CYTOSKELETON: Structure, Function, Evolution

The cytoskeleton is unique to eukaryotic cells. It is a network of protein fibers supporting cell shape and anchoring organelles within the cell. The three main structural components of the cytoskeleton are microtubules (formed by tubulins), microfilaments (formed be actins) and intermediate filaments. All three components interact with each other non-covalently.It is a dynamic three-dimensional structure that fills the cytoplasm. This structure acts as both muscle and skeleton, for movement and stability. The long fibers of the cytoskeleton are polymers of subunits.

Cytoskeleton functions to:

- establishing cell shape
- providing mechanical strength
- locomotion
- chromosome separation in mitosis and meiosis
- intracellular transport of organelles

A cytoskeleton can **provide support and shape for a cell** like skeleton supports and shapes body. In a cell, a cytoskeleton can anchor a cell in one place or allow a cell to move. The parts of cells' cytoskeletons can also help structures move independently within cells. A cytoskeleton can position cell structures in specific places within the cell, or it can move cell structures from one end of the cell to the other.

A cell's cytoskeleton is comprised of three different types of fibers: microtubules, intermediate filaments, and microfilaments.

The cytoskeleton is the overall name given to protein filaments and motor proteins (also called molecular motors) in the cell. These protein filaments form an enormous three dimensional (3D) meshwork. Filaments can be cross linked to other similar filaments, and to

membranes, by means of accessory proteins. This inter-linking greatly increases rigidity. Some filaments are used s trackways for motor proteins to transport cargoes.

All cells, except those of most bacteria, contain components of the cytoskeleton.

They help the cell remain rigid but also help it move and change its shape when instructed to do so.

Components of the cytoskeleton also enable cilia, flagella and sperm to move, cell organelles to be moved and positioned, and muscles to function.

During cell division these components also assist by pulling the daughter chromosomes to opposite ‘poles’ in the dividing process.

Throughout the life of the cell various molecules and cargo containing vesicles are transported around the cell by **motor proteins**.

These move along the protein filaments using them as trackways rather like a railway locomotive runs on rail tracks.

In the early evolution of eukaryote cells the compartmentalisation of cell functions into membrane bounded structures, was accompanied by the evolution of a system that positioned and anchored them.

• This system therefore contributes to the architecture of the cell, its rigidity and in some cases to its ability to move.

• It also contributes by providing a physical transport system that enables cargo filled vesicles, some individual molecules, and even some cell organelles to be moved within the cell.

• The cytoskeleton is a dynamic entity with some cytoskeletal components being assembled and disassembled to meet the changing needs of the cell.

Mover - Shaper Concept in Cell

Shapers (protein filaments) come in three sizes

The variable shape and rigidity of the cell and its ability to move is largely dependent on three groups of cytoskeletal protein filaments:

- Microtubules – size: about 25nm external diameter
- Intermediate filaments – size: about 10nm external diameter
- Actin filaments – size: about 8nm external diameter

All three groups of protein filaments are polymers made up of protein sub-units.

Movers (motor proteins) come in three models

The most fascinating proteins that associate with the cytoskeleton are the molecular motors called motor proteins. These remarkable proteins bind to a polarized cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move steadily along it. Dozens of different motor proteins coexist in every eucaryotic cell. They differ in the type of filament they bind to (either actin or microtubules), the direction in which they move along the filament, and the "cargo" they carry. Many motor proteins carry membrane-enclosed organelles—such as mitochondria, Golgi stacks, or secretory vesicles—to their appropriate locations in the cell. Other motor proteins cause cytoskeletal filaments to slide against each other, generating the force that drives such phenomena as muscle contraction, ciliary beating, and cell division.

Cytoskeletal motor proteins that move unidirectionally along an oriented polymer track are reminiscent of some other proteins and protein complexes discussed elsewhere in this book, such as DNA and RNA polymerases, helicases, and ribosomes. All of these have the ability to use chemical energy to propel themselves along a linear track, with the direction of sliding dependent on the structural polarity of the track. All of them generate motion by coupling nucleoside triphosphate hydrolysis to a large-scale conformational change in a protein.

The cytoskeletal motor proteins associate with their filament tracks through a "head" region, or *motor domain,* that binds and hydrolyzes ATP. Coordinated with their cycle of nucleotide hydrolysis and conformational change, the proteins cycle between states in which they are bound strongly to their filament tracks and states in which they are unbound. Through a

mechanochemical cycle of filament binding, conformational change, filament release, conformational relaxation, and filament rebinding, the motor protein and its associated cargo move one step at a time along the filament (typically a distance of a few nanometers). The identity of the track and the direction of movement along it are determined by the motor domain (head), while the identity of the cargo (and therefore the biological function of the individual motor protein) is determined by the tail of the motor protein.

Molecules and cargo containing vesicles, and sometimes organelles, are moved around the cell by motor proteins. There are three main groups of motor proteins, all powered in an efficient way by adenosine triphosphate (ATP)

- Kinesin
- Dynein
- Myosin

Like all the components of the cell, members of the cytoskeleton work in conjunction with other parts of the cell as a dynamic whole.

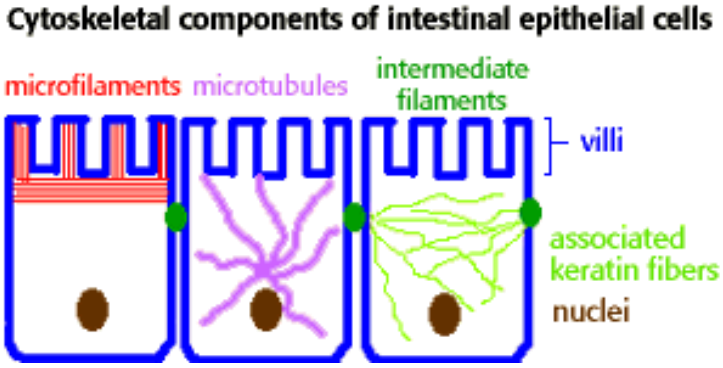
Examples of the cytoskeleton in epithelial cells

Microfilaments project into the villi, giving shape to the cell surface.

Microfilaments project into the villi, giving shape to the cell surface.

Microtubules grow out of the centrosome to the cell periphery.

Intermediate filaments connect adjacent cells through desmosomes.



Protein biogenesis/quality control pathways

1. Translation

    a. Following translation, nascent actin and tubulin undergo folding via:

    i. PFD (Prefoldin) folding pathway.

    1. Assisted by PFD and substrate delivery.

    ii. PFD-independent pathway.

2. Folding

    a. Both PFD and PFD-independent folding pathways lead to formation of CCT (cytosolic chaperonin).

    b. CCT-mediated folding follows, leading to:

    i. Formation of near-native alpha- and beta-tubulin.

    ii. Formation of near-native actin.

3. Assembly, Disassembly, and Polymerization

    a. Near-native alpha- and beta-tubulin assembles into a complex.

    i. Complex forms folded tubulin heterodimer.

    ii. Folded tubulin heterodimer polymerizes into a microtubule.

    b. Near-native actin is folded into a folded actin monomer.

    i. Folded actin monomer polymerizes into a microfilament.

4. Degradation

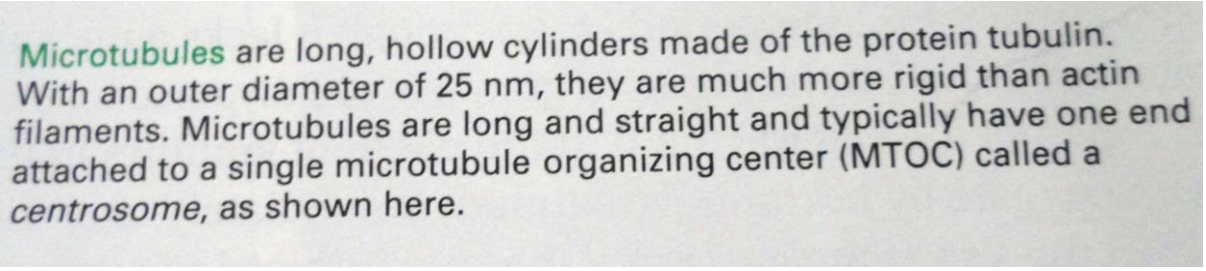
    a. Free tubulin and free actin not used in polymerization undergo ubiquitylation into proteasome.

    i. Proteasome is then degraded.

Degradation of cytoskeletal proteins

Part of the quality control process includes the degradation of proteins. Damaged proteins and misfolded proteins that cannot be refolded with the help of chaperones are removed in this process. Unfortunately, the pathway in which the degradation occurs is not as well studied as the biogenesis process.[

Microtubules



Microtubules are cylindrical tubes, 20-25 nm in diameter. They are composed of subunits of the protein tubulin--these subunits are termed alpha and beta. Microtubules act as a scaffold to determine cell shape, and provide a set of "tracks" for cell organelles and vesicles to move on. Microtubules also form the spindle fibers for separating chromosomes during mitosis. When arranged in geometric patterns inside flagella and cilia, they are used for locomotion.

Microtubule lengthening is brought about by the polymerisation of tubulin molecules, depolymerization brings shortening. Microtubules are assembled linearly from building blocks of tubulin molecules grouped into pairs called a dimer. Dimers are joined end-to-end by the process of polymerisation to form a linear polymer called a protofilament. Thirteen protofilaments lying in parallel are formed into a circular tube with the duct running down the middle.

Microtubule associated proteins (MAPs) in the cytoplasm regulate the lengthening and shortening process. They can bind to a microtubule at both its ends and along its length. In so doing MAPs can restrict lengthening and help attach microtubules to organelles and membranes within the cell.

Microtubules are very unstable and can disassemble very quickly. On first thoughts this would appear to be very inefficient but during mitosis and in circumstances that demand a rapid change of cell shape, quick disassembly and assembly are useful assets. This process is called ‘dynamic instability’ and is mainly directed and controlled by the chemical guanosine 5’ triphosphate (GTP).

Microtubules

- grow at the **plus end** by the polymerization of tubulin dimers (powered by the hydrolysis of GTP), and
- shrink by the release of tubulin dimers (depolymerization) at the same end.
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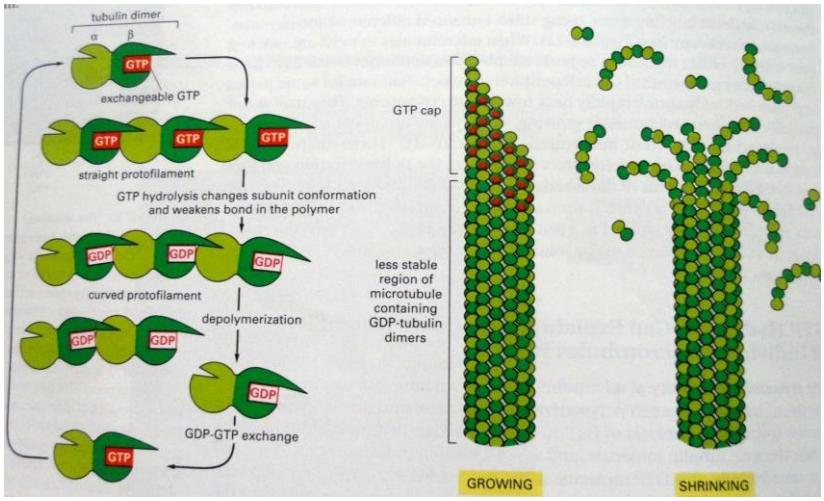


Most microtubules are attached to, and initially arise from, an organising centre (MTOC); in animal cells this is generally the centrosome. When cells divide the centrosome also divides. Microtubules are attached to the centrosome at their ‘minus’ end, the end that is slowest growing. Centrosomes often lie close to the cell nucleus and microtubules radiate from here in all directions towards the edge of the cell (plasma membrane).

The ‘plus’ end of the microtubule is furthest away from the centrosome. This is where microtubules rapidly lengthen or shorten in response to signals.

Plant cells do not have a centrosome and hence no single observable nucleation site from which new microtubules are produced.

In plant cells there are many small nucleation sites and these and the microtubules they initiate are located in the cell on the cytoplasm side of the plasma membrane and just below it. They are aligned parallel to one another but are closely interlinked to form a network layer running parallel to the plasma membrane throughout the cell.

Intriguingly plant cell microtubules can re-align themselves in response to chemical stimulation. Microtubules in cells near the root tip are found at right angles to the direction of the growth of the root. Further back from the root tip the microtubules rotate through a right angle and align themselves to become parallel to the direction of root growth. The importance of microtubules in plant cells should not be underestimated. Depolymerisation of microtubules causes cellulose to be laid down in a disorganised way. The root tip becomes a mass of such cells and although they expand they cannot elongate and the tissue grows in a distorted manner. Chemicals such as colchicine inhibit polymerisation and hence stop the production of microtubules. Some synthetic weedkillers bring about microtubule depolymerisation.

Function:

Microtubules function to support the cell shape, but they also help cell division. Microtubules can be used like train tracks in the cell along which vesicles and structures can be transported by riding across them. A fourth important function of microtubules is in cell movement. Some cells have flagella and cilia made of microtubules. Flagella are long, snake-like whips that drive cell movement. Sperm cells are an example of cells that have flagella, which allows them to swim. **Cilia** are multiple short, hair-like structures that beat to move liquid around a cell. Cilia are found in the cells that line your respiratory track, where they rhythmically beat to help you move mucous when you're sick. Both flagella and cilia are made with microtubule structures surrounded by a plasma membrane.

Microtubules participate in a wide variety of cell activities. Most involve motion. The motion is provided by protein "motors" that use the energy of ATP to move along the microtubule.

Microtubule motors

There are two motor proteins that assist organelles to move along the microtubules:

- Kinesin (most of these move toward the plus end of the microtubules), which moves things away from the nucleus.
- Dynein (which move toward the minus end), which moves things towards the nucleus.

Kinesin is a motor protein that moves along microtubules. It was first identified in the giant axon of the squid, where it carries membrane-enclosed organelles away from the neuronal cell body toward the axon terminal by walking toward the plus end of microtubules. Kinesin is similar structurally to myosin II in having two heavy chains and two light chains per active motor, two globular head motor domains, and an elongated coiled-coil responsible for heavy chain dimerization. Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the only common element. The yeast *Saccharomyces cerevisiae* has six distinct kinesins. The nematode *C. elegans* has 16 kinesins, and humans have about 40.

As in the myosin superfamily, only the motor domains are conserved. Conventional kinesin has the motor domain at the N-terminus of the heavy chain.

There are at least ten families of **kinesin-related proteins**, or **KRPs**, in the kinesin superfamily. Most of them have the motor domain at the N-terminus of the heavy chain and walk toward the plus end of the microtubule. A particularly interesting family has the motor domain at the C-terminus and walks in the opposite direction, toward the minus end of the microtubule. Some KRP heavy chains lack a coiled-coil sequence and seem to function as monomers, analogous to myosin I. Some others are homodimers, and yet others are heterodimers. At least one KRP (BimC) can self-associate through the tail domain, forming a

bipolar motor that slides oppositely oriented microtubules past one another, much as a myosin II thick filament does for actin filaments. Most kinesins carry a binding site in the tail for either a membrane-enclosed organelle or another microtubule. Many of the kinesin superfamily members have specific roles in mitotic and meiotic spindle formation and chromosome separation during cell division.

The dyneins are a family of minus-end-directed microtubule motors, but they are unrelated to the kinesin superfamily. They are composed of two or three heavy chains (that include the motor domain) and a large and variable number of associated light chains. The dynein family has two major branches (Figure 16-56). The most ancient branch contains the *cytoplasmic dyneins,* which are typically heavy-chain homodimers, with two large motor domains as heads. Cytoplasmic dyneins are probably found in all eucaryotic cells, and they are important for vesicle trafficking, as well as for localization of the Golgi apparatus near the center of the cell. *Axonemal dyneins,* the other large branch, include heterodimers and heterotrimers, with two or three motor-domain heads, respectively. They are highly specialized for the rapid and efficient sliding movements of microtubules that drive the beating of cilia and flagella. A third, minor, branch shares greater sequence similarity with cytoplasmic than with axonemal dyneins but seems to be involved in the beating of cilia.

Like myosin II and kinesin, cytoplasmic dynein is a two-headed molecule. The ciliary dynein shown has three head.

Dyneins are the largest of the known molecular motors, and they are also among the fastest: axonemal dyneins can move microtubules in a test tube at the remarkable rate of 14 μm/sec. In comparison, the fastest kinesins can move their microtubules at about 2–3 μm/sec.

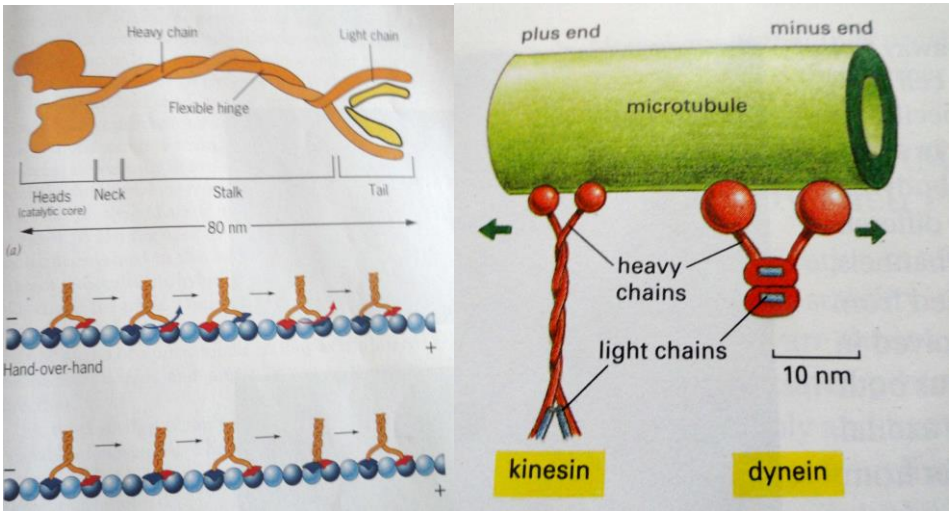
Some examples:

- The rapid transport of organelles, like vesicles and mitochondria, along the axons of neurons takes place along microtubules with their plus ends pointed toward the end of the axon. The motors are kinesins.
- The migration of chromosomes in mitosis and meiosis takes place on microtubules that make up the **spindle fibers**. Both kinesins and dyneins are used as motors.

- **Vincristine**, a drug found in the Madagascar periwinkle (a wildflower), binds to tubulin dimers preventing the assembly of microtubules. This halts cells in metaphase of mitosis.

- **Taxol®**, a drug found in the bark of the Pacific yew, prevents depolymerization of the microtubules of the spindle fiber. This, in turn, stops chromosome movement, and thus prevents the completion of mitosis.

Because the hallmark of cancer cells is uncontrolled mitosis, both vincristine and Taxol are used as anticancer drugs

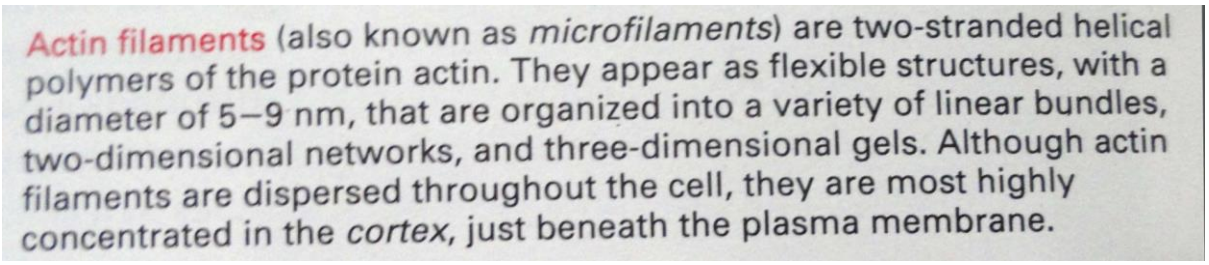


Kinesin. (*a*) Structure of a conventional kinesin molecule, which consists of (1) two heavy chains that wrap around each other as a coiled coil in the region of the stalk and (2) two light chains associated with the globular ends of the heavy chains. The force-generating heads bind to the microtubule, and the tail binds to the cargo being transported. Having a molecular mass of approximately 380 kDa, kinesin is considerably smaller than the other motor proteins, myosin (muscle myosin, 520 kDa) and dynein (over 1000 kDa). (*b*) Schematic diagram of a kinesin molecule moving along a microtubular track. Two alternate models are shown. In the hand-over-hand model, the two heads carry out identical but alternating movements, not unlike that of a person walking through a garden on a linear path of stepping stones. In the inchworm model, the leading head always advances to a new site on the track and the lagging head then catches up.

A major function of cytoskeletal motors in interphase cells is the transport and positioning of membrane-enclosed organelles. Kinesin was originally identified as the protein responsible for fast axonal transport, the rapid movement of mitochondria, secretory vesicle precursors, and various synapse components down the microtubule highways of the axon to the distant nerve terminals. Although organelles in most cells need not cover such long distances, their polarized transport is equally necessary. A typical microtubule array in an interphase cell is oriented with the minus ends near the center of the cell at the centrosome, and the plus ends extending to the cell periphery. Thus, centripetal movements of organelles toward the cell center require the action of minus-end-directed motor proteins such as cytoplasmic dynein, whereas centrifugal movements toward the periphery require plus-end-directed motors such as kinesins.

The role of microtubules and microtubule motors in the behavior of intracellular membranes is best exemplified by the part they play in organizing the endoplasmic reticulum (ER) and the Golgi apparatus. The network of ER membrane tubules aligns with microtubules and extends almost to the edge of the cell, whereas the Golgi apparatus is located near the centrosome. When cells are treated with a drug that depolymerizes microtubules, such as colchicine or nocodazole, the ER collapses to the center of the cell, while the Golgi apparatus fragments and disperses throughout the cytoplasm. *In vitro*, kinesins can tether ER-derived membranes to preformed microtubule tracks, and walk toward the microtubule plus ends, dragging the ER membranes out into tubular protrusions and forming a membranous web very much like the ER in cells. Likewise, the outward movement of ER tubules toward the cell periphery is associated with microtubule growth in living cells. Conversely, dyneins are required for positioning the Golgi apparatus near the cell center, moving Golgi vesicles along microtubule tracks toward minus ends at the centrosome.

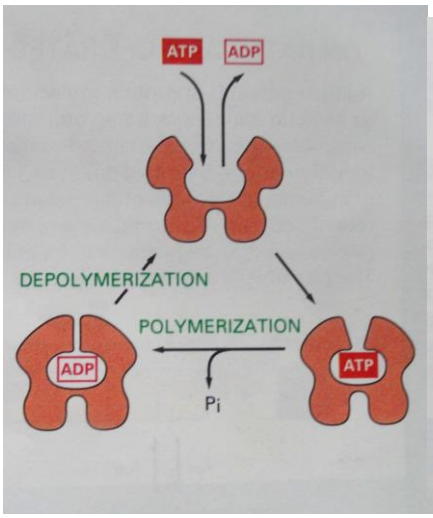
Microfilaments

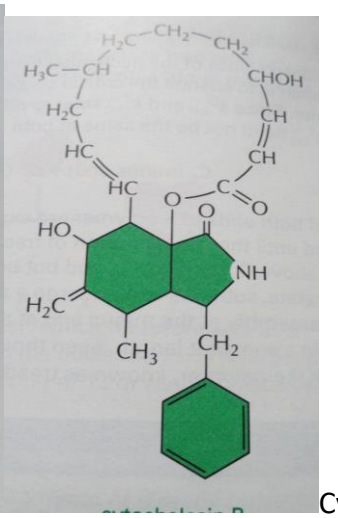

Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

Microfilaments are fine, thread-like protein fibers, 3-6 nm in diameter. They are composed predominantly of a contractile protein called actin, which is the most abundant cellular protein. Microfilaments' association with the protein myosin is responsible for muscle contraction. Microfilaments can also carry out cellular movements including gliding cntraction, and cytokinesis.

Monomers of the protein actin polymerize to form long, thin fibers. These are being the thinnest of the cytoskeletal filaments, are also called **microfilaments**. (In skeletal muscle fibers they are called "thin" filaments.) Some functions of actin filaments:

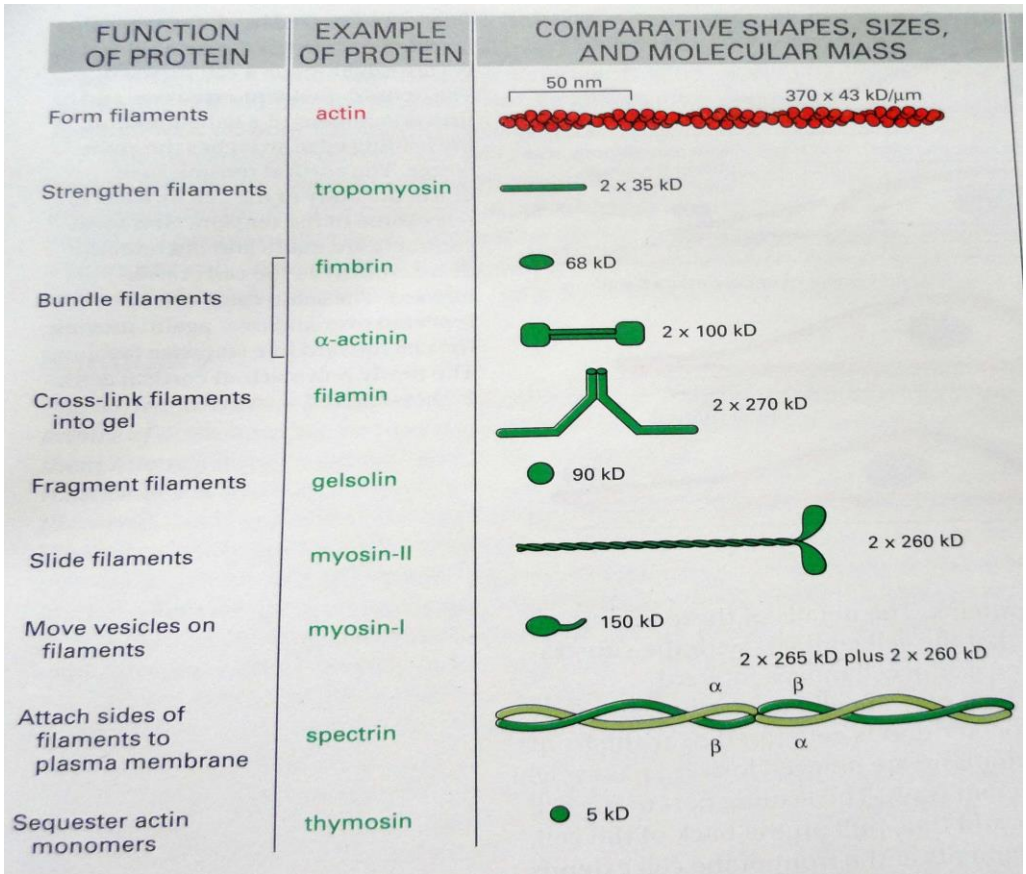
- form a band just beneath the plasma membrane that
    - provides mechanical strength to the cell
    - links transmembrane proteins (e.g., cell surface receptors) to cytoplasmic proteins
    - pinches dividing animal cells apart during cytokinesis
- generate cytoplasmic streaming in some cells
- generate locomotion in cells such as white blood cells and the amoeba
- interact with myosin ("thick") filaments in skeletal muscle fibers to provide the force of muscular contraction



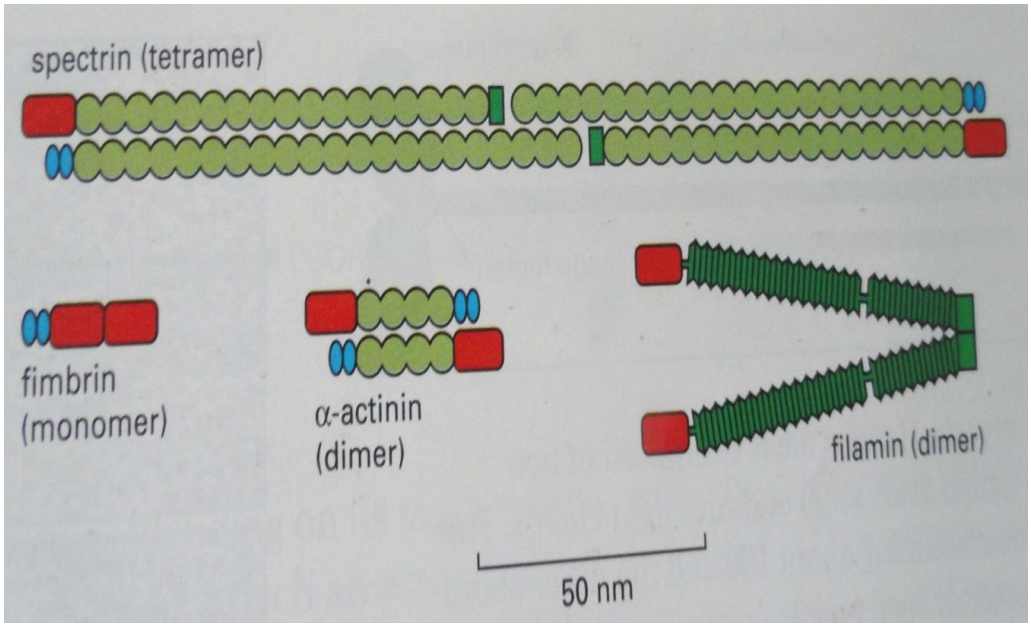


Cytochalasin B, depolymerizing agent

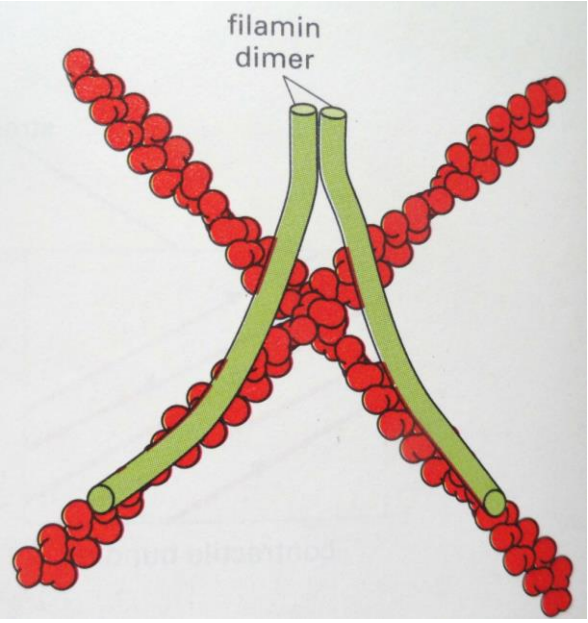
Actin Binding Proteins:

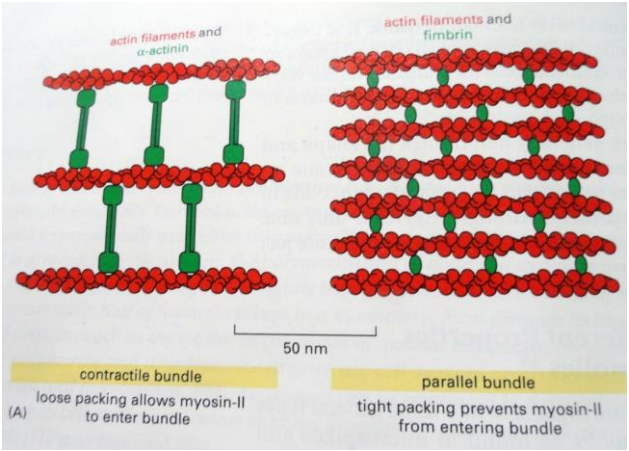


Pattern of interaction of Four Actin Binding Proteins



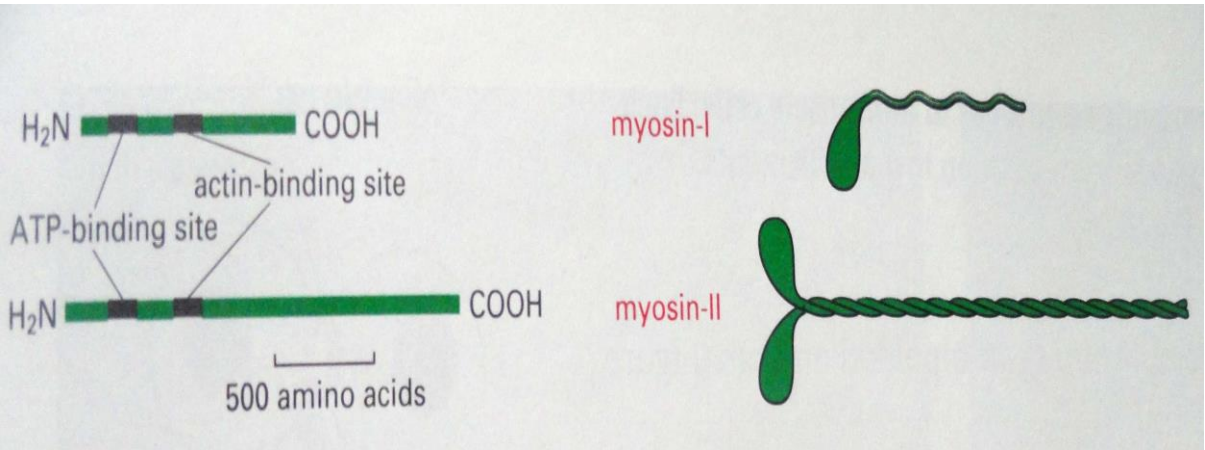
The modular structures of four actin-binding proteins. Each of the proteins shown has two actin-binding sites (*red*) that are related in sequence. Fimbrin has two directly adjacent actin-binding sites, so that it holds its two actin filaments very close together (14 nm apart), aligned with the same polarity (see Figure 16–66). The two actin-binding sites in α-actinin are more widely separated and are linked by a somewhat flexible spacer 30 nm long, so that it forms actin filament bundles with a greater separation between the filaments (40 nm apart) than does fimbrin. Filamin has two actin-binding sites that are very widely spaced, with a V-shaped linkage between them, so that it cross-links actin filaments into a network with the filaments oriented almost at right angles to one another (see Figure 16–67). Spectrin is a tetramer of two α and two β subunits, and the tetramer has two actin-binding sites spaced about 200 nm apart. The spacer regions of these various proteins are built in a modular fashion from repeating units that include α-helical motifs (*light green*), β-sheet motifs (*dark green*), and Ca^{2+}-binding domains (*blue ovals*).

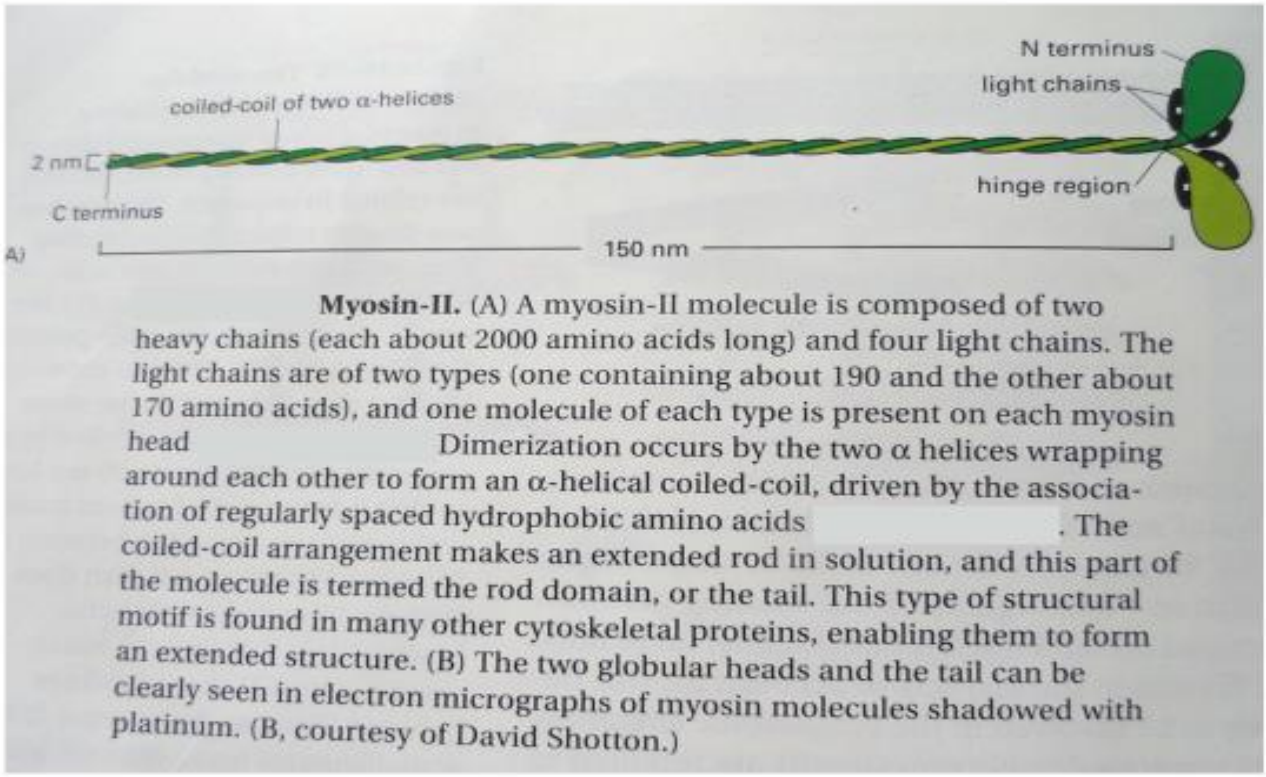




Filamin cross-links actin filaments into a three-dimensional network with the physical properties of a gel. Each filamin homodimer is about 160 nm long when fully extended and forms a flexible, high-angle link between two adjacent actin filaments. Filamin can constitute 1% of the cell protein, or about one molecule per 50 actin monomers.

Actin based motor proteins





Myosin-II. (A) A myosin-II molecule is composed of two heavy chains (each about 2000 amino acids long) and four light chains. The light chains are of two types (one containing about 190 and the other about 170 amino acids), and one molecule of each type is present on each myosin head Dimerization occurs by the two α helices wrapping around each other to form an α-helical coiled-coil, driven by the association of regularly spaced hydrophobic amino acids . The coiled-coil arrangement makes an extended rod in solution, and this part of the molecule is termed the rod domain, or the tail. This type of structural motif is found in many other cytoskeletal proteins, enabling them to form an extended structure. (B) The two globular heads and the tail can be clearly seen in electron micrographs of myosin molecules shadowed with platinum. (B, courtesy of David Shotton.)

Actin-based Motor Proteins Are Members of the Myosin Superfamily

The first motor protein identified was skeletal muscle **myosin**, which is responsible for generating the force for muscle contraction. This myosin, called myosin II is an elongated protein that is formed from two heavy chains and two copies of each of two light chains. Each of the heavy chains has a globular head domain at its N-terminus that contains the force-generating machinery, followed by a very long amino acid sequence that forms an extended coiled-coil that mediates heavy chain dimerization. The two light chains bind close to the N-terminal head domain, while the long coiled-coil tail bundles itself with the tails of other myosin molecules. These tail-tail interactions result in the formation of large bipolar "thick filaments" that have several hundred myosin heads, oriented in opposite directions at the two ends of the thick filament.

Each myosin head binds and hydrolyses ATP, using the energy of ATP hydrolysis to walk toward the plus end of an actin filament. The opposing orientation of the heads in the thick filament makes the filament efficient at sliding pairs of oppositely oriented actin filaments past each other. In skeletal muscle, in which carefully arranged actin filaments are aligned in "thin filament" arrays surrounding the myosin thick filaments, the ATP-driven sliding of actin filaments results in muscle contraction. Cardiac and smooth muscle contain myosins that are similarly arranged, although they are encoded by different genes.

When a muscle myosin is digested by chymotrypsin and papain, the head domain is released as an intact fragment (called S1). The S1 fragment alone can generate filament sliding in vitro, proving that the motor activity is contained completely within the head.

It was initially thought that myosin was present only in muscle, but in the 1970's, researchers found that a similar two-headed myosin protein was also present in nonmuscle cells, including protozoan cells. At about the same time, other researchers found a myosin in the freshwater amoeba *Acanthamoeba castellanii* that was unconventional in having a motor domain similar to the head of muscle myosin but a completely different tail. This molecule seemed to function as a monomer and was named myosin I (for one-headed); the conventional myosin was renamed myosin II (for two-headed).

Subsequently, many other myosin types were discovered. The heavy chains generally start with a recognizable myosin motor domain at the N-terminus, and then diverge widely with a variety of C-terminal tail domains. The new types of myosins include a number of one-headed and two-headed varieties that are approximately equally related to myosin I and myosin II, and the nomenclature now reflects their approximate order of discovery (myosin III through at least myosin XVIII). The myosin tails (and the tails of motor proteins generally) have apparently diversified during evolution to permit the proteins to dimerize with other subunits and to interact with different cargoes.

Some myosins (such as VIII and XI) have been found only in plants, and some have been found only in vertebrates (IX). Most, however, are found in all eucaryotes, suggesting that myosins arose early in eucaryotic evolution. The yeast *Saccharomyces cerevisiae* contains five myosins: two myosin Is, one myosin II, and two myosin Vs. One can speculate that these three types of myosins are necessary for a eucaryotic cell to survive and that other myosins perform more specialized functions in multicellular organisms. The nematode *C. elegans*, for example, has at least 15 myosin genes, representing at least seven structural classes; the human genome includes about 40 myosin genes.

All of the myosins except one move toward the plus end of an actin filament, although they do so at different speeds. The exception is myosin VI, which moves toward the minus end.

The exact functions for most of the myosins remain to be determined. Myosin II is always associated with contractile activity in muscle and nonmuscle cells. It is also generally

required for cytokinesis, the pinching apart of a dividing cell into two daughters, as well as for the forward translocation of the body of a cell during cell migration. The myosin I proteins contain a second actin-binding site or a membrane-binding site in their tails, and they are generally involved in intracellular organization and the protrusion of actin-rich structures at the cell surface. Myosin V is involved in vesicle and organelle transport. Myosin VII is found in the inner ear in vertebrates, and certain mutations in the gene coding for myosin VII cause deafness in mice and humans.

Intermediate filaments

Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and carrying the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell-cell junction to another.

