Genetics & molecular biology

◉ Sheet ○ Slide

Number:	29
Done by:	Maryam Ali
Corrected by:	Sarah Jaar
Doctor:	Diala AbuHassan

The Nucleolus

The nucleolus is the most prominent of the nuclear bodies. And although it may seem a prominent part of the nucleus when examined with the microscope, it is continuous with the nucleus , and it doesn't have a surrounding membrane.

- Nucleoli are the cell factories for the manufacture of rRNA and other RNA molecules, in addition to fulfilling the function of ribosome subunits assembly.
- The nucleolus is associated with chromosomal regions that contain about 200 copies of the genes for 5.8s , 18s and 28s rRNAs to synthesize large amounts of ribosomes. The 5.8s , 18s and 28s rRNA genes are transcribed as a single unit by polymerase I in the nucleolus. These genes are separated by spacers (non-coding sequences) which provide protection for the genes from deletion or damages on the sequence of rRNA (during cutting process). 18s rRNA is part of the small ribosomal subunit (40s); whereas the large ribosomal subunit (60s) has the 5.8s and 28s rRNAs , in addition to the 5s rRNA (which is produced outside the nucleolus by RNA polymerase III).
- Ribosomal subunits preparation:
  After the transcription of the genes mentioned above (with the spacers to separate the three genes from each other (18s, 5.8s, 28s)) there will be the cutting process.

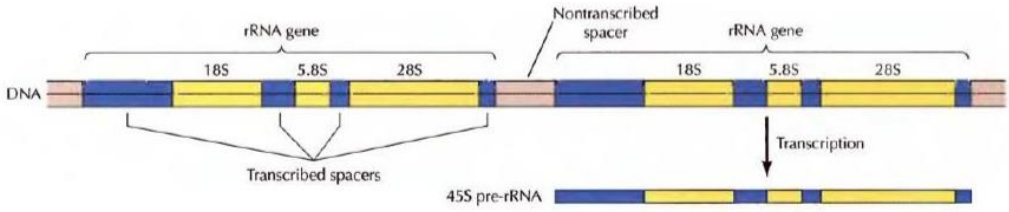


FIGURE 9.25 Ribosomal RNA genes Each rRNA gene is a single transcription unit containing the 18S, 5.8S, and 28S rRNAs as well as transcribed spacer sequences. The rRNA genes are organized in tandem arrays, separated by nontranscribed spacer DNA.

Functions of the nucleolus:

1-rRNA synthesis

2-Ribosome production

3-RNA modification and the assembly of ribonucleoprotein particles

4-Small RNA production such as tRNA , snRNA , RNase P RNA (the catalytic part of the tRNA processing enzyme) , SRP (targets proteins to ER)

5-Cell division and responding to stress

snoRNA:

- Small nucleolar RNAs, localized to the nucleolus
- They complex with proteins to form snorRNPs (small nucleolar ribonucleoproteins)

FUNCTION:

It recognizes the region between the spacers and rRNA (18s , 28s , 5.8s)
It will attach to this region by **complementarity** (A-U / G-C) (the snoRNAs contain around 15 nucleotides complementary to pre-rRNA), and this binding will be marked by **methylation** (adding CH3). Then it will be recognized by endonuclease to cut the pre-RNA into 18s, 28s and 5.8s (like spliceosomes of pre-RNA)

- ✓ Without the methyl group the endonuclease will try to cut the pre-RNA randomly until it fits to the right position, but by having methyl group the cutting process of the pre–RNA will be much easier and faster.

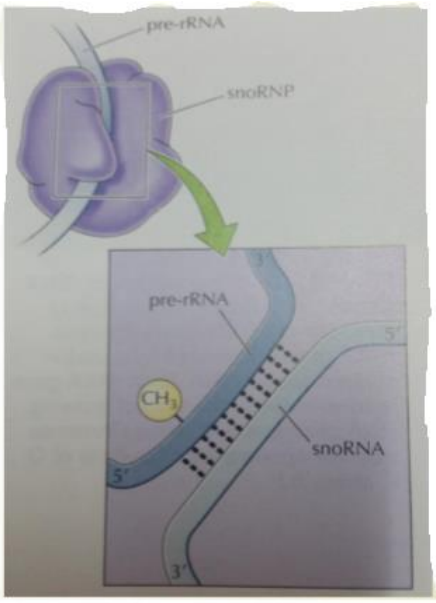


FIGURE 9.30 Role of snoRNAs in base modification of pre-rRNA The snoRNAs contain short sequences complementary to rRNA. Base pairing between snoRNAs and pre-rRNA targets the enzymes that catalyze base modification (e.g., methylation) to the appropriate sites on pre-rRNA.

- ## Processing of pre-rRNA:

It includes the removal of the spacers (internal transcribed spacers [ITS] - colored blue) and (external transcribed spacers [ETS] - colored red).

Steps:

1. Cleavage within the external transcribed spacer (ETS) near the 5'end
2. Removal of the ETS at the 3'end
3. Base modification (methylation of ribose and some bases)

The steps are not required!!!

BUT, we need to know that at the **end of the process** we will end up with 18s , 28s , 5.8s **separated from each other**, that we can attach to them ribosomal protein.

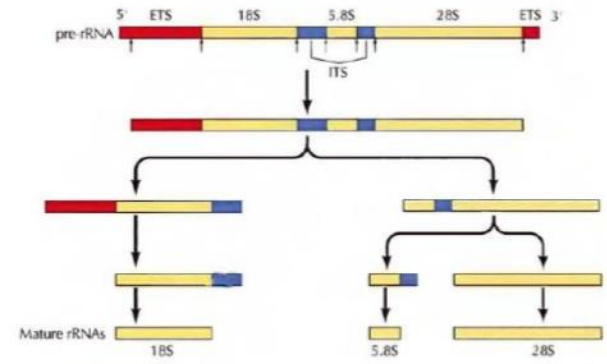


FIGURE 9.29 Processing of pre-rRNA The higher eukaryote 45S pre-rRNA transcript contains external transcribed spacers (ETS) at both ends and internal transcribed spacers (ITS) between the sequences of 18S, 5.8S, and 28S rRNAs. The pre-rRNA is processed via a series of cleavages to yield the mature rRNA species.

- **Ribosome assembly:**

This process takes place early on, that means while cutting and processing of pre-rRNA is going on .

Small subunit (40s) is formed by: 18s + proteins

Big subunit (60s) is formed by: 28s + 5.8s + 5s + proteins

The ribosomal proteins are transcribed outside the nucleolus by polymerase II then translated in the cytoplasm.

- **NOTE**

5S rRNA is transcribed outside the nucleolus by polymerase III

5.8S , 18S , 28S rRNAs are transcribed inside by polymerase I

Small subunit processing is **simpler** by **4 endonuclease** cleavages in the **nucleus**.

Large subunit (28s , 5.8s and 5s rRNAs) processing is **more complex with extensive nuclease** cleavage in **the nucleolus.**

Ribosomal subunit maturation: pre-ribosomal particles are **exported to the cytoplasm** to form the **active** 40s and 60s subunits of ribosomes

Then they will combine once mature-mRNA binds to the small subunit and then the large subunit will bind forming the ribosome structure and after that translation will occur.

The cytoskeleton and cell movement (Actin microfilaments):

What is the cytoskeleton?

A dynamic network of protein filaments extending throughout the cytoplasm (cell) to perform their function:

1- Structural framework for cells
2- Determines cell shape , movement and division
3- Determines positions of organelles
4- Determines overall organization of cytoplasm
5- Regulates internal movement of organelles and other structures such as mitotic chromosomes

Three types of protein filaments : actin microfilaments , microtubules , intermediate filaments

These functions can be **specific** for one type of protein filaments **or** can be done by **all** of them

The actin filaments (microfilaments)

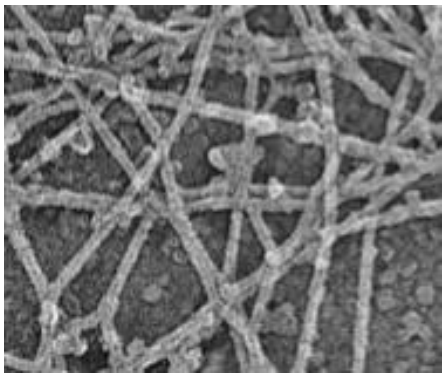
Thin **flexible fibers** , 7nm diameter and several µm in length; that's why they are flexible (they're **considered the smallest filaments** according to diameter). The largest is the microtubules , and the intermediate is in between .

They are organized into higher-order structures , forming **bundles or three-dimensional networks.**

They form **semisolid gels** (because they are the smallest)

Their **assembly**, **disassembly, cross-linking** and association with cellular structures are regulated by a variety **of actin-binding proteins**.

They are abundant **beneath the plasma membrane** to form **a network** that provides **mechanical support, determines cell shape and allows cell movement.**



The actin proteins

Polymer structures, made of monomers (globular-actin i.e. **G-actin**) which are **coded for by 6 different genes;** these give variability for the actin.

1-**FOUR** of these genes are expressed in different types of **muscle**

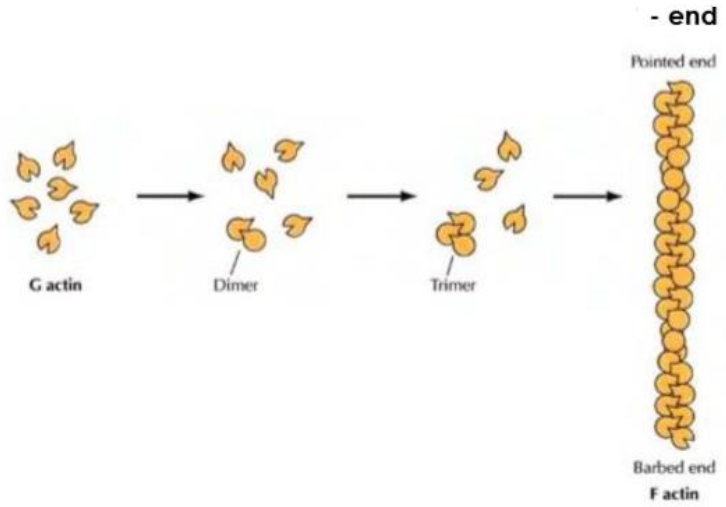
2-**TWO** of these genes are expressed in **nonmuscle** cells

Also, these G-actin monomers have their orientation (dimer, trimer) that they are tightly bound to <u>two other actin monomers</u> having a head-to-tail interactions

<u>G-actin monomers polymerize to form filamentous [F] actin.</u>

Actin filaments have distinct polarity and **2 different ends**: **the barbed (plus) end** [elongation of F actin] and **pointed (minus) end** [starting point, there will not be adding any more G-actin].

Polarity affects actin assembly and the direction of myosin's movement relative to actin



Formation of filaments:

These actin filaments are considered as **dynamic structures** even if the cell doesn't move , they are dynamic (between assembly and disassembly) and sometimes they have the **same speed** that will lead to have the **same length,** so there will be **equilibrium between assembly and disassembly**.

<u>BUT,</u> sometimes I need to have much more assembly than disassembly under certain conditions that will be discussed later on. And sometimes there will be **collapse, much more disassembly**.

<u>Treadmilling:</u> a process of **adding ATP-actin to the barbed end** (plus end), while **ADP-actin dissociates from the pointed end** (minus end)

This needs to be **controlled by binding the G-actin to ATP/ADP** to have **different conformations;** that allows distinguishing which conformation is to be considered for assembly or disassembly.

ATP-actin → much more stable and strong; used for the assembly process
ADP-actin→ when ATP is hydrolyzed and gives ADP; used for disassembly

Steps for the formation of filament:

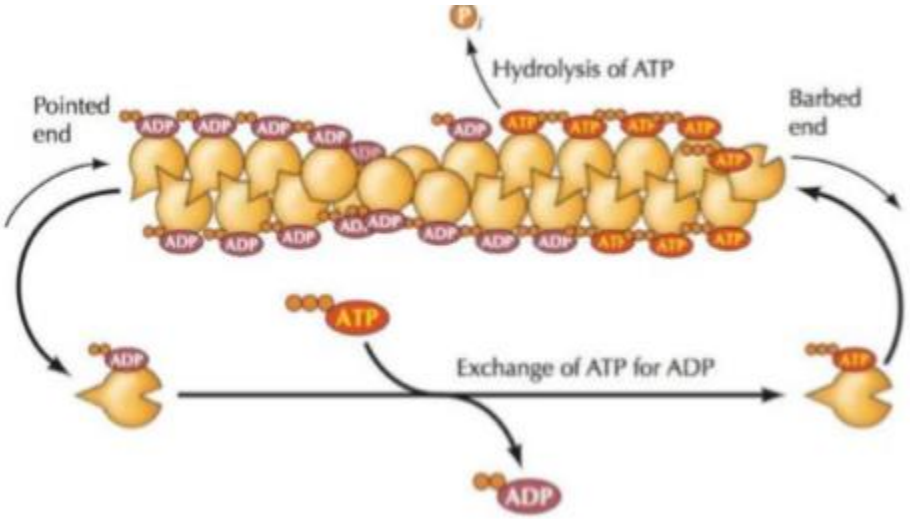
1-**Nucleation**: formation of a stable 'actin nucleus' which is an aggregate of 3 actin monomers

2- Filament growth by adding additional monomers to both ends (barbed and pointed), but faster at barbed ends (since ATP-actin has a higher affinity for the barbed end)

3-The monomers are bound to ATP, which **isn't required for nucleation**. **But still, ATP:**

1- **Is hydrolyzed into ADP following assembly**
2- **Speeds polymerization**
3- **Stabilizes binding**

* Actin filament depolymerization happens when all monomers become bound to ADP



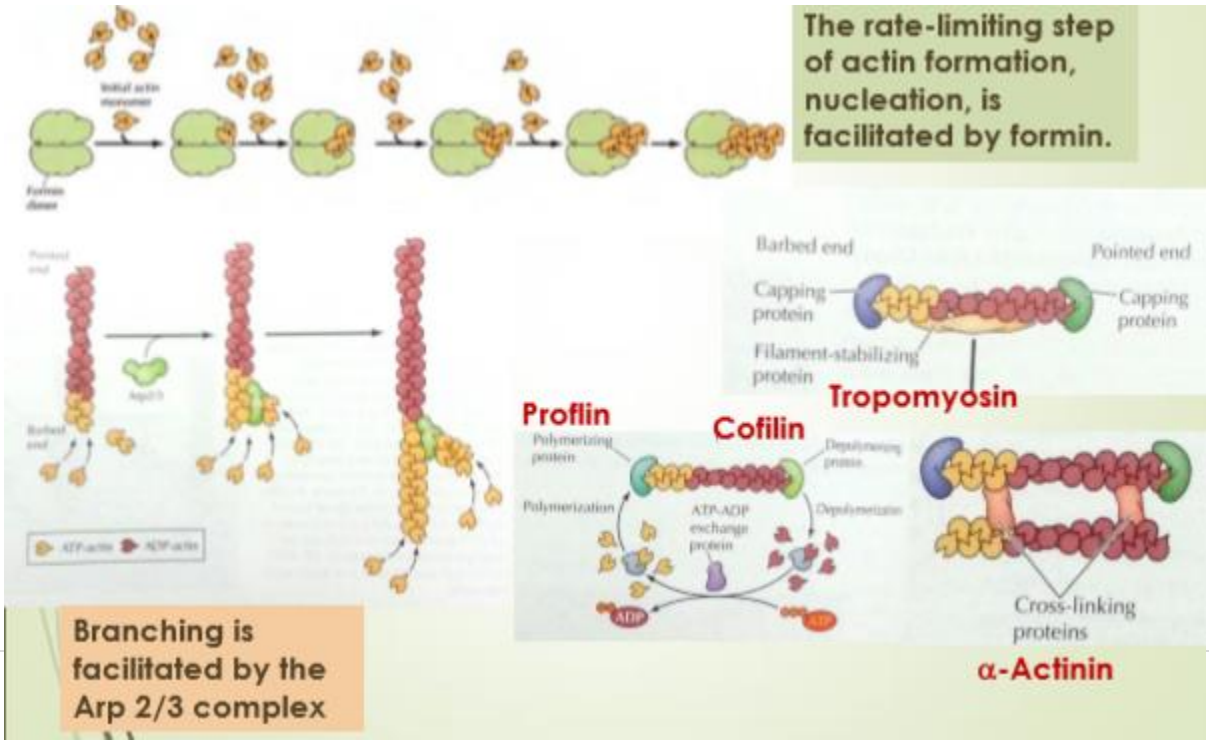
Actin-binding proteins:

These accessory proteins regulate actin assembly and disassembly, as well as the stability of actin cytoskeleton. Such bindings increase the variety and the stability of actin filaments (only the examples in rectangles in the below table are for memorization)

Cellular Role	Representative Proteins
Filament initiation and polymerization	Arp2/3, formin
Filament stabilization	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
End-capping	CapZ, tropomodulin
Filament severing/depolymerization	ADF/cofilin, gelsolin, thymosin
Monomer binding	Profilin, twinfilin
Actin filament linkage to other proteins	α-catenin, dystrophin, spectrin, talin, vinculin

The following points discuss the previous table:

- **The rate limiting step of actin formation**, which is nucleation, is facilitated by **formin**
- **Formin** initiates the **formation of an actin nucleus (three monomers)**
- **Profilin** enhances the **elongation of the actin filaments** (make the actin filaments longer by bringing more ATP-actin)
- **Branching is facilitated by the ARP2/3 complex** (on its two sides it'll bind to ATP-actin)
- **Cofilin facilitates the disassembly process** by removing ADP-actin from the minus end. Also, **cofilin** stimulates the exchange of ADP for ATP; this provides active monomers for assembly into filament.
- **Tropomyosin facilitates the stabilization** of the filaments (makes the filament inaccessible to destabilizing and depolymerizing agents) and giving strength
- **α-actinin** aids in **filament cross-linkage** which makes them **stronger**

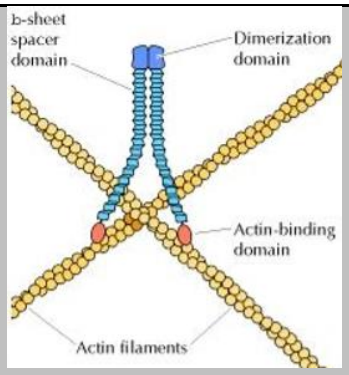
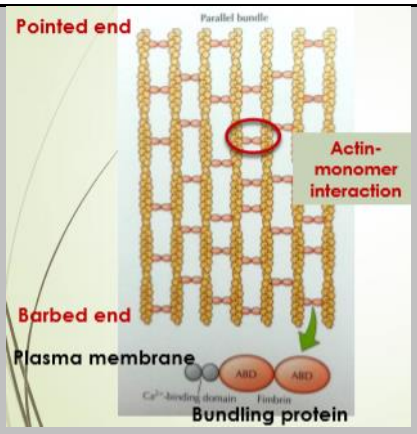
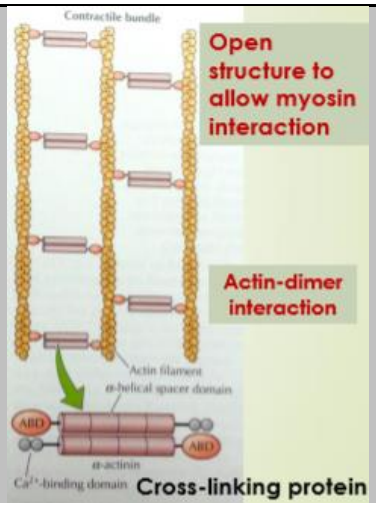


Actin filaments arrangements

Different arrangements of actin filaments are required for actin to perform its various functions in the cell. The are two main arrangements of actin filaments: **bundles** and **networks. Each of these arrangements involve different actin-binding proteins to construct a cross-linkage.**

These arrangements differ in flexibility , size and shape of the cross-linking proteins.

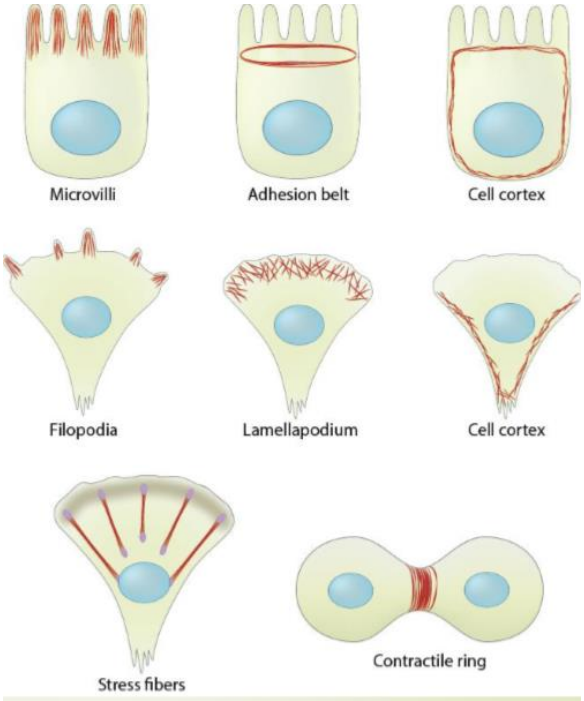
--- In muscles, the cross-linking proteins are far from each other; that allows the muscle to relax and contract easily. BUT, if they were near each other, the structure will be very rigid ------ so you can't get relaxation and contraction in an easy way.

	Networks	**First type of bundle (Parallel Bundles)**	**Second type of bundle (Contractile Bundles)**
illustration			
Cross-linking protein example	Large actin-binding proteins: **filamin**, binds actin as a flexible dimer (they dimerize to allow some sorts of movements)	**Fimbrin;** actin-**monomer** interaction	**Alpha-actinin;** actin-**dimer** interaction
Features	Actin filaments are crosslinked in a **perpendicular** way. **Flexible**, loose 3D meshwork, **supports the cell membrane**	Actin filaments are crosslinked in a **parallel** way. **Closely packed, rigid,** supports the membrane projections like microvilli	Actin filaments are crosslinked in a **parallel** way. **More widely spaced and flexible** than first type of bundle**,** allows contact and interaction (for example myosin)
Location	**Under plasma membrane**	**Microvilli** in intestinal epithelium	**Muscle cells**

Where we can find Actin fibers in the cell ?

Not only we can find them in the muscle cells, but each cell has actin filaments in different regions:

1- **Cell cortex / cortical cytoskeleton**: found under the cytosolic part of the plasma membrane to give special shape for the cell
2- **Contractile ring** : for constriction of the cell during cell division
3- **Stress fiber** : network of the actin bundles that form under specific condition, increase in stress fibers will lead to increase in the rigidity that takes place in a pathologic conditions (e.g. glaucoma)
4- **Lamellipodia** : movement of organelles
5- **Filopodia** : movement of organelles (projections from lamellipodia and are smaller than them)
6- **Microvilli** : bundles; projections to increase surface area
7- **Adhesion belt** : to hold the epithelial cell together in adhesion



Actin filaments and the plasma membrane

Cell cortex or cortical cytoskeleton :

The 3D network of actin filaments and associated actin-binding proteins at the periphery that determines cell shape and assists in cellular activities such as movement .

Studies are performed on RBCs BECAUSE:

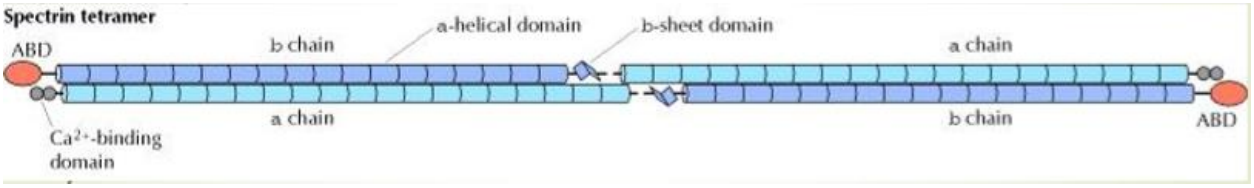
1- **The don't have other cytoskeletal structures**
2- **They don't have a nucleus or other organelles (e.g. mitochondria) so no contamination**
3- **The cytoskeleton is uniform with no specialized regions like in other cells.**

Spectrin as a structural component of cortical cytoskeleton

Cortical cytoskeleton needs to be connected to protein to give support , such as the nuclear lamina that attaches emerin to the nuclear envelope.

The major protein that provides the structural basis for the cortical cytoskeleton in erythrocytes is **Spectrin**; it's an actin binding protein.

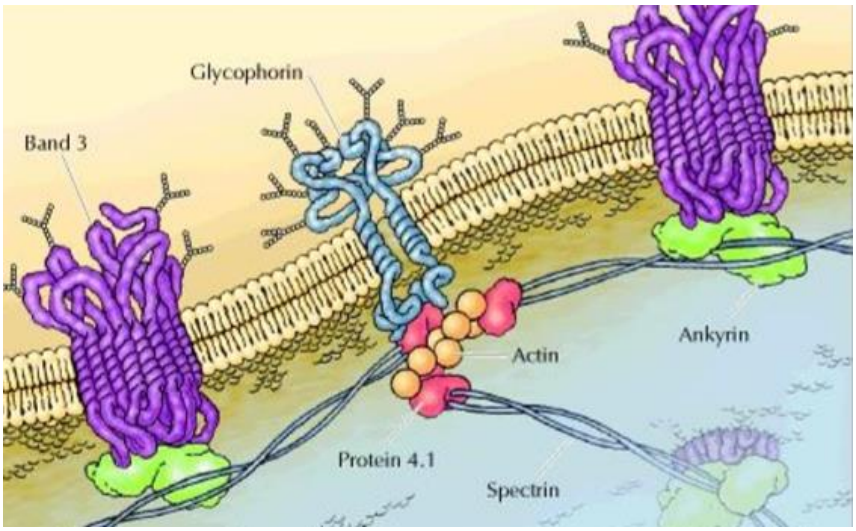
Spectrin is tetramer of two alpha and beta polypeptides, with the alpha chain having 2 Ca^{2+} binding domains at its C-terminus and the beta chain having the actin binding domain (ABD).



Actin filaments-plasma membrane interaction

The ends of the spectrin tetramers then associate with actin filaments, resulting in the spectrin-actin network that forms the cortical cytoskeleton. The spectrin-actin network is linked to membrane by:

1- **Ankyrin** , which binds to both spectrin & the abundant **transmembrane protein band 3**
2- **Protein 4.1** , that binds to **glycophorin** (another transmembrane protein) **to increase the stability of the structure**
3- **Phospholipids**

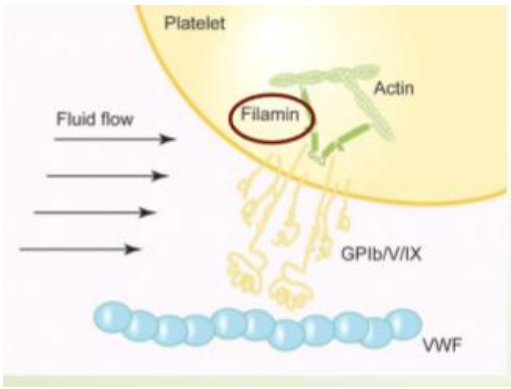


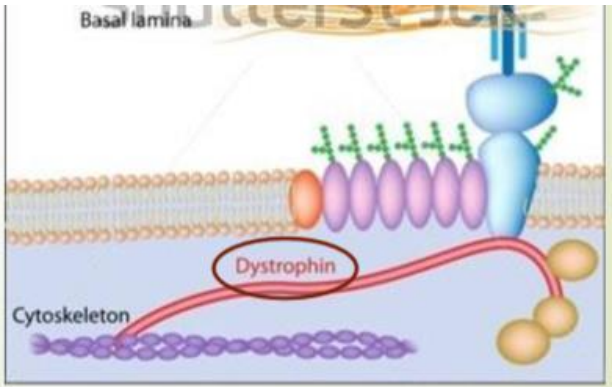
Other linkage to plasma membrane in other cells:

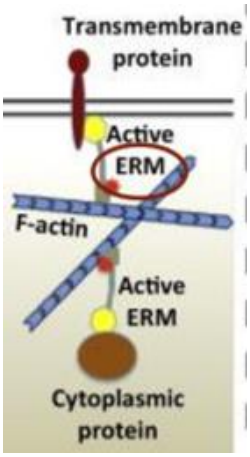
The ERM proteins (protein 4.1 – related) link actin filaments to plasma membranes of different kinds of cells

Filamin (spectrin-related) links actin filaments with the plasma membrane of blood platelets

Dystrophin **(spectrin related)** links actin filaments to transmembrane proteins of the muscle cell plasma membrane and the latter link the cytoskeleton to the ECM. **This maintains cell stability during muscle contraction**

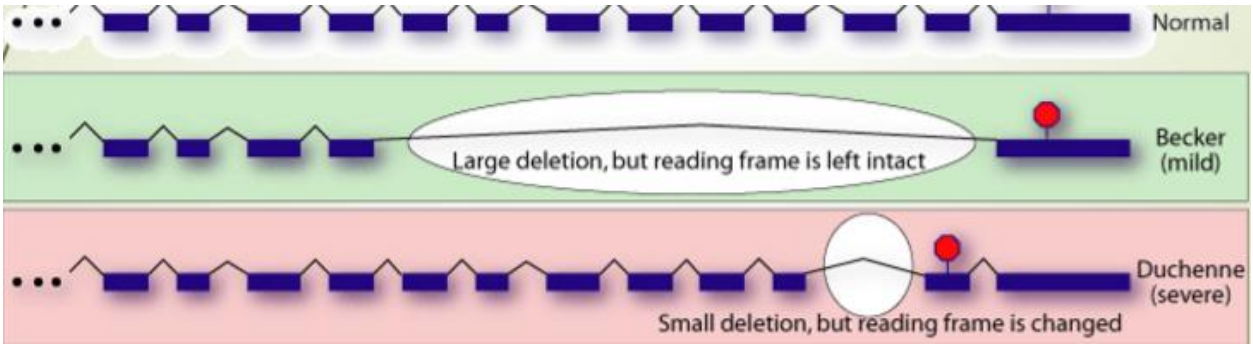






***Mutation** in Dystrophin **leads to muscular dystrophies**

- The dystrophin gene encodes a large protein (427 kd)
- Mutation in the gene causes **2 types** of muscular dystrophy: **Duchenne's** (severe) which has a **small deletion** but leads to a frameshift mutation resulting in changing the protein, and **Becker's** (moderate) which has **large deletion** in the gene.
- X – linked inherited diseases
- Progressive degeneration of skeletal muscle
- **Patients with Duchenne's muscular** dystrophy usually die in their teens or early twenties



- **Patients with muscular dystrophies have** :
  - **deformities** in their muscular skeletal muscle , such as having **curves** in their bodies , **protrusion** of the abdomen
  - Usually , they **can't walk**
  - Some of them are of **size abnormal (relatively small)**
  - No treatment for them until now

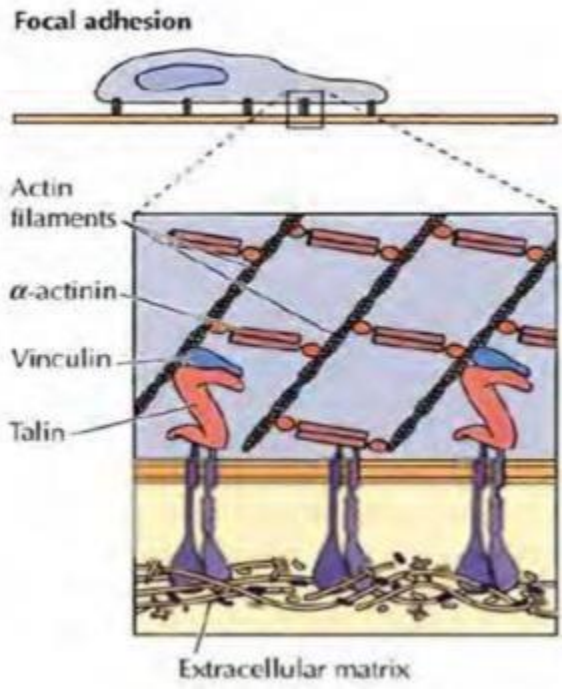
Focal adhesion

Focal adhesions are specialized local regions that serve as attachment sites for bundles of actin filaments (stress fibers) that anchor the cytoskeleton (and cells) to areas of cell contact or to extracellular matrix via the binding of transmembrane proteins (called integrins) to the extracellular matrix. Focal adhesion , like other cell-ECM connections, has a great role in the stabilization of cells and providing them with the needed mechanical strength, especially for epithelial cells.

Integrins are transmembrane proteins that have 2 subunits (heterodimer): alpha and beta. The alpha subunit is diverse in different cells and is usually denoted by numbers .

Integrins' attachment with the cell cortex isn't directly but it is through proteins **(talin, then vinculin which has an actin binding domain) ------ this is called focal adhesion**

- **Stress fibers are contractile bundles of actin filaments**
- **The stress fibers are cross linked by alpha-actinin.**

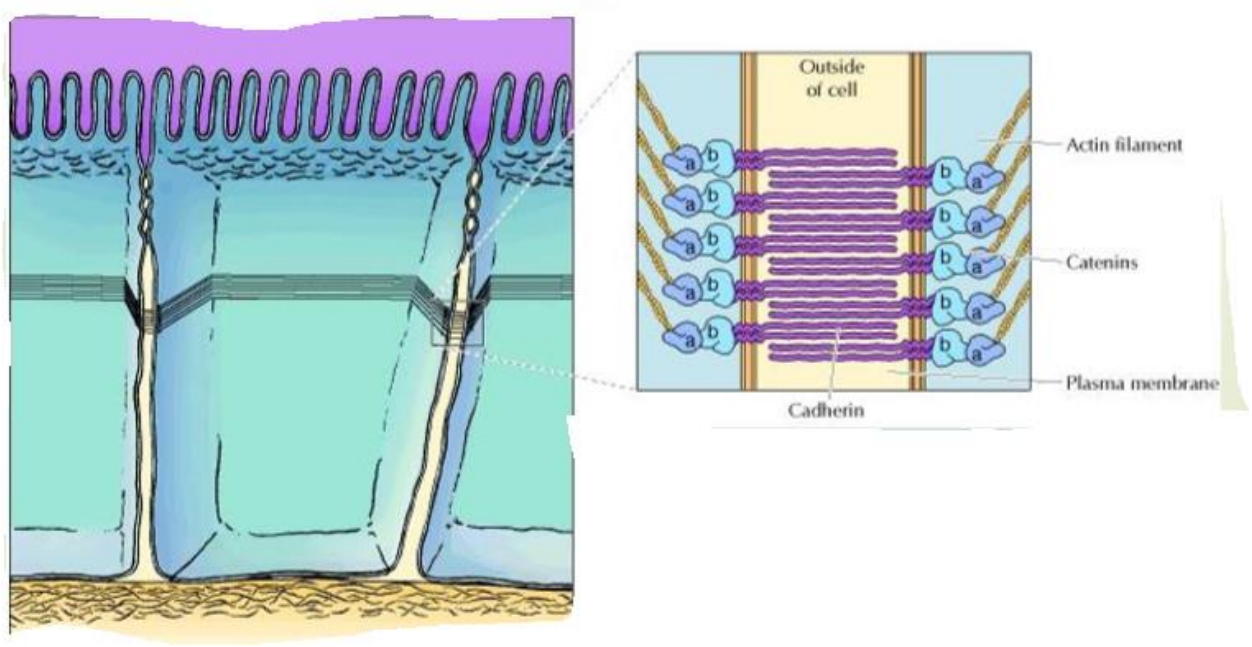


Adherens junctions

Another type of junction that includes actin filaments

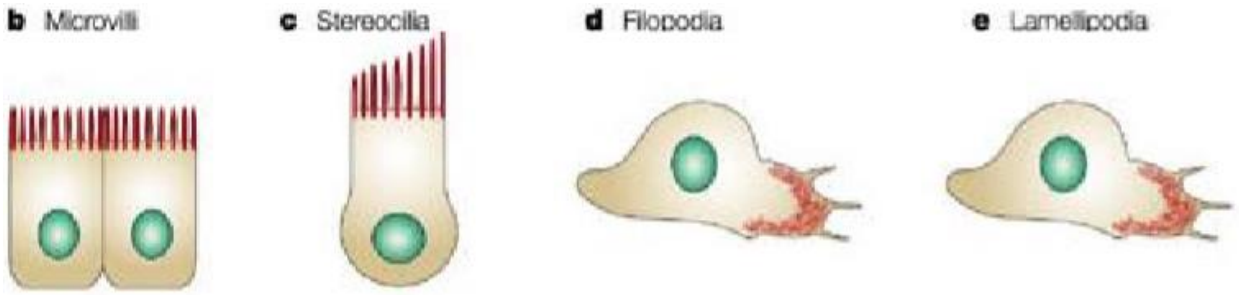
- Regions of **cell-cell contact (epithelial cells)** to which actin cytoskeleton is anchored.
- They form a continuous **beltlike structure (adhesion belt) around each cell** in which an underlying contractile bundle of actin filaments is linked to the plasma membrane.

- **Contact between cells** is mediated **by cadherins (transmembrane proteins)** that form a complex with **cytoplasmic proteins called catenins** (has 2 subunits: alpha-binds with actin & beta-binds with cadherin), which associate with actin filaments.



Protrusions of the cell surface

- A variety of protrusions or extensions are present on cell surfaces
- Cell surface protrusions are involved **in cell movement , phagocytosis , or specialized functions such as absorption of nutrients**
- Most of these **cell surface extensions are based on actin filaments** organized into either relatively permanent or rapidly rearranging bundles or networks.
- **Examples : microvilli , stereocilia , filopodia , lamellipodia**



Microvilli

- **Ridge** structure , **bundles** , found in the **small intestine**
- Fingerlike extensions of the plasma membrane that are particularly abundant on the surfaces of cells involved in **absorption , such as the epithelial cells lining the intestine**
- They form a layer on **the apical surface (called a brush border)** to increase the exposed surface area available for absorption.

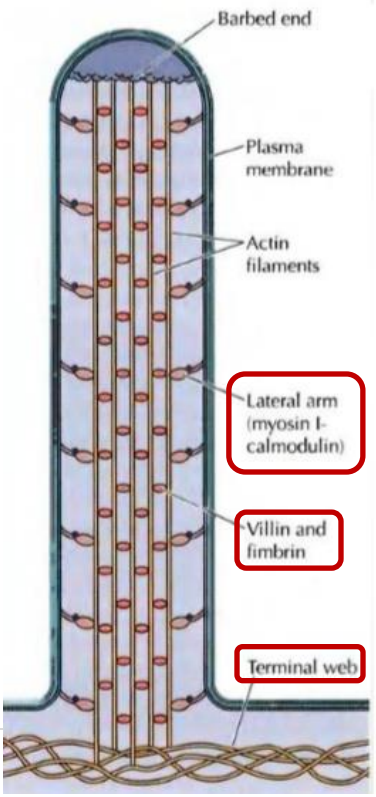
Stereocilia

- **Specialized forms of microvilli** on the surface of **auditory hair cells**
- Responsible for **hearing** by detecting sound vibrations
- The **form bundles**
- Don't mix it up with cilia, which are found in **the respiratory cells**

Organization of microvilli

- 20-30 closely packed parallel **bundles**
- Filament bundles are **linked by linker proteins villin (major) and fimbrin**
- Attachment to **plasma membrane is mediated by a lateral arm made of calmodulin and myosin I** to assist in movement (here to provide better anchoring)
- Filaments are linked to the **cortex** at the base via a **spectrin-rich region** called the **terminal web**

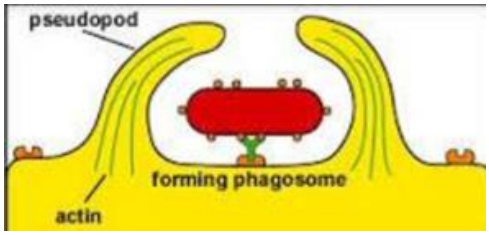
***Notice:** that the structure of microvilli is stabilized by many mechanisms ; which include the **closely-packed bundle of actin filaments ; the interaction with the cortex of the membrane ; and having myosin interactions.**



Other protrusions:

pseudopodia

Extensions of moderate width responsible for phagocytosis



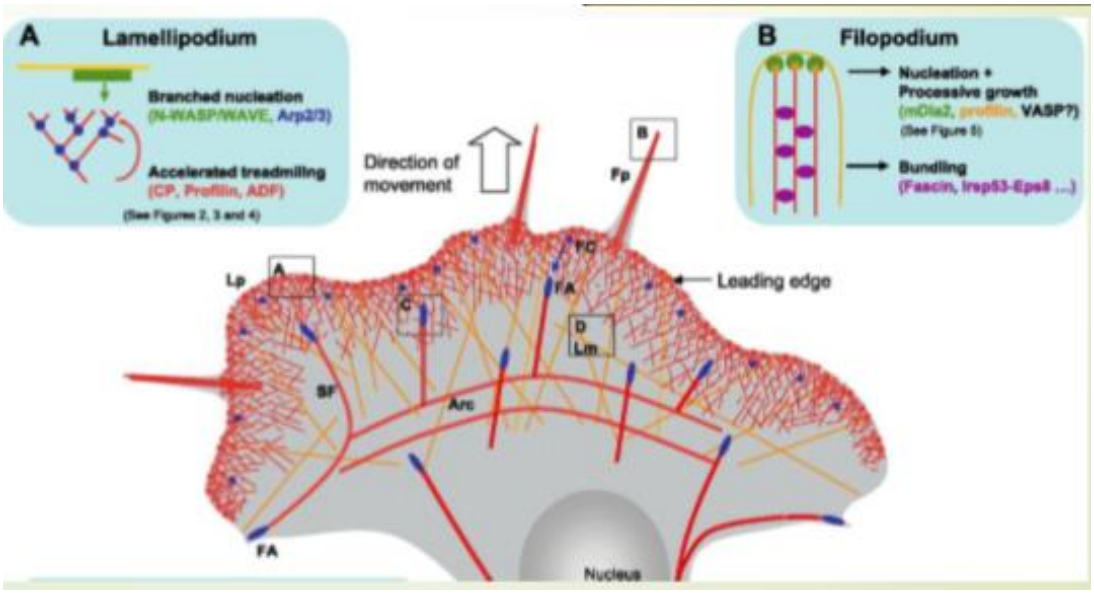
****lamellipodia****

Broad sheet-like networks of actin leading edge of moving fibroblast

****filopodia****

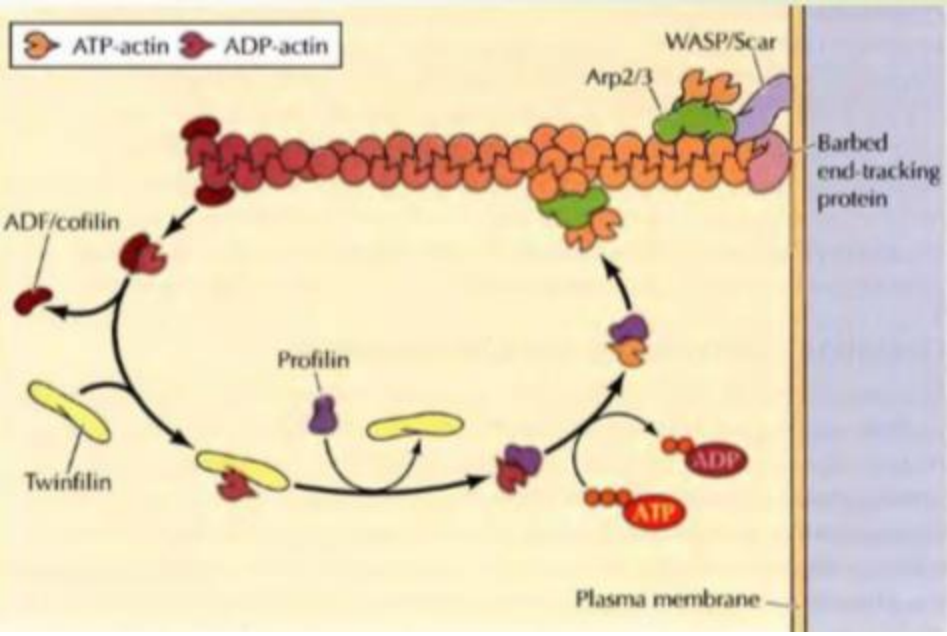
Thin projections **extending from lamellipodia**

- **At the point where the cell wants to extend the lamellipodia and filopodia – there will be more actin filaments & more proteins that are needed for the assembly process .**



Dynamic of actin filaments at the leading edge

- Certain signals lead to the recruitment of **Arp 2/3** (responsible for the branching) , **WASP/Scar** , and **barbed-end tracking proteins** to the leading edge (Why we need branching? to increase the width of the protrusions)
- **WASP/Scar activates Arp2/3, initiating filament branching** to provide more force to push against the membrane
- At the pointed end , ADP-actin **is disassembled by ADF-cofilin**
- ADP-actin monomers are carried to the leading edge **by twinfilin and reactivated by profilin**

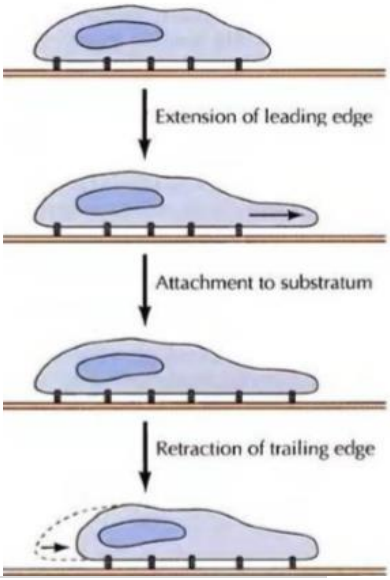


Cell migration

Cells tend to move in many circumstances ; such as the attraction of inflammatory cells such as the leukocytes and the macrophages , under the effect of specific agents. It is just like human movements

The steps:

1- Developing **polarity** via specialization of the plasma membrane or the cell cortex
2- Extend **protrusions** (lamellipodia, filopodia, pseudopodia) at the **leading edge** via the force of branching and polymerization of actin
3- Attach to **substratum** (e.g. focal adhesion), leading to create mechanical stress in the trailing edge which leads to breaking the focal adhesion that was normally found in order to move the trailing edge
4- The **trailing edge dissociates from substratum** (due to the mechanical stress mentioned above) and retracts into the cell body

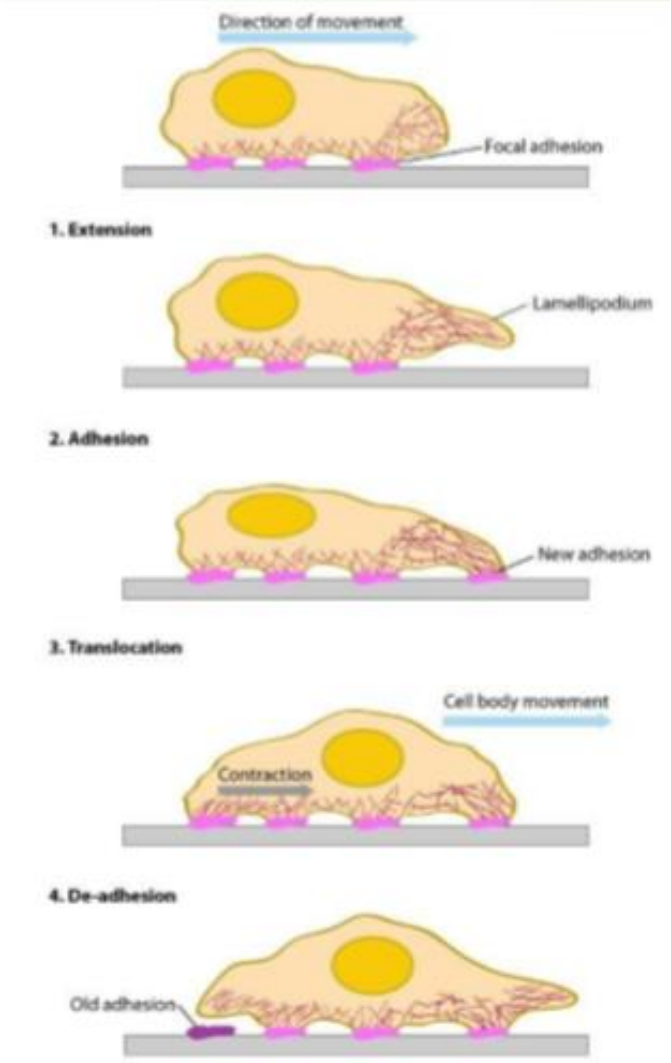


the movement of the cell is considered to have the changing of the focal adhesion

Modification of focal adhesions

- Cell-substratum attachment is initiated via transporting actin-bundling proteins and focal adhesion proteins **(vinculin** and **talin)** to **the leading edge** in connection with integrins
- **At the trailing end** , focal adhesions are broken down

This is true for slow-moving cells like fibroblasts and epithelial cells, but rapidly-moving cells like macrophages form diffuse contacts with the substratum, whose composition is unknown



Good luck