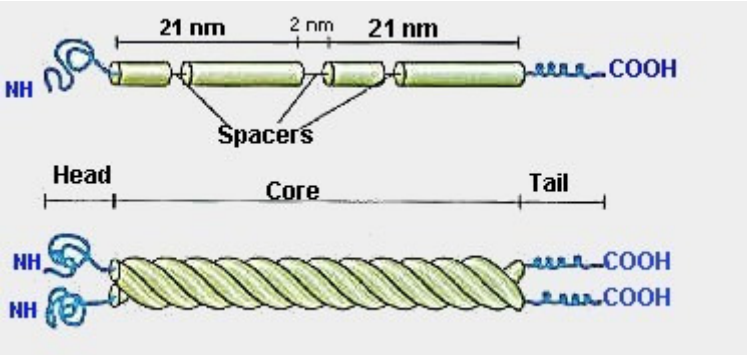

Figure 2: Intermediate filaments: Diagramed showing the Formation of the protofilaments(cytochemistry).

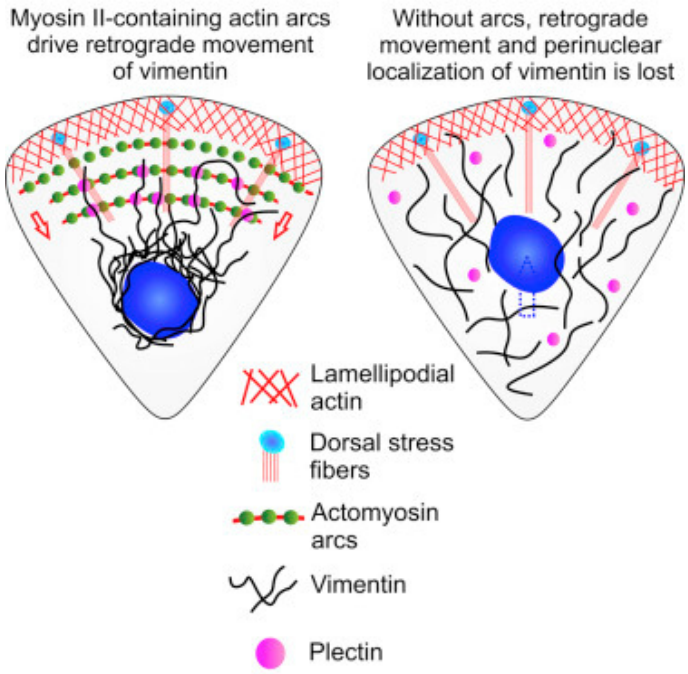

Figure 3: Vimentin: Organization of the vimentin intermediate filaments inside the cell (Research gate).

The intermediate filament proteins are encoded by roughly 70 distinct human genes. However, common traits among IFs of various types include: In general, they are all

polymers that, when completely assembled, have a diameter of between 9 and 11 nm[13]. Based on commonalities in amino acid sequence and protein structure, animal IFs are divided into six types: Acidic and neutral types I and II of keratins Intermediary strands of keratin (red-stained) surrounding epithelial cells. Cytokeratin proteins make up type I (acidic) and type II (basic) IF proteins and are the most varied among IFs. The numerous isoforms are split into two categories: Epithelial keratins (about 20) and trichophytic keratins (about 13), which are the building blocks of hair, nails, horns, and reptilian scales, are found in epithelial cells[13] Figure 4. Keratins are either acidic or neutral, depending on the group. A keratin filament is created when heterodimers of acidic and basic keratins bind to one another to produce an acidic-basic heterodimer[13].

Cytokeratin filaments laterally join to form a thick bundle with a radius of about 50 nm. The interaction of long-range electrostatic repulsion and short-range hydrophobic attraction determines the ideal radius of such packages[13]. These bundles would then cross each other at intersections to create a dynamic network that covered the cytoplasm of epithelial cells. Desmosomes and other cell organelles, such as desmin IFs, are connected to the cytoskeleton by the sarcomeres in muscle cells [13]. In astrocytes and other glia, there is a protein called glial fibrillary acidic protein (GFAP). Fibroblasts, leukocytes, and blood vessel endothelial cells all contain the IF protein known as vimentin, which is the most extensively distributed of all IF proteins (Figure 3). They provide structural support for the cell membranes, maintain some organelles in a fixed location within the cytoplasm, and send messages from membrane receptors to the nucleus[14]. The type IV family of intermediate filaments, known as type IV Alpha-internexin Neurofilaments, is abundantly present along the axons of mammalian neurons. Lamins are fibrous proteins that serve a morphological purpose in the nucleus of the cell. A and B-type lamins exist in metazoan cells, and they vary in length and pI. In the summary different types of the intermediated filaments class are listed here (Figure.4).

Class	IF protein	Cell type	Molecular weight (Kd)
I	Type I. acidic keratins	Epithelia	52.5–68
II	Type II. basic keratins	Epithelia	40.0–64
III	Vimentin	Mesenchyma	54
	Desmin	Muscle	52
	GFAP	Glial cells: Astrocytes	53
	Peripherin	Peripheral nervous system	57
	Plasticin	Retinal ganglion cells	52.6
IV	Neurofilament proteins L, M, H.	Central nervous system	68, 145, 200
	α-internexin	CNS neuronal cells	66
V	Lamins A, B, C	Nuclear membrane	60, 67, 70
VI	Nestin	Neuroepithelial cells	200

Figure 4: Differetn intermediate filaments: Diagrame Showing the details of the different types of the intermediated filaments (Research gate).

Three genes in human cells are differently regulated. There are B-type lamins in every cell. Lamin B1 and B2 of the B type are expressed from the corresponding LMNB1 and LMNB2 genes located on 5q23 and 19q13, respectively. A-type lamins only manifest themselves after gastrulation. The two most prevalent A-type lamins, lamin A and lamin C are splice variations of the LMNA gene, which is located at 1q21[14]. The nuclear lamina, a proteinaceous structure layer next to the inner surface of the nuclear membrane, and the nucleoplasmic veil throughout the nucleoplasm are the two locations where these proteins are

found in the nuclear compartment. The lamins have an additional 42 residues (six heptads) in coil 1b when compared to vertebrate cytoskeletal IFs. The nuclear localization signal (NLS), an Ig-fold-like domain, and, most frequently, an isoprenylated and carboxymethylated carboxy-terminal CaaX box are all found in the c-terminal tail region (lamin C does not have a CAAX box). The final 15 amino acids and their farnesylated cysteine are taken out of lamin A through additional processing. Lamins are phosphorylated by MPF during mitosis, which causes the lamina and the nuclear membrane to separate .

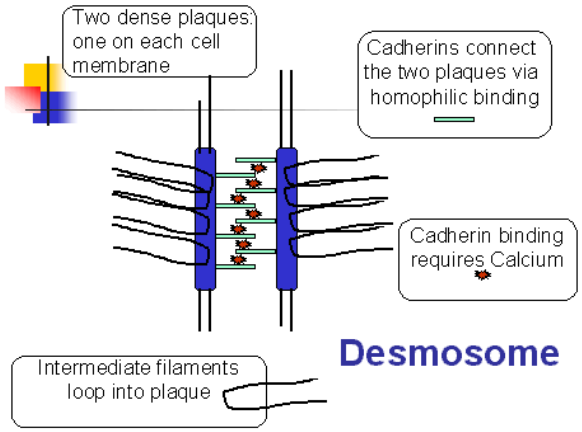

Figure 5: Desmosome: Diagramed showing the role of the intermediated filaments in the desmosome formation (cell biology and cytochemistry).

Some keratins or desmin engage at the plasma membrane through adapter proteins with desmosomes (cell-cell adhesion) and hemidesmosomes (cell-matrix adhesion) (Figure.5). In epidermal cells, filaggrin attaches to keratin fibers. Vimentin is connected to other vimentin strands, microfilaments, microtubules, and myosin II by lectin. Vimentin and tubulin may be connected by motor proteins, according to studies on kinesin. Desmosomes, which bind the cytoskeleton together, are connected to keratin filaments in epithelial cells via plakoglobin, desmoplakin, desmogleins, and desmocollins; desmin filaments are similarly linked in heart muscle cells (Figure 5).

CONCLUSION

The cytoskeleton proper in mammalian tissues is made up, depending on the cell type, of proteins of the intermediate filament (IF) supergene family. All IF proteins exhibit a distinctively organized, extended-helical conformation that is prone to create two-stranded coiled coils, which are the fundamental building blocks of these extremely flexible, stress-resistant cytoskeletal filaments. Since IF proteins are strongly charged, they are polyampholytes that can serve a variety of purposes. As our main examples, we provide a summary of the molecular and structural characteristics of vimentin, keratins, and nuclear lamins. These, in turn, show that IF proteins can assemble into distinctive, highly diverse supramolecular assemblies and biomaterials that are found, for instance, at the inner nuclear membrane, throughout the cytoplasm, and in extremely complicated extracellular appendages, like hair and nails, of vertebrate animals. The types of intermediated filaments and their purpose in cellular function are covered in the chapter summary.

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CHAPTER 7

DRUGS' IMPACT ON THE CYTOSKELETON

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ABSTRACT:

The cytoskeletal protein polymer microtubules and actin strands are essential for cell growth and division, signaling, and the establishment and preservation of cell structure. Because of their significance, they could be a focus for anti-cancer medications. The most common mode of action is suppression of cell proliferation through mitosis blockade with modification of microtubule dynamics. Recent years have seen the discovery of several new natural products that can be used to investigate the dynamics and functionality of the cytoskeleton. They include many hopeful novel chemotherapeutic medications. Designing safe medicines will be made possible by a growing understanding of antitumor effects and mechanisms of action. In this piece, we address medications that affect the cytoskeleton with a focus on paclitaxel and other molecules that share many of paclitaxel's biological properties.

KEYWORDS:

Actin-strand, Actin-cytoskeleton, Cytochalasin D, Cytoskeleton drug, Vinca alkaloids.

INTRODUCTION

Small compounds known as cytoskeletal drugs engage with actin or tubulin. There are three primary methods by which these medications can affect a cell's cytoskeletal elements. Some cytoskeletal medications, like taxol, which stabilizes microtubules, and phalloidin, which stabilizes actin strands, each stabilize a different part of the cytoskeleton. Others attach to actin monomers and stop them from polymerizing into strands, like Cytochalasin D. Demecolcine is one such medication that works by speeding up the depolymerization of already-made microtubules. Some of these medications affect the cytoskeleton in various ways. For instance, latrunculin stops actin polymerization while also speeding up its rate of depolymerization. The majority of the time, medications that target the microtubule are found in medical settings where they are effectively employed in the management of certain cancer types [1]. These medications have unacceptably undesirable off-target effects when used in animals because they lack selectivity for a particular variety of actin (i.e., they cannot differentiate between cardiac, smooth muscle, muscle, and cytoskeletal types of actin).

The actin-targeting compounds can nevertheless be applied on a cellular level to help advance our knowledge of how this intricate component of the cells' internal apparatus functions. For instance, the filamentous actin in frozen samples can be seen using phalloidin that has been coupled with a fluorescent sensor[1]. Both cytochalasin D and latrunculin are thought to be poisons produced by specific fungi and sponges to encourage filament depolymerization. Particularly, Latrunculin is a toxin produced by sponges, whereas Cytochalasin D is a fungal alkaloid. Despite having distinct processes, they both cause depolymerization. The addition of new components is prevented by cytochalasin D's binding to the (+) end of F-actin. In contrast, Latrunculin attaches to G-actin and sequesters it, stopping it from joining the filament end of F-actin. Cytochalasin D and Latrunculin break down the actin cytoskeleton when added to active cells, which prevents motility [2].

The roles of Cytochalasin D and Latrunculin are contrasted by other toxins produced by sponges, such as jasplakinolide and phalloidin (phallotoxins), which were identified from Amanita phalloides, or the "death cap" mushroom [3]. By promoting nucleation, one of the initial stages of G-actin polymerization [4], jasplakinolide binds to and stabilizes actin dimers while reducing the critical concentration, or the minimal concentration required to make filaments[4]. Phalloidin binds to and locks together F-actin components to stop strands from polymerizing. Phalloidin paralyzes a cell, which causes the cell to die[4].

A phalloides, a species of fungus, has been the source of phallotoxin isolates that have been linked to fatal instances of mushroom poisoning. Ingestion of the toxin most frequently results in liver and renal damage in humans, which can lead to symptoms like jaundice and convulsions, to name a few, and eventually mortality. Amatoxins, phallotoxins, and virotoxins are three categories of poisons that can be extracted from A. phalloides. These poisons have a 2–8-hour mortality time window. The virotoxins bind with actin and inhibit filament depolymerization similarly to the phallotoxins. In the end, these poisons disable the cytoskeleton's functions, crippling vulnerable cells[3].

Drugs can dismantle the dynamic cytoskeletal network of microtubules and actin strands. Drugs that affect the monomer-polymer balance change the size and quantity of macromolecular crowders inside cells, which is how cytoskeletal medicines function. Taxanes, vinca alkaloids, and epothilones are examples of microtubule inhibitors (MTI), which strengthen or disrupt microtubules. This suppresses the microtubule dynamics necessary for normal mitotic function, essentially stopping cell cycle advancement and inducing apoptosis. Taxanes are microtubule-binding medications that specifically target regions of polymerized microtubules' interiors. They work by attaching to GDP-bound -tubulin molecules and converting them to the more stable GTP-bound -tubulin structure, which stabilizes the GDP-bound tubulin molecules.Here, we provide an overview of the main reproductive events, such as gamete maturation, activation, fertilization, and early embryo development, as well as the structure, functions, and dynamics of microfilaments and microtubules[5]. We also discuss how cytoskeleton components are involved in these processes and how drugs affect the cytoskeletal network. In this chapter, we demonstrate how research on drugs may aid in understanding the function of the cytoskeleton in cells.

LITERATURE REVIEW

The actin cytoskeleton is known to have an impact on the mechanical characteristics of cells. In this paper, tether extraction and rheology studies using optical tweezers are used to explore the effects of drugs that interact with the actin cortex. On the dynamic characteristics of the cell, the effects of Blebbistatin, Cytochalasin D, and Jasplakinolide are assessed. Contrary to popular belief regarding jasplakinolide, the findings demonstrate that all three medications and therapies weaken the actin cytoskeleton, thereby reducing the strain in the cell membrane. When Cytochalasin D disrupted the actin cytoskeleton, the cell membrane bending elasticity increased. Jasplakinolide and blebbistatin did not have this impact. Only cytochalasin D was able to change the actin network into a more fluid-like structure, even though all medications reduced the cell's viscoelastic moduli by a factor of two. The findings can be explained by the interaction of the cytoskeleton's actin network and myosin dispersion as actin cross-linkers. By highlighting the roles that each one plays in these properties, this knowledge may help to improve comprehension of how the membrane and cytoskeleton are engaged in cell mechanical properties[6].

It has been challenging to determine how the various actin structures are assembled in cells and how they regulate cell behavior due to the high degree of the structural and molecular

complexity of the actin-based cytoskeleton, as well as its capacity to reorganize quickly and locally in response to stimuli and its force-generating properties. The targeted pharmacological disruption of actin structures is a clear method for examining the connections between actin organization, kinetics, and functions. The majority of our understanding regarding the role of actin in fundamental cellular processes was based on the widespread use of cytochalasins, which were the only agents that could interfere with cellular functions by binding to actin up until recently. The latrunculins, jasplakinolides (jaspamides), swinholide A, misakinolide A, halichondramides, and pectenotoxin II are just a few of the actin-targeted aquatic natural products that we have discovered in recent years (**Table 1**). These uncommon macrolides, which are all obtained from marine sponges, can be grouped into several large groups, each of which has a unique chemical structure. We outline the current state of knowledge regarding these compounds' actin-binding abilities and demonstrate how each drug class alters actin distribution patterns differently. We also demonstrate how even within a chemical class, structurally similar compounds can have different biochemical characteristics and cellular effects. We also talk about how these novel medications affect the development of fenestrae in liver endothelial cells as an illustration of their potential utility as instruments to specifically reveal actin-mediated dynamic processes [7]. The cytoskeleton of neurons gives them structure and rigidity, and cytoskeletal changes are essential for neuronal development and synaptic plasticity. The function of the cytoskeleton in forming drug memories has garnered greater attention in recent years due to the growing understanding that drug abuse results from improper learning of highly reinforcing cues[8].

Only about three decades ago, actin was discovered in non-muscle cells, and around the same time, it was discovered that malignantly changed cells had actin strands that were disrupted. All mammalian cells have an essential structural and functional structure called the actin network, despite its complexity. The fundamental framework for preserving cell shape and activities like adhesion, movement, exocytosis, endocytosis, and cell division is provided by actin fibers. Actin remodeling, or variations in actin polymerization, appears to play a crucial role in controlling the morphologic and phenotypic events of a malignant cell, according to mounting data from this group and others. Ras and Src, among other oncogenic actin signaling pathways, as well as the loss of several crucial actin-binding proteins with tumor suppressor roles can both cause actin rearrangement (e.g., gelsolin). Some of these genes exhibit distinctive protein expression patterns in cancer and the development of increasing malignant processes. Since it is now clear that the interaction of the small GTPases Rac, Rho, and Cdc42, which are members of the Ras superfamily, controls actin dynamics, attempts are being made to create particular inhibitors of these small GTPases as anticancer medications. This overview will cover the functional importance of actin change concerning malignant phenotypes, how actin remodeling is altered during the malignant transformation process, and methods of targeting actin remodeling for the creation of chemopreventive and chemotherapeutic drugs. It will be described how to directly modify actin polymerization using natural products, how to use tiny G protein actin pathway inhibitors, and how to use actin-binding protein gene augmentation[9].

At the moment, cardiovascular disease (CVD) medication development has mainly concentrated on tackling the immunopathology and inflammation elements common to different CVD traits like cardiac fibrosis and coronary artery disease. Recent research, however, points to novel biochemical mechanisms for controlling the cytoskeletal and extracellular matrix (ECM) in a variety of CVDs, such as the control of the cellular microenvironment by matricellular proteins like tenascin-C. Further evidence that the cardiac

cytoskeleton and ECM offer potential therapeutic possibilities comes from the effectiveness of anti-inflammatory medications like colchicine, which targets microtubule polymerization.

Another potential therapeutic target is microtubules. They take part in a variety of biological functions, such as cell reproduction and transport. Microtubule inhibitors (vincristine and vinblastine, alkaloids from the Madagascar periwinkle, also known as the Catharanthus roseus and formerly known as Vinca rosa), which were approved by the FDA in 1963 and 1965, and stabilisers Taxol (Paclitaxel) and Taxotere (Docatexel), which were approved in 1992 and 1996, respectively, are the two classes of microtubule medications. Additionally, vindesine and vinorelbine, two vincristine semi-synthetic compounds, are both microtubule inhibitors. Epothilones belong to a different family of anticancer medications that work on microtubules. Epothilones A and B are 16-membered macrolides that are spontaneously made by the myxobacterium Sorangium cellulosum or Myxococcus xanthus. Eribulin is a different all-natural substance that affects tubulin. It was extracted from Pacific seaweed and prevents tubulin from polymerizing. Combretastatin, another naturally occurring regulator of tubulin polymerization, is obtained from the root wood of the Combretum caffrum tree. Maytansine is another medication that attaches to microtubules. Microtubule formation is hampered[10].

For more than 50 years, vinca alkaloids have been recognized as effective antitumor medications. They are categorized as cytotoxic chemotherapy medicines that target rapidly proliferating cancer cells because they work during cellular mitosis. To escape the negative effects and general toxicity of "cytotoxic chemotherapies," like the vinca alkaloids, novel "targeted" therapies have emerged as a result of the growth of cancer drug research. Many people have forgotten that vinca alkaloids, taxanes, and similar medicines do have a particular molecular target: tubulin, as a result of their initial categorization. They remain some of the most effective anticancer medications, possibly as a result of their effects on the microtubule network, which go far beyond just stopping cells in mitosis and include inducing death at all stages of the cell cycle. In this overview, we emphasize the numerous cellular effects of altering microtubule dynamics, adding to our understanding of microtubule destabilizing substances and presenting fresh possibilities for their application in the treatment of cancer[11].

Taxanes, vinca alkaloids, and epothilones, which target the dynamic instability of microtubules and block spindle function, are common and efficient chemotherapeutic medicines. There is a need to find new antimitotic medications that can be used as anticancer agents because the manufacturing, solubility, effectiveness, and undesirable toxicities of these drugs are constrained.

Microturbines (Microtubule Inhibitors), a novel class of small synthetic compounds that target tubulin to inhibit microtubule polymerization, arrest cancer cells primarily in mitosis, activate the spindle assembly checkpoint, and cause apoptotic cell death, have been discovered and characterized by our team.

It's significant to note that the Microtubins do not challenge known vinca or colchicine binding locations. Additionally, we have discovered that particular modifications to the Microtubin phenyl ring can either trigger or inhibit its bioactivity through chemical synthesis and structure-activity association research. Together, these findings identify the Microtubins as a novel family of drugs that interfere with microtubule assembly to suppress the growth of cancer cells. These drugs may be used to create fresh cancer treatments[12](Figure 1).

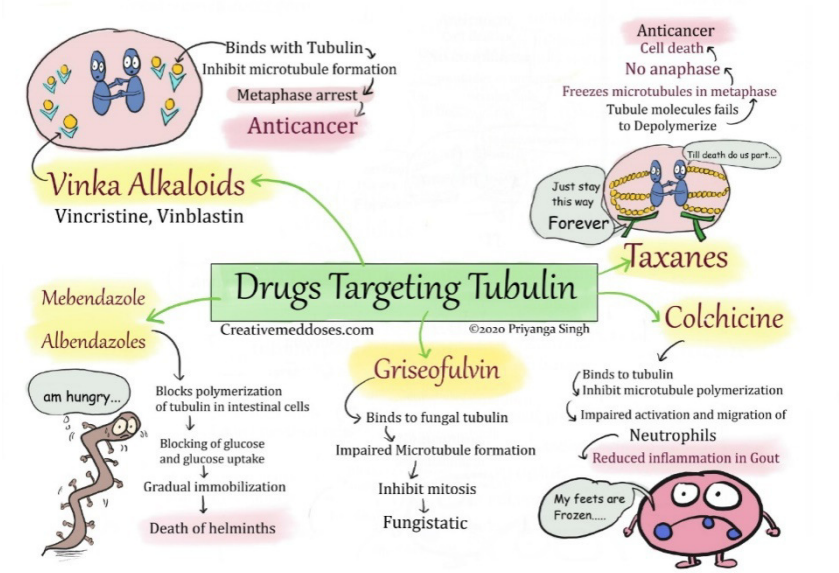

Figure 1: Drugs used against the microtubules. Cartoon diagram showing the different drugs against the microtubules treatment (Creative med doses).

Key elements of chemotherapeutic treatments for different solid tumors include antimitotic drugs that target the dynamic balance between the microtubule polymer and tubulin heterodimers. Based on how they affect microtubule polymerization and the number of microtubule polymers, these substances can be split into two main classes: those that block polymerization, like vinca alkaloids, and those that maintain microtubules, like taxanes and epothilones. The first antimicrotubule drugs authorized for use in solid tumors were the taxanes paclitaxel (Taxol) and docetaxel (Taxotere), but their utility is frequently constrained by the emergence of drug resistance. The epothilones are structurally and physiologically distinct from the taxanes, and it has been demonstrated that they are more potent than the taxanes in vitro and animal models. The epothilones have demonstrated action against taxane-resistant cancers and are immune to P-glycoprotein-mediated efflux. Other natural products with potential pharmacologic characteristics for stabilizing microtubules are also available. The causes of drug resistance are covered in this paper, along with the scientific and clinical evidence that suggests new microtubule-stabilizing drugs may be able to achieve wide antitumor effectiveness without developing drug resistance. At the time of presentation and in the presence of taxane resistance, the capacity of epothilones and other microtubule-stabilizing agents to lessen the formation of resistance may offer additional therapeutic choices[13].

Reorganization of the actin cytoskeleton is crucial for many cellular functions, including force production, cell movement, and preservation of cell structure. Small compounds known as cytoskeletal medicines influence cytoskeletal elements by either stabilising or destabilising them. Actin-binding medication swinholide A is produced from the sea sponge. Swinholide A breaks filaments and binds actin dimers. Finding out how Swinholide A modifies actin filament assembly dynamics in the presence of macromolecular crowding is the primary goal of this research. Swinholide A-mediated actin filament disassembly and severing is immediately visible using total internal reflection fluorescence (TIRF) microscope imagery. Actin filament lengths and the length distribution regulated by Swinholide A are calculated to assess filament disintegration and breaking. This research contributes to our understanding of the basic process by which Swinholide A influences the kinetics of actin assembly and disassembly. Further studies will enable the investigation of novel therapeutic strategies for a

variety of illnesses, including cystic fibrosis, which has pathogenetically high amounts of filamentous actin, as well as a medication to inhibit the rapid growth of cancers.

Table 1: List of the drugs used against the cytoskeleton.

Drugs	Target cytoskeleton	Effect	Application
Colchicine	Microtubule	Prevents polymerization	Used to treat gout
Cytochalasins	Microtubule	Prevents polymerization	None
Demecolcine	Actin	Prevents polymerization	Chemotherapy
Latrunculin	Microtubule	Depolymerizes	None
Jasplakinolide	Actin	Prevent polymerization, enhance depolymerization	None
Nocodazole	Actin	Enhances polymerization	None
Paclitaxel (taxol)	Microtubule	Prevents polymerization	Chemotherapy
Phalloidin	Microtubule	Stabilizes microtubules and therefore prevents mitosis	None
Swinholide	Actin	Stabilizes filaments	None
Vinblastine	Actin	Sequesters actin dimers	Chemotherapy
Rotenone	Microtubule	Prevents polymerization	Pesticide

Synaptic function alterations that last over time are linked to cocaine addiction. Synaptic plasticity is influenced by the cycling of actin between its polymerized [F (for filamentous)] and depolymerized forms, and the acute and chronic effects of cocaine discontinuation caused reversible and persistent increases in F-actin in the nucleus accumbens, respectively. Actin binding proteins (ABPs), which co-segregate with F-actin, experienced alterations in composition or phosphorylation after 3 weeks of repetitive cocaine use.

Increases in mammalian Enabled, phosphorylated (p)-cortactin, p-vasodilator-stimulated phosphoprotein, and actin depolymerization (e.g., decreased LIM (Lin11/Isl-1/Mec3)-kinase and p-cofilin) were all present in the changed APB profile, which was compatible with the formation of filopodia. Contrary to recurrent cocaine use, acute cocaine administration led to decreased actin cycling and depolymerization, which led to a rise in F-actin. The possibility that long-term cocaine use causes a rise in actin cycling The resumption of cocaine seeking in rats that had previously been taught to self-administer cocaine was investigated by either accelerating actin depolymerization with a LIM-kinase inhibitor or blocking actin polymerization with intra-accumbens latrunculin A. Actin cycling disruption by either method increased the resumption of drug use brought on by cocaine administration but had no effect on the locomotor reaction. The actin-ABP complexes are restructured as a result of repetitive cocaine use, increasing actin cycling and possibly modifying the resumption of drug use caused by cocaine[14].

CONCLUSION

New therapeutic approaches have been proposed as a result of recent insights into tumor signaling networks by cell- and molecular biology, which have also helped to advance the creation of innovative anticancer drugs. Oncological research is a highly dynamic area. Smart medicines, also known as so-called molecular mechanism-based medications that have been created to specifically target these signaling pathways may have potent antitumor effects. Because chemotherapy has poor cancer cell selectivity and a history of severe side effects, it may therefore seem theoretically inferior to other cancer treatments. Combination strategies, or regimens that combine the chemotherapeutic high killing potential with the molecular cause-targeting selectivity of new anticancer agents, maybe a rational synergic treatment, as well as allow the drug dose to be lowered with a corresponding decrease in drug-induced toxicity.

Single-agent anticancer therapy, even when carried out with "smart drugs," is frequently insufficient to eradicate the disease. In various clinical studies, various smart drug combos with chemotherapeutics are being investigated to encourage the re-expression of genes involved in cell division and the apoptotic process, eradicating the malignant disease by endogenous monitoring mechanisms. Several anti-cytoskeleton medications are currently being examined for use in tumor treatment. In addition to having a stronger anticancer potential by focusing on microtubule assembly dynamics, benomyl, griseofulvin, and some sulfonamides—drugs used to treat mycosis and bacteria—also show more constrained toxicity when compared to taxanes and Vinca alkaloids. To better understand microtubule/actin structure and dynamics to create new cytoskeleton-targeted drugs for more successful cancer treatment, will be a fascinating task for the upcoming years.

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CHAPTER 8

DISEASE-RELATED TO A MUTATION IN THE ACTIN

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ABSTRACT:

Nearly all cellular cells produce the protein actin, which is broadly distributed. There are six distinct genes in people and each produces a distinct actin variant. It has been found that other proteins may interact with actin to create compounds that can change cells' function when they are under cellular stress. These complexes might start or help the development of illnesses due to their fast creation.Each of these has been linked to disease-causing changes, the majority of which are missense. Mutational foci are identified through analysis of the location of the ensuing altered acids in the protein.Many of these take place in areas crucial for the synthesis of actin. With an emphasis on ventricular actin mutations, In this chapter quickly address the difficulties in identifying the consequences of these actin mutants.

KEYWORDS:

Actin Monomer, Missense Mutation, Thin Filaments, Nemaline Myopathy, Smooth Muscle.

INTRODUCTION

Actin, which can take the form of a globular protein (G-actin) that congregates into filaments (F-actin), is essential for cell growth, intracellular growth, muscular tension, and a variety of other processes. The human DNA contains six actin properties. Three of them are responsible for encoding the skeletal, smooth, or heart-actin variants (ACTC1, ACTA1, and ACTA2, separately). Two genes that specifically target smooth muscle and one that is broadly expressed (ACTG1) both encode -actin (ACTG2). The most prominent -actin is encoded by the final grade (ACTB). At the protein level, these actin subtypes are extremely well retained (>90%). Actin is a protein that can interact with a wide range of other proteins and is also open to a wide range of post-translational modifications.Actin is a protein that can associate freely with many other proteins and is also prone to a variety of unique post-translational modifications [1].

Actin's monomeric and filamentous forms have both been lit multiple times, with the latter drawing on developments in cryo-electron imaging. Furthermore, structures of F-actin have been found in complexes with a myosin engine cavity and/or with cofilin and tropomodulin, two F-actin strand authoritative proteins [2]. According to these structures, an apparent cleft connecting a nucleotide and a cation (Mg2+), the actin monomer is split into two sections (the internal and exterior regions).Each portion is divided into two areas, one of which contains subdomains 1 and 2, and the other of which contains subdomains 3 and 4. On the outside of the actin filament, subdomains 1 and 2 are located. Each actin monomer (subunit) in the actin fiber makes clever linear connections inside a protofilament along the actin fiber and along the side (between the two protofilaments), allowing each monomer to interatomically connect to its three surrounding subunits [3].

The currently known structures of G- and F-actin provide a wealth of information for understanding how actin interacts with myosin, how the actin monomer forms fibers, how it interacts with a variety of actin-authoritative proteins, and how disease-causing changes to actin affect its normal function.Each of the six actin characteristics has been broken down into disease-causing changes, highlighting how crucial actin is for normal cell function and behavior in a variety of cell types. The majority of these (>90% for five of the six actin characteristics) lead to missense changes in the protein, and frequently these transformations are severe [4]. The type of disease that changes in a particular actin quality typically induce mirrors its expression design, as discussed in detail below. Additionally, each actin component changes the entire cluster.any specific clusters or frequently used regions in the encoded proteins that contain these missense variants. The main goal of this chapter is to describe these missense mutations, identify any mutational "hotspots," and determine how a mutation in these genes causes disease [4].

LITERATURE REVIEW

Hereditary myopathies are clinically and genetically diverse diseases with varying degrees of skeletal muscle weakening. Actin myopathy, intranuclear rod myopathy, and nemaline myopathy are the three main types. The most prevalent of these myopathies, nemaline myopathy, is further split into seven categories based on its intensity, a propensity to advance, and age at the start. Congenital myopathies are currently related to five genes. These include troponin T (TNNT1), nebulin, -actin (ACTA1), and -tropomyosin (TPM3 and TPM2), and (NEB). All of their protein byproducts are parts of the sarcomere's narrow thread. The abnormalities found in these genes affect protein structure differently and result in various hereditary myopathies [5].

In both healthy and sick cells, cytoplasmic-actin promotes basic cellular functions such as cell attachment, motility, cytokinesis, and preservation of cell polarity. Cytoplasmic -actin is encoded by the gene ACTB, and mutations in this gene cause serious diseases with a variety of symptoms. Patients with congenital abnormalities, blindness, juvenile-onset dystonia (p.R183W), and neutrophil malfunction were found to have the two dominant heterozygous gain-of-function -actin variants p.R183W and p.E364K. (p.E364K). Actin's nucleotide-binding region is close to Arg183. The replacement of a tryptophan residue at position 183 creates an unusual stacking interaction with Tyr69, according to our findings from biochemical studies and molecular dynamics simulations. This interaction disturbs the release of nucleotides from actin monomers and the behavior of polymerization by causing a closed-state conformation. It appears that the substitution of a lysine residue for Glu364 acts as an allosteric trigger event that favors the development of the closed state [6]. Genetic compensation has been found as the absence of a mutant trait in homozygous mutant people caused by compensating gene expression initiated ahead of protein function. The existence of homozygous loss of function mutants in healthy human people indicates that compensation may not be limited to this paradigm, even though this interesting mechanism has been identified in zebrafish. Nemaline myopathy is caused by the loss of skeletal actin [7].

Discovery of the -cardiac actin gene (ACTC) in a family with hereditary hypertrophic cardiomyopathy as a new disease gene (FHC). With load values ranging from -2.5 to -6.0, linkage studies eliminated all of the previously described FHC loci as potential disease markers in the family under investigation. With a maximum load score of 3.6, ACTC was

found as the most probable disease gene through additional linkage studies of viable candidate genes that were strongly expressed in mature human hearts. In exon 5, near to two missense mutations were newly reported to produce the hereditary type of idiopathic dilated cardiomyopathy, ACTC mutation analysis identified an Ala295Ser mutation (IDC). The first sarcomeric gene for which two distinct cardiomyopathies are caused by alterations was ACTC [8].

End-stage renal failure is the result of the deadly nephrotic condition known as focal segmental glomerulosclerosis (FSGS) (ESRF). Inverted formin 2 (INF2) mutations have lately been linked to a hereditary cause of FSGS-associated nephrotic syndrome. INF2 is a member of the formin family of actin-regulating proteins. Actin strands can be polymerized and depolymerized by a special type of protein called INF2. It is unclear how INF2 variations cause illness. In the current research, we demonstrate that the FSGS-related variants E184K, S186P, and R218Q decrease INF2 auto-inhibition and boost interaction with monomeric actin [9].

The gene that produces the isoform of -actin that is almost exclusively expressed in skeletal muscle, ACTA1, has the most notable amount of minute alterations (over 220). Changes to ACTA1 are present throughout the cluster, and more than 92% of these changes result in one destructive amino acid replacement in the protein. Nemaline Myopathy is a non-progressive skeletal muscle infection that frequently manifests early in life, with extreme instances being identified at delivery. They are a prevalent source of this condition. This disease typically results from muscular weakness, particularly in the respiratory muscles, which can make respiration difficult but, in more severe instances, can also make swallowing difficult [10].One variant of -actin that ACTA2 encodes is strongly expressed in particular smooth muscle cells connected to the vasculature. Perhaps not unexpectedly, ACTA2 is the most frequently mutated gene in familial thoracic aortic aneurysms and is highly linked with changes in this gene. Cerebral arteriopathy is an uncommon but serious complication of ACTA2 mutations, with Arg179 being the most frequently mutated nucleotide [11].The extensively expressed gene ACTB, which encodes -actin, has about 70 known mutations.

Only 50% of the changes in this instance are missense variants. Given its extensive-expression, mutations cause a variety of defects, such as a particular facial look, cerebral impairment, hearing loss, cardiac and renal defects, brain anomalies, defects in neuronal migration, and muscular wasting, which are characteristic of the Baraitser-Winter syndrome. Growth retardation, developmental delay, and organ abnormalities are among the many consequences of the ensuing haploinsufficiency of -actin, though this phenotype is thought to be different from the signs and symptoms of Baraitser-Winter syndrome. Mutations in ACTB have also more recently been associated with bleeding disorders [12].

The gene ACTG1 encodes the second protein that is extensively produced, -actin. This is in line with the expression and function of -actin in the sensitive epithelium cells of the inner ear, where -actin and -actin work together to form the stereocilia. For sound to be detected, stereocilia distortion is necessary. The study also showed that actin (ACTG1) is not necessary for growth, possibly as a result of rising amounts of actin that helped make up for -actin loss. Baraitser-Winter syndrome is caused by a significant percentage of the residual ACTG1 mutations, which is in line with the extensive-expression of -actin in various organs. Due to the distribution of the affected residues for both kinds of illness, mutations in ACTG1 seem

to have a more restricted impact than other mutations.Only smooth muscle cells in the intestine, prostate, bladder and adrenal gland show this variant of actin. changes in The condition known as persistent intestinal pseudo-obstruction, visceral myopathy (or degenerative leiomyopathy), and Megacystis microcolon-Intestinal hypoperistalsis syndrome are all caused by ACTG2. These are all issues with gastrointestinal smooth muscle activity [4].

There are common mutational regions where the number of mutations tends to be greater than elsewhere, according to a study of the missense mutations for each of the actin isoforms, even though they are present throughout the genome. Skeletal-actin mutations account for a sizable portion of the overall number of missense mutations. In subdomain 2, the DNAse-1 loop (or D-loop) is a significant center for changes (SD2). Its capacity to attach to DNAse-1, which prevents the formation of F-actin, gave rise to its moniker, and this interaction was crucial in producing the first crystal structure for G-actin. Actin polymerization depends on the D-loop, which is also the focus of many actin-binding proteins. These include cofilin, which breaks actin filaments, and tropomodulin, which covers the pointed end of actin filaments to inhibit polymerization and depolymerization. The D-loop is crucial for polymerization because it participates in lateral (between the two protofilaments) and longitudinal (along the protofilament) interactions between actin monomers and because the occupancy of the nucleotide-binding site affects the shape of the D-loop [13].The D-loop receives information about the nucleotide state from the residues Ser14 and the modified His73, which can then change its location.

One of the two residues in the D-loop that are oxidized by MICAL (the protein engaging with CasL) is Met47. The other residue is Met44. The actin strand is rapidly and catastrophically depolymerized as a result, and the resulting monomers do not polymerize as effectively as actin monomers that have not been changed. Thus, in addition to the function of actin breaking proteins like cofilin, oxidation of Met44 and Met47 is another method for controlling actin polymerization. A longitudinal actin-actin M37-O-T351 contact is eliminated by changing Met47 to Leu, which stops the disastrous filament disintegration. All three -actin types as well as -actin have a mutation in Met47. Therefore, it might be anticipated that disease-related changes in this residue that prevent its oxidation would stabilize actin strands [4].

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Arg256, located in a helix in subdomain 4, and Pro70, located at the beginning of the sensor (His73) loop at the boundary, are two additional heavily mutated residues. Arg256 is also near to and probably interacts with Ile191 in H5. Actin-binding proteins are believed to attach to this network of interactions, which then signals the remainder of the actin molecule.Except for cardiac and -smooth actin, all actin isoforms have mutations in Pro70, which is located at

the beginning of the sensor loop (His73). Ophthalmic coloboma and Baraitser-Winter syndrome are both caused by mutations to Leu in -actin.In four of the six variants, Gly268 mutations are located in the hydrophobic plug (FIGM). One of only two lateral contacts between actin monomers in the filament, in which the hydrophobic plug engages with the D-loop, is crucial for the hydrophobic plug.

The interactions between Gly268 and His40 were mentioned above. Two additional changes (to Asp or Cys) in skeletal -actin also cause nemaline myopathy. This residue is changed to Arg in both skeletal and smooth -actin, resulting in nemaline myopathy and aortic disease, respectively. Deafness and Baraitser-Winter syndrome are caused by similar mutations in - and -actin, respectively. Gly268 mutation is expected to reduce the lateral contact between adjacent monomers and impair the actin filament's stability.Deafness and Baraitser-Winter syndrome are caused by similar mutations in - and -actin, respectively. Gly268 mutation is prone to decrease the horizontal contact between neighboring monomers, causing the actin filament to become unstable. Intriguingly, different actin proteins have mutations of His40 and Gly268 [4].

Even though changes can have predictable effects, speculating on those effects remains a difficult task. Consider a few instances of changes in cardiac -actin that are thought to be linked with myosin and how they lead to illness as an illustration of this difficulty. These include modifications to segment 3, the exterior location of the D-loop, and the N-terminal region of actin (between buildups 311 and 335). The exterior location of the D-loop is a part of the "Milligan" contact, in which actin SD1 and the adjacent actin subunit's D-loop are interatomic partners with myosin loop 3 (H551-G576) within the L50 area. However, each myosin essentially links to SD1 buildups, SD2 (such as the D-loop of the adjacent actin), and SD3 intuitive connections.

The actin region that engages with the cardiomyopathy loop in myosin and the tropomyosin binding site in the absence of Ca2+ is where the DCM-causing mutation A331P (Ala331Pro) is located. However, this variant appears to affect myosin attachment indirectly by altering tropomyosin's behavior rather than directly. Recent research using the baculovirus/insect cell expression method and recombinant cardiac -actin demonstrated that it polymerizes more quickly than wild-type actin, but that the contact between myosin and actin in reassembled thin filaments is weakened. It was hypothesized that this mutation would impact how tropomyosin interacts with adjacent residues, especially residues D25, R28, and P33, which together determine tropomyosin's location on actin when it is in the "off" position.

By decreasing the probability that tropomyosin will shift from its location in the "off" state to the "on" state, A331P may increase the potential for tropomyosin to suppress the actomyosin interaction, which would account for the reduced myosin interaction with the reconstituted thin filament. Myosin binding protein C (MYBPC) C0C2 monomers' binding propensity to actin was also lowered by A331P. To control contraction, C0-C1 engages with actin and tropomyosin. According to confidential research from our lab, eGFP-A331P was able to integrate into muscle sarcomeres, and although we did not detect a substantial impact on contraction, this may rely on the amounts of expression [4].

The HCM-causing mutation E361G (Glu361Gly) is near the C-terminal myosin binding area of actin. However, it appears to work by influencing the thin filament's Ca2+ sensitivity, which in turn affects how myosin binds to actin. In transgenic rodents, E361G binds to fine

filaments in the cardiac muscle. In addition, our unpublished research demonstrates that, when produced using an adenoviral system in isolated cardiomyocytes, eGFP-E361G integrates properly into thin filaments and has no effect on cardiomyocyte contraction. In vitro motility experiments using thin filaments isolated from the transgenic mice with E361G expressed at 50% of the total actin demonstrate normal myosin-driven motility, and the animals have normal myosin-driven locomotion.The transgenic mice have a very mild phenotype, and thin filaments separated from the animals with E361G expressed at 50% of the total actin exhibit typical myosin-driven motility in vitro motility tests. The phosphorylation of troponin I do not, however, affect the sensitivity of heart muscle in these animals[4].

Protein kinase A (PKA) phosphorylates the heart troponin-I N-terminal peptide sequence in reaction to 1-adrenergic signaling Through its association with troponin C, this lowers troponin C's propensity for binding Ca2+, speeding up Ca2+ dissociation and raising the rate of twitch relaxation. As a result, the heart rate can rise, increasing power production. Therefore, the usual connection between 1 adrenergic signaling, Ca2+ sensitivity, and troponin I phosphorylation is broken by the actin E361G mutant.Last but not least, it is also possible that the mutation Arg312His (R312H), which causes DCM, will tangentially impact myosin binding to actin by altering the actin-tropomyosin association. In in vitro motility tests, the myosin-driven velocity of reconstituted actin R312H strands is decreased at high Ca2+ concentrations but increased at low Ca2+ concentrations [14].

Strong myosin attachment to the mutant actin was unchanged, though.Last but not least, it is also possible that the mutation Arg312His (R312H), which causes DCM, will tangentially impact myosin binding to actin by altering the actin-tropomyosin association. In in vitro motility tests, the myosin-driven velocity of reconstituted actin R312H strands is decreased at high Ca2+ concentrations but increased at low Ca2+ concentrations. Strong myosin attachment to the mutant actin was unchanged, though.Recombinant actin made from insect cell lines was used in both experiments. In our private research, we discovered that eGFP-R312H could integrate into muscle sarcomeres in isolated adult rat cardiomyocytes with no impact on contractility. The consequences of this mutation may be more likely to be explained by a shift in Ca2+ sensitivity, mediated through troponin/tropomyosin, given that the mutant actin appears to be able to form thin strands in cells [15].

CONCLUSION

The difficulties of attempting to comprehend the effects of these mutations are illustrated by the studies of a limited number of mutations in cardiac -actin described in the preceding section. The fact that only a small number of actin mutations have been thoroughly characterized despite the high number of actin mutations described may not come as a surprise then.

The consequences of many of these mutations, especially those in smooth and non-muscle actin isoforms, have not been thoroughly investigated even though disease-causing mutations are found in all six actin isoforms. Although the effects of these mutations can be anticipated based on where they are located in the structure, experimental testing is still necessary to fully grasp their possibly complicated effects. It may be worthwhile to investigate straightforward mammalian expression systems to analyze the effects of these mutations due

to the prevalence of mutations in comparable areas of the genome across the various actin isoforms and their probable effects on filament stability.

