Cytoskeleton III: Microfilament

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Lesson: Cytoskeleton III: Microfilament
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Introduction

Microfilaments also known as actin filament are the cytoskeletal elements which are 7 nm in diameter and composed of globular protein **actin** which is one of the most abundant intracellular protein found in eukaryotic cells. Actin was first isolated in 1942 and accounts for 1-5 % of the total cell protein in non-muscle cells and 10 % in muscle cell. Actin has molecular weight of 43 Kd and is found to be highly conserved (Yeast and mammals share 90% similarity in their amino acid sequence). Organisms like yeast and amoeba have two actin genes, humans have six genes coding for isoforms of actin. Out of six isoforms, four isoforms designated as α-actin are found in muscle cells while two isoforms β- and γ – are found in non-muscle cells. Plants have more than sixty actin genes which are mainly pseudogenes.

Actin exists in two forms: monomeric form known as **Globular** or **G actin** and polymeric form known as **Filamentous** or **F actin** (Fig. 1).

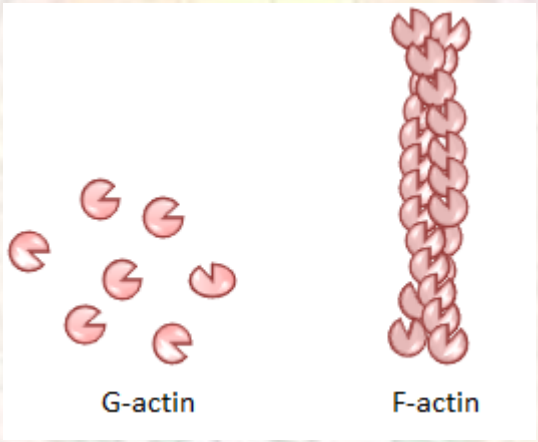

Fig. 1: Two forms of actin: monomeric G-actin and Filamentous or F-actin formed by polymerization of G-actin. **Source: Authors**

The G-actin contains two lobes which are separated by a cleft having a Mg^{2+} ion complexed with either ATP or ADP. Soon after polymerization the ATP bound to G-actin is hydrolyzed to ADP. So the filament mostly comprises of ADP–F-actin and some amount of ATP–F-actin found at the end. However, hydrolysis of ATP is not required for polymerization. Each G-actin is rotated by 166^{o} in the filament giving the appearance like double-stranded helix. All monomers in the F-actin are oriented in the same direction, giving the microfilaments polarity. The ends of the actin filaments were identified in the decoration experiments. These experiments are based on the ability of myosin to bind preferentially to actin. Purified myosin is obtained and cleaved by proteolytic enzyme to obtain fragments. One such fragment S1 when mixed with actin filaments coats the actin filament with arrowheads pointing at one end of the filament. This end is known as pointed end (also called "minus" end) and other end is known as barbed end (also called "plus" end). The plus end or barbed end elongates 5–10 times faster as compared to the minus or pointed end.

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Microfilament assembly and disassembly

The actin polymerization studies done in vitro suggest that the addition of ions like Mg^{2+}, K^{+} induces the polymerization of G- actin to F-actin. If the ionic strength is lowered, there is depolymerization of F-actin to G-actin making it as reversible process. In vitro polymerization of G- actin to F-actin involves three distinct phases:

1. **Nucleation:** It is the slowest step in the formation of an actin filament and requires the monomers to come in proper orientation (Fig. 2). During this phase, G-actin aggregates into dimers and trimers which act as a stable seed for polymerization.

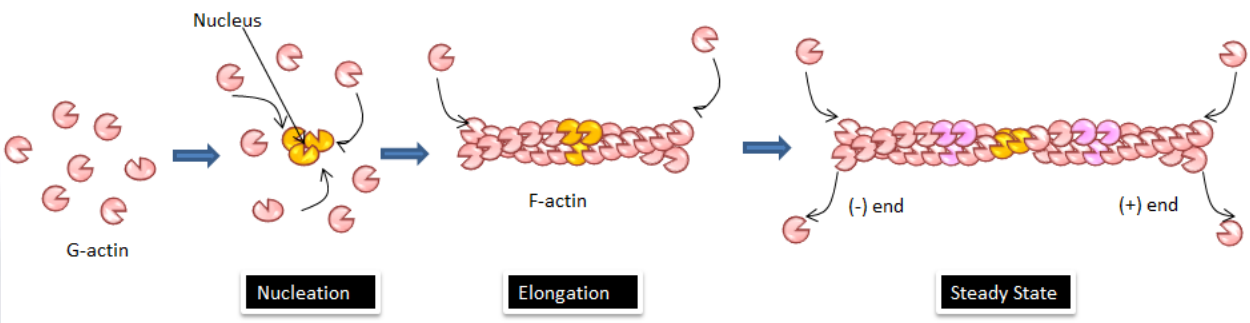

Fig. 2: Assembly of Actin filaments from G-actin occurs in three steps: nucleation, elongation and termination. **Source: authors**

2. **Elongation:** Once the seed or nucleus is formed, it rapidly grows by the addition of actin monomers to both the ends. As the elongation continues there is decline in the free monomer concentration. During this, the monomers keep on adding to the barbed or plus end and there is continued loss of the subunits from pointed or minus end.
3. **Steady-state:** If the concentration of monomers decreases further, the addition of the monomers to the barbed or plus end of is balanced by their dissociation from the pointed or minus end. During this phase, an equilibrium is reached between filaments and monomers.

Under steady state, the concentration of the monomers present is called the **critical concentration (C_c)**. It is therefore defined as the concentration of G-actin when the addition of the monomers to the filament is balanced by their dissociation from the filament. So, at the concentration higher than C_c, there is polymerization of G-actin to F-actin and below C_c, there is depolymerization of F-actin to G-actin. So under steady-state, the filament exhibit **treadmilling** where monomers added at plus or barbed end migrate through the filament to reach the minus or pointed end from where they are dissociated from the filament (Fig. 3). There is subsequent hydrolysis of ATP to ADP and before reincorporation, the monomers exchange ATP or ADP.

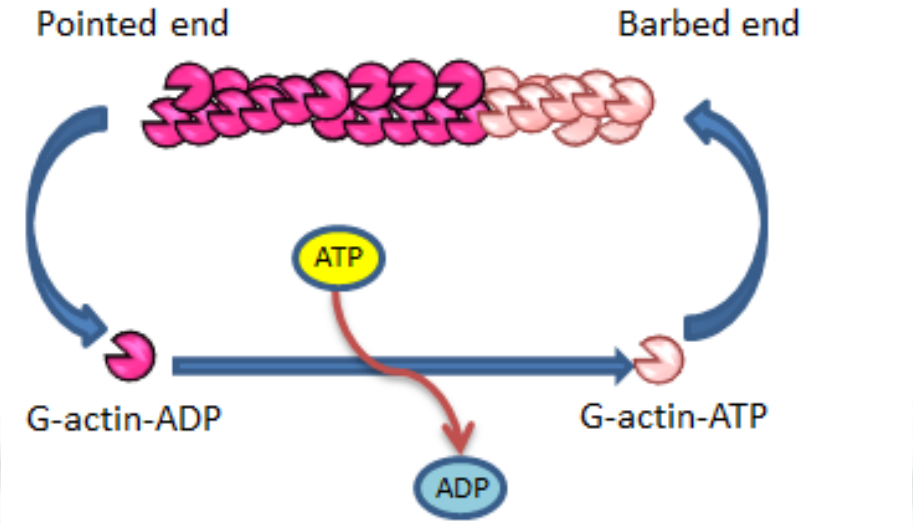

Fig. 3: Treadmilling in actin filaments. The monomers are added from barbed end removed from pointed end. There is no change in the overall length of the filament. **Source: authors**

As already mentioned, the plus end or barbed end elongates 5–10 times faster as compared to the minus or pointed end. This is due to the difference in the critical concentration (C_c) at the two ends of the filament which can be about six times lower for polymerization at the plus or barbed end than for addition at the minus or pointed end. So, if the concentration of ATP-G-actin is below C_c for plus end, no filament growth is seen. If the concentration of ATP-G-actin is between C_c for plus and minus end, filament grows only at the plus end. At the concentration of ATP-G-actin higher than C_c for minus end, filament grows by addition of subunits at both the end. At ATP-G-actin intermediate between plus and minus end, steady state is reached.

Do you know??

A number of naturally occurring toxins have been identified which are known to affect the stability of actin filaments. These toxins include cytochalasin D (a fungal alkaloid), latrunculin (a sponge toxin) and phalloidin (toxin obtained from deathcap mushroom). Cytochalasin D depolymerizes actin filaments by binding to the barbed or plus end, blocking the addition of subunits. Latrunculin binds ATP-G-actin and prevents its addition to the filament. Phalloidin prevents depolymerization of actin filaments by binding tightly at the interface between subunits, preventing their dissociation from the filament.

Actin-Binding Proteins

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Within a cell the assembly and disassembly of actin filaments is regulated by diverse group of proteins called actin-binding proteins. More than 100 different actin-binding proteins have been isolated from various cell types. Actin-binding proteins can promote assembly and stability by:

1. nucleating the assembly of the filament
2. exchanging ATP for ADP promoting the polymerization,
3. binding along the length of the actin filament
4. capping the ends preventing addition or dissociation of the subunits from the filament.

Actin-binding proteins can also promote disassembly by:

1. fragmenting the actin filament
2. sequestering the monomers preventing their re-polymerization

These proteins are classified based on their function as nucleating proteins, capping proteins, filament-depolymerizing proteins, monomer-binding proteins, Monomer-polymerizing proteins, Filament-severing proteins, Cross-linking proteins and Membrane-binding proteins.

Nucleating proteins

As already mentioned nucleation is the slowest step in the formation of an actin filament and requires the monomers to come in proper orientation. Two proteins, **formin** and **Arp2/3 complex** (**a**ctin-**r**elated **p**rotein) are known to accelerate the formation of actin filaments by aiding their nucleation. Formin is a barbed-end tracking protein which is responsible for formation of long and unbranched actin filaments found in stress fibers, filopodia and in muscles (Fig. 4).

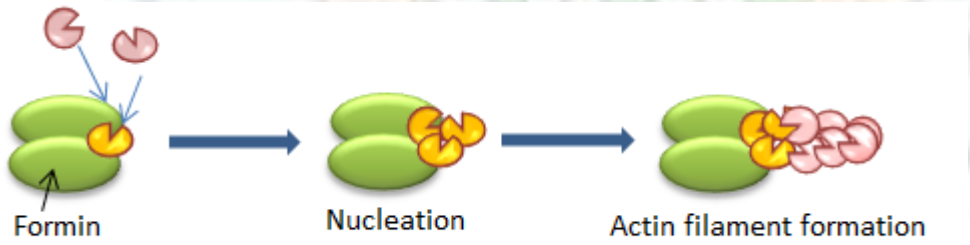

Fig. 4: Formin is responsible for nucleating the assembly of the actin filament. It orients the actin monomers to mediate the formation of dimers and trimers which seed the filament formation. **Source: Authors**

Arp2/3 complex which is a complex of seven proteins nucleates the formation of actin branches rather than forming unbranched filaments like formin. Arp2/3 complex binds at 70° to the barbed end of the filament and nucleates the formation of a branch (Fig. 5).

These branches are required for pushing the membrane forward resulting in cell movement. The Arp2/3 complex is activated by WASP complex and is controlled by Rho GTPases.

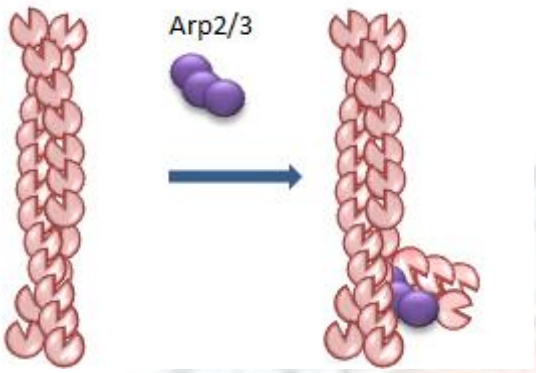

Fig. 5: Arp2/3 complex mediates the formation of branches of actin filaments. **Source: Authors**

Capping proteins

The actin capping proteins stabilize the filaments by capping their ends, thus preventing the dissociation of subunits. The examples of capping proteins are **capZ** and **tropomodulin**. CapZ caps the plus or barbed end of the actin filament while tropomodulin caps the minus or pointed end. Tropomodulin is thought to act as a complex with tropomyosin in stabilizing the actin filaments. This is because of the fact that there is enhancement in the capping activity of tropomodulin in the presence of tropomyosin. The capping of both ends is required at the places where the stable actin filaments are required like in sarcomere where the thin filaments are capped at their barbed end by capZ and at their pointed end by tropomodulin (Fig. 6).

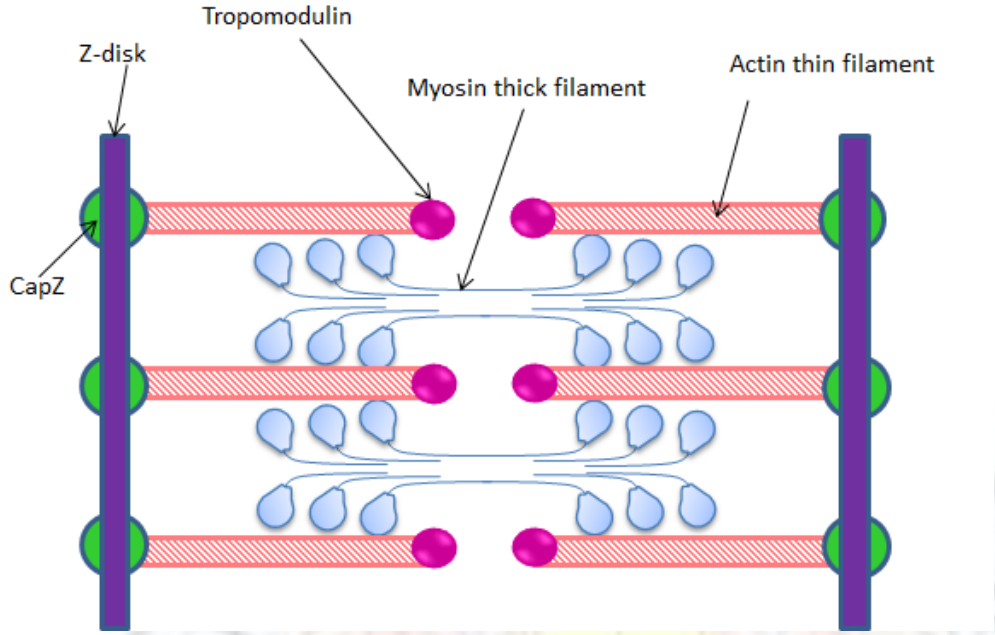

Fig. 6: Role of capping proteins in maintaining stable actin filaments in sarcomere. The barbed ends are capped by capZ and pointed ends by tropomodulin. **Source: Authors**

Filament-depolymerizing proteins

Members of **ADF/cofilin** (**A**ctin **d**epolymerizing **f**actor) family bind to the actin monomers at the pointed end and increase the rate of their dissociation from the filament. Apart from promoting depolymerization, it can also fragment the filament. The activity of these proteins are required for rapid turnover of the actin filament required for processes like cell locomotion and phagocytosis.

Monomer-binding proteins

The monomer binding proteins bind to the G-actin monomers and either prevent or promote their assembly. Proteins like **Thymosins** bind to ATP-G-actin and sequester them, thus preventing their polymerization. Another protein **twinfilin** binds to ADP-G-actin and play an important role in carrying them to the barbed end equired for their re-incorporation into the growing filament as seen during cell locomotion.

Monomer-polymerizing proteins

Monomer-polymerizing proteins promote the polymerization of the monomers by employing several mechanism. **Profilin** is one such protein which binds ATP-actin monomers in 1:1 ratio. They bind to the same site as thymosin i.e. opposite to the ATP-binding end. But unlike thymosin, it does not sequester the monomers, it promote the assembly of actin filaments. Profilin acts as a nucleotide-exchange factor by exchanging ATP for ADP. By doing this it replenishes the pool of ATP actin monomers that can be re-incorporated into the

growing filaments. Profilin is also known to promote the addition of actin monomers to the barbed or plus end of the filament. Profilin also controls the assembly of actin filaments at the plasma membrane by interacting with cell signaling components found in the membrane.

Filament-severing proteins

Severing proteins fragment the actin filament by straining the inter-subunit bond. The severing proteins like **cofilin** and **gelsolin** are known to break the actin filament. Once the filament is fragmented, the severing protein remain associated with the plus or barbed end, thus acting as capping protein. The minus or pointed end is gradually shortened. The severing activity of these proteins is important for rapid turnover of acting filament required for cellular processes like cell locomotion.

Cross-linking proteins

Cross-linking proteins play an important role in organizing the actin filaments in the cell. The actin filaments can be cross-linked to form bundles or networks. The formation of actin bundles and networks is mediated by cross-linking proteins. Proteins that mediate formation of actin bundles are small rigid proteins known as bundling proteins. Proteins that cross link actin filaments into network are large, flexible proteins. Majority of actin cross-linking proteins belong to the calponin homology domain (CH-domain) superfamily. These proteins have actin binding domains which bind with the actin filaments. The actin binding domains share sequence homology with a muscle protein, calponin and are separated by α-helical or β-sheet domains.

Two different types of actin bundles are formed by bundling proteins: closely spaced and widely spaced. **Fimbrin**, a 68 Kd protein is responsible for forming closely spaced actin bundles (Fig. 7). First isolated from microvilli, Fimbrin has two actin binding domains and acts as monomer. It can bind and hold two parallel filaments with the distance of 14 nm between the two filaments.

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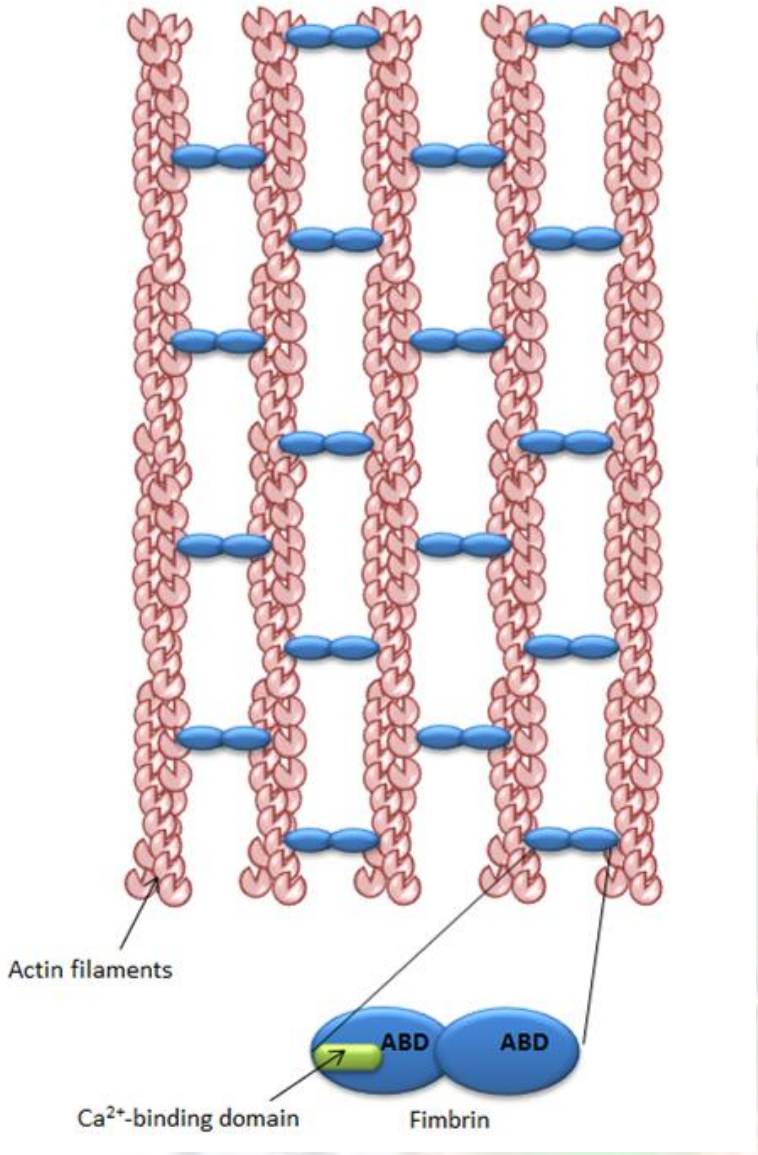

Fig. 7: Actin bundling protein fimbrin has two actin binding domains and acts as monomer and holds two parallel filaments with the distance of 14 nm between the two filaments. **Source: Authors**

Microvilli are the finger-like projections of the plasma membrane supported by actin cytoskeleton and are found in the intestinal epithelial cells. There can be about one thousand microvilli per epithelial cells forming a layer called **brush border**. These increase the surface area for absorption. A single microvillus contains about 20-30 parallel bundles of actin filaments cross-linked by fimbrin and **villin** (Fig. 8). Villin (92 Kd protein) is the major actin bundling protein in microvilli. The actin bundles are attached to the plasma membrane by lateral arms consist of myosin I and calmodulin, a calcium binding protein. The barbed ends are embedded in the unidentified capping protein while the pointed ends are embedded in a spectrin-rich region called the **terminal web**.

