9.3 ACTIN FILAMENT OR MICROFILAMENT

Actin is a globular monomer with a central groove or cleft for binding to ATP molecule (Fig. 9.4b). Actin molecules are arranged in a head-to-tail fashion to form a chain-like protofilament. The protofilament is usually made of two chains intertwined to form a helix of 5 to 9 nm diameter. The ATP-binding cleft forms the minus end and the other end forms the plus end (Fig. 9.4a). Within the cell, actin filaments are organized into linear bundles, linear networks, and three-dimensional gels. They are predominant in the cortical region of the cytoplasm (Fig. 9.5).

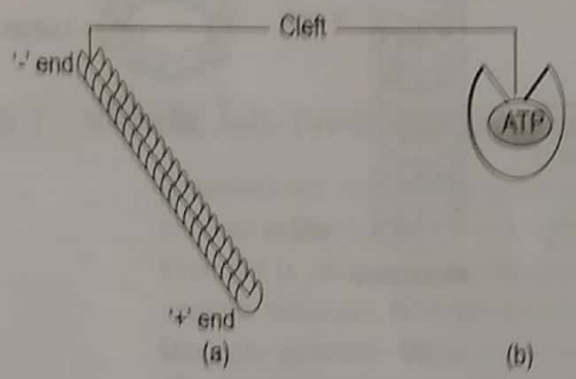

FIGURE 9.4 (a) Actin protofilament formed from polymerization of actin monomers (the end of the filament exposing the cleft forms the minus end) (b) Actin monomer with a cleft is projected (ATP molecule bound to the cleft of the monomer is shown as a shaded cloud)

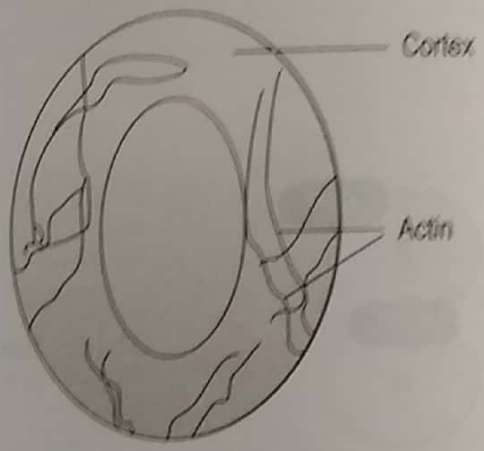

FIGURE 9.5 Distribution of actin filaments within the cell—the network is predominant in the cortical region (shaded region) of the cytoplasm

9.3.1 Functions of Actin Filaments

Actin filaments:

- serve as bridging elements between the transmembrane proteins and the cytoplasmic proteins.
- are anchors for centrosomes during the process of mitosis.
- function as partitions to segregate the two dividing cells during the process of cytokinesis.
- help in mechanical movement in leucocytes.
- promote cytoplasmic streaming and gliding.

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9.4 INTERMEDIATE FILAMENTS

Intermediate filaments are present in the cells of metazoans, that is, vertebrates, nematodes, and molluscs. They are present in a few cell types and not in others. The individual unit of intermediate filament is a polypeptide, a monomer which forms lateral contact with another monomer forming a dimer. Two dimers subsequently associate to form a tetramer. Eight tetramers are associated to form a protofilament. Several protofilaments associate in a head-to-tail fashion forming a rope-like structure. Formation of intermediate filament from a single polypeptide is schematized in Fig. 9.6.

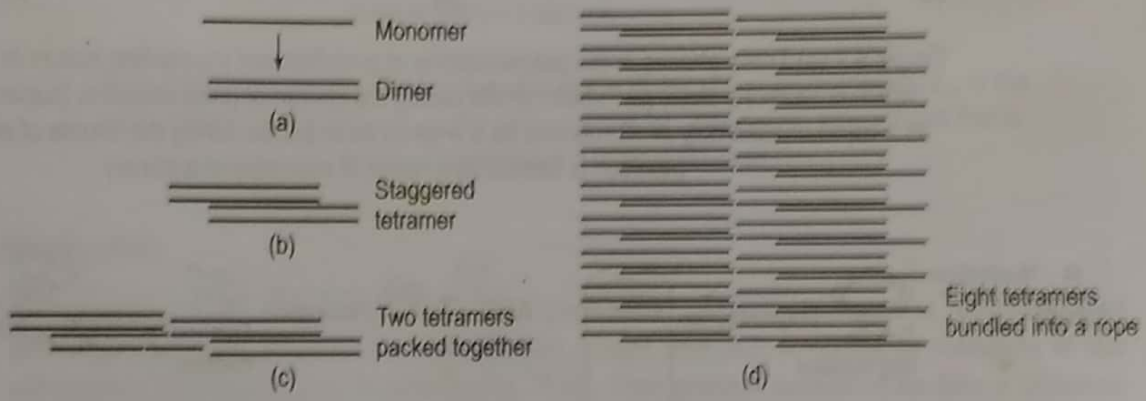

FIGURE 9.6 Formation of intermediate filament from a single polypeptide helix

Intermediate filaments are found to be easily deformed and withstand excess stress and strain without rupture. Thus, they are responsible for maintaining the cellular integrity. Intermediates filaments are distributed in different types of cells. They are shown in Table 9.1. Intermediate filaments act as a supporting framework and provide tensile strength to the cells.

9.5 NUCLEATION AND POLYMERIZATION

Formation of protofilaments for subsequent building of cytoskeletal network occurs by the process of polymerization, that is, by assembly of protein monomers into filaments. In vitro studies indicate that the polymerization takes place in three different stages or phases: (i) Lag phase or stationary phase, (ii) growth phase or elongation phase, and (iii) steady-state or equilibrium phase as shown in Fig. 9.7. This process is called triphasic polymerization.

TABLE 9.1 Types of intermediate filaments

Name of the filament	Cells expressing the filament
Lamin	Nucleus
Vimentin	Mesenchymal cells
Desmin	Myocytes
Glial fibrillary acidic protein	Glial cells
Peripherin	Neurons
Keratin	Epethilial cells, hair, and nails
Neurofilaments	Neurons

Lag phase represents the time course for a process called *nucleation*. Nucleation is a process in which two or more subunits bind to form an oligomer, which remains stable without dissociation and acts as a nucleus for further polymerization. For example, for the polymerization of tubulin protofilament, at least

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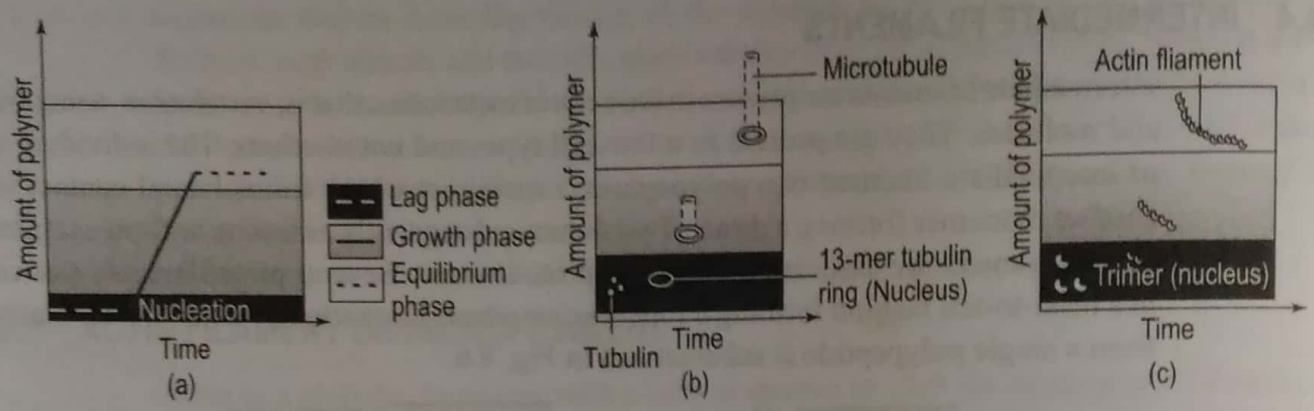