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CHAPTER 9

FUNCTIONAL ORGANIZATION OF THE MICROTUBULES

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ABSTRACT:

Microtubules are hollow tubes with a component of the cytoskeleton of eukaryotes. When accessory proteins engage with microtubules to form larger structures like the mitotic spindle, such as "railways" for motor-driven intracellular transport, the remainder of the cell is given an organizational framework. Microtubules' "dynamic" nature is essential to these processes. Dynamic instability, a surprising behavior associated with microtubule turnover, includes individual polymers switching stochastically between growth and depolymerization. Microtubules can explore intracellular space and remodel in reaction to intracellular and extracellular cues thanks to dynamic instability. Numerous proteins engage in interactions with microtubules inside the cell and are crucial for processes like microtubule development, stabilization, and destabilization as well as interactions with chromosomes during cell division. Microtubules are used as transport routes by the motor proteins kinesin and dynein, which are also involved in cell division.

KEYWORDS:

Microtubules, Cell, Cytokinesis, Dynamics, Eukaryotes.

INTRODUCTION

Early microscopists like Leeuwenhoek observed processes like cell locomotion that were controlled by tubulin and microtubules (1677). However, it wasn't until the development of better light microscopes, two centuries later, that the fibrous nature of flagella and other structures was realized. The electron microscope and biochemical research in the 20th century verified this discovery [1] .Research on microtubule motor proteins like dynein and kinesin is done in situ by fluorescently labelling a microtubule, attaching the microtubule or motor protein to a microscope slide, and then using video-enhanced microscopy to observe the slide and track the movement of the motor proteins [2]. This enables the motor proteins to travel along microtubules or the microtubules to move over the motor proteins.

Microtubules are one of the three major classes of cytoskeletal filaments in eukaryotic cells, along with actin and intermediate filaments. All known eukaryotic organisms contain microtubules. Microtubules were therefore present in the last common ancestor of eukaryotes, along with the dynein and kinesin motors that control the microtubule cytoskeleton [3]. The most prevalent gene that encodes FtsZ, a protein that forms polymers implicated in cytokinesis, is found in many prokaryotes. It is homologous to the tubulin gene and is present in many prokaryotes. The tubulin gene family may have evolved very early, possibly in the last common ancestor of all life on Earth, according to these findings [4]. Even without knowing anything else about microtubules, the fact that these structures and the proteins that make them up have survived over such a long period of time and in such a wide

range of organisms suggests that they play a crucial part in eukaryotic cell biology [5]. The various cellular shapes that can be found in parasitic kinetoplastids are defined by the subpellicular microtubule array. However, little progress has been made in figuring out the molecular processes underlying array differentiation during their life cycle or the apparent stability and longevity of array microtubules. Morphological studies have described array organization [6].

Using cryoelectron microscopy and image processing, construct intact microtubules in three dimensions at a resolution of about 8, which was adequate to resolve most of the secondary structure. The interactions between adjacent protofilaments, which are crucial for microtubule stability, are better understood thanks to this study. It also raises the possibility that some structural characteristics of microtubules differ from those of the zinc sheets used to model the tubulin structure [7]. All eukaryotes require microtubules, a type of polymer, for a variety of reasons, including cell growth and transport.

The exact spatial and temporal pattern of microtubules that is seen throughout the cell cycle is the outcome. The study of microtubule function and regulation as well as the mechanism of action of antimitotic medicines that alter typical microtubule behaviour have both benefited from recent high-resolution analyses of the structures of tubulin and the microtubule [8]. Microtubules disassemble (prophase) and a mitotic spindle is formed up (metaphase) during mitosis to handle the chromosome separation that follows (anaphase). Following the disintegration of the microtubular cytoskeleton, the rough endoplasmic reticulum vesiculates, the partial degeneration occurs, and the stacks of Golgi cisternae are dispersed.

The radiating microtubule pattern is restored and the rough endoplasmic reticulum and Golgi complex regain their typical interphase structure following the completion of the nuclear division (telophase) [9]. Microtubules are hollow tubes with a diameter of about 25 nm that are a component of the cytoskeleton of eukaryotes. They are made of -tubulin heterodimers that join to create protofilaments that run lengthwise along the microtubule wall, with the orientation of the -tubulin subunit towards the microtubule plus end indicating structural polarity. The tubulins and are very stable. The nucleation and assembly of microtubules are regulated by -tubulin, a third component of the tubulin family. There is evidence that additional tubulin family members participate in microtubule formation [5]. Microtubules are primarily made of "GDP-tubulin," which is stabilised at the plus end by a short "cap," after the hydrolysis of GTP linked with -tubulin occurs in conjunction with microtubule assembly. Dynamic instability, which is defined by growth that is haphazardly interrupted by pauses and shrinkage, is a crucial characteristic of microtubules. Numerous proteins engage in interactions with microtubules inside the cell and are crucial for processes like microtubule development, stabilization, and destabilization as well as interactions with chromosomes during cell division[5].

LITERATURE REVIEW

Microtubules structure:

Microtubules are long, hollow cylinders found in eukaryotes that are composed of polymerized beta- and beta-tubulin dimers. The lumen refers to the interior region of hollow microtubule tubes. At the amino acid level, the and -tubulin subunits are 50% similar and both have a molecular weight of about 50 kDa. These /-tubulin dimers polymerize end-to-end

into linear protofilaments that join laterally to create a single microtubule. This microtubule can then be lengthened by the addition of additional /-tubulin dimers. Although microtubules made of fewer or more protofilaments have been seen in different species as well as *in vitro*, thirteen protofilaments typically form a parallel association to form microtubules. The biological role of microtubules depends on their distinct polarity. The -subunits of one tubulin dimer interact with the -subunits of the following dimer as tubulin polymerizes end to end. As a result, in a protofilament, one end will have the visible -subunits while the other end will not. The (-) and (+) ends, accordingly, are given these designations. In a microtubule, there are two ends, the (+) end and the (-) end, both of which have only visible -subunits because the protofilaments bundle parallel to one another with the same polarity. Although microtubule elongation can occur at both the (+) and () extremities, it happens at the (+) end much more quickly Figure 1.

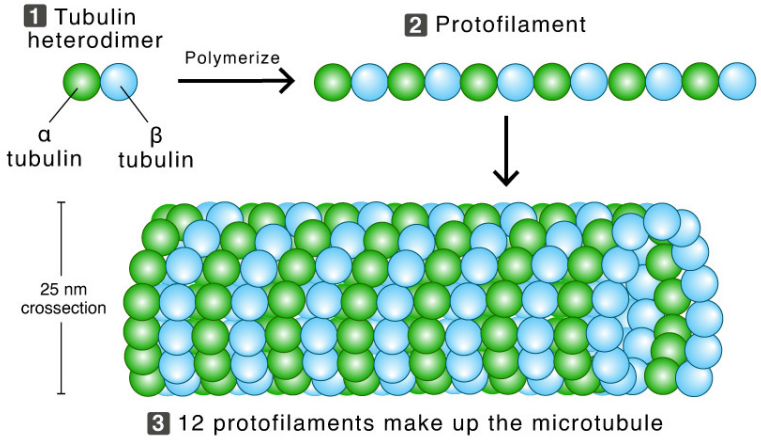

Figure 1: Structural organization of microtubules: Schematic diagramed showing the organization of the microtubules (Science fact).

A pseudo-helical structure is created by the lateral association of the protofilaments, and one turn of the helix contains 13 tubulin dimers, each from a distinct protofilaments (Figure.1). Due to the helicity of the turn, the 13th tubulin dimer engages with the next tubulin dimer in the most typical "13-3" architecture with a vertical offset of 3 tubulin monomers. Other alternative designs with a much lower frequency have been identified, including 11-3, 12-3, 14-3, 15-4, and 16-4. In protist creatures like foraminifera, microtubules can also change into other shapes, such as helical filaments. The A-type and B-type lattices are two different kinds of interactions that can take place between the lateral protofilament subunits within the microtubule. The lateral associations of protofilaments in the A-type lattice involve the interaction of neighbouring and -tubulin subunits (i.e., a -tubulin subunit from one protofilament engages a -tubulin subunit from an adjacent protofilament). The and -tubulin subunits of one protofilament engage with the and -tubulin subunits of the adjacent protofilament, respectively, in the B-type lattice. According to experimental research, the B-type lattice is the predominant configuration within microtubules. The majority of microtubules do, however, contain a seam where tubulin subunits communicate with one another.

Thus, the precise order and molecular make-up of microtubules can be summarized as follows: Since they are heterodimers (made up of two different polypeptides, -tubulin and -tubulin), a -tubulin connects with a -tubulin in the setting of an absent covalent bond [10].

Therefore, following the formation of the heterodimers, they unite to create long chains that ascend allegorically in one direction (e.g. upwards). Protofilaments are created when these heterodimers are connected in a specific orientation. Now that these lengthy strands (protofilaments) have grown close to one another over time, a tube-like structure with a tube-like lumen has developed. As a result, the exterior wall of the microtubules is composed primarily of 13 protofilaments.

Additionally, it's crucial to remember that the heterodimers have a positive and negative end, with beta-tubulin constituting the positive end and alpha-tubulin acting as the negative end. There is always a negative and positive end because the heterodimers are piled on top of one another. Heterodimers are added to microtubules at the plus end to increase their size [10]. In some Prosthecobacter species, microtubules are also present. Similar to eukaryotic microtubules, these bacterial microtubules have a hollow tube-like shape made of protofilaments assembled from heterodimers of bacterial tubulin A (BtubA) and bacterial tubulin B. (BtubB)[11]. Features of both - and -tubulin are shared by BtubA and BtubB. Bacterial microtubules can coil on their own, unlike eukaryotic microtubules Bacterial microtubules only have five protofilaments as opposed to the 13 protofilaments found in human microtubules [11].

Microtubule polymerization:

Nucleation

The process that starts microtubule creation from the tubulin dimer is known as nucleation. Typically, microtubule-organizing centres are the organelles that nucleate and arrange microtubules (MTOCs). Another form of tubulin, -tubulin, which is distinct from the - and - subunits of the microtubules themselves, is located within the MTOC. The "tubulin ring complex" (-TuRC), which is made up of the -tubulin and several other related proteins, resembles a lock gasket. This complex serves as a template for the polymerization of /-tubulin dimers and caps the (-) end of the microtubule as it grows away from the MTOC in the (+) orientation [12].

Most cell kinds' main MTOC is the centrosome. Microtubules can, however, also form at other locations. For instance, the basal bodies of cilia and flagella are MTOCs. Golgi-associated microtubule nucleation may enable the cell to establish asymmetry in the microtubule network because nucleation from the centrosome is inherently symmetrical[13]. It has been demonstrated to bind with -TuRC and raise the density of microtubules near the mitotic spindle origin. Some cell types, like plant cells, don't have MTOCs that are clearly characterized. Microtubules in these cells form at specific locations in the cytosol. A MTOC is present in other cell types as well, but it is always located at the base of the flagellum in trypanosomatid pathogens. In this instance, a canonical centriole-like MTOC is not responsible for the nucleation of microtubules for structural functions and for the generation of the mitotic spindle[6].

Polymerization

The growing polymer needs to be supplemented with tubulin monomers after the original nucleation event. The process of adding or deleting monomers is dependent on the ratio of the concentration of -tubulin dimers in solution to the critical concentration, or the steady-state dimer concentration at which there is no longer any net assembly or disassembly at the end of

the microtubule. The microtubule will polymerize and expand if the dimer concentration is higher than the critical concentration (Figure 2). The length of the microtubule will shorten if the concentration falls below the crucial concentration [5].

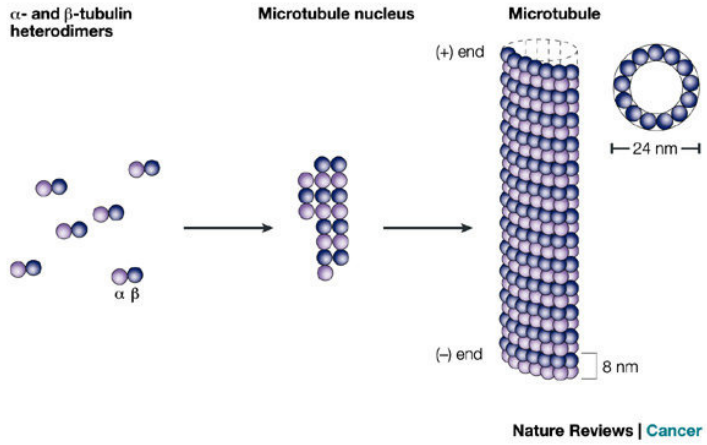

Figure 2: Microtubule growth: Diagramed showing the microtubule nucleation and polymerization (Nature reviews).

Microtubule dynamics:

Dynamic instability

The cohabitation of assembly and disassembly at the ends of a microtubule is referred to as dynamic instability. In this region, the microtubule can dynamically transition between the expanding and contracting phases. Two GTP molecules can be bound by tubulin dimers, one of which can be cleaved after the dimer is formed. The tubulin dimers are in a GTP-bound condition during polymerization [5].When GTP is attached to -tubulin, it is stable and serves a structural purpose. However, soon after assembly, the GTP attached to -tubulin may be hydrolyzed to GDP. Since GDP-tubulin is more likely to depolymerize than GTP-tubulin, its assembly characteristics are distinct from those of the latter (Figure 3).

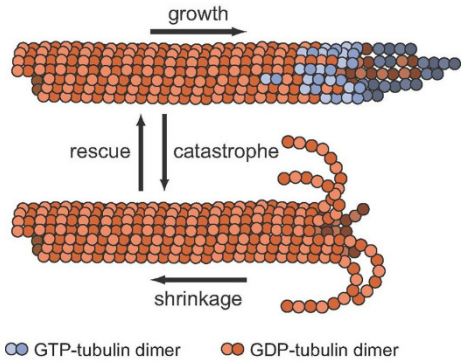

Figure 3 : Microtubules dynamics: Microtubules structure is highly dynamins, growth and shrinkage regulates the microtubules dynamics(Research gate).

Although a GDP-bound tubulin subunit in the centre of a microtubule cannot spontaneously extrude from the polymer, the GDP-bound tubulin subunit at the tip of a microtubule tends to fall off. It is suggested that a cap of GTP-bound tubulin exists at the tip of the microtubule to

prevent it from disassembling because tubulin adds onto the end of the microtubule in the GTP-bound state. Hydrolysis starts a fast depolymerization and shrinkage when it reaches the tip of the microtubule. A catastrophe is the change from development to shrinkage. Tubulin that is GTP-bound can start re-adding to the microtubule's tip, creating a fresh cap that prevents the microtubule from contracting. "Rescue" is the term used for this[5].

Functions of microtubules

The plus extremities of microtubules are frequently confined to specific structures. Microtubules are disproportionally oriented from the MTOC towards the location of polarity, such as the leading edge of migrating fibroblasts, in polarised interphase cells. This arrangement is believed to facilitate the transfer of microtubule-bound vesicles from the Golgi to the polarity site (Figure 4). The majority of crawling mammalian cells must migrate, and this is also dependent on the dynamic instability of microtubules. It can be said that microtubules function to create directionality and to impede cell movement [8].

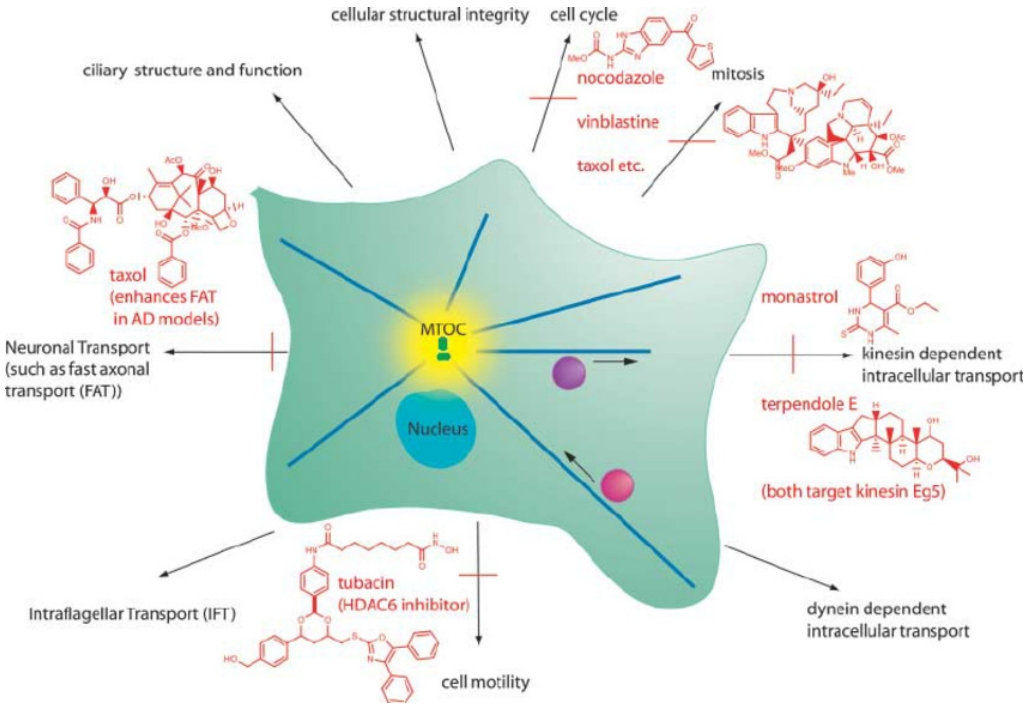

Figure 4: Functions of the microtubules inside the cell. The function of microtubules in different cellular processes is presented schematically (Science direct).

Eukaryotic cilia and flagella are largely structurally dependent on microtubules. Always, cilia and flagella stretch straight from the basal body of an MTOC. The various microtubule strands that travel along a cilium or flagellum can bend and produce force for swimming, moving extracellular material, and other functions thanks to the activity of the dynein motor proteins on these strands. The microtubules are rearranged so that their (-) ends are situated in the lower portion of the oocyte as a result of signals sent between the follicular cells and the oocyte, polarizing the structure and causing the appearance of an anterior-posterior axis[8]. Mammals also exhibit this participation in the structural makeup of the body. In higher

vertebrates, where the dynamics of tubulin and its associated proteins, such as microtubule-associated proteins, are carefully regulated during the formation of the nervous system, microtubules play a key role in this process. The cellular cytoskeleton is a dynamic system with a wide range of activities. It can affect gene expression in addition to providing the cell a specific shape and facilitating the transport of vesicles and organelles. There is little knowledge of the signal transduction processes used in this communication[8]. However, the connection between the specific expression of transcription factors and the drug-mediated depolymerization of microtubules has been described, which has given information on the various ways that genes are expressed based on the presence of these factors[8].

CONCLUSION

Eukaryotic cells' cytoskeleton is made up of tubulin molecules called microtubules, which give eukaryotic cells their shape and structure. Microtubules are necessary for a number of vital procedures like cell motility, mitosis, and intracellular transfer.

The dynamic characteristics of microtubules make this feasible. Numerous microtubule plus-end-binding proteins, also known as + TIPs, closely regulate many of these characteristics. These proteins are in the ideal position to regulate microtubule dynamics because they are aware of the distal end of microtubules. Microfilaments play a part in cytoplasmic streaming, which is the movement of cytosol (cell fluid) throughout the cell, as well as the partition of cytoplasm during cell division. Microtubules are smaller than intermediate filaments but larger than microfilaments. They offer structural support and aid in giving the cell its form.

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CHAPTER 10

HISTORY OF THE CYTOSKELETON

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ABSTRACT:

In all life domains, the primary structure is a network of intracellular filaments that is essential for cell shape, division, and function. Prokaryotes' simple cytoskeletons are surprisingly plastic in their makeup; none of the essential filament-forming structures are conserved across all groups. On the other hand, eukaryotic cytoskeletal function has undergone significant gene duplication and specialisation, the addition of accessory proteins, and so on. Before the last common ancestor of eukaryotes, a large portion of this diversity developed. The probable prokaryotic line that underwent this eukaryotic transition is constrained by the spread of the cytoskeletal filaments.

KEYWORDS:

Archaea, Cytoskeleton, Conserve, Eukaryotes, Prokaryotes,

INTRODUCTION

In 1903, it was proposed that the shape of cells was determined by a network of tubules that he termed the cytoskeleton. The concept of a protein mosaic that dynamically coordinated cytoplasmic biochemistry was proposed by Rudolph Peters in 1929 [1]. while the term (cytosquelette, in French) was first introduced by French embryologist Paul Wintrebert in 1931[1]. When the cytoskeleton was first introduced, it was thought to be an uninteresting gel-like substance that helped organelles stay in place [2].

Much research took place to try to understand the purpose of the cytoskeleton and its components. With the help of a researcher, it was discovered that microtubules vibrate within neurons in the brain, suggesting that brain waves come from deeper microtubule vibrations [3]. This discovery demonstrated that the cytoskeleton is not just a gel-like substance and that it has a purpose.

Initially, it was thought that the cytoskeleton was exclusive to eukaryotes but in 1992 it was discovered to be present in prokaryotes as well. This discovery came after the realization that bacteria possess proteins that are homologous to tubulin and actin; the main components of the eukaryotic cytoskeleton [4]. The bacterial cell division protein FtsZ, which also hydrolyzes GTP, was found to have a seven-amino-acid sequence that is nearly similar to the "tubulin signature sequence" by three separate groups. In a similar manner to the straight protofilaments of the microtubule wall and the tubulin rings that separate from microtubules during disassembly, tubulin, and FtsZ have nearly identical structures at the level of protein folding, any issue of homology was answered [4].

The mystery surrounding tubulin and FtsZ: despite being one of the eukaryotes' most slowly evolving proteins, tubulins are so different from their bacterial homolog FtsZ that they are essentially indistinguishable. While among very diverse species, bacterial FtsZs share 40–

50% of their sequence. Similar levels of similarity can be seen between bacterial and archaeal FtsZs. Contrarily, most animals, plants, and fungi exhibit 75–85% sequence similarity in tubulins [5]. The ancestral complexity of the cytoskeleton in eukaryotes leaves a huge gap between prokaryotes and the earliest eukaryote.

LITERATURE REVIEW

Prokaryotic cytomotive strands gave rise to the eukaryotic cytoskeleton. The structural and dynamic intricacy of prokaryotic filament systems is mind-boggling, and in many ways, they are a precursor to the eukaryotic cytoskeleton's self-organizing characteristics. The function and evolution of organelles networks are explored concerning the dynamic properties of the prokaryotic and eukaryotic cytoskeleton [6]. The development of molecular motors and novel aspects of filament dynamics in eukaryotes transformed the eukaryotic cytoskeleton into a self-organizing "active gel," the dynamics of which can only be explained by computational models.

The origin of the self-organizing cytoskeleton in early eukaryotes and its involvement in the evolution of novel eukaryotic functions, such as amoeboid motility, mitosis, and ciliary swimming, may be better-understood thanks to advances in modeling and comparative genomics [2].

In all 3 realms of life, the cytoskeleton is a network of intracellular filaments that is essential for cell shape, division, and function. Because none of the essential filament-forming proteins are conserved across all lineages, the simple cytoskeletons of prokaryotes exhibit a makeup that is surprisingly plastic [7]. On the other hand, the eukaryotic cytoskeletal function has undergone significant gene duplication and specialization, the addition of auxiliary proteins, and so on. Before the last common ancestor of eukaryotes, a large portion of this complexity developed. The location of cytoskeletal filaments places limitations on the most probable prokaryotic line that underwent this embryogenesis-related transition [7].

Following a short recapitulation of how the current theory has been developed over the past few decades, some overlooked or rarely remembered forerunners of current views on biological motion and its structural foundations are briefly described. It is demonstrated that as the idea of fibers as the primary components of biological matter evolved, scientists in the 18th century began to speculate about microscopic structures that closely resembled microtubules [4].

At the start of this time, it was thought that muscle contraction was caused by fibers gliding over one another and being propelled by inserted moving components. A description of the myofibril contraction process from the next century showed longitudinal displacements of submicroscopic rodlets that contained myosin. In the second half of the 19th century, the presence of fibrils in the protoplasm of non-muscle cells was a hotly contested topic that was essentially dismissed as unimportant or unreliable 100 years ago.

Nearly 20 years before intracellular filamentous structures were first seen with electron microscopy, the problem reappeared in the early 1930s as a speculative hypothesis the cytosquelette. Although under new interpretations with a much broader significance, including modulation of gene expression, morphogenesis, and even consciousness, the role initially assumed for such fibrils as signal conductors are currently being reevaluated. Since all of the aforementioned ancestors' beliefs were ultimately rejected, the corresponding modern viewpoints are, to some degree, recurring [8].

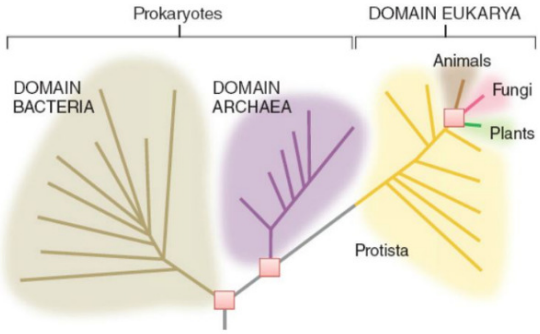

Figure 1: Eukaryotic and prokaryotic domain structure. Evolutionary tree displaying how prokaryotes and eukaryotes are organized for understanding their evolution Eukaryotic and prokaryotic domain structure (chegg).

One of the biggest unsolved questions in contemporary biology concerns the origin of the multicellular organism. The lost eukaryotic ancestors gave rise to particular biological processes that are common to all eukaryotes.

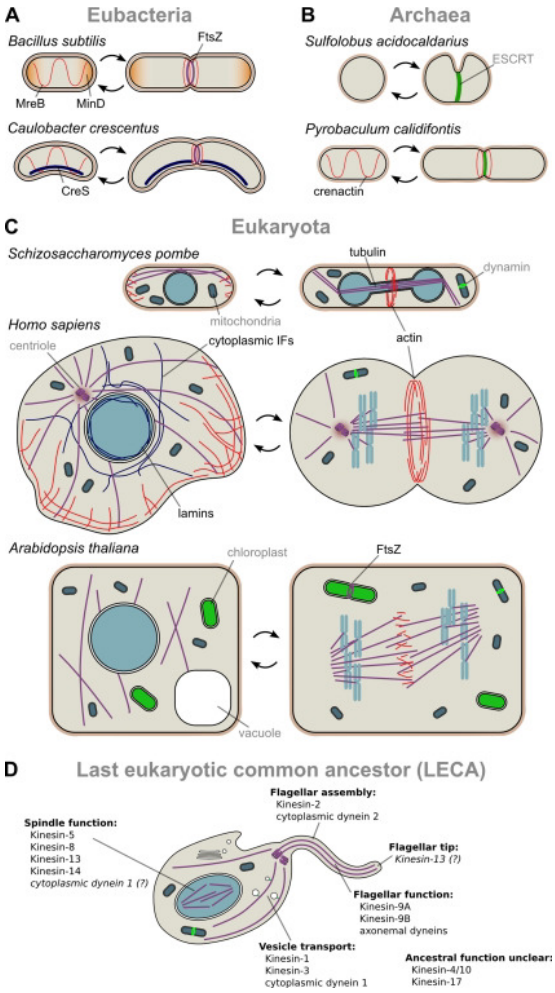

Figure 2: Cytoskeletons of bacteria, archaea, and eukaryotes: Diagrame showing the organization of the actin filament in the different organism (Semanatic scholar).

The actin cytoskeleton is one of these distinguishing characteristics that characterize eukaryotes. Asgard archaea have recently been sequenced, characterized, and isolated,

opening a fascinating window into the pre-eukaryotic cell [2]. The organization of the cytoskeleton in dividing and non-dividing cells is depicted schematically for a select few model organisms from each of the three realms of life (A–D) (right and left of each pair, respectively). Similar colors characterize homologous strands. The LECA's (D) potential cytoskeleton organization is also displayed, emphasizing the ancestry groups of microtubule motors.First, sequencing of anaerobic sediments led to the discovery of Asgard archaea, a collection of uncultured organisms with genes that are homologous to eukaryotic signature genes. Second, it was shown that Asgard archaea have biological processes that are similar to those of humans by characterizing the proteins that these genes produce. Ultimately, the isolation of an Asgard archaeon has resulted in a model organism that can be used to study the morphological effects of eukaryotic-like processes. Here, we examine the implications for the Asgard actin cytoskeleton and the development of a regulated actin system during the archaea-to-eukaryotic transition (Figure.3)[6].

Eukaryotic cells can be distinguished from their prokaryotic (bacterial or archaeal) "cousins" by the existence of a sophisticated cytoskeletal system. No extant prokaryote that has been examined thus far lacks the prominent cytoskeletal proteins that are shared by all eukaryotes, such as actin or tubulin. The capacity to form filaments and limited sequence similarity to some cytoskeletal elements have been identified in several proteins, though. The FtsZ family of bacterial and archaeal tubulin-related proteins involved in cell division4 and an intermediate filament-like protein (crescentin) from Caulobacter are among them [9]. This part covered a thorough examination of the cytoskeleton's evolutionary history. We discuss how filament-associated proteins differ significantly between species and how bacteria harbour similar proteins and filaments, even though it is presently still exploring how bacteria evolved from prokaryotes to eukaryotes [10].

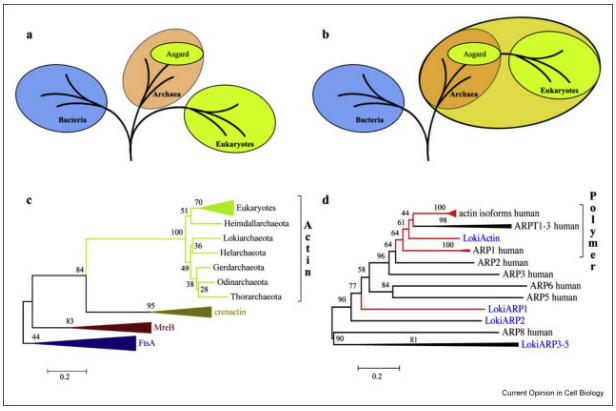

Figure 3 :Mythical beginnings of the cytoskeletal:Representing the effects of the shift from archaea to eukaryotes on the Asgard actin cytoskeleton and the evolution of a regulated actin system(Science direct.com).

Beginning with the proteins that make up the three systems actin filaments, intermediate filaments, and microtubules the reviews in this compilation describe the cytoskeleton's structures and operations. Additional related reviews, a subset of which is mentioned above, explain how cells put these proteins together to form useful supramolecular structures [11]. They also explain how these assemblies give cells their mechanical integrity, aid in adhesion to extracellular molecules and other cells, transport materials inside cells, move entire cells, move their cilia, separate chromosomes during mitosis, and divide cells in half during cytokinesis [12]. The actin and tubulin genes both developed in the common ancestor of life on Earth and have since diverged in intriguing ways. Prokaryotes and eukaryotes of today make distinct polymers with distinct functions from homologous proteins. Over the past 500

million years, the genes for intermediate filaments have evolved through gene duplication and divergence, particularly in mammals.

These genes first appeared in early eukaryotes [12][13]. This part covered a thorough examination of the cytoskeleton's evolutionary history. We discuss how filament-associated proteins differ significantly between species and how bacteria harbor similar proteins and filaments, even though it is presently still exploring how bacteria evolved from prokaryotes to eukaryotes [12].

CONCLUSION

The cytoskeleton is an internal support system found in all prokaryotic and eukaryotic cells, including yeast, plants, and mammals. Our understanding of the cytoskeleton's evolutionary process has undergone significant change in the last 20 years [13]. It has been established that the prokaryotic cytoskeleton is dynamic and varied in addition to existing. Unexpectedly, it has also proven to be disposable, at least in its canonical versions. It appears likely that more surprises will surface as more biological data are collected and sequences from a wider variety of genomes can be analyzed. Some of the prokaryotes' performers may be still waiting to take the stage [10].

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CHAPTER 11

A BRIEF INTRODUCTION OF CYTOSKELETONPRESENT IN THE CELL

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ABSTRACT:

The capacity of a eukaryotic cell to stand up to distortion, to intracellular cargo, and to alter shape amid development depends on the cytoskeleton, an interconnected organization of filamentous polymers and administrative proteins. Later work has illustrated that both inner and outside physical powers can act through the cytoskeleton to influence nearby mechanical properties and cellular conduct. Consideration is presently centered on how the cytoskeleton system creates, transmits, and reacts to mechanical signals over cellular conduct. Consideration is presently centered on how both brief and long timescales. A critical knowledge arising from this work is that long-lived cytoskeletal structures may act as epigenetic determinants of cell shape, work, and fate.

KEYWORDS:

Actin Filaments, Cytoskeleton Structure, Cellular Conduct, Living Cells Intermediate Filaments.

INTRODUCTION

In a 1960 address, cell, and formative scientist Paul A. Weiss energized his group of onlookers to think of the cell as a coordinates entire "lest our fundamental and exceedingly distraction with cell parts and divisions darken the reality that cell isn't fair an inert playground for a couple of all- powerful planning atoms, but could be a system, a progressively requested framework, of commonly forbid species of atoms, atomic groups, and supramolecular substances; which life through cell life, depends on the arrange of their interactions" [1]. The cytoskeleton carries out three wide capacities; it spatially organizes the substance of the cell; it interfaces the cell physically and biochemically to the outside environment; and it create facilitated strengths that empower the cell to move and alter its shape. To realize these capacities, the cytoskeleton coordinates the action of a large number of cytoplasmic proteins and organelles. Despite the essence of the word 'skeleton' the cytoskeleton isn't a settled structure whose function can be caught on in segregation. Or maybe, it could be an energetic and versatile structure whose component polymers and administrative proteins are in steady flux [1].

Numerous fundamental building squares of the cytoskeleton have been distinguished and characterized broadly in vitro, and analysts are presently utilizing progressed light microscopy to decide, with incredible spatial and worldly accuracy, the areas and flow of these cytoskeletal proteins amid forms such as cell division and motility. For case, more than 150 proteins have so distant been found to contain official spaces for the protein actin, which polymerizes to create one of the key cytoskeletal fibers in cells [2]. One set of actin controllers shapes macromolecular shapes an outfit called the WAVE complex that advances the gathering of actin filaments systems at the driving edge of motile cells [3]. High-resolution light microscopy of quickly slithering leukocytes uncovered that the WAVE

complex shapes exceedingly coherent voyaging waves whose development relates to cell protrusion [4]. Such perception in living cells fortify the arrangement of point-by-point theories for how atom collaborate to make useful cytoskeleton structures, but to test these theories absolutely, the components must be separated from cells and filtered.

Surprisingly, test that combine a little number of filtered proteins have illustrated that numerous complex cytoskeletal structures watched in cells can be reconstituted *in vitro* from filtered components. For illustration, as it were three proteins are required to effectively track and transport cargo on the developing conclusion of microtubules, which are shaped by the polymerization of subunits comprising αβ-tubulin heterodimers and are another key cytoskeletal fiber in cells [5]. Even though the list of proteins related to the cytoskeleton proceeds to develop, the extreme objective remains understanding how the intuitive of the personal particles of the cytoskeleton deliver rise to the large-scale cellular practices that depend on them[6].

In this report, we discuss the progress toward a coordinated understanding of the cytoskeleton. In specific, we center on the mechanics of cytoskeletal systems and the parts that mechanics have in numerous cell natural forms. Instead of centering on one cellular prepare or cytoskeletal fiber, we describe a set of fundamental concepts and interface them to work in a few cytoskeleton-related areas [6]. We start with a brief presentation of the major polymers that constitute the cytoskeleton and after that moving center from particles to more complex structures, emphasizing three concepts that resound Weiss's 1960 challenge to see cells as a coordinate entirely.

The primary concept is that long-range arrangement emerges from the controlled self-assembly of components guided by spatial signals and physical limitations[6]. The moment is that past basic composition, it is the design of the cytoskeleton that controls the physical properties of the cell. And the third is that the cytoskeleton joins to the outside microenvironment and can intercede with both briefs and timescale changes in cellular conduct [6]. We wrap up by talking about the interesting and under-appreciated address of whether long-lived cytoskeletal structures can work as a cellular 'memory' that coordinates past intelligence with the mechanical microenvironment and impacts future cellular conduct[6].

LITERATUYRE REVIEW

Contraction, cell motility, organelle and vesicle movement through the cytoplasm, cytokinesis, intracellular cytoplasm structure, creation of cell polarity, and numerous other processes carried out by the cytoskeleton are necessary for cellular balance and life [7]. The system of fibrillar structures in eukaryotic cells'(Figure. 1A) cytoplasm is known as the cytoskeleton "ticking cells Microtubules, actin filaments, and intermediate filaments are the three major kinds of fibrils that make up these structures (Figure. 1B). "The term "cytoskeleton" is unquestionably appropriate because it refers to the interior structural support system of cells. But this is just one of its many functions. All motions made by and occurring inside eukaryotic cells, such as muscular contraction, cilia and flagella beating, chromosome movement during mitosis, and many more, are carried out by cytoskeletal filaments. Therefore, the cytomuscular system could justifiably be used to describe this network of nerves. However, the words are a little misleading when compared to the organ systems of the entire mammal and these intracellular structures. The cytoskeletal systems are exceptional because of how active they can be. Many of these structures, such as the mitotic spindle and the extended pseudopod's actin cortex, can be completely created de novo in a brief amount of time before dissipating once they have served their purpose. Imagine an

animal growing a new leg in a matter of minutes, developing a complicated skeleton and muscles, and then dissolving it just as quickly! The two major molecular reactions that cause the fast rearrangement of cytoskeletal structures are the polymerization of fibres from protein subunits and the reversible process, depolymerization.

The fact that the cytoskeleton is the only component of the cell that directly touches every other cellular organelle, from the nucleus to the outer membrane, further distinguishes it from other structures. Numerous different kinds of other cellular structures can be reversibly connected to cytoskeletal fibres. As a moving cytoplasmic framework that envelops and embeds other internal structures, the cytoskeleton can therefore be thought of as such. The form of the entire cell as well as the location and motion of other cellular components may be governed by this matrix. The metabolic activity of other organ- elles as well as the activity of the entire cell may be dynamically regulated by this network. "The idea of a cytoskeleton is both very ancient and very new in terms of human history [7]. It is very new because the contemporary understanding of the cytoskeleton has only been established over the past two decades. Since the first theories regarding the existence of dynamic fibrillar structures in the cytoplasm were created more than a century ago, when the first fibrillar structures were discovered, the idea is extremely ancient."

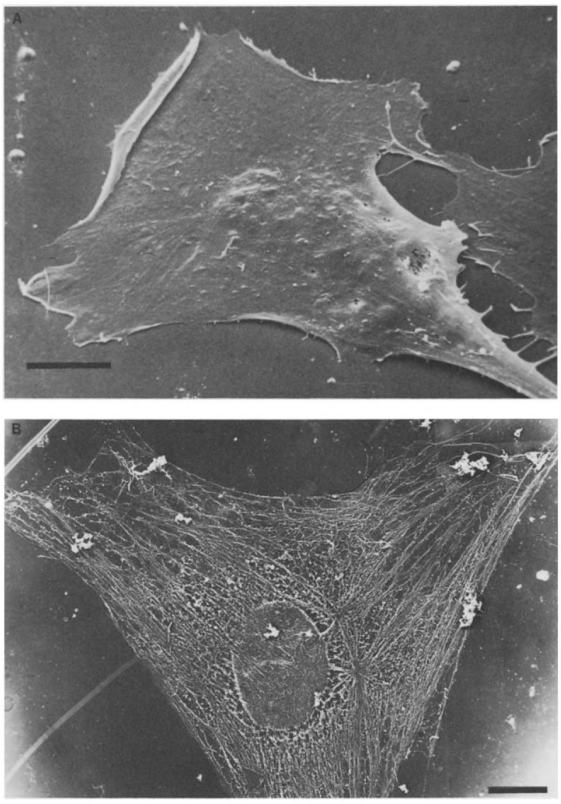

Figure 1 Structure of a fibroblast cell, (A) Mouse fibroblasts in intact culture are dispersed on the ground. (B) Detergent-cultured fibroblast membrane. The cytoplasm and the area around the nucleus are filled with a cytoskeleton framework (book.google).

THE OPENING "as well as by numerous other researchers. With very few exceptions, they had demonstrated that each eukaryotic cell contains all three morphological kinds of cytoskeletal strands. It was also demonstrated that the majority of cytoskeletal structures were kept in cells from which the membranes and liquid components had been removed using glycerol- or nonionic-detergent solutions. When properly set up and provided with ATP, these so-called "cell models" (Figure. 2A) display a variety of motion phenomena, such as

flagella pounding, contraction, and mitotic movements of chromosomes. The first researcher to create the glycerol-extracted moving models of different cells was H. Hoffman-Berling. The study of the cytoskeleton's shape and function was made easier by the use of cell models. The novel way of morphological investigation of the three-dimensional cytoskeleton was made possible by a combination of extraction techniques and more advanced electron microscopic clone preparation (Figure. 2B). When similar cytoskeletal protein types were isolated from various cell types, it was finally demonstrated that the cytoskeleton is universal and that each type of cytoskeletal fibre can be polymerized in vitro from the corresponding protein subunits: microfilaments from actin, microtubules from tubulin, and intermediate filaments from a variety of related protein types, including cytokeratins and vimentin. Additional proteins linked to the extremities and side surfaces of fibrils have also been identified. At the moment, study is focused on isolating cytoskeletal proteins and analysing their structures. Additionally, the majority of contemporary techniques for studying the cytoskeleton are based on the separation of pure proteins[8]. In specific, in vitro studies that examine the interactions of purified proteins are used to simulate the processes of cytoskeletal structure formation and function in the cell. The molecular makeup of different cytoskeletal components is examined using polyclonal and monoclonal antibodies against these proteins[9]."

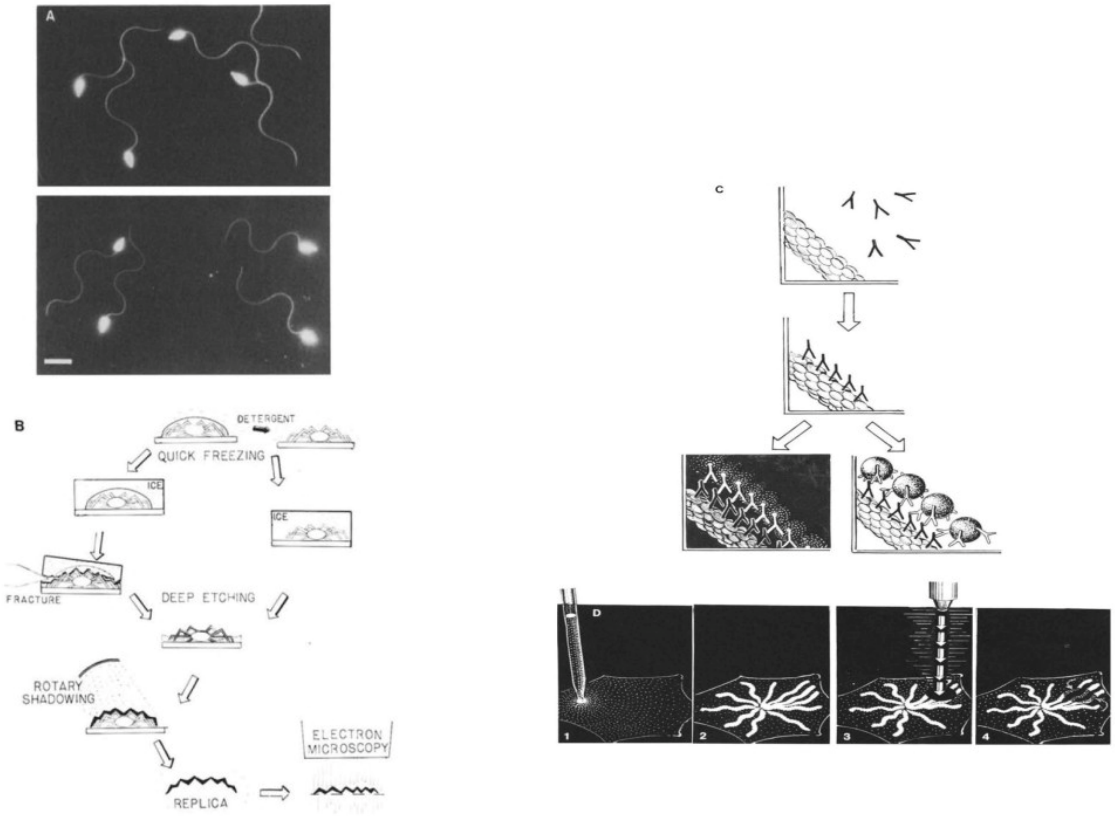

Figure 2:History of technology for cytoksleton: Diagrame showing the initial technolofy which were used for the determination of the cytoksleton (book.google).

(A). Technique for determining the structure of the cytoskeleton Sperm moving at the bottom of the overservation disc is depicted in (A) a dark-field photograph. (B) The preparation of cytoskeletal copies for electron imaging. (C) The cytoskeleton structure's immunomorphological analysis preparation. (D) The kinetics of the cytoskeletal structure was determined by injecting labelled protein and photobleaching.Selectively interacts with

tubulin and prevents microtubule polymerization " The development of these techniques has greatly advanced our understanding of the cytoskeleton over the past two decades. This information has already fundamentally altered our understanding of how cells are organised, and there is cause to believe that we will soon comprehend the molecular underpinnings of some puzzling cellular functions like cell directionality, cell shape changes, and mitosis [8]. "Despite all of the advancements, our understanding of the cytoskeleton's structure and function is still very limited. Numerous crucial cytoskeleton molecules and the processes governing cytoskeleton movements in cells are subjects we know very little about. We are only now starting to understand that in addition to the three traditional categories of cytoskeletal structures, the cell may contain additional structures. We still don't fully understand the cytoskeleton's capabilities, particularly in relation to how it controls cell development, division, and metabolism [10]."