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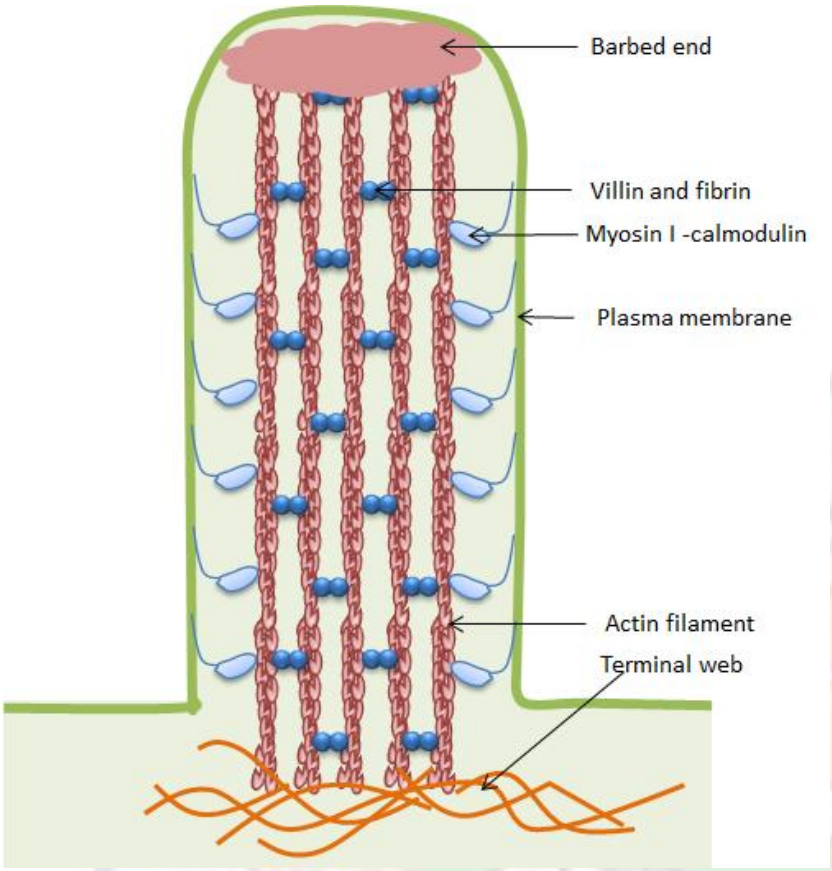

Fig. 8: Structure of microvilli. Actin filaments cross-linked by fimbrin and **villin** and are attached to the plasma membrane by lateral arms consist of myosin I and calmodulin. The barbed ends are embedded in the unidentified capping protein while the pointed ends are embedded in **terminal web**. **Source: Authors**

Widely spaced actin filament bundles are created by **α-actinin** which is a large protein of 102 Kd in size and has a single actin binding domain (Fig. 9). α-actinin has four α-helical spacer domains and acts as a dimer, crosslinking the actin filaments with distance of 40 nm between the two filaments. Such widely spaced actin filaments are found in contractile bundles like in stress fibers and in sarcomeres (explained later in muscle contraction). Both fimbrin and α-actinin have Ca^{2+} binding domains.

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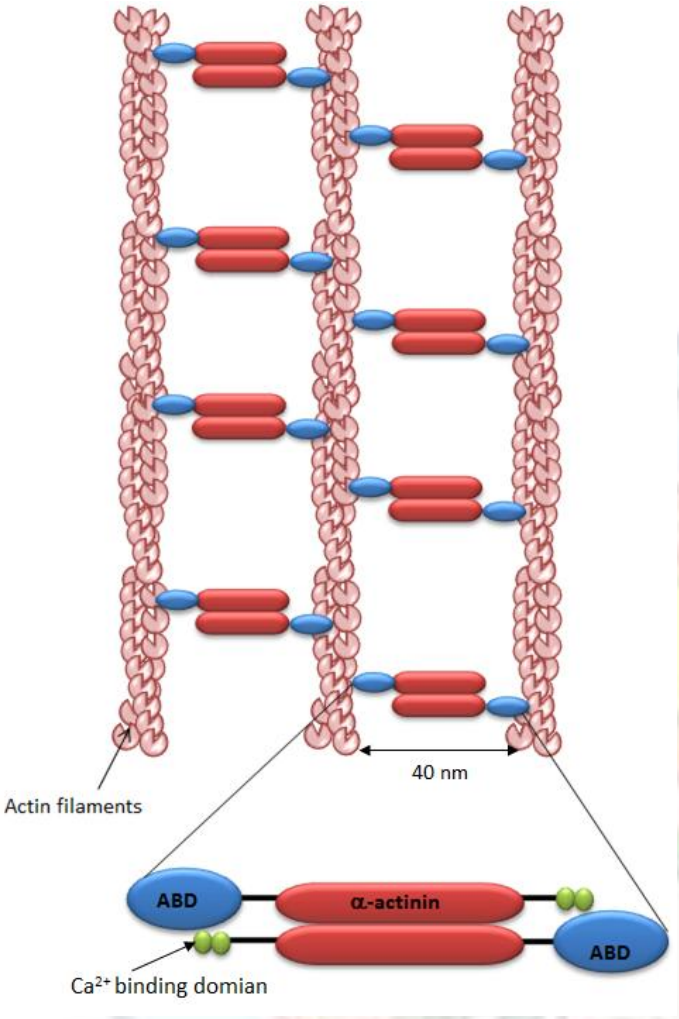

Fig. 9: Actin bundling protein α-actinin has four α-helical spacer domains and acts as a dimer, crosslinking the actin filaments with distance of 40 nm between the two filaments. **Source: Authors**

The actin filaments are cross-linked to form network by **filamin**, a 280 Kd protein acting as a dimer (Fig. 10). The dimerization is mediated by dimerization domain located at the end of the protein. Actin binding and dimerization domains are separated by a flexible β-sheet spacer domain which offers flexibility to the protein, allowing it to form networks. Such networks formed by filamin is found beneath the plasma membrane.

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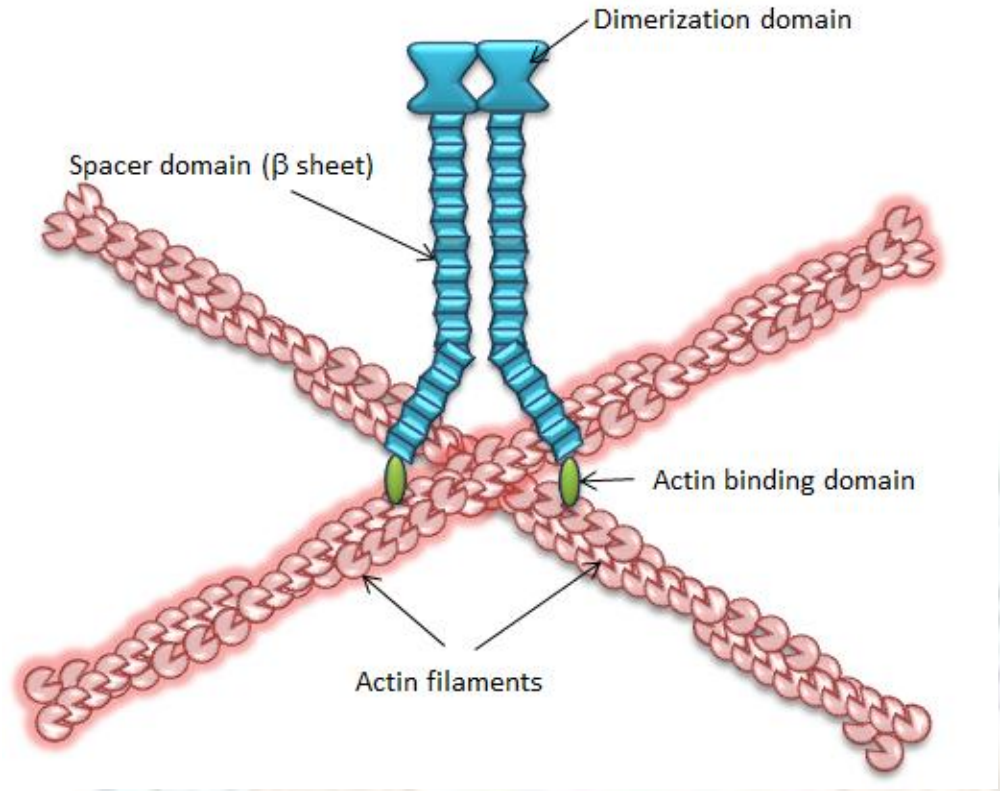

Fig. 10: Actin cross-linking protein filamin. Filamin acts as a dimer. The dimerization is mediated by dimerization domain located at the end of the protein. **Source: Author**

Membrane-binding proteins

Membrane-binding proteins play an important role in linking the plasma membrane with the actin cytoskeleton in non-muscle cells like erythrocytes, and also at focal adhesions and adherens junctions. The examples of such membrane linking proteins are **spectrin** (found in erythrocytes), **vinculin** and **talin** (found at focal adhesions) and **catenins** (found in adherens junctions).

Spectrin is a tetramer of α and β chains with molecular weight of 240 Kd and 220 Kd, respectively. It is the major actin binding protein found in the cortical cytoskeletal of erythrocytes. α and β chains associate laterally to form dimers and then associate head to head forming tetramer (Fig. 11).

The β chain has a single actin-binding domain at N-terminal while α chain lacks actin-binding domain but has Ca^{2+} binding domain. The two actin binding domain in a tetramer are separated by 200 nm. Another protein, **ankyrin** binds spectrin and to the cytosolic domain of the erythrocyte transmembrane protein band 3 (Fig. 11). Additional links are provided by **protein 4.1** which binds spectrin-actin network and cytoplasmic domain of glycophorin.

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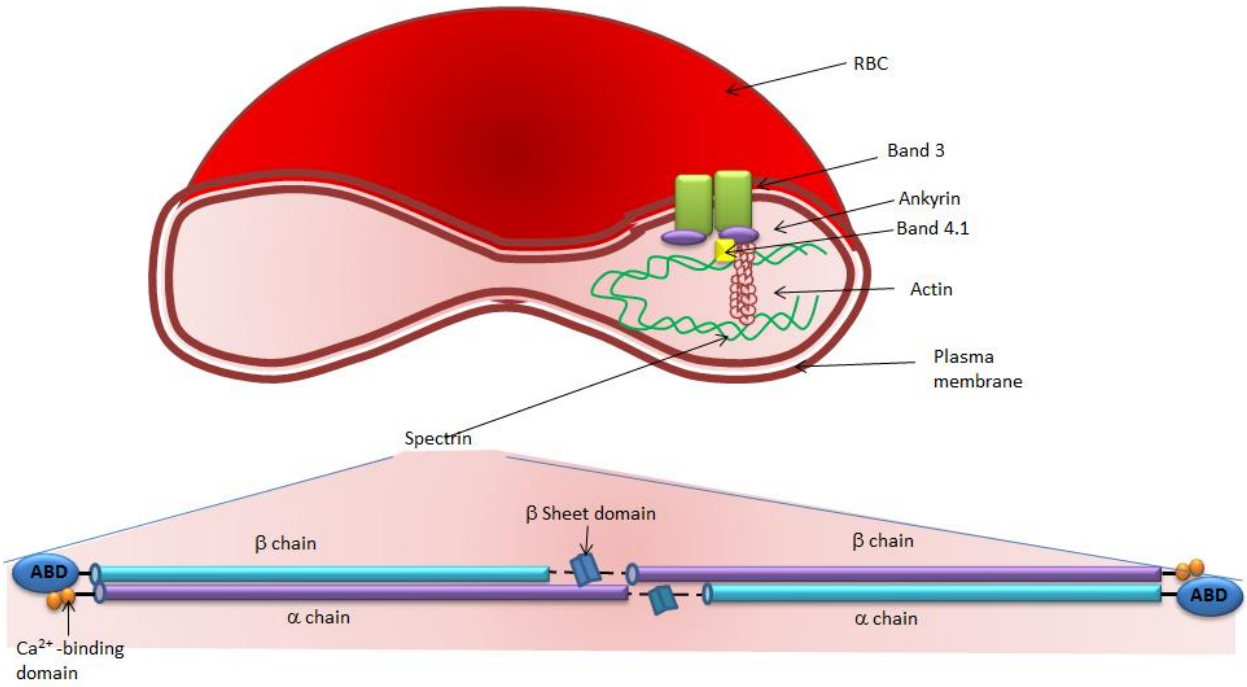

Fig. 11: Spectrin is major actin binding protein found in the cortical cytoskeletal of erythrocytes. It is a tetramer of α and β chains which associate to form dimers and tetramer. **Source: Authors**

In muscles, the major membrane-binding protein is **dystrophin** which links actin filaments to the sarcolemma. Dystrophin is a large protein of 427 Kd and has a single actin-binding domain at N-terminal and membrane binding domain at C-terminal.