Major types of Intermediate Filaments

Major Types of Intermediate Filament Proteins in Vertebrate Cells

Type of IF	Component Polypeptides (mass in daltons)	Cellular Location
Nuclear lamins	lamins A, B, and C (65,000–75,000)	nuclear lamina of eucaryotic cells
Vimentinlike proteins	vimentin (54,000)	many cells of mesenchymal origin, often expressed transiently during development
	desmin (53,000)	muscle
	glial fibrillary acidic protein (50,000)	glial cells (astrocytes and Schwann cells)
	peripherin (66,000)	neurons
Keratins	type I (acidic) (40,000–70,000) type II (neutral/basic) (40,000–70,000)	epithelial cells and their derivatives (e.g., hair and nails)
Neuronal intermediate filaments	neurofilament proteins NF-L, NF-M, and NF-H (60,000–130,000)	neurons

Intermediate filaments have a diverse role in structure and support within the cell. One specialized group of intermediate filaments has an important role in the nucleus, where they can make up the nuclear lamina. Here they are essentially the cytoskeleton of the nucleus, specifically supporting a structure of your cells like the bones supporting your hands. Here, intermediate filaments provide support to the nuclear lamina and aid in regulating nuclear

processes. They also extend from the nuclear envelope, helping the nucleus keep its position within the cell.

The evolution of cytoskeletal proteins

The evolution of cytoskeletal proteins required novel biogenesis machinery. The cytoskeleton in eukaryotes enhances intracellular trafficking and cell division. These functions were once believed to be the distinguish elements of eukaryotes from prokaryotes because bacterial cytoskeleton also composed of proteins that similar to actin and tubulin. The actin-like and tubulin-like proteins in bacteria form filamentous structures which imply in the division of genetic material and maintenance of cell shape.[2] However, actins and tubulins in eukaryotes are distinct from similar prokaryotic proteins in the way that they maintain inovative properties which is critical for eukaryogenesis (the origin of the eukaryotic condition/the evolution of the eukaryotic condition).[3]

Both eukaryotes and bacteria have a cytoskeleton. The bacterial proteins homologous to actin are MreB and ParM, and the bacterial proteins homologous to tubulin are FtsZ. However, actin and tublin differ from MreB, ParM, and FtsZ in the sense that they have properties important for eukaryogenesis.

Actins and tubulins in eukaryotes formed microfilaments and microtubules which unite with their complementary molecular motors (myocin, kinesin, dynein) to be used for phagocytosis. Phagocytosis enables endosymbiosis and also cilia's development which supports mobility and sensory. The inception of actins and tubulins in eukaryotes is an important factor in facilitating their efficient folding and assembly which is called cytoskeletal protein biogenesis machinery (CPBM). The CPBM includes molecular chaperones which assist folding of chaperonin containing tailless complex polypeptide-1 (CCT), prefoldin (PFD) - phosducin-like proteins that regulate CCT functions, and five other cofactors. In addition, post-translational modifications and proteasomal degradation are required in regulating the function of actins and tubulins which is also unique to eukaryotes.

On the other hand, in prokaryotes, the cytoskeletal protein biogenesis machinery is absent.

Autoregulation of cytoskeletal protein synthesis

Actin and tubulin concentrations are strickly controlled due to their critical effects on cytoskeletal dynamics. In animal cells, tubulin synthesis is regulated by an autoregulatory feedback mechanism that can sense the concentration of tubulin heterodimer in order to regulate the stability of α-tubulin, and β-tubulin mRNAs. Similar to animals, the synthesis of tubulin in metazoans is also autoregulated due to its critical influences. Researches have shown that overexpression of β-tubulin in Saccharomyces cerevisiae leads to abnormal microtuble function and slow growth.

Actin overexpression causes by an incompletely characterized feedback mechanism that is sensitive to the concentration of actin monomers is preventable by the presence of the 3' untranslated region of actin mRNA.

Biogenesis of cytoskeletal proteins

The cytoskeleton protein biogenesis machinery is found in eukaryotes but not in prokaryotes. It includes chaperonin containing CCT (complex polypeptide-1) and PFD (prefoldin), phosductin-like proteins, and five cofactors. The chaperonin molecules help actin and tubulin proteins to fold. Challenges to the protein biogenesis machinery include:

(1) the high concentrations of actin and tubulin in cells,

(2) the tendency of actin and tubulin to self-associate,

(3) the inability of actin and tubulin to fold without the help of other molecules,

(4) the fact that actin and tubulin compete with one another for the same folding space.

Biogenesis of cytoskeletal proteins faces a lot of difficulty which mainly due to the abundant of actin and tubulin concentrations, self-associate tendency, and the inability in folding independently. In addition, the competing for access to limited folding space is also a challenge to biogenesis of cytoskeletal proteins.[16] Fortunately, the action of specific chaperonin cofactors can account for the regulation of chaperonin-mediated cytoskeletal protein folding.

Eukaryotic cytosolic chaperonin

Eukaryotic cytosolic chaperonin is a unique ability to assist the folding of actin and tubulin. Molecular chaperones can interact with the newly synthesized polypeptide chains to become stable during the folding process form a linear monomer amino acids chain into a more complex and functional protein. There are many types of chaperones that direct the folding of new proteins, refolding of stress-denatured proteins, unfolding of proteins, and transporting proteins.

Chaperonin, an important family of molecular chaperones, has a barrel-like structure with two multimeric stacked rings of 60kDa (Dalton's atomic mass unit). Chaperonins undergo ATP-dependent conformational changes during its folding cycle: facilitate substrate binding, encapsulation and release. In eukaryotes, the cytosolic chaperonin is the tailless complex polypeptide 1 ring complex (CCT) which is required for viability in yeast and worms.

CCT is crucial for the biogenesis of actin and tubulin. CCT is composed of eight related subunits - α, β, ɣ, δ, ε, j, ŋ, θ - which show up twice in each oligomer. CCT is closely related to archaeal chaperonin thermosome but quite different from bacterial chaperonin GroEL. CCT is known to have a more specific binding profile than bacterial GroEL. As a chaperonin, CCT undergoes ATP-dependent conformational changes during its folding cycle. Due on the copiousness of actin and tubulin, they occupy significant proportion of CCT complexes. Beside actin and tubulin, there are other CCT substrates also have key roles in progression of the cell cycle. Researches show that CCT function in vivo is regulated by several dedicated cofactors, including PFD and phosducin-like proteins.

PFD, a jellyfish-shaped molecular chaperone required for stabilization of new cytoskeletal proteins, is a CCT co-chaperone for the biogenesis of actin and tubulin.

Phosducin-like proteins, regulators of the folding of actin and tubulin in association with CCT, are thioredoxin domain-containing proteins with homology to phosducin, a regulator of retinal G-protein signaling.

Tubulin folding cofactors

Scientists believed that actin is released from CCT in a native, assembly-competent state, but cyclase associated protein might interact with and stabilize near-native or unstable forms of actin in close association with the chaperonin [28]. On the other hand, functional tubulin is an obligate α-β heterodimer, and evolved in a folding pathway linked to dimer assembly[29].

Roles of each cofactor:

1. Tubulin cofactor A (TBCA): collects unassembled beta-tubulin
2. Tubulin cofactor D (TBCD): assists beta-tubulin down the assembly pathway
3. Tubulin cofactor B (TBCB): binds to alpha-tubulin after chaperonin release
4. Tubulin cofactor E (TBCE): receives alpha-tubulin from TBCB and processes it further
5. Tubulin cofactor C (TBCC): promotes GTP hydrolysis in beta-tubulin if in the presence of a stable supercomplex (formed by the joining of beta-tubulin-TBCD and alpha-tubulin-TBCE complexes)

    a. also facilitates the release of native alpha-beta-tubulin heterodimer

Diseases linked to the cofactors:

It is believed that if TBCB is not degraded properly, a neurological disease called giant axonal neuropathy may result. This disease is related to decreased density of the microtubule of cells. Mutations of TBCE are associated with hypoparathyroidism (a developmental disorder), mental retardation, and facial dysmorphism (HRD).

PFD

Prefoldin (PFD) is a CCT co-chaperone required for stabilizing nascent cytoskeletal proteins. It consists of two alpha-type and four beta-type subunits which collectively make its structure resemble the shape of a jellyfish. The six subunits form a cavity shaped like a rectangle that attaches to newly formed actin and tubulin as the chains leave the ribosome. PFD then delivers the actin and tubulin to CCT, probably via a docking and substrate-release mechanism (supported by electron microscopy analysis of PFD-actin complexes). It is also possible that PFD improves the efficiency of actin and tubulin protein folding by navigating partially folded molecules back towards the CCT for more folding. This idea is supported by

the fact that yeast cells without PFD were observed to fold actin and tubulin more slowly than wild-type cells. Furthermore, Pfd1 knockout mice showed signs of dysfunction of cytoskeletal proteins, neuronal loss, neuromuscular defects, and defective development of lymphocytes. These Pfd1 knockout mice were only viable for five weeks.

Phosducin-like proteins

Phosducin-like proteins regulate the folding of actin and tubulin. Three such proteins have been termed PhLP1, PhLP2, and PhLP3 as they are CCT-binding proteins. PhLP1 assists in assembling heterotrimeric G-proteins by CCT; this process is regulated by PhLP1 phosphorylation. PhLP2 and PhLP3 are involved in the biogenesis of cytoskeletal proteins. It is believed that there is a specificity of PhLP2 for actin biogenesis and of PhLP3 for tubulin biogenesis. In other words, disruption of PhLP2 function caused severe actin cytoskeletal defects whereas disruption of PhLP3 function alters normal tubulin biogenesis.

When studied in vitro, it is observed that an excess of PhLP2 and PhLP3 prevents actin and tubulin from being folded via the CCT-mediated folding pathway. It is believed that this is due to the reduced activity of CCT ATPase. However, when the study in done on yeast cells, it appears that PhLP2 stimulates actin folding by purified yeast CCT. The researchers of this study concluded that amino acids of mammalian PhPL2 that were not present in yeast PhLP2 are responsible for preventing actin and tubulin from being folded. This also supports the idea that higher eukaryotes evolved with more regulation of cytoskeletal proteins.

Protein biogenesis/quality control pathways

1. Translation
    a. Following translation, nascent actin and tubulin undergo folding via:
    i. PFD folding pathway.
    1. Assisted by PFD and substrate delivery.
    ii. PFD-independent pathway.
2. Folding

a. Both PFD and PFD-independent folding pathways lead to formation of CCT (cytosolic chaperonin).

b. CCT-mediated folding follows, leading to:

i. Formation of near-native alpha- and beta-tubulin.

ii. Formation of near-native actin.

3. Assembly, Disassembly, and Polymerization

a. Near-native alpha- and beta-tubulin assembles into a TBCE-TBCD complex.

i. Complex forms folded tubulin heterodimer.

ii. Folded tubulin heterodimer polymerizes into a microtubule.

b. Near-native actin is folded into a folded actin monomer.

i. Folded actin monomer polymerizes into a microfilament.

4. Degradation

a. Free tubulin and free actin not used in polymerization undergo ubiquitylation into proteasome.

i. Proteasome is then degraded.

Post-translational modification (PMT) of cytoskeletal proteins

For actin, post-translational modification is known to affect only folding. Tubulin, on the other hand, is affected by PTM in such a way that allows native proteins to turn on and off activity in a reversible and regulatory manner. Tubulin can be modified in a number of ways such as acetylation, detyrosination, and glutamylation. These tubulin modifications occur on microtubules, and it has been hypothesized (though not well-tested) that the free tubulin heterodimer is the substrate responsible for reversing the modifications. Much study, however, has been dedicated to Tubulin PTMs in general. Tubulin acetylation, for example, has been shown in recent studies to be linked to a human neurodegenerative disease called amytrophic lateral sclerosis (ALS).

Degradation of cytoskeletal proteins

Part of the quality control process includes the degradation of proteins. Damaged proteins and misfolded proteins that cannot be refolded with the help of chaperones are removed in this

process. Unfortunately, the pathway in which the degradation occurs is not as well studied as the biogenesis process.

Turn-over rates and steady-state concentrations of all cellular proteins is regulated in the following ways:

1. protein degradation through the ubiquitin-proteasome system (UPS)

2. lysosomal degradation

3. another proteolytic mechanism

“Proteostasis” is the regulatory process in which damaged or incorrectly folded proteins that cannot be repaired by charperones are removed by proteolysis. Not much work has been put into the study of actin and tubulin degradation, but tubulin has been known to rapidly degrade in the presence of microtubule-destabilizing drugs such as colcemid. Such drugs make tubulin more soluble.

Parkin

Parkin is a ubiquitin-protein ligase important to tubulin degradation. Parkin is mutated in patients with autosomal recessive juvenile Parkinson disease (PD). Its normal function is to interact with HSP70-interacting protein (CHIP) to make stress-denatured proteins undergo ubiquitylation. Parkin is also believed to stimulate ubiquitylation and proteasomal degradation of alpha-tubulin and beta-tubulin. Cells that over-express mutant alpha-synuclein, a toxic inclusion-forming protein in Parkinson disease, reveal increased concentrations of alpha-tubulin and insoluble parkin. These two traits are also observed in patients affected by Lewy body disease.

Cofactor E-like

E-like (COEL), a tubulin folding cofactor, is a protein that destabilizes tubulin. Cells without human COEL contain excess amount of stable microtubules while microtubule disassembly

and the degradation of α-tubulin is observed in cells with excess COELs. The degradation of tubulin caused by the presence of COEL is countered by a negative regulator of microtubule called stathmin that isolates tubulins. Overall, COEL is important for three reasons: 1. it can remove misfolded tubulin 2. regulate the concentration of tubulin 3. control tubulin isotype.

Actin Degradation

In metazoan cells, the concentration of tubulin is reduced when CCT or PFD does not function properly. The concentration of actin, however, is not affected significantly. This suggests that the quality control for actin differs from that of tubulin. Relative to the control tubulins, there seems to be less of a need to remove misfolded β-actins. Still, there are incidences when actin degradation is necessary. In the case of ischemic oxidative damage, α-actin specific to the heart is degraded by proteasome. It is also found that α-actins are degraded by lysosomes when muscle contraction in cardiomyocytes is inhibited by the use of drugs. It has been recently observed that TRIM32, an ubiquitin that ubiquitylates α-actin in vitro, when unusually expressed in the human embryonic kidney cells, reduces the concentration of cytoplasmic β-actins. TRIM32 mutations have been found in Bardet-Biedl syndrome and muscular dystrophy, although the actual role TRIM32 plays in these diseases remains unknown.