"In these studies (Figure.2C), morphological preparations are first incubated with an antibody directed against specific cytoskeletal proteins, and the distribution of the first antibody is then made visible by a second antibody linked to a label detected by either indirect immunofluores- cence microscopy (indirect immunofluores- cence method) or indirect immunogold or immunoferritin microscopy (indirect immunogold or immunoferritin methods) or electron microscopy. These approaches, particularly indirect immunofluorescence, which were developed by E. Lazarides and K. Weber in 1974 for the study of the cytoskeleton, are now the norm for examining the molecular morphology of different cells. These "molecular pictures" stayed motionless up until lately because only nonliving fixed and extracted cells could be looked at. However, it has now been feasible to investigate the molecular dynamics of the cytoskeleton in living cells thanks to the creation of specialised video cameras that enhance the fluorescent pictures. To achieve this, specific fluorochrome-labeled proteins are introduced into living cells. Video cameras are then used to monitor the kinetics of the label's incorporation into different cytoskeletal structures. A unique technique known as photobleaching can be used to numerically evaluate the mobility of injected proteins that have been integrated into different structures (Figure. 2D). The roles of these proteins in different cellular processes are revealed using antibodies that inhibit cytoskeletal proteins and nonfunctional analogues of these proteins that are injected into the living cell. A different more traditional approach that is frequently employed for the same"

CONCLUSION

In this chapter, we go over the key information and issues concerning the composition and operation of the cytoskeleton. Three distinct kinds of protein components make up the cytoskeleton. The microfilaments (actin filaments), intermediate filaments, and microtubules are arranged from smallest to broadest. Myosin is frequently related to microfilaments. They enable cellular motions and give the cell rigidity and form. The nucleus and other organelles are anchored in position by tension-bearing intermediate filaments. Microtubules assist the cell in resisting compression, act as vesicle-moving tracks for motor proteins, and draw duplicated chromosomes to the opposing extremities of dividing cells. They serve as the anatomical foundation for cilia, flagella, and centrioles.

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CHAPTER 12

A KINESIN PLAY IMPORTANT ROLE IN MICROTUBULE DEPOLYMERIZING

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ABSTRACT:

The Kinesin superfamily is a sizable collection of molecular motors that control their association with the microtubule cytoskeleton by regulating the turnover of ATP. A kinesin's 350 residue motor domain, which controls most of its action, is adequate to identify a kinesin as a member of a specific family. These motors utilise the coupled connection between nucleotide turnover and microtubule binding in several ways, which enables them to perform a wide range of cellular tasks. A class of specialised microtubule depolymerizing motors is known as Kinesin-13. Members of this family control chromosome segregation, cilia upkeep, and neuronal growth through their microtubule destabilising action. Here, we discuss the family of kinesins' structure as it is currently understood, as well as how various sections of these proteins affect the kinesins' ability to depolymerize microtubules and perform other functions.

KEYWORDS:
Depolymerizing activity, Kinesin 13-family, Motor domain, Microtubules ends, Kinesin superfamily

INTRODUCTION

Tubulin heterodimers, which are made up of - and -tubulin polypeptides, serve as the foundation for the microtubule cytoskeleton (Nogales, 2000). The changing characteristics of microtubules contribute to their significance in biological processes. The nucleotide attached to -tubulin regulates the oscillation of microtubules between stages of growth and shrinkage: GTP-tubulin favours polymerization while GDP-tubulin favours depolymerization. In vivo, this behaviour, known as dynamic instability, is controlled and managed, especially in the intricate processes of the mitosis spindle [1][2]. Members of the kinesin group are molecular engines that move cargo along microtubule pathways throughout the cell using the energy from ATP. The extremely conserved motor centre of this superfamily, which includes both ATP and microtubule binding sites, serves as its distinguishing feature.

Because of the close connections between these sites, the nucleotide state controls how the motor interacts with its track and vice versa. Kinesins can be categorised based on where the motor is located within their protein strands . KinN kinesins have their motor core at their N terminus and move towards the plus ends of microtubules, whereas KinC kinesins have their motor core at the C terminus and move towards the minus ends of microtubules. Each kinesin subclass's movement is controlled by class-specific neck linker segments [3].

The motor centre of the third family of kinesins, known as KinI, is found internally within the polypeptide sequence. Since KinIs have ATP-dependent microtubule depolymerizing activity, these proteins display characteristics that set them apart from other, motile members of the group. kinIs are primarily found in the mitotic spindle of separating cells and are

crucial during mitosis. In contrast to purified tubulin, the mitotic spindle has more dynamic properties, but recent research revealed that these dynamic properties could be easily recreated by combining tubulin with the KinI XKCM1 and the microtubule-associated protein XMAP215. These findings point to KinI motors as important contributors to spindle motion [4].

How do KinI motors use the ATP-fuelled depolymerization of microtubules? The central location of the KinI motor within the native dimeric protein was initially proposed to be an important clue to the depolymerizing mechanism because the motor cores of the entire kinesin superfamily are highly conserved. This was done by analogy with the different directions taken by KinNs and KinCs [5]. Recent research has demonstrated that this is false, as versions of MCAK (another KinI) missing only the motor core and neck linker exhibit depolymerizing activity comparable to that of dimeric molecules. Thus, although dimerization and localisation to the centromere are mediated by the protein's N- and C-terminal domains, respectively, these functions can be wholly distinguished from KinI depolymerizing activity [6]. Regarding how KinI motors and tubulin work at the molecular level, nothing is known. The drastically different behaviour of KinIs indicates that this subgroup of kinesins may choose to engage with microtubules in a very different way. If the sequence conservation of the kinesin motor core stretches to any functional similarity, it can only be determined directly by visualising a KinI-tubulin complex [7].

LITERATURE REVIEW

KinI kinesins are microtubule depolymerizing enzymes as opposed to the majority of kinesins, which move along microtubules. Unexpectedly, we discovered that an incomplete KinI segment that only contains the motor core is able to undergo ATP-dependent depolymerization. In all nucleotide states, the motor attaches to microtubules, but in the presence of AMPPNP, microtubule depolymerization also takes place. The results of AMPPNP-induced destabilization's structural analysis provided a glimpse of the disassembly mechanism in action as it exactly deformed a tubulin dimer. KinIs use the energy of ATP binding to stretch the underlying protofilament, whereas normal kinesins use it to perform a "power stroke." Therefore, an essentially conserved form of association with microtubules is modulated by the KinI motor core's class-specific differences, which results in a distinctive depolymerizing activity [7].

The renegade kinesins of the kinesin family are the KinI kinesins, which have the capacity to depolymerize microtubules (MTs). Here, we present the in vitro depolymerization-sufficient 1.6 crystal structure of a KinI motor core from Plasmodium falciparum. The loop regions L6 and L10 (the plus-end tip), L2 and L8, and switch II (L11 and helix4) of the pKinI structure vary from all other published kinesin structures in that nucleotide is not present. Other than these variations, the pKinI structure is very similar to earlier kinesin structures. The impacts of alanine on kinI-conserved amino acids on ATP breakdown and depolymerization were investigated. Interestingly, rather than generally MT binding or ATP breakdown, mutation of three residues in L2 appears to mainly impact depolymerization. The findings of this research support the hypothesis that loop 2 is crucial for KinI operation and show that KinI is uniquely specialised to hydrolyze ATP after starting depolymerization[8][9]. The KIF19A kinesin-8 motor builds up at the ends of the cilia and regulates cilium length. Due to unusually long cilia, defective KIF19A causes hydrocephalus and feminine sterility. KIF19A is the only kinesin with the dual abilities of microtubule depolymerization and movement along ciliary microtubules. We solved the crystal structure of its motor domain and identified its

complicated structure from cryo-electron microscopy with a microtubule to clarify the molecular processes underlying these functions.

On its microtubule-binding side, KIF19A has a concentration of characteristics that allow its dual role. Surprisingly, switch II and L8 destabilisation works together to allow KIF19A to adapt to both straight and bent microtubule protofilaments. The microtubule is attached by the L2 and L12 basic groups. The long L2 efficiently maintains the curved shape of microtubule ends thanks to its distinctive acidic-hydrophobic-basic pattern. KIF19A, therefore, makes use of a variety of techniques to carry out its dual tasks of movement and microtubule depolymerization by ATP hydrolysis[10]. Within the kinesin superfamily of motor proteins, kinesin-13s are a separate subgroup that supports microtubule depolymerization but does not have motile action. It is still unknown how kinesin-13s depolymerize microtubules and are modified to carry out a function that appears to be very distinct from that of other kinesins.

Here, we present the structures of Drosophila melanogaster kinesin-13 KLP10A protein constructs attached to bent or straight tubulin in various nucleotide states using cryo-electron microscopy (cryo-EM). These structures demonstrate how the movement of the kinesin-13-specific loop-2 in conjunction with nucleotide-induced conformational changes near the catalytic site cause tubulin bending and microtubule depolymerization. The findings emphasise a modular structure that enables the use of related kinesin core motor domains for various tasks, including movement or microtubule depolymerization [11].

Important microtubule regulators are known as kinesin-13s cause microtubule disintegration in an ATP-dependent way. We present the crystal structure of a working construct of the kinesin-13 Kif2C/MCAK attached to the -tubulin heterodimer in an ATP-like state, simulating the species that dissociates from microtubule ends during catalytic disassembly, to shed light on their mechanism. Our findings show that Kif2C maintains a bent tubulin shape. When tubulin binds to the Kif2C 4-L12-5 region, an extraordinary 25° twist occurs that targets the -tubulin hinge. While the neck and the KVD motif, two distinct components of kinesin-13s, target the distal end of -tubulin, this movement causes the 5a-5b motif to engage with it. When combined with research on Kif2C mutants, point to the stabilisation of curved tubulin as a key component of the Kif2C process [12].

By twisting tubulins at microtubule ends, the kinesin-13 family of motors catalyses microtubule depolymerization. The kinesin-13 motor core has inherent depolymerization activity, but the activity of the core alone is very modest when compared to constructs that also contain a conserved neck region. The full-length dimeric motor locates microtubule ends and deployments effectively. It also diffuses across the microtubule matrix. The data at hand is consistent with the concept of a universal process for kinesin-13-catalyzed depolymerization. To allow a variety of cellular functions, kinesin-13 motor activity is carefully localised and controlled in vivo. These proteins play a role in the overall regulation of microtubule movements.

Additionally, they are confined to the mitosis and meiotic spindles, where they aid in the establishment and upkeep of spindle bipolarity as well as chromosomal congression, attachment rectification, and chromatid separation. Kinesin-13 motors appear to operate on particular groups of microtubules in interphase cells through complex and subtle processes. These kinesins are effective, multi-tasking molecular movers due to their meticulously regulated localization and regulation. The Kinesin-13 family is a collection of kinesins that

depolymerize microtubules and play an essential role in controlling microtubule length. Members of this family are essential for cilium length regulation, chromosome sorting, spindle formation, and neuronal growth in both mitotic and meiotic cell division. We developed a synthetic primordial Kinesin-13 motor domain to better comprehend the development of microtubule depolymerization activity in the Kinesin-13 family.

This sequence is expected to have been present in the common ancestor of the Kinesin-13 family according to phylogenetically derived ancestral motor domain analysis (Figure 1A). Here, we demonstrate that the primordial Kinesin-13 motor depolymerises stabilised microtubules more quickly than any depolymerase that has been examined to date. This powerful activity enables the ancestral Kinesin-13 to depolymerise doubly-stabilized microtubules that are unaffected by MCAK and is more than an order of magnitude quicker than the most extensively studied Kinesin-13, MCAK. These findings imply that a "super depolymerizer" was the progenitor of the Kinesin-13 family and that members of the Kinesin-13 family have developed away from this extreme depolymerizing activity to provide more regulated microtubule depolymerization activity in living cells[13].

Microtubules are depolymerized by kinesin-13 proteins in an ATP hydrolysis-dependent way. It's still unknown how these two pursuits relate to one another. investigated the function of the KVD motif at the tip of loop 2, which is unique to the kinesin-13 subgroup (Figure 1B,1C). Kif2C mutants with reduced microtubule-stimulated ATPase and defective depolymerization capacity were produced by shortening the loop, switching the lysine for the glutamate, and also substituting another Val for Ser. Based on a structural model of the Kif2C-ATP-tubulin complex created from newly discovered structures of kinesin-1 attached to tubulin, we explained these findings. According to this hypothesis, Kif2C experiences a conformational shift upon microtubule binding that is in part controlled by the interaction of the KVD motif with the tubulin interdimer interface.

The conserved glutamate residue of the switch 2 nucleotide binding motif was then changed to an alanine. This mutation prevents ATP metabolism and suppresses motile kinesins in the post-conformational shift state. This Kif2C mutation still produced clusters of one Kif2C and two tubulin heterodimers and depolymerized microtubules. These findings show that, in contrast to ATP breakdown, the structural modification of Kif2C-ATP that occurs upon attachment to microtubule ends is adequate to cause tubulin release. Overall, our results indicate that kinesin-13s adopt a conformation that is similar to that of tubulin-bound, ATP-bound, motile kinesins upon tubulin attachment, but that this conformation is tailored to microtubule depolymerization[14]. The tubulin-removal conformation of the motor is isolated by a point mutation in the mitotic centromere-associated kinesin/Kif2C (E491A) family member, which is different from all previously known kinesin-13 conformations obtained from nucleotide analogues. The E491A mutant is able to effectively liberate from attached tubulin dimers to recycle them catalytically, but it is unable to do so from stabilised MTs.

The mutant can only catalytically remove tubulin dimers from stabilised MTs in adenosine diphosphate (ADP), as lattice-bound tubulin has a higher propensity for the mutant's preference for detached tubulin dimers in ADP. The engine can renew as a result of additional rounds of disassembly. By using the mutant, we demonstrate that kinesin-13 motors release tubulin at the ATP hydrolysis transition state, demonstrating a substantial difference in their linkage to ATP turnover compared to motile kinesins. recognise the mechanism by which the Kip3D protein regulates microtubule structure. When it attaches to microtubules, Kip3D, a microscopic motor, utilises the energy from ATP to carry out action. Kip3D pulls microtubules apart using ATP energy (depolymerisation). This is crucial for the

effective completion of mitosis because the organisation of the microtubule cytoskeleton is continuously changing during this process. To observe how Kip3D works with microtubules, we'll use an electron microscope, a very potent type of microscopy that can produce images at extremely high magnification. Understanding how Kip3D molecules use ATP to depolymerise microtubules will be made easier by looking at the shape of Kip3D attached to microtubules [15].

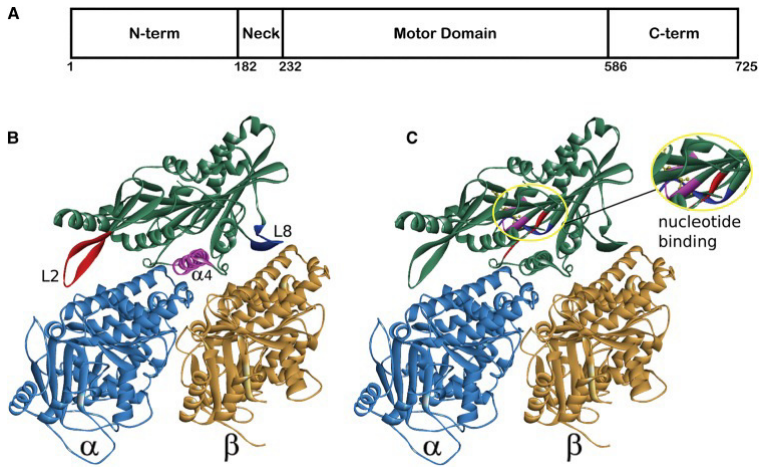

Figure 1: Domain organization and motor domain structure of the kinesin-13:(A) Diagram showing the domina organization of the kinesin-13. (B) Ribbon diagrame showing the organization of the motor domain of kinesins-13(Research gate).

Recent structures of Kinesin-13 neck-motor constructs show the neck helix to bind across tubulin subunits, indicating a possible additional role at the location of depolymerization. These shapes suggest that the total input of the Kinesin-13 neck to microtubule depolymerization is still unclear. Each member of the Kinesin-13 family has cellular functional specialisation thanks to the non-motor portions that regulate their cellular location. But the emergence of long-range intramolecular interactions that alter the shape of full-length Kinesin-13s suggests that these regions serve another purpose. These associations between the N- and C-terminal domains and the motor and neck domains regulate how Kinesin-13s associate with microtubules, just like they do with other kinesins like CENP-E, Kinesin-1, and KIF17. Kinesin-13's non-motor regions are also involved in facilitating the formation of the homodimers that perform Kinesin-13's cellular activities. Since monomeric constructs of Kinesin-13s can display similar rates of microtubule depolymerization to full-length versions in vitro, it is still unknown why these kinesins operate as dimers in the physiological setting. One hypothesis holds that the two motor sections of the active molecule enhance the processivity of tubulin component removal from the microtubule end. The justification for the requirement for dimerization is still unclear, though. The primary sequence of the typical Kinesin-13 has the unique kinesin motor domain in the centre.

The Kinesin-13 that has received the most study, MCAK, contains the N-terminal domain, the neck, the central motor domain, and the C-terminal domain. This basic domain arrangement is also present in other members of the Kinesin-13 family. The motor section identifies a member of the Kinesin group. It serves as both the nucleotide-binding site and the main microtubule interaction area. The capacity of Kinesin-13 MCAK motor domain-only truncation forms to depolymerise microtubules demonstrates that the Kinesin-13 motor domain alone has microtubule depolymerase activity. All four structures provide evidence for

the Kinesin-13 motor domain's location close to tubulin (Figure 1B). The 4-helix is seen to be resting in the space between - and -tubulin known as the intermemer gap. The Loop 8 portion is situated at the distal end of the -tubulin subunit, and the family-specific long Loop 2 connects at the interdimer groove, which is situated at the longitudinal junction between - and -tubulin. The microtubule-binding face of the Kinesin-13 motor domain contains two family-specific nucleotide sequences. The Loop 2's Loop 4 helix and hairpin exhibit these patterns.

According to each of the most recent structures of the Kinesin-13 motor domain in combination with tubulin, the 4 helix, which can be found in the intradimer groove, is the centre axis of the tubulin-binding interface (Figure 1B). The fourth helix contains the family-specific, extremely conserved motif KECIR. A number of these residues are necessary for MCAK to depolymerize microtubules, and more recent studies have revealed that they are also necessary for MCAK to recognise and bind to the microtubule end. Additionally, it has been shown that Cdk1 activation at a location right next to the 4 helices' C-terminus lessens microtubule end identification. According to the most current structures of the Kinesin-13 motor domain in combination with the tubulin heterodimer, the loop connecting H11 and H12 in -tubulin is in contact with the residues K, E, and R of this family-specific motif. They are in a position to notice conformational changes at the intradimer interface between - and -tubulin thanks to the suggested technique of microtubule end detection. The Loop 2 of the kinesin motor domain is formed by members of the Kinesin-13 family, which have a significantly longer -hairpin than other kinesins.

Since Loop 2 engages with each of the most recent configurations of Kinesin-13-tubulin complexes at the -tubulin side of the heterodimer in the interdimer cleft, it would be guided towards the minus end of a microtubule (Figure 1B). The importance of this loop and the family-specific KVD motif it contains for microtubule depolymerization activity cannot be overstated. The precise molecular mechanism underlying Loop 2's crucial function in microtubule depolymerization is still unclear. This area may simply contribute to the tubulin curvature that diminishes the microtubule structure, as shown by the structure of KIF2A in conjunction with tubulin , or it may actively take part in disrupting longitudinal contacts between tubulin heterodimers.

It has been established that the Kinesin-13, KLP10A, has an additional tubulin-binding site on the motor domain side that is distinct from the major tubulin-binding interface. This additional tubulin-binding site mediates the formation of the oligomeric tubulin bands and spirals that are observed by the cryoEM of depolymerizing microtubules. The residues that make up this secondary microtubule-binding region have a distinct electrical potential in the Kinesin-13 family than they do in other kinesin families. Most kinesins have a negative charge in this region. But in the Kinesin-13 family, it has the propensity to be positively charged, which would make it simpler for it to interact with the negatively charged surface of microtubules. This second microtubule-binding region's disruption in the D. melanogaster raises the possibility that it functions in cell division. In cultured cells, kinesin-13, also known as KLP10A, hinders mitosis.[15].

CONCLUSION

The motor domain of this Kinesin family is specially adapted for microtubule depolymerization as evidenced by the Kinesin-13 motor domain alone having strong microtubule depolymerizing action. Uncertainty exists regarding the chemical traits that give the Kinesin-13 family specialised depolymerizing action. However, the tubulin-binding interface appears to be specially tailored to recognise the microtubule end, according to the

structures and mutational experiments reported here. Due to the greater conformational flexibility provided by this adaptation, which is not accessible to subunits embedded in the lattice, this adaptation probably gives the ability to detect curved tubulin conformations that appear at or near microtubule ends. When compared to the other members of the superfamily, family-specific sequence patterns on the tubulin-binding surface significantly alter how the Kinesin-13 motor domain interacts with tubulin.The Kinesin-13 family's non-motor sections control their cellular localization, giving each member of the family cellular functional specialisation. However, the development of long-range intramolecular contacts that change the conformation of full-length Kinesin-13s indicates that these areas play an additional function.

These associations between the motor and neck domains, the N- and C-terminal domains, and the Similar to other kinesins like CENP-E, Kinesin-1, and KIF17, Kinesin-13s are associate with microtubules (reviewed in ref. [64]). The non-motor sections of Kinesin-13 also play a part in mediating the assembly of the homodimers that carry out Kinesin-13's cellular functions. It is still unclear why these kinesins function as dimers in the physiological context since monomeric constructs of Kinesin-13s can exhibit comparable rates of microtubule depolymerization to full-length versions in vitro. According to one theory, the active molecule's two motor regions increase the processivity of the elimination of tubulin subunits from the microtubule end. However, the rationale behind the demand for dimerization is still not clear.

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CHAPTER 13

A BRIEF OVERVIEW; HOW MICROTUBULES FUNCTION IN THE CANCER CELL

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ABSTRACT:

Microtubules are extremely active structures made of - and -tubulin heterodimers that play a role in mitosis, intracellular transport, and cell motility. The tubulin family of proteins is known to be a target for tubulin-binding chemotherapeutic drugs that induce mitotic arrest and cell mortality by inhibiting mitotic spindle movements. It's significant to note that various tumors have been linked to altered post-translational changes, changed expression of various tubulin isotypes, and altered microtubule stability. In solid and hematological cancers, these alterations were associated with a bad outcome and chemotherapy tolerance. The processes underlying these findings are still not completely known, though. Increasing evidence points to the possibility that tubulin and microtubule-associated proteins may be involved in a variety of biological reactions to stress, giving cancer cells a survival edge. The significance of protein microtubule networks in the control of significant cellular processes in reaction to stress will be the main topic of this study. The understanding of microtubule function in this setting may lead to the development of novel cancer treatments.

KEYWORDS:

Breast Cancer, Microtubule Dynamics, Tubulins Isotypes, Cancer Cells, Cell Death, Stress Response.

INTRODUCTION

Microtubules (MTs) are highly dynamic key components of the cytoskeleton composed of alpha- and beta-tubulin heterodimers. Microtubule dynamics are tightly regulated by the “tubulin code”, various microtubule-associated proteins, kinases, and phosphatases. Proper regulation of MT dynamics is not only important for mitosis and faithful chromosome segregation but also for cell signaling, trafficking, cell migration, and ciliogenesis. Defects in spindle assembly or the separation of the duplicated chromosomes into daughter cells may lead to cell death or genomic instability causes for diseases such as developmental disorders and cancer. Given their indispensable role in cell division and diverse cellular processes, MTs have served for decades as pharmacologically validated and attractive targets for cancer therapy. Understanding how the MT cytoskeleton is formed and regulated in somatic and malignant cells will help us to improve treatment strategies for cancer patients.

For this purpose, the identification of proteins that modulate the MT network could lead to a better understanding of chromosome instability and tumor progression, and provide additional prognostic information for the selection of adequate anti-cancer therapies for

patients to significantly optimize clinical outcomes. Therefore, this Special Issue focuses on how microtubules and their MT-associated proteins (MAPs) regulate MT nucleation, migration, chromosome congression, apoptosis, and autophagy in various cancer entities as well as how these structures can be used as future therapeutic targets.Microtubules, together with microfilaments and intermediate filaments, form the cell cytoskeleton. The microtubule network is recognized for its role in regulating cell growth and movement as well as key signaling events, which modulate fundamental cellular processes. Emerging evidence also suggests that it is critically involved in cell stress responses. This chapter will focus on the role of microtubules in the context of cancer. Microtubules are composed of α- and β-tubulin heterodimers that associate to form hollow cylindrical structures [1].

They are highly dynamic and are constantly lengthening and shortening throughout all phases of the cell cycle. During interphase, microtubules are nucleated at the centrosome (minus end) and radiate toward the cell periphery (plus end). Interphase microtubules are involved in the maintenance of cell shape and the trafficking of proteins and organelles [1]. Motor proteins translocate cell components on microtubule tracks,protein–protein interactions with other adaptor proteins coordinate this process. Tubulin heterodimers also exist in soluble form in cells, and protein interactions with this tubulin population regulate microtubule behavior. The addition and removal of soluble tubulin heterodimers to dynamic microtubule ends is a highly regulated process. Tubulin dimers are nucleotide-binding proteins, with β-tubulin also possessing GTPase activity. How tubulin heterodimers are orientated in microtubules gives rise to a polar molecule that differs in both structure and kinetics at each end of the microtubule.

The dynamics of tubulin addition and release are much slower at the minus end of the microtubule, which terminates with α-tubulin proteins, compared with the plus end of the microtubule, which terminates with β-tubulin [2] Proteins. The addition of a tubulin heterodimer to a microtubule activates the GTPase activity of β-tubulin, locking the β-tubulins in the microtubule in a GDP-bound state. The βtubulins exposed to the solvent at the end of the microtubule form a GTP cap that is important in preventing microtubule depolymerization. Therefore, the binding of GTP at the microtubule plus end imparts structural and kinetic polarity to microtubules and is an important regulator of microtubule stability. It is believed that the polymerized and soluble tubulin pools interact with different signaling networks, however, the dynamic exchange of tubulin subunits between these pools makes it difficult to distinguish the functional roles of soluble and polymerized tubulin experimentally[2].

The reader is referred to several excellent reviews for more detailed information on microtubule structure and dynamics. During mitosis, microtubules form the spindle to enable correct chromosomal segregation[3]. Tubulin-binding agents (TBAs; e.g., taxanes, vinca alkaloids, epothilones, and eribulin) are important chemotherapeutic drugs that suppress spindle dynamics, causing subsequent mitotic arrest and cell death in rapidly dividing cells [3]. Recent evidence suggests that the induction of cell stress in interphase cells also contributes significantly to TBA-mediated cell death [4], highlighting the importance of tubulin in cell stress responses in cancer. In humans, microtubules are composed of combinations of eight α-tubulin isotypes and seven β-tubulin isotypes, with the different tubulin isotypes possessing specific tissue and developmental distributions. The members of the tubulin family share a high degree of structural homology and are distinguished from one

another by highly divergent sequences at their carboxy-terminal (C-terminal) tail.The C-terminal tails of tubulin are also thought to mediate protein–protein interactions and act as sites of post-translational modifications to confer unique functionality to each isotype [5].

LITERATURE SURVEY

For anticancer treatment, the tubulin/microtubule system is a crucial target. Vinca alkaloids and taxanes are two of these compounds' most therapeutically useful subgroups. New tubulin-binding drugs have recently entered experimental or commercial testing. In phase III clinical studies, one of these classes of drugs, the epothilones, has shown a lot of potentials. All of these substances have one thing in common: they attach to -tubulin and interfere with the way microtubules work during mitosis. This causes mitotic arrest and cell death. These substances can also prevent growth. Drug resistance, regardless of its efficacy, can be a significant therapeutic issue [6]. This review of the processes underlying cellular target-related tubulin-binding agent resistance includes a discussion of solutions to this significant clinical issue[3].

Due to its excessive aggressiveness, invasiveness, late detection, and absence of effective treatments, pancreatic cancer has a very bad prognosis. Agents that target microtubules are thought to be the most hopeful of all the medications used to treat this form of cancer. Although they do so through various methods, such as preventing cell division, causing apoptosis, etc., they suppress cancer cells. Thus, a thorough investigation of the roles of microtubule cytoskeletal proteins in tumor cells and the impact of microtubule-targeting drugs on pancreas cancer is conducted[7]. The human lung cancer cell line A549 was used to identify the taxol-resistant A549-T12 and -T24 cell lines. They require modest amounts of Taxol for proliferation but are 9- and 17-fold resistant to Taxol. Discovered that cells immune to taxol had considerably more dynamic microtubule instability. In contrast to A549 cells, microtubule dynamics rose by 57% in A549-T12 cells when added Taxol was not present.

Speed and length reduction both went up by 75 and 59%, respectively. A549-T24 cells further boosted these metrics, with total dynamics rising by 167% over parental cells. Thus, the enhanced microtubule dynamics of these cells can account for their decreased susceptibility to taxol. A549-T12 cells were stopped at the metaphase/anaphase transition and displayed abnormal mitotic spindles with chromosome non-congression when cultured without Taxol. Cells developed properly in the presence of 2–12 nM taxol, indicating that excessive microtubule dynamics are what causes the mitotic halt. These findings imply that microtubule dynamics, both excessive and repressed, affect mitotic spindle function and prevent growth and that they contribute significantly to Taxol resistance[8]. Taxanes and vinca alkaloids, two drugs that influence microtubule dynamics, have been a staple of leukemia and solid tumor therapy for many years. Clinical studies for new, more potent microtubule-targeting drugs are still being conducted, and some, like the epothilone ixabepilone, have already received usage approval.

Contrarily, several additional medications in this family that had positive preclinical evidence subsequently turned out to be useless or intolerable in animal models or human trials. For various microtubule-targeting drugs at varying phases of development, we address the molecular processes as well as the preclinical and clinical outcomes in this overview. We also address which microtubule-targeting drugs, based on their availability, effectiveness, and toxic profile, are suitable for further research[9].Small compounds that disrupt microtubule dynamics, like Taxol and Vinca alkaloids, are frequently employed in cell biology studies

and as antitumor medications in humans. They cannot, however, restrict their activities to particular target cells, which has detrimental side effects when used in chemotherapy. In this study, photostatins, inhibitors that can be visually controlled to regulate microtubule dynamics in vivo.

Photostatins regulate mitosis in living organisms with single-cell spatial accuracy and influence microtubule dynamics with subsecond reaction times. Photostatins are up to 250 times more harmful when activated with blue light than when left in the dark in longer-term uses in cell culture. Photostatins, a novel family of precision chemotherapeutics whose toxicity can be spatiotemporally limited by the use of light, are thus useful tools for cell biology [10]. A growing body of research suggests that a family of cellular proteins that interact with microtubules and change microtubule dynamics may influence how sensitive cancer cells are to drugs that target microtubules as well as how resistant tumor cells are to these drugs. Apoptosis regulators, tumor suppressors, and oncogene products all belong to the expanding family of microtubule-associated proteins (MAPs), which suggests that the alteration of microtubule dynamics may be one of the crucial processes in tumorigenesis and tumor development.

The purpose of this study is to bring together information on these unconnected proteins with a shared function, investigate their significance for microtubule-targeted treatments, and emphasize MAPs-tubulin-drug interactions as a novel route for discovering new drugs. We propose that rational microtubule-targeted therapies should, in the ideal case, involve proteomic profiling of tumor MAPs before the administration of microtubule-stabilizing/destabilizing agents, preferably in combination with agents that modulate the expression of relevant MAPs. This is based on the evidence currently available. The treatments that are currently accessible do not completely help metastatic breast cancer, a fatal complication. The report state that ATIP3 is a powerful protein that stabilizes microtubules, and that its reduction enhances microtubule dynamics. Results support the idea that ATIP3 regulates the capacity of microtubule tips to reach the cell cortex during migration by lowering microtubule dynamics, a process that could explain why cancer cell movement and spread are inhibited.

Discovery of a working ATIP3 domain that interacts with microtubules and reproduces ATIP3's impacts on microtubule dynamics, cell growth, and migration is intriguing. the research represents a significant advance in the creation of novel tailored therapies for metastatic breast cancers that lack ATIP3 expression [11]. The most common reason for mortality from advanced colon cancer is tumor metastases. In colon cancer, upregulation of KIAA1199 was found to be associated with worse results, increased cell motility, and tumor metastasis, the causes of which were not completely understood. Here, we demonstrate that, in an orthotopic graft colon cancer tumor model, KIAA1199 silencing reduces tumor spread. Crucially, we discovered that PP2A's phosphatase activity is increased as a result of an interaction between PP2A's C-terminal region and KIAA1199, which is necessary for KIAA1199-mediated cell motility.

Furthermore, locate stathmin, a protein that destabilizes microtubules, as being downstream of the KIAA1199-PP2A complex. The dephosphorylation of stathmin brought on by KIAA1199 causes microtubule instability and boosts cell movement. Additionally, the microtubule-stabilizing medication paclitaxel could suppress tumor metastasis in vivo and in

vitro in colon cancer, as well as shield against KIAA1199-induced microtubule destabilization. Together, these findings indicate that KIAA1199 may be a potential target for the prevention of colorectal cancer metastasis because it facilitates the metastasis of colorectal cancer cells through microtubule destabilization controlled by the PP2A/stathmin pathway[12]. The most extensively studied microtubule alteration associated with cancer is altered tubulin isotype expression, which has been noted in both solid and blood malignancies.

These changes have a poor outcome and are often associated with resistance to chemotherapy (Table 1). Since TBAs bind to the -tubulin subunit to exert their toxic effects, -tubulin isotypes have attracted more interest than -tubulin isotypes in this context. This is primarily because isotype-specific antibodies are readily available. In addition, III-tubulin is the isoform that has been most thoroughly studied in a variety of tumors. Various post-translational modifications (PTMs) can be applied to tubulins. Most tubulin PTMs are extremely diverse and little is known about how they are regulated or what their effects are. Post-translational modifications are thought to control how proteins interact with the microtubule cytoskeleton and influence cellular communication. Most of these changes are confined to the tubulin C-terminus and may confer unique functional properties to different tubulin isotypes[6].A wide variety of proteins are known to be associated with tubulins. Microtubule stability and dynamics are influenced by interactions between tubulin and MAPs, which are also known to influence chemotherapy sensitivity and tumor development in cancer.

Abnormal expression of mainly neuronal MAPs such as Tau and MAP2 was found in non-neural cancer tissue. For example, overexpression of tau is associated with poor prognosis in breast cancer and may affect sensitivity to taxanes by reducing drug affinity for β-tubulin. Taxane resistance is also associated with altered MAP2 expression with significant implications for primary and metastatic melanoma. It is well known that microtubules play a key role in the movement of messenger proteins between different cellular compartments to facilitate efficient signal transduction. But the assembly data point to a specific function for microtubule dynamics, tubulin isotypes, and MAPs in controlling the timing, intensity, and kinetics of MAPK signaling.

Microtubule movements control p53 levels[5]. TBA therapy increases p53 levels and its nuclear accumulation at concentrations that inhibit microtubule dynamics without altering microtubule network organization. Additionally, MAP1B interacts with p53, reducing its activity and blocking the death that doxorubicin causes in neuroblastoma cells. Production of tubulin isotypes and MAPs, microtubule dynamics, and reorganization can all be influenced by p53 signaling. Taken together, microtubules have a substantial effect on p53-mediated stress response signaling by controlling p53 levels and translocation[5].

Table 1: (Semantic scholar) Alternation of the microtubule in cancer.

Microtubule alteration	**Observation**	**Effect**	**Cancer**
Altered isotype expression	High Bl-tubulin	Poor response to docetaxel treatment	Breast cancer

	High Bill-tubulin expression	Favorable response to taxane treatment	Ovarian (clear cell adenocarcinoma)
		Poor response to taxane treatment	Pancreatic ductal adenocarcinoma
		Correlates with disease stage	Glioblastoma
		Correlates with disease stage	Colorectal cancer
		Localized to invasive edge	Uterine serous carcinoma
		Poor response to taxane/platinum treatment	Gastric cancer
		Poor response to taxane treatment	Prostate cancer
		Aggressive disease, patient outcome	Ovarian cancer
		Poor response to taxol treatment	Hepatocellular carcinoma
	Low Bll-tubulin expression	Favorable response to taxane treatment	NSCLC
	High BlVa-tubulin expression	Histological grade	Hepatocellular carcinoma
	High 3V-tubulin expression	Poorly differentiated	Medulloblastoma
Altered post-translational modification	High A2a-tubulin	Poor response to vinca alkaloid treatment	Advanced NSCLC
	High detyrosinated tubulin	Disease aggressiveness	Breast cancer
	Active	Favorable patient	Neuroblastoma

	tyrosination cycle	outcome	

In solid tumors, hypoxic areas are formed due to rapid cell proliferation and insufficient vascular development. Hypoxia-inducible factor 1 (HIF1), which is upregulated in most solid tumors, is thought to be a master regulator of the cellular response to hypoxia. Direct contacts between III-tubulin and glutathione S-transferase 4 were observed in ovarian cancer cells, indicating that tubulins are involved in mediators of the oxidative stress response. DNA excision repair protein excision repair group 1 (ERCC1) and III-tubulin interact to influence how well patients respond to a combination of taxane and paclitaxel therapy. Early research showed that detyrosinated microtubule plus ends become unstable with ATP depletion, suggesting that microtubules may function as a sensor of the cell's energy state. Through CLIP170 phosphorylation, AMPK affects microtubule dynamics and is a key sensor for the metabolic state of the cell [5].

By making paclitaxel more soluble in tubulin, CLIP170 changes the sensitivity of breast cancer cells to the drug. Inhibition of axonal microtubule development by AMPK activation under metabolic stress in neuronal cells provides further evidence for the involvement of microtubules in early metabolic stress signaling processes. In mouse brain preparations, the primary neuronal tubulin, IItubulin, was also found to be a downstream target of AMPK. Microtubules and tubulins have long been hypothesized to play an important role in modulating mitochondrial respiration. Recent research has shown that tubulin can engage and inhibit VDAC, control the compartmentalization of ATP and metabolites, and enhance the Warburg effect. As this interaction is mediated by the tubulin C-terminus, post-translational modifications and different tubulin isotypes may differentially drive VDAC dynamics to affect cancer metabolism by reprogramming.

Enzymes of the tricarboxylic acid cycle and glycolysis interact with tubulins, specifically III-tubulin. Tubulin works with a variety of glycolytic enzymes, including pyruvate kinase, phosphofructokinase, aldolase, hexokinase, GAPDH, and lactate dehydrogenase, according to in vitro experiments in reduced systems. Because these enzymes engage the -tubulin C-terminus rather than the tubulin core, interactions with some of them may be isotype-specific. Cells can be triggered to undergo macroautophagy, also known as autophagy, in response to various stresses, such as metabolic stress and ER stress. A catabolic process called autophagy enables the separation and recycling of organelle components and proteins by enclosing them in vacuoles for later lysosomal breakdown [5].

homeostatic processes are impacted by changes in the production of tubulin isotypes, tubulin post-translational modifications, and the association of microtubules with MAPs seen in cancer. As a consequence of improved cell survival in the challenging tumor microenvironment, resilience to chemotherapy, and the emergence of more aggressive disease, microtubules may work to coordinate stress reactions across the cell. In addition, it serves as a key quality control procedure by allowing the elimination of proteins and structures that have been damaged by oxidative stress and protecting cells from such damage. Because autophagic activity can help cells maintain ATP levels, it is inextricably linked to metabolic stress responses. Microtubules have been known for many years to be essential for

autophagic flux; however, it has recently become clear that they are also crucial for lysosome initiation, trafficking, and assembly (Figure.1). Protein degradation, insufficient chaperone activity, and problems with protein-handling mechanisms can all lead to misfolded proteins. The ER is extremely sensitive to cellular conditions and is responsible for ensuring the proper packaging of membrane and secretory proteins [5].

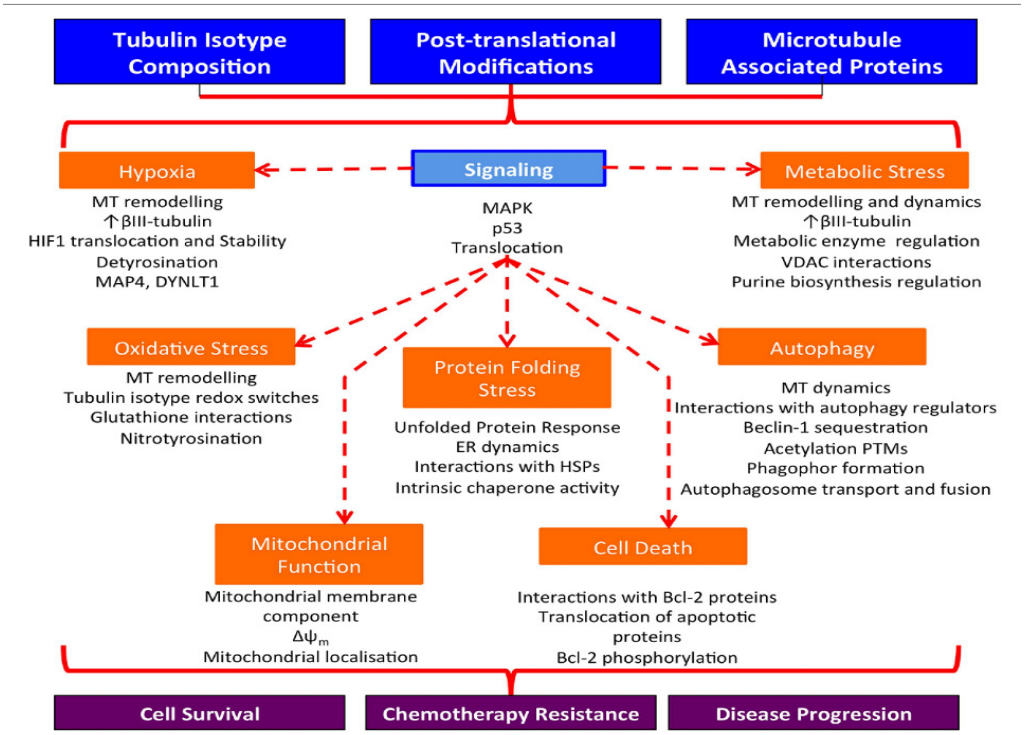

Figure 1: Cancer cells regulation: Microtubules regulate and coordinate various cellular stresses response in cancer cells (Semantic scholar).

Unfolded proteins can accumulate inside the ER, and the unfolded protein response can begin when any number of factors are slightly altered (UPR). In a concerted effort to reduce the burden of misfolded proteins, the UPR signifies the activation of the ER-associated degradation machinery, which enables the transfer of unfolded proteins to cytoplasmic proteasomal systems, inhibition of translation, and upregulation of chaperones. When the UPR is initiated, ER stress is reduced or cell death is initiated. The UPR plays an important role in the growth and stabilization of tumors and is upregulated in many malignancies. Topoisomerase inhibitors are among the many chemotherapeutics to which ER stress makes cells more susceptible. Hsp27, a microtubule-interacting heat shock protein, promotes microtubule formation away from the centrosome and changes microtubule shape. TBAs cause Hsp27 to be phosphorylated in MCF-7 cells via the p38 signaling pathway, with microtubule stabilizers and destabilizers causing different phosphorylation patterns on this protein. However, uncertainty surrounds the physiological effects of these phosphorylation sites (Figure.1).

By interacting with the tubulin C-terminus, Hsp70 also binds to tubulin, and this association may be facilitated by MAP1B. The involvement of Bcl-2 in TBA-mediated cell death is another indication of crosstalk between microtubules and apoptosis networks. Upregulation of Bcl-2 inhibits the TBA apoptotic response in leukemia cell lines without requiring G2/M arrest or microtubule structural changes. High numbers of Bcl-xL may protect cells from the

stress that taxol causes. Direct associations of Bcl-2 and tubulin may be responsible for these results. The dynein light chain binds to the Bcl-2 interacting cell death mediator (Bim), which is then confined to microtubules and prevents the initiation of apoptosis signaling (Figure.1). Bim interacts with Bcl-2, Bcl-xL, or Bax to promote apoptosis after being released from microtubules and translocated to mitochondria[5].

CONCLUSION

The role of tubulins, microtubules, and their interacting partners in maintaining cellular homeostasis and executing cellular stress responses is increasingly recognized. A growing body of research shows that altered tubulin isotype composition, post-translational modifications, and MAP expression in cancer affect various cellular processes and promote cell survival in the face of oxidative, hypoxic, and protein stress. Through the transit of molecules and organelles, scaffolding for protein-protein interactions, control of enzyme activity, and sequestration of stress response regulators, microtubules and tubulins impact protein signaling networks. Developing a detailed spatiotemporal knowledge of the specific function of tubulin isotypes, their post-translational modifications and the proteins with which they associate represents a major challenge and is a necessary basis for understanding the role of the microtubule network in the regulation and execution of stress responses.

By influencing various cellular stress responses, microtubules are well-positioned to act as coordinators of cellular function in response to stress. In addition, the crosstalk between different signaling events of the stress response means that microtubule engagement in this context can have profound consequences for various cellular functions.

A better understanding of how tubulins and microtubules function in cancer cell stress responses has significant therapeutic benefits. Finding the communication networks that the microtubule cytoskeleton effects could lead to the discovery of new anticancer drugs. It should also be possible to use current therapies more successfully if we better understand the function of the microtubule cytoskeleton in response to cellular stress, particularly chemotherapy stress.

Chemotherapy regimens known to induce specific stress conditions could be chosen to exploit altered stress response signaling in cancer by analyzing tubulin and microtubule aberrations in tumors. Through these pathways, a deeper understanding of the function of the microtubule cytoskeleton in stress responses has the potential to lead to expanded therapeutic windows, reduced resistance to chemotherapy, and more effective cancer treatments with fewer side effects.

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CHAPTER 14

THE ROLE OF THE MICROTUBULE IN THE ORGANIZATION OF THE MTOCS INSIDE THE CELL

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ABSTRACT:

In order to organise microtubules properly during mitosis and ensure correct chromosome division, the centrosome nucleates microtubule polymerization. Cancer cells commonly exhibit defects in centrosome number and function, and centrosome proteins are possible candidates for therapeutic action. Recent research indicates that some centrosome proteins may also play a role in cellular division and that abnormalities in centrosomal components may contribute to a number of human developmental diseases. Despite these findings, the molecular mechanisms governing the operation of centrosomes and other MTOCs remain a mystery. Although the molecular specifics are mainly unclear, acentrosomal MTOCs, in addition to centrosomes, also contribute to microtubule nucleation and organisation both during mitosis and during cell division. In order to comprehend how centrosomal and acentrosomal MTOCs are formed and regulated, as well as how they contribute to microtubule nucleation and organisation, this chapter concentrates on the -tubulin ring complex, the primary nucleator of microtubule polymerization and important component of MTOCs.