FIGURE 9.7 (a) Three phases in the polymerization of protofilament (nucleation occurs in the lag phase, which is relatively slow) (b) Tubulin profile during the course of polymerization (nucleus is formed as a result of assembly of 13 subunits as a ring) (c) Actin profile during the course of polymerization (nucleus is formed as a result of assembly of a trimer)

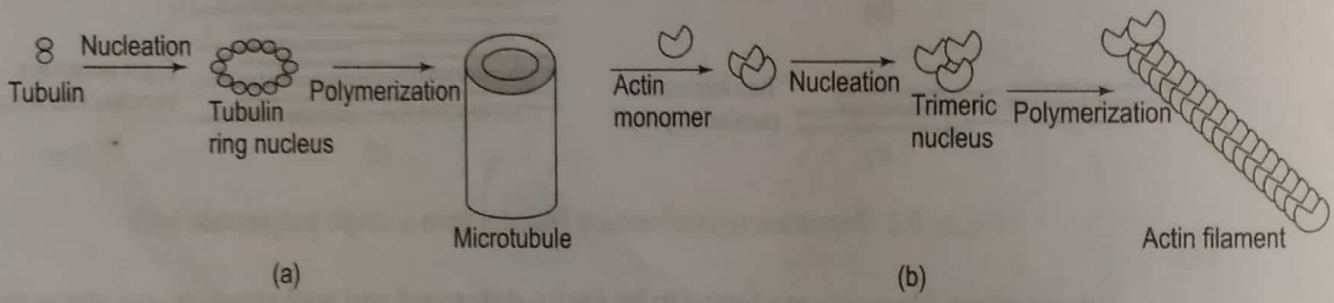

FIGURE 9.8 Nucleation of (a) tubulin and (b) actin (an oligomer of 13 tubulin subunits is the nucleus for the formation of microtubule; a trimer of actin functions as a nucleus for the formation of microfilament. In either case, the nucleus remains stable enough to continue polymerization, but the process of nucleation is relatively slow.)

13 monomers should assemble as a ring to function as a nucleus (Fig. 9.8a). In the case of actin protofilament, a trimer can be stable enough to drive polymerization (Fig. 9.8b). If the number of monomer is reduced to less than 13 or 3 for tubulin and actin, respectively, the structure will not be stable, leading to disassembly and inhibition of polymerization. Nucleation is a relatively slow process. The lag phase representing the nucleation process can be decreased or skipped if preformed nuclei exist. Cells make use of proteins to catalyse the nucleation at a specific site to form the cytoskeleton at that site. Thus, it is clear that nucleation is the rate-limiting step in the formation of cytoskeleton and hence the cell depends on it to regulate its own shape and movement.

Elongation phase represents the formation of a polymer from the stable nucleus. In this phase, the protein subunits add rapidly to elongate the filaments. At the end of the process, a balance is achieved between the addition of monomer and the dissociation of monomer at the filament ends. This phase is called the equilibrium phase or steady-state phase, where the filament remains constant or the concentration of the subunit remains unchanged. At the equilibrium phase, the concentration of the free subunits left in the solution is called as the critical concentration, represented as C_c. C_c is equal to the ratio of the rate constants for subunit dissociation and subunit addition, that is, $C_c = k_{off}/k_{on}$. The addition and dissociation of subunit in a protofilament and the corresponding rate

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constants are shown in Fig. 9.9. The rate of addition is called 'on rate' and the rate of dissociation is called 'off rate'.

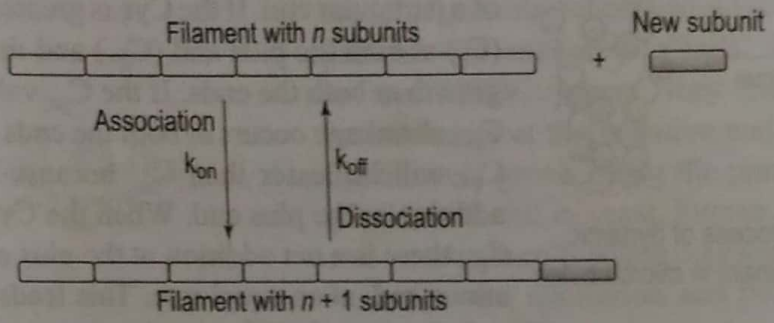

FIGURE 9.9 Addition and dissociation of subunits at the end of protofilament (k_{on} is the rate constant for addition; k_{off} is the rate constant for dissociation. The subunit that is added or dissociated is shaded for clarity.)

9.6 TREADMILLING

Treadmilling is a process by which the cytoskeletal polymer filament is maintained at constant length by the addition of subunits at one end and severing of subunits at the other end. This process is shown in Fig. 9.10. This process seems to be like a filament migrating across the stratum or the cytosol. The treadmilling process predominates in actin filaments.

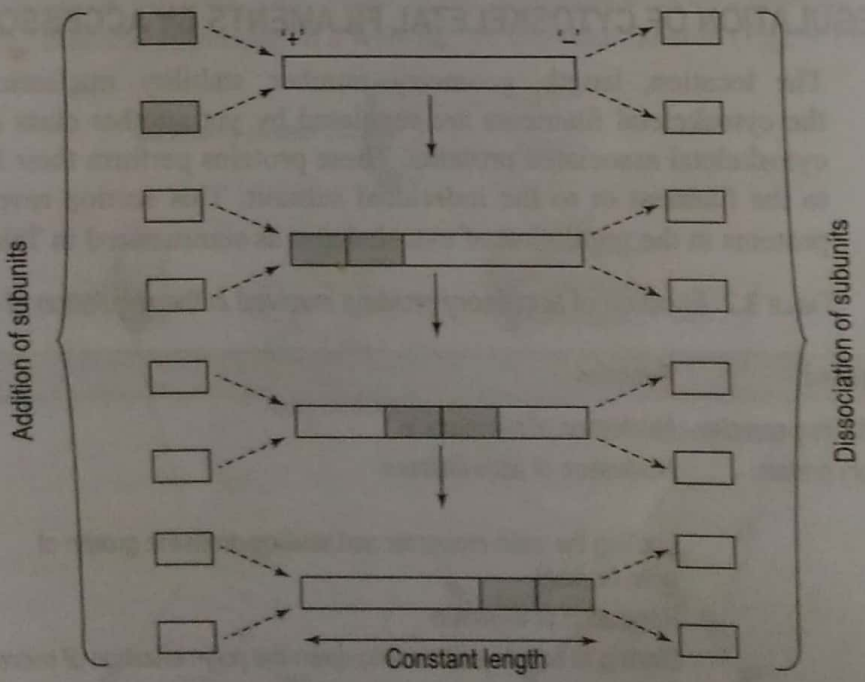

FIGURE 9.10 Process of treadmilling [Addition of subunits (dotted arrows) occurs at the plus end and dissociation of subunits (dotted arrows) occurs at the minus end. The subunits that are added initially are shaded to enable understanding the seeming motion of the subunits along the length of the filament. Bold arrow indicates that the net result of treadmilling is the constant filament length maintained due to equal rate of addition and dissociation.]

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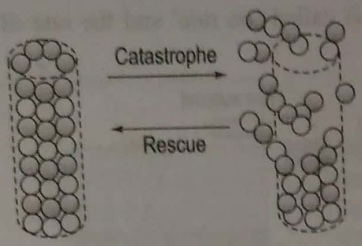

FIGURE 9.11 Process of dynamic instability predominant in microtubules

The cytosolic concentration (Cyc) of the free subunits of the filament determines the status (the shrinking status or growth status) of a particular end. If the Cyc is greater than the critical concentration (C_c) at both the plus end (C_{c+}) and the minus end (C_{c-}), it results in growth at both the ends. If the C_{yc} value is lesser than both Cc+ and C_{c-}, shrinkage occurs at both the ends. However, in the general case, C_{c+} will be lesser than C_{c-} because of the probability of subunit addition at the plus end. When the Cyc value falls between C_{c+} and C_{c-}, there is a net addition at the plus end and net disassembly at the minus end, at an equal rate. This leads to a constant filament length and, thus, treadmilling predominates.

9.7 DYNAMIC INSTABILITY

Dynamic instability is the process of abrupt transformation of a filament from growth stage to shrinkage stage or vice versa (Fig. 9.11). When the cytosolic concentration of a free monomer subunit is intermediate between C_c for the plus end and C_c for the minus end, any plus end will grow and any minus end will shrink. Sometimes, a single filament can grow for a while and then suddenly begin to shrink rapidly even when the free subunit concentration is constant. This sudden interconversion is called dynamic instability. Transformation of a filament to rapid shrinkage state is called a catastrophe, whereas the transformation to rapid growth is called a rescue. Dynamic instability predominates in microtubules.

9.8 REGULATION OF CYTOSKELETAL FILAMENTS BY ACCESSORY PROTEINS

The location, length, geometry, number, stability, nucleation, and the dynamics of the cytoskeletal filaments are regulated by yet another class of diverse proteins called cytoskeletal associated proteins. These proteins perform their function by binding either to the filament or to the individual subunit. This section reveals the role of accessory proteins in the regulation of cytoskeleton as summarized in Table 9.2.

TABLE 9.2 Function of accessory proteins involved in the regulation of cytoskeleton

Accessory protein	Function	Binding site
Gamma tubulin ring complex	Nucleation of microtubule	End of the filament
Actin regulatory protein complex	Nucleation of actin filament	Sides of the filament
Thymosin	Locking the actin monomer and slowing down the growth of actin filament	Sides of the filament
Profilin	Regulation of thymosin	Sides of the filament
Stathmin	Binding to tubulin and slowing down the polymerization of microtubule	Sides of the filament
Microtubule-associated proteins (MAPs)	Stabilization of microtubules Inhibition of disassembly and prevention of the dynamic instability Promote the interaction of microtubule with other cellular components	Ends of the filament
Tropomyosin	Regulates the binding of myosin head to actin filament Regulates muscle contraction	Sides of the filament
Cofilin	Severes and depolymerizes actin filament	Sides of the filament

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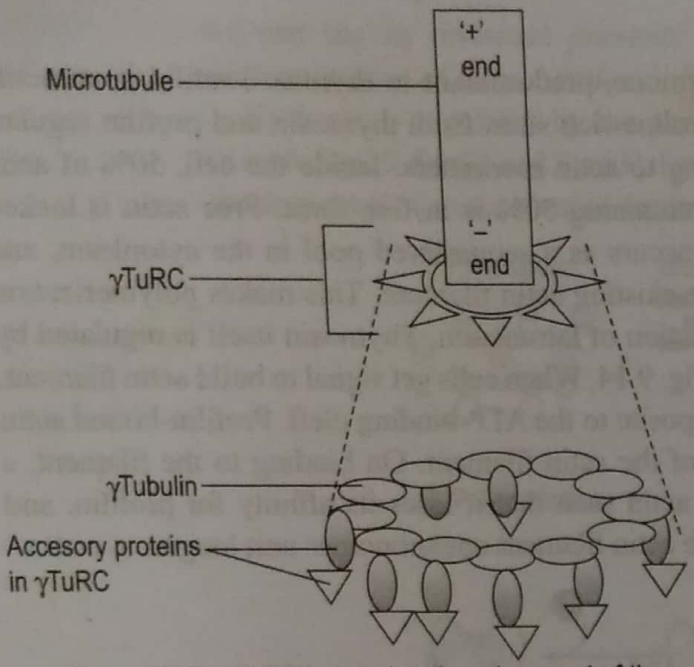

FIGURE 9.12 γTuRC located at the minus end of the microtubule (upright rectangle) is enlarged to show the presence of γ tubulin ring and other accessory proteins

9.8.1 Gamma Tubulin Ring Complex

Gamma tubulin ring complex (γTuRC) is a ring made of gamma tubulin to which several other proteins are anchored. γTuRC is concentrated at the MTOC where they nucleate tubulin heterodimers at the negative end. Subsequently, the filament grows along the plus end direction. γTuRC is found in yeast, human beings, insects, and vertebrate cells where they serve as a template to initiate the nucleation and polymerization of microtubules. The ring complex attached to the microtubule is shown in Fig. 9.12.

9.8.2 Actin Regulatory Protein Complex

The actin regulatory protein (ARP) complex is made of ARP2, ARP3, and an assembler protein (Fig. 9.13a). ARP2 and ARP3, which have 45% structural identity to actin monomer itself, are held or assembled by the assembler protein in the cortical region of the cytoplasm, almost beneath the plasma membrane. At this region, the complex initiates the nucleation of the minus end leading to the elongation of the plus end of the filament (Fig. 9.13b). The complex remains more active if it is bound to the side of the pre-existing actin filament. This results in the formation of actin filaments at 70° angle to the original filament appearing in a web-like or tree-like fashion (Fig. 9.13c).

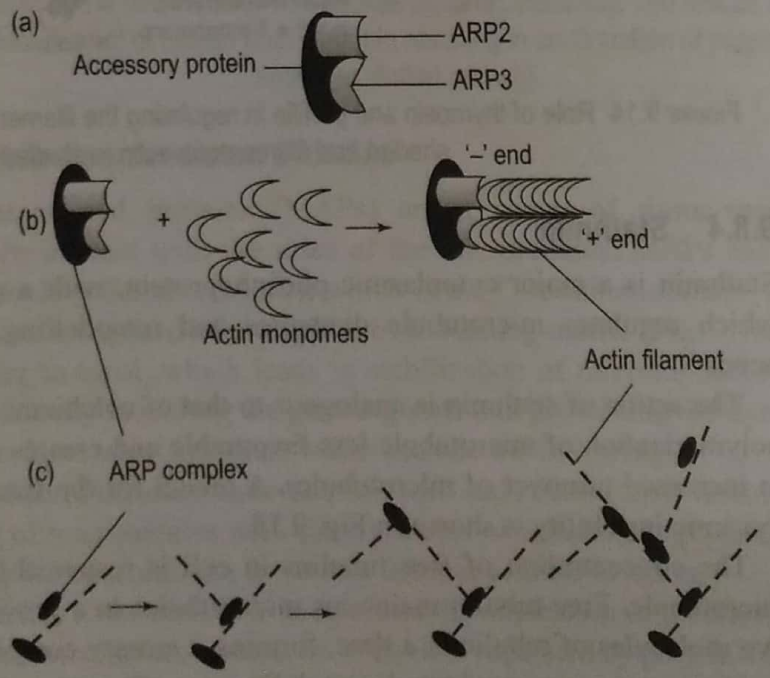

FIGURE 9.13 (a) Structure of actin regulatory protein complex (ARP2 and ARP3 are shaded) (b) Nucleation of actin at the minus end and elongation at the plus end of the filament (c) Formation of actin filament web, like a tree (This occurs when the ARP is attached to a pre-existing filament; the actin monomers in the filament are simplified as dark bands and the ARP complex are shown as dark ovals.)

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9.8.3 Thymosin and Profilin

Thymosin is a small polypeptide hormone, predominant in thymus. Profilin is an actin-binding protein with specificity for proline-rich sites. Both thymosin and profilin regulate the actin filament assembly by binding to actin monomers. Inside the cell, 50% of actin is in filamentous form, whereas the remaining 50% is in free form. Free actin is locked by thymosin. Thymosin-bound actin occurs as a sequestered pool in the cytoplasm, and it cannot bind to either ends of the pre-existing actin filament. This makes polymerization less favourable; thus, mimicking the action of latrunculin. Thymosin itself is regulated by profilin, whose role is schematized in Fig. 9.14. When cells get signal to build actin filament, profilin binds to the actin monomer opposite to the ATP-binding cleft. Profilin-bound actin can readily bind to the free plus end of the actin filament. On binding to the filament, a conformational change takes place in actin such that it loses its affinity for profilin, and profilin detaches from actin, leaving the actin filament one monomer unit longer.

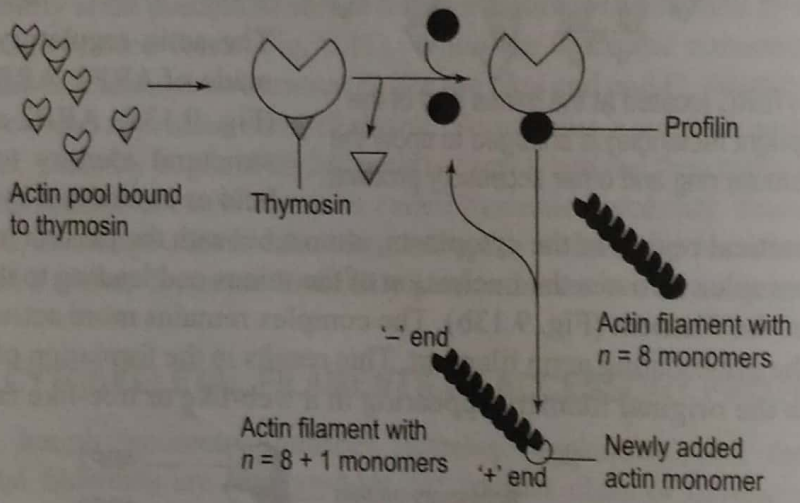

FIGURE 9.14 Role of thymosin and profilin in regulating the filamentation of actin (Free actin is not shaded and filamentous actin is shaded dark)

9.8.4 Stathmin

Stathmin is a major cytoplasmic phosphoprotein, with a molecular weight of 17 kDa, which regulates microtubule dynamics and remodelling, depending on the cellular needs.

The action of stathmin is analogous to that of colchicine. That is, stathmin makes the polymerization of microtubule less favourable and creates dynamic instability resulting in increased turnover of microtubules. A model for the function of stathmin in creating dynamic instability is shown in Fig. 9.15.

The concentration of free tubulins in cell is responsible for the polymerization of microtubule. Free tubulin maintains microtubules in a growing state. Stathmin binds to two molecules of tubulin at a time, forming a ternary complex represented as T2s, thus, building up a sequestered tubulin pool. Because of sequestration, concentration of the free tubulin shrinks. Consequently, the elongation of microtubule slows down (the process is called catastrophe). Consequently, the GTP cap at the plus end will be hydrolyzed at a rapid rate, shifting the microtubule from the growing state to shrinking state. Thus,

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we can say *as thymosin prevents the polymerization of actin, stathmin prevents the polymerization of microtubule*.

Stathmin is regulated by the phosphorylation. Phosphorylation of the serine residues (Ser16, Ser25, Ser38 and Ser63) detatches the protein from the tubulin, thereby increasing the free tubulin concentration leading to polymerization.

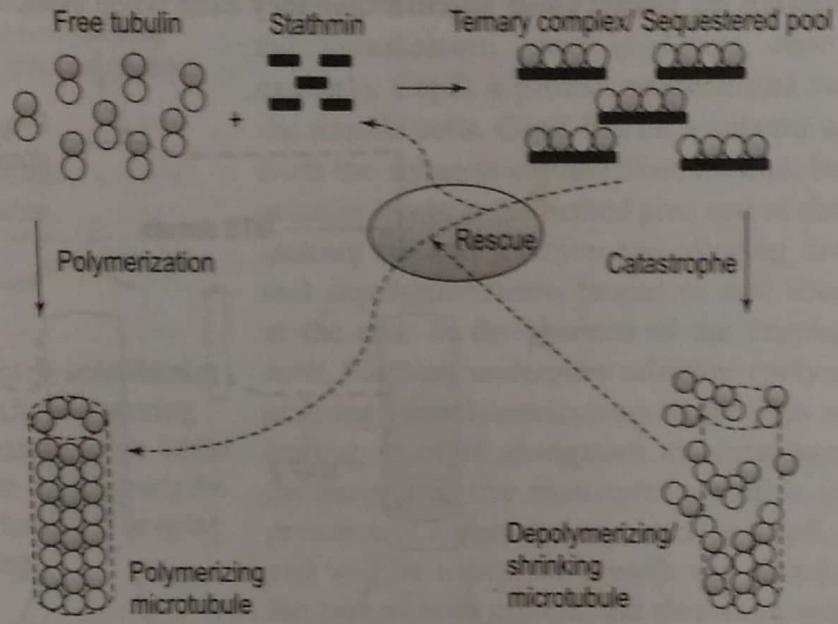

FIGURE 9.15 A model for the function of stathmin in the regulation of microtubule dynamics (Stathmin is shown as a dark rectangle. Binding of stathmin to free tubulin results in catastrophy, leading to depolymerization of the microtubule. This causes dynamic instability. The rescue process, which results in detachment of tubulin from stathmin resulting in continuation of polymerization, is shown as dotted arrows)

9.8.5 Microtubule-associated Proteins

Microtubule-associated proteins (MAPs) are a group of tissue-specific regulatory proteins. MAPs interact with the sides of the microtubules. MAPs have two domains: microtubule-binding domain (MBD), which is the C-terminal domain, and a projecting domain. MBD is composed of multiple tubule-binding motifs (Fig. 9.16a). Functionally, MAP is similar to taxol, which leads to stabilization of microtubules and inhibition of disassembly, thereby favouring the growing state and preventing the dynamic instability. MAPs also perform other functions which include the following:

- Association of different microtubules with each other (cross linking)
- Linking of microtubules with other proteins or cellular components
- Steering the microtubules towards a definite cellular location

In vitro studies show that MAP accelerates the nucleation of tubulins, which is critical for polymerization of microtubules. MAP itself is regulated by protein kinase enzymes via phosphorylation. Phosphorylated MAPs cannot accelerate nucleation and polymerization leading to dynamic instability, which is observed during the mitosis phase.

Examples for MAPs are MAP2 and tau protein, both present in vertebrates, and XMAP 215, which is a *Xenopus*-associated protein with molecular weight 215, present in all

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eukaryotes. MAP2 has a long projection domain capable of binding to other microtubules present nearby (Fig. 9.16b). Because of the length of the projection domain, the microtubules in a bundle linked by MAP2 are not spaced closer (Fig. 9.16c). In contrast to MAP2, tau protein has a shorter projection domain. Both the MBD and the projection domain of tau protein are attached to the same tubule filament forming a loop. Therefore, the microtubular bundles are spaced closer together than they exist when associated with MAP2.

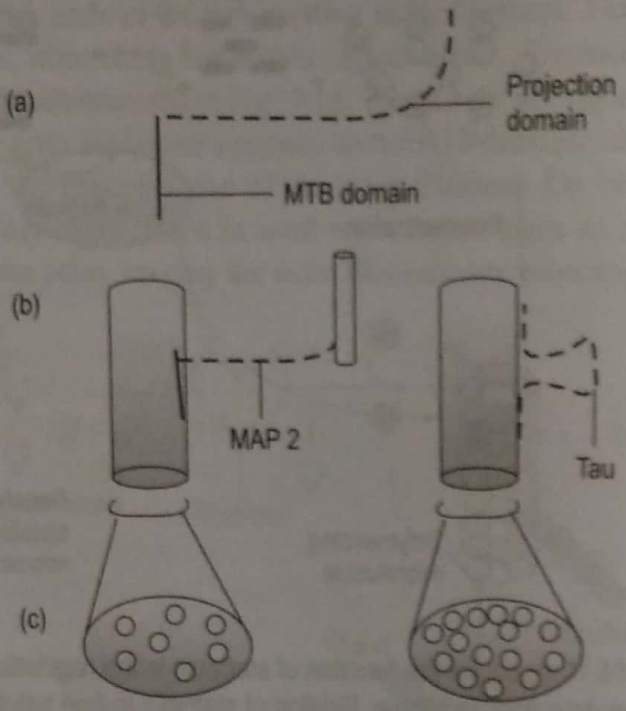

FIGURE 9.16 (a) Domains of MAPs (projection domain is shown as dotted line and MTB domain as solid line) (b) Association of MAP2 and tau protein with the microtubule showing the difference in the domain length (c) Hypothetical representation of the cross section of microtubule bundles showing the spatial distribution [tubule associated with MAP2, spaced apart (shown on left), and tau protein, spaced closer together (shown on right)]

9.8.6 Tropomyosin

Tropomyosin is a class of accessory protein that binds to the side of actin filaments, spanning approximately seven actin subunits at a time. Tropomyosin regulates the binding of myosin to actin in association with troponin, thereby regulating muscle contraction.