Focal adhesions are the site of attachment of a cell to the extracellular matrix by means of transmembrane proteins called **integrins**. These sites also bind large bundles actin filaments called **stress fibers**. The proteins like talin and vinculin mediate the attachment of these stress fibers with the plasma membrane (Fig. 12). As already mentioned, the contractile bundles in stress fibers are cross-linked by α-actinin. Both talin and vinculin bind the actin filament, but only talin can bind with integrins.

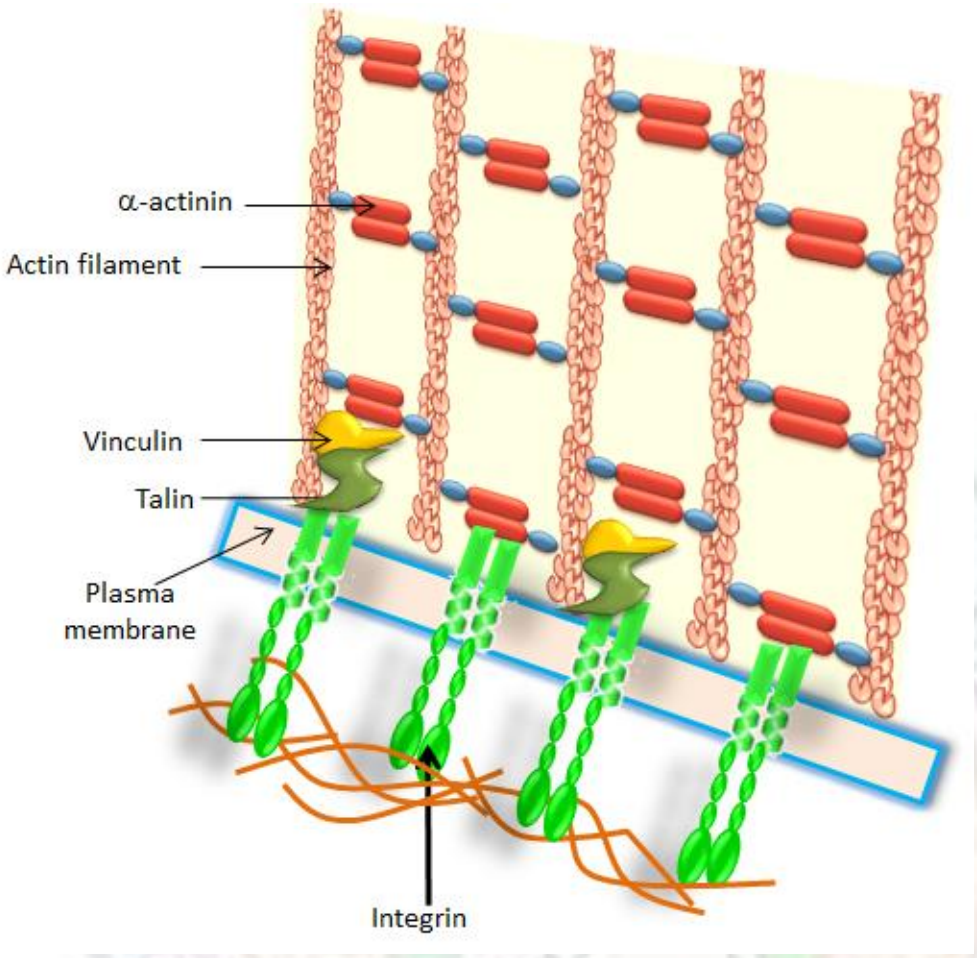

Fig. 12: Focal adhesions and stress fibers. Integrins mediate the attachment of a cell to the extracellular matrix. The proteins like talin and vinculin mediate the attachment of stress fibers which are bundles of actin filaments with the plasma membrane. **Source: Authors**

Adherens junctions are the regions of cell-cell contact found between the sheets of epithelial cells, where they formed continuous belt known as **adhesion belt**. The contact between two epithelial cells is mediated by transmembrane protein called **cadherins** (Fig. 13). The cadherins bind **β-catenin** which in turn binds **α-catenin**. α-catenin anchor actin bundles to the plasma membrane.

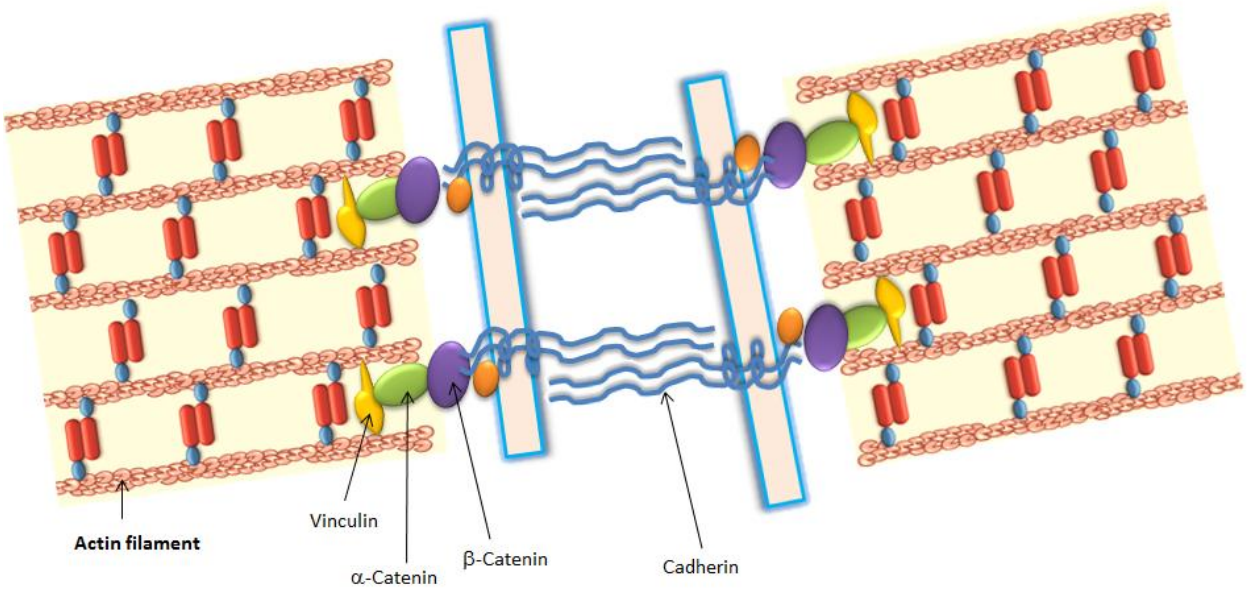

Fig. 13: Adherens junctions are the regions of cell-cell contact found between the sheets of epithelial cells mediated by transmembrane protein cadherins. The cadherins bind β-catenin which binds α-catenin. α-catenin anchor actin bundles to the plasma membrane. **Source: Authors**

Actin, myosin and muscle contraction

Myosin was first isolated from skeletal muscles of mammals. Now it is well known that apart from forming the contractile assemblies in skeletal muscles, they are also found in cardiac and smooth muscle cells and also in other non-muscle cells of eukaryotes. All known mysoins contain two heavy chains which are organized into head, neck, and tail domains and several light chains. The myosin head contains an ATP binding site and an actin binding site. The myosin head therefore forms a motor domain just like kinesins and dyneins which are microtubule-associated motor proteins. Due to their role, the myosin head domains are found to be similar across different myosins. The human genome encodes 40 different myosins including 16 different myosin II heavy chains.

Till date about 18 different classes of myosin have been identified which are broadly divided into the **conventional** (or **type II**) **myosins**, and the **unconventional myosins** (includes type I and types III–XVIII). Many of these classes are expressed in all eukaryotic cells but some are found to be restricted like myosin VIII and XI are found only in plants while myosin X is present only in vertebrates. Majority of known myosins are dimers except myosin I.

Conventional or type II myosins: These were first identified in muscle cells, and later on were found to be present in various non-muscle cells. Myosin II is a dimer of two heavy chain (200 Kd each) and also contain two pairs of light chains (20 Kd each) denoted as essential light chain (ELC) and regulatory light chain (RLC). The heavy chains have long α-helical tail which associate to form coiled-coil structure. The light chains are found to be associated with the neck domain of each heavy chain (Fig. 14). The regulatory light chain plays an important role in smooth muscle contraction. Type II myosins are found in muscle

cells and slides over the actin filaments to produce muscle contraction. Other functions of type II myosins are cytokinesis and cell movement.

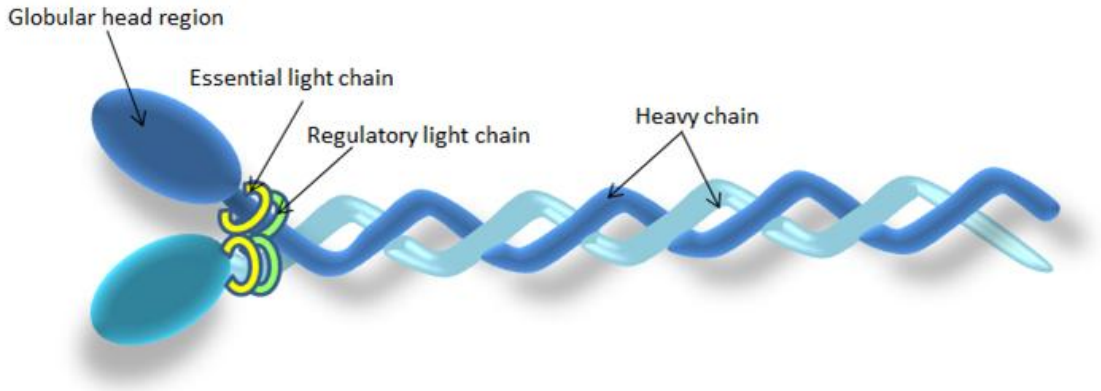

Fig. 14: Structure of Mysoin II. Myosin II is a dimer of two heavy chains and also contain essential light chain (ELC) and regulatory light chain (RLC). The heavy chains have long α-helical tail which associate to form coiled-coil structure. **Source: Author**

Unconventional myosins: The unconventional myosins do not play any role in muscle contraction but are responsible for transport of vesicles and several other functions. Among the unconventional myosins, myosin I and V are best studied. Myosin I is a small protein (110 Kd), first isolated from *Acanthamoeba*, a common soil amoeba in 1973 by Thomas Pollard and Edward Korn. Subsequently, many isoforms of myosin I were identified in *Acanthamoeba* like myosin IA (associated with small cytoplasmic vesicles) and Myosin IC (found at the plasma membrane and contractile vacuole). Myosin I lacks long tail domain and therefore is not able to form dimer. Myosin I appears to transport membrane vesicles and organelles along the microfilaments. Myosin I also forms the lateral arms found in microvilli and helps in its movement. Myosin V, like myosin II is a dimer of two heavy chains but has long neck domain, three times that of myosin II. The myosin V also contains light chains which are **calmodulin,** a Ca^{2+} binding protein. All known myosins move towards the barbed-end of the actin filament except myosin VI which moves towards the pointed end.

Do you know???

Myosin V requires a peripheral membrane protein called Rab27a and melanophilin which serve as adaptors and help in linking the tail domain of heavy chain to the vesicle membrane.