KEYWORDS:

Microtubule organization center, Centrosomes component, Eukaryotes cells, Basal bodies, Non centrosome.

INTRODUCTION

In the 1960s, around the same time that the first pictures of eukaryotic cells were captured with a transmission electron microscope, comparative anatomy of the microtubular cytoskeleton in eukaryotes began. The most current analysis of the eukaryotic flagellar system in microalgae was released more than ten years ago [1], but a lot has changed since then, particularly our understanding of novel eukaryotic cell lineages and how they fit into the larger eukaryotic tree. We present a fairly thorough review of the eukaryotic flagellar apparatus within a contemporary molecular phylogenetic framework in an effort to comprehend general patterns of cytoskeletal homology in eukaryotes. In order to deduce ancestral characteristics and later trait changes during the evolutionary history of the flagellar apparatus, we address the known cytoskeletal variety within the main groups of eukaryotes presented above [2].

The microtubule-organizing centre (MTOC), a component of eukaryotic cells, is where microtubules emerge. The two main functions of MTOCs are the organisation of eukaryotic flagella and cilia as well as the construction of the mitotic and meiotic spindle machinery, which divides the chromosomes during cell division. The immunohistochemistry identification of -tubulin makes it possible to observe the MTOC, a crucial site of microtubule formation, in vivo. For MTOCs, the different phyla and regions display distinctive morphological characteristics. The basal bodies, which are linked to cilia and

flagella, and the centrosome, which is involved in spindle creation, are the two most important MTOC types in mammals [3]. Microtubule-organizing centres function as both a hub for microtubule free ends to congregate and as the place where microtubule construction begins. Cells may contain microtubule-organizing centres in a diversity of arrangements. The basal bodies are composed of a group of microtubules that can arrange themselves into a pinwheel structure, which can lead to the formation of microtubule clusters in the cytoplasm or the 9+2 axoneme.

The spindle pole bodies of fungi and mammalian chromosomal kinetochores are examples of other shapes (flat, laminated plaques). MTOCs can be widely dispersed throughout the cytoplasm or can be centralised as centres. The two most noticeable MTOCs are the interphase centrosome and the mitotic spindle poles [4]. The cell's centrioles can act as markers for MTOCs. Centrioles that are evenly distributed throughout the cytoplasm can aggregate to create MTOCs during differentiation. Centrosomes can operate as an MTOC even in the absence of centrioles, but they can also cluster around a solitary one [4]. Most animal cells have one MTOC during interphase, which is generally located nearby and is usually closely connected to the Golgi apparatus. Two centrioles make up the central portion of the MTOC, and PCM, which is essential for the creation of microtubules, surrounds the centrioles. The minus extremities of microtubules anchor them at the MTOC while the plus ends continue to stretch into the cell's periphery.

The polarity of the microtubules is critical for cellular transport because the motor proteins kinesin and dynein typically move preferentially in the "plus" and "minus" directions along a microtubule, allowing vesicles to be directed to or from the endoplasmic reticulum and Golgi apparatus, respectively. The overall layout and position of the Golgi in the cell are influenced by structures linked to the Golgi apparatus, in particular, as they move in the direction of a microtubule's negative end [5]. The centrosome's actions control the motion of the microtubules. Following the end of mitosis, each offspring cell contains one primary MTOC. Before cell division, the interphase MTOC splits into two distinct MTOCs (now typically referred to as centrosomes).

During cell division, these centrosomes move to the extremities of the cell opposing each other and initiate microtubules that aid in the formation of the mitotic/meiotic spindle. If the MTOC does not replicate, mitosis is prematurely terminated and the spindle cannot form. Microtubules are created by interactions between the tubulin molecule at the negative end of the microtubule and the centrosome protein tubulin. Y-description tubulin's of the orientation of the microtubules regulates how they are arranged at the MTOC, or centrosome in this case [4]. In epithelial cells, MTOCs also contain and organise the microtubules that make up the cilia. These MTOCs function similarly to the centrosome by stabilising and directing microtubule movement, enabling the cilium to advance in a single direction rather than being crossed by vesicles.

In yeasts and some cyanobacteria, the MTOC performs the role of a spindle pole body. MTOCs from yeast and fungi lack centrioles. The nuclear membrane does not degrade in these species, and the spindle pole body links the cytoplasmic and nucleus microtubules during mitosis [6]. The three sections that make up the disc-shaped spindle pole body are the centre plaque, inner plaque, and outer plaque. The centre plaque is embedded in the membrane, the outer plaque is a layer located in the cytoplasm, and the inner plaque is an irregular intranuclear layer. Except for their flagellate male gametes, conifers and flowering plants don't have centrioles or spindle pole bodies at all. Instead, the nuclear membrane itself appears to be the main MTOC for microtubule formation and spindle organisation during plant cell division.

The MTOC reorients itself during signal transmission, primarily during immune responses or wound repair. The MTOC is relocated to an area between the cell's edge and its nucleus in cells like macrophages, fibroblasts, and endothelial cells. Microtubules can grow or contract in response to transduction signals, which also make the centrosome motile and aid in the MTOC's rapid reorientation. While the negative ends of microtubules are confined in the MTOC, which is located in a perinuclear location, the positive ends of microtubules rapidly grow towards the cell's border. The MTOC and Golgi apparatus move in concert to simulate a polarised signal being sent by the cell. When mounting an immune response in response to antigen-specific antigen-presenting cells, immune cells like T cells, natural killer cells, and cytotoxic T lymphocytes position their MTOCs close to the contact zone between the immune cell and the target cell. The MTOC is reoriented for T cells as a result of the T cell receptor signalling response, which shortens the microtubules and moves it closer to the T cell receptor contact [7].

LITERATURE REVIEW

Controlling chromosome partition during cell division, placement and movement of various organelles, as well as cell polarity and morphogenesis, all depend on the structure of microtubule networks. The location and activity of the locations where microtubules are nucleated and where their minus ends are attached have a significant impact on the shape of microtubule arrays. The centrosomes in mammals and spindle pole bodies in fungi are two examples of the microtubule-organizing regions where these sites are frequently grouped. Other microtubules can also nucleate, stabilise, and attach microtubule minus ends, as can membrane regions like the cell nucleus, the Golgi apparatus, and the cell cortex. Microtubule-nucleating elements, such as -tubulin-containing complexes and their activators and receptors, as well as microtubule minus end-stabilizing proteins and their binding partners, are necessary for these activities [4].

The connectivity and processing properties of a neuron are determined by the wiring arrangements of its dendrite and axon arbour. These dendrite and axon arbours' identities are produced by unequal polarisation of their microtubule arrays, and the lengthening and structuring of these arrays produces their intricacy and pattern. We outline several molecularly unique microtubule organising centre (MTOC) processes that work to produce and organise dendrite and axon microtubules during neuron differentiation. Arbor cabling is generated, patterned, and diversed by these MTOCs' temporal and spatial structure [8]. The essential cellular process of microtubule separation from microtubule organising centres is necessary for typical cell growth. Microtubule turnover rises as cells begin mitosis, and microtubule detachment also increases at the same time. It has been demonstrated that MCAK, a protein linked to kinesin that is most abundant in the early phases of mitosis, controls microtubule detachment. Cell division is hindered and spindle function is interfered with by abnormal rises or reductions in detachment frequency. The inhibition of microtubule detachment from centrosomes by medicines that can promote microtubule assembly, such as paclitaxel and epothilones, has been demonstrated to stop cell growth.

On the other hand, cytotoxic levels of medications that destabilise microtubules (such as vinblastine and nocodazole), tubulin mutations that result in paclitaxel tolerance, and particular -tubulin isotypes raise the incidence of microtubule detachment [9]. The -tubulin protein, which is present in the -tubulin ring complex (TuRC) that nucleates microtubules, is necessary for microtubule assembly by microtubule-organizing centres like the centrosome. Tubulin and the proteins that make up the tubulin complex make up the majority of the ring-shaped macromolecular complex known as the TuRC. Despite the recent discovery of new TuRC components, little is known about the complex's molecular make-up and regulation

characteristics [10]. Eukaryotic cells can be organised spatiotemporally thanks to the microtubule cytoskeleton. Microtubule organising centres (MTOCs) are usually the only locations where new microtubules can form, and these locations also need -tubulin, which assembles into multisubunit complexes of different sizes.

The effective microtubule nucleators known as tubulin ring complexes (TuRCs) are linked to numerous targeting, activating, and modifying proteins. Microtubules can form at conventional MTOCs like centrosomes and spindle pole bodies as well as additional locations like the Golgi apparatus, nuclear envelope, plasma membrane-associated sites, chromatin, and the surface of pre-existing microtubules. Although much has been learned about the structure of -tubulin complexes and the characterization of interacting components with the TuRC, the regulatory processes governing microtubule nucleation remain poorly known (Figure 1A-1C). In this article, we discuss current research on the variables and control mechanisms that affect the nucleation of centrosomal and non-centrosomal microtubules [11].

The unique spatial structure of the microtubule cytoskeleton, whose configuration is particular to cell type, is necessary for the process of cellular differentiation. Microtubule segmentation is given by specific subcellular locations known as microtubule-organizing centres, not haphazardly (MTOCs). Since the finding of MTOCs fifty years ago, the centrosome has been the primary subject of their research. Centrosomes serve as MTOCs during mitosis in all mammal cells. The MTOC function is shifted to non-centrosomal sites in many differentiated cells, though, to produce non-radial microtubule structure better adapted for novel cell functions, like mechanical support or intracellular transport. the present knowledge of non-centrosomal MTOCs (ncMTOCs) and the processes by which they develop in mammal cells differentiating [12].

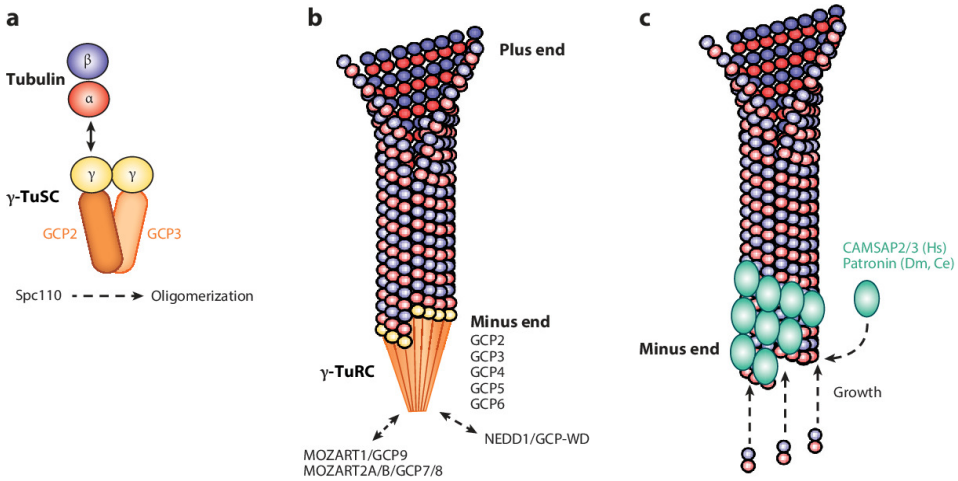

Figure1: Proteins that attach to microtubule minus ends. Showing the example of the different protein attached to the plus ends (Journal of biology).

Different types of simple epithelial cells organise microtubules into apicobasal arrays, with the plus points pointing towards the basal surface and the minus ends anchored close to the apical surface. Although apicobasal arrays in Caenorhabditis elegans, Drosophila, and mammals share some similarities, different processes can be used to produce geometrically comparable arrays, according to a study of these arrays. Consequently, even though -tubulin is delocalized from centrosomes and then relocalizes to just below the apical surface in all of these cells, the methods by which they each carry out this appear to be different. Microtubules develop longitudinal arrays and a stationary lattice as myoblasts fuse to create

myotubes; in this lattice, the dynamic plus ends trace along pre-existing microtubules while the minus ends are linked to the Golgi and nuclei (Figure 1). Centrosomal proteins like pericentrin, ninein, and -tubulin delocalize from centrosomes during myoblast development. Later on, some of these are attracted to the Golgi and the nuclear envelope [13].

Although array geometry is extremely cell type particular, comparable arrays are formed by similar cells in different species. Illustrated are a few sample cell kinds for various array shapes (Figure 2). The pericentriolar material (PCM), which surrounds the two centrioles and is responsible for microtubule formation as well as the anchoring and attachment of microtubule minus ends, forms the centrosome. These two centrioles are known as the mother and daughter. Nine microtubule pairs make up the centre of the symmetric circular shape of centrioles. Centrioles act as the foundation for cilia and flagella in addition to contributing to centrosome development. Here, the topic of great discussion has been the processes of centriole formation and duplication. Importantly, centrosomes are frequently lost when the activity of structural centriolar components like the microtubule-binding protein Sas-4 [also known as CPAP (centrosomal P4.1-associated protein) or CENPJ in humans] or of key factors necessary for centriole duplication, such as Plk4, is disrupted either pharmacologically or genetically. Astral microtubule creation also requires centrosomes.

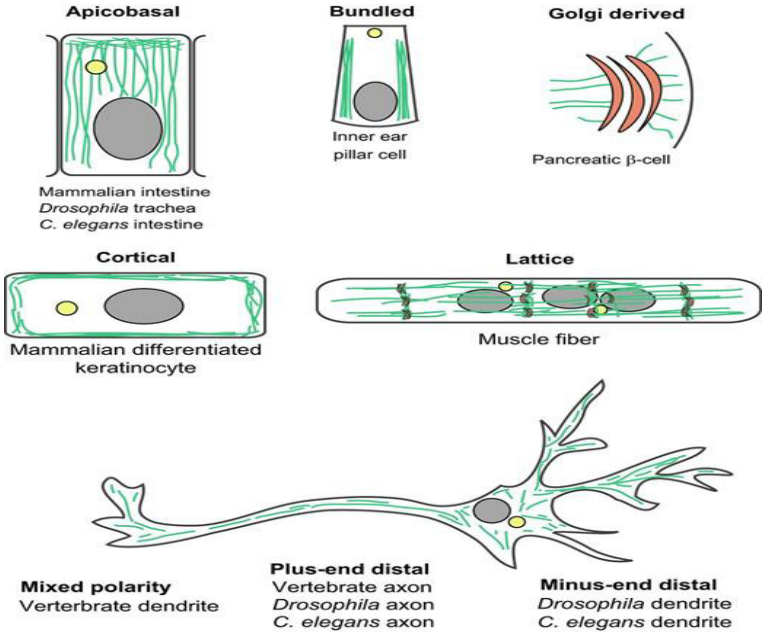

Figure 2: Organization of the different non-mammals cells: Different non-centrosomal MT arrangements are formed by differentiated mammal cells (Secemtic science).

Accordingly, mutations in many centriolar and centrosome components do not cause lethality but rather result in serious developmental defects. Centrosome activity is crucial but not necessary for various cellular functions. Primary autosomal recessive microcephaly, also known as hereditary microcephaly, is a main subtype of this developmental disease. Patients with this condition have tiny brains at birth, simplified gyri, and varying degrees of cerebral impairment. Primordial dwarfism, a disease where patients are born with tiny brains and short height, is also caused by some mutations in centrosome proteins. For instance, pericentrin gene mutations can cause dwarfism, microcephaly, and mental impairment, while CDK5RAP2 gene changes can result in Seckel syndrome, which is marked by prenatal proportionate low height, serious microcephaly, and cerebral disability.

In addition, microcephaly is caused by abnormalities in the genes that encode the centrosomal protein CEP152, the centriole assembly proteins CPAP, STIL, and CEP135. A reduced amount of cells in the brain or the entire body can be the outcome of the spindle misorientation and mitotic delay brought on by centrosome abnormalities. These conditions may also cause progenitor cells to undergo apoptosis. The fact that most tissues grow in these individuals fairly regularly leads one to believe that the centrosome is more crucial for regulating cell quantity than for determining cell polarity and differentiation. Having more centrosomes can also lead to the creation of multipolar spindles, which, while usually fleeting and evolving into bipolar spindles through the clustering of additional poles, can impair mitosis fidelity and cause genome instability, which is frequently linked to cancer. Additionally, raising the amount of centrosomes while maintaining MTOC activity encourages the invasive nature of tumor cells [4].

CONCLUSION

Numerous findings have been made that reveal the processes governing microtubule reorganisation as a result of the recent revival of interest in the creation of non-centrosomal microtubule arrays. However, we think that a few unanswered issues should receive extra attention in the future. It will be fascinating to investigate whether conserved mechanisms connect the cell cycle to centrosome inactivation in various tissues since differentiation undoubtedly causes microtubule rearrangement in many cell types and in many species. Second, the continued creation of in vivo live imaging instruments will contribute to the resolution of lingering issues regarding microtubule nucleation and reorganisation. The idea that reorganisation of microtubules necessitates a momentary spike in dynamics that is eventually repressed intrigues us. Ongoing research on the roles played by non-centrosomal microtubules in living things is also necessary. In order to address these questions and advance from straightforward phenotypic accounts to a mechanistic knowledge of microtubule function, new methods to alter microtubule dynamics, organisation, and polymer levels in the organism will need to be developed. Additionally, it should be noted that correct microtubule function in vivo may rely on a complicated interaction between controlled microtubule dynamics, post-translational modifications, and cell-specific MAPs in addition to the spatial location of filaments. A significant unanswered issue is how all of these various components are combined within the cell to produce effective non-centrosomal arrays.

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CHAPTER 15

MICROTUBULE'S ROLE IN NEURON DEVELOPMENT

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ABSTRACT:

Microtubules are essential for the shape and operation of neurons, which are exquisitely polarised cells. Microtubule structure and microtubule-associated proteins vary between signal-receiving dendrites and signal-sending axons. These variations work together to control intracellular transport, morphology, and function at the local level, along with microtubule post-translational modifications. The control of non-centrosomal microtubule arrays in neurons, the connection between microtubule acetylation and mechanosensation, and the spatial patterning of microtubules that controls motor activity and cargo transport in axons and dendrites are all subjects of recent research. The combination of these novel studies advances our comprehension of the localization of microtubule function to the specialized functions involved in signal transmission and reception.

KEYWORDS:

Dendrites Outgrowth, Microtubule Dynamics, Neuron Growth, Regulation of Microtubules, Minus Ends, Morphological Function.

INTRODUCTION

The function of the cell depends on the polarity and structure of the cell. The range of functions that neurons perform is reflected in the diversity of morphologies that they exhibit. No matter how simple or complex they are in shape, all neurons have unique signal-receiving dendrites and signal-sending axons and are highly compartmentalized [1]. The underlying microtubule cytoskeleton, which shapes the neuronal structure and facilitates the movement of RNAs, proteins, vesicles, and organelles that support neuronal activity, is essential for both a neuron's morphology and function. It is not unexpected that microtubule organization and function are affected by the distinct compartmentalization of neuronal activity.both within and between the divisions of axons and dendrites. Understanding how microtubules are locally regulated to correspond with particular neuronal processes is a difficult task. Here, we highlight new research on the patterning of microtubules that regulates molecular motor transport and diversifies microtubule function regionally [1].

The different roles of signal transmission and reception are carried out by axonal and dendritic projections, which are shaped and supported by the neuronal microtubule cytoskeleton. The polarity, structure, post-translational modifications, and microtubule-binding proteins of microtubules in axons and dendrites distinguish them. Due to the head-to-tail assembly of a- and b-tubulin dimers (the a- and b-tubulin ends are referred to as the plus end and negative end, respectively), microtubules have an inherent polarity [2].

This polarity, which is believed to be a key determinant of neural polarity, is read out by molecular motors and other proteins. In dendrites, microtubule polarity is mixed to varying

degrees based on location within the dendritic arbor, neuronal type, and organism, in contrast to axons, where microtubules are uniformly arrayed with their plus-end positioned distal towards the axon terminal. The molecular motors dynein and kinesin, which are essential in building the tracks that microtubules use for movement, control microtubule orientation in part [3]. The mammalian tripartite motif-containing (TRIM) protein TRIM46 is one more protein that controls microtubule direction, though its precise molecular function is still unknown [4]. The consistent alignment of axonal microtubules is hampered by TRIM46 loss.

However, dendritic carriers like AMPA receptors retain their normal locations. This suggests that changing the functional polarity of neurons may require more than just interfering with microtubule polarity.In contrast to other cell types, neurons' microtubules are not usually anchored at a specific microtubule organizing center (MTOC). While mammals' post-mitotic neuronal centrosome primarily facilitates ciliary function, some organisms, like fruit flies, have neurons that are completely devoid of a centrosome [5]. Microtubule nucleation, growth, and organization are locally controlled in the lack of a central MTOC. Recent research has illuminated the roles played by the TPX2 and SSNA1 microtubule-binding proteins, the CAMSAP microtubule minus-end-binding proteins, and the augmin complex in the control of gamma-tubulin-mediated nucleation [5].

Augmin boosts microtubule density to allow efficient transport in both axons and dendrites by recruiting gamma-tubulin to microtubules to amplify existing arrays [5]. Loss of augmin function in mammals alters the orientation of axon microtubules, potentially by allowing gamma-tubulin to become uncoupled from preexisting microtubules. However, in dendrites, loss of augmin alone does not affect microtubule polarity, and studies in flies indicate that centrosomin/CDK5RAP2 limits augmin activity [6]. Centrosomin/CDK5RAP2 is thought to link gamma-tubulin-mediated microtubule nucleation to dendritic Golgi outposts, which act as local MTOCs in developing neurons [6], along with GM130 and AKAP450.

A recent worm research revealed that dynein, which tethers microtubule plus-ends to restrict their growth, locally regulates the growth of existing microtubules in dendrites in addition to shaping microtubule networks through microtubule nucleation [7]. As a result, neurons employ a variety of methods to adjust the microtubule cytoskeleton in both space and time. Because of this local control over nucleation, growth, and organisation, microtubules are able to mediate a variety of actions and adapt quickly to changes in neuronal function[7].

In the growing brain, correct neuronal alignment depends on neuronal migration, which requires the coordinated activity of cellular elements, including cytoplasmic MTs [8]. Excitatory neurons that are born in deep layers of the brain and then migrate radially toward the brain surface have been characterized as migrating in three different ways during the development of the cerebral cortex[8]. Somal translocation, multipolar migration, and glial-guided radial migration or locomotion are some of these mechanisms.

Leading process extension, nucleokinesis (i.e., nuclear translocation into the leading process), and cell posterior retraction are the three sequential stages that makeup radial migration. The centrosome is situated in advance of the nucleus in moving neurons (a phenomenon called N–C coupling). Given that an N-C coupling is disturbed in neurons with migration defects, this arrangement is thought to be essential for nucleokinesis [9].Dynein activity is necessary for the development of consistently oriented MTs in axons in Drosophila DA neurons, and MT-

based transport of Rab5-endosomes by dynein and kinesin is necessary for the morphogenesis of dendritic branches.

Short branches don't have as many retrogradely developing MT plus ends as longer dendrites do, and Golgi outpost MT organizing centers play a role in the formation of MT polarity[10]. These results collectively imply that MT structure and the development of neuronal processes depend on a complex interaction between MTs, motor proteins, and membrane organelles [10]. Golgi outposts are located at dendritic branching sites in mammalian hippocampal neurons, but MT orientation nucleated from Golgi outposts has not been studied. After retraction of the process tip, we saw the formation of retrogradely growing MTs in slice-cultured mouse neocortical neurons, which suggests that MT severing and/or catastrophe near the tip of processes adds to the nucleation of mixed-polarity MTs [11].

LITERATURE REVIEW

Microtubule (MT) activity is required for neuronal migration and polarisation, two important processes in brain morphogenesis [12]. Because of the imbalance of the -tubulin heterodimer, MTs have inherent polarity. A slow-growing minus end, where -tubulin subunits are exposed, and a fast-growing plus end, where -tubulin subunits are exposed, are the two different ends that characterize MTs. A cellular process's MT network polarity has an impact on both its directed transport along MTs and its dynamic character [12]. By attaching -tubulin heterodimers to the -tubulin ring complex on the exterior of an MT organizing center, such as the centrosome, cytoplasmic MT formation is started [13]. A polarised cytoskeleton is created by MT elongation through the attachment of tubulin heterodimers to the plus end. Dynamic instability is a behavior that occurs when fibers are forming and involves periods of growth and shortening [13]. Large cellular protrusions called leading processes, axons, and dendrites are formed by neurons and are involved in circuit creation and neuronal migration. The MT cytoskeleton is present in these processes, and dynamic MT changes are what cause their expansion and retraction. Additionally, the MT cytoskeleton must preserve the stability of neuronal processes in the growing brain [13].

For MT-based motors to allow directional movement of intracellular cargos within processes, the highly polarised MT structure provides tracks. In migratory neurons, the minus-end-directed dynein motor complex is crucial for nucleokinesis. KIFs, the majority of which are plus-end-directed motors, have a variety of impacts on the dynamics of MTs and neuronal morphogenesis. For instance, kinesin-1 (KIF5) promotes axon formation and elongation by transporting cargos like membrane vesicles and the CRMP2-tubulin complex, whereas kinesin-2 (KIF3) allegedly polarises the Par3 complex leading to axon specification (Figure 1A-1D).

Short MT transport is adversely regulated by the mitotic MT-associated motor proteins kinesin-5 (Eg5, KIF11), kinesin-12 (KIF15), and this restricts axonal growth and neuronal migration. According to reports, MT organisation in axons and dendrites is allegedly regulated by kinesin-6 (CHO1, MKLP1, KIF23) and kinesin-12 (HKLP2, KIF15). It is known that other members of the kinesin family, including kinesin-8 (Kip3) and kinesin-13 (MCAK), regulate dynamic instability by encouraging MT disaster [13].

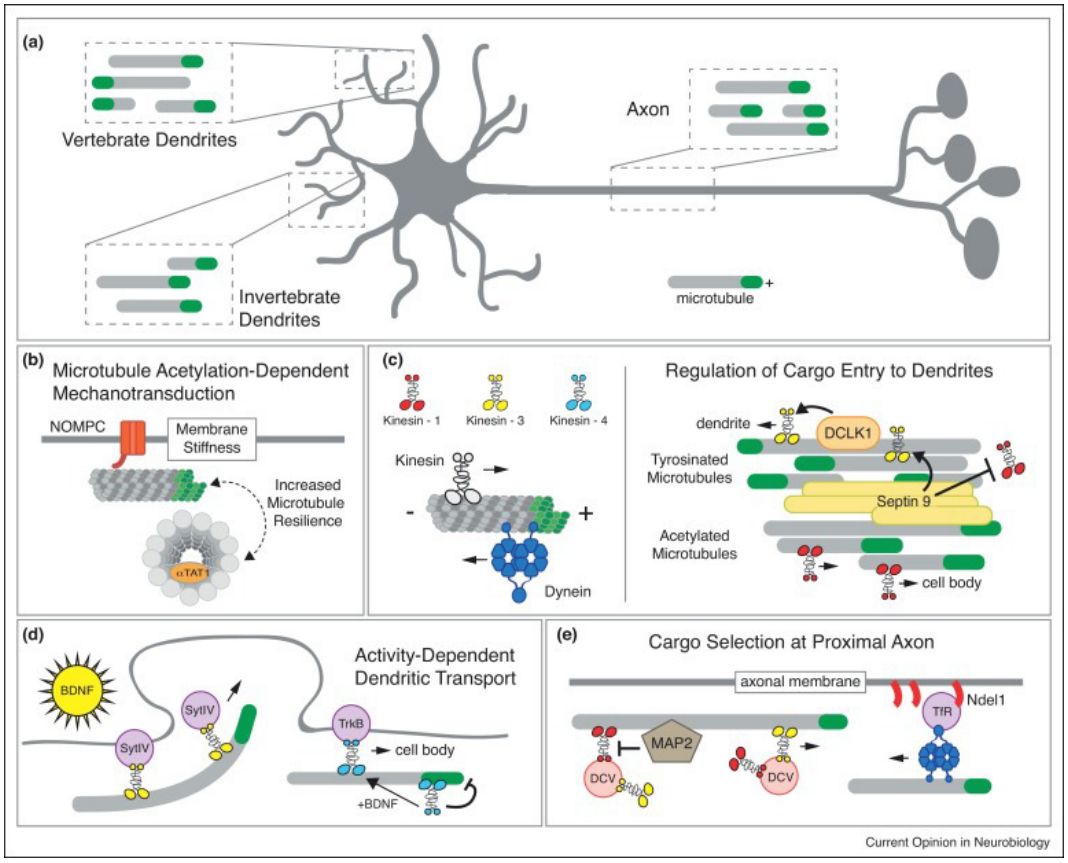

Figure 1 : Function of the microtubules: Schematic diagram showing the different functions of the microtubules in the nervous system (current opinion in neurobiology).

The development and enlargement of the growth cone, a dynamic structure at the developing axon's tip that drives axon elongation and branching, is a significant shift that happens after axon specification. Neuronal growth cones explore the extracellular world and interact with a variety of external stimuli, which causes the axon to be guided in a specific path. The protrusion, engorgement, and consolidation are the three different morphological stages that the growth cone passes through using the cytoskeletal machinery (Figure 1D,1E)[1].

To advance through these phases, a variety of dynamic MTs enter the growth cone's central and periphery and exhibit a variety of behaviors, including splaying, looping, and bundling. For MTs to probe the growth cone periphery in quest of guidance signals, which trigger growth cone advancement and turning, this remodeling is necessary. The interaction between actin filaments and dynamic MTs, which together encourage the extension of lamellipodia and filopodia at the tip of the axon, is also necessary for growth cone formation and advancement. The accumulation of actin filaments that will eventually create axonal filopodia along the axon shaft during axon branching necessitates cytoskeletal remodeling as well [1].

Shortly after, the normally bundled MT array is localized splayed as MTs start to infiltrate the actin-rich filopodia. The axonal MTs' invasion of the filopodia enables them to mature into collateral branches as they continue to expand.Other neurites start to turn into dendrites after axon formation, causing significant alterations to the MT network. Although dendrites branch more widely than axons do, the activity of MTs during this process is still not completely understood. The orientation of their MTs is one of the most noticeable characteristics that sets dendrites apart from axons.

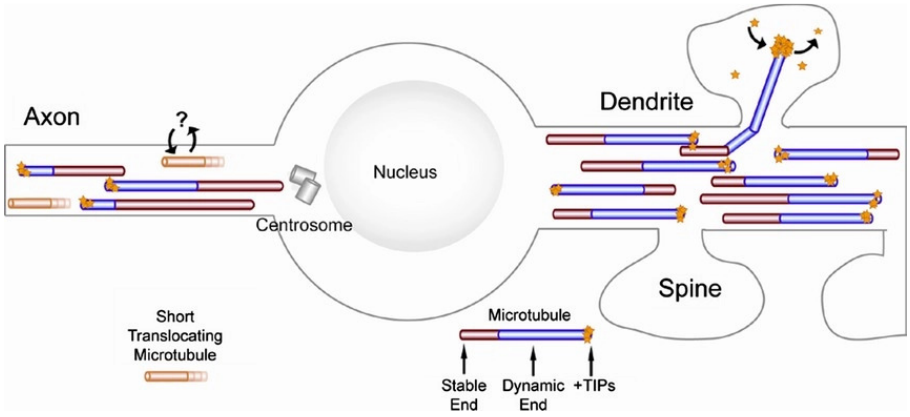

Figure 2: Microtubules in neuron: Microtubules in the axon and dendrite as information bearers. Diagram of a stylized neuron with microtubules in the axon and dendrite (Wiely online library).

While dendrites contain a community of mixed-polarity MTs, where both plus and minus ends are directed toward the cell body, axons exhibit uniform plus-end distal MTs[14].Dendritic spines, which serve as the postsynaptic locations of excitatory synapses, form and mature as the next stages in neuronal growth. Throughout development and into adulthood, the brain constantly rewires the connections between an axon and a dendrite of nearby neurons. This rewiring happens in reaction to novel stimuli (Figure 2). For a while, it was believed that actin was the primary regulator of spine shape and dynamics related to synaptic plasticity and that dendritic spines lacked dynamic MTs[14].

Neuronal microtubules contain both stable and extremely dynamic regions, some of which continue to exist as short mobile polymers after episodes of severing. Microtubule plus-end tracking proteins known as +TIPs, which are concentrated at the plus ends of the highly dynamic areas, can interact with a variety of other proteins and structures important to the neuron's plasticity. The idea that short, mobile microtubules might also carry information as they move around the neuron is also thought to be controversial. Microtubules may therefore serve a role in the neuron as "information carriers" in addition to their well-known conventional roles in sustaining neuronal architecture and organelle transport. There is the possibility that neuronal microtubules serve as "information carriers," transporting biochemical elements within axons and dendrites. Longer microtubules have more dynamic regions that are associated with information-rich proteins called +TIPs at their plus ends [14]. These +TIPs can engage or deposit on other proteins or structures that the microtubule encounters as it assembles. Short, steady microtubules that move through the axon (and, presumably, the dendrite) may also be used to transport this data. Microtubules are well-known for their functions in architecture and transport, and we propose that a third significant function of microtubules in neurons may be acting as information carriers.

End-binding protein 3 (EB3) was used in cultured hippocampal and Purkinje neurons to perform the first study to demonstrate dynamic microtubule polymerization in dendrites. This study showed that while microtubule polymerization in neuronal cells is slower than in non-neuronal cells, the link of +TIPs with microtubules is still present in both types of cells. This

research also provided evidence for the widespread occurrence of microtubule polymerization and, by extension, microtubule dynamic instability within the axonal and dendritic arbors [15].

The observation of "moving comets" that appear at the plus ends of developing microtubules was another astounding feature of imaging +TIPs. Interestingly, further research revealed that the +TIP proteins that make up these comets interact with the plus ends of developing microtubules only momentarily. As a result, +TIP proteins are continually being exchanged and concentrated at microtubules' plus ends. What pulls +TIP proteins to the expanding microtubule tip? It seems that EB proteins' calponin homology domain connects microtubule protofilaments and binds near the GTP-binding site. Recent research also suggests that the GTP cap on microtubules in cells is quite large (> 700 tubulin subunits) and binds nearly 300 EB1 dimers. What impacts on cellular structure and function might the localized concentration and release of +TIP proteins have? It is widely believed that +TIP proteins, including EB proteins, adenomatous polyposis [15]coli, cytoplasmic linker proteins (CLIPs), and CLIP-associated proteins, stabilize microtubules against depolymerization by encouraging microtubule growth and preventing or minimizing catastrophe.

In addition, EB proteins can attach other +TIP proteins as well as microtubules directly. Numerous investigations have demonstrated that microtubule plus ends specifically target adhesions between cells and between cells and the cell matrix. It's interesting to note that dynein, functioning as a +TIP protein, binds to NCAM180 to secure dynamic microtubule plus ends, maintaining the density of synapses along the dendritic arbour [15].

The tethering of microtubules to NCAM180 at synapses raises the prospect that material can be specifically transported to a given synapse to increase synapse stability. Additionally, cell Rho GTPase pathway regulators can attach to microtubules. It's interesting to note that one study found that GEF-H1 (Lfc) release from dendrite microtubules enables it to enter spines and activate RhoA, which causes changes in actin polymerization that lead to shorter spines and denser spines. As a result, microtubules can transport activators of Rho GTPases locally to particular areas of the neuronal cell membrane[15].

CONCLUSION

MTs have distinctive structural and physical characteristics and are necessary for cellular polarisation and movement. Immature processes must be differentiated into axons or dendrites through modification of their structural characteristics. Molecular motors, which regulate neuronal morphology and function, produce force, which is translated by molecular transporters (MTs), which act as tracks for directed transport.

The discovery of mutations in the genes encoding MT-related proteins and tubulin in individuals with brain defects or disorders emphasizes the significance of MT structure. Although MT polarity within structural elements of migrating neurons and polarised neurons have recently been characterized, more research is needed to understand how this organization is controlled. The primary cellular organizing center for MT, the centrosome, exhibits changing behavior during the polarisation and migration of neurons. The instructive function of cells is presently being reevaluated through the comparison of various cellular systems.

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CHAPTER 16

MIGRATION, INVASION AND MALIGNANT FEATURES OF INTERMEDIATE FILAMENTS

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ABSTRACT:

Intermediate filaments (IF) proteins make up the largest family of cytoskeletal proteins in metazoans and are traditionally known for their places in fostering structural integrity in cells. Remarkably, individual IF genes are tightly regulated in a fashion that reflects the type of towel, its experimental and isolation stages, and the natural environment. In cancer, IF proteins serve as individual labels, as excrescence cells incompletely retain their original hand expression of IF proteins. still, there are also characteristic differences in IF gene expression and protein regulation. The use of high outturn analytics suggests that excrescence-associated differences in IF gene expression have prognostic value. resemblant exploration is also showing that IF proteins directly and significantly impact several crucial cellular parcels, including proliferation, death, migration, and invasiveness, with a demonstrated impact on the development, progression, and characteristics of colorful excrescences. In this chapter, we study concentrated on IF proteins most associated with cancer(keratins, vimentin, nestin, etc.) to punctuate how intermediate filaments are important in cancer. The substantiation formerly in hand establishes that IF proteins serve beyond their classical places as labels and serve as effectors of tumorigenesis.

KEYWORDS:

Cancer cell, Cell migration, Intermediate filaments, Cell proliferation, cytoskeleton protein

INTRODUCTION

The intermediate hair(IF) family of proteins is composed of 73 genes, making it the most different family of cytoskeletal proteins, which also includes microtubules and actin fibers. The intermediate fibers entered its name due to their 10 nm hair range being intermediate between those of 25 nm wide microtubules and 6 nm wide actin fibers. Each gene within the intermediate hair family is distributed into one of six major subtypes, grounded on primary amino acid sequence similarity, with expression being in nearly all eukaryotic cell types. Even though the family contains many genes, each cell in the body only produces a portion of them. therefore, the expression of each member is tightly regulated in a towel, isolation, or environment-dependent manner. Remarkably, all IF proteins partake in an analogous structure in that a largely conserved central α- spiral rod sphere is adjoined by further divergent head and tail disciplines[1]. It's the central rod sphere that enables the tone-assembly into oligomers and conformation of 10 nm fibers[2].

Since they are cytoskeletal proteins IF proteins play a crucial part in preserving the structural integrity of cells and organs. This is demonstrated by IF gene mutations, which cause aberrant filament formation and directly contribute to a variety of uncommon human illnesses that show symptoms of disturbed tissue integrity, such as skin blistering or atypical muscle fiber formation[3]. Interestingly, research investigating the role of IF in both normal tissue homeostasis and human illnesses has shown that IF proteins perform a variety of non-

mechanical support activities. Nowadays, it is generally acknowledged that IFs control several biological functions, including cell migration, apoptosis, and growth. A change in the levels of IF proteins play a critical role in altering cellular processes to facilitate disease progression in diseases like alcoholic and nonalcoholic steatohepatitis and psoriasis, where an aberrant expression of IF proteins can also be seen in addition to mutations seen in many diseases, as discussed above.

The roles of IF proteins in cancer will be discussed here, with an emphasis on a subset of IFs—keratins, vimentin, and nestin that have been researched the most concerning cancer [2]. In response to mechanical and non-mechanical stressors, keratins, which are members of the IF protein family, play a critical structural support role in all epithelium cells. Pairwise control is needed at the transcriptional and post-translational levels for the 54 keratin genes with 28 type I and 26 types II sequences for Type I and II keratins to heterodimerize and further polymerize to create filaments. Not all keratins are produced in a particular cell, just as it is for the entire IF family. Only a small portion is, however, produced in a tissue-specific, differentiation-dependent, and developmentally-regulated way. Epithelial cell categorization is therefore made possible by the profile of keratin expression.K5, and K14, for instance, are produced in basal epithelial cells, whereas K8, K18, and K19 are found in basic epithelial cells.

Upon various mechanical and non-mechanical inputs, keratin filaments can actively reshape and experience rearrangement to control a variety of cellular processes, including cell signaling and migration [2]. A 57 kDa type III IF protein called vimentin is primarily found in mesenchymal cell types like fibroblasts, blood cells derived from bone marrow, and endothelial cells. Vimentin, a significant IF protein found in mesenchymal cells, is essential for a variety of biological processes, including cell adhesion, motility, and communication. The epithelial-to-mesenchymal transition (EMT), which is essential for cancer spread, is where vimentin is most commonly used as a marker of mesenchymal cell types in cancer[2].

Nestin is a class VI IF protein that was first identified in neuroepithelial stem cells. It is still used as a marker for neural stem and precursor cells in the central nervous system, but it is also found in other tissue types, such as endothelial cells. Nestin, a large protein with a long C-terminal end that weighs >170 kDa, links with other IF proteins to create filaments because it is unable to do so on its own. Nestin has been discovered to be present in cancer cells with weak differentiation and cancer stem-like cells[4]. Patients with cancer may profit from therapeutic agents against IFs and therapeutic methods against its related signaling network given the clinical association and the function of IF proteins in tumor development. Tumour development and metastasis were inhibited in several rodent models by withaferin-A, a bioactive substance that targets vimentin and causes vimentin cleavage. Although it affects several other cellular components, withaferin-A is the only small molecule that suppresses IF construction and function. Therefore, it will be crucial to create small compounds that specifically target IF proteins to modify their roles in malignancy. Clinically speaking, creating such a reagent and figuring out the processes underpinning IF-mediated tumor progression will be extremely beneficial for cancer patients[2].

LITERATURE REVIEW

Intermediate filaments (IF) proteins make up the largest family of cytoskeletal proteins in metazoans and are traditionally known for their places in fostering structural integrity in cells. Remarkably, individual IF genes are tightly regulated in a fashion that reflects the type of towel, its experimental and isolation stages, and the natural environment. In cancer, IF proteins serve as individual labels, as excrescence cells incompletely retain their original

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A

IF Type	Protein		Gene	Mol Wt (kDa)	Primary Cell Types
I	Keratin	K9, 10, 12-20, 23-28, 31-40	*KRT9, 10, 12-20, 23-28, 31-40*	40 – 57	Epithelial cells
II		K1-8, 71-86	*KRT1-8, 71-86*	50 – 70	
III	Vimentin		*VIM*	57	Mesenchymal cells
	Glial Fibrillary Acidic Protein		*GFAP*	50	Glial cells
	Peripherin		*PRPH*	57	Neurons
	Desmin		*DES*	53	Muscle
	Synchoilin		*SYNC*	54	Muscle
IV	Neurofilament proteins (NF-L, NF-M & NF-H)		*NEFL, NEFM & NEFH*	60 – 110	Neurons
	a-internexin		*INA*	66	Neurons
	Synemin		*SYNM*	>150	Muscle & Neurons
	Nestin		*NES*	>170	Neural stem/progenitor cells
V	Lamins A, B & C		*LMNA, LMNB1 & LMNB2*	67 – 70	Nucleus
VI	Filensin		*BFSP1*	100	Lens
	Phakinin		*BFSP2*	49	Lens

B

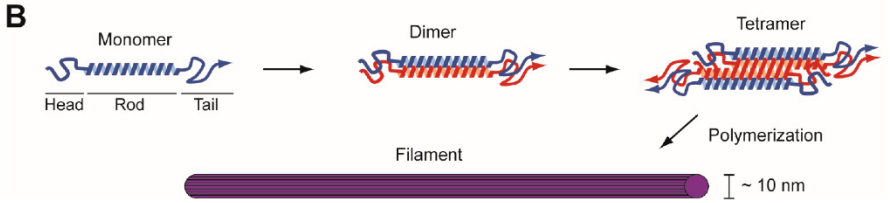

Figure 1: Intermediate Filament (IF) Family of Proteins: (A) List of the class classes, gene names, molecular weights, and expression profiles of the IF proteins. (B) The domains of the intermediate filaments are shown schematically(MDPI).

Intermediate fibers constitute the third element of the cellular shell. Unlike actin and microtubule cytoskeletons, the intermediate fibers are composed of a wide variety of structurally related proteins showing distinct expression patterns in keratin and cell types (Figure. 1A). Changes in the expression patterns of intermediate fibers are frequently associated with cancer progression; in particular with phenotypes leading to increased cellular migration and irruption. In this review, we will describe the part of vimentin intermediate fibers in cancer cell migration, cell adhesion structures, and metastasis conformation [5].

The vimentin (decoded by VIM) is one of the 70 mortal intermediate fibers(IFs), erecting largely dynamic and cell- type-specific web networks in the cytoplasm (Figure.1B). hardihood mice parade process blights associated with cell isolation, which can have counteraccusations for understanding cancer and complaint. The main focus of the discussion is on vital signaling pathways associated with how VIF coordinates irruption cells and migration. The current exploration will open up multiple processes to probe the function of VIF and other IF proteins in cellular and molecular biology, and they will lead to essential perceptivity into different VIF situations for invasive metastatic cancer cells. Enrich GO databases used Gene Ontology and Pathway Enrichment Analysis.

Estimation with STRING online was to prognosticate the functional and molecular relations of proteins – protein with Cytoscape analysis to search and select the master genes. Using

Cytoscape and STRING analysis, we presented eight genes, RhoA, Smad3, Akt1, Cdk2, Rock1, Rock2, Mapk1, and Mapk8, as the essential protein–protein commerce with vimentin involved in the irruption. The relations between a cancer cell and its extracellular matrix(ECM) have been the focus of an adding quantum of disquisition. The part of the intermediate hair keratin in cancer has also been coming into focus of late, but further exploration is demanded to understand how this piece fits in the mystification of the cytoskeleton-intermediated irruption and metastasis. In Panc- 1 invasive pancreatic cancer cells, keratin phosphorylation in confluence with actin inhibition was set up to be sufficient to reduce cell area below either treatment alone. By anatomized cutting keratin and actin filaments in the cytoskeleton of cyclically stretched cells and setting up no directional correlation.

The part of keratin association in Panc- 1 cellular morphological adaption and directed migration was also anatomized by cultivating cells on cyclically stretched polydimethylsiloxane(PDMS) substrates, nanoscale grates, and rigid pillars. In general, the reorganization of the keratin cytoskeleton allows the cell to come more ‘ mobile ’- flaunting briskly and more directed migration and exposure in response to external stimulants. By combining keratin network anxiety with a variety of physical ECM signals, we demonstrate the connected nature of the armature inside the cell and the scaffolding outside of it and punctuate the crucial rudiments easing cancer cell- ECM relations [6]. The main value of intermediate filaments (IF) in biological and applied research is their high cell and tissue specificity.

This is particularly well illustrated by the expression of keratin (K) in various oral epithelia. Although the original IF class is usually preserved in tissues after neoplastic transformation, epithelia tend to change their keratin expression pattern in a way that, although not precisely predictable, can sometimes have diagnostic or prognostic significance. This report compares keratins in normal oral epithelium, which show predominantly site-dependent expression, with keratins in squamous epithelium. The main changes in the latter are the presence of simple epithelial keratins K8 and K18 (sometimes K7), reduced expression of differentiation-related keratins (K1, K10, K, and K13), and a tendency to downregulate primary keratins. K5 and K1 . Moderate and severe dysplasias also usually have K8 and K18 and co-expression of differentiation-related keratins [7].

Nestin is a class VI intermediate filament (IF) expressed in 30% of cases of pancreatic ductal adenocarcinoma (PDAC) and its expression in PDAC is positively correlated with peripancreatic invasion. An expression vector carrying a short hairpin RNA (shRNA) targeting nestin was stably transfected into human pancreatic cancer cells PANC-1 and PK-5H, which express high levels of nestin. Changes in the morphology and alignment of actin filaments and α-tubulin were examined by phase contrast and immunocytochemistry. Effects on cell growth, migration in scratch and Boyden chamber assays, invasion, cell adhesion and in vivo growth were determined.

Differences in mRNA levels were examined by group. Cells transfected with nestin shRNA showed reduced expression of nestin, a disc-like appearance with tight cell-cell adhesion, increased expression of filamentous F-actin and E-cadherin, and impaired migration and invasion, both of which improved upon re-expression. of nestin, Nestin downregulation did not alter the α-tubulin expression or in vitro cell growth and adhesion, while liver metastases were reduced. Thus, nestin plays an important role in the migration, invasion, and metastasis of pancreatic cancer cells by selectively modulating the expression of actin and cell adhesion molecules, and may therefore be a new therapeutic target in PDAC[8].Intermediate filaments (IFs) are assembled from a diverse group of evolutionarily conserved proteins and are defined in the body in a tissue-, cell-type, and context-dependent manner. IFs are involved in a

variety of cellular processes that are critical for maintaining cell and tissue integrity and for responding to and adapting to various stresses, such as a variety of debilitating clinical disorders caused by inherited mutations in IF coding sequences.

Consequently, the activities, composition, and organization of investment funds are strictly regulated. Migration is a relevant example of a cell-based phenomenon in which IFs participate as both effectors and regulators. With a specific focus on vimentin and keratin, here we investigate how the contributions of IFs to cell mechanical properties, cytoarchitecture and adhesion, and regulatory pathways together significantly affect cell migration [9].Cell migration is a multi-step process based on the coordination of basic cellular structures in space and time. Although the role of actin and microtubules has been studied in detail, the lack of inhibitors and imaging tools and the large number of proteins that form intermediate filaments (IFs) have delayed the characterization of IF functions during migration. However, a large body of evidence has gradually indicated changes in IF composition as an important parameter in regulating cell motility properties during both development and tumor invasion. Recent comprehensive analyzes show that IFs are dynamically reorganized to participate together with microfilaments and microtubules in key steps leading to cell migration [10].

Interactions between a cancer cell and its extracellular matrix (ECM) have been increasingly studied. Recently, the role of intermediate filament keratin in cancer has also received attention, but more research is needed to understand how this piece fits into the puzzle of cytoskeleton-mediated invasion and metastasis. In Panc-1 invasive pancreatic cancer cells, inhibition of keratin phosphorylation together with actin was found to be sufficient to reduce cell surface area under either treatment alone. We then analyzed the cross-linking of keratin and actin filaments in the cytoskeleton of cyclically stressed cells and found no direct correlation. The role of keratin organization in morphological adaptation and directed migration of Panc-1 cells was then analyzed by culturing cells on cyclically strained polydimethylsiloxane (PDMS) substrates, nanoscale grids, and rigid pillars. In general, reorganization of the keratin cytoskeleton allows the cell to become more "mobile" and exhibit faster and more directed migration and orientation in response to external stimuli. By linking keratin network perturbations to various physical ECM signals, we demonstrate the interconnected nature of intracellular architecture and extracellular scaffolds and highlight key elements that facilitate interactions between cancer cells and the ECM [6].

Metastasis is the leading cause of death in cancer patients. In recent decades, significant progress has been made in understanding the molecular and cellular basis of this cancer-killing process. This report summarizes some of the most important advances in this field and discusses the role of cell junctions, cell adhesions, epithelial-mesenchymal transition, angiogenesis,lymphangiogenesis, and organ-specific metastasis [11].

Most cancer deaths are caused by metastases, not the primary tumor. Cancer cells invade normal tissue such as epithelial sheets or single cells, inducing the expression of programs characteristic of developmental processes. Depending on the tissue of origin, cancer cells then spread to separate target organs, where they seed secondary tumors (metastases). A recent report indicates that the formation of metastases requires changes not only in cancer cells but also in the tumor microenvironment and the location of the metastatic target. For example, a premetastatic niche is formed in target organs that attract cancer cells. Understanding the different mechanisms used by cancer cells to form metastases allows a better evaluation of patients and the design of innovative therapies[12]. The α6β integrin is a receptor of the laminin family of extracellular matrix proteins and is widely expressed in most epithelial tissues and Schwann cells. Most epithelial tumors exhibit increased

expression of this integrin, indicating a function for 64 in the development of these tumors. Through functional and physical interactions with other receptors, the tumor milieu is also known to increase the signaling efficacy of 64. In this overview, we go over the biological processes by which integrin 64 encourages mammary tumor development by promoting carcinoma cell invasion and motility [13].

The capacity of tumor cells to transition between mesenchymal and amoeboid (bleb-based) migration allows them to invade remote locations. Because of this distinction, metastasis inhibitors need to consider each method of migration. Vimentin's function in the amoeboid movement has not yet been identified, though. Because vimentin is known to have a significant impact on the mechanical characteristics of cells and because amoeboid leader bleb-based migration (LBBM) takes place in constrained areas, the researcher proposed that a malleable vimentin network is necessary for rapid amoeboid migration. As a well-established indicator of the epithelial-to-mesenchymal transition, vimentin makes a perfect target for a metastatic suppressor. With the aid of a previously created polydimethylsiloxane slab-based method for cell confinement, RNAi-based vimentin silencing, vimentin overexpression, pharmaceutical treatments, and measurements of cell stiffness, we discovered that vimentin overexpression and simvastatin-induced vimentin bundling inhibit fast amoeboid migration and proliferation. It is significant to note that these effects were unaffected by variations in actomyosin contractility. According to the findings, a malleable vimentin intermediate filament network encourages the LBBM of amoeboid cancer cells in restricted spaces, and vimentin bundling interferes with cancer cells' mechanical characteristics and prevents them from becoming invasive [14].

When normal cells transform into cancer cells, the original expression signatures of IF proteins are largely preserved. Since most cancer cells originate from epithelial cells, staining of IF cells, especially keratins, has proven to be a useful tool for the pathologist in identifying tumor and cell types. The phosphoinositide 3-kinase/Akt pathway is one of the main oncogenic signaling pathways activated in human cancers and regulates various cellular processes, including cell proliferation and migration. selected keratins were found to be required for the activation of Akt and its downstream signaling molecule rapamycin (mTOR). K19 has also been shown to regulate the Notch signaling pathway for cell proliferation, albeit with different effects on different cancer cell lines [2]. Huh7 hepatocellular carcinoma cells expressing K19 shRNA showed reduced cell proliferation and decreased levels of NOTCH1, JAG1, DTX1 and TGFBR1, as well as phosphorylated SMAD2 and SMAD3.

However, knockdown of K19 in MDA-MB-231 and MCF7 breast cancer cells increased proliferation by regulating the Notch signaling pathway. All these data suggest that targeting nestin may be a viable therapeutic option in cancer treatment. Regarding nestin's underlying tumor growth mechanisms, Wnt signaling appears to be necessary, as nestin depletion by siRNA transfection reduced Wnt/β-catenin activation, which is critical for human breast cancer stem cell proliferation. In cancer, IFs can enhance tumorigenesis by inhibiting tumor suppressors. For example, K17 interacts with the tumor suppressor and cell cycle inhibitor p27KIP1 and promotes its nuclear export for degradation in human cervical cancer cell lines and this K17-dependent regulation of p27KIP1 increases cell proliferation. Interestingly, another tumor suppressor, NF1, a protein regulating Ras activity, has been shown to associate with K1 during skin development in the basal layer of the epidermis , suggesting that K1 may regulate its tumor suppressor function[2]. Resistance to cell death is an important property of tumor cells that allows them to withstand countless challenges to proliferate, including chemotherapy. Clinical associations between altered IF protein expression and resistance to chemotherapy and radiotherapy have been observed in several cancer types, and new data

confirm the effect of IF proteins on resistance to cell death. In addition to nestin and vimentin, keratin also plays a key role in resisting cell death. For example, the use of gemcitabine and cisplatin, a standard chemotherapy regimen for advanced bladder urothelial cancers, in mouse xenograft tumors showed that chemotherapy-resistant bladder cancer stem cells express K1[2].

As for vimentin, it is required for cell sprouting in endothelial cells. Vimentin was required for membrane localization and proper activation of MT1-MMP, which in turn is required for endothelial sprouting. Similarly, human umbilical vein endothelial cells expressing vimentin shRNA showed reduced expression of focal adhesion kinase, which forms a complex with vimentin and RACK1 for cell budding during endothelial cell invasion. vimentin regulates Notch ligand signaling activity for angiogenesis, as vimentin binds to proangiogenic Notch ligands and regulates their endocytosis. The IF requirement for metastasis manifests at two levels that are not mutually exclusive and may in fact be complexly interrelated. First, as polymerized filaments, IF proteins provide mechanical elements that allow cells to invade and migrate through surrounding tissues in the early stages of tumor metastasis[2]. This element of IF proteins likely includes other cytoskeletal proteins, as the mechanical properties and dynamics of intermediate filaments are related to those of microtubules and actin filaments for cell propulsion. Second, IF proteins have been found to regulate cell migration signaling pathways through their interacting partners. Of IF cells, vimentin and Cells nestin are positive regulators of cell migration and invasion, partly due to their role in signaling events during EMT and stem cell maintenance, as mentioned. Several animal studies have shown that vimentin is required for tumor metastasis. Interestingly, however, a previous study in a teratocarcinoma model using injection of vimentin-null embryonic stem cells showed that vimentin did not affect tumor growth[2].

CONCLUSION

In conclusion, IF proteins actively participate in the initiation, growth, and spread of cancer. There are many parallels between IF proteins despite variations in expression patterns and some cellular activities, so information acquired from one IF protein may very well be applied to other IF proteins. For instance, the pro-tumorigenic IF proteins vimentin, nestin, and a subgroup of keratins, including K17, all exhibit the characteristic of being induced in response to tissue damage and wounding when cells proliferate excessively.In the future, it will be crucial to understand how various intermediate filament proteins that are present in a particular tumor organize their activities to influence tumor development. Multiple keratins are found in the same tumors, and vimentin is co-expressed with keratins in some cancer kinds. These two intermediate filament networks communicate and are engaged in crosstalk, which is crucial for cell migration. Different intermediate filament proteins, such as vimentin and keratins, assemble into distinct filament networks with varying dynamics. Understanding how different intermediate filament proteins collaborate or compete with one another's functions would be important for predicting a patient's prognosis for cancer because intermediate filament proteins' production and functions are context-dependent.

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CHAPTER 17

MICROTUBULE CYTOSKELETON'S FUNCTION IN NEURODEVELOPMENTAL DISEASES

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ABSTRACT:

For a variety of functions during early embryonic development, including cell division and migration, intracellular trafficking, signal transduction, correct axon guidance, and synapse formation, neurons are dependent on the highly dynamic microtubule (MT) cytoskeleton. For freshly formed neurons to migrate properly and form neural connections, MT coordination, and support are essential. MTs offer structural integrity and support to maintain neural connectivity throughout development once connections have been established. Developmental problems can result from abnormalities in neural migration and connectivity caused by genetic mutations of MT-associated proteins. There is growing evidence linking these mutations to a variety of neurodevelopmental disorders, such as intellectual disabilities (ID) and autistic spectrum disorders (ASD). In this review article, we emphasized the critical function of the MT cytoskeleton in neurodevelopment and list genetic mutations of different MT-related proteins that may cause or be a factor in neurodevelopmental disorders.

KEYWORDS:

Microtubule Dysfunction, Neurodegenerative Disorder, Alzheimer's Disease, Microtubule Associated Protein.

INTRODUCTION

Since their discovery in the 1950s, it has become evident that microtubule function is critical for the development and maintenance of the nervous system in a variety of animal species, including nematodes, fruit flies, frogs, and rodents [1]. It is not surprising that this is also true of how the human brain develops. Microtubules promote neurogenic division, promote neuronal migration, and they are necessary for neuronal differentiation and circuit creation. With a focus on human diseases brought on by mutations in these genes, we explore the function of the various tubulins in these processes here [2].Brain development necessitates significant neuronal migration and proliferation. The MT cytoskeleton and several MT-associated proteins play a critical role in the support and coordination of this carefully orchestrated movement of neurons to their ultimate location within the brain.

Different neurodevelopmental diseases and downstream flaws in neural connectivity can result from abnormalities in proliferation or migration[3]. Understanding the dynamic structure and function of the MT cytoskeleton and its associated regulators during neuronal migration has been greatly aided by research on human and mouse genetics. In the sections that follow, we go over several well-known neuronal migration disorders and the different MT-associated genetic mutations that cause these disorders when the brain is developing [3].Congenital or acquired neurodegenerative illnesses are both possible. Their main

processes include I protein aggregate accumulation that impairs neuronal function, neuroinflammation, dysfunctions of mitochondria and other organelles, and demyelination. They all result in the loss of neurons due to cell death and the irreversible loss of neuronal processes.Type I lissencephaly ("smooth brain"), also referred to as classic lissencephaly, is one of the most well-known neuronal migration diseases.

The absence or reduction of cortical folds (gyri) and grooves (sulci), which cause sections of the brain's surface to appear smooth, is one of a range of cortical abnormalities that define this malformation. Agyria refers to the complete loss of cortical folds (type I lissencephaly), whereas pachygyria refers to the reduction of cortical folds (type I lissencephaly), or to regions of heterotopic bands of grey matter within the cortex([4]. Instead of the typical six-layered cortex, which is caused by post-mitotic neurons reaching their correct locations, a disorganized and thickened four-layer cortex results in agyria and pachygyria. In SBH, neurons move atypically within the cortex, forming an extra layer of cells beneath the grey matter. Children with type I lissencephaly are usually identified within the first few months of life, and patients frequently experience a wide range of symptoms, such as epilepsy, ID, developmental delays, and motor function issues [5].

Study shows that pathophysiological changes in microtubule dynamics result in neurodegeneration, network remodeling, and relative impact on synaptic transmission. Recent studies have examined the phosphorylation status of microtubule-associated proteins, like tau, in neurological disorders and epileptic states, as well as the impact of microtubule-active substances on the cytoskeleton's stability in epilepsy models. The modulation of hyperexcitability was discovered to be effective with microtubule polymerization manipulation. The significance of microtubules and associated neurotrophic factors during neural development was also taken into account because they are crucial for the development of a correctly functional neuronal network. Otherwise, this may result in neurodevelopmental abnormalities, hyperexcitability phenomena, and cognitive deficits.

The importance of microtubule dynamics in the transport of mitochondria, cellular components that fulfill the energy requirements for neuronal activity, and a putative influence on cannabinoid-mediated neuroprotection were all taken into account as we assessed the impact of microtubule dynamics on neuronal efficiency. Polymicrogyria (PMG), a spectrum of diseases marked by excessive cerebral cortex folding and malformations of cortical layering, is another well-known neuronal migration disorder. Early embryonic development may be affected by non-genetic factors such as hypoxia, congenital infections, microvascular inflammation, and mitochondrial diseases[6]. These factors may result in cortical anomalies linked to PMG. Consistent classification of PMG is challenging due to its heterogeneous clinical manifestations, which result in a broad variety of developmental disabilities.

Although PMG has been linked to both hereditary and environmental factors, our knowledge of this cortical malformation is still insufficient at this time. It is debatable whether PMG is truly caused by a defect in neuronal migration or by a post-migrational defect, with abnormalities developing after neurons are correctly positioned to form the cortical layers when it comes to defining its characteristics[6]. Primary microcephaly (MCPH), a neurodevelopmental disorder caused by abnormal prenatal brain growth, is defined by a smaller-than-normal head size. This smaller head size results from neural stem cells' insufficient proliferation or increased apoptosis, which reduces the number of neurons and

impairs neurogenesis during development. In addition to seizures, ID, poor motor function, abnormal craniofacial features, and this condition are frequently present in those who have it[7]. Cases of microcephaly have been associated with a variety of causes, including genetic mutations, chromosomal abnormalities, infections passed vertically, and other environmental factors[7].

Complex neurodevelopmental disorders known as ID are a major public health concern that impacts a sizeable percentage of the general population. ID is characterized by impaired intellectual and adaptive functioning that impacts day-to-day living and is defined by an IQ score below 70. Seizures, craniofacial abnormalities, and microcephaly are a few examples of additional medical or behavioral signs that may coexist with ID. Both genetic and environmental variables can contribute to ID, with genetic causes accounting for up to 50% of all cases. Pathogenic copy number variants (CNVs) and single gene mutations have both been linked to ID, and several of the implicated genes are essential for the operation of the MT [8].

ASD is a diverse group of disorders distinguished by a broad array of signs and impairments that can differ in severity. Deficits in verbal and nonverbal communication, issues with social interactions, repetitive behaviors, and constrictive interests are some of these signs. Additional medical problems like epilepsy, motor function issues, ID, anxiety, and sleep disorders can also manifest in people with ASD. The bulk of ASD cases lack a known cause, and the pathogenesis of the disorder is still poorly understood. Nevertheless, increased research has uncovered several genetic mutations related to ASD, including several MT-associated genes[9].

LITERATURE REVIEW

Neurodegenerative diseases are a class of various illnesses characterized by a gradual loss of neurons in the central nervous system (CNS). The aetiology, pathomechanism, and localization of pathological processes in the brain or other parts of the CNS determine the specific symptoms of a given illness. There are hundreds of neurodegenerative diseases, many of which are uncommon, but the most prevalent and well-known ones are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS)[10].Intraneuronal neurofibrillary tangles made of the tau microtubule-associated protein are present in Alzheimer's disease (AD) (MAPT). Similar tau deposits can be found in other neurodegenerative diseases (progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, etc.) in the lack of extracellular deposits. In some types of Parkinson's disease (PD) and prion diseases, tau pathology is also frequently observed. Mutations in tau link abnormal tau to the start of neurodegeneration in genetic types of FTD.

In FTD, there are deposits especially in temporal and frontal lobes, regions that are very important for behavior and executive function. To contemplate creating any treatment strategies, it is imperative to comprehend how tau becomes pathogenic. The main microtubule-associated protein (MAP) of a fully developed cell is tau. The neural MAPs MAP1 and MAP2 are the others[11]. These three MAPs all comparably support microtubule assembly and stability. In the pig brain, tau protein was identified as a microtubule-associated factor. It was discovered to be a protein that co-purified with tubulin and could encourage microtubule formation in a test tube. 2-3 moles of phosphate per mole of tau protein are

present in the normal mature human brain. The biological action of tau is suppressed by hyperphosphorylation of tau. In familial instances of frontotemporal dementia, the tau gene has been linked to nearly 80 diseases through intronic and missense mutations (FTD). Autism spectrum disorders (ASD) are characterized by deficiencies in social interactions, constrained interests, and repetitive behaviors. The growing corpus of research indicates that cerebellar changes play a part in the pathology of ASD[11].

Some research results imply a link between cerebellum damage and ASD symptoms like motor issues as well as social deficits, repetitive behaviours, and mental rigidity. Future research is necessary to fully comprehend the role of this brain structure in the pathogenesis of ASD, though. Therefore, in this research, we created a rodent model of ASD by giving pregnant rats a single dose of valproic acid (VPA), followed by studies of the offspring's cerebellar morphology that concentrated on changes to important cytoskeletal components. The expression (Western blot) of actin-crosslinking II-spectrin, neurofilament light polypeptide (NF-L), key neuronal MT-associated proteins (MAP) such as MAP-Tau and MAP1B, MAP2, and MAP6 (STOP), as well as /-tubulin was examined. We discovered a substantial reduction in the protein levels of /-tubulin, MAP-Tau, MAP1B, MAP2, and II-spectrin following maternal exposure to VPA. Furthermore, it was suggested that critical Tau-kinases were activated as well as excessive MAP-Tau phosphorylation at (Ser396). The loss of Purkinje cells and chromatolysis in the cerebellum of autistic-like rats revealed by immunohistochemical staining cast light on one of the potential molecular mechanisms underlying altered neuroplasticity in the ASD brain. TUBA1A, an isotype of -tubulin that is only expressed in the developing nervous system, is necessary. It has been demonstrated that mice with heterozygous TubA1A mutations exhibit abnormal neuronal migration and lamination abnormalities that are comparable to the human phenotype. The molecular cause of these changes is still unknown, but subsequent patient studies have found several TUBA1A mutations in regions predicted to disrupt interactions with known binding partners, such as LIS1, DCX, and other tubulins. These mutations are thought to cause cortical migration defects [12].

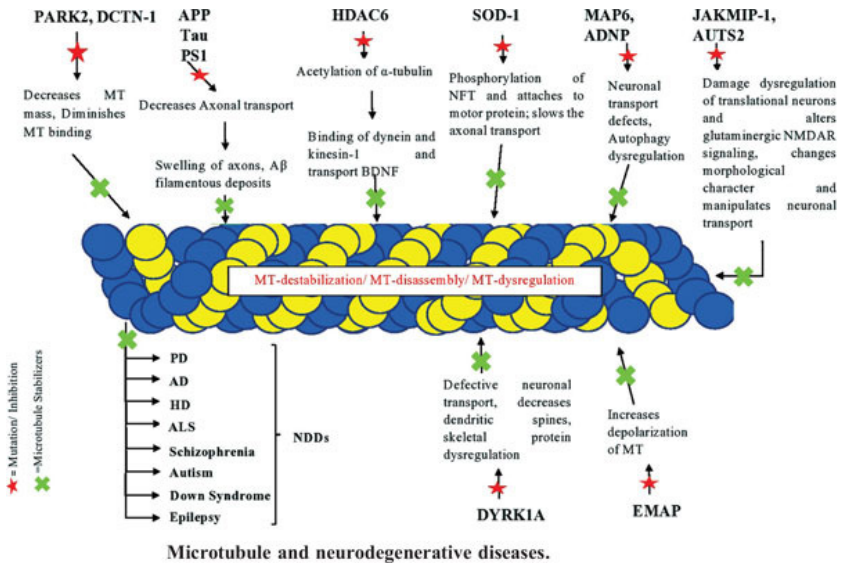

Figure 1: Microtubules-related disease. Diagram showing the specific site of the mutation in the microtubules (Bentham Science publisher).

Studies conducted over the past few years have discovered new PMG-related mutations in genes encoding MT motor proteins, such as the kinesin family members KIF5C and KIF2A and a dynein-associated protein, DYNC1H1. Although each mutation causes a different trait, all of these genes are essential for controlling the MT cytoskeleton in neurons. Both KIF5C and KIF2A, which encode members of the kinesin superfamily and are involved in the intracellular transport of cargo along MTs, are strongly expressed in the developing nervous system [3]. Additionally, Dync1h1 reduction by RNA interference impairs neuronal migration in a manner that is analogous. Numerous candidate genes, including transcription factors, signaling molecules, and different cytoskeletal elements, such as various tubulin isotypes and members of the kinesin family, have been implicated in genetic research as being related to PMG[3]. PMG has been associated with the -tubulin genes TUBA1A and TUBA8, as well as the -tubulin genes TUBB2B and TUBB3.Particularly in the developing brain, ASPM is necessary for mitotic spindle pole organization and normal operation.

Recent research suggests that the misregulation of this process may result in microcephaly because ASPM can also enlist Katanin to promote MT severing and disassembly. Two additional genes, PRUNE1, and KIF20B have lately been found to be associated with MCPH. A member of the DHH (Asp-His-His) phosphodiesterase protein superfamily necessary for cell movement is encoded by prune exopolyphosphatase 1 (PRUNE1). As MT polymerization, cell migration, and proliferation were all negatively impacted by PRUNE1 mutations, these processes may be fundamentally regulated by PRUNE1 throughout cortex development. Kinesin Family Member 20B (KIF20B) is an MT plus end-directed motor that controls cell polarity in neurons and is necessary for the conclusion of cytokinesis (Figure.1).

Loss of Kif20b impairs neurite outgrowth and branching, as well as brain cortex growth and cell polarization [3]. A novel mutation in CAP-Gly domain-containing linker protein 1 (CLIP1), which encodes a +TIP, CLIP-170, that localizes to the ends of growing MTs, was found in a recent NGS analysis of big consanguineous Iranian families affected by ID. CLIP1 engages in MT-mediated transport in neurons and controls MT behavior. Cell lines derived from these ID patients lacked the protein encoded by CLIP1, indicating that loss of CLIP1 activity can impair cognition. Additionally, it has been demonstrated that CLIP-170 and LIS1 may interact to control MT dynamics and facilitate dynein recruitment to MTs. It's conceivable that CLIP1 and LIS1's interaction is crucial for correct neuronal migration during brain development. ASD has also been related to the dysregulation of several MT-associated kinases [3].

The RNA-binding protein Janus kinase and MT interacting protein 1 (JAKMIP1) are strongly expressed in glutamatergic neurons and have been shown to alter MT polymers and affect MT dynamics. Additionally, it has been proposed that JAKMIP1 interacts with the MT cytoskeleton to modify the intracellular transport of GABA receptors. MARK1 is important for neuronal polarisation and migration and assists in the control of mitochondrial trafficking along MTs in both axons and dendrites. Cell migration and synaptic function have both been shown to be compromised by MARK1 overexpression or decrease. It's conceivable that MARK1 mutations alter the phosphorylation activity of MAPs, altering MT dynamics abnormally and impairing normal neural development (Figure.1). ADNP is involved in the recruitment of Tau to MTs and has been linked to tau mRNA splicing, possibly preventing the buildup of free Tau that ultimately results in neurodegenerative disorders.

It has been demonstrated that ADNP interacts directly with MT EBs EB1 and EB3 to encourage neurite sprouting and the development of dendritic spines. Together, these results suggest that ADNP variants may change how it interacts with a number of MT-associated proteins, with detrimental downstream consequences that change MT dynamics and impede various neuronal processes in the early stages of development[3].

CONCLUSION

In terms of both chemical and functional characteristics, MTs are one of the most complex structures found within the cell. This intricacy is produced by the coordinated action of regulatory mechanisms that control the expression of various tubulin isotypes across time and tissues, their chemical modification, and their interactions with other proteins and cellular effectors. MT functions are hampered by changes to these regulatory systems. Numerous studies have shown that MT dysfunction can either add to or cause neurodegenerative processes.Microtubule mass is frequently reduced in neurodegenerative illnesses, and there is a chance that microtubule polarity patterns and transport are affected. Whether or not these negative effects are the disease's main cause, therapeutics that can correct these microtubule abnormalities have great potential to improve the condition of the nervous system's deteriorating tissues. In cellular and animal models of various neurodegenerative diseases, substances that regulate MT stability or increase tubulin acetylation have demonstrated several advantages, some of which have been applied in clinical studies. These findings have sparked a lot of interest in this area of study. However, a deeper comprehension of MT dysfunctions is required, for example through an examination of the changing state of MTs in various neurodegenerative conditions. Even though MT-stabilizing substances can help to reestablish MT stability, taking too many of these medications can damage MT integrity and encourage over-stabilization. Alternatively, because inhibitors of tubulin deacetylases have numerous targets, it is necessary to create drugs with higher selectivity.

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CHAPTER 18

NEURONAL AND GLIAL INTERMEDIATE FILAMENT DYSFUNCTION IN DISORDER

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ABSTRACT:

Most eukaryotic cells, including those in the nervous system, contain intermediate filaments (IFs), which are common structures. The neurofilament triplet proteins and internexin are the main elements of neuronal IFs in the Brain. Peripherin, a fifth neuronal IF protein, is also found in the peripheral nerve system. Glial fibrillary acidic protein (GFAP) makes up the majority of the IFs in astrocytes, though some immature and mature astrocytes also produce vimentin. In this chapter, we concentrate on the IFs of neurons and glial cells (primarily GFAP) as well as how these IFs relate to various neurodegenerative disorders.

KEYWORDS:

Intermediate filaments, Neurodegenerative disorders, Motor neurons, Parkinson's disease, Mutations genes

INTRODUCTION

Microfilaments, microtubules, and intermediate filaments are three widely distributed groups of fibrillary proteins that make up the cytoskeleton. The word "intermediate filament protein" refers to a protein whose diameter lies halfway between that of microfilaments and that of microtubules[1]. Their high number, distribution in the cytoplasm and nucleus, diverse primary structure, nonpolar architecture, relative insolubility, and nucleotide-independent dynamics set them apart from actin microfilaments and tubulin microtubules. Microtubules, which are 24–26 nm in diameter, and microfilaments, which are 6–8 nm in size, together with intermediate filaments (IFs), which are 8–10 nm structures, make up the cytoskeleton found in virtually all eukaryotic cells. IFs are found in the nervous system in neurons and astrocytes, but not in oligodendrocytes, which in the CNS make the myelin sheath[2]. IF proteins belong to a large family of proteins that also contain keratins and nuclear lamins and are structurally related to one another.

It is important to remember, though, that protein buildup in neurons was already a known phenomenon before the development of genetics. When dementia patients' brains were autopsied, silver stains created by Camillo Golgi in 1873 which rely on the so-called "black reaction" and improved by David Bodian 60 years later showed the existence of protein tangles and accumulations. Later, it was discovered that these clusters contained particular proteins that build cytoskeletal polymers known as neurofilaments (NFs)[3]. Within a few years, NFs were revealed to overlap with tau neurofibrillary tangles in the brains of AD patients, within Lewy bodies in dopaminergic neurons suffering from Parkinson's disease, and in skeins and aggregates in the dystrophic neurites of motor neurons suffering from ALS.

Hirano bodies, which are crystalloid structures identified in the nuclei of neurons in several degenerative diseases, including ALS and AD, are also stained intensely for NFs[4].

Glial fibrillary acidic protein (GFAP) is the predominant IF protein in astrocytes, though nestin, vimentin, and synemin are present in smaller amounts. Multiple GFAP isoforms have been found recently, and they may express differently in reactive versus quiescent astrocytes[2]. The most prevalent isoform of GFAP and the leukodystrophy/neurodegenerative disorder Alexander disease are currently the only links between any of these astrocytic IF proteins and specific diseases. The neurofilament triplet proteins (NFTPs), which include the low-molecular-weight neurofilament subunit (NFL) (68 kDA), middle-molecular weight neurofilament subunit (NFM) (160 kDA), and high-molecular-weight neurofilament subunit (NFH) (205 kDA), as well as -internexin, are the main neuronal IF proteins in the CNS. Peripherin is produced in the peripheral nervous system (PNS) alongside NFTPs [2]. A growing amount of research supports the idea that abnormal protein production, processing, or misfolding, followed by accumulation in the nervous system, is the most prevalent mechanism of chronic neurodegenerative diseases[5]. Numerous neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth disease, giant axonal neuropathy, neuronal intermediate filament inclusion disease (NIFID), Parkinson's disease (PD), diabetic neuropathy, dementia with Lewy bodies, and spinal muscular atrophy are frequently linked, either directly or indirectly, to changes in the metabolism and/or organisation of neuronal IFs [5]. While recent studies using transgenic mouse models suggested that IF disorganization itself can also produce deleterious effects and therefore could contribute to the neurodegeneration process, it is also possible that IF abnormalities in neurodegenerative disorders simply reflect a pathological result of neuronal dysfunction. Neurodegenerative disorders are also caused by mutations in glial IF, more specifically GFAP in astrocytes. While nestin, vimentin, and synemin are among the IF proteins expressed by astrocytes, GFAP is the most prevalent. A type III IF protein called GFAP exists in various spliced versions. These GFAP transcripts' relative abundance varies and may be influenced by the position of the astrocytes or their pathological conditions [6]. Alexander disease (AXD), an uncommon leukodystrophy, is brought on by GFAP mutations that result in GFAP protein accumulations [6].Discuss how the pathogenesis of neurodegenerative disorders and the development of intermediate filamentous accumulations are affected by mutations in the genes that code for the IF protein found in glial cells and neuronal cells (primarily GFAP).

LITERATURE REVIEW

It is well known that the buildup of disease-specific proteins plays a role in several neurodegenerative diseases, such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis. Less well-known are the buildups of neuronal intermediate filaments (NFs), a different group of proteins that have been linked to these diseases for decades (figure.1). In addition to determining axonal caliber, which controls signal conduction, NFs also belong to the family of cytoskeletal intermediate filament proteins (IFs), which give cells their shape. They also control synaptic vesicle transport and modulate synaptic plasticity by interacting with neurotransmitter receptors. Numerous rare diseases have been identified in the past 20 years that are brought on by changes in the genes that encode NFs or control their metabolism. These uncommon neurological conditions are shedding new light on the function of NF aggregation in more frequent neurological conditions [7].Alexander disease is a rare

leukodystrophy (a disorder characterized by failure or loss of myelin, leading to progressive degeneration of the brain's white matter) of unknown incidence that almost always results from dominantly acting mutations in the GFAP coding region. Alexander's disease was first described in 1949.

Before they turn two years old, the majority of patients begin to exhibit their first signs, such as seizures or developmental delays[8]. Patients experience progressive deterioration and die before the age of six due to the illness, which is characterized by a dramatic loss of white matter in the frontal lobes. Although this is the most prevalent form of Alexander disease, there are also later onset and milder types of the condition that occasionally have no white matter abnormalities at all. The appearance of Rosenthal fibers, protein aggregates, in the cytoplasm of astrocytes, particularly those in subpial, subependymal, and perivascular regions, is the pathology's defining characteristic. Inclusions of complicated ubiquitinated stress proteins, these fibers additionally contain mutant GFAP and an as-yet-unidentified number of other constituent proteins [9]. Both the proteasomal and the autophagic pathways are responsible for the degradation of other proteins crucial for neurodegenerative illness, such as -synuclein. Although it was previously believed that autophagy did not add to the degradation of GFAP, it now appears that this pathway is strengthened in the context of mutant GFAP forms linked to Alexander's disease and by GFAP accumulation.

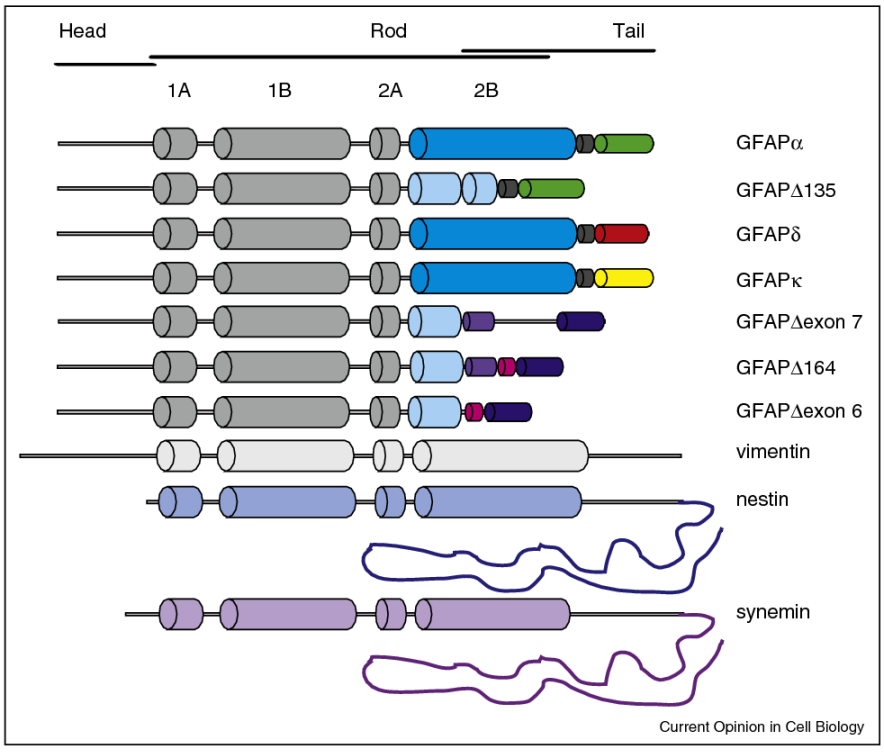

Figure 1 : Intermediate filament protein in astrocytes: Diagram showing the types of the intermediate filament protein present in the astrocytes (Sementic scholar).

These alterations were visible not only in transfected cell lines but also in the brains of patients with Alexander disease as well as tissues and cells from mouse knockin models of the illness. Rosenthal fibers are surrounded by membrane-bound structures that resemble autophagosomes and autolysosomes according to their morphology and microscopy (EM)

studies of cell lines, mouse tissues, and one patient with Alexander disease have disclosed these structures. Recent research has also demonstrated that autophagy is an adaptive reaction in cardiomyopathy caused by mutations in B-crystallin that result in the aggregation of desmin, another IF protein [10].

The mutant forms of GFAP linked to Alexander disease show a change in equilibrium from soluble (i.e., monomers to small oligomers) towards insoluble (assembled filaments or aggregates) pools, and they pull IF-associated proteins like B-crystallin and most likely plectin in the same direction. GFAP, one of the minor isoforms of GFAP, appears to be predominantly expressed in the subpial and periventricular astrocyte populations that have the greatest number of Rosenthal fibers. Recent research demonstrates that GFAP- changes how B-crystallin binds to GFAP filaments. Less than 10% of the overall GFAP in the normal human spinal cord is made up of GFAP. It is unclear whether GFAP- is abnormally elevated in tissue affected by Alexander disease, which could further impact filament solubility and association with IF-associated proteins. In people with Alexander's disease, the stress reaction that is triggered in astrocytes may have both harmful and beneficial effects [10].

Studies in cell culture indicate that these astrocytes are less able to react to additional stress, such as that caused by camptothecin or hydrogen peroxide. Recent research, however, suggests that enhancing astrocytic B-crystallin upregulation can provide a dramatic rescue from the otherwise fatal consequences of GFAP mutation and excess[10]. The NFTPs are the primary neuronal IF proteins in both the Brain and the PNS (NFL, NFM, and NFH) (Figure.1). While peripherin, another neuronal IF protein, is produced in the PNS, the CNS also expresses -internexin abundantly in the same filament system as the NFTPs . Axon diameter and neuronal IF content are correlated, with neuronal IFs constituting the main structural component in the axon. Large accumulations of neuronal IFs, also known as spheroids, have been seen in both the cell bodies and axons of neurons in several neurodegenerative disorders [10]. The neuronal IF inclusion disease is a newly identified condition that affects neuronal IFs (NIFID)[11]. This illness can be distinguished microscopically by neural inclusion bodies (IFs) that don't contain either synuclein or tau. NIFID can thus be easily distinguished from other conditions that also involve filamentous inclusions, such as motor neuron disease, synucleinopathies (such as Parkinson's disease and multiple system atrophy), tauopathies (such as Alzheimer's disease, frontotemporal dementia, and parkinsonism linked to chromosome 17 [FTDP-17]), and tauopathies. The inclusions in NIFID are unique because -internexin has been identified as a significant constituent.

Despite being seen in other neurodegenerative disorders, internexin immunoreactivity is typically a small part of the pathological neuronal inclusions in these disorders [10]. The ventral anterior and posteromedial centers of the thalamus, as well as the large pyramidal neurons of the neocortex, develop neurofilaments inclusions when internexin is overexpressed in transgenic mice. The cerebellum of these rodents, however, exhibits the most glaring pathology, with multiple swellings of the proximal portions of Purkinje cell axons being noted. These enlargements, also called astorpedoes, are packed with enormous, disorganized neural IFs. The morphological alterations in Purkinje cells are correlated with the mice's impairment in motor coordination. Furthermore, depending on the degree of internexin overexpression, neurofilaments inclusions cause a gradual loss of neurons in old transgenic mice. Other associations between torpedoes and cerebellar disorders, mainly in animal models, have been made. It is intriguing to note that a subtype of essential tremor, a

syndrome marked by a steadily progressing postural and/or kinetic tremor, which is present in as many as 23% of people over 65 years old, has an increased number of cerebellar torpedoes . This profusion of cerebellar torpedoes may eventually result in the loss of Purkinje cells, indicating that studies of neural IF dysfunctions may be interested in essential tremors [12].

Up until lately, there was no consensus on the causal link between neurodegeneration and mutations in the genes encoding the neuronal IF proteins. Therefore, the correlation between NEFL mutations and Charcot-Marie-Tooth disease (CMT), a neurodegenerative condition, was of great concern. The inherited peripheral neuropathy known as CMT has been related to numerous gene mutations. In the general population around the globe, it affects about 1 in 2,500 individuals and is a highly prevalent sensory and motor neuropathy. Based on nerve-conduction velocity (NCV), the disease was initially divided into CMT1 and CMT2: CMT1 patients have a reduced NCV, whereas CMT2 patients have a comparatively normal NCV. Typically, CMT1 is an axonal neuropathy, whereas CMT2 is a demyelinating neuropathy. There is a connection between demyelination and axonal degeneration, and the clinical overlap between the various types of CMT indicates a common pathogenic mechanism. Both of these types of CMT are slowly progressing bilateral neuropathies with a distal preponderance; as the nerves that supply the extremities deteriorate, patients lose the ability to use their limbs normally [10]. Both in terms of clinical appearance and genetic level, patients exhibit a high degree of heterogeneity. CMT1 as well as CMT3 and CMT4 (CMT3 is an especially severe demyelinating form of CMT, whereas CMT4 is an autosomal recessive form) have generally been identified as being caused by mutations in several genes crucial for myelin formation and maintenance. These include changes to the genes encoding the transcription factors early growth response 2 (EGR2), which attaches to the GJB1 promoter, peripheral myelin protein 22 (PMP22), myelin protein 0 (P0), gap junction membrane channel protein 1, also known as connexin 32, and myelin protein 0 (P0).

NEFL was the first gene discovered to be connected to CMT2, and this association was seen in two distinct families as a consequence of two distinct mutations. Table 4 contains a summary of additional mutations that lead to CMT2[10]. These first two NEFL mutations linked to CMT alter NFL's rod domain (Q333P) and head domain (P8R). In transfected nonneuronal cells with the two mutations, filament assembly was observed to be disrupted. Since wild-type NFL could not correct the assembly defect, this impact was dominant. Additionally, the two mutations in transfected cultured neuronal cells impacted mitochondrial localization and transport, as well as slow and rapid anterograde and retrograde axonal transport. Additionally, mutant NFL led to Golgi apparatus disintegration and accelerated neuritic degeneration. The dominant effects are consistent with the illness, even though these studies were conducted in cells overexpressing the mutant proteins. This suggests that generalized defects in axonal transport may be to blame for this neuropathy[10].

Since the first two NEFL mutations associated with CMT were reported, additional NEFL mutations have been found in families and individual CMT cases. The head and rod domains are the primary areas affected by these mutations. In the tail region, two described mutations were found to not be pathogenic. Due to a significantly reduced NCV more resembling CMT1 than CMT2, patients with NEFL mutations are typically categorized as having CMT2E (although some have been classified as having CMT1F). CMT has also been linked to mutations in the genes that code for two proteins that can bind with neurofilaments. It has been noted that HSPB1 (also known as Hsp27) interacts with several IF proteins and affects

how they are assembled. According to reports, CMT2F, a new subtype of the axonal variety of CMT, is brought on by mutations in HSPB1. Mutant HSPB1 was discovered to have an inhibitory impact on NFL assembly in transfected cells during cotransfection experiments. Following research in non-neuronal cells, additional studies in cultured motor neurons revealed that the coexpression of wild-type HSPB1 reduced the aggregation of CMT mutant NFL, whereas the expression of mutant HSPB1 caused progressive degeneration of motor neurons as well as disruption of the neurofilament network. Myotubularin-related protein 2 (MTMR2) is a second protein that interacts with NFL and has been associated with CMT. CMT4B is a subtype of CMT caused by mutations in MTMR2, and mutant MTMR2 causes aberrant NFL assembly in transfected cells. It should be noted that mice lacking MTMR2 acquire a CMT-like neuropathy with several dysmyelination-related symptoms. Following MTMR2 inhibition specific to Schwann cells, the same phenotype was seen, whereas inactivation specific to neurons did not produce a clear phenotype. As previously mentioned, NFL-deficient rodents do not display a neuropathy similar to CMT [8].

It is therefore conceivable that the NFL assembly defects brought on by disease-linked MTMR2 mutations could contribute to the pathogenesis of CMT4B in humans. Nevertheless, despite their seemingly normal appearance, they do show a loss of motor neurons and noticeably smaller axon diameters.In the perikarya and axons of ALS patients' motor neurons, protein spheroids known as neuronal IFs gather. Numerous studies have looked for mutations in the genes encoding NFTPs due to the presence of neuronal IFs in these spheroids, and there are reports of codon deletions in the tail domain of NFH in sporadic ALS patients as well as some potential mutations in the tail domain of NFM [10]. However, there have been no accounts of assembly studies in cells transfected with the alleged sporadic ALS NFH and NFM mutants, and no mutations in the genes encoding NFTPs have been found in familial cases of ALS. Superoxide dismutase 1 (SOD1) is mutated in 10% of familial instances of ALS, and various transgenic mouse models have been used to study the relationship between neurofilaments and SOD1.

Peripherin and neurofilaments have been discovered in motor neuron disease spheroid. In addition, motor neuron perikarya of 9 of 40 ALS cases showed peripherinpositive Lewy body-like inclusions, a form of cytoplasmic inclusion that frequently contains -synuclein and is common in patients with Parkinson's disease (and not in controls). These inclusions don't exhibit B-crystallin, NFTPs, actin, synuclein, interexin. Transgenic rodents with peripherin overexpression develop late-onset motor neuron disease. If the rodents are also deficient in NFL, this defect is exacerbated.With a prevalence of about 2% among persons over 65, Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease (AD) [2]. The substantia nigra pars compacta's dopaminergic melanin-containing neurons are lost as a result of this illness, and the striatum loses dopamine as a result. Another significant pathological trait is the existence of Lewy bodies, which are eosinophilic cytoplasmic inclusion bodies made up of -synuclein, NF proteins, ubiquitin, and proteasome subunits and are particularly common in substantia nigra pars compacta neurons. Numerous characteristics set apart NFs in PD, such as incorrect Lewy body phosphorylation and proteolysis, decreased NFL and NFH mRNA levels, and decreased NFL and NFM protein levels. In a case of PD with an early onset, a point mutation in the NEFM gene was described. This mutation involved changing Ser to Gly at residue 336 in the rod domain 2B of NFM, a highly conserved area that was thought to interfere with NF assembly (Figure.2).

The G336S mutation, however, does not affect the distribution and assembly of NFs in vitro and screenings of PD patients with the same or distinct ethnic backgrounds failed to find this mutation, disproving the idea that this NEFM mutation contributes to the pathogenesis of PD.

It's interesting to note that studies have shown that PD patients have substantially higher serum levels of anti-NF protein antibodies and that changes in NFL levels in the cerebrospinal fluid may be used as biomarkers for the diagnosis of PD. Finally, it appeared that age and the duration of the illness were strongly correlated with the serum level of NFs in PD patients. These results provide evidence that cytoskeleton proteins are released as a result of axonal injury, and that variations in serum NF concentrations are probably correlated with the degree of axonal injuries[10]. ALS has been associated with a newly discovered mouse peripherin splice variant that produces an aberrant peripherin protein. Intron 4 is spliced out of the protein's most common form while maintaining the reading frame, creating a larger form of peripherin (Per61) in this form of peripherin. Both the filamentous inclusions in the SOD mutant rodent model of ALS and the axonal spheroids in tissue from people with ALS are stained by an antibody that is specific for a peptide encoded by this intron. The latter finding is unexpected because, absent a frameshift, the inclusion of human intron 4 would result in a truncated protein.

Later research reported a novel human peripherin transcript (Per28) that retains both introns 3 and 4, which are spliced out of the most prevalent form of human peripherin. In an instance of human ALS, Per28 was elevated at both the protein and mRNA levels, and an anti-Per28 antibody stained the filamentous inclusions. According to these findings, the disease may result from peripherin misplacing. These are very interesting findings that merit further investigation. Peripherin mutations have also been linked to ALS in additional studies. The peripherin gene has been sequenced in both sporadic and familial ALS cases as part of these investigations. In one research, two variants were found only in ALS cases, while 18 polymorphic variants were found in both ALS and control populations. One of these variations results in a peripherin protein with 85 amino acids because of a single base pair deletion in exon 1 of the peripherin gene. Similar to the CMT-linked shortened NFL mutant previously discussed, this truncated peripherin impairs the assembly of neuronal IF in transfected cells [10]. Peripheral neuropathy is primarily brought on by diabetes globally. Neuropathy affects 60 to 70 percent of diabetics in some way. Nerve problems can occur in diabetics at any moment, but the risk increases with age and the duration of the disease (Figure.2).

The dorsal root ganglia and sensory nerves are more commonly affected by diabetic neuropathies than motor fibers. Neurovascular factors that cause blood vessel damage, autoimmune factors, lifestyle factors, and inherited traits that increase susceptibility to nerve disease are all potential causes of nerve damage. Metabolic factors (such as high blood glucose and abnormal blood fat levels) may also contribute to nerve damage. Diabetic neuropathy is defined by slower conduction velocity, impaired axonal transport, axonal atrophy, and reduced ability for nerve regeneration, even though its pathogenesis is not fully understood. All of these aspects of nerve function are reliant on NFs and the health of the neuronal cytoskeleton.

IF protein	Phenotype
Mice lacking neuronal IF proteins	
NFL	Absence of axonal IFs, decrease in axon caliber; abnormal axon conductance. Decreases in both NFM and NFH. Loss of motor neurons (approximately 20%) and delayed maturation of regenerating myelinated axons. Increase in microtubule content.
NFM	Decrease in radial growth of large myelinated axons. Decrease in IF content. Decrease in NFL; increase in NFH. Atrophy of motor neurons and hind limb paralysis with aging. Increase in microtubule content.
NFH	Small reduction in radial growth. Little reduction in spacing between neurofilaments.
NFM and NFH	More severe reduction in radial growth than lack of NFM alone. Unassembled NFL in perikarya.
Peripherin	Reduced number of unmyelinated sensory axons. Increased levels of α-internexin in ventral roots.
α-Internexin	No effect on axonal caliber. No neuronal defects.
Mice expressing truncated neuronal IF proteins	
NFH-tailΔ	No effect on axonal diameter; slower radial growth. No change in interfilament spacing.
NFM-tailΔ	Reduction in radial growth similar to NFM deficiency. Reduced spacing between neurofilaments.
Mice overexpressing neuronal IF proteins	
NFL (mouse)	Massive accumulations of neurofilaments in ventral horn motor neurons accompanied by increased axonal degeneration, proximal axon swelling, and severe skeletal muscle atrophy.
NFM (mouse)	Reduction in radial growth. No change in interfilament distance.
NFH (mouse)	Reduction in radial growth. No change in interfilament distance.
NFL + NFM; NFL + NFH	Increase in radial growth.
NFL (human)	Perikaryal accumulations in the thalamus.
NFM (human)	Neuronal dysfunction and motor neuron loss. Neurofilamentous swellings (depending on level).
NFH (human)	Abnormal neurofilamentous swellings in cell bodies and proximal axons. Skeletal muscle atrophy. Motor dysfunction. No motor neuron loss.
Peripherin	Selective degeneration of motor axons during aging. Overexpression of peripherin in NFL null mice leads to early-onset formation of IF inclusions and selective death of spinal motor neurons
α-Internexin	Cerebellar torpedoes/Purkinje cell degeneration.
Mutant NFL (rod domain mutation)	Degeneration of motor neuron. Abnormal axonal and perikaryal accumulations of IFs. Atrophy of skeletal muscles.
Mutant NFL (3′ UTR mutation)	Impairment of motor function. Atrophy of nerve fibers in the ventral root.

Figure 2: Mice missing neuronal IF proteins and mice overexpressing neuronal IF proteins exhibit different phenotypes (JCI).

This is supported by the discovery of numerous NF biology anomalies in diabetes model organisms. In rats with streptozotocin-induced diabetes and BioBreeding rats (a model of spontaneous type I diabetes), there was an impairment of the axonal transport of NFs, actin, and tubulin along with a proximal rise and a distal decrease of axonal calibers. In addition, accumulations of highly phosphorylated NF epitopes are found in proximal axonal segments of dorsal root ganglia sensory neurons from diabetic patients. The distal axonal shortening is accompanied by a concurrent NF loss in this area. In lumbar dorsal root ganglia from rodent models, NF phosphorylation increased and was associated with JNK activation. Finally, there were decreased NF numbers and densities within large myelinated sensory of long-term diabetic models, as well as a significant drop in the mRNA levels of all three NF subunits[10].

CONCLUSION

In this chapter, we have discussed some of the most recent data supporting the possibility that neuronal and glial cell IF dysfunction contributes to the development of neurodegenerative diseases. Numerous studies into the functions of IF proteins are currently being conducted. amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth disease, giant axonal neuropathy, neuronal intermediate filament inclusion disease (NIFID), Parkinson's disease (PD), diabetic neuropathy, dementia with Lewy bodies, and spinal muscular atrophy are examples of intermediate filament proteins that have been linked to neuronal human disease, it is still very likely that mutations in the genes responsible for some or all of these proteins could cause human disease. It will be discovered that each of these proteins is connected to specific human illnesses. With numerous mutations in non-intermediate filament genes still to be

identified, the phenotype of a specific intermediate filament-associated illness may be genetically heterogeneous. Improvements in prenatal and postnatal diagnostic capabilities, honed the use of intermediate filament proteins as markers of disease progression, improved understanding of environmental, genetic, and epigenetic modifiers, and therapeutic manipulation of these proteins' expression are likely to be clinically relevant areas of growth in research related to intermediate filament proteins. In terms of the framework, Well-tailored therapeutic approaches can be created to overcome the limitations of existing treatments as the regulatory processes of intermediate filament proteins are revealed, assisted in part by pertinent animal models.

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CHAPTER 19

PROFILIN IS AN ACTIN AND MICROTUBULE MODULATOR

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ABSTRACT:

Profilins, which can interact with a variety of acting binding proteins and connect membrane lipids to cytoskeleton elements, are emerging as crucial controllers of actin dynamics after being initially discovered as G-actin sequestering proteins. Lately, it was discovered that profilin contains residues specifically suited for attaching to microtubules in addition to its actin, poly-proline, and phosphatidylinositol binding regions. Here, we will address the growing body of evidence that suggests profilins are key intermediaries in the interplay between actin microfilaments and microtubules. We'll also talk about the research's open issues, like how profilin interacts with microtubules and how that affects microtubule behavior. Our knowledge of how various cytoskeleton elements are integrated within cells is deepened by these new findings.

KEYWORDS:

Actin filaments, Actin dynamics, Actin microtubules,Barbed ends,Actin polymerization.

INTRODUCTION

The specifics of the interaction between the actin and microtubule cytoskeletons, as well as the processes by which the two systems work together to regulate cell dynamics and design, are just beginning to become clear. Despite having comparable structures, the actin-binding proteins Pfn1 and Pfn2 have been shown to have distinct preferences for their well-known binding partners in a landmark study using affinity chromatography of a brain tissue extract on profilin columns[1]. For instance, Pfn2 attaches PLP-containing proteins more tightly than Pfn1. Pfn2's potential PLP-binding domain is structurally different from Pfn1's in that it has a larger region of aromatic residues, which could account for the difference in binding affinities [2].

As a result, unique associations between various profilin variants and microtubules may be discovered. It is interesting to note that tubulin could only be collected from the Pfn1 column, indicating that Pfn2 is unlikely to be a part of the molecular interactome between the actin and microtubule networks. Because direct Pfn1-tubulin binding was not shown in these experiments, the idea that the interaction might instead be indirectly acquired importance. Following in vitro experiments using isolated proteins demonstrated, as is described below, that tubulin and Pfn1 can be co-immunoprecipitated, confirming the interaction of Pfn1 with microtubules. The binding of Pfn1 to microtubules has likely gone unnoticed for years due to its broad cellular dispersion as seen by immunofluorescence and the oversimplified notion that it would only function as a regulator of actin dynamics, as was recently brought to light [3].

Profilin antibodies produce a dot-like pattern and abundance at the leading cell edge and perinuclear area, which may be consistent with some of the proteins colocalizing with microtubules. Later, it was shown that profilin does, in fact, partly co-distribute with microtubules in human fibroblasts, and this was most recently verified in rodent platelets and fibroblasts [4]. These results raised the possibility that actin-bound profilin complexes could be transported along microtubules to cellular areas where active actin polymerization necessitates a higher abundance of G-actin, as is the case with other cytoskeleton elements like intermediate filaments. Recent research discovered that glutathione S-transferase (GST)-Pfn2b binds tubulin in non-neuronal cells and that green fluorescent protein (GFP)-Pfn2b is recruited to spindles and asters during mitosis in HeLa cells, in contrast to earlier findings showing a lack of tubulin binding to a Pfn2 affinity column[5].

As a result, the Pfn1 profilin variation might not be the only one that attaches tubulin. One of the most prevalent proteins in non-muscle cells is profilin. Although profilins exhibit significant differences at the amino acid level, they fold in a 3D shape that is remarkably well preserved from amoebas to mammals. The profilin family of proteins in animals includes the widely expressed profilin1 (Pfn1), the brain-specific Pfn2 the testis-specific Pfn3, and Pfn4. Pfn1 was first discovered as a G-actin binding protein in purified preparations of bovine non-muscle actin. Its potential function as an actin monomer sequester quickly appeared.

Profilins promote actin filament nucleation and elongation by interacting with formins, the Arp2/3-dependent Wiskott-Aldrich syndrome protein (WASP)/WASP family verprolin-homologous protein (WAVE) family, and Ena/vasodilator-stimulated phosphoprotein (VASP), which inhibits actin filament spontaneous nucleation/polymerization by sequestering G-actin. Notably, profilin facilitates ADP/ATP exchanges on monomeric actin, allowing a pool of polymerizing competent ATP-actin to be added to the rapidly expanding end of an existing actin filament. Profilins interact with poly-proline (PLP) segments, which are found in many actin-binding proteins, such as Ena/VASP, formins, and the WASP/WAVE family, in addition to their wide binding site to actin [6].

Profilin's ability to join G-actin is therefore essential for its activity. However, it also has the ability to associate with other proteins thanks to its PLP binding. The spatial-temporal control of actin polymerization depends heavily on the association of profilin with proteins containing PLP. In addition, profilins have two phosphatidylinositol lipid-binding regions that connect them to the plasma membrane. Notably, profilin competes with phosphoinositide-specific phospholipase C to bind PI-(4,5)-bisphosphate (PIP2), blocking PIP2 hydrolysis by this enzyme. Another possibility is that by interacting with PIP2, profilin may be able to directly contend with other PIP2 binding proteins, such as cofilin, affecting local actin dynamics[7]. Certain sections of the various profilin binding domains overlap due to their tiny size (12–15 kDa), indicating a high degree of regulatory intricacy.

Furthermore, because of their poor genetic homology, profilins' affinities for their various ligands can vary by orders of magnitude between those from the same organism and those from different realms. Several biological processes, including early development (Verheyen & Cooley, 1994; Witke, Sutherland, Sharpe, Arai, & Kwiatkowski, 2001), cell growth, and motility, particularly cell edge advancement (Le Clainche & Carlier, 2008; Pollard & Borisy, 2003), are actively influenced by profilins in a variety of organisms. Profilins have been described as important molecular controllers of actin polymerization dynamics that link

membrane lipids and cytoskeleton components, despite being initially thought of as actin sequestering proteins. Pfn1 has recently been shown to have particular acids that allow it to associate directly with microtubules (Henty-Ridilla, Juanes, & Goode, 2017). (Figure 1a). This discovery fueled profilins' intricacy and significance as proteins that can engage with various cytoskeleton elements. Here, we will address the growing body of evidence indicating that profilins, in addition to being essential controllers of the actin cytoskeleton, may also have a significant impact on microtubule dynamics, thereby modulating cytoskeletal integration.

LITERATURE REVIEW

The regulation of polarised actin filament development is necessary for both cell movement and actin homeostasis. By attaching G-actin, profilin, a plentiful modulator of actin dynamics, aids filament formation at barbed ends. Here, we show that profilin regulates motility, cell migration, and actin balance by binding and weakening filament barbed ends at normal amounts. Profilin makes strand length variations more pronounced. Profilin and Capping Protein fight at the barbed ends, resulting in less profilin-actin production than would be anticipated if the barbed ends were securely capped. Profilin suppresses filament branching by the WASP-Arp2/3 complex by competing with barbed end polymerases like formins and VopF for filament barbed ends, which accounts for its as-yet-unknown effects on motility and metastatic cell migration found in this concentration range. In summary, profilin is a key coordinator of regulated polarised actin filament development. Profilin, in particular, forms a compound with cellular monomeric (G)-actin, which prevents spontaneous actin nucleation but promotes the formation of actin filaments (F-actin) by elongation-promoting factors (formins, Ena/VASP).

In contrast, site-specific F-actin oxidation by Mical encourages F-actin disintegration and the production of Mical-oxidized (Mox)-G-actin, which is polymerization-impaired. Here, we discover a connection between these two diametrically opposed mechanisms that helps to coordinate actin/cellular reorganisation. With regard to this, we discover that profilin binds Mox-G-actin, but that these compounds do not support elongation factors' mediated F-actin assembly instead they block polymerization and encourage further Mox-F-actin disassembly. We demonstrate analogous profilin-Mical links exist in vivo using the Drosophila model system, where they are responsible for the F-actin/cellular remodeling that follows Semaphorin-Plexin cellular/axon repulsion. Profilin and Mical work together to support F-actin disassembly and inhibit F-actin assembly, which simultaneously promotes cellular reorganization and plasticity [8]. Plasmodium sporozoites move quickly through the epidermis during the transfer of malaria-causing parasites from mosquito to mammal to enter the circulation and attack the liver.

A myosin motor's short tracks are provided by highly dynamic actin filaments and the unique gliding motion is dependent on the backward movement of membrane proteins. We previously proposed that actin filaments create macromolecular compounds with plasma membrane-spanning adhesins to produce force during migration using laser forceps and parasite mutants. Loss of actin-binding also corresponds with a loss of force generation and motility, according to mutations in the actin-binding region of profilin, a protein that binds to actin almost universally. Here, we demonstrate that various profilin mutants that do not impair actin binding in vitro still result in less force being produced during Plasmodium

sporozoite movement. Increased retrograde flow is inversely correlated with lower force production, indicating that the main fundamental principle regulating Plasmodium gliding motility is the slowing of flow to produce force, just like in mammalian cells [9].

The regulation of polarised actin filament development is necessary for both cell movement and actin homeostasis. By attaching G-actin, profilin, a plentiful modulator of actin dynamics, aids filament formation at barbed ends. Here, we show that profilin regulates motility, cell migration, and actin balance by binding and weakening filament barbed ends at normal amounts. Profilin makes strand length variations more pronounced. Profilin and Capping Protein fight at the barbed ends, resulting in less profilin-actin production than would be anticipated if the barbed ends were securely capped. The as-yet-unknown effects of profilin on motility and metastatic cell migration observed in this concentration range can be attributed to its competition with barbed end polymerases, such as formins and VopF, and inhibition of filament branching by the WASPArp2/3 complex by competition for filament barbed ends.

As a result of rivalry between filament branching machinery, barbed end cappers, trackers, and destabilizers, profilin is a key organizer of the polarised development of actin filaments[10][11]. In eukaryotic cells, profilin regulates the mechanisms of actin formation and construction. For filament development, profilin: actin is recruited by actin nucleation and elongation-promoting factors (NEPFs) like Ena/VASP, formins, and proteins from the WASP family. Actin polymerization from microtubule-related platforms is made feasible because some of these are discovered to be microtubule-associated. Actin and microtubule systems are coupled, as shown by the role of microtubules in the formation of cell polarity, the recycling of focal adhesions, and migration. Here, we show that profilin and formins effectively associate with microtubules, with formins serving as key intermediaries in this relationship.

To come to this result, we merged various fluorescence microscopy methods, such as super-resolution microscopy, with siRNA-mediated profilin expression regulation and medication to disrupt actin dynamics. Our research demonstrates that a portion of profilin actively interacts with microtubules, contributing to the equilibrium of actin assembly during homeostatic cell development and influencing microtubule dynamics. Consequently, profilin serves as an actin control component in addition to being a modulator of microtubule (+)-end recycling[9][3].In the eukaryotic cell, membrane layers, and cytoskeleton movements are closely related. The chemical processes taking place at this junction have recently received increasing attention. The actin cytoskeleton can interact with membranes through different distinct membrane domains, according to numerous studies. It has been demonstrated that the actin-binding protein profilin inhibits actin polymerization and encourages F-actin extension.

This depends on a variety of variables, including the proportion of profilin to G-actin and the cell's electrical milieu. Theoretically, this allows profilin the chance to interact with membranes, and a large number of studies have verified this potential. Additionally, profilin has specialized domains that interact with phosphoinositides and poly-L-proline-rich proteins. Profilin I is widely expressed in rodents, whereas profilin II is only highly expressed in the brain. Profilin I and II can assemble compounds with endocytosis, synaptic vesicle recycling, and actin assembly regulators in rodent brain extract. Several profilins I and profilin II .identified ligands from rodent brain lysates using mass spectrometry and database searches,

including dynamin I, clathrin, synapsin, Rho-associated coiled-coil kinase, the Rac-associated protein NAP1, and a member of the NSF/sec18 family. In neuronal and dendritic processes in vivo, profilins co-localize with dynamin I and synapsin. r research firmly suggests that the brain's profilin I and profilin II complexes connect the movement of endocytic membranes and the actin cytoskeleton, guiding the assembly of actin and clathrin to specific membrane regions[1].

In addition to being involved in the inclusion and dissociation of /-tubulin heterodimers, the microtubule (+)-ends serve as a focal point for a network of related proteins called the microtubule plus endtracking proteins (+TIPs), which control the (+)-end dynamics. A number of +TIPS, such as the adenomatous polyposis coli and end-binding (EB) proteins, engage with various factors that promote actin nucleation and elongation, such as formins linked to diaphanous (Dia). Therefore, via formins, TIPs can crosslink the cytoskeletons of the microtubule and actin. There are at least 15 distinct formin genes in mammals, making it a very big family. The homodimeric formin homology 2 (FH2) domain of formins engages with the barbed extremities of actin filaments to promote nucleation and unbranched extension. FH1 domains have PLP segments that bind profilin next to the FH2 domains [12]. Actin elongation is hastened by the actinmonomer binding protein, whereas formins are processive both in the presence and lack of profilin. Although formins were originally thought to be actin filament nucleators, their FH2 domain also allows them to associate with microtubules, improving their stability [13].

Formin carboxy-terminal sections may also associate with microtubules in specific circumstances. Conversely, formins can aggregate at microtubule plus-ends where they nucleate and encourage de novo actin polymerization by interacting indirectly with microtubules through the +TIP proteins EB1 and cytoplasmic linker protein 170 (CLIP-170)[14]. Total internal reflection fluorescence (TIRF) imaging has demonstrated that CLIP-170-mDia1 complexes colocalize at growing actin filament ends in vitro and are attracted by EB1 to growing microtubule ends. When affixed to the microtubule surface, these complexes promote the polymerization of actin filaments and offer a method by which developing microtubule plus ends control fast actin assembly (Figure 1). Curiously, formins also cause actin-microtubule co-alignment by joining the two filament systems (Figure 1). Considering that formin-profilin complexes further enhance actin elongation in the presence of profilins, they are likely key actors in modulating the interaction between the microtubule and actin cytoskeleton (Figure 1).

Recent research has given some of the first proof that profilin regulates microtubule growth dynamics and contributes to the coordination of the actin and microtubule systems through an indirect interaction mediated by formins. Profilin dynamically interacts with microtubules in the murine melanoma cell line B16, and the microtubulebound profilin portion controls microtubule dynamics, it has been demonstrated. Reduced profilin cellular content in these cells was associated with increased microtubule development as observed after EB3-enhanced green fluorescent protein (EGFP) transfection and increased tubulin stability as determined by higher acetylated tubulin levels. Based on these findings, it was determined that profilin inhibits microtubule development by increasing the incidence and/or pausing of catastrophic microtubule (+)-end events. This would slow the pace at which new tubulin molecules are added to the microtubules' +TIP. Data using mouse platelets and fibroblasts

were previously reported, providing additional evidence for a potential indirect association of profilin with microtubules.

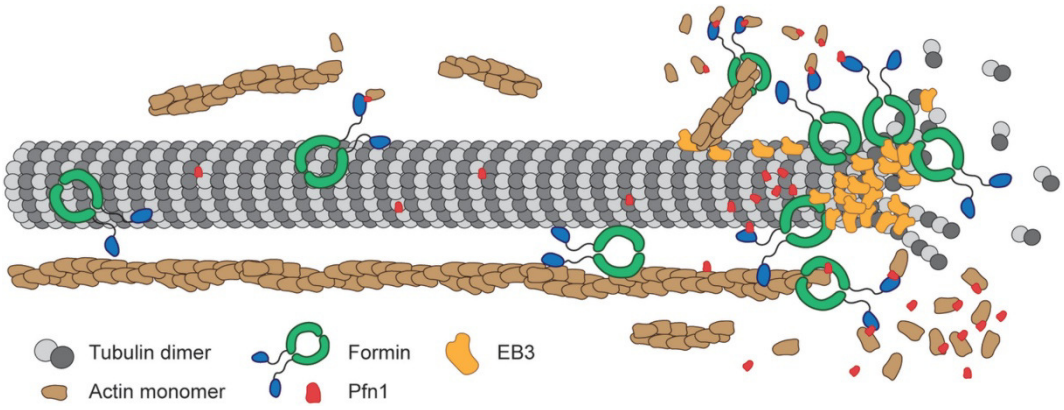

Figure 1: Binding of profilin with actin Actin- and microtubule-based cytoskeletal contacts are mediated by the molecular receptor Pfn1 (online libeary).

Similar to B16 cells, irregularly arranged and hyperstable microtubules with elevated amounts of acetylation were also discovered in profilin-deficient platelets. Despite the fact that Pfn1 and platelet microtubules colocalized in this research, no evidence of direct Pfn1 attachment to microtubules was found. Therefore, it has been hypothesised that Pfn1 may be tangentially connected to microtubules by an unidentified protein complex[3]. Strangely, the potential PLP binding site of profilin, which facilitates the contact between profilin and formin, is a long way from the residues involved in microtubule binding. As a result, one hypothesis is that profilin may associate with the microtubule polymer in a complex after being attached to PLP-containing proteins, similar to how formins do. Pfn1 may therefore be attracted to areas that are abundant in FH2-bound microtubules by formins through FH1 domains. Pfn1 was overexpressed in rat neuroblastoma (N2A) cells to examine the molecular applicability of the in vitro findings.

Pfn1 can assign specific actin or microtubule nets to fundamental cytoskeleton components. Pfn1 attaches to the microtubule structure directly or via formins, and it also promotes formin-mediated actin polymerization. Through EB1/3 binding, formins may also enhance microtubule polymerizing ends, ultimately recruiting Pfn1 for dynamic microtubule tips and enhancing actin coupling (Figure.1).Microtubule growth pace was nearly tripled by monitoring microtubule (+)-ends, and microtubule entry into filopodia was significantly enhanced. As mentioned above, the results found in melanoma cells contrast with the impact of Pfn1 in promoting microtubule growth rates in vitro and in neuroblastoma cells [3]. Surprisingly, higher microtubule growth speed was also achieved when Pfn1 was depleted from N2A cells, though to a lesser degree than that caused by Pfn1 overexpression[15]. It was therefore determined that, despite the lack of a proposed mechanism to account for this apparent discrepancy, microtubule growth rates are probably extremely sensitive to Pfn1 levels in either way.

CONCLUSION

A growing amount of evidence indicates that profilin is a dual regulator of actin dynamics and microtubule (+)-end turnover. There is still much to learn about the function of profilin in these situations, even though Pfn1 possesses the characteristics necessary to facilitate actin-

microtubule contacts and operate as a modulator of pressures produced by polymer assembly dynamics. Many questions, in particular, anticipate answers that will undoubtedly come from future studies, including the following: Does profilin attach to microtubules directly in vivo, or does this relationship involve formins or other factors that aid in actin nucleation and elongation? Does the sort of cellular structure, environment, and compartment affect how directly or indirectly profilin interacts with microtubules? What specific biochemical specifics underlie profilin's disruption of microtubule dynamics? How can we explain the ability of profilin to accelerate microtubule development speed in the same biological setting whether it is overexpressed or downregulated? Is Pfn1 the only protein that profilin interacts with microtubules? Earlier studies have shown that Pfn2b can associate with tubulin and be recruited to spindles and asters during mitosis in HeLa cells, despite early research showing that only Pfn1 can bind tubulin. The idea that profilin may typically be involved in the control of the microtubule cytoskeleton is further supported by the fact that different profilin variants display the same potential key microtubule residues. Additionally, even though formins were first discovered to control actin and microtubule dynamics in non-neuronal cells, it has recently been demonstrated that this process is preserved in neurons. Despite these developments, it is still unknown how formins affect the neural actin-microtubule interaction. Regarding the role of profilin in the relationship between actin and microtubules, it is important to note that profilin mutants linked to ALS have no impact on actin binding, organizations, or dynamics, allowing researchers to identify the microtubule-binding residues on Pfn1. Together, the new information indicates further research into profilin's potential function in neural biology.

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CHAPTER 20

REGULATION OF THE MICROTUBULES IN THE CELLULAR ENVIRONMENTS

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ABSTRACT:

All eukaryotic cells require microtubule dynamics and their control in order to operate normally and divide. Dynamic microtubule tips, which can bind to different intracellular targets, produce mechanical forces, and couple with actin microfilaments, are largely responsible for this wide range of functions. Microtubules are necessary for the development of cell polarity, polarised cell migration, the transfer of intracellular vesicles, and the segregation of chromosomes during mitosis. Microtubules (MTs) are nonequilibrium polymers of /-tubulin heterodimers in which assembly is followed by GTP hydrolysis on the -tubulin component. Proteins that bind either tubulin dimers or assembled microtubules play a significant role in controlling the equilibrium between dynamically unstable and stable microtubules.A large number of MT motor proteins, as well as non-motor proteins, support MT motion enable tubulin dimer incorporation into the expanding plus end, promoting MT assembly. The association of the protein with particular MT motors as well as other proteins that can affect MT stability and dynamics have been shown to change as a result of various regulatory processes, such as post-translational modifications to tubulin.

KEYWORDS:

Microtubule, Dynamics, Regulation, Post-Translation, Motor Protein.

INTRODUCTION

By acting as a scaffold for intracellular transport, signalling, and organelle placement, microtubules regulate the structure of the cell. Microtubules are inherently polarised, and they react to and contribute to cell polarity through their orientation, density, and post-translational modifications. While stably polarised cells frequently develop non-centrosomal microtubule networks attached to the cell cortex, nucleus, or other structures, animal cells that can quickly reorient their polarity axis, such as fibroblasts, immune cells, and cancer cells, contain radially organised microtubule arrays anchored at the centrosome and the Golgi apparatus [1]s. The dynamics of their plus ends have a significant impact on the density, longevity, and post-translational changes of microtubules. Cortical assemblies that integrate cytoskeletal organisation, cell adhesion, and secretion and are controlled by microtubule-dependent feedback regulation frequently contain elements that regulate microtubule plus-end dynamics.

Last but not least, microtubules can physically support cell elongation, which may be crucial for cells with dense microtubule arrays developing in soft environments [1].MAPs regulate the dynamics of microtubules both in vitro and in vivo by attaching to their sides. Microtubules are stabilized by many MAPs, including conventional MAPs [2]. Traditional MAPs were typically found primarily in the nerve system. Compared to the majority of

interphase cells, the microtubules in neurons are typically much more robust. It follows that the stabilization of microtubules against disassembly is one of the key roles played by conventional MAPs. As previously mentioned, the C-terminal three or four pseudorepeats of tau, MAP2, and MAP4 are conserved and act as a microtubule-binding region [2].

Here are some of the ways that the centrosomal proteins may control the integrity and dynamics of microtubules. I Centrosomal proteins have the capacity to post-translateally modify microtubules and change their stability. By either sequestering the soluble fraction or by inhibiting microtubule polymerization/depolymerization, centrosomal proteins like fibroblast growth factor receptor 1 (FGFR1)oncogene protein (FOP)-like protein of molecular mass of 20kDa (FOR20) may change the equilibrium between levels of soluble and polymeric tubulin in the cell cytoplasm [3].

The Ras-like GTPase Ran-GTP, the microtubule plus end-directed proteins, the microtubule-associated proteins (MAPs), and tubulin-binding proteins are just a few of the microtubule effectors that control microtubule dynamics. The cyclin-dependent kinase Cdk1 (or Cdc2), which functions with cyclin B as a cognate partner, is a crucial enzyme for mitosis entrance and is required for spindle morphogenesis [4]. Different degrees of information complexity can arise as a result of PTMs. Simple modifications like acetylation or detyrosination/tyrosination produce binary signals, but due to differences in side-chain lengths and modification of either a- or b-tubulin, or both, polyglutamylation, polyglycylation, or polyamination can produce more graded signals [5]. Most importantly, based on where these modification sites are located, tubulin PTMs can influence different functional roles of microtubules. While most other tubulin PTMs change the carboxy-terminal tails of tubulin, which are found at the outer surface of microtubules, acetylation, for example, is found at the luminal surface of the microtubules .

The carboxy-terminal tail is believed to be a hotspot for the selective regulation of microtubule-MAP interactions because it is a key interaction site for many MAPs. We go over the different tubulin PTMs and how they affect microtubule activities in this primer [5]. For many different biological functions, the movement of motor proteins on biopolymers is crucial. Motor proteins can travel along one-dimensional tracks made of actin, microtubules, and nucleic acids. For motors to fulfil their biological functions, filaments must accumulate them at an adequate density. Regulation of MT overlaps is crucial for mitotic spindle activity and cytokinesis because the spindle midzone contains arrays of overlapping antiparallel MTs[6].

Control of the Kinesin and dynein motors move a variety of organelles and vesicles along the long pathways provided by microtubules.

Cargoes are attached to cytoskeletal strands by motor protein complexes, which aids in their communication and interaction. Subsets of motors can identify a specific microtubule identity thanks to the development of biochemically distinct microtubule subpopulations, enabling further cytoplasmic organization [6]. Multiple mechanisms, including acute modification of both motor-cargo and motor-track associations by various physiological signals, are used to spatiotemporally control both transport and tethering. In specialized cell types like neurons, strict control of intracellular transport is crucial. Here, we go over some basic mechanisms for controlling the movement of cargo as well as some specific instances where this has happened[6].

LITERATURE REVIEW

Dynamic microtubule tips, which can bind to different intracellular targets, produce mechanical forces, and couple with actin microfilaments, are largely responsible for this wide range of functions [7]. Many protein involved in the regulation of the microtubules. It has been demonstrated that MAPs, or microtubule-associated proteins, are essential for controlling microtubule movements *in vivo*. Depending on the microtubule-associated proteins (MAPs) that are present, different microtubule polymerization, depolymerization, and catastrophe speeds occur. Based on their molecular weight, the initially discovered MAPs from brain tissue can be divided into two categories. The term "tau" protein refers to MAPs in the first class that have a molecular weight between 55 and 62 kDa or less (Figure 1).

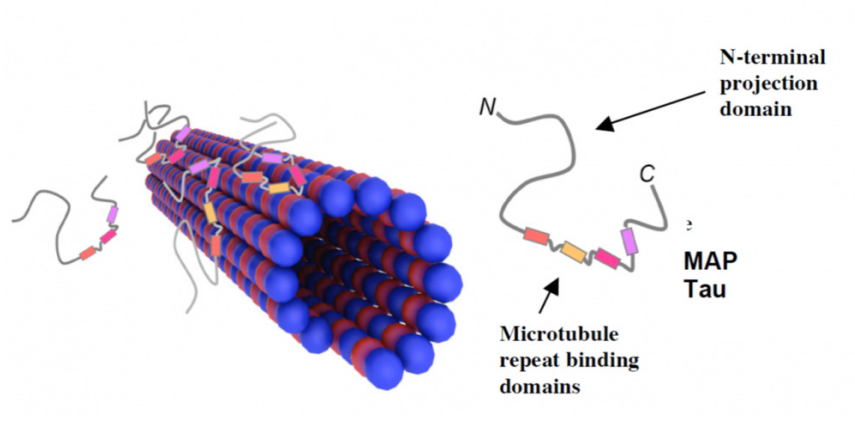

Figure 1: Microtubule binding protein: Showing the interaction of the microtubules binding protein with the microtubules (Open scholar).

The induction of parallel groups and the promotion of nucleation and prevention of disassembly are all effects of tau proteins in vitro [8]. Tau proteins have also been linked to Alzheimer's disease and have been shown to support microtubules in axons[9].Four different kinds of MAPs with molecular weights between 200 and 1000 kDa make up the second class. three, two, one, and four. Three distinct proteins, A, B, and C, make up the MAP-1 protein family. The C protein, also referred to as cytoplasmic dynein, is crucial for the backward movement of vesicles. Both the dendrites and the body of neurons contain MAP-2 proteins, which interact with other cytoskeletal strands there (Figure2).

Most cells contain the MAP-4 proteins, which help to keep microtubules stable. Other MAPs, in addition to those that stabilise the structure of microtubules, can destabilise it by cleaving microtubules or by causing them to depolymerize. The quantity and length of microtubules have been found to be regulated by the destabilising actions of three proteins known as katanin, spastin, and fidgetin. Additionally, it is expected that KIAA1211L will be found in microtubule[10].

Plus-end tracking proteins (+TIPs) :

Plus end tracking proteins are MAP proteins that attach to the growing microtubules' tips and are crucial in controlling the dynamics of these structures. For instance, it has been discovered that +TIPs take role in the interactions between chromosomes and microtubules during mitosis. A function in microtubule depolymerization rescue events has been demonstrated for CLIP170 (cytoplasmic linker protein), the first MAP to be recognised as a

+TIP. EB1, EB2, EB3, p150Glued, Dynamitin, Lis1, CLIP115, CLASP1, and CLASP2 are additional instances of +TIPs[8] Figure2.

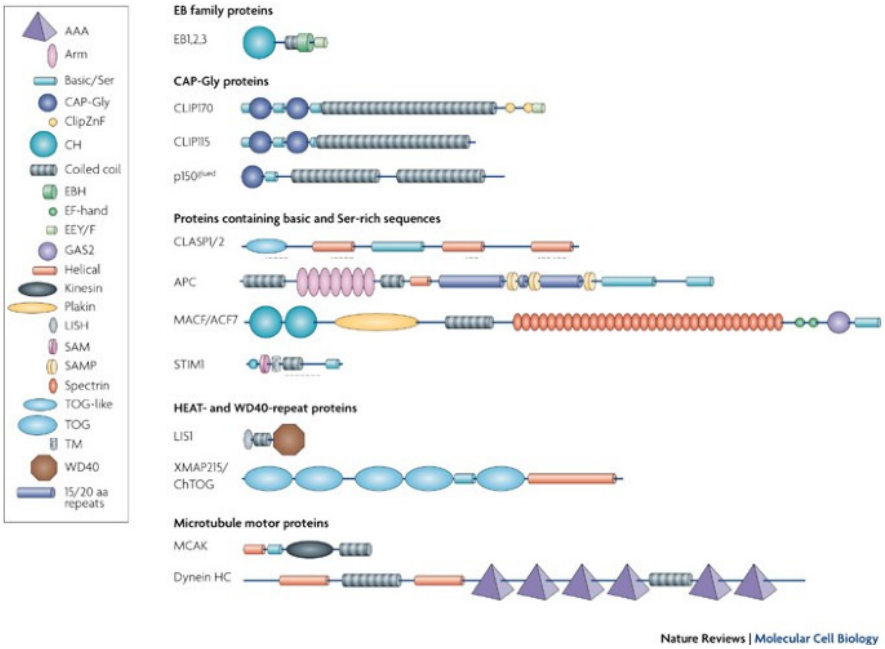

Figure 2: Microtubules ends tracking protein:Diagramed showing the list of the protein involve in the microtubule regulation (Nature).

Microtubule motor protein:

Motor proteins that are engaged in crucial cellular processes like vesicle trafficking and cell division can act as substrates on microtubules. Motor proteins use the energy from ATP hydrolysis to produce mechanical activity that moves the protein along the substrate, in contrast to other microtubule-associated proteins[6]. Kinesin, which typically moves towards the (+) end of the microtubule, and dynein, which typically moves towards the (-) end, are the two main motor proteins that associate with microtubules (Figure.3). Two identical heavy chains, which together form two sizable spherical head domains, make up dynamin, along with an unknown number of intermediate and light chains. The (+) end of the microtubule moves towards the (-) end during dynein-mediated transport [6].

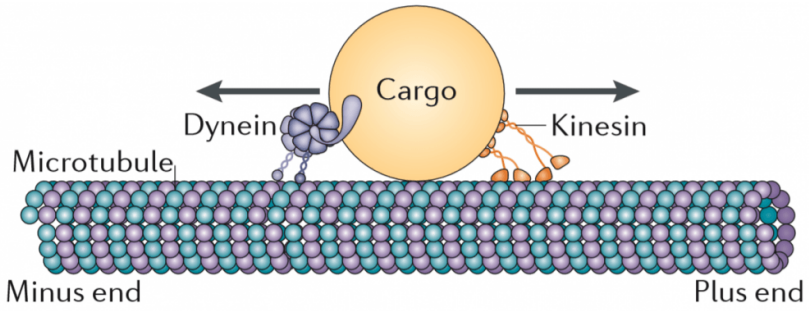

Figure 3: Motor protein: Showing the diagrammed of the motor protein which are involve in transport and regulating the microtubules dynamics (sites of Penn state).

The globular head domains, which resemble the AAA+ (ATPase associated with different cellular activities) protein family, are where ATP hydrolysis takes place. Through the microtubule-binding domains, ATP breakdown in these domains is connected to movement along the microtubule. Vesicles and cells are transported by dynein throughout the cytoplasm.

In order to accomplish this, dynactin is a component of a protein complex that binds dynein molecules to organelle membranes. Kinesin and dynein share a comparable structure. A number of intracellular cargoes, such as vesicles, organelles, protein complexes, and mRNAs, are transported by kinesin in the direction of the microtubule's (+) end[11].

The pivotal characteristics of MTs must be adjustable and closely controlled in order to accomplish this. This is made possible by a wide range of tubulin posttranslational modifications, which modify MT properties either directly or indirectly by altering the structural properties of the MT lattice or by altering the MT interaction partners. Acetylation of lysine 40 (K40) of a-tubulin is the tubulin change that has received the most research to date. This modification site's oddity stems from where it is located—at the luminal surface of microtubules [5].

This makes it more likely to control the binding of luminal proteins to the inner surface of microtubules rather than the binding of MAPs and motors, which are less likely to be affected by it. The ciliary, flagellar, and neuronal microtubules are highly acetylated, as shown by the anti-K40-acetylation antibody's staining of distinct microtubule populations in interphase cells. The acetyl transferases aTAT/Mec-17 and Atat-2 (Atat-2 has only been discovered in Caenorhabditis elegans thus far) cause the acetylation of K40, and the deacetylases HDAC6 and SIRT2 carry out the deacetylation Figure 4.

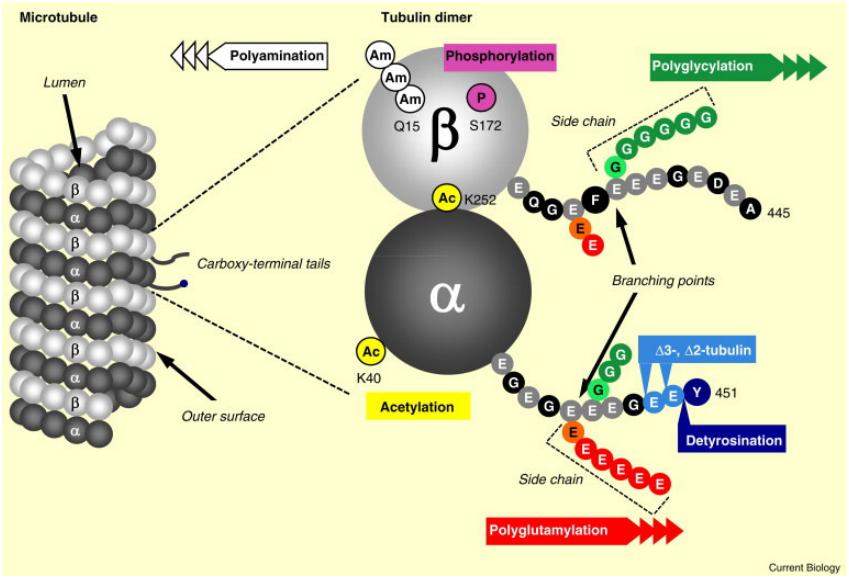

Figure 4: Tubulin posttranslational modifications.Diagramed showing the different localization of the post translation modification for microtubules regulation (Science direct.com).

The uses of K40 acetylation are not well understood. The Sun acetyltransferase catalyses the acetylation of K252, in contrast to aTAT (the a-tubulin K40 acetylase), which modifies free tubulin dimers and inhibits their formation into microtubules. A-tubulin-b-tubulin dimers, which create hollow tubes made of 13 protofilaments, are used to construct microtubules. While the carboxy-terminal tails of the tubulins decorate the outer surface of microtubules (Figure.4).The discovery of numerous potential acetylation sites on both a- and b-tubulin in a whole-proteome mass spectrometry study suggested that tubulins may be prone to more complicated acetylation processes [5]. It is thought that microtubule polyglutamylation

controls electrostatic microtubule-MAP contacts by altering the charges on the carboxyterminal tails of tubulins. It is thought that microtubule polyglutamylation controls electrostatic microtubule-MAP contacts by altering the charges on the carboxyterminal tails of tubulins [12]. Similar to polyglutamylation, polyglycylation modifies tubulin by adding side chains of glycine to target proteins, possibly using the same glutamate residues as the donor. Both a- and b-tubulins are modified by glycylation, which also produces chains of various lengths and has a number of alteration sites within the carboxyterminal tubulin tails [12].

Microtubule cross-linking protein: Cross-linkers and bundlers Microtubules are horizontally linked together by bundlers and cross-linkers. Proteins referred to as MAP65/Ase1/PRC1 selectively bundle antiparallel microtubules, an action crucial for the mitotic spindle. Although most stabilisers exhibit some bundling activity, it is unknown whether this activity has any physiological significance. Bundling can happen when tau peptides are used to simply cover negatively charged microtubules, as well as when crowding agents like polyethylene glycol are added [8]. Proteins with caps capping proteins can prevent both dimer association and separation by binding to the microtubule plus or minus end. it is known about microtubule capping proteins, possibly due to the larger and more complicated microtubule tip. For instance, rather than being single proteins, the only known minus-end capping proteins are huge complexes. The related complexes -TuRC and -TuSC, which not only cap minus ends but also nucleate microtubules and microtubule-associated proteins, are the best-characterized instances [13].

CONCLUSION

Microtubules are dynamic and unstable structures. Microtubule dynamics in vitro and in vivo by binding along the sides of the microtubules. Many cellular protein are involved in the regulation of the microtubules. *In vitro* analysis of microtubule dynamic instability is thus critical in studying the mechanism of action of MAPs or new therapeutics that act directly or indirectly on microtubules.Microtubules are stabilises by many MAPs, including classical MAPs. Classical MAPs were typically found primarily in the nervous system. In tau, one of these repeats binds to the tubulin C-terminus, while others bind to another internal tubulin site. As a result, these tandem repeats are likely to bind to microtubules in a way that cross-links adjacent tubulin subunits while also stabilising microtubules against disassembly. Phosphorylation regulates these bindings. Tau and MAP2 both contain numerous phosphorylatable residues. This chapter address the proteins and processes involved in microtubule regulation. The role of these proteins will provide an insight into the overall mechanism of microtubule regulation.

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CHAPTER 21

MUSCLE CONTRACTION IN THE PRESENCE OF THE ACTIN CYTOSKELETON

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ABSTRACT:

The actin cytoskeletal dynamics in smooth muscle provide a brand-new concept for the regulation of smooth muscle contraction. Myosin and actin are two proteins that are present in all types of muscle tissue. Thick myosin filaments and small actin filaments combine to control muscle tension and movement.

The chemical energy of ATP is transformed into mechanical energy by a sort of molecular drive known as myosin. The actin filaments are then drawn along by mechanical energy, which causes the muscle fibers to contract and generate movement. Calcium induces constriction by preventing the interaction of actin and myosin in the absence of regulatory proteins. The light chains are thought to function by sterically obstructing myosin sites when calcium isn't present, and the two myosin heads need to cooperate for myosin to be in their "off" state. A wide range of organisms has the ability to regulate actin. Many animals' muscles contain this type of regulation. Although the existence of *in vivo* actin control in mammalian smooth muscles where actin is the main source of muscle contraction cannot be ruled out.

KEYWORDS:

Actin Polymerization, Contractile Stimulation, Cytoskeletal Signaling, Smooth Muscle, Sarcomere Organization.

INTRODUCTION

Luigi Galvani found in 1780 that a spark of electricity caused the muscles in dead frogs' legs to twitch. One of the earliest ventures into the area of bioelectricity continues to investigate the electrical patterns and signals in tissues like nerves and muscles[1]. Excitation-contraction coupling is the name for the physiological mechanism that transforms an electrical stimulus into a mechanical response. It was first used in 1952 [2]. This process, where the mechanical reaction is contraction and the electrical stimulus is typically an action potential, is essential to the physiology of muscles. In many illnesses, the coupling of excitation and contraction can be dysregulated. Although excitation-contraction coupling has been known for more than 50 years, the biomedical study is still being done in this area[2]. To depolarize the cell membrane, an action potential is thought to generally occur. This depolarization causes a rise in cytosolic calcium, known as calcium transient, through muscle-type-specific mechanisms.

Calcium-sensitive contractile proteins are activated by this rise in calcium, and they use ATP to shrink cells as a result. Scientists have struggled to understand the process of muscle contraction for a long time, and their understanding needs to be updated[3]. Both Hugh

Huxley and Jean Hanson and Andrew F. Huxley independently developed the sliding filament hypothesis. Their research was presented in two articles that were published back-to-back in Nature on May 22, 1954, with the common title "Structural Changes in Muscle during Contraction." Striated and smooth muscles have distinct mechanisms for controlling contraction, which can serve as the foundation for specific pharmacological changes to these muscle types' contractility [3].

The development of our knowledge of the tropomyosin-troponin regulation system of striated muscle between the early 1970s and the early 1990s is discussed, along with the critical ideas needed to comprehend this intricate system. The new development of the putative contractile regulatory proteins of smooth muscle, caldesmon, and calponin, is also covered in this review. The actin-linked regulatory mechanisms of striated and smooth muscle are contrasted[4].Myosin and actin engage cyclically during muscle contraction, and this interaction is fueled by the simultaneous hydrolysis of adenosine triphosphate (ATP). The molecular structures of the individual proteins were combined with the low-resolution electron density maps of the complex produced by cryo-electron imaging and image analysis to create a model for the rigor complex of F actin and the myosin head [5]. A working theory for the cross-bridge cycle is suggested by the spatial relationship between the ATP binding pocket on myosin and the major contact area on actin, which is consistent with earlier independent structural and biochemical investigations[5].

Isometric force can be produced by smooth muscles over a very broad spectrum of cell lengths. This phenomenon's molecular causes are unknown, but it is said to be reflected in the smooth muscle cells' "mechanical flexibility." Here, plasticity is defined as a steadfast alteration of cellular composition or operation in reaction to a shift in the external environment. Chemical (such as neurotransmitters, aracoids, and cytokines) and external mechanical cues are significant environmental factors that cause muscle plasticity (e.g., applied stress and strain). Ionic and protein kinase signaling pathways are likely responsible for converting both types of signals into changes in the cytoskeleton and contractile system as well as in gene expression patterns [6]. Actomyosin cross-bridge cycling is acknowledged as the basic process for tension development and shortening in all forms of muscle, as well as in contractile nonmuscle cells, thanks to the role of filamentous actin in the activation of myosin ATPase activity and cross-bridge cycling. Through the ATPase activity of the myosin head, myosin filaments can crawl along actin filaments when myosin is activated by a contractile stimulus, which causes the cell to shorten or produce tension [7].

This widely accepted model of smooth muscle contraction has been predicated on the idea that during a contractile event, the structure and organizations of filamentous actin remain largely constant, and that actin filaments anchored at adhesion sites at the plasma membrane and at dense bodies within the cytosol provide a stable and fixed network on which the myosin or thick filaments move during shortening and tension development [7].In addition to actomyosin interaction and cross-bridge cycling, there is growing evidence that smooth muscle movement necessitates actin filament polymerization and a number of other cytoskeletal processes. The actomyosin system is activated concurrently with a complex collection of cytoskeletal events that seem to be crucial to the mechanical response of the muscle tissue [7]. This has led to the creation of new paradigms for the contraction of smooth muscle that takes into account findings that the actomyosin system is not the only cellular mechanism involved in the control of smooth muscle contraction and tension development

[7]. Many smooth muscle tissues possess unusual adaptive qualities that allow them to modify their contractile and mechanical properties to handle changes in their surrounding environment. These dynamic cytoskeletal processes may be the basis for these abilities. Growing evidence indicates that tension generation in smooth muscle requires a more complex range of physiological processes than previously believed and that the cytoskeletal processes that take place during the contractile activation of smooth muscle cells may share significant similarities with the cytoskeletal mechanisms that regulate cell motility and migration [7].

LITERATURE REVIEW

The most prevalent protein in cells is actin, which can be found in both a liquid and filamentous form. Asymmetric bilobed 42-kDa actin monomers are arranged into a double-stranded helical array to form filamentous actin, a polymeric structure. Actin strands contain actin monomers that are constantly exchanging with soluble actin monomers [7]. In smooth muscle cells, where the cytoskeleton connects to the extracellular matrix, actin filaments are anchored to the membrane via a complex of adhesion proteins that interact with the cytoplasmic tails of integrin proteins. Additionally, actin filaments adhere to smooth muscle cells' cytosolic dense bodies, which are mainly made of the actin cross-linking protein – actinin [7].

Numerous studies that assessed the effects of inhibiting the actin polymerization process on tension generation in response to contractile stimulation evaluated the crucial role of actin polymerization in tension development in smooth muscle tissues and cells. The effects of inhibiting actin polymerization on contractile responses to agonist stimulation have been extensively studied in a variety of smooth muscle tissue and cell types using the pharmacologic agents' latrunculin and cytochalasin, which inhibit actin polymerization by sequestering G-actin monomers and capping actin filaments, respectively [7]. Studies on the smooth muscles of the uterus, the intestinal tract, the airways, and the vascular system have all demonstrated that short-term exposure of smooth muscle tissues to inhibitors of actin polymerization causes a profound suppression of tension development and inhibition of shortening or constriction. Studies showing that molecular constructs or peptides that disrupt particular steps in the actin polymerization process also inhibit tension development in smooth muscle tissues in response to contractile stimuli provide additional evidence that actin polymerization plays a crucial role in the process of mechanical tension development in smooth muscle[7].

According to cellular imaging studies, the contractile apparatus's organizations or integrity are not disrupted when interventions that prevent actin polymerization reduce tension formation. Collectively, these numerous studies offer convincing proof that dynamic modifications to the actin cytoskeleton are essential for controlling tension formation during smooth muscle contraction. However, it is presently unknown how actin polymerization controls the emergence of tension in smooth muscle[7]. Actin cytoskeletal remodeling and myosin activation are both necessary for smooth muscle movement. Muscles are made up of muscle fibers [7]. Muscle fiber bundles make up the muscular tissue. Long, slender cells called muscle fibers can grow up to several inches long and, in the case of skeletal muscle, may have multiple nuclei (Figure.1). Myofibrils, which are long, thread-like structures found in the cytoplasm of muscle fibers and are composed of bundles of dense, myosin filaments

and thin actin filaments, are found there. The sarcoplasmic reticulum (SR), a network of tubules that stores calcium ions, surrounds the actin and myosin fibers. The SR is crucial in the transmission of electrical impulses. Neurons transmit these electrical impulses to the muscle cells [7].

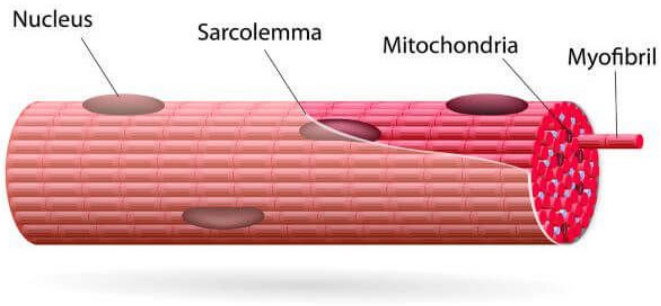

Figure 1:Muscle fiber: Diagramed showing the organization of the muscle fiber (biology dictionary).

By improving intercellular mechanical transduction and force transmission between the contractile unit and the extracellular matrix (ECM), actin cytoskeletal reorganization helps smooth muscle contract. The actin cytoskeleton could be thought of as the smooth muscle's "transmission system," whereas myosin could be considered it's "motor" for contraction [7].

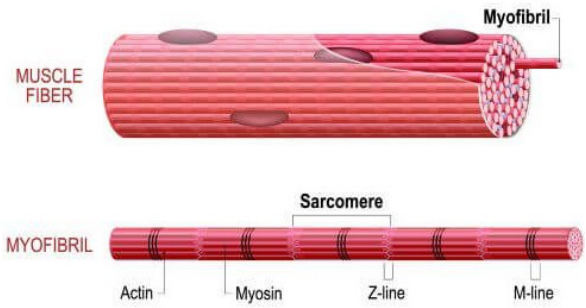

Figure 2 : Structure of muscle: Muscle fibers are made up of the bundle of the muscle fiber. Muscle fiber origination accomplished actin and myosin which made the structure sarcomere (biology dictionary).

The sarcomere needs to shrink for a muscle cell to contract. Sarcomeres' constituent dense and thin filaments, however, do not shorten (Figure. 2). Instead, they move past one another, shortening the sarcomere while keeping the strands at their original length[8]. The named bands on the sarcomere were found to vary at various levels of muscle contraction and relaxation, leading to the development of the sliding filament theory of muscle contraction. Myosin's binding to actin creates cross-bridges that cause filament migration during contraction[8].

Some areas of a sarcomere shrink while others remain the same size. A sarcomere is described as a space between two adjacent Z discs or Z lines; this space is shrunk when a muscle contracts. Only thick strands are present in the H zone, which is the center of the A zone, and it shortens during contraction (Figure.3) [8]. Only thin strands can be found in the I band, and it also shortens. Although the A band does not shrink during contraction it stays the

same length it does get closer together before disappearing altogether. Up until the Z discs get close to the thick filaments, thin filaments are drawn toward the middle of the sarcomere by the thick filaments. As the thin filaments travel inward, the zone of overlap where thick and thin filaments share the same space grows[8].

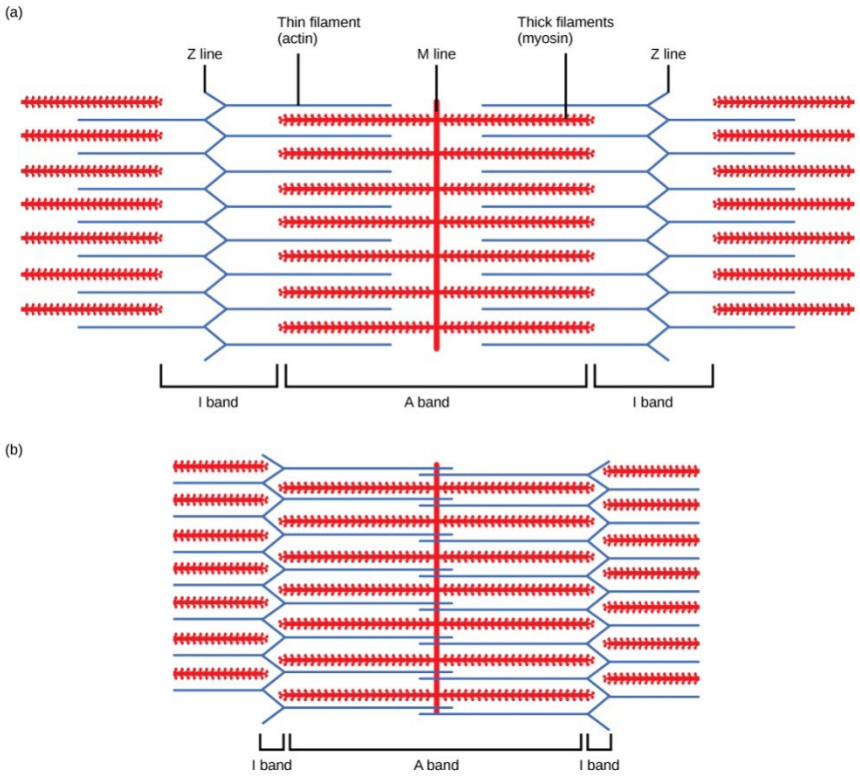

Figure 3. Organization of the sarcomere in contracts and relax muscle: When (a) a sarcomere (b) contracts, the Z lines move closer together and the I band gets smaller. The A band stays the same width and, at full contraction, the thin filaments overlap (med libra texts).

Myosin heads attach to actin and draw the actin inward, shortening the muscle. ATP supplies the necessary energy for this activity (Figure.4). At a binding site on the globular actin protein, myosin attaches to actin. Another ATP-binding site on myosin is where enzymatic action converts ATP to ADP, releasing energy and an inorganic phosphate molecule[9]. Actin and myosin can separate from one another when myosin releases actin in response to ATP binding. The freshly bound ATP is then changed into ADP and inorganic phosphate, Pi, as a result. ATPase is the name of the enzyme located at the myosin binding region[9].

The myosin head's angle becomes "cocked" as a result of the energy produced during ATP hydrolysis. The myosin head now has the potential energy to travel further, but ADP and Pi are still firmly attached. The myosin will stay in the high energy configuration with ATP hydrolyzed but still attached if actin binding sites are blocked and inaccessible [9]. A cross-bridge will develop if the actin-binding sites are exposed; in this case, the myosin head will span the space between the actin and myosin molecules. The stored energy is then released as Pi, enabling myosin to undergo a conformational change [9]. The actin is drawn along by the myosin head as it travels in the direction of the M line (Figure.4).

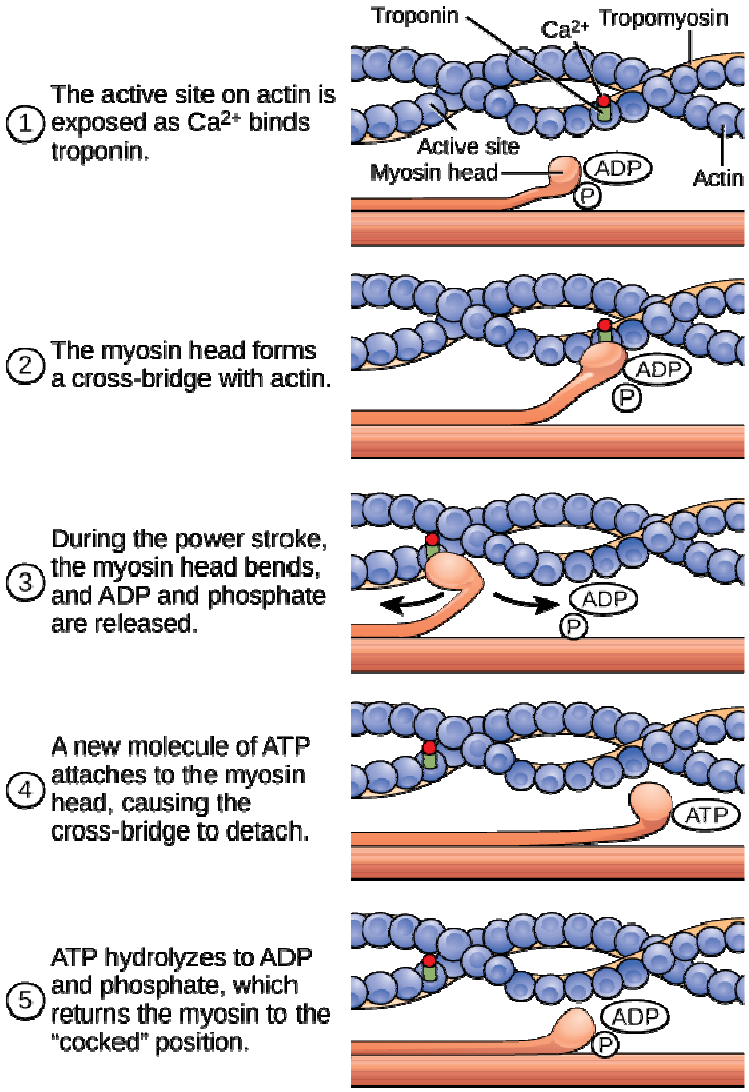

Figure 4. Muscle contraction:The Ca2+ attachment to the actin active site initiates the cross-bridge muscle contraction cycle, which is displayed. Actin shifts in relation to myosin during each muscle cycle (med libra texts).

The filaments migrate towards the M line by about 10 nm as the actin is pulled. The power stroke is the name given to this motion because it is where energy is generated. The sarcomere shortens and the muscle contracts as the actin are drawn towards the M line [9]. The myosin head has energy and is in a high-energy shape when it is "cocked." The myosin head uses up this energy as it moves through the power stroke, and after the power stroke, it is in a low-energy state. ADP is released after the power stroke, but actin and myosin are still connected by the cross-bridge that was created (Figure.4). Once ATP has attached to myosin, the cross-bridge cycle can begin once more, resulting in additional muscular contraction [10].

CONCLUSION

Actin and myosin filaments cooperate to produce energy. This energy results in muscle cell contractions, which make it easier for muscles and subsequently bodily structures to move. A growing amount of evidence supports the idea that smooth muscle cells' actin cytoskeletons are dynamic structures that play a crucial role in controlling the growth of mechanical tension and the material characteristics of smooth muscle tissues. It has been conclusively shown in

numerous smooth muscle tissues and cells that the proportion of filamentous actin increases in response to the stimulation of smooth muscle cells and that stimulus-induced actin polymerization and cytoskeletal dynamics play a crucial role in the generation of mechanical tension.

Actin polymerization plays a functional role in contraction that is distinct from and regulated independently from the actomyosin cross-bridge cycling process. To determine the molecular underpinnings of actin polymerization regulation and its functional functions in various kinds of smooth muscle cells and tissues, however, a lot more information will be required. Smooth muscle, is consistent with the idea that contractile stimulation triggers a signaling pathway at the membrane that is mediated by proteins in cytoskeletal/extracellular matrix adhesion complexes, which in turn coordinates the polymerization and organization of a network of actin filaments below the plasma membrane. The membrane may be strengthened by the cytoskeletal network to better transmit the force produced by the contractile machinery. This cytoskeletal network's dynamic reorganization may also be what allows smooth muscle cells to adjust and conform to their surrounding environment.

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CHAPTER 22

STRUCTURE AND FUNCTION OF THE ACTIN INSIDE THE CELLULAR ENVIRONMENT

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ABSTRACT:

The most prevalent protein in the majority of cellular cells is Actin. It participates in more protein-protein interactions than any other known protein and is extremely conserved. Actin is an essential component of many cellular processes, from cell motility and the preservation of cell shape and polarity to the control of transcription.

These characteristics, as well as its capacity to switch between monomeric (G-actin) and filamentous (F-actin) states under the control of nucleotide hydrolysis, ions, and a large number of actin-binding proteins, make it a key player in these processes. In addition, the foundation of muscle contraction is the interaction of filamentous actin and myosin. The actin cytoskeleton is also disturbed or seized upon by numerous pathogens due to its crucial function in the cell. The structures of G-actin and F-actin are discuss here, along with some of the relationships that regulate actin polymerization and disassembly.

KEYWORDS:

Actin, Cytoskeleton, Eukaryotic, Filaments, Monomer.

INTRODUCTION

The most prevalent protein in the majority of living cells is actin. It participates in more protein-protein interactions than any other known protein and is extremely conserved. Actin is an essential component of many cellular processes, from cell motility and the preservation of cell shape and polarity to the control of transcription. These characteristics, as well as its capacity to switch between monomeric (G-actin) and filamentous (F-actin) states under the control of nucleotide hydrolysis, ions, and a large number of actin-binding proteins, make it a key player in these processes[1]. In addition, the foundation of muscle contraction is the interaction of filamentous actin and myosin. The actin cytoskeleton is also disturbed or seized upon by numerous pathogens due to its crucial function in the cell. The polymerization and disassembly of actin are the method which used by the actin for maintaining the dynamics [2].

MreB is one of the prokaryotic actin-like proteins that contributes to the preservation of cell structure. Genes for actin-like proteins are found in all non-spherical bacteria, and they create a helical network that directs the proteins involved in cell wall biosynthesis beneath the cell membrane [3]. Some plasmids contain the genes for a distinct mechanism that uses the actin-like protein ParM. Dynamically unstable ParM filaments may divide plasmid DNA into the dividing daughter cells using a method akin to how microtubules function during eukaryotic mitosis [4].It was widely find that the evolving actin cytoskeleton matrix was unique to the cytosol of vertebrates and had evolved before the cell nucleus appeared to support uptake, chemotaxis, and the complex operation of the endomembrane system within the cytosol[5].

chemotaxis, and the complex operation of the endomembrane system within the cytosol[5]. All these dynamics are regulated by the dynamics nature of actin. One of the major driving forces behind actin dynamics is the hydrolysis of ATP by actin. However, a variety of factors, including a group of actin-binding proteins, also regulate in vivo dynamics [6].Actin is now known to be a very abundant protein (typically 5 to 10% of total protein) in all kinds of eukaryotic cells. However, the amino acid sequences of all actin are remarkably similar and have been extremely conserved throughout the evolution of eukaryotes. For instance, the actin of mammalian cells and yeast actin share 90% of their amino acid composition [7].Eukaryotes actin present in the cytosol as well as in the nucleus also. The upkeep of the nuclear structure and the disintegration of the nuclear envelope are both influenced by nuclear actin. Additionally, it plays a role in the chromatin remodeling and nucleosome and chromatin mobility required for DNA recombination, repair, and the start of transcription. Additionally, it attaches RNA polymerases to facilitate transcription. The future task will be to further define nuclear actin's functions in different cellular processes and diseases due to its multifaceted role [8].

LITERATURE REVIEW

In 1990, Kenneth Holmes, Wolfgang Kabsch, and their coworkers mapped the three-dimensional structures of actin filaments and individual actin molecules. The globular proteins that make up individual actin monomers have 375 amino acids (43 kd). Actin monomers polymerize to create filaments (filamentous [F] actin) because each actin monomer (globular [G] actin) has tight binding sites that mediate head-to-tail interactions with two other actin monomers (Figure.1)[9]. In the filaments, each monomer is rotated by 166 degrees, giving the filaments the look of a double-stranded helix. Actin filaments have a unique polarity and can be distinguished from one another by their ends (known as the plus and minus ends) because all of the actin monomers are oriented in the same direction [10]. The actin filaments' polarity is crucial for both their assembly and for determining the specific direction in which myosin moves in relation to actin.

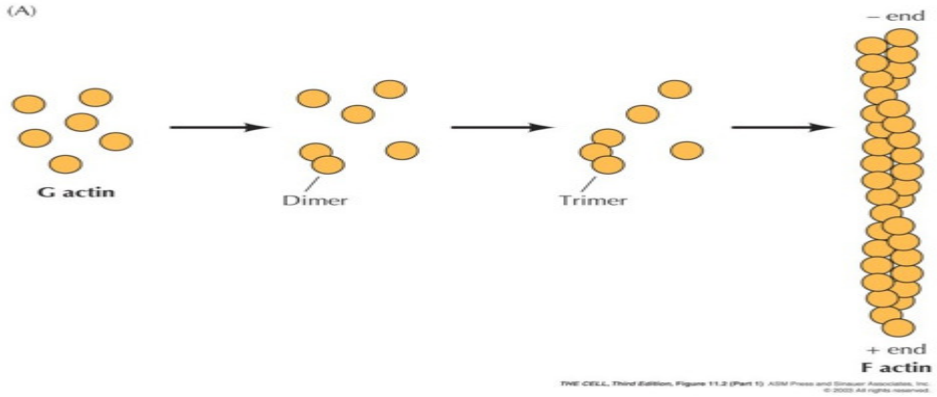

Figure 1: Dynamicity of the actin: Actin changes from G-actin to F-actin are shown schematically (Quizlet).

The two ends of an actin filament grow at varying rates, as previously mentioned; monomers are added to the fast-growing end (the plus end) five to ten times faster than to the slow-

growing end (the minus end). The critical concentration of monomers required for polymerization at the two ends differs because ATP-actin dissociates less easily than ADP-actin (Figure.2). Treadmilling, a phenomenon that demonstrates the dynamic behaviour of actin filaments, can arise from this different [6]. The concentration of free actin monomers must lie halfway between the critical concentrations needed for polymerization at the plus and minus ends of the actin filaments for the system to be in an overall steady state. In these circumstances, there is a net loss of monomers from the negative end, which is counterbalanced by a net increase to the positive end. ADP-actin dissociates from the minus end of strands while ATP-actin polymerizes at the plus end, necessitating ATP for treadmilling[6]. Treadmilling may represent the dynamic assembly and disassembly of actin filaments necessary for cells to move and change form.

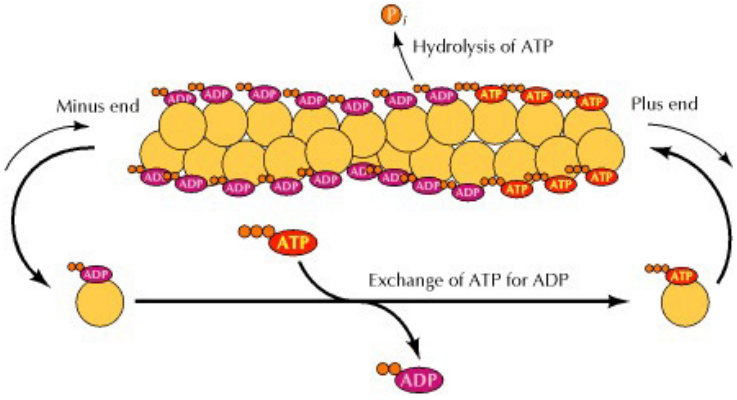

Figure2: Actin dynamics: Diagrame showing the growth of the actin filaments bby theexchange of the ATP-ADP.

A complex network of dynamic polymers known as the actin cytoskeleton is essential for the maintenance of cell shape, polarity, cell division, cell migration, endocytosis, vesicular trafficking, and mechanosensation, among other basic cellular processes. The concerted action of about 100 highly conserved accessory proteins, which nucleate, elongate, cross-link, and sever actin filaments, controls the precise spatiotemporal assembly and disassembly of actin structures. The present understanding of actin dynamics and function has been shaped by both in vitro studies of purified proteins and in vivo studies in a variety of organisms, from yeast to metazoans [11]. In conjunction with biochemical analysis, advanced real-time imaging, genome-wide functional analysis, molecular genetics, and ultrastructural research, yeast has emerged as a promising model to study the actin cytoskeleton, its molecular dynamics, and physiological function. The universal process governing actin assembly and disassembly in eukaryotes has been largely defined by research on the yeast actin cytoskeleton. Here, we discuss some of the significant discoveries made through research on the actin cytoskeleton in two significant yeast models: the fission yeast Schizosaccharomyces pombe and the budding yeast Saccharomyces cerevisiae [11].

The Asgard archaea are thought to be the eukaryotes' nearest living cousins. Numerous eukaryotic signature proteins (ESPs) found in their genes have sparked theories about how eukaryotic cells evolved1, It has been hypothesized that ESPs play a part in the development of a complex cytoskeleton and complex cellular structures, but this has never been demonstrated [12]. There is only one membrane and intricate surface structures that make up the cell wall. Throughout the cell bodies, protrusions, and constrictions is a long-range

cytoskeleton. The filaments' twisted double-stranded structure is compatible with F-actin. According to immunostaining, the strands contain Lokiactin, one of the Asgard archaea's most highly conserved ESPs. We suggest that an intricate actin-based cytoskeleton, which existed before the first eukaryotes, played a key role in the development of the Asgard phylum by supporting complicated cellular structures [12].

Actin monomers create filamentous cables with a diameter of 6 nm, which are the building blocks of microfilaments, which are made up of two intertwined strands of actin. Actin filaments and motor proteins like myosin cooperate to cause animal muscular contraction or some eukaryotic microbes' amoeboid movement [13]. Actin exists in two different configurations in ameboid organisms: a more rigid, polymerized gel form and a more flexible, unpolymerized soluble form. The ectoplasm, the gel-like region of cytoplasm just inside the plasma membrane of ameboid protozoans, is stabilised by actin in its gel state. Cell motility is generated by the forward passage of soluble actin filaments into the pseudopodia, followed by the cycling of the actin filaments between the gel and the sol, which results in the temporary extensions of the cytoplasmic membrane known as pseudopodia (meaning "false feet")[14].

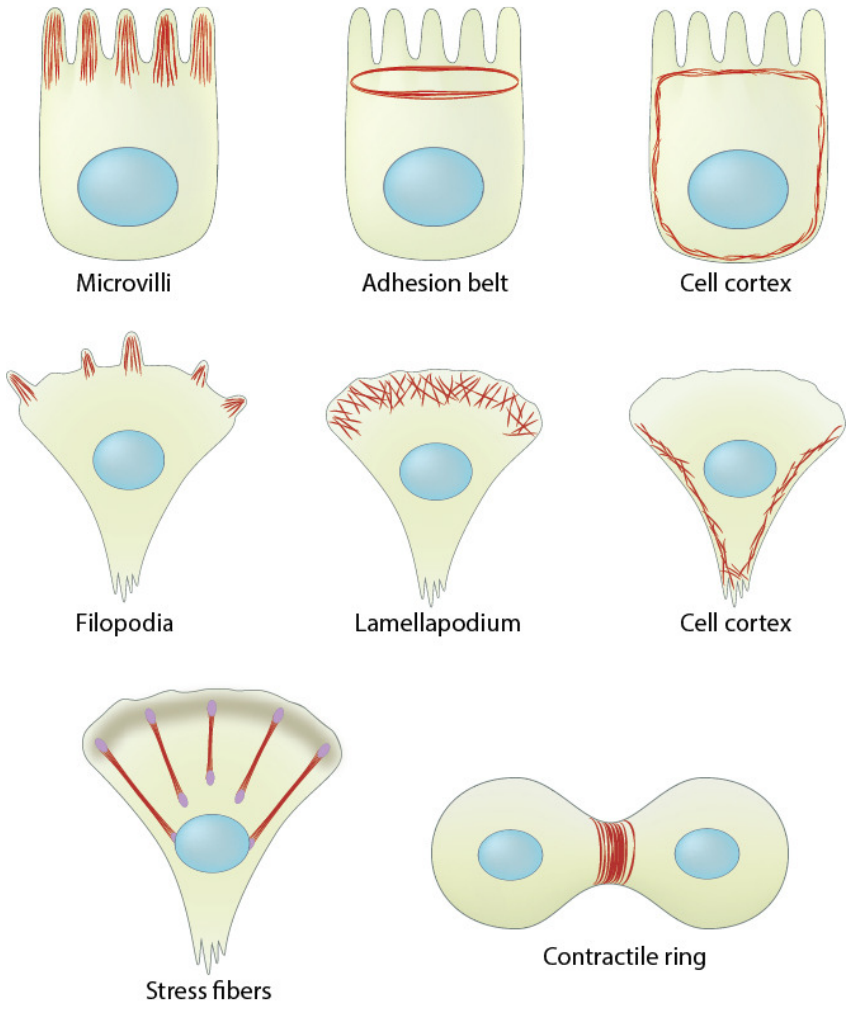

Figure 3: Functions of the actin: Diagramed showing different cellular function of the actin (MBinfo).

The leftover cytoplasm flows up to join the leading edge after the cytoplasm has extended outward and formed a pseudopodium, resulting in forward locomotion (Figure.3). Microfilaments play a role in a number of eukaryotic cell functions aside from amoeboid movement, such as cytoplasmic streaming (the movement or circulation of cytoplasm within the cell), the creation of cleavage furrows during cell division, and animal muscle movement (Figure.3)[14]. These functions are the result of interactions between molecular motors in various types of eukaryotic cells and the dynamic properties of microfilaments, which can polymerize and depolymerize relatively easily in reaction to cellular signals [14].

Visualization of the structural details of such a conformational change has come from a comparison of crystal structures of the actin monomer (G-actin) in the ATP and ADP states. ATP-actin structures have been determined from complexes with actin-binding proteins that keep actin in a monomeric state: DNase I, profilin , gelsolin, and vitamin D-binding protein ,ADP-actin, on the other hand, was crystallized in a monomeric state after binding tetramethylrhodamine-5-maleimide (TMR)1 to Cys-374, which blocks polymerization [10]. A comparison of the structures in the two states reveals how the release of the nucleotide γ-phosphate triggers a sequence of events that propagate into a loop to helix transition in the DNase I-binding loop in subdomain 2. However, a proper comparison of the ATP- and ADP-bound states of actin would require for the two structures to be determined under similar conditions [10]. The ADP-bound TMR-labeled actin structure (yellow and green) docked into the electron microscopic reconstruction of the ADP-bound F-actin. Rendered surface of the docked ADP-bound TMR-labeled structure. The surface has been rendered at Þ 22 Å to match the resolution of the electron microscopic data. Electron microscopic reconstruction of F-actin in ADP state (Figure.4). Arrows point to the closed and open nucleotide-binding cleft that is apparent in these low- resolution surfaces (Figure.4).

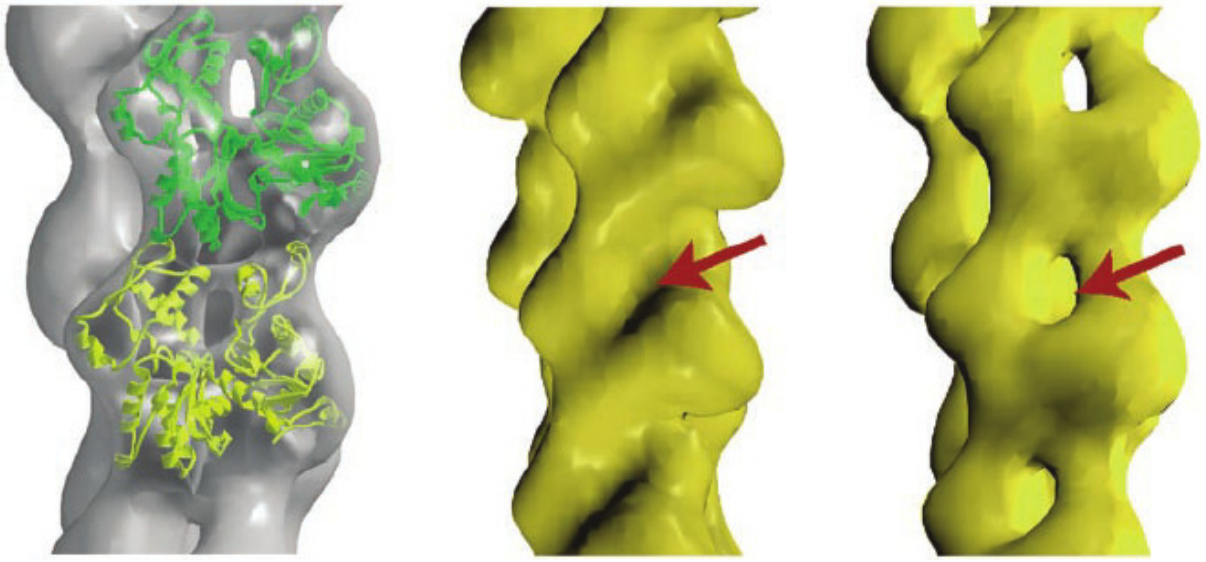

Figure 4: Shows the nucleotide's location and conformation within each actin molecule: The two structures of TMR-actin with bound ADP and AMPPNP are shown in this figure (Research gate).

To create the dynamic cytoskeleton, which connects the interior of the cell with its environment and provides structural support for cells. Signaling pathways translate and communicate forces operating on the actin cytoskeleton to transmit knowledge about the external environment. To permit cell movement. For instance, by way of the development and operation of Filopodia or Lamellipodia. Motor proteins move intracellular organelles to the daughter cells along actin wires during mitosis.Actin filaments are oriented in muscle cells, where myosin proteins exert forces on the filaments to promote muscle contraction[7]. Thin filaments are the name given to these structures. Actin filaments in non-muscle cells create a track system for the transportation of freight, which is propelled by unconventional myosins like myosin V and VI[7]. Non-traditional myosins move cargo (like vesicles and organelles) at speeds that are much faster than diffusion by using the energy from ATP hydrolysis. As a consequence, the theory that actin first appeared in the first cell and that microfilaments then emerged before the eukaryotic cytoskeleton has taken shape [7]. The identification of nuclear actin provided a fresh viewpoint on the subject by indicating that the nucleus activities of actin are a reflection of the roles of ancestor actin-like proteins. Our findings support the notion that the cytoskeleton attained major eukaryotic innovations before the tandem evolution of the cytoskeleton and nucleus took place because both ancient and eukaryotic features of the actin world can be detected in the nucleus today[5].

CONCLUSION

The actin system's genes share a common ancestor, so evolution should be able to help unravel the intricate processes. It should be possible to identify the underlying molecular mechanisms for each actin-based function as well as more general principles with continued emphasis on tractable model systems. The list of components is continually expanding, and novel interactions are being discovered through research. More research is needed on how dynamics control actin and how actin interacts with other cellular systems like membranes. Technological developments should be crucial. Additionally, improvements in light and electron microscopy enable the localization of actin protein components at the nanometer scale as well as the determination of both the global and local concentrations of molecules inside living cells. These chapter address the composition, structure, and presence of actin in various forms in various organisms.

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CHAPTER 23

KINETOCHORE-MICROTUBULE INTERACTION IS CHANGEABLE

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ABSTRACT:

Chromosome division in mitosis and meiosis is powered and regulated by the kinetochore, a control mechanism. The microtubule-kinetochore junction is highly fluid, with the microtubules expanding and contracting where they are attached to the kinetochore. Additionally, the kinetochore itself is very dynamic, altering as cells initiate mitosis and as they come into contact with microtubules. The structure continues to be mysterious because active kinetochores have not yet been separated or recreated. However, thanks to recent developments in genetic, bioinformatic, and imaging technology, we are now starting to comprehend how kinetochores organise, attach to microtubules, and release them when the links formed are inappropriate as well as how they impact the behaviour of microtubules. Many kinetochore components are extremely dynamic, and some alternate between kinetochores and spindle poles along microtubules, according to recent research on the route of kinetochore assembly in animal cells. Additional research on the kinetochore-microtubule interface is instructive. It sheds light on the following topics: the function of the Ndc80 complex and Ran-GTPase system in microtubule attachment, force production, and microtubule-dependent inactivation of kinetochore spindle checkpoint activity; the role of chromosomal passenger proteins in the correction of kinetochore attachment errors; and the role of microtubule in chromosome division.

KEYWORDS:

Budding Yeast, Chromosome Division, Spindle Pole, Sister Kinetochore, Mitotic Spindle.

INTRODUCTION

The kinetochores, which are structures that create the interface between the chromosomes and the mitotic spindle's microtubules, start, regulate, and watch the remarkable motions of the chromosomes during mitosis. Animal cell kinetochores can be split into two areas. The inner kinetochore typically develops on extremely repetitive DNA segments and comes together to create a particular type of chromatin that is present for the duration of the cell cycle. Only during mitosis does the outer kinetochore, a proteinaceous structure with numerous dynamic components, organise and perform its tasks. Chromosome attachment to spindle microtubules, monitoring of those attachments, activation of a signalling (checkpoint) mechanism to halt cell-cycle advancement in the event of defects, and assistance in chromosome mobility on the spindle are all kinetochore activities. We start by going over the kinetochore's chemical structure and building process. Dynamic instability is a condition in which microtubules, which are metastable polymers of - and -tubulin, alternate between periods of growth and contraction [1]. We go over how chromosome movement and segregation are accomplished by combining the highly dynamic behaviour of microtubules

with kinetochore activity in the sections below. More information on the dynamics and physics of the microtubule plus end, spindle assembly processes, and the role of spindle checkpoints is discussed elsewhere [1].

The animal kinetochore is the subject of this article, and in particular, the junction between the exterior kinetochore region and the spindle microtubules. First discovered by traditional fixation and staining techniques for electron microscopy the kinetochore is made up of several distinct layers. More recently, fast freezing/freeze substitution has allowed researchers to better understand these layers[1]. The chromatin arrangement with the most nucleosomes, which have at least one specific histone, support proteins, and DNA, is known as an inner plate. One of the least known characteristics of the kinetochore in animal cells is the composition and structure of this DNA. Throughout the cell cycle, the inner plate persists as a distinct heterochromatin region. An outer layer made mainly, if not entirely, of protein surrounds it [1].

This structure develops on the chromosome's surface around the time that the nuclear membrane breaks down[1]. Budding yeast's outer plate only has one end-on attachment site, whereas the outer plate of mammalian kinetochores has about 20 end-on attachment sites for the plus extremities of microtubules (known as kinetochore microtubules, or kMTs). By standard electron imaging, the kinetochore's topmost areas can be seen as a fibrous corona, though typically only when microtubules are absent. The spindle checkpoint, microtubule attachment, and behaviour control are all regulated by this dynamic network of permanent and transient components[1].

In animal cells, the nuclear envelope is broken down, and highly active centrosome-nucleated microtubules constantly seek for and seize chromosomes in the cytoplasm. When a microtubule comes into contact with a kinetochore, it stabilises; otherwise, it depolymerizes [2]. Chromosome alignment is started by the trapped chromosome moving quickly in a poleward direction while interacting laterally with the microtubules' surface via a single microtubule that emerges from the centrosome. The cytoplasmic dynein's minus-end-directed motor activity, which is highly concentrated at unattached kinetochores, is most likely the mechanism causing this movement [3]. As chromosomes gain kMTs, poleward migration slows down and is now controlled by variations in kMT length. The microtubules that are connected to the kinetochore have special characteristics. In comparison to microtubules with unattached plus ends, kMTs are significantly more immune to depolymerization brought on by chilly therapy, high hydrostatic pressure, or exposure to calcium. Furthermore, kinetochore microtubules depolymerize quickly when a chromosome is surgically detached from them, and they rotate much more slowly than astral and spindle microtubules that have open plus end in vivo [4]. After it was established that dynein and CENP-E are not required for the creation of kMTs, the hunt for additional proteins required for stable kMT attachment got underway. The significance of the Ndc80 protein complex for kMT adhesion was discovered by groundbreaking genetic research in budding and fission yeast[1]. The four parts of the growing yeast Ndc80 complex are Ndc80p, Nuf2p, Spc24p, and Spc25p.

Without completely losing the kinetochore structure, yeast strains missing the Ndc80 complex show loss of kinetochore-microtubule attachment. Contrarily, mutants that eliminate kinetochore assembly, such as Ndc10 mutants in budding yeast, are defective in both microtubule attachment and their checkpoint response. This is most likely because

kinetochores function as a platform for organising the response. The Ndc80 complex has been discovered in S. pombe, C. elegans, Xenopus, poultry, and humans. It is extremely conserved. Hec1 (highly enhanced in cancer cells 1), the human equivalent of Ndc80, has been demonstrated to be crucial for chromosome alignment and mitotic development and to engage with cohesin and condensin complex components.

The Ndc80 complex is essential for the stable kinetochore-microtubule attachments required to maintain the centromere tensions necessary to achieve proper chromosome alignment in higher eukaryotic cells, according to several recent studies. Cells with impaired Ndc80 complex function (caused by RNAi, gene disruption, or antibody microinjection) have elongated spindles, lose tension across sister kinetochores, fail to align their chromosomes, and have few or no kMTs at temperatures low enough to depolymerize non-kMTs selectively. Ran can play a significant part in the formation of the mitotic spindle, especially in cells like Xenopus oocytes that lack centrosomes. Importins attach to and sequester a number of proteins during interphase, including TPX2 and NuMA, which are necessary for the creation of spindle poles and the assembly of spindle microtubules. When Ran-GTP attaches to the importins during mitosis, TPX2 and NuMA are freed to perform their role in spindle construction.

In this instance, RCC1 attached to the chromatin produces Ran-GTP close to the chromosomes. When kinetochores accidentally come into contact with microtubules during mitosis and meiosis, adhesion mistakes frequently occur [5]. Chromosomes originally become mono-oriented by either one (monotonic attachment) or both (syntelic attachment) sister kinetochores following nuclear envelope disintegration. The metaphase plate is a dynamic collection of aligned chromosomes that is formed when chromosomes are attached to both spindle poles (biorientation). The sister kinetochores need to accomplish amphibolic attachment, where one sister is only attached to microtubules from one pole and the other sister is only attached to microtubules from the opposite pole, for correct chromosome segregation. A merotelic attachment occurs when one or both of the sibling kinetochores have microtubule links to both poles. Microtubule-plus-end-binding proteins and kinetochore proteins both control kinetochore mobility by altering the kinetics of kMT plus ends [6]. However, given how changeable the kinetochore-microtubule interaction is, several of these proteins seem to be genuine elements of both structures. KinI kinesin motors, which serve as depolymerase, and microtubule plus-end-tracking proteins (+TIPs), which encourage polymerization, perhaps by competing with the depolymerase, are two groups of proteins that stand out as being especially significant [1].

LITERATURE REVIEW

It is mainly unclear how kinetochore proteins interact dynamically with microtubules. The 10-protein Dam1 complex in budding yeast is a target of Aurora kinase and is crucial for preserving the integrity of the mitotic spindle and controlling contact with the kinetochore. Here, we looked into the pure Dam1 complex's molecular characteristics. Around microtubules, the compound oligomerized into bands. Microtubules helped to promote ring creation, but it was still possible without them. Reduced microtubule binding or partly formed complexes were caused by mutant genes. The C termini of both Dam1 and alpha beta-tubulin facilitate the contact between rings and microtubules. Microtubule construction, stabilisation against disintegration, and bundling are all facilitated by ring formation. The

complex's favoured binding partner is a GTP-tubulin lattice, and Dam1 rings can move laterally on microtubules. These findings point to a process by which the kinetochore can identify and cling to microtubule plus ends [7].

The creation of links between microtubule polymers and each sister chromatid is essential for accurate chromosome division. The macromolecular structure known as the kinetochore, which assembles at each chromosome's centromere during mitosis, acts as a bridge between the DNA and the microtubules. The behaviours and molecules involved in producing kinetochore-microtubule attachments, including the early phases of lateral kinetochore-microtubule interactions and development to stable end-on attachments, are covered in this Cell Science at a Glance piece and the associated exhibit. We also investigate the characteristics that enable the kinetochore to be tracked by moving microtubules[8]. Kinetochores and microtubules work together dynamically to segregate chromosomes. First, microtubules effectively catch kinetochores. When chromosomes are positioned on the spindle's centre, flexible interactions between kinetochores and microtubules enable correct orientation.

Lastly, chromosomes are pulled towards the spindle poles by microtubules that are firmly connected to kinetochores. As a result of these processes, kinetochore structure and makeup as well as microtubule dynamics shift from lateral to the end-on attachment as the method of interaction of kinetochores with microtubules. In recent years, it has become increasingly clear how steady kinetochore-microtubule adhesion is mediated at the molecular level. However, the process governing the dynamic control of kinetochore-microtubule contact in early mitosis, which is essential for accurate chromosome segregation, is still a mystery[9]. A heterodecameric part of the kinetochore required for precise chromosome division is the yeast DASH complex. DASH creates tight circles with significant space between them and the microtubule cylinder. We identified potential polypeptide expansions involved in creating the DASH-microtubule interface by characterising the microtubule-binding characteristics of restricted proteolysis products and DASH subcomplexes.

Tubulin monomers' acidic C-terminal appendages are not required for DASH binding. Using scanning transmission electron microscopy, we also calculated the molecular weight of DASH rings on microtubules and discovered that each ring is made up of roughly 25 DASH heterodecamers. The kinetochore may be able to move along the microtubule surface due to the dynamic association and repositioning of numerous flexible DASH appendages[10]. The heterodecameric DASH complex in yeast functions as a crucial point in keeping this connection. DASH can create load-bearing attachments with the ends of polymerizing and depolymerizing microtubules in vitro by forming oligomeric regions and bands. In living cells, DASH is mainly found at the kinetochore and is important for ensuring proper chromosome and spindle attachment in Saccharomyces cerevisiae and Schizosaccharomyces pombe. Recent research has started to explain how DASH works with other exterior kinetochore elements to form a dynamic, controlled kinetochore-microtubule interface [11].

Large protein structures called kinetochores to connect sibling chromatids to the spindle and translate the motion of microtubules into chromosome movement. The plus end-associated Dam1 complex and the kinetochore-resident Ndc80 complex in budding yeast create the kinetochore-microtubule interface, but it is unclear how they function together or whether a physical link between them is important for chromosome segregation. In this article, we

describe the structural components necessary for the Ndc80-Dam1 association and examine how they work in vivo. A new ndc80 allele with abnormalities in development and chromosome segregation was specifically defective in Dam1 binding. Its lethality when combined with an N-terminal truncation showed that the Ndc80 N-tail and Ndc80-Dam1 interaction played crucial but somewhat overlapping functions. However, Dam1 did not restore the kinetochore function in the case of changes in the calponin homology region of Ndc80 [12].

Centromere, kinetochore, and spindle microtubule interactions are intricately regulated and necessary for the faithful division of chromosomes during mitosis. The design of this interaction is elusive despite its significance. The natural morphology of the kinetochore-microtubule interface in human U2OS cells at various phases of mitosis was here visualised using in situ cryo-electron tomography. We discover that the centromere shapes kinetochore microtubules into a pocket-like structure. Within this centromeric compartment, two physically different fibrillar densities link to the plus ends of microtubules end-on and side-on, respectively.

Kinetochores, mechanochemical organelles that form on mitotic chromosomes to link them to spindle microtubules, are necessary for the segregation of the copied DNA during cell division. The DNA-proximal framework for kinetochore formation is composed of the conserved structural protein CENP-C and the histone H3 variation CENP-A. We discovered KNL-1, a new kinetochore protein, using RNA interference-based genomics in Caenorhabditis elegans. KNL-1's loss, like that of CeCENP-A or CeCENP-C, results in a "kinetochore-null" trait. In a straight assembly structure, CeCENP-A and CeCENP-C are below KNL-1. The C. elegans homologs of Ndc80p/HEC1p and Nuf2p—two highly conserved outer kinetochore components show substoichiometric interactions with CeCENP-C and form a near-stoichiometric complex with CeNDC-80 and HIM-10 in embryonic preparations.

Even though their suppression results in chromosome missegregation and prevents the creation of a mechanically stable kinetochore-microtubule interface, CeNDC-80 and HIM-10 are not functionally identical to KNL-1. The fact that KNL-1 must target several elements of the peripheral kinetochore, including CeNDC-80 and HIM-10, may explain its higher functional significance. As a result, KNL-1 is essential for converting CeCENP-A and CeCENP-kinetochore C's assembly start into the development of a useful microtubule-binding interface[13]. Sister kinetochores' capacity to cling to spindle microtubules is necessary for faithful chromosome division. Early in mitosis, the exterior layer of kinetochores briefly swells to create a fibrous corona before compacting after microtubule capture. Here, we demonstrate how the RZZ (ROD-Zwilch-ZW10) complex and the dynein adaptor Spindly cause kinetochore expansion in a dynein-independent way.

Spindly undergoes structural changes as a result of C-terminal farnesylation and MPS1 kinase activity, which aid in the oligomerization of RZZ-Spindly complexes in cells and in vitro to create a filamentous meshwork. By enlisting dynein via three preserved short linear motifs, Spindly potentiates kinetochore compaction concurrent with kinetochore growth. Expanded kinetochores that are unable to compress associate extensively and persistently with lateral microtubules, which causes merotelic attachments and incorrect chromosome sorting in anaphase. Thus, a singular Spindly-based mechanism that supports initial microtubule capture

and later proper maturation of attachments coordinates dynamic kinetochore size control in mitosis [14].

The microtubule cytoskeletal network of a human cell breaks down and then reassembles to create a bipolar structure called the mitotic spindle as the cell gets ready to split. The mitotic spindle's microtubules divide the DNA into two equal groups as they engulf the chromosomes (Figure 1). The correct connection between the chromosome and microtubule is crucial for precise division. The kinetochore is a submicron-sized macromolecular structure that promotes chromosome-microtubule adhesion. In electron microscopy, the kinetochore appears as a three-layered structure with an interior plate formed on centromeric chromatin and an outer layer that touches microtubules. In people, several microtubules interact with the kinetochore, and the microtubules that are attached to the kinetochore are collected into k-fibers. It is not only intriguing but also crucial for therapeutic purposes to comprehend how microtubules are connected to kinetochores. To precisely target the characteristics of cancer cells exhibiting chromosomal instability, we could take advantage of some redundant regulatory processes in chromosome-microtubule attachment. Soon after the nuclear envelope is ruptured, when chromosomes are first revealed to the cytoplasm, human kinetochores become accessible for acquisition by microtubules. End-on conversion, a multi-step procedure, describes how kinetochores are originally captured along the lateral walls of microtubules and then attached to microtubule-ends.

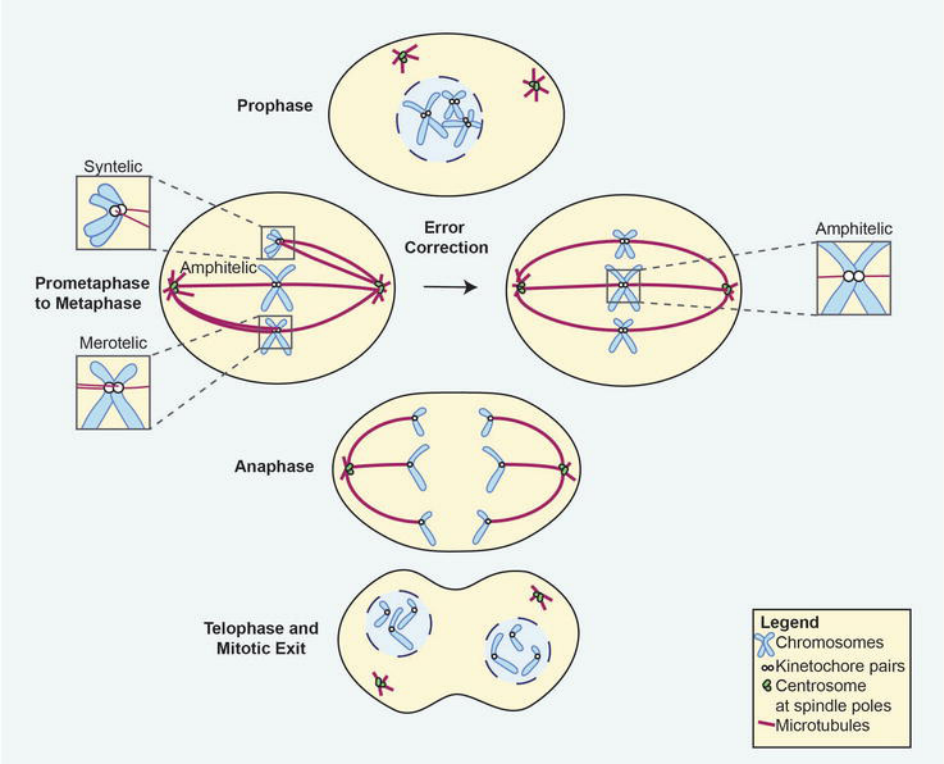

Figure1 : Chromosome segrgration: Diagrame showing the role of the kinetochore in the chromosome segrgration (intechopen).

This shift in the kinetochore-microtubule attachment axis is a significant occurrence. The expansion and contraction of microtubules can only be converted into pressing or dragging forces that move chromosomes when kinetochores are attached to the ends of the microtubules. The orientation of the attachment, which requires that one sister kinetochore of

a pair be attached to one spindle pole and the other sister kinetochore of the pair be attached to the opposite spindle pole, is just as important as the correct plane of kinetochore-microtubule attachment. Biorientation or an amphitelic attachment is the term used to describe this manner of chromosome-microtubule attachment (Figure 1). Sister chromatids can be simultaneously pulled apart into two daughter sets when the cell enters anaphase, which occurs when all kinetochores are connected in an amphitelic manner.

CONCLUSION

We are beginning to write down in great detail what happens when kinetochores engage with microtubules at various phases of mitosis. We are still at a point where questions are arising more quickly than solutions, though, when one takes into account the fundamental processes. It has been 37 years since Inoue suggested the dynamic equilibrium model for spindle assembly and chromosome movement, and 110 years since Metzner first characterised the presence of substructures responsible for chromosome movement. Currently, we are moving towards creating a preliminary list of the protein elements responsible for attaching microtubules to kinetochores, detecting when attachment has taken place, releasing microtubules from incorrect attachments, and coupling polymerization and depolymerization to force production. All of this knowledge is currently applicable to the relationships between individual microtubules and kinetochores. Kinetochores are typically connected to clusters of 20 or fewer kMTs in mammalian cells. Kinetochore directional instability most likely entails the synchronised change of all of these kMTs' polymerization states simultaneously. As we attempt to advance to the next level of knowledge of the dynamic interactions between kinetochores and microtubules, we still have many questions about how choices to change the polymerization state are transmitted laterally through the bundle of keys.

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CHAPTER 24

IN NATURE DIFFERENT ACTIN SUBTYPES ARE PRESENT

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ABSTRACT:

Actin is frequently conceived of as a single protein, but in mammals, six different isoforms are each encoded by a different gene. There are relatively few isoforms. With only minor differences in amino acid sequence, each isoform is comparable to the others. The actin supergene family encodes a variety of structurally similar but possibly functionally different protein isoforms that control the contractile potential in muscle tissues and aid in regulating the shape and motility of non-muscle cells.Actin in the cytoplasm of mammals interacts with typical non-sarcomeric and members of the nonmuscle myosin-2 family. Numerous cellular processes, such as cytokinesis, cell polarity preservation, cell adhesion, migration, and mechano-electrical transduction, are supported by these interactions.The discovery of a new class of actin isoforms termed actin-related proteins provides evidence that the actin gene and protein isoform family is more diverse than previously believed. Here, we describe how an actin isoform in an *in vivo* environment may affect the cytoskeletal function of cells.

KEYWORDS:

Actin, Cytosolic, Isoform, Nuclear, Mammals.

INTRODUCTION

Actins found in different amoebas, yeast, and slime molds are similar to cytoplasmic actins found in vertebrates [1][2]. In comparison to vertebrate muscle actin isoforms, invertebrate muscle actin is more closely linked to vertebrate cytoplasmic actins [3]. Primitive chordates were the first organisms to develop actin isoforms unique for striated muscle tissue [3]. In their primitive muscles, urochordate and lampreys still produce an isoform that is similar to the alpha heartbeat. This gene is most likely duplicated at the level of early amphibians or stem reptiles, giving rise to current alpha-skeletal and alpha-cardiac isoactins [3]. The smooth muscle isoactins are likely descended from early skeletal muscle actin and are thought to have evolved during the later evolution of warm-blooded vertebrates [4]. From diverse muscle sources, over 30 different actins have been identified in total, some of which play very specific roles [5].

The family of actin multigene is very stable. Over a total of 375 residues in the bovine, only four amino acids separate the alpha-skeletal and alpha-cardiac actin sequences[6]. This is one of the vertebrate actins with the greatest conservation rates [6]. Skeletal alpha-actin is eight amino acids and six amino acids different from the beta-smooth muscle type. The amino acid pattern of cytoplasmic actin differs, from the non-muscle actin isoforms. The amino terminus of the protein is where these variations are primarily found[7]. It is crucial for cytoarchitecture and other protein-protein interactions as the location of the majority of actin-

myosin interactions[7].The gene structures of the two striated muscle isoactins show a high degree of sequence continuity. Five introns are located at similar locations in skeletal alpha-actin and alpha-cardiac actin[8]. Even the non-coding regions of the two genes and between various species are remarkably similar [8]. The human and rat alpha-skeletal actin genes share 92% and 85% homology in two significant segments that make up the majority of the 3' untranslated regions (UTR) [9]. Rat and human DNA sequences typically only share 37–45% of their 3' UTRs. This high degree of similarity suggests a strong selective drive to preserve the sequence given the early separation of the two genes.

These conserved UTRs may have an impact on the transcripts' stability, ability to be translated, and intracellular position, further influencing how the protein is expressed [9]. The production of the various actin isoforms is temporally and tissue-specifically controlled during development. The cytoplasmic actins are typically present in all cell kinds and at all stages of development. The expression of the other two sets of actin isoforms in striated (cardiac and skeletal actin) and smooth muscle (two isoforms of smooth muscle actin) muscles has been demonstrated[10]. The striated and smooth muscle pairs are coexpressed during growth, according to earlier research [10]. Since the regulatory sequences for these genes are also highly conserved, this coexpression is not particularly unexpected[10]. Cardiac alpha-actin, which makes up the majority of the actin isoforms in the adult heart, has also been demonstrated to be the major form during the early stages of muscle formation in mice, humans, and the majority of cultured cell lines[10].

It has been proposed that this concerted co-expression takes place to promote quick protein accumulation. Alpha-skeletal actin, which makes up more than 95% of all striated muscle actin isoforms, takes over as the main isoform in adult tissue as alpha-cardiac actin expression is downregulated in later development[10]. The muscle of the mammalian heart expresses skeletal alpha-actin as well. Alpha-skeletal actin may still account for nearly half of the total amount of striated muscle actin content even though alpha-cardiac actin becomes the predominant isoform during the late period of fetal development. Similar outcomes in the poultry heart have been demonstrated [10]. Alpha-skeletal actin and alpha-cardiac actin both increase in content in a coordinated way as the somites of the amphibian Xenopus laevis embryos develop. However, in amphibians, only one isoform appears to be expressed in smooth muscle while at least three different isoforms appear to be expressed in striated muscle [10]. Studies showing that alpha-smooth muscle isoactin is expressed during early cardiac and skeletal muscle development in a range of myofibroblast-like cells have further complicated this developmental paradigm.

The contentious issue of whether this co-expression represents a truly regulated expression or rather is the result of a persistent "leakage" from the initial high expression during early myogenesis has not yet been adequately addressed by substantial evidence. Cross-responsiveness to cellular or environmental stimuli may also be caused by "leakage" arising from partially diverged regulatory elements[10]. It is assumed that at least these two actin isoforms are functionally redundant based on the high sequence similarity and the lack of pathology in a mutant mouse model that produces high levels of alpha-skeletal actin in the heart[11]. These two actins' functions most likely have less to do with their protein coding and more to do with how they are differentially regulated in space and time [11]. Although numerous studies have previously clarified the precise expression pattern of alpha-skeletal actin in a model organism, there hasn't yet been any analysis of various species. Rodent

model data show supporting the idea that various actin isoforms perform specific cellular tasks and discussing potential ways in which actins might have different effects on cells [12].

LITERATURE REVIEW

There are numerous types of actin, each with slightly different structures and functions. Actin is only found in muscle fibers, though it is prevalent in other cells as well. Due to their high turnover rate, the latter types are mostly located outside of permanent structures. Other than muscle cells, three different kinds of microfilaments have been identified [12]. The cytoskeleton of actin is essential for yeast endocytosis, cytokinesis, cell orientation determination, and morphogenesis. These mechanisms depend on actin, but also on 20 or 30 associated proteins, all of which have a high degree of evolutionary conservation, as well as numerous signaling molecules. These components work together to enable a spatially and temporally modulated assembly that establishes a cell's reaction to both internal and exterior stimuli. Patches, cables, and rings are the three major components of actin found in yeasts[13].

Due to ongoing polymerization and depolymerization, these structures, despite not being around for very long, are prone to a dynamic equilibrium. Aip1, a cofilin cofactor that promotes microfilament disassembly, Srv2/CAP, a process regulator related to adenylate cyclase proteins, profilin with a molecular weight of roughly 14 kDa that is related to/associated with actin monomers, and twinfilin, a 40 kDa protein involved in patch organization, are among the accessory proteins they possess[13]. The actin gene family contains protein isovariants, according to research on the plant genome. There are numerous myosins, six profilins, and ten different kinds of actin in the model organism Arabidopsis thaliana[14]. The evolutionary necessity of having variants with subtly different expressions in time and space accounts for this diversity. The bulk of these proteins was expressed in combination in the tissue under study.

Cells that have been grown in vitro have cytoplasm that is covered in actin networks[14]. A concentration of the network, which is highly dynamic and undergoes constant polymerization and depolymerization, is located around the nucleus and connected to the cellular cortex via spokes[14].

Even though the majority of plant cells have a cell wall that determines their morphology, these cells' microfilaments can still produce enough energy to carry out a variety of cellular functions, like the cytoplasmic currents produced by myosin and the microfilaments.

Additionally, actin plays a role in organelle mobility and cellular morphogenesis, which includes cell division as well as cell elongation and differentiation. Fimbrin, which can recognize and unite actin monomers and is involved in the formation of networks (by a different regulation process from that of animals and yeasts), formins, which can act as an F-actin, and villain, which is related to gelsolin/Severin and has the ability to cut microfilaments and bind actin monomers in the presence of calcium cations are some of the most notable proteins associated with the actin cytoskeleton in plants[14]. In 1977, Clark and Merriam discovered and first characterized nuclear actin.

Report describe a protein found in the nuclear portion of Xenopus laevis oocytes that resembles skeletal muscle actin in appearance. Since then, there have been numerous scientific studies about the nucleus's actin structure and functions. Actin is able to play a

significant part in a variety of crucial nuclear processes thanks to the nucleus' ability to regulate the amount of the protein, its interactions with actin-binding proteins (ABP), and the existence of various isoforms [12].

Actin isoforms:

Actin exists in various variants in the cell nucleus. Actin isoform levels may alter in reaction to transcriptional activity and/or cell proliferation being inhibited or stimulated. Isoform beta is the main center of research on nuclear actin. However, the use of antibodies against various actin isoforms enables the identification of alpha- and gamma-actin in specific cell types in addition to cytoplasmic beta in the cell nucleus [12]. Since the amount of each isoform can be independently controlled, the presence of various isoforms of actin may significantly affect how it functions in nuclear processes[12].

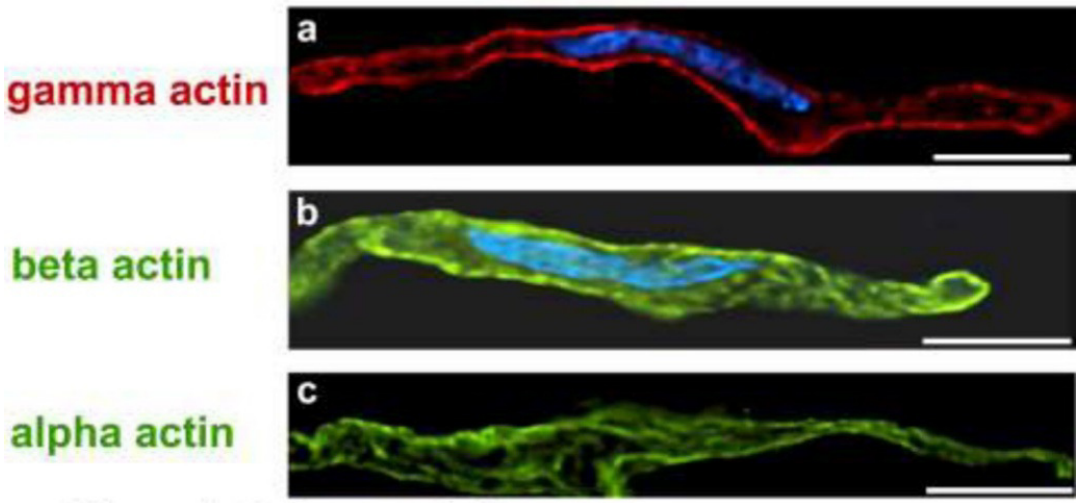

Figure 1: Actin types: Structure and dynamics of the actin-based smooth muscle contractile and cytoskeletal apparatus(EUROPE PMC).

A cytoskeletal protein known as actin is widely produced in eukaryotic cells. Cell motility, muscle contraction, and cytoskeleton maintenance are a few examples of actin activities. Additional research has demonstrated the significance of actin in a variety of cellular processes, including gene transcription and chromosome morphology, control of the cell cycle, modulation of several membrane responses, translation of many mRNA species, and modulation of enzyme activity and localization within the cell [12].

In higher vertebrates, there are six main actin isoforms: beta-cytoplasmic (ACTB), alpha-skeletal (ACTA1), alpha-cardiac (ACTC1), alpha-smooth muscle (ACTA2), gamma-smooth muscle (ACTG2), and gamma-cytoplasmic isoactin (ACTG1) (Figure.1A). Actins can be categorized into three pairs: two cytoplasmic isoforms, two smooth muscle isoforms (alpha-smooth muscle is primarily found in vascular tissue and -smooth muscle in the gastrointestinal and genital systems), and two isoforms of striated muscle (skeletal and cardiac tissue)[15].

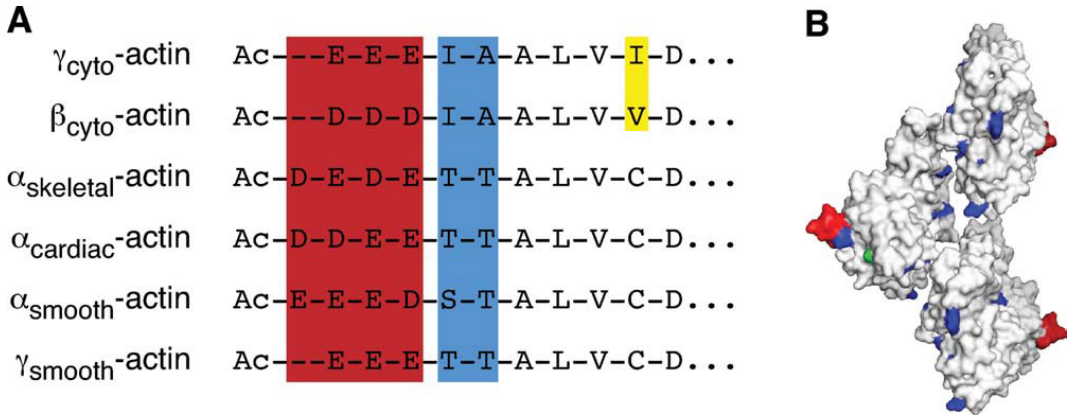

Figure 2: Illustrate the A: The six human actin isoforms' N-terminal ends are in alignment,and B: Variations in amino acids are matched to the structure of F-actin (Research gate).

The red residues differ the most between and within the muscle and cytoplasmic versions. The blue residues mainly differ between the muscle and cytoplasmic versions. Green denotes substitutions between various muscle isoforms, while yellow shows differences between β_{cyto}-actin and Ccyto-actin(Different actin isoforms) (Figure. 2B). Two widely accepted theories describe how various isoactins might carry out various cellular tasks. First, a subset of actin-binding proteins may interact with just one isoform by binding exclusively to that isoform. Several proteins have been identified that distinguish between muscle and cytoplasmic actin isoforms, including cofilin, l-plastin, and profiling [12]. Additionally, annexin 5a may attach to ccyto-actin rather than bcyto-actin preferentially. The second hypothesis is that various subcellular locations for actin isoforms are determined, possibly as a result of different interactions with actin-binding proteins or by a mechanism that specifically targets transcripts. Actin isoforms are localized differently in various cell types, though there is some disagreement among reports regarding the exact localization patterns of the various actins [12].

A skeletal actin is restricted to the sarcomeric thin filaments in skeletal muscle, which appears to be a clear illustration of the differential localization of muscle and nonmuscle actin isoforms. However, cytosolic -actin, which has the best-described localization pattern in muscle, does not appear to be present in narrow filaments but is present in other muscle cell structures [12]. αskeletal-actin was first found in filamentous formations near the sarcolemma and in the vicinity of mitochondria. In later studies, it was discovered that the only actin species found at costameres which are structures located between the sarcolemma and the z-disk is ccyto-actin. In other recent investigations, γcyto –acitn was found in costameres and a novel zone next to the z-disk[12]. Finally, various groups have found that γcyto-actinis only present in z-disks and not in costameres.

In various cell types, βcyto-actin modulation by ZBP1 has significant functional effects. Growth cones subjected to an attractive cue in neurons cultured from X[12]. laevis require targeted βcyto-actin transcripts and freshly made βcyto -actin protein for normal turning behavior. Growth cones do not turn when neurons are treated with antisense oligos to βcyto-actin, and βcyto-actin is enriched relative to ccyto-actin on the side of the growth cone exposed to attractant. Because inhibiting ZBP1 activity changes cell morphology and migration, Zbp1-mediated targeting of bcyto-actin transcripts seems to be crucial in other cell types, such as fibroblasts and adenocarcinoma cells[12]. According to several studies, βcyto-

actin is enriched at the leading edge of cultured fibroblasts and myoblasts compared to stress fibers found in the cell's center, which is consistent with the targeting of βcyto-actin transcripts. In comparison, ccyto-actin in fibroblasts seems to be evenly distributed throughout all actin-containing structures[12]. The intuitive simplicity of differential localization as a method to explain the various roles of actin isoforms makes it an appealing theory.

How relative richness affects actin function, though, is still a mystery. According to recent biochemical research, the characteristics of F-actin may differ depending on the mixture of isoforms present in the filament[12]. They discovered that βcyto -actin behaved more dynamically than ccyto-actin under calcium-bound circumstances, with faster rates of polymerization and depolymerization. It's interesting to note that biochemical tests showed that βcyto -actin and ccyto-actin easily copolymerize and that the rates of polymerization and depolymerization of the resulting filaments depend on the ratio of βcyto -actin to ccyto-actin. Different combinations of actin isoforms may exhibit unique biophysical characteristics or associations with stability regulators like AIP1 or cofilin in addition to polymerization dynamics.To modify the cytoskeleton for many functions, changing the actin mixture may be a useful strategy. By comparison, steel is an alloy made up of various metals. The ratio of each component determines the characteristics of the specific type of steel, which is engineered to meet the intended trade-offs between properties like weight, tensile strength, and cost[12]. The ability to manipulate alloys is crucial because a wide variety of steel are needed to satisfy the various requirements of machines and structures. The cellular steel is actin. To adjust a filament's characteristics to suit the needs of various cells or subcellular structures, the ratio of actin isoforms within the filament can be changed[12].

CONCLUSION

In almost all eukaryotic cells, the cytoskeletal protein actin performs a wide variety of tasks. Alpha-skeletal, alpha-cardiac, alpha-smooth muscle, gamma-smooth muscle, beta-cytoplasmic, and gamma-cytoplasmic isoactin are the six main actin isoforms distinguishable in higher vertebrates. Although the mechanisms and the precise differences are still poorly known, the expression of these actin isoforms is tightly regulated both temporally and tissue-specifically during vertebrate development. Because of the high level of conservation among all members of the actin multigene family, these proteins are likely under strong selective pressure. Every eukaryotic cell exhibits a widespread expression of beta- and gamma-actin in the cytoplasm. which restores cytoskeletal organization and imparts resistance to external perturbation when two heterologous actin variants are expressed, each of which is specifically suited for assembling a different network. Therefore, while species using single actin have homeostatic actin networks, actin assembly pathways in species using multiple actin isoforms may operate more independently.

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CHAPTER 25

TYPES OF CYTOSKELETON PRESENT IN THE CELLS

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ABSTRACT:

The cytoskeleton is a 3D network of filamentous proteins that connects all areas and parts of the cell and is extremely active. All cells, including bacteria, have some type of cytoskeleton. This network serves multiple purposes, including supporting the cell's structural integrity, acting as a structure for active transport mechanisms, and acting as a system for the production and transmission of mechanical force. The steel framework of a high-rise building and the railroad tracks that link different areas of a metropolis are both metaphors for the cytoskeleton. By connecting numerous cellular components, the cytoskeleton preserves cellular structure. It facilitates communication throughout the entire cell, which has a significant effect on cellular processes. The cytoskeleton is made up of three major parts. These could be thought of as distinct networks with various compositions that perform slightly different but interdependent tasks. Which are intermediate filaments, microfilaments, and microtubules.

KEYWORDS:

Actin filaments, Cytoskeleton, Intermediated filaments, Microtubules, Myosin.

INTRODUCTION

All cells, including bacteria and archaea, contain the cytoskeleton, a dynamic, complicated network of interconnecting protein filaments, in their cytoplasm [1]. In different organisms, it connects the cell nucleus to the cell membrane and is made up of identical proteins. It is made up of three major parts in eukaryotes: microfilaments, intermediate filaments, and microtubules. Depending on the needs of the cell, these parts can rapidly grow or disassemble [2].The cytoskeleton is capable of performing a wide range of tasks. Its main job is to give the cell structure and mechanical resistance against deformation. Additionally, it stabilises whole tissues by interacting with extracellular connective tissue and other cells [2][3].

Additionally, the cytoskeleton has the ability to constrict, which causes the cell and its surroundings to change and permits cell migration [3]. Additionally, it functions as a scaffold to organise the contents of the cell in space and in intracellular transport (for instance, the movement of vesicles and organelles within the cell) as well as in many cell signalling pathways and the uptake of extracellular material (endocytosis),[2] the segregation of chromosomes during cellular division,[4] the cytokinesis stage of cell division, [5] and can be a template for It can also create complex structures like flagella, cilia, lamellipodia, and podosomes. Actin strands are displayed in red, and beta tubulin-containing microtubules are displayed in green. based on the organism and sort of cell [5][6].The cytoskeleton can alter even within a single cell due to interactions with other proteins and the network's past behaviour [7].

Muscle movement is one prominent instance of a cytoskeleton-mediated process. Teams of highly specialized cells collaborate to carry this out. The microfilament is a crucial part of the

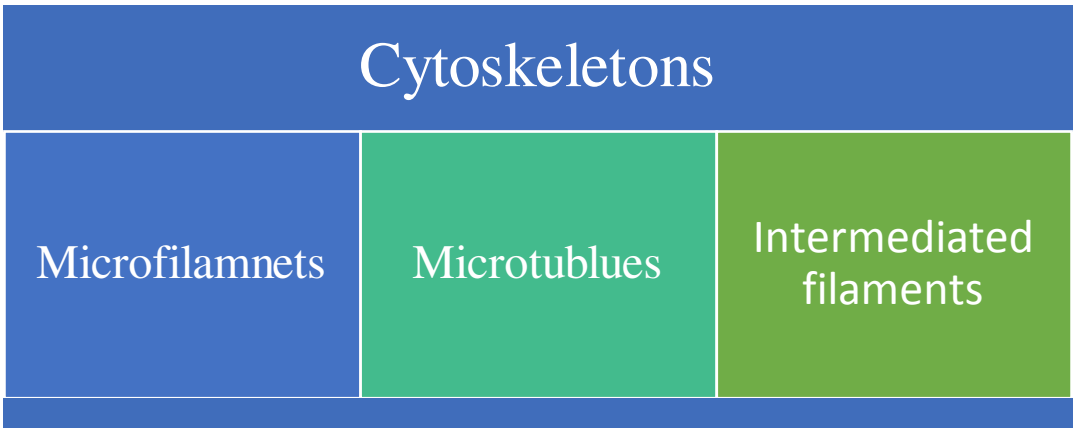
cytoskeleton that aids in illuminating the real purpose of this muscle contraction. Actin, the most prevalent cellular protein, makes up microfilaments [8]. Myosin molecular motors collectively apply forces on parallel actin filaments within muscle cells during contraction. Nerve impulses trigger muscle contraction, which results in the discharge of more calcium from the sarcoplasmic reticulum. Tropomyosin and troponin, two proteins, aid in the initiation of muscle activation in response to increases in calcium in the cytosol [9]. Tropomyosin inhibits the interaction of actin and myosin, whereas troponin detects calcium increases and relieves the inhibition [2]. By contracting one muscle cell simultaneously with many other muscle cells, this action also contracts the complete muscle.

Muscle movement is one prominent instance of a cytoskeleton-mediated process. Teams of highly specialised cells collaborate to carry this out. The microfilament is a crucial part of the structure that aids in illuminating the real purpose of this contraction. Actin, the most prevalent cellular protein, makes up microfilaments. In each muscle cell, myosin molecular motors work together to pull parallel actin strands together when a muscle contracts [10]. Nerve signals trigger muscle movement, which results in the discharge of more calcium from the sarcoplasmic reticulum. Tropomyosin and troponin, two proteins, aid in the initiation of muscle activation in response to increases in calcium in the cytosol [10].When the calcium level rises, troponin detects the increase and breaks the inhibition that tropomyosin had been holding in place between actin and myosin [10]. By contracting one muscle cell simultaneously with many other muscle cells, this action also contracts of the muscle.

LITERATURE REVIEW

Microfilaments, microtubules, and intermediate filaments are the three primary types of cytoskeletal strands found in eukaryotic cells (Table. 1). Neurofilaments are the name for the intermediate strands in neurons [11]. Thus every variety has its own distinctive structure and intracellular distribution and is created by the polymerization of a particular kind of protein subunit. Microfilaments have a width of 7 nm and are polymers of the protein actin. Tubulin makes up microtubules, which have a width of 25 nm. Depending on the type of cell they are located in, intermediate filaments are made up of different proteins and typically measure 8–12 nm in diameter [4]. The cell's cytoskeleton gives it form and structure, and by keeping macromolecules out of some of the cytosol, it increases the density of these molecules in this compartment [4]. Membrane proteins and cytoskeletal components engage in extensive and close communication [4].

Table 1: Presenting the types of the cytoskeleton presents in the cell.



Actin filaments, also known as microfilaments, are made up of linear polymers of the G-actin protein. When the filament's growing (plus) end presses up against a wall, like the cell membrane, it produces force. Myosin molecules attach to the microfilament and "walk" along them, using them as tracks for their mobility. Actin is typically the main protein or component of microfilaments. A polymer is created when the G-actin monomer combines to make the microfilament (actin filament). Then, these subunits combine to form two strands that entwine to form what are known as F-actin chains [12]. Actomyosin fibres, which are present in both muscle and the majority of non-muscle cell types, produce contractile forces when myosin motors along F-actin strands [12].Actin filaments, microtubules, and a collection of polymers collectively known as intermediate filaments are the three major categories of cytoskeletal polymer. These polymers work together to regulate the mechanics and structure of eukaryotic cells. All three play crucial roles in setting up and preserving the integrity of intracellular compartments.

They are arranged into networks that resist deformation but can reorganize in reaction to externally applied forces. Together with molecular motors that move along the actin filaments and microtubules, directed forces produced by the polymerization and depolymerization of actin filaments and microtubules cause changes in cell shape and direct the arrangement of cellular components[7]. Several classes of regulatory proteins influence the architecture of the networks that cytoskeletal polymers form, including nucleation-promoting factors, which cause filament formation, capping proteins, which stop filament growth, polymerases, which encourage faster or more sustained filament growth, depolymerizing factors, which disassemble filaments, and crosslinkers, which organise and reinforce higher-order n-dimensional networks. The local arrangement of the filaments in the networks can be impacted by mechanical forces that originate from either inside or outside the cell.

The three main cytoskeletal polymers differ from one another most significantly in terms of their mechanical stiffness, the dynamics of their assembly, their polarity, and the kinds of molecular motors they associate with. These differences determine the architecture and function of the networks they form (Figure.1)[7]. In addition to the actin gene, the common ancestor of all species on Earth also had a gene for a protein that was similar to tubulin. The origin of the - and -tubulin genes in organisms is unknown. However, because microtubule-based axonemes have been a distinctive characteristic of single-celled eukaryotes for more than one billion years, they were present in the very early eukaryotes [7].

Usually made up of 13 protofilaments, which are themselves polymers of alpha and beta tubulin, microtubules are hollow cylinders measuring about 23 nm in width (lumen diameter is roughly 15 nm)(Table.2) (Figure. 2). They behave in a very active way by attaching GTP to initiate polymerization. The centrosome frequently organises them[13].

The cytoskeleton of many eukaryotic organisms is made up of intermediate filaments (Figure. 2). These filaments, which have an average width of 10 nanometers, are heterogeneous components of the cytoskeleton and more stable (strongly bound) than microfilaments. By supporting tension, they serve the same purpose as actin filaments in maintaining cell structure (microtubules, by contrast, resist compression but can also bear tension during mitosis and during the positioning of the centrosome). A cell's interior tridimensional structure is organised by intermediate filaments, which also act as structural elements of the nuclear lamina and anchors for organelles. They take role in some junctions between cells and between cells and the matrix. All tissues and mammals have nuclear lamina. The fruit fly is one of many species that lacks cytoplasmic intermediate filaments. These filaments are tissue-specific in animals that produce cytoplasmic intermediate filaments[7].

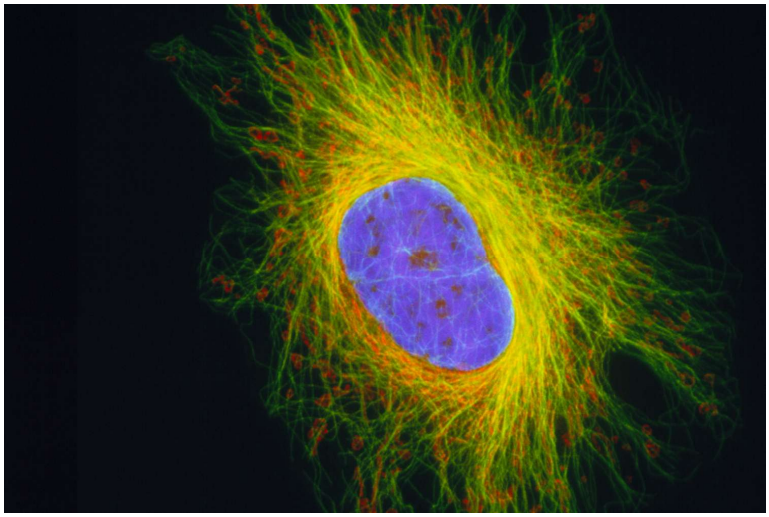


Figure 1: Anatomy of cytoskeleton: A microscopic representation of the arrangement of the various kinds of cytoskeleton in a cell serves as the cytoskeleton's anatomy (ThougthCo).

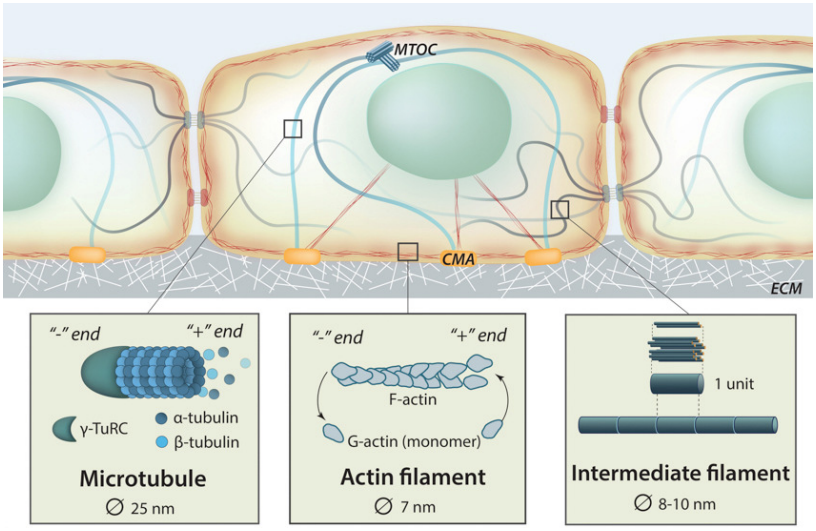


Figure 2: Graphical represtation of the cytoskeleton: microfilaments, microtubules, and intermediate filaments are the three major elements of the cytoskeleton, which is depicted graphically (MBInfo).

By adhering to nearby cells or the extracellular matrix, the cytoskeleton shape is changed (ECM). The sort and strength of these adhesions play a crucial role in controlling how the cytoskeleton's constituent parts assemble and disassemble. Cellular movement is made possible by this dynamic characteristic and is controlled by forces (both internal and external) [7]. Mechanosensors pick up on this information, which is then transmitted through the cytoskeleton to trigger chemical signalling and an appropriate reaction. All three filament systems' subunits are found throughout the cell, but due to variations in the subunit structures and the attractive forces that exist between them, each system has its own unique mechanical characteristics and a range of stabilities. These traits account for their distribution in specific cell structures and/or areas. The spatial and temporal distribution of the cytoskeleton is also regulated by a large number of proteins that are linked with the cytoskeleton. For the majority of cellular functions, the organization and assembly of one filament system is affected by the others in a coordinated manner [14].

Table 2: Demonstrating the contrast between the various cytoskeletons.

Cytoskeleton type	Diameter (nm)	Structure
Actin	6	Double helix
Microtubules	10	Two antiparallel helices forming teramers

CONCLUSION

This collection's first part describes the different types of cytoskeleton, beginning with the proteins that make up the three systems actin filaments, intermediate filaments, and microtubules. Additional related reviews, a portion of which is cited above, explain how cells put these proteins together to form useful supramolecular structures. They also explain how these assemblies give cells their mechanical integrity, aid in adhesion to extracellular molecules and other cells, transport materials inside cells, move entire cells, move their cilia, separate chromosomes during mitosis, and divide cells in half during cytokinesis. A cell's cytoplasm contains a structure of filaments and fibres known as the cytoskeleton. The cytoskeleton organises the various components of the cell, regulates its structure, and is also in charge of the motility of the cell and the movement of the numerous organelles that are found inside. The strands of the cytoskeleton are so tiny that their existence was only discovered thanks to the electron microscope's greater resolving power. The three main types of filaments that make up the cytoskeleton are actin filaments, intermediate filaments, and microtubules. The actin filaments, which are present in cells as meshworks and spirals of parallel strands, help regulate the cellular structure and its capacity to adhere to surfaces. Actin filaments are constantly changing arrays that help a cell move and regulate some internal processes, such as cell division during mitosis. Together with actin, two other cytoskeletons were praised for controlling how cells operate.

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