9.8.7 Cofilin

Cofilin is an actin-binding protein of molecular weight 14 kDa, smaller than its target protein actin. Cofilin is known to depolymerize the minus end of the actin filament and to prevent further reassembly. Both the filamentous and free forms of actin are the targets for cofilin. Cofilin binds preferentially to the ADP-containing actin at 1:1 stoichiometry, making the twist of the filament tighter, thereby inducing a mechanical stress. Mechanical stress leads to (i) decrease in the distance spanned by the filament, that is, decrease in length of the filament and (ii) dissociation of ADP–actin from the minus end of the filament, followed by severing of actin filament and depolymerization. This results in a net increase in the rate of treadmilling. Influence of cofilin on length of the actin filament

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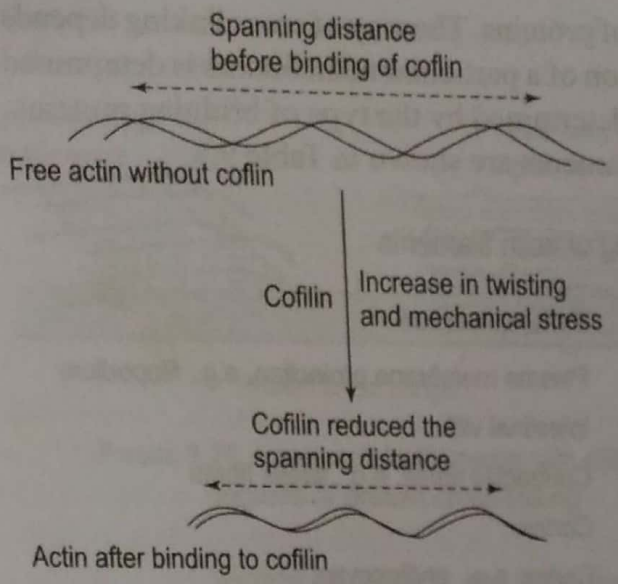

FIGURE 9.17 Influence of cofilin on actin filament (Actin is shown as a double helix. Spanning length of actin is indicated by dotted arrow. Cofilin decreases the spanning length by increasing the twist and mechanical stress leading to severing and treadmilling.)

is schematized in Fig. 9.17. Because of its function, cofilin is also called actin-depolymerizing factor. ATP-containing actin are resistant to cofilin-induced severing.

9.8.8 Plus-end Capping Protein—CapZ

Proteins that bind to the end of the filaments can regulate the cytoskeleton even at lower concentrations, for example, CapZ, a protein predominant in the Z band of the muscle cells. CapZ has an α subunit and a β subunit. Both the subunits cap the filament end, but the β subunit actually binds to the barbed plus end of the actin filament, making the end inactive and slowing down the growth and depolymerization processes that usually take place at the end. In the absence of the capping proteins, the actin filament undergoes addition (polymerization) and severing (depolymerization) at the plus and minus ends leading to rapid elongation or shrinkage depending on the amount of free monomers available. However, in the presence of a plus-end cap, such as CapZ, only the minus end will be active for growth and shrinkage. Therefore, the rate of both growth and shrinkage will be slow at all monomer concentrations. The rate of filament growth and shrinkage in the presence and absence of a plus-end cap is represented in Fig. 9.18.

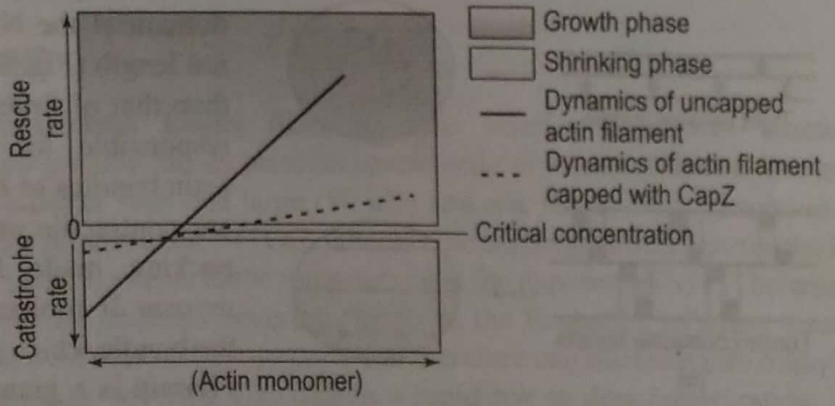

FIGURE 9.18 Effect of a cap protein on filament dynamics

9.8.9 Catastrophin

Catastrophin is a capping protein capable of binding to the ends of microtubules. It lowers the normal activation energy barrier which blocks dismantling of protofilaments. Consequently, it induces catastrophes, resulting in shrinking of the tubule.

9.9 PROTEIN CROSS BRIDGE

Several proteins cross link cytoskeletal filaments into parallel arrays or bundles and form cross bridges, which add to the tensile strength and mechanical properties of the cell. Protein cross bridges are found in actin filaments and intermediate filaments.

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Actin filaments are cross linked by a range of proteins. The way of cross linking depends on the subcellular location of actin. The function of a particular actin bundle is determined by the type of cross linking, which in turn is determined by the type of bridging proteins. Proteins responsible for cross linking actin filaments are shown in Table 9.3.

TABLE 9.3 Proteins used in cross linking of actin filaments

Protein	Type of cross link	Cellular location
Fimbrin	Tight parallel bundle	Plasma membrane projection, e.g., filopodium
Villin	Tight parallel bundle	Intestinal villi
α Actinin	Loose contractile bundle	Contractile fibres, e.g., stress fibres
Filamin	Gel-like network	Cortex
Spectrin	Web-like network	Cortex, e.g., erythrocytes

Fimbrin is a monomer with a tiny actin-binding domain of 14 nm and 27 kD. It is found in intestinal epithelial cells, leucocytes, and mesenchymal cells of solid tissues. It cross links actin filaments into tight parallel bundles with plus ends aligned at one end (Fig. 9.19 a). This type of cross link is found in projections of plasma membrane, such as filopodia, which help cell to explore its surroundings. Villin is a protein similar to fimbrin in its function. Both fimbrin and villin cross link actin filaments in microvilli in the intestinal epithelial cells, which is responsible for increasing the absorptive surface area. α Actinin is an anti-parallel dimer with actin-binding domain at the N-terminal ends. It spans 30 nm length (Fig. 9.19b), comparatively larger than that of fimbrin (14 nm). This length is responsible for making loose contractile actin bundles as found in stress fibres, which are contractile and exert stress. The looser packing made by actinin freely allows myosin II molecules to accommodate into the bundle which gives the ability to contract. Filamin is a giant cytoplasmic protein with molecular weight of 250 kD. It has two actin-binding sites interconnected by a 'v' shaped domain, spanning approximately 25 nm. The structural feature of filamin enables cross linking of actin filaments, almost at right angles to each other resulting in a highly viscous gel-like or web-like network, usually an orthogonal network predominant in the cortex of the cytoplasm. The network enables the cell membrane to project into a structure called lamellipodia with which the cells can crawl. Spectrin is another bundling

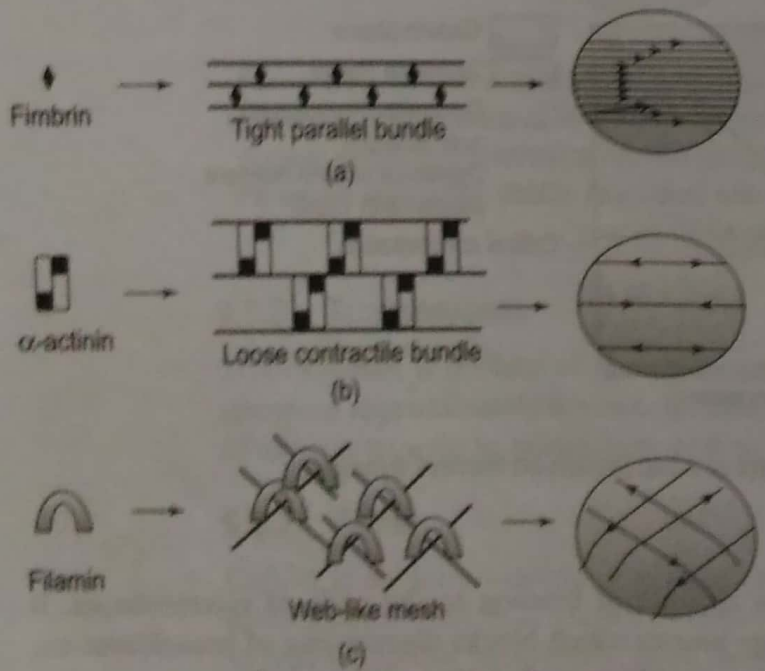

FIGURE 9.19 (a) Fimbrin monomer links filaments with close spacing to form tight parallel bundles (b) a Actinin, a dimer links filaments with wide spacing to form loose contractile bundles (c) Filamin dimer cross links filaments at almost right angles to each other forming a gel-like network (orientation of filament is shown in shaded circle)

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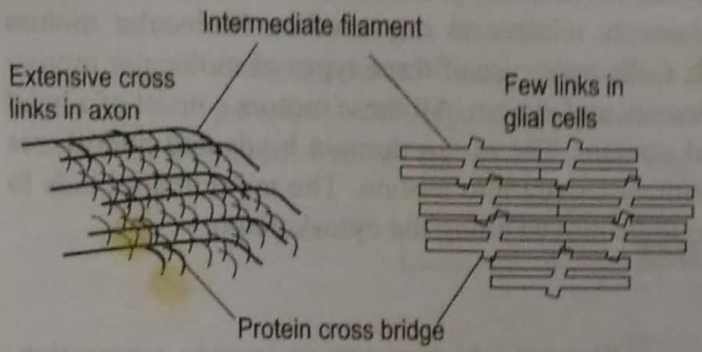

FIGURE 9.20 Intermediate filaments with different degrees of protein cross linking

protein, analogous to filamin in that it forms a gel-like actin network in red blood cells. The structure of this protein is discussed in Chapter 7. Spectrin–actin network helps to maintain the red cell integrity during circulation.

The extent of cross linking among intermediate filaments depends on the function of the cells. For example, the axon of neural cells shows high degree of cross linking among intermediate filaments resulting in high tensile strength. Whereas in glial cells, few cross linkings are found (Fig. 9.20).

Plectin is a huge protein with a molecular weight of 500 kD and is composed of a central alpha coil linked to two globular domains, the latter of which is responsible for cross linking. Plectin is peculiar since it cross links intermediate filaments to other intermediate filaments, microtubules, actin arrays, and myosin thick filaments. It also links cytoskeleton to junctions existing in the plasma membrane. Plectin is responsible for providing cells with necessary tensile strength needed to withstand mechanical stress. Another protein capable of forming cross bridge is flaggrin. Flaggrin is a predominant protein in keratohyalin granules. This protein is rich in basic amino acids, such as arginine, lysine, and histidine. Flaggrin bundles keratin filaments, the structural unit of epidermis, to give toughness to the outermost layer of the skin.

9.10 SEVERING PROTEINS

Severing proteins break longer filaments into smaller fragments. Katanin severes microtubules, and gelsolin family proteins severe actin filaments. Katanin (meaning 'sword' in Japanese) is a dimer with one large (80 kD) and one small (60 kD) subunit. The larger subunit directs the protein to the MTOC, whereas the smaller subunit severes the longitudinal bonds in the 13 protofilaments in the microtubule at the expense of ATP. The smaller subunit is also called ATPase domain. Severing results in the formation of many small filaments which have their own plus and minus ends and therefore can nucleate into many other larger filaments. Sometimes, severing also causes a rapid rise in depolymerization, leading to a change in physical and mechanical properties of the cytoplasm.

Members of gelsolin super family severe actin in the presence of high calcium ion as the signal, and without the input of energy by ATP. The protein has two domains. One domain binds to the external surface of the actin filament and remains there for encountering a thermal fluctuation which generates a small fissure between the neighbouring subunits of the protofilament. The protein insinuates its other domain into the fissure, severing the filament. Local rise in PIP2 concentration detaches the severing protein from the filament.

9.11 MOLECULAR MOTORS

Molecular motors are cellular protein molecules that harness energy from ATP to perform mechanical work. Basically two types of mechanical work are carried out: (i) movement

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of cellular contents, such as lipids, synaptic vesicles, proteins, and cell organelles (e.g., mitochondria) and (ii) sliding of filaments relative to one another. Molecular motors function along the cytoskeleton track. Cells make use of three types of molecular motors for moving the contents—myosin, kinesin, and dynein. All these motors consist of a head domain (or motor domain) and a tail domain. The motor domain binds and hydrolyzes ATP to convert its energy into mechanical energy into motion. The tail domain binds to the cellular component (called cargo) to be moved along the cytoskeleton.

9.11.1 Myosin

Myosin is the molecular motor for actin filament which results in muscle contraction. Myosin has three structural domains: dead domain, neck domain, and tail domain. The head domain has a binding site for ATP and is known as the moor domain. It is responsible for binding to the actin filament and causes hydrolysis of ATP through its ATPase activity. This results in the conversion of the energy derived from ATP to mechanical force leading to the movement of myosin head over the actin filament.

Myosin in muscle contraction

A signal is passed from the motor nerve to the muscle cell in the form of action potential. This results in the depolarization of the T tubule in the muscle cell membrane, which in turn opens the calcium channel present in the membrane of the sarcoplasmic reticulum. Consequently, calcium ions effluxes from the sarcoplasmic reticulum into the cytosol. Calcium mediates the movement of tropomyosin present on the surface of the actin filament, which otherwise blocks the myosin-binding site presents in the actin filament. Myosin head slides past the actin filament, both the components not undergoing any change in length (Fig. 9.21). This sliding motion is the muscle contraction, which occurs in the presence of ATP. Muscle contraction involves the following steps (Fig. 9.22):

- One molecule of ATP binds to the cleft at the back side of the myosin head, that is, behind the actin-binding site.
- ATP binding induces a conformational change in the actin-binding site, thereby reducing the affinity between actin and the myosin head.
- Using the energy derived by ATP hydrolysis, the head moves along the filament by a distance of approximately 5 nm, thus, taking a new position in the filament. The products of hydrolysis, ADP and Pi, still remain with the head.
- Weak binding of head to the new position results in the release of Pi, concomitantly leading to a tight binding.
- Release of Pi triggers a 'power stroke' which help myosin to regain its original conformation with the simultaneous release of ADP.
- Then the next sliding cycle starts with binding of another ATP molecule as before and the cycle continues.

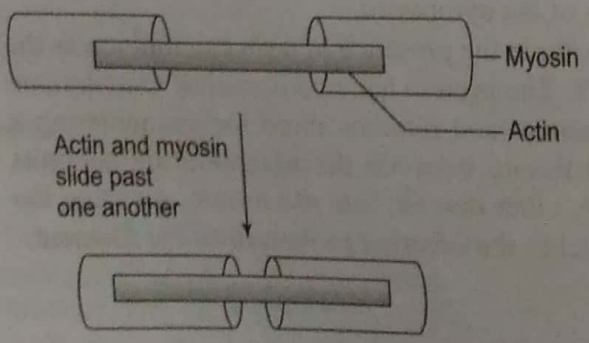

FIGURE 9.21 Sliding motion of myosin over actin filament—The muscle contraction

9.11.2 Kinesin

Kinesin is made of two motor domains with bound ATP and two cargo-binding domains. These two

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domains are linked by a long stalk (Fig. 9.23). The entire structure falls between 70 to 80 nm in length. Kinesin is responsible for the movement of cellular material or cargo along the microtubules, away from the nucleus, that is, from the centre of the cell to the periphery. This type of movement is called anterograde transport. Kinesin moves towards the plus end of the microtubule (Fig. 9.24).

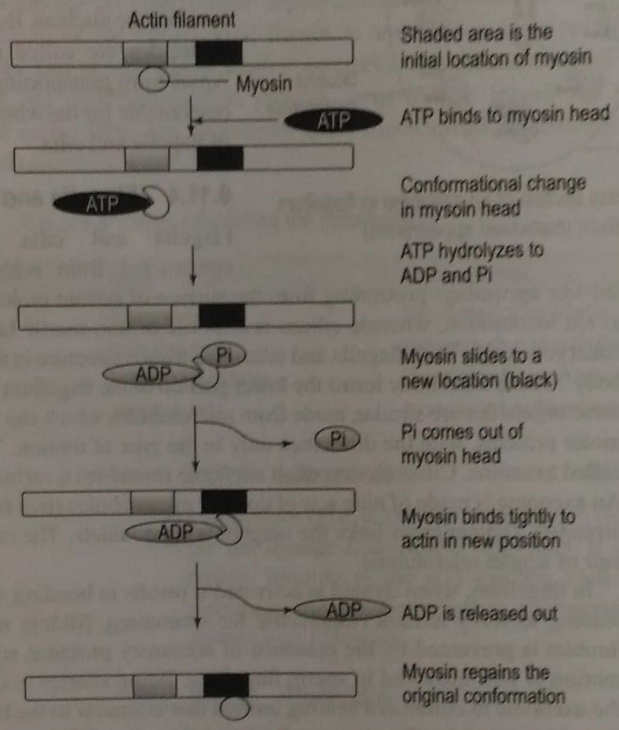

FIGURE 9.22 Steps involved in sliding movement of myosin over actin at the expense of ATP to cause muscle contraction

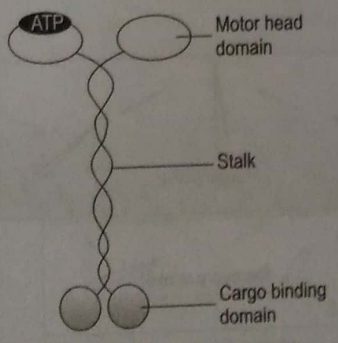

FIGURE 9.23 Structure of kinesin

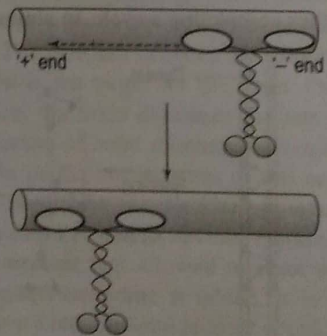

FIGURE 9.24 Association of kinesin with microtubule (dotted arrow indicates movement of kinesin towards the plus end of the tubule)

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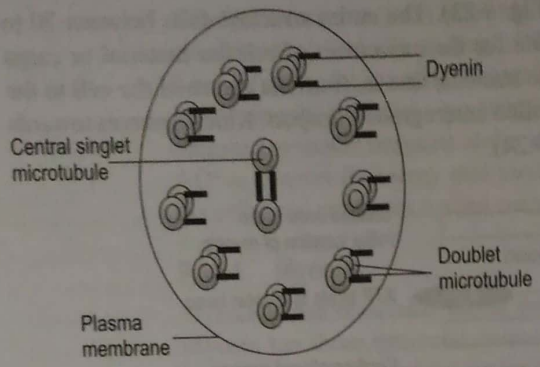

FIGURE 9.25 Cross section of an axenome in flagellum and cilium (cartwheel appearance)

9.11.3 Dynein

Dynein functions as a molecular motor and moves towards the minus end of the microtubules, which is usually directed towards the centre (nucleus) of the cell. Thus, dynein transports the cellular content towards the nucleus. Because of its function, dynein molecules are called minus-end-directed motors. Apart from transporting cargo in the cell, it is also responsible for the whipping and propelling motion of flagella and cilia.

9.11.4 Flagella and Cilia

Flagella and cilia are motility organelles constructed from microtubules. Flagellum is a tail-like appendage protruding from the surface of certain prokaryotic and eukaryotic cells to aid locomotion, whereas cilium is a motile or non-motile hair-like projection found in eukaryotic cells. Both flagella and cilia arise from a structure in the cell surface called 'basal body'. Thus, basal body forms the lower portion of the flagellum and cilia. Structurally, both these organelles are similar, made from microtubules which can be moved mechanically by motor proteins, with the difference only in the type of motion. The core of the structure is called axenome. Cross section of an axenome resembles a cartwheel as shown in Fig. 9.25. An axenome is made of nine sets of doublet microtubules (two fused microtubules) radially arranged. Dynein cross links the neighbouring doublets. The central portion is made of a pair of singlet microtubule.

In flagellum, when dynein is activated it results in bending of microtubule (Fig. 9.26), leading to wavy motion responsible for swimming. Sliding motion of the microtubule doublet is prevented by the presence of accessory proteins, such as nexin. This type of motion is well observed in sperm flagellum. Being shorter in comparison, the motion of the axenome in cilium is a beating motion that is similar to the breast stroke in swimming. Dynein generates a force perpendicular to the axis of the axenome which leads to a fast power stroke during which the fluid is driven over the surface of the cell, followed by a slow recovery stroke during which the cilium returns to the original position (Fig. 9.27), thus completing a cycle, in almost 0.1 to 0.2 s.

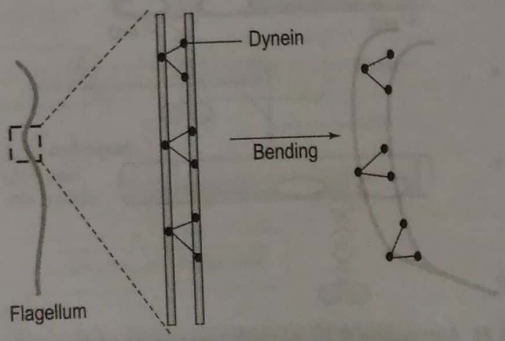

FIGURE 9.26 Role of dynein in bending of flagellum to create wavy motion

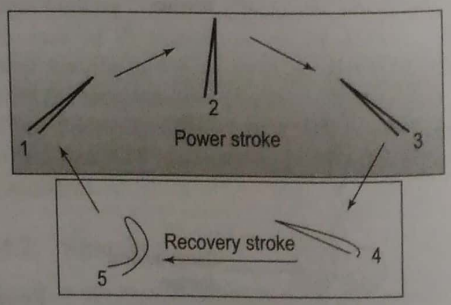

FIGURE 9.27 Bending of cilium by dynein to create beating motion

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9.12 DRUGS INFLUENCING CYTOSKELETON

Cytoskeletal filaments are targets for various drugs and toxins (Table 9.4). Two groups of drugs influence the polymerization process of cytoskeletal filaments. One group of drugs prevents polymerization and the other group leads to enhancement in polymerization. A balance between the polymerization–depolymerization processes is essential for the proper organization of the cytoskeletal filaments and proper functioning of the cells. Therefore, drugs and toxins which induce assembly and disassembly of filaments by polymerization and depolymerization process, respectively, impair the structure and function of the cells. Table 9.4 represents the various drugs influencing the filament assembly and disassembly.

TABLE 9.4 Drugs influencing the cytoskeleton

Drugs	Target	Mode of action
Lantrunculin	Actin monomers	Depolymerization of actin filaments
Phalloidin	Actin filaments	Polymerization of actin filaments
Colchicine	Tubulin heterodimer	Depolymerization of microtubules
Taxol	Microtubules	Increase in tubulin polymerization

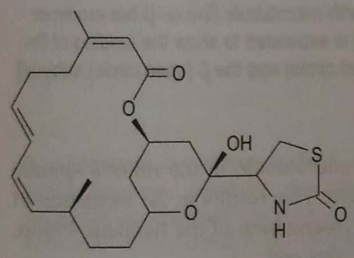

FIGURE 9.28 Structure of latrunculin

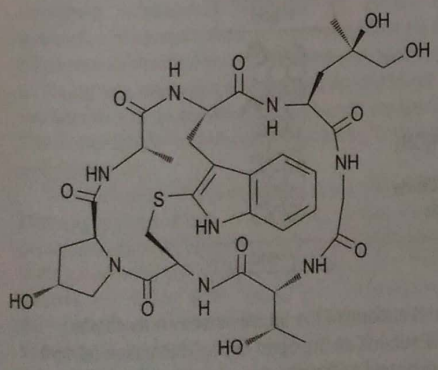

FIGURE 9.29 Structure of phalloidin

9.12.1 Latrunculin

Latrunculin (Fig. 9.28) is a natural toxin produced from the sea sponge *Latrunculia magnifica*. It is capable of rapidly binding to the ATP-binding cleft of the actin monomer and prevents further polymerization of the subunit into filaments.

9.12.2 Phalloidin

Phalloidin is a bicyclic, heptapeptide toxin produced by the fungus *Amanita phalloides*. The cyclic structure of phalloidin is shown in Fig. 9.29. Phalloidin binds to actin filament with much high affinity than free actin monomer. The interface between the monomeric unit on the filament is the target for the toxin. On binding to the interface, the toxin decreases the rate constant for the dissociation of actin monomers from the filament. This results in the stabilization of the actin filament. Affinity of phalloidin to actin filament is used to image the actin within live and fixed cells. Phalloidin is tagged with a fluorescent tag, allowed to react with the cell. When diaminobenzindine is added, it is oxidized by eosin to form a red-coloured product which can be made electron dense and viewed in the cytoplasm by electron microscopy.

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9.12.3 Colchicine

Colchicine is a secondary metabolite (Fig. 9.30) produced by the plants belonging to the genus Colchicum. It binds to the tubulin protein at the α–β interface, forming a tubulin–colchicine complex (Fig. 9.31). Consequently, the toxin copolymerizes into the microtubule lattice, suppressing microtubule dynamics, and prevents the polymerization of tubulin into microtubular filament. Also, higher concentration of colchicine causes depolymerization of microtubules. The net result is the inhibition of microtubule formation. As microtubule is essential for the spindle formation during mitosis and cell division, colchicine causes inhibition of mitosis. Hence, colchicine has the potential to function as an anti-cancer agent, but its toxicity matters.

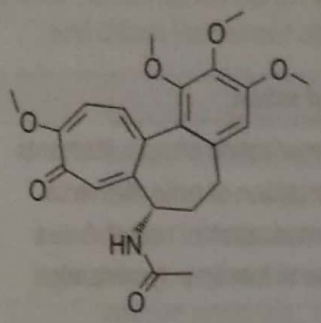

FIGURE 9.30 Structure of colchicine

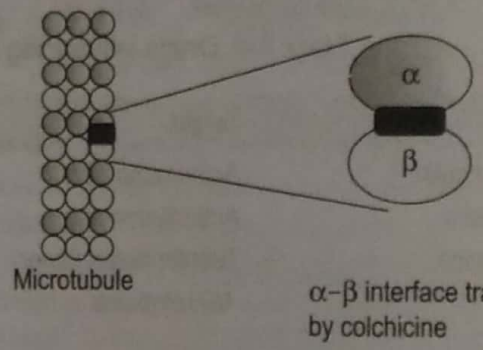

FIGURE 9.31 Interaction of colchicine with microtubule [the α–β het erodimer targeted by colchicine (black rectangle) is expanded to show the binding of the drug to the interface between α (shaded circle) and the β (open circle) subunit]

9.12.4 Vinblastine

Vinblastine (Fig. 9.32) binds to the plus end of the microtubule in the mitotic spindle and kinetochore present in the M phase of the cell cycle. This results in the formation of vinblastine–tubulin paracrystal, resulting in the depolymerization of the filament. Figure 9.33 shows the vinblastine bound to the tubulin at the plus end.

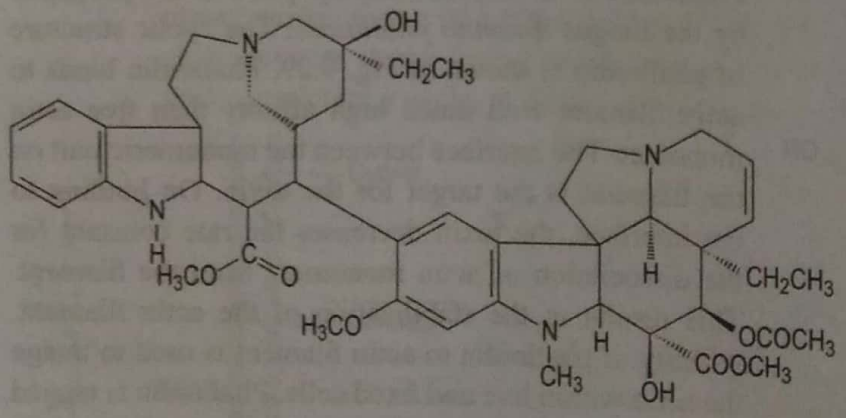

FIGURE 9.32 Structure of vinblastine

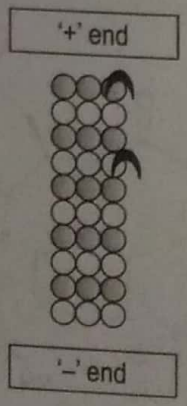

FIGURE 9.33 β subunit of the tubulin is shown as shaded circle and the α subunit as the open circle (vinblastine (shown as dark arc) bound to the microtubule at the plus end)

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9.12.5 Taxol

Taxol is a plant alkaloid (Fig. 9.34) which targets and binds to the pocket in the β tubulin on the inner surface of the microtubule filament. Drug binding counteracts the effects of GTP hydrolysis occurring on the other side of the monomer. This results in the stabilization of microtubule against depolymerization associated with an inhibition in the tubule dynamics (growth and shrinkage). As a result, the drug blocks the cell cycle by interfering with the G1 or M phase. Hence, this is used as an anti-cancer agent or anti-mitotic agent.

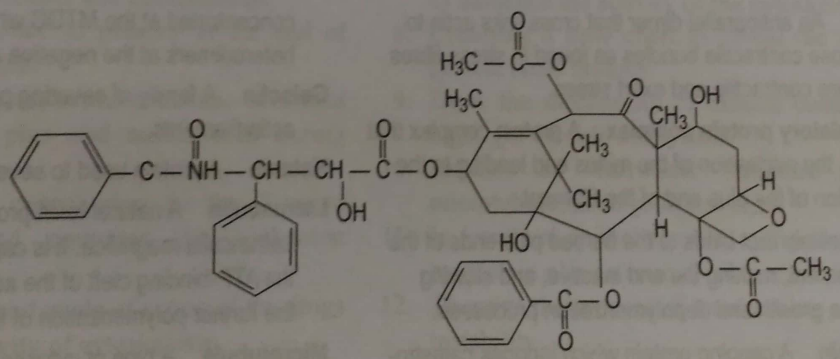

FIGURE 9.34 Structure of taxol

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