The unconventional myosins perform many functions like vesicle transport (myosin I, V, and VI), hearing and stereocilia structure (myosin VI, VII, and XV), transport of pigment

granules (myosin Va) and cytoplasmic streaming (myosin XI). Myosin XI is said to be the fastest myosin of all the known myosins.

Do you know???

Mutations in gene coding for different myosins has been implicated in various human diseases. For example mutation in gene coding myosin Va results in a rare disorder called Griscelli syndrome. Individuals with Griscelli syndrome exhibit partial albinism. Mutations in myosin VIIa results in Usher 1B syndrome, characterized by deafness and blindness.

Muscle contraction

There are three different types of muscle cells in vertebrates:

1. **Skeletal or voluntary muscles:** These muscles are anchored to bones and are responsible for all voluntary movements.
2. **Smooth muscles:** found in visceral organs like stomach, intestine and uterus, responsible for involuntary movements. These muscles are under the control of autonomic nervous system.
3. **Cardiac muscles:** found in heart, responsible for contraction of heart which pumps the blood into the blood vessels.

Skeletal muscle cell, also known as **myofiber** or **muscle fiber** is 10 to 100 µm in diameter, more than 100 mm in length covered by **sarcolemma**, the membrane of muscle fiber. The membrane is folded at many places to form **transverse tubules or T-tubules** which terminate in proximity to **sarcoplasmic reticulum (SR)**, the specialized endoplasmic reticulum of muscle fibers. Sarcoplasmic reticulum regulate the Ca2+ concentration and play an important role in muscle contraction.

Each myofiber is made by fusion of large numbers of **myoblasts** (mononucleated pre muscle cells) in the embryo and are therefore multinucleated. A myofiber is made up of several **myofibrils** which itself consists of specialized repeating contractile units called **sarcomere**. The sarcomere has banding pattern responsible for characteristic striated appearance and is made up of thick filaments of myosin and thin filaments of actin. Within each sarcomere, there are dark bands known as **A-band** (anisotropic band) alternate with light bands known as **I-band** (isotropic band). The center of A band has a lightly staining **H zone** which has a densely staining **M line** in its center. The A band consists of both thick and thin filaments while I band consists of only thin filaments. H zone has only thick filaments and therefore appear light. The barbed or plus ends of actin filaments are attached to the **Z discs**. The area between two Z discs forms a sarcomere which is the contractile unit of muscle fibers (Fig. 15). The thin filaments contain two other proteins: **tropomyosin** and **troponin**. Tropomyosin is a rod-shaped, elongated protein which binds with seven actin subunits along the actin filaments. Tropomyosin is associated with is a globular protein troponin which is a complex of three polypeptides: troponin T (tropomyosin-binding), troponin C (Ca^{2+} binding) and troponin I (inhibitory). Troponin C is

similar to calmodulin and myosin light chains. Troponin binds both the actin filaments and tropomyosin. The complex of troponin and tropomyosin blocks the myosin binding sites on the actin filaments in resting muscle fiber.

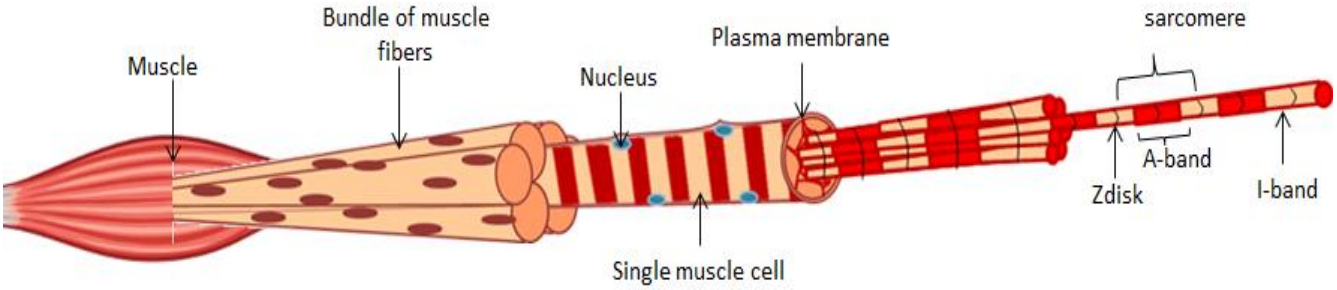

Fig. 15: The organization of skeletal muscle. Skeletal muscle cell, also known as myofiber are made up of several myofibrils which consists of repeating contractile units called sarcomere. The sarcomere consists of dark bands or A-band alternate with light bands or I-band. The center of A band has a lightly staining H zone which has a densely staining M line in its center. The barbed or plus ends of actin filaments are attached to the Z discs. The area between two Z discs forms a sarcomere which is the contractile unit of muscle fibers. **Source: Authors**

Each thick filament consist of several hundred myosin II molecules associated with their tail domains. The globular head domains of myosin binds and slides on the actin filaments. The polarity of myosin II molecules in the thick filaments is reversed at the M line. The globular head domains bind ATP, hydrolysis of which provides energy for sliding the filaments producing muscle contraction.

Muscle also contains other proteins required for maintaining its structure and stability. The thin filaments are capped at their barbed end by capZ and at their pointed end by tropomodulin. A protein **nebulin** is associated with actin filaments and is thought to regulate actin assembly. Another protein **titin** which is the third most abundant protein of vertebrate skeletal muscles, extents from M line to Z disc (Fig. 16).

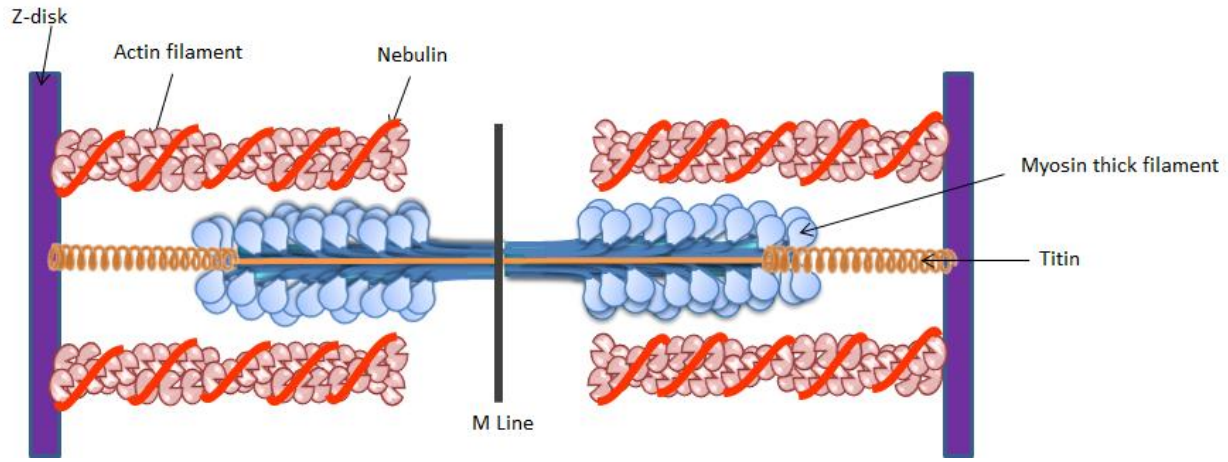

Fig. 16: Additional proteins required for stable sarcomeres. **Nebulin** is associated with actin filaments and is thought to regulate actin assembly while Titin is a large which is thought to keep myosin filament placed at center of the sarcomere. **Source: Authors**

Titin is an extremely large (containing more than 38,000 amino acids) and highly elastic protein which is thought to keep myosin filament placed at center of the sarcomere.

The muscle fibers are organized into groups known as **motor units**, innervated by a single motor neuron. The axon terminal of a motor neuron contacts with the muscle fiber at place called **neuromuscular Junction**.

The Sliding filament model

The sliding filament model of muscle contraction was proposed independently in 1954 by Andrew Huxley and Rolf Niedergerke, and Hugh Huxley and Jean Hanson. According to this model, the muscle contraction results from the sliding of actin and myosin filaments past each other. As a result, the sarcomere is shortened bringing the two Z discs closer which causes I band and H zone to disappear completely, but there is no change in the width of A-band (Fig. 17).

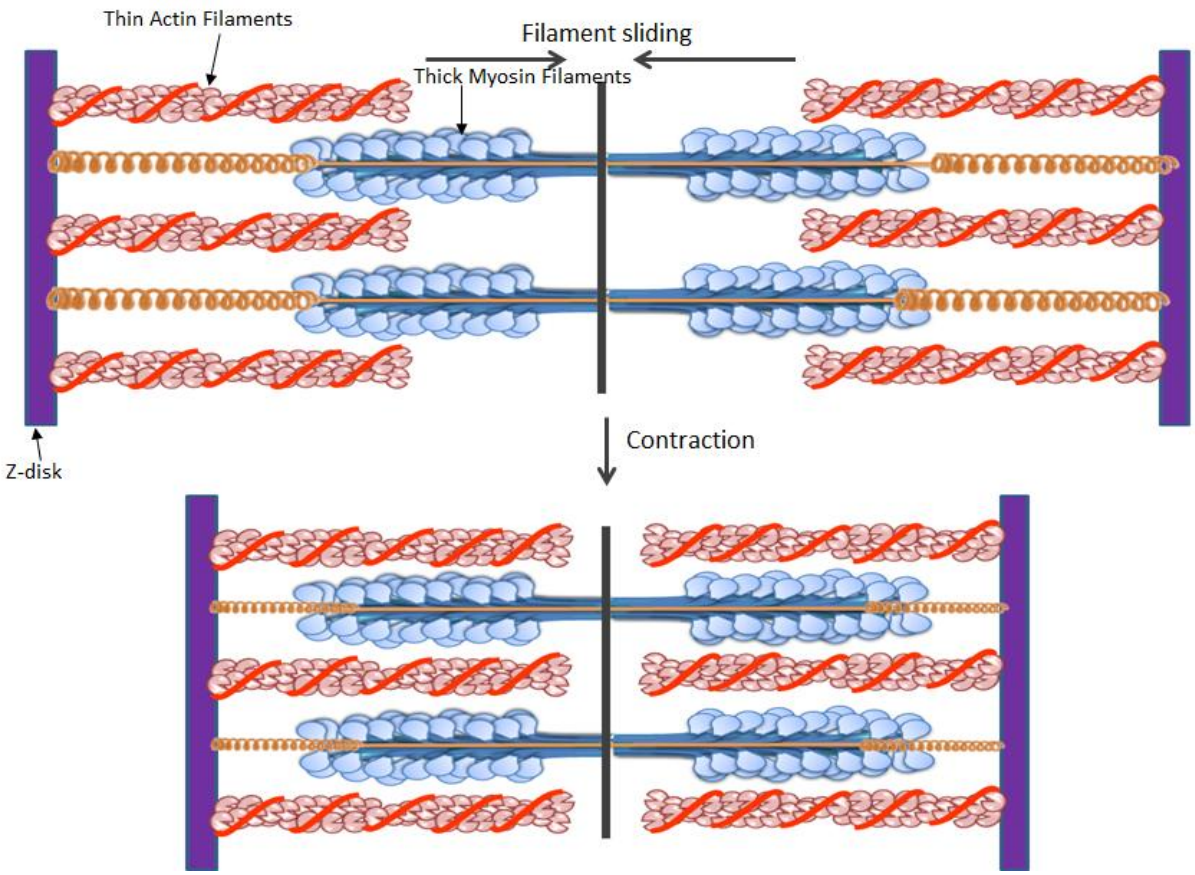

Fig. 17: Sliding filament theory of muscle contraction. The muscle contraction results from the sliding of actin and myosin filaments past each other resulting in the shortening of the sarcomere which causes I band and H zone to disappear completely. **Source: Authors**

Events during muscle contraction

When the nerve impulse from motor neuron reaches the neuromuscular junction it is carried to the interior of the muscle fibers by T-tubules to the Sarcoplasmic reticulum (SR). This leads to the opening of the voltage-gated Ca^{2+} channels and release of Ca^{2+} from SR increasing the cytosolic Ca^{2+} concentration. This increase in Ca^{2+} concentration triggers the events leading to the muscle contraction. The Ca^{2+} concentration in the cytoplasm of a relaxed muscle fiber is approximately 0.2 μM but after the arrival of a nerve impulse, it rises up to 50 μM.

In resting muscle fiber, the myosin binding sites on actin filaments are bounded by the complex of tropomyosin and troponin. When the levels of Ca^{2+} increase due to stimulation, Ca^{2+} bind to troponin C resulting in the conformational changes in the troponin complex which results in shifting of the tropomyosin (Fig. 18). This shifting in the location of tropomyosin exposes the myosin binding sites on actin filaments allowing myosin heads to bind the actin filaments.

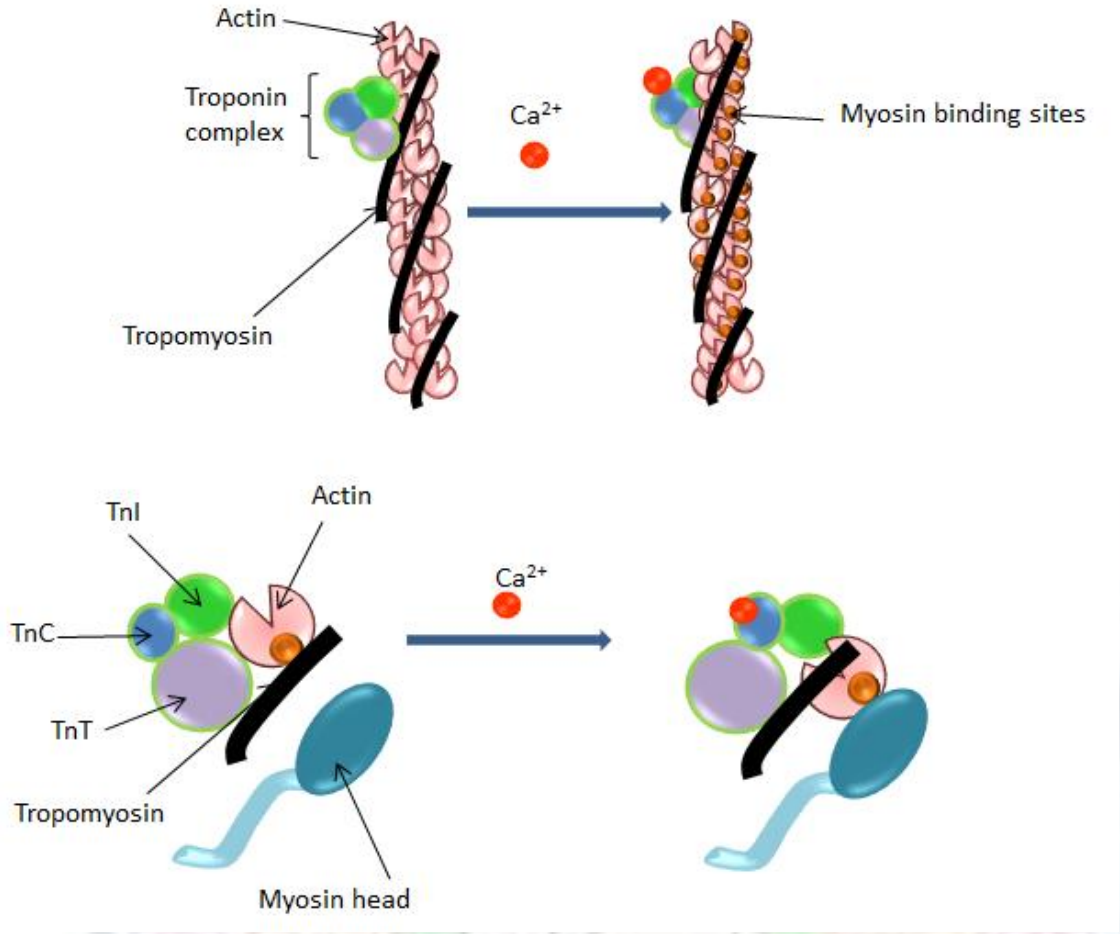

Fig. 18: Tropomyosin binds with along the actin filaments and is associated with is a globular protein troponin which is a complex of three polypeptides. The complex of troponin and tropomyosin blocks the myosin binding sites on the actin filaments in resting muscle fiber. During contraction, Ca^{2+} bind to troponin C resulting in the conformational changes in the troponin complex which results in shifting of the tropomyosin exposing the myosin binding sites allowing myosin heads to bind the actin filaments. **Source: Authors**

Do you know???

Sydney Ringer, an English physician in 1882 first demonstrated the importance of calcium in muscle contraction while working with the isolated frog heart. He formulated "Ringer's solution" which has been used to study the heart beat in situ.

The myosin head binds and hydrolyses ATP which results in change in conformational changes in myosin. The hydrolysis of ATP coupled with the conformational changes and filament sliding have been explained in a model known as "**swinging cross-bridge**

model". This model suggests that during muscle contraction the myosin head binds tightly to the actin filaments forming a cross-bridge between two filaments. The myosin II which is the myosin found in muscle is a non-processive motor which remain in contact with the actin filament for only a small fraction of time.

The contraction cycle begins with binding of an ATP molecule to the myosin head which results in dissociation of the cross-bridge from the actin filament. This is followed by the hydrolysis of ATP to ADP and P_i which remain bound to the myosin head. At this energized state the myosin head binds to the actin filament and the bound P_i is released. This results in power stroke that slides the thin actin filament 10 nm toward the M-line of the sarcomere and release of ADP to restart the new cycle. This model assumes the conformational changes in the head by ATP hydrolysis. The elongated neck is thought to acts as a "lever arm" that are responsible for sliding of action filaments. So it can be seen that the displacement of myosin head requires ATP, in the absence of which the head remains tightly bound to the actin filament.

Once the muscle contraction has taken place, the Ca^{2+}-ATPase in SR membrane removes the excess of Ca^{2+} which decreases their concentration. The decrease in Ca^{2+} results in their dissociation from troponin and movement of tropomyosin to their original position of actin filament blocking the myosin binding sites.

Do you know???

ATP is required for displacement of myosin head from actin filaments. In the absence of ATP, the head remain tightly associated with the actin filaments. After death, there is depletion of ATP and as a result the myosin head remain bound with the actin filaments resulting in a condition known as rigor mortis, characterized by stiffening of the body.

Contraction of smooth muscle

The contraction of smooth muscle cells is regulated by phosphorylation of the regulatory light chain of myosin. The contraction of smooth muscle in part is also regulated by Ca^{2+} levels. Increase in the cytosolic Ca^{2+} concentration results in their binding to a Ca^{2+} binding protein, calmodulin. The calmodulin binds and activates myosin light chain kinase (MLCK) which phosphorylates myosin regulatory light chain resulting in muscle contraction (Fig. 19).

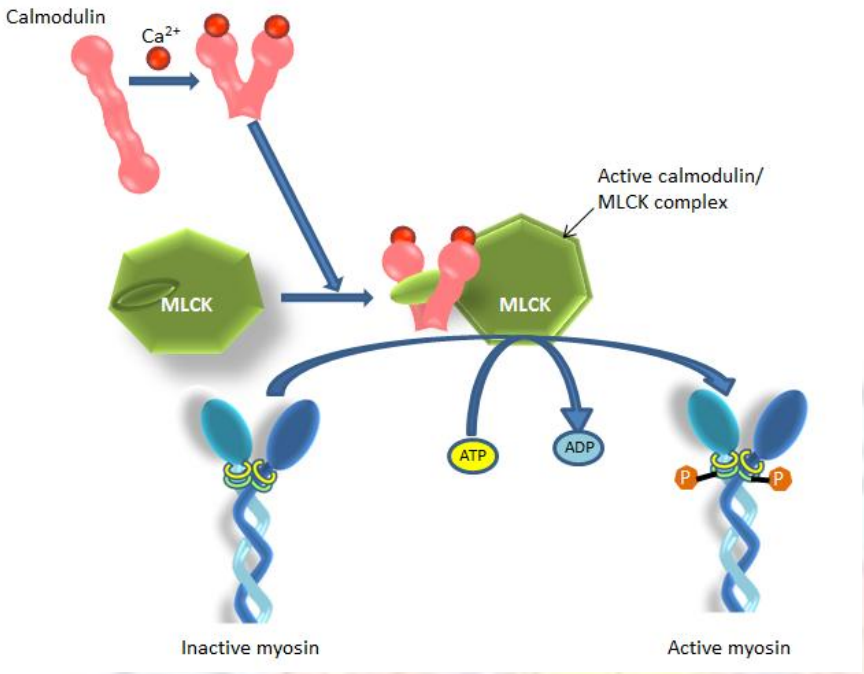

Fig. 19: Regulation of smooth muscle contraction. Ca^{2+} binds to calmodulin which binds and activates myosin light chain kinase (MLCK). MLCK phosphorylates myosin regulatory light chain resulting in muscle contraction. **Source: Author**

Myosin light chain phosphatase dephosphorylates the myosin regulatory light chain, resulting in muscle relaxation. The contraction of smooth muscle cells is also regulated by Rho kinase which phosphorylates myosin LC phosphatase and inhibits its activity. Rho kinase can directly phosphorylate the regulatory light chain.

Contractile assemblies of non-muscle cells

Actin is one of the most abundant proteins of non-muscle cells. The example of contractile assemblies found in non-muscle cells are focal adhesions, adherens junction and contractile ring. Focal adhesions and adherens junction have already been discussed (see section: membrane binding proteins).

Contractile ring consists of actin and myosin II formed during cytokinesis in animal cells. The contractile ring is assembled just beneath the plasma membrane by membrane-bound myosin. The subsequent contraction of this ring pulls the plasma membrane inwards, dividing the cell into two.

Cell locomotion

Cell locomotion is employed for many activities like embryonic cell migration during tissue and organ development, blood vessels formation, movement of WBCs, wound healing and

metastasis of cancerous cells. The cell movement requires various coordinated steps which are repeated (Fig. 20):

1. Development of initial polarity and formation of membrane protrusions like lamellipodia or filopodia to establish leading edge of the cell
2. Attachment of the membrane protrusions to the substratum
3. Retraction of the rear end

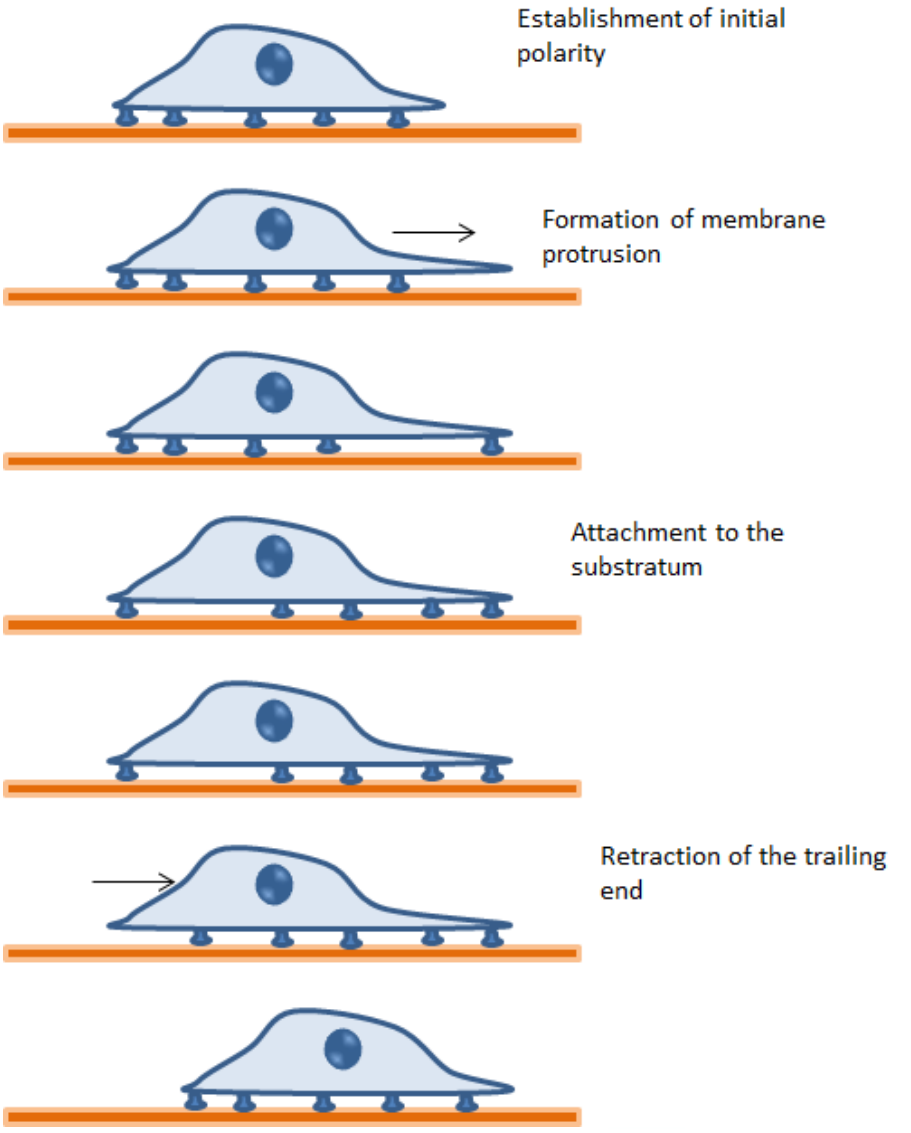

Fig. 20: Steps in cell movement. The cell movement involves three steps: formation of membrane protrusions, attachment of the membrane protrusions to the substratum and retraction of the rear end. **Source: Authors**

Cytoskeleton III: Microfilament

The formation of membrane protrusion requires polymerization of actin filaments that pushes plasma membrane forward. Various signals are responsible for cell movement and lead to the activation of receptors. In response to the signals, actin binding proteins are recruited to the membrane. These proteins include Arp2/3 complex and its activator WASP family proteins. Once activated by WASP family proteins, Arp2/3 complex binds near the barbed end of the actin filament and initiate the formation of a branch. The polymerization of ATP-actin is mediated by profilin. As new filaments grow, disassembly of the existing filaments is promoted by ADF/cofilin (Fig. 21). The ADP-actin monomers are carried to the barbed end by twinfilin. These monomers are activated by profilin by exchanging ATP for ADP. The formation of several filaments and branches provides the pushing force resulting in the formation of membrane protrusion.

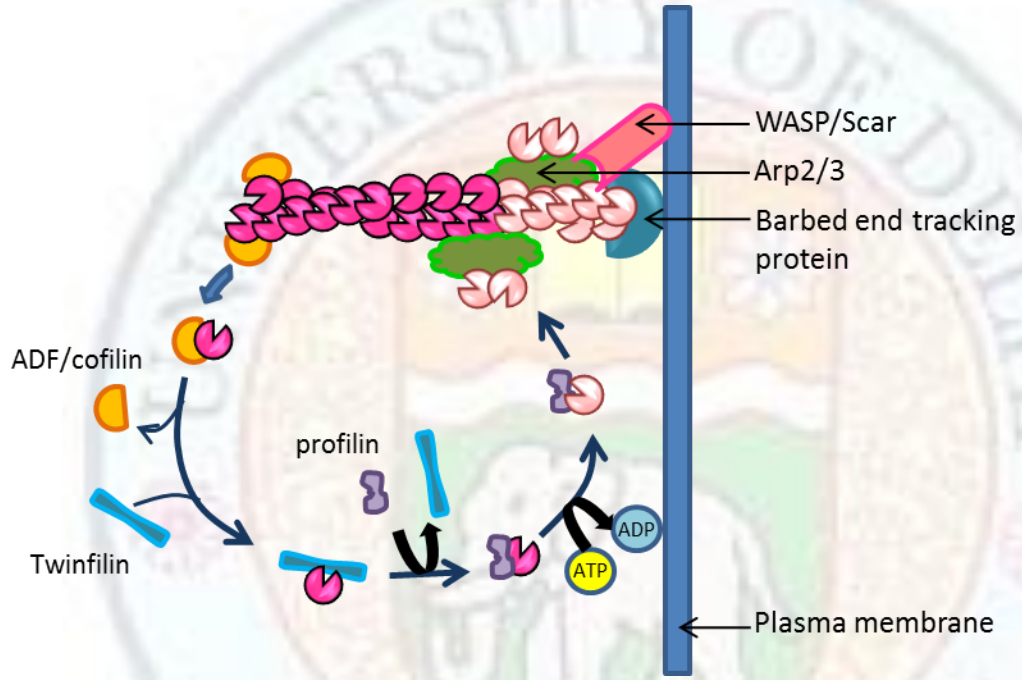

Fig. 21: Formation of membrane protrusion by branching of actin filaments. The branching is initiated by Arp2/3 complex, disassembly of the existing filaments is promoted by ADF/cofilin, ADP-actin monomers are carried to the barbed end by twinfilin and reactivated by profilin. **Source: Authors**

The formation of membrane protrusion is followed by the attachment of cell to the substratum. These sites of attachment are known as focal adhesions containing stress fibers attached to the plasma membrane via talins and vinculins. The final step involves the retraction of rear end which is thought to be regulated by ARF and Rho family proteins.

The forces involved in cell movement also called "traction forces" are generated at the adhesion sites. The sliding of myosin II bound to the actin filaments and stress fibers, generates the contractile forces responsible to pull the rear end in to the advancing end.

Cytoskeleton III: Microfilament

Regulation of Actin cytoskeleton

The actin cytoskeleton is regulated by small GTP binding proteins belonging to **Rho subfamily** like **Rho, Rac** and **Cdc42**. These proteins are activated by either integrins or by growth factors. The role of these proteins was first demonstrated in cultured fibroblast where it was found that Rho is responsible for formation of stress fibers, Rac is responsible for formation of lamellipodia and Cdc42 is required for filopodia formation.

Rac and Cdc42 are known to activate WASP family members which are the activator of Arp 2/3 complex responsible for branching of the existing actin filament. Rho activates formin required for formation of unbranched stress fibers. Rho also activates a protein serine-threonine kinase, **ROCK** which phosphorylates myosin regulatory light chain and inhibits myosin light chain phosphatase. Thus activating myosin II and promoting the formation of stress fibers and focal adhesions.

Integrins are also known to activate downstream signaling pathways through non-receptor protein-tyrosine kinases, FAK (focal adhesion kinase) localized at focal adhesions. The actin binding proteins like capping and severing proteins ae also regulated by signaling pathways. The severing proteins like cofilin and gelsolin are regulated by phosphatidylinositol 4,5-bisphosphate (PIP_2). Binding of these proteins to PIP_2 inhibits their severing activity. Their activity is promoted by phospholipase C mediated hydrolysis of PIP_2. The severing activities of cofilin and gelsolin are also regulated by phosphorylation and cytosolic Ca^{2+} concentration, respectively.

Summary

Microfilaments also known as actin filament or F-actin are 7 nm in diameter and are composed of globular protein actin. Actin has molecular weight of 43 Kd and is one of the most abundant and highly conserved intracellular protein found in eukaryotic cells. Actin exists in two forms: monomeric form known as Globular or G actin and polymeric form known as Filamentous or F actin. The G-actin binds ATP which is hydrolyzed soon after their polymerization into filaments. Each G-actin is rotated by 166^{o} in the filament giving the appearance like double-stranded helix. All monomers in the F-actin are oriented in the same direction, giving the microfilaments polarity with pointed end (also called "minus" end) and barbed end (also called "plus" end). The plus end or barbed end elongates 5–10 times faster as compared to the minus or pointed end.

In vitro polymerization of G- actin to F-actin involves three phases: Nucleation, elongation and steady-state. Critical concentration (C_c) is the concentration of G-actin when the addition of the monomers to the filament is balanced by their dissociation from the filament. So, at the concentration higher than C_c, there is polymerization of G-actin to F-actin and below C_c, there is depolymerization of F-actin to G-actin. So under steady-state, the filament exhibit treadmilling where monomers added at plus end migrate through the filament to reach the minus end from where they are dissociated from the filament.

Within a cell the assembly and disassembly of actin filaments is regulated by diverse group of proteins called actin-binding proteins. More than 100 different actin-binding proteins have been isolated from various cell types and are grouped according to their function. Nucleating proteins like formin and Arp2/3 complex (actin-related protein) are known to accelerate the

formation of actin filaments by aiding their nucleation. Formin is responsible for formation of long and unbranched actin filaments found in stress fibers, filopodia and in muscles. Arp2/3 complex nucleates the formation of actin branches rather than forming unbranched filaments like formin. The actin capping proteins like capZ and tropomodulin stabilize the filaments by capping their ends, thus preventing the dissociation of subunits. Filament-depolymerizing proteins like members of ADF/cofilin (Actin depolymerizing factor) family bind to the actin monomers at the pointed end and increase the rate of their dissociation from the filament. Apart from promoting depolymerization, it can also fragment the filament. The monomer binding proteins like Thymosins and twinfilin bind to the G-actin monomers and either prevent and promote their assembly, respectively. Monomer-polymerizing proteins like profilin promote the polymerization of the monomers by employing several mechanism. Severing proteins like cofilin and gelsolin are known to break the actin filament by straining the inter-subunit bond. Cross-linking proteins play an important role in organizing the actin filaments in the cell. The actin filaments can be cross-linked to form bundles or networks. Fimbrin is responsible for forming closely spaced actin bundles with the distance of 14 nm between the two filaments. Widely spaced actin filament bundles are created by α-actinin crosslinking the actin filaments with distance of 40 nm between the two filaments. The actin filaments are cross-linked to form network by filamin. Such networks formed by filamin is found beneath the plasma membrane. Membrane-binding proteins play an important role in linking the plasma membrane with the actin cytoskeleton in non-muscle cells like erythrocytes, and also at focal adhesions and adherens junctions. The examples of such membrane linking proteins are spectrin (found in erythrocytes), vinculin and talin (found at focal adhesions) and catenins (found in adherens junctions).

Myosin was first isolated from skeletal muscles of mammals and contain two heavy chains which are organized into head, neck, and tail domains and several light chains. The myosin head contains an ATP binding site and an actin binding site. Till date about 18 different classes of myosin have been identified which are broadly divided into the conventional (or type II) myosins, and the unconventional myosins (includes type I and types III–XVIII). The conventional myosins are present in contractile assemblies of the muscle cells and are responsible for muscle contraction. The unconventional myosins do not play any role in muscle contraction but are responsible for transport of vesicles and several other functions.

Skeletal muscle cell consists of dark bands known as A-band band consisting of both thick and thin filaments alternate with light bands known as I-band consisting of only thin filaments. The center of A band has a lightly staining H zone which has a densely staining M line in its center. The barbed or plus ends of actin filaments are attached to the Z discs. The area between two Z discs forms a sarcomere which is the contractile unit of muscle fibers. A complex of troponin and tropomyosin blocks the myosin binding sites on the actin filaments in resting muscle fiber. The thin filaments are capped at their barbed end by capZ and at their pointed end by tropomodulin. Nebulin is thought to regulate actin filament assembly in muscle and titin is thought to keep myosin filament placed at center of the sarcomere. The muscle fibers are organized into groups known as motor units, innervated by a single motor neuron. The axon terminal of a motor neuron contacts with the muscle fiber at place called neuromuscular Junction. The sliding filament model of muscle contraction proposes that the muscle contraction results from the sliding of actin and myosin filaments past each other.

In response to the nerve impulse, the release of Ca^{2+} from SR takes place increasing the cytosolic Ca^{2+} concentration. Ca^{2+} bind to troponin C resulting in the conformational changes

in the troponin complex which results in shifting of the tropomyosin allowing myosin heads to bind the actin filaments. The hydrolysis of ATP coupled with the conformational changes and filament sliding have been explained in a model known as "swinging cross-bridge model". This model suggests that during muscle contraction the myosin head binds tightly to the actin filaments forming a cross-bridge between two filaments.

The contraction of smooth muscle cells is regulated by phosphorylation of the regulatory light chain of myosin by myosin light chain kinase (MLCK). The contraction of smooth muscle cells is also regulated by Rho kinase which phosphorylates myosin LC phosphatase and inhibits its activity. Rho kinase can directly phosphorylate the regulatory light chain.

The cell movement requires various coordinated steps like development of initial polarity and formation of membrane protrusions to establish leading edge of the cell, attachment of the membrane protrusions to the substratum and retraction of the rear end. The formation of membrane protrusion requires polymerization of actin filaments that pushes plasma membrane forward mediated by Arp2/3 complex. As new filaments grow, disassembly of the existing filaments is promoted by ADF/cofilin. The ADP-actin monomers are carried to the barbed end by twinfilin. These monomers are activated by profiling by exchanging ATP for ADP. The formation of several filaments and branches provides the pushing force resulting in the formation of membrane protrusion.

The actin cytoskeleton is regulated by small GTP binding proteins belonging to Rho subfamily like Rho, Rac and Cdc42.Integrins are also known to activate downstream signaling pathways through non-receptor protein-tyrosine kinases, FAK (focal adhesion kinase) localized at focal adhesions.

Exercise/ Practice

A. Multiple choice questions:

1. Which of the following does not affect the stability of actin filaments?
   a) cytochalasin D (b) latrunculin (c) phalloidin (d) vinblastine

2. Actin bundling protein found in stress fibers is
   (a) villin (b) fimbrin (c) α-actinin (d) filamin

3. The protein linking actin filament to the membrane in the erythrocyte is
   (a) spectrin (b) vinculin (c) talin (d) catenins

4. Myosin responsible for cytoplasmic streaming is
   (a) myosin VI (b) myosin VII (c) myosin XI (d) myosin XV

5. The light chain of myosin V is
   (a) calmodulin (b) Essential LC (c) Regulatory LC (c) intermediate LC

6. The dark band consists of
   (a) thin filaments (b) thick filaments (c) both thick and thin filaments (d) none

7. The elastic protein that keeps myosin filament placed at center of the sarcomere
   (a) tropomyosin (b) titin (c) troponin (d) nebulin

8. The contraction of smooth muscle cells is not regulated by
   (a) calmodulin (b) MLCK (c) myosin LC phosphatase (d) phospholipase C
9. Contractile ring is responsible for _____ in animal cells
   (a) cell movement (b) cytokinesis (c) muscle contraction (d) vesicle transport

10. Which of the following does not regulate the stress fibers
    (a) Rho (b) ROCK (c) myosin regulatory light chain (d) PIP_2

B. Fill in the blanks:

1. The slowest step in the formation of an actin filament and requires the monomers to come in proper orientation _____
2. _____ and _____ accelerate the formation of actin filaments by aiding their nucleation.
3. In sarcomere the thin filaments are capped at barbed end by _____ and at pointed end by _____
4. The protein that exchanges ATP for ADP in actin monomers is _____
5. The examples of filament severing proteins are _____and _____
6. Major actin bundling protein found in microvilli is _____
7. The actin filaments are cross-linked to form network by _____
8. The proteins vinculin and talin are found at _____
9. The regions of cell-cell contact between the sheets of epithelial cells are_____
10. The activator of Arp2/3 complex is _____

C. True/False

1. The barbed end elongates 5–10 times faster as compared to the pointed end.
2. Fimbrin is responsible for forming widely spaced actin bundles.
3. Filamin is a major actin bundling protein.
4. Stress fibers are found at focal adhesions.
5. The contact between two epithelial cells is mediated by transmembrane protein called cadherins.
6. Unconventional myosins play an important role in muscle contraction.
7. Titin is thought to regulate actin assembly in muscle cells.
8. The contraction of smooth muscle cells is regulated by phosphorylation of the regulatory light chain of myosin.
9. Contractile ring helps in cell locomotion.
10. ATP is required for muscle contraction and relaxation.

Cytoskeleton III: Microfilament

D. Expand the following

1. G actin
2. F actin
3. C_c
4. Arp
5. ADF
6. ELC
7. RLC
8. T-tubules
9. SR
10. A-band
11. I-band
12. PIP_2

Glossary

A-band: anisotropic band, dark bands seen in the muscle fibers

Adherens junctions: regions of cell-cell contact found between the sheets of epithelial cells

Cardiac muscles: found in heart, responsible for contraction of heart

Critical concentration (C_c): the concentration of G-actin when the addition of the monomers to the filament is balanced by their dissociation from the filament

F actin: G actin polymerizes to form filaments known as Filamentous or F actin

Focal adhesions: site of attachment of a cell to the extracellular matrix by means of transmembrane proteins called integrins

G actin: monomeric form of actin

I-band: isotropic band, light bands seen in the muscle fibers

Sarcomere: area between two Z discs, contractile unit of muscle fibers

Sarcoplasmic reticulum (SR): Specialized endoplasmic reticulum of muscle, stores Ca^{2+}

Skeletal muscles: muscles anchored to bones and are responsible for all voluntary movements

Smooth muscles: muscles found in visceral organs, responsible for involuntary movements.

Stress fibers: large bundles actin filaments attached to the integrins at focal adhesions

T-tubules: also called transverse tubules infoldings of sarcolemma, helps in transmitting the nerve impulse to the interior of the muscle

Cytoskeleton III: Microfilament

References/ Bibliography/ Further Reading

Berg, J. S., B. C. Powell, and R. E. Cheney. 2001. A millennial myosin census. Mol. Biol. Cell 12: 780–794.

Borisy, G. G., and T. M. Svitkina. 2000. Actin machinery: pushing the envelope. Curr. Opin. Cell Biol. 12: 104–112.

Bresnick, A. R. 1999. Molecular mechanisms of nonmuscle myosin: II. Regulation. Curr. Opin. Cell Biol. 11: 26–33.

Buss, F., J. Luzio, and J. Kendrick-Jones. 2002. Myosin VI, an actin motor for membrane traffic and cell migration. Traffic **3**:851–858.

Condeelis, J. 2001. How is actin polymerization nucleated in vivo? Trends Cell Biol. 11: 288–93.

Cooper, J. A., and D. A. Schafer. 2000. Control of actin assembly and disassembly at filament ends. Curr. Opin. Cell Biol. 12: 97–103.

Field, C., R. Li, and K. Oegema. 1999. Cytokinesis in eukaryotes: a mechanistic comparison. Curr. Opin. Cell Biol. 11: 68–80.

Furukawa, R., and M. Fechheimer. 1997. The structure, function and assembly of actin filament bundles. Intl. Rev. Cytol. 175: 29–90.

Geeves, M. A. and Holmes, K. C. 1999. Structural mechanism of muscle contraction. Ann. Rev. Biochem. 68: 687-728.

Goode, B. L. and Eck, M. J. 2007. Mechanisms and functions of formins in the control of actin assembly. Ann. Rev. Biochem. 76: 593-627.

Gregorio, C. C., H. Granzier, H. Sorimachi, and S. Labeit. 1999. Muscle assembly: a titanic achievement? Curr. Opin. Cell Biol. 11: 18–25.

Higgs, H. N., and T. D. Pollard. 2000. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Ann. Rev. Biochem. 70: 619–676.

Kwiatkowski, D. J. 1999. Functions of gelsolin: motility, signaling, apoptosis, cancer. Curr. Opin. Cell Biol. 11: 103–108.

McGough, A. 1998. F-actin-binding proteins. Curr. Opin. Struc. Biol. 8: 166–176.

Mermall, V., P. L. Post, and M. S. Mooseker. 1998. Unconventional myosin in cell movement, membrane traffic, and signal transduction. Science 279: 527–533.

Pollard, T. D., and G. G. Borisy. 2003. Cellular motility driven by assembly and disassembly of actin filaments. Cell 112: 453–465.

Pollard, T. D., L. Blanchoin, and R. D. Mullins. 2000. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. Ann. Rev. Biophys. Biomol. Struc. 29: 545–576.

Rayment, I. 1996. The structural basis of the myosin ATPase activity. J. Biol. Chem. 271: 15850–15853.

Theriot, J. A. 1997. Accelerating on a treadmill: ADF/cofilin promotes rapid actin filament turnover in the dynamic cytoskeleton. J. Cell Biol. 136: 1165–1168.

Welch, M. D., A. Mallavarapu, J. Rosenblatt, and T. J. Mitchison. 1997. Actin dynamics in vivo. Curr. Opin. Cell. Biol. 9: 54–61.

Answers

A. Multiple choice questions

1. (d) vinblastine
2. (c) α-actinin
3. (a) spectrin
4. (c) myosin XI
5. (a) calmodulin
6. (c) both thick and thin filaments
7. (b) titin
8. (d) phospholipase C
9. (b) cytokinesis
10. (d) PIP_2

B. Fill in the blanks

1. Nucleation.
2. formin and Arp2/3 complex
3. capZ, tropomodulin
4. Profilin
5. Cofilin and gelsolin
6. villin
7. filamin
8. focal adhesions
9. Adherens junctions
10. WASP family proteins

C. True/False

1. True
2. False, they are required for closely forming closely spaced actin bundles
3. False, filamin forms networks of actin filaments
4. True
5. True

6. False, conventional myosins play an important role in muscle contraction
7. False, nebulin regulates actin assembly in muscle cells
8. True
9. False, it is required for cytokinesis
10. True

D. Expand the following

1. Globular actin
2. Filamentous actin
3. critical concentration
4. actin-related protein
5. Actin depolymerizing factor
6. essential light chain
7. regulatory light chain
8. transverse tubules
9. sarcoplasmic reticulum
10. anisotropic band
11. isotropic band
12. phosphatidylinositol 4,5-bisphosphate