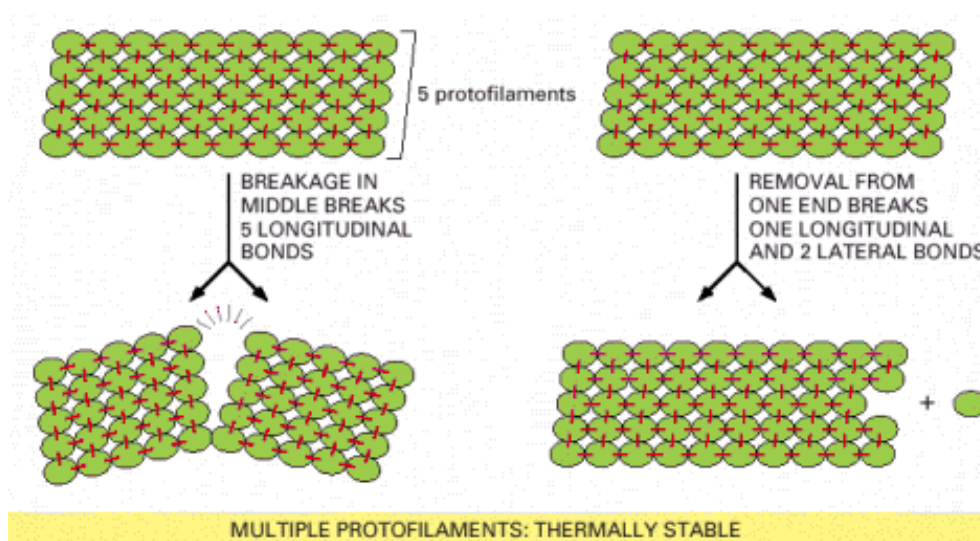


## Introduction to the cytoskeleton

All the highly developed spatial and mechanical functions in eucaryotic cells, depend on the remarkable system of filaments called the **cytoskeleton**. They are responsible for the different cell types and shapes found in the human body. All cells utilized a cytoskeleton, which is a dynamic and adaptable structure. The cytoskeleton consists of 3 main molecular filament systems, as well as a large number of associated proteins. These three are the **microfilaments**, **microtubules** and **intermediate filaments**. The cytoskeleton has many important functions in the cell. They are very important for Cell migration, intracellular traffic of organelles, mechanical strength, chromosome separation during mitosis, cytokinesis. It is also important in determining the shape of the cell and functions of cell protrusions (Filapodia and Stereocilia). It can also be important in giving functionality to certain tissues, such as muscle contractions, skin integrity, bone rigidity and neuronal circuitry.

Filaments are composed of small protein subunits, in the case of microfilaments; there is an assembly of actin, which are protein units. Microtubules are made up of tubulin proteins and intermediate filaments are made up of larger heterogeneous proteins. All these will be covered later in more detail. The units that form filaments are named protofilaments, and they are assembled like string of subunits joined end-to-end. Protofilaments provide thermal stability, especially during the initial parts of the assembly. It is important to understand how filaments are dynamic, there is a rapid addition and also loss of subunits at the ends of these filaments. As it is depicted in the figure, we can notice that the presence of 5 protofilaments bound together makes each filament more stable, which is what provides this thermal stability which are particularly important during nucleation.



The **nucleation** is the rate-limiting step in filament polymerization. Stabilization occurs when many subunits aggregate together, and this is what we refer to as nucleation. Short oligomers of only a few subunits assemble spontaneously, but are unstable, this is a

relatively slow process, filament elongation occurs rapidly from a nucleation origin. This stabilization happens with the assistance of specialized proteins that control where and when a new filament will form.

This filament assembly is determined by two constants,  $K_{on}$ , rate constant of addition ( $M^{-1}sec^{-1}$ ) and  $K_{off}$ , rate constant of removal ( $M^{-1}sec^{-1}$ ). The number of monomers that add to the polymer per second is proportional to the concentration of the free subunits ( $K_{on} C$ ). The subunits will leave the polymer at a constant rate that does not depend on the concentration ( $K_{off}$ ).  $C_c$  is the critical concentration, where free subunit concentration is at an equilibrium and polymerization stops.

