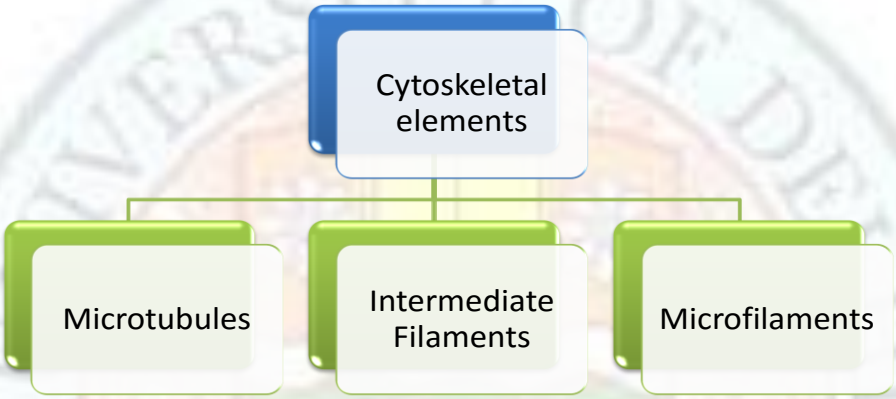
Cytoskeleton I

Paper: Cell Biology
Lesson: Cytoskeleton I
Author Name: Dr. Devi Lal , Dr. Mansi Verma
College/ Department: Ramjas College, Sri Venkateswara College
Department of Zoology , University of Delhi

Cytoskeleton I

Introduction

Just like the organisms have skeleton to provide the shape and support to the body, cells have cytoskeleton. The cytoskeleton is composed of microtubules, microfilaments, and intermediate filaments which together form a collaborative network. If a cell is treated with a nonionic detergent, soluble cytosolic proteins diffuse away leaving membrane-bound cell organelles and cytoskeletal elements. The three type of cytoskeletal elements can be distinguished on the basis of their diameter, monomeric units and their arrangements.



Microtubules are rigid, hollow, tubular structures having outer diameter of 25 nm and wall thickness of 4nm. They are composed of globular protein **tubulin.** A microtubule is a heterodimer of 55 kd protein **α- and β-tubulin** arranged in longitudinal rows forming **protofilament.**

Microfilaments also known as actin filament are 7 nm in diameter, composed of globular protein **actin** which exists in two forms: monomeric form known as **Globular** or **G actin** and polymeric form known as **Filamentous** or **F actin**.

Intermediate filaments (IFs) are rope like with diameter of 10 nm and are thought to contain more than 70 proteins.

The cytoskeleton is found to be highly conserved feature found in eukaryotes. Comparative structural and sequence analysis from the cytoskeletal monomers in yeast and humans suggest a small difference between these elements. This conservation is due to the critical role of cytoskeletal elements in various cell functions. Comparative analyses have also identified proteins in bacteria that are similar to eukaryotic actin and tubulin. The bacterial homolog of tubulin is FtsZ protein which plays an important role in cell division. In bacteria FtsZ forms a contractile ring (also known as Z ring) responsible for fission. In vitro studies purified FtsZ have demonstrated that it can assemble into protofilaments but cannot form microtubule-like structure. Homologs of bacterial FtsZ protein are found in chloroplast designated as FtsZ1 and FtsZ2. Both these proteins play important role in forming the contractile ring responsible for chloroplast fission.

Homolog of eukaryotic actin has also been found in bacteria. A bacterial protein MreB, has been found to be similar in structure and sequence to actin. MreB is found to be present only in rod-shaped bacteria and is thought to control the width in these bacteria.

No bacterial homologs have been identified for intermediate filament proteins suggesting these appear later during evolution. It has been proposed that the first intermediate filament proteins to be evolved were probably lamins that form nuclear lamina.

Organization of cytoskeleton

The cytoskeletal elements in a cell are organized either forming the bundles or networks. The arrangement of cytoskeletal elements in these two types of organization is different. In bundles, the filaments of cytoskeleton are arranged in closely packed parallel arrays while in network these elements are cross-linked. The network forms a web-like structure and is also responsible for gel-like properties of the cytosol.

Visualizing cytoskeleton

Study of cytoskeletal elements has been facilitated by the revolutions in the microscopic techniques. Now it is possible to look at a live cell using the fluorescence microscope in an approach known as live-cell imaging. Live-cell imaging allows researchers to directly visualize various molecular and biochemical processes going on inside a living cells. In order to study cytoskeleton, the subunits are fluorescently labeled using fluorescent dye. For example, is someone is interested in looking at the organization of microtubules, the tubulin monomers are labeled and are injected into a live cell. These labeled subunits are incorporated into the growing cytoskeletal elements allowing there visualization using a fluorescent microscope.

An alternative approach to visualize the location of a particular cytoskeletal element is to use fluorescently labeled antibodies directed against the cytoskeleton proteins. In another approach, drugs that bind to cytoskeletal elements are fluorescently labeled. The fluorescently labeled drugs like phalloidin is used to visualize actin cytoskeleton.

Functions of Cytoskeleton

The cytoskeletal elements serve many important functions inside a cell like:

1. The cytoskeletal elements are responsible for providing support and determine the cell shape.
2. The cytoskeletal elements are also responsible for separating the chromosomes during cell division and cytokinesis.
3. The cytoskeletal elements form an internal framework which help in positioning various organelles.
4. The cytoskeletal elements form network of tracks like railways tracks that are responsible for transporting membrane bound vesicles, organelles and other cargo like RNA and proteins within cell.

5. The cytoskeletal elements are also responsible for cell locomotion by the aid of cilia and flagella or by producing the tension on the substratum.
6. The actin filaments found in muscle help in muscle contraction.

Overview of three cytoskeletal elements

The three cytoskeletal elements play different roles in a cell and are characterized by differences in the subunit composition, structure and distribution. Table 1 provides a tabulated summary of the three cytoskeletal elements.

Table 1: Summary of key properties of the three cytoskeletal elements:

Properties	Microtubules	Microfilaments	Intermediate filaments
Distribution	All eukaryotes	All eukaryotes	animals
Dimensions	outer diameter 25 nm, wall thickness 4nm	7 nm in diameter	8-10 nm in diameter
Structure	rigid, hollow, tubular structures	Flexible and helical	Tough, flexible and rope-like
Subunits	Heterodimer of GTP bound α and β tubulin	Monomeric ATP α-actin in muscle cells β- and γ- in non-muscle cells	More than 70 known proteins
Presence of polarity	Yes, plus (+) end and minus (-) end "+" end is fast growing end	Yes, barbed end or plus end, pointed end or minus end. The plus end elongates 5–10 times faster as compared to minus end	No polarity
Site of subunit incorporation	Both + and - end	Both + and - end	Internal association
Enzymatic activity	GTPase	ATPase	None
Associated proteins	Microtubule-associated proteins (MAPs)	Actin-binding proteins	Plackins
Associated motor proteins	Kinesins, dyneins Majority of kinesins move towards + end, some towards – end and some do not move	Myosin, all myosins move towards the barbed-end of the actin filament except myosin VI which moves towards the pointed end	None

	All dyneins move towards – end		
Subunit Treadmilling	yes	Yes	No
Subcellular localization	Cytoplasm	Cytoplasm	Cytoplasm and nucleus
Primary functions	Intracellular transport of vesicles and cell organelles; Cell movement by cilia and flagella; separation and movement of chromosomes during mitosis	Intracellular transport of vesicles, muscle contraction and cell locomotion	Structural support

Cooperation between different cytoskeletal elements

The various cytoskeletal elements cooperate with each other to form a network inside a cell. It has been found that both actin and microtubule cytoskeleton act as railway tracks that help in transporting cargos. These cargos are usually membrane bound vesicles but can vary with the type of cytoskeleton. The microtubule associated motor proteins are **kinesins** and **dyneins** while microfilament associated motor protein is **myosin**. Several experimental evidences suggests that microtubule and microfilament associated motor proteins can bind to and transport the same vesicles. Experiments conducted on extruded cytoplasm from a squid giant axon suggest that vesicles that travel along microtubules can move in the microfilament rich region lacking microtubules. Several other experimental evidences have clearly demonstrated that a vesicle can move on a microtubule or a microfilament by binding two motor proteins, myosin and kinesin or myosin or cytosolic dynein. Such cooperation between microtubule and microfilaments have been studied in neuron and pigment cells. In neurons, the synaptic vesicles are first transported through axon along microtubule tracks by kinesin and then at the terminals along actin filaments by myosin. Similarly in pigment cells, the pigment granules are first transported by kinesin along microtubule tracks and then by myosin V along the actin cytoskeleton to the periphery of the pigment cell. The cytoskeletal elements also carry the subunits and binding proteins of the other cytoskeletal elements like myosin V can transport intermediate filaments along the actin filaments and kinesin can transport actin-binding proteins along microtubules tracks.

Intermediate filaments

Intermediate filaments are chemically heterogeneous cytoskeletal elements. Unlike microtubules and microfilaments which are made up of only one type of monomeric units, intermediate filaments are composed of more than 70 different kinds of proteins.

Cytoskeleton I

Intermediate filaments are rope-like and provide mechanical strength to the cells. Till date, intermediate filaments have only been identified in animal cells and are 10 times more abundant as compared to microfilaments or microtubules. Intermediate filaments have diameter of 10-12 nm which is intermediate between microtubules (24 nm) and microfilaments (7 nm) and hence their name. Intermediate filaments are more stable as compared to microtubules and microfilaments and unlike them intermediate filaments do not bind or hydrolyze ATP or GTP. Intermediate filaments do not contribute to cell motility like microtubules and microfilaments.

Structure and assembly of intermediate filaments

Though intermediate filaments are composed of more than 70 different kinds of proteins, all share similar structural organization. All intermediate filament proteins have a central α-helical rod domain of 310-350 amino acids flanked by globular N-terminal head and C-terminal tail domains of variable size and structure (Fig. 1). The rod domain is found to be conserved in all intermediate filament proteins and consists of four α-helices.

The rod domain of two polypeptides associate to form coiled-coil dimers. The dimer then associate side by side in antiparallel orientation forming a tetramer. Tetramers associate end to end to form protofilament and eight protofilaments associate laterally to produce an intermediate filament (Fig. 1). The tetramers are thought to act as immediate subunit for assembly of intermediate filaments. Since the intermediate filaments are assembled from end to end association of tetramers, they lack polarity like microtubules and microfilaments.

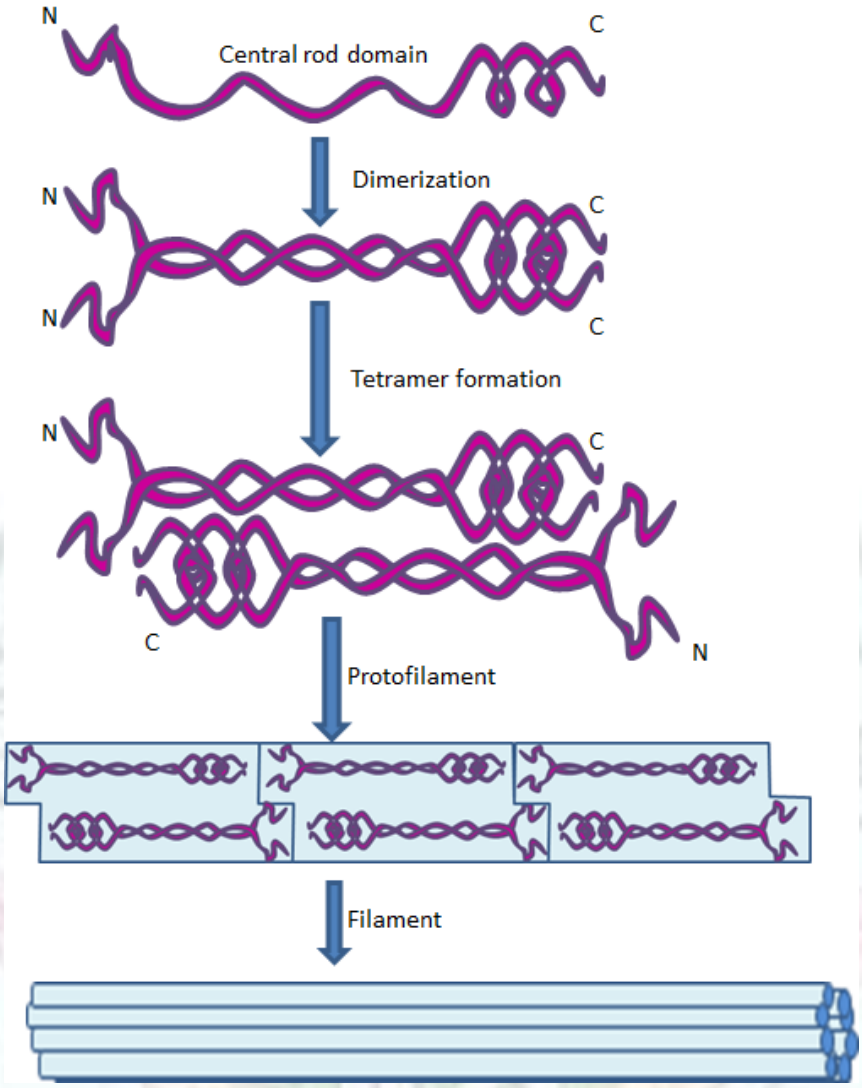


Fig. 1: Assembly of intermediate filaments. The rod domain of two polypeptides associate to form coiled-coil dimers which associate side by side in antiparallel orientation forming a tetramer. Tetramers associate end to end protofilament and eight protofilaments associate laterally to produce an intermediate filament.

The assembly of intermediate filaments does not involve use of ATP or GTP. As the head and tail domains of different intermediate filament proteins vary in size and structure, they were thought to play no role in filament assembly. However, studies with truncated proteins demonstrated that if the N- or C-terminal domain is removed the assembly into filament is inhibited. This suggest that these domains are also required for assembly into filaments and are thought to mediate lateral interactions within an intermediate filament.

Dynamic nature of intermediate filaments

Cytoskeleton I

Although intermediate filaments are more stable as compared to microtubules and microfilaments, studies have revealed that they can also show dynamic properties. Experiment done with cultured fibroblasts using a biotin-labeled type I keratin demonstrated the incorporation of labeled keratin into already existing keratin cytoskeleton. Moreover the incorporation was not at the ends of the filaments like seen in microtubules and microfilaments but into the interior. The intermediate filament's assembly and disassembly is controlled by phosphorylation and dephosphorylation of the constituent proteins. The excellent example of regulation of assembly and disassembly of intermediate filament is provided by breakdown of the nuclear envelope during mitosis. Nuclear lamins, a type of intermediate filament proteins support the nuclear membrane by forming a meshwork. In the beginning of mitosis, nuclear lamins are phosphorylated by Cdc2, a cyclin-dependent kinase which results in their disassembly and also prevents reassembly. The removal of phosphate group by phosphatases after mitosis promotes their reassembly to form nuclear membrane. Similarly vimentin, another intermediate filament protein is phosphorylated by protein kinase-A promoting its disassembly.

Classification of intermediate filament proteins

The intermediate filament proteins have been classified into five groups based on amino acid similarity (Table 2).

Type I and II intermediate filament proteins

Type I and II consists of acidic and basic keratins, respectively expressed in epithelial cells. The assembly into keratin filaments require both type I and type II keratin which associate in 1:1 to form heterodimers. Keratins are the only intermediate filament proteins that can assemble without the head and tail domains. There are 28 type I and 26 type II keratin found in humans. Genes for all type I keratin (except K18) are found on human chromosome 17 while for 26 type II keratins and K18 are located on human chromosome 12.

The central rod domain of keratin has a pseudorepeat of 7-residues, with position 1 and 4 occupied by non-polar residues which promote side by side association with other helix. Keratins have also been classified as **α keratins** which occur in mammals and **β keratins** which are found in birds and reptiles. **α keratins** are rich in cysteine that form disulfide bonds with adjacent polypeptide chains. This makes **α keratins** insoluble and resistance to stretching. Mammals have more than 30 keratin genes expressed in tissue-specific manner. Large number of keratin isoforms have been identified which makes it one of the most diverse group of intermediate filament proteins. The isoforms are divided into **hard keratins** which have high sulfur content and are found in hair, nails, wool and horn; and **soft keratins** which have low sulfur content and are found in the epithelial cells that line internal body cavities. Soft keratins are also known as **cytokeratins**.

Table 2: Classification of various intermediate filament proteins into five groups

Type	IF protein	Size (Kd)	Filament form	Distribution

I	Acidic keratins	40-57	Heteropolymers	Epithelial cells
II	Basic keratins	53-67	Heteropolymers	
III	Vimentin	57	Homo and heteropolymer	Fibroblasts
	Desmin	53	Homo and heteropolymer	Muscle
	Glial fibrillary acidic protein	50	Homo and heteropolymer	Astrocytes, Glial cells,
	Peripherin	57	Homo and heteropolymer	Peripheral neurons
IV	Neurofilaments NF-L NF-M NF-H	 62 102 110	 Homopolymer Heteropolymer Heteropolymer	Peripheral and central Neurons
	α-internexin	66	Homopolymer	Developing neurons
	Nestin*	200	Homopolymer	Stem cells
	Synemin*	150-230	-	Skeletal, heart and smooth muscle
	Syncoilin	54	-	Skeletal and heart muscle
V	Nuclear lamins Lamin A Lamin B Lamin C	 70 67 67	Homopolymer	Nucleus

*nestin and synemin sometimes classified as type VI rather than type IV

Do you know???

Keratins are tough and insoluble fibrous proteins which are resistant to digestion. This is evident from the fact that any hair we ingest is not digested and comes out as such in feces. Certain insects like clothes moth larva has high concentration of mercaptans in their digestive system. Mercaptans can reductively cleave disulfide bonds. This makes keratin vulnerable to digestion.

Type III intermediate filament proteins

Type III proteins form both homo and heterodimers. This group includes vimentin, desmin, glial fibrillary acidic protein and peripherin. Of all these IF proteins, vimentin is more widely distributed. It is found in a variety of cell types like smooth muscles, white blood cells, endothelial cells, epithelial cells and fibroblasts. Vimentin forms a network inside a cell that extends from nucleus to the cell's periphery. Vimentin is often associated with microtubules.

Vimentin filaments help in keeping nucleus and other organelles in place and also support cell membrane.

Desmin is found in muscle cells and connects Z-disc to the other contractile elements thus stabilizing the sarcomeres. Desmin is expressed prior to the expression of other muscle proteins like titin, nebulin, actin and myosin. Cardiac muscles contain more desmin as compared to skeletal muscle.

Glial fibrillary acidic protein is expressed in glial cells which surround neurons; and Peripherin is found in the neurons of the peripheral nervous system.

Type IV intermediate filament proteins

Type IV intermediate filament proteins include neurofilaments, α-internexin and nestin. Three different neurofilament polypeptides are known and designated as NF-L (light), NF-M (medium) and NF-H (heavy). They are abundantly found in mature neurons particularly motor neurons. Neurofilaments play an important role in supporting the long axons and are also responsible for the radial growth. Neurofilaments thus have important role in determining the axonal diameter.

α-internexin is expressed in the early stages of neuron development when the neurofilaments have not yet expressed. Nestins are also expressed in the embryonic stages in stem cells.

Synemin is a large protein found in muscle and few non-muscle cells. Though originally identified in avian erythrocytes, it is found to be present in human lens cells, chicken gizzard and astrocytes. Synemin cannot assemble into filaments by itself but requires other intermediate filament proteins like desmin and vimentin.

Syncoilin is named because it is present at the neuromuscular **syn**apse and has **coil**ed-coils structure. Syncoilin is highly expressed in skeletal and cardiac muscle. It is required for the maintenance and maturation of the synapse.

Type V intermediate filament proteins

Type V intermediate filament proteins involve nuclear lamins which form nuclear lamina. Mammals express three lamin genes which are designated as A, B and C. The nuclear lamina forms a framework that supports the nuclear envelope and serves as a site of attachment of chromatin fibers. The framework is composed of cross-linked lamin A and lamin C filaments, attached to the inner nuclear membrane by lamin B. It is also known to play poorly understood role in DNA replication. The lamin proteins bind to specific proteins of the inner nuclear membrane like emerin and lamin B receptor (LBR) (which interacts with lamin B) (Fig. 2). This binding helps in attachment of nuclear lamins to the nuclear membrane. Lamins also bind histones H2A and H2B facilitating their attachment with chromatin.

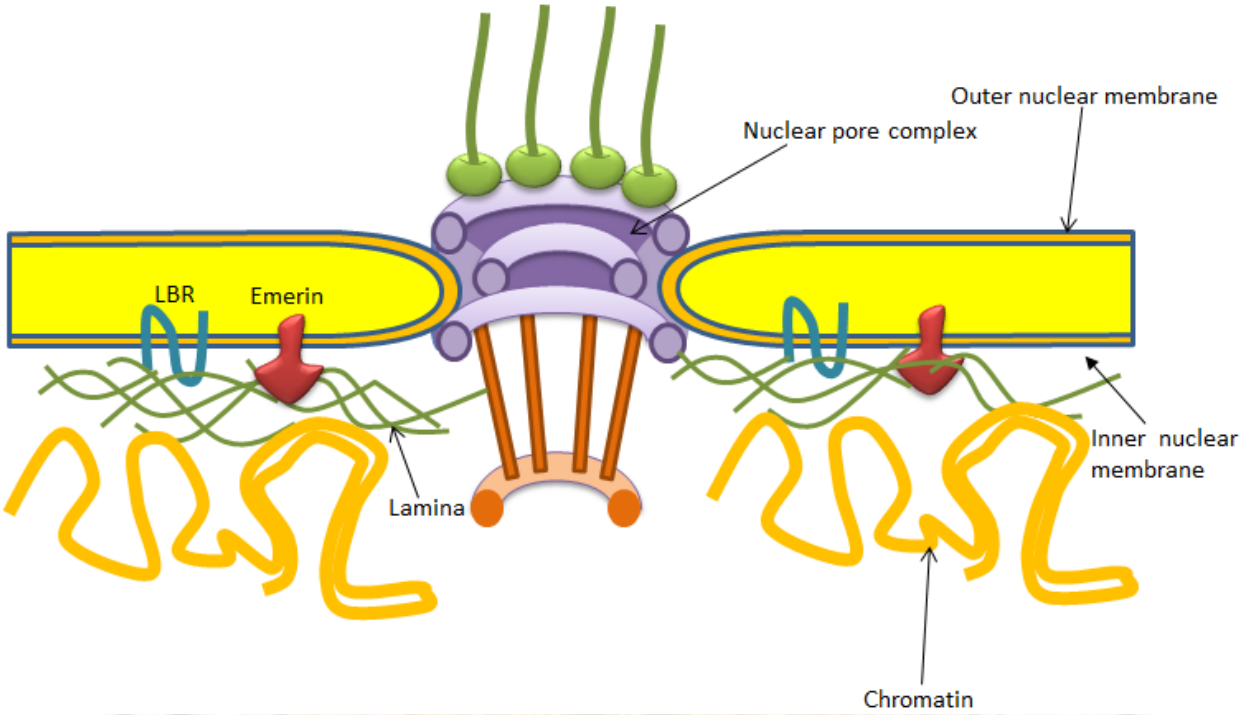


Fig. 2: The nuclear lamina. The nuclear lamina forms a framework that supports the nuclear envelope and is composed of cross-linked lamin A and lamin C filaments, attached to the inner nuclear membrane by lamin B. The lamin proteins bind to specific proteins of the inner nuclear membrane like emerin and lamin B receptor (LBR).

Intermediate filament–associated proteins (IFAPs)

Like actin-binding proteins or microtubule-associated proteins (MAPs), Intermediate filament–associated proteins (IFAPs) play an important role in organizing intermediate filaments within the cell. But unlike actin-binding proteins or microtubule-associated proteins (MAPs), these proteins do not sequester intermediate filament proteins, and sever or cap intermediate filaments. Till date no such intermediate filament associated motor proteins have been identified suggesting these proteins have no role in cargo transport. IFAPs mainly help in organizing the intermediate filaments into networks or bundles and also mediate their interaction with microfilament and microtubule cytoskeletons. These proteins also attach intermediate filaments with nuclear and plasma membranes. Members of plakin family are responsible for linking intermediate filaments with other cytoskeletal elements and plasma membrane. A member of plakin family, **plectin** has been studied in details. Plectin is a 500 Kd protein and has a calponin-homology (-CH) domain at the N-terminus. The calponin-homology (-CH) domain is also found in the other members of plakin family and in actin cross-linking proteins. Plectin cross-links intermediate filaments with microtubules and microfilaments and also interacts with actin-binding proteins and microtubule-associated proteins. The members of plakin family like plectin and desmoplakin attach intermediate filaments to the plasma membrane of epithelial cells.

Do you know???

Studies have found that the intermediate filaments and microtubules are physically linked in a cell. Treatment of cell with high concentration of drugs like colchicine results in complete disassembly of microtubules leaving only vimentin filaments. The vimentin filaments are found to be clumped into disordered bundles near the nucleus. This clearly indicates that within a cell the organization of vimentin filaments depends on microtubules. Similarly, the presence of calponin-homology (-CH) domain at the N-terminus of the members of IFAPs like plectin indicate that intermediate filament binding proteins link intermediate filaments with actin filaments.

Cellular organization of intermediate filaments

One of the chief roles of intermediate filament proteins is to provide mechanical support. This is quite evident in case of nuclear envelope which is supported by nuclear lamina composed of nuclear envelope. Intermediate filaments also form an extensive network that extends from nucleus to the cell periphery. The key intermediate filament which forms a network in the cytosol is vimentin. Vimentin is attached with the plasma membrane through plectin and ankyrin, an actin-binding protein.

Intermediate filament protein, desmin is found in the muscle cells at the periphery of Z-disk and encircling it (Fig. 3). The desmin filaments are attached to the sarcolemma by many IFAPs like paranemin. The importance of desmin in the integrity of the sarcomere is seen in transgenic mice that lacks desmin. In such mice the muscles are misaligned and there is overall disruption of the muscular structure. The desmin filaments encircle the Z disk and are also connected to neighboring Z disks. These connections cross-link myofibrils into bundles.

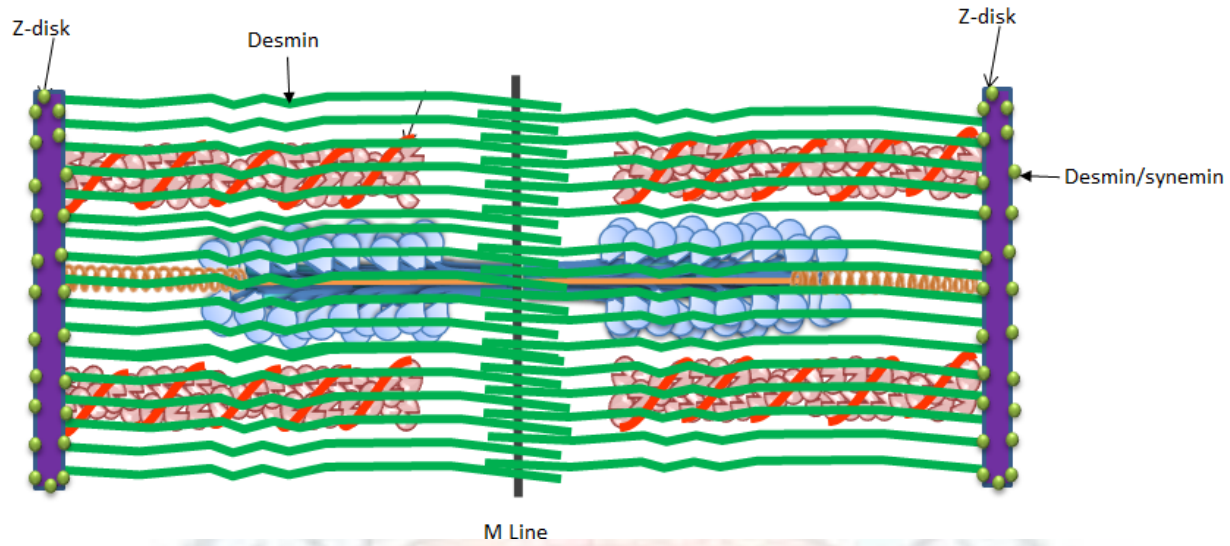


Fig. 3: Desmin is found in the muscle cells where it encircles the Z disk. The desmin filaments are attached to the sarcolemma by many IFAPs like paranemin and are held at proper orientation with the sarcomere by desmin/synemin collar at Z disk.

The organization of keratin filaments are seen in epithelial cells where they are connected to the plasma membrane by IFAPs in **desmosomes** and **hemi-desmosomes**. Desmosomes are the regions of cell-cell contact where the interaction between adjacent epithelial cells are mediated by transmembrane proteins like desmoglein and desmocollin (Fig. 4). The keratin filaments are attached to the plasma membrane and transmembrane proteins by desmoplakin (a member of plakin family), plakophillin and plackoglobin.

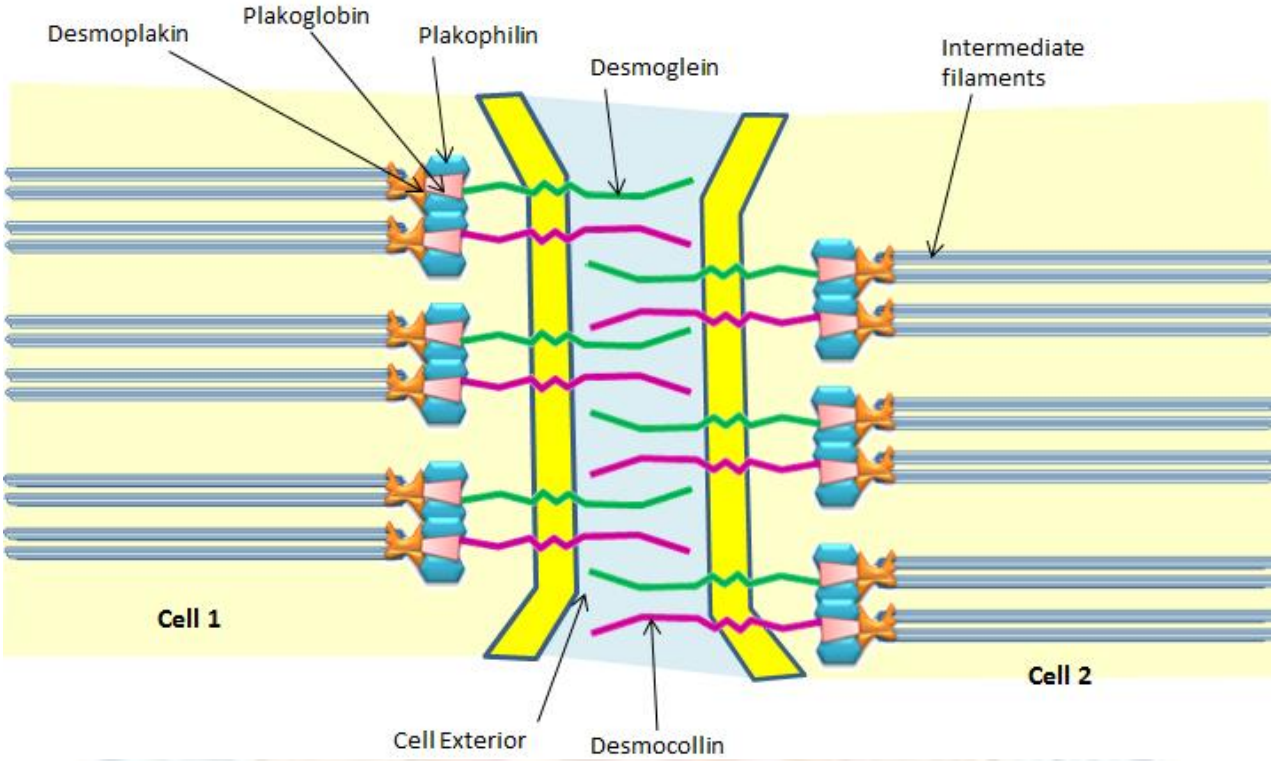


Fig. 4: Attachment of intermediate filaments to desmosomes. The interaction between adjacent epithelial cells are mediated by transmembrane proteins desmocollin and desmoglein. The intermediate filaments are attached to the plasma membrane and transmembrane proteins by desmoplakin, plakophillin and plackoglobin.

Hemi-desmosomes are the regions of epithelial cell contacts with the underlying connective tissue. The integrin links the extracellular matrix with the keratin filaments through plectin (Fig. 5). Other proteins like BP180 and BP230 play important role in regulating the assembly and stability of the hemi-desmosomes.

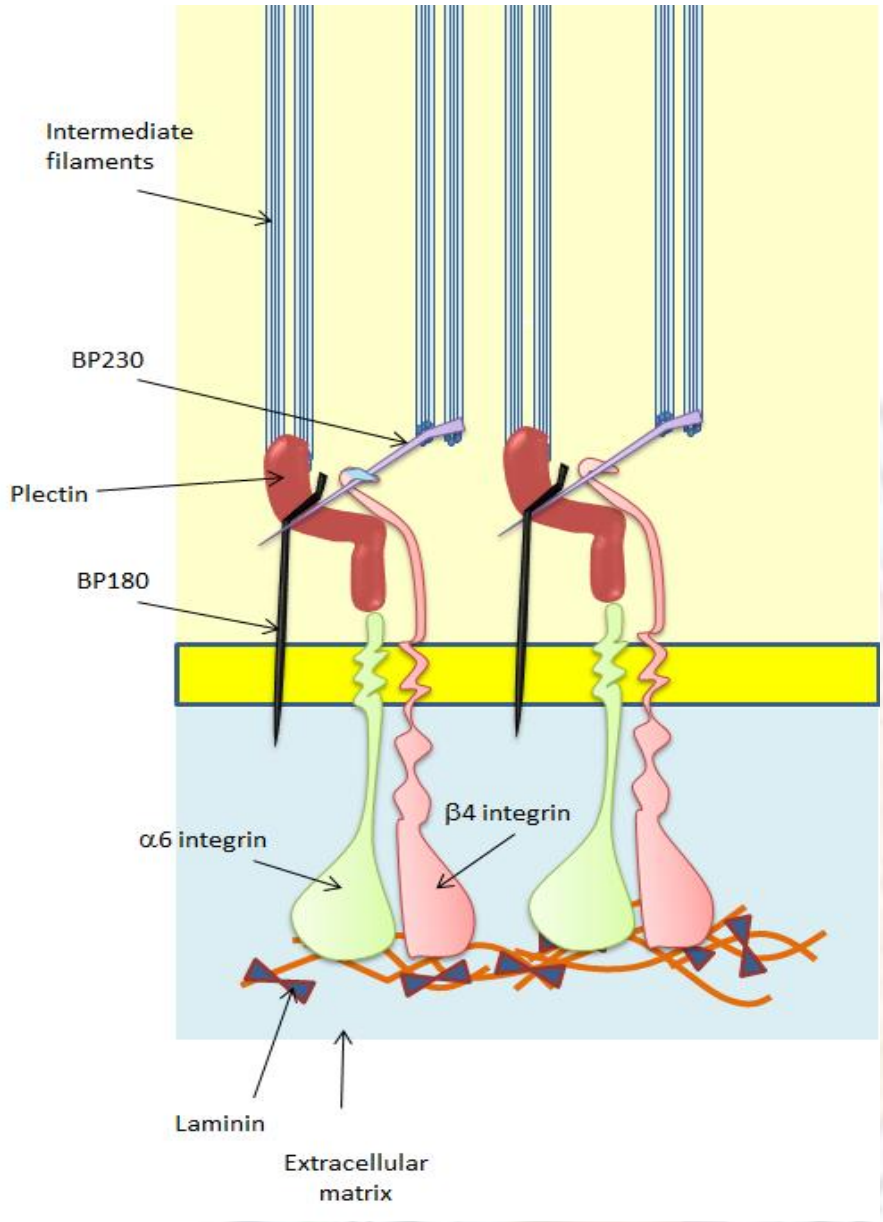


Fig. 5: Attachment of intermediate filaments to hemi-desmosomes. The integrins links the extracellular matrix with intermediate filaments through plectin. The additional interactions are provided by BP180 and BP230 which also regulate the assembly and stability of the hemi-desmosomes.

Disorders associated with intermediate filaments

Intermediate filaments are known to provide mechanical strength and support to the cell. It was earlier thought that these are not required for normal cell growth. This was evident from the experiments using antibodies against vimentin which cause disorganization of vimentin filaments without affecting the cell growth. Elaine Fuchs and colleagues in 1991 first gave the experimental evidences for the role of keratin in cellular organization. They

created transgenic mice with mutant keratin (a mutant K14 with deletions in N- or C-terminal). The transgenic mice showed skin abnormalities like skin blistering due to epidermal cell lysis. This resembles a human genetic condition known as **epidermolysis bullosa simplex (EBS)**. These experimental evidences indicate the role of keratin filaments in maintaining the structural integrity of epithelial cells. The keratin mutants cannot assemble into protofilaments and therefore the mechanical strength is lowered resulting in skin blistering due to mild mechanical trauma.

Similarly mutations in lamin genes have been implemented in a number of human genetic conditions. Mutations in lamin gene *LMNA* have been associated with a rare form of muscular dystrophy called **Emery-Dreifuss Muscular Dystrophy (EDMD)**. In this condition, there is gradual wasting of muscles and development of heart diseases at quite young age. A similar type of muscular dystrophy is caused by mutation in emerin which links lamins to the inner nuclear membrane. Earlier it was thought that Emery-Dreifuss Muscular Dystrophy is caused only by mutation in emerin but subsequently the role of mutations in lamin gene was identified.

Another disease, **Hutchinson-Gilford progeria syndrome (HGPS)**, is also found to be associated with mutations in *LMNA.* HGPS is characterized by premature aging and death at young age. Subsequent studies demonstrated that the mutations in *LMNA* is not only associated with Emery-Dreifuss Muscular Dystrophy and Hutchinson-Gilford progeria syndrome, but also with other genetic conditions like Dunnigan-type partial lipodystrophy, and Charcot-Marie tooth disorder. Mutations in lamin B receptor are also responsible for genetic conditions like Pelger-Huet anomaly.

Another intermediate filament protein, demin plays a significant role in maintaining the alignment of the myofibers. Studies done on transgenic mice that do not produce desmin demonstrate the structural role of desmins in muscles. In humans, mutations in desmin results in **desmin related myopathy** characterized by skeletal muscle weakness and congestive heart failure.

Studies done on transgenic mice model suggest the abnormalities in neurofilaments to be associated with a condition known as **Amyotropic lateral sclerosis (ALS) or Lou Gehrig's disease**. The disease is the outcome of progressive loss of motor neurons resulting in muscular atrophy and paralysis. Abnormal neurofilament assembly is thought to be responsible for ALS. These disorders indicate the tissue-specific functions of various intermediate filament proteins.

Summary

The cytoskeleton provides shape and support to the cells. The cytoskeleton is composed of three cytoskeletal elements: microtubules, microfilaments, and intermediate filaments which together form a collaborative network. The three type of cytoskeletal elements can be distinguished on the basis of their diameter, monomeric units and their arrangements. Microtubules have outer diameter of 25 nm and are composed of globular protein tubulin. Microfilaments also known as actin filament or F-actin are 7 nm in diameter, composed of globular protein actin. Intermediate filaments (IFs) are rope like with diameter of 10 nm and are thought to contain more than 70 proteins. Comparative analyses have identified bacterial proteins similar to eukaryotic actin and tubulin. The bacterial homolog of tubulin is

Cytoskeleton I

FtsZ protein which plays an important role in cell division. While homolog of actin is MreB, found only in rod-shaped bacteria and is thought to control the width in these bacteria. No bacterial homologs have been identified for intermediate filament proteins suggesting these appear later during evolution. The cytoskeletal elements in a cell is organized either forming the bundles or networks. In bundles, the filaments of cytoskeleton are arranged in closely packed parallel arrays while in network these elements are criss-cross-linked. To study cytoskeleton, fluorescently labeled subunits or fluorescently labeled antibodies are used and visualized in fluorescence microscope. The cytoskeletal elements serve many important functions inside a cell like providing support and determine the cell shape; for separating the chromosomes during cell division and cytokinesis; help in positioning various organelles, transporting membrane bound vesicles, organelles and other cargos within cell, cell locomotion by the aid of cilia and flagella or by producing the tension on the substratum. The cytoskeletal elements cooperate with each other to form a network inside a cell. Several experimental evidences suggests that microtubule and microfilament associated motor proteins can bind to and transport the same vesicles. Experimental evidences have clearly demonstrated that a vesicle can move on a microtubule or a microfilament by binding two motor proteins, myosin and kinesis or myosin or cytosolic dynein. Such cooperation between microtubule and microfilaments have been studied in neuron and pigment cells.

Intermediate filaments are important cytoskeletal elements that differ from microtubules and microfilaments as they are composed of more than 70 different kinds of proteins. Intermediate filaments have diameter of 10-12 nm which is intermediate between microtubules (24 nm) and microfilaments (7 nm). Intermediate filaments are more stable as compared to microtubules and microfilaments.

All intermediate filament proteins have a central α-helical rod domain of 310-350 amino acids flanked by globular N- terminal head and C-terminal tail domains of variable size and structure. The rod domain of two polypeptides associate to form coiled-coil dimers. The dimer than associate side by side in antiparallel orientation forming a tetramer. Tetramers associate end to end protofilament and eight protofilaments associate laterally to produce an intermediate filament which lack polarity like microtubules and microfilaments. Studies have revealed that intermediate filaments can also show dynamic properties like microtubule and microfilament. The intermediate filament's assembly and disassembly is controlled by phosphorylation and dephosphorylation of the constituent proteins.

The intermediate filament proteins have been classified into five groups based on amino acid similarity. Type I and II consists of acidic and basic keratins, respectively expressed in epithelial cells. The assembly into keratin filaments require both type I and type II keratin which associate in 1:1 to form heterodimers. Keratins have also been classified as α keratins which occur in mammals and β keratins which are found in birds and reptiles. α keratins are rich in cysteine that forms disulfide bonds with adjacent polypeptide chains. This makes α keratins insoluble and resistance to stretching. Type III includes vimentin, desmin, glial fibrillary acidic protein and peripherin. Vimentin is more widely distributed, found in a variety of cell types like smooth muscles, white blood cells, endothelial cells, epithelial cells and fibroblasts. Vimentin filaments help in keeping nucleus and other organelles in place and also support cell membrane. Desmin is found in muscle cells, Glial fibrillary acidic protein is expressed in glial cells which surround neurons and Peripherin is found in the neurons of the peripheral nervous system. Type IV intermediate filament proteins include neurofilaments, α-internexin and nestin. Neurofilament are abundantly found in mature neurons particularly motor neurons. Neurofilaments play an important role

in supporting the long axons and are also responsible for the radial growth. α-internexin is expressed in the early stages of neuron development when the neurofilaments have not yet expressed. Nestins are also expressed in the embryonic stages in stem cells. Type V intermediate filament proteins involve nuclear lamins which form nuclear lamina. The nuclear lamina forms a framework that supports the nuclear envelope.

Intermediate filament–associated proteins (IFAPs) help in organizing the intermediate filaments into networks or bundles and also mediate their interaction with microfilament and microtubule cytoskeletons. Intermediate filaments form an extensive network that extends from nucleus to the cell periphery. The organization of keratin filaments are seen in epithelial cells where they are connected to the plasma membrane by IFAPs in desmosomes and hemi-desmosomes. Desmosomes are the regions of cell-cell contact where the interaction between adjacent epithelial cells are mediated by transmembrane proteins like desmoglein and desmocollin. Hemi-desmosomes are the regions of epithelial cell contacts with the underlying connective tissue where integrin links the extracellular matrix with the keratin filaments through plectin.

Many disorders associated with intermediate filament proteins have been identified. These disorders indicate the tissue-specific functions of various intermediate filament proteins. A human genetic condition known as epidermolysis bullosa simplex (EBS) is caused by mutant keratin, characterized by skin blistering due to mild mechanical trauma. Mutations in lamin genes have been implemented in a number of human genetic conditions like Emery-Dreifuss Muscular Dystrophy (EDMD). Resulting in gradual wasting of muscles and development of heart diseases, Hutchinson-Gilford progeria syndrome (HGPS) characterized by premature aging and death at young age, Dunnigan-type partial lipodystrophy, Charcot-Marie tooth disorder and Pelger-Huet anomaly. In humans, mutations in desmin results in desmin related myopathy characterized by skeletal muscle weakness and congestive heart failure. The abnormalities in neurofilaments are associated with a condition known as Amyotropic lateral sclerosis (ALS) or Lou Gehrig's disease. The disease is the outcome of progressive loss of motor neurons resulting in muscular atrophy and paralysis.

Exercise/ Practice

A. Multiple choice questions:

1. The most stable cytoskeletal element is

   a) Microtubules b) Microfilaments c) Intermediate Filaments d) none

2. The function of intermediate filaments is

   a) transporting membrane bound vesicles b) cell locomotion c) chromosomes separation d) mechanical support

3. The diameter of intermediate filaments is

   a) 24 nm b) 7 nm c) 4 nm d) 10 nm

4. IF share the following characteristic with other cytoskeletal elements

   a) polarity b) dynamic behavior c) treadmilling d) associated motor proteins

5. The immediate subunit for assembly of intermediate filaments is
   a) monomer b) trimer c) tetramer d) protofilament

6. The conserved feature of all intermediate filament proteins is
   a) central rod domain b) N- terminal head c) C-terminal tail d) none

7. Which of the following intermediate filament proteins form homopolymers
   a) acidic keratins b) basic keratins c) Nuclear lamins d) NF-H

8. The most widely distributed intermediate filament protein is
   a) desmin b) keratin c) nestin d) vimentin

9. Which of the following is the disorder related to keratin filaments
   a) Muscular Dystrophy b) epidermolysis bullosa simplex c) progeria d) amyotropic lateral sclerosis

10. A member of plakin family found in desmosomes is
    a) desmoglein b) desmoplakin c) plakophillin d) plackoglobin

B. Fill in the blanks:

1. The bacterial homolog of tubulin is _____
2. α keratins which occur in _____ and β keratins which are found in _____
3. The bacterial homolog of actin is _____
4. Hard keratins have high _____ content.
5. Of all these IF proteins, _____ is more widely distributed and is found in a variety of cell types like smooth muscles, white blood cells, endothelial cells, epithelial cells and fibroblasts.
6. IF protein found in glial cells is _____
7. The nuclear lamina forms a framework which is composed of lamin _____
8. Example of intermediate filament associated protein is _____
9. At desmosomes the keratin filaments are attached to the plasma membrane and transmembrane proteins by _____
10. _____ connects Z-dics to the other contractile elements.

C. True/False

1. Bacteria do not have any protein similar to IF proteins but have homologs of microtubules and microfilament.
2. The tetramers act as immediate subunit for assembly of intermediate filaments.

3. IF have polarity like microtubules and microfilament.
4. Intermediate filaments are more stable as compared to microtubules and microfilaments.
5. Keratins are one of the most diverse groups of intermediate filament proteins. True
6. IF do not show dynamic behavior.
7. Keratins are the only intermediate filament proteins that can assemble without the head and tail domains.
8. The central α-helical rod domain IF has variable size and structure.
9. IF proteins like microtubules and microfilaments are globular in nature.
10. The assembly and disassembly of IFs is controlled by phosphorylation and dephosphorylation of the constituent proteins.

D. Expand the following

1. IF
2. NF
3. LBR
4. IFAP
5. CH domain
6. EBS
7. EDMD
8. ALS
9. GFAP

Glossary

α-internexin: type IV IF protein expressed in the early stages of neuron development

Cytokeratins: another name for soft keratins

Desmin: type III IF protein found in muscle cells, connects Z-dics to the other contractile elements thus stabilizing the sarcomeres.

Desmosomes: the regions of cell-cell contact between adjacent epithelial cells

FtsZ: a bacterial homolog of tubulin, forms a contractile ring which plays an important role in bacterial cell division

Glial fibrillary acidic protein: type III IF protein expressed in glial cells which surround neurons

Hard keratins: keratins having high sulfur content and are found in hair, nails, wool and horn

Hemi-desmosomes: the regions of epithelial cell contacts with the underlying connective tissue

MreB: A bacterial homolog of actin, present only in rod-shaped bacteria

Nestins: type IV IF protein expressed in the embryonic stages in stem cells.

Neurofilament: type IV IF protein abundantly found in mature neurons, play an important role in supporting the long axons

Nuclear lamins: type V IF proteins which form nuclear lamina, mammals express three lamin genes which are designated as A, B and C

Peripherin: type III IF protein found in the neurons of the peripheral nervous system

Soft keratins: keratins having low sulfur content and are found in the epithelial cells that line internal body cavities.

Syncoilin: type IV IF protein present at the neuromuscular junction, required for maintenance and maturation of the synapse

Synemin: type IV IF protein found in muscle and few non-muscle cells

Vimentin: widely distributed type III IF protein, helps in keeping nucleus and other organelles in place and also support cell membrane

References/ Bibliography/ Further Reading

Brown, R. H. Jr. 1995. Amytrophic lateral sclerosis: Recent insights from genetics and transgenic mice. Cell 80: 687-692.

Coulombre, P. A., and Omary, B. M. 2002. "Hard" and "Soft" principles defining the structure, function, and regulation of keratin intermediate filaments. Curr. Opin. Cell Biol. 14: 110–122.

Epstein, E. H., Jr., Bonifas, J. M., and Rothman, A. L. 1991. Epidermolysis bullosa simplex: Evidence in two families for keratin gene abnormalities. Science 254: 1202-1205.

Fuchs, E. and Raghavan, S. 2002. Getting under the skin of epidermal morphogenesis. Nature Rev. Genet. 3: 199-209.

Fuchs, E. and Yang, Y. 1999. Crossroads on cytoskeleton highways. Cell 98: 547-550.

Fuchs, E., and Cleveland, D. W. 1998. A structural scaffolding of intermediate filaments in health and disease. Science 279: 514–519.

Graumann, P. L. 2007. Cytoskeleton elements in bacteria. Ann. Rev. Microbiol. 61: 589-618.

Herrmann, H., and Aebi, U. 2004. Intermediate filaments: Molecular structure, assembly, mechanism, and integration into functionally distinct intracellular scaffolds. Ann. Rev. Biochem. 73: 749-789.

Herrmann, H., Bar, H., Kreplak, L., Strelkov, S. V. and Aebi, U. 2007. Intermediate filaments: from cell architecture to nanomechanics. Nature Revs. Mol. Cell Biol. 8: 562–573.

Leung, C. L., Green, K. J. and Liem, R. K. 2002. Plakins: a family of versatile cytolinker proteins. Trends Cell Biol. 12: 37–45.

Schwarz, M. A., Owaribe, K., Kartenbeck, J. and Franke, W. W. 1990. Desmosomes and hemidesmosomes: Constitutive molecular components. Ann. Rev. cell Biol. 6: 461-491.

Stuurman, N., Heins, S. and Aebi, U. 1998 Nuclear lamins: their structure, assembly, and interactions. J. Struc. Biol. 122: 42–66.

Vassar, R., Coulombe, P. A., Degenstein, L., Albers, K. and Fuchs, E. 1991. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin condition. Cell 64: 365-380.

Answers

A. Multiple choice questions

1. c) Intermediate Filaments
2. d) mechanical support
3. d) 10 nm
4. b) dynamic behavior
5. c) tetramer
6. a) central rod domain
7. c) Nuclear lamins
8. d) vimentin
9. b) epidermolysis bullosa simplex
10. b) desmoplakin

B. Fill in the blanks

1. FtsZ protein
2. mammals, birds and reptiles
3. MreB protein
4. sulfur content
5. Vimentin
6. Glial fibrillary acidic protein
7. A and C filaments
8. plectin
9. desmoplakin
10. Desmin

C. True/False

1. True

2. True
3. False, they lack polarity
4. True
5. True
6. False; they can also undergo assembly and disassembly like other cytoskeletal elements
7. True
8. False; it is a conserved feature of all IF proteins.
9. False, they belong to fibrous proteins
10. True

D. Expand the following

1. Intermediate filaments
2. Neurofilament
3. lamin B receptor
4. Intermediate filament–associated proteins
5. calponin-homology domain
6. epidermolysis bullosa simplex
7. Emery-Dreifuss Muscular Dystrophy
8. Amyotropic lateral sclerosis
9. Glial fibrillary acidic protein