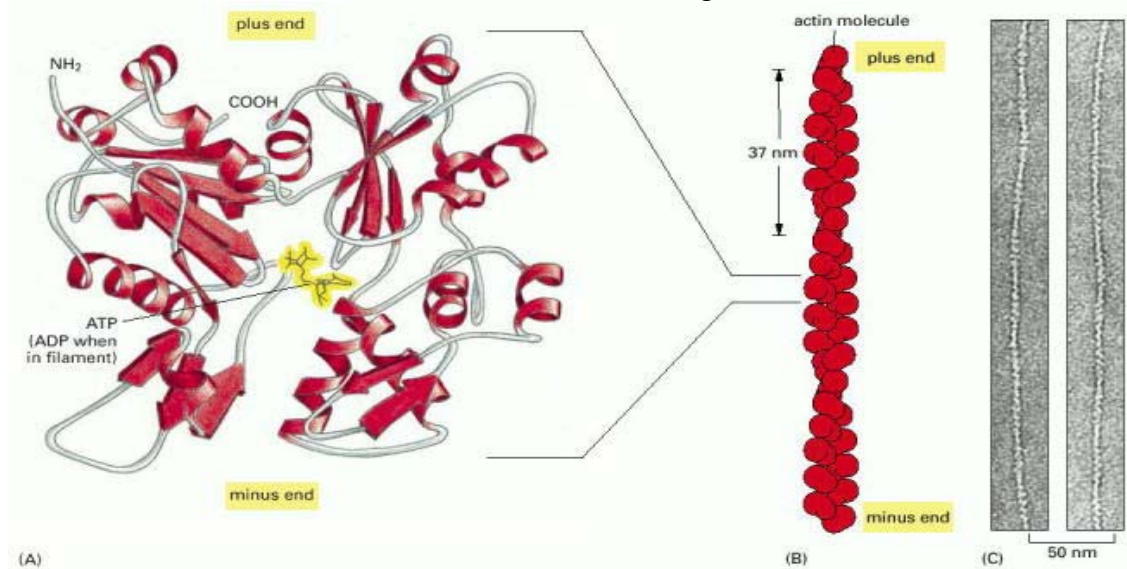
$$K_{on} C = K_{off} \rightarrow C_c = (K_{off} / K_{on}) = 1 / K$$

Where  $K$  is the equilibrium constant for subunit addition.

During filament assembly, Actin + ATP is hydrolyzed to Actin + ADP soon after its addition to the polymer. Similarly Tubulin + GTP is hydrolyzed to Tubulin + GDP. This hydrolysis reduces the binding affinity to neighbouring subunits and makes it more likely to dissociate from each end. So the basic idea to know here is that the incoming subunits bind to the end of the filament and at this stage they are in the T form (ATP or GTP), this allows for more subunits to bind to the filament since the T form has a higher affinity for other subunits. This faster addition results in what we call a “cap” of subunits containing ATP or GTP, which have stronger bonds than subunits in the D form. This usually only happens in one end of the filament.

## Microfilaments

Also known as actin filaments, it is a two-stranded helical polymer of the protein actin. Microfilaments are flexible??? structures with a diameter of 5-9 nm (smallest in diameter). They are found organized as: linear bundles, 2D networks or 3D gels. They are mostly concentrated in the cortex of the cell, which is the area beneath the plasma membrane, whereas the monomers are scattered throughout the cell.



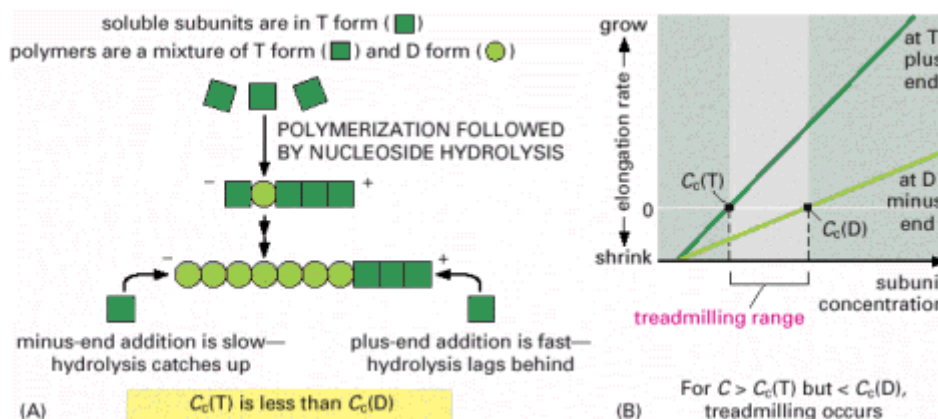
Each subunit has a binding pocket for the nucleotide ATP (or ADP). Actin monomers assemble head to tail generating structural polarity. Two protofilaments are held together with lateral contacts to form a helical structure with a twist every 37nm, which is repeated throughout the filament.

There are 3 phases in the assembly of microfilaments:

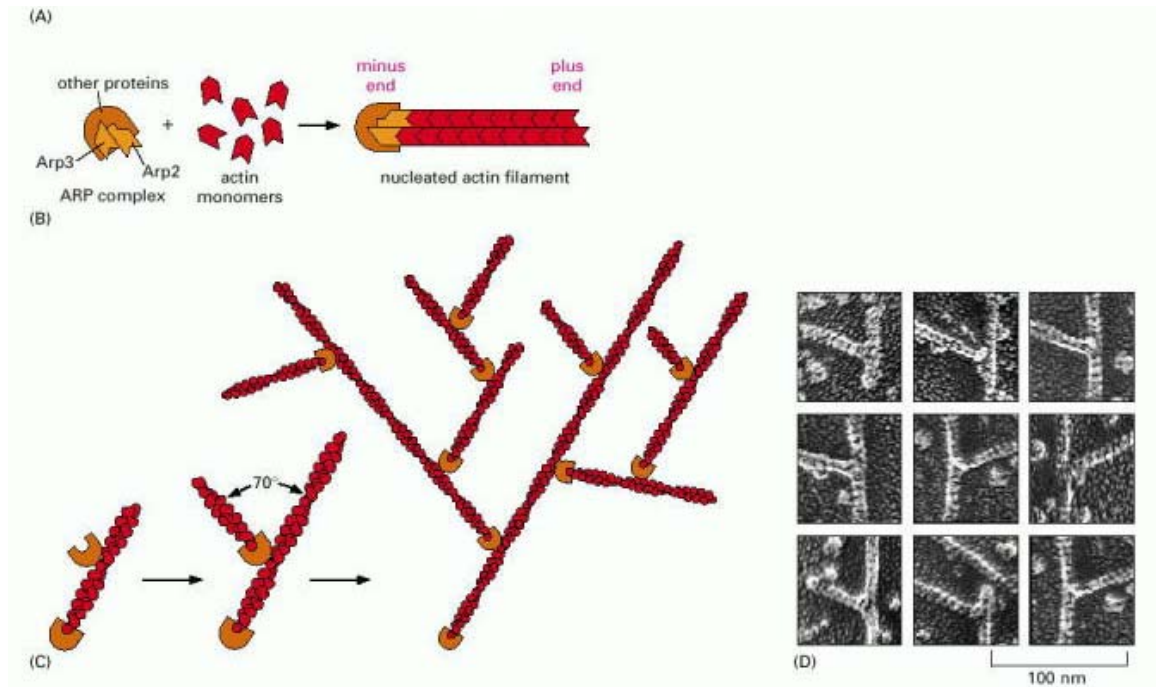
1. Lag phase – Time taken for nucleation to occur, which we already discussed, is the slower part of the process. Actin subunits produce oligomers. After nucleus is stable growth begins.
2. Growth phase – Addition of monomers to exposed ends of the growing filament. Elongation happens from both ends, however it is stronger at the plus end.
3. Equilibrium phase – Monomer addition and removal are equal, therefore no net growth.

**Plus end** = The end of a microtubule or actin filament at which addition of monomers occurs most readily; the “fast-growing” end of a microtubule or actin filament. The plus end of an actin filament is also known as the barbed end.

As we have seen ATP hydrolysis causes a weakening of the bond, therefore it causes the critical concentration at both ends of the microfilament to be different. The rate of subunit addition at the end of a filament is the product of the free subunit concentration and the rate constant  $k_{on}$ . The  $k_{on}$  is much faster for the plus end of a filament than for the minus end because of a structural difference between the two ends. At an intermediate concentration of free subunits, it is therefore possible for the rate of subunit addition to be faster than nucleotide hydrolysis at the plus end, but slower than nucleotide hydrolysis at the minus end. In this case, the plus end of the filament remains in the T conformation, while the minus end adopts the D conformation. As just explained, the D form has a higher critical concentration than the T form; in other words, the D form leans more readily toward disassembly, while the T form leans more readily toward assembly. If the concentration of free subunits in solution is in an intermediate range (higher than the critical concentration of the T form (that is, the plus end), but lower than the critical concentration of the D form (that is, the minus end)) the filament adds subunits at the plus end, and simultaneously loses subunits from the minus end. This leads to the remarkable property of filament **treadmilling** (also in microtubules).



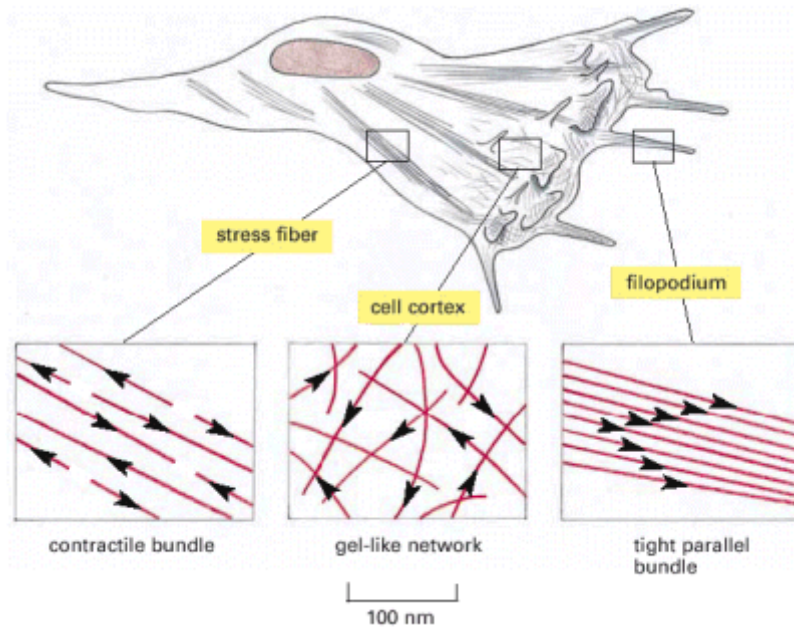
Another part of the assembly of actin filament is the **nucleation**. This occurs primarily at the cell cortex, catalyzed by two **actin related proteins** (ARPs). ARP complex nucleates growth of actin filaments from the  $\ominus$  end, as a result of stopping the net disassembly at the  $\ominus$  end; there is a rapid elongation at the  $\oplus$  end.



ARP complex dependent nucleation, is the process where the ARP complex assists the process of nucleation facilitating the rapid growth at the positive end. The figure above shows a web like structure that is an arrangement found in the cortex of the cell, which is produced from this kind of nucleation. The ARP complex nucleates filaments more efficiently when it is bound to the side of a pre-existing actin filament. The result is a filament branch that grows at a  $70^\circ$  angle relative to the original filament. Repeated rounds of branching nucleation, result in a treelike web of actin filaments.

There are ways in which it is possible to regulate the growth of filaments. Actin monomers bound to **thymosin**, sterically blocks the elongation of filaments, not allowing the monomer to bind to the  $\oplus$  end. On the other hand it is possible to increase the affinity of the actin monomers, **profilin** shuttles speed up the elongation process. Thymosin and profilin cannot bind to the same monomer at the same time.





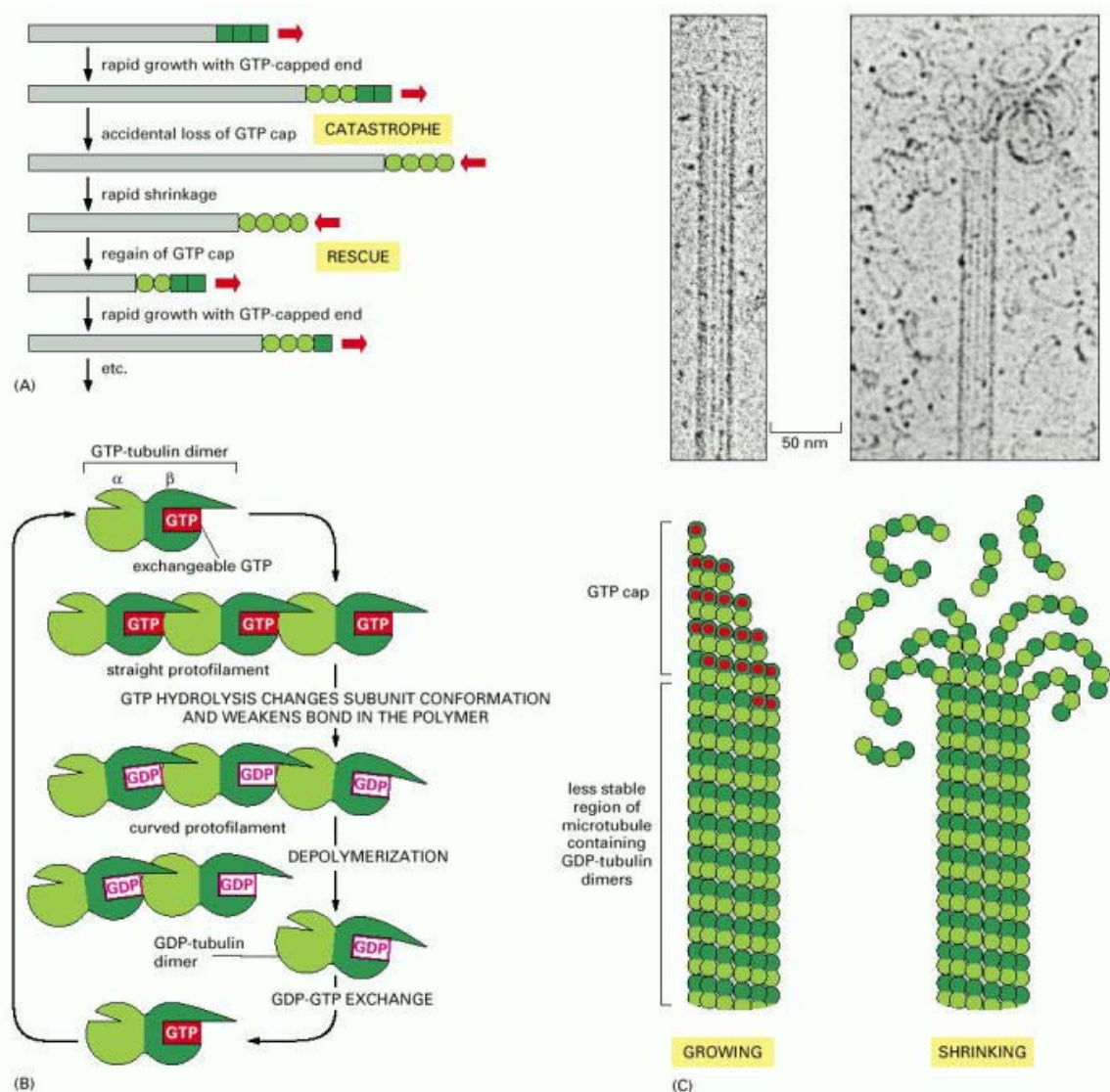
*Figure 16-38.* Actin arrays in a cell. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in *red*, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments. The cortex underlies the plasma membrane.

Microvillus are another good example of the use of microfilaments, they are important in the gut, to increase surface area. There they are found in bundles to maintain they're overall structures intact even under harsh conditions.

## Microtubules

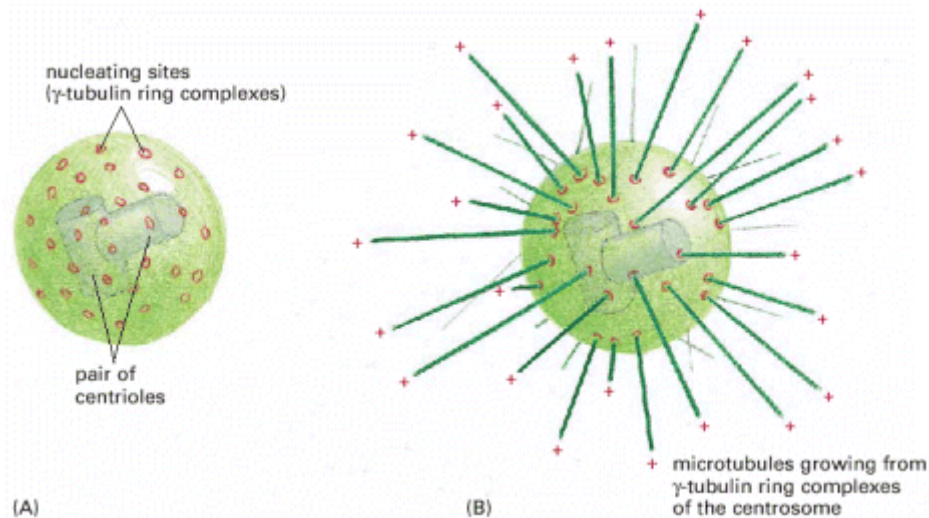
They are long hollow cylinders made up for 13 parallel protofilaments. Each protofilament is a heterodimer subunit of  $\alpha$ -tubulin and  $\beta$ -tubulin proteins. Similarly to microfilaments, they also have a polar nature. Each protofilament subunit (dimer) points in the same direction, and both tubulin monomers in the dimer have a bound GTP. However only the GTP from the  $\beta$ -tubulin can be hydrolyzed or exchanged. Microtubules are in general 25nm in outer diameter, and are more rigid than microfilaments.

An important concept to know is **Dynamic Instability**. Single microtubules can switch between the growing state and the shrinking state. Growing microtubules contain a GTP cap, which can be easily understood with the knowledge we have about the higher affinity of GTP bound monomers. However, if nucleotide hydrolysis proceeds more rapidly than subunit addition, the cap is lost and the microtubule begins to shrink, this event is called a "catastrophe". Microtubule depolymerization is over 100 times faster at a tubulin-GDP end. Basically dynamic instability is the alteration between slow and rapid disassembly. (Figure A below)



In this same figure (B), we can notice that the addition of tubulin-GTP subunits is easily packed into straight protofilaments. GTP hydrolysis changes the conformation of subunits, which forces a curved shape of protofilament (less stable). This is depicted in the picture, where the curved protofilament is more susceptible to breaking. Again note that the GTP of the beta unit is hydrolyzed but the alpha unit is also GTP bound.

Similarly to microfilaments, microtubules undergo a nucleation process. In all eukaryotic cells microtubule organizing center (MTOC) assist the assembly of these filaments. Centrosomes are located near the nucleus and is the main MTOC. Microtubules are nucleated at their minus ends, and the plus end grows out to the periphery. Centrosomes contain 2 centrioles (modified microtubule + associated proteins).



**Figure 16-23.** The centrosome. (A) The centrosome is the major MTOC of animal cells. Located in the cytoplasm next to the nucleus, it consists of an amorphous matrix of protein containing the  $\gamma$ -tubulin ring complexes that nucleate microtubule growth. This matrix is organized by a pair of centrioles, as described in the text. (B) A centrosome with attached microtubules. The minus end of each microtubule is embedded in the centrosome, having grown from a  $\gamma$ -tubulin ring complex, whereas the plus end of each microtubule is free in the cytoplasm.

We should also know about **MAPs**, microtubule associated preteins. MAPs are important in the regulation of microtubules, since they stabilize microtubules enhancing growth, suppressing the frequency of catastrophes, resulting in a longer and less dynamic microtubule. On the other hand we have another protein called **catastrophin**, which as you can imagine increases the frequency of catastrophes, resulting in a shorter and more dynamic microtubule.

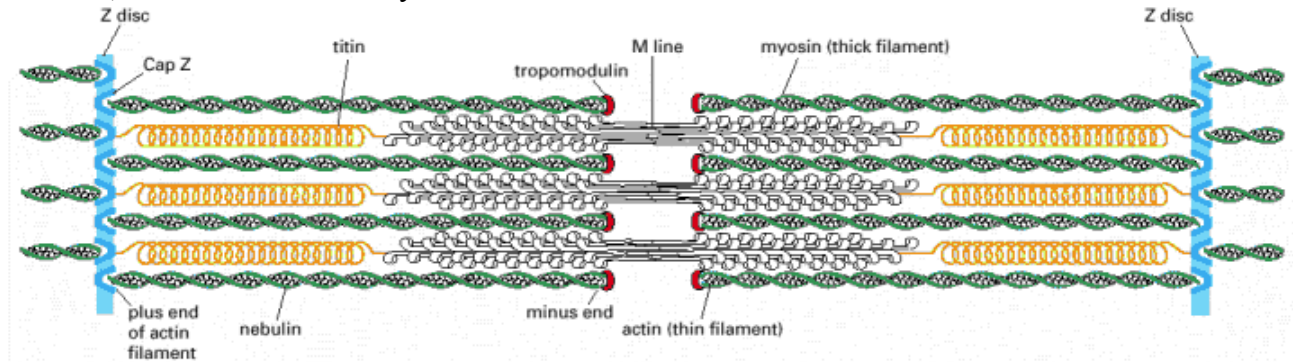
There are also molecular motors that can bind specific polarized filaments, and use ATP hydrolysis to propel along filament in one direction. This process is able to carry membrane-enclosed organelles, such as mitochondria and Golgi stacks. This is also a way to cause filaments to slide against each other to generate force, such as in muscle contraction and cell division.

Myosin proteins are a good example of a motor protein. Myosin II proteins are composed of 2 heavy chains and 2 copies of each of two light chains. Long coiled-coil tail of heavy chains bundle together into a thick filament. The N-terminus globular domain of the heavy chains contain a force generating machinery.

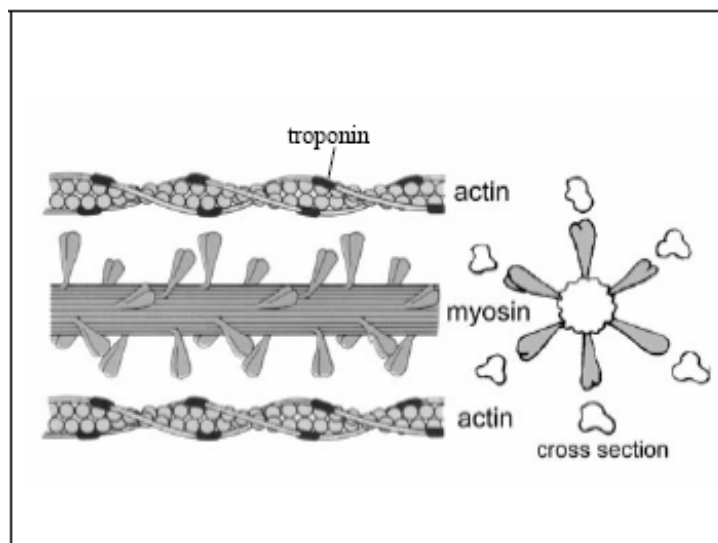
Evidence of the motor activity of the myosin head comes from an experiment done in a lab, where a glass slide was attached to myosin heads. Labeled actin filaments were added and allowed to bind to the myosin heads. After the addition of ATP the filaments began to glide, indicating that indeed there was movement produced from just the heads.

Similar to this experiment is the event of muscle contraction. Muscle cells (or muscle fibers) are multinucleated cells formed from the fusion of myoblasts. Myosin II interaction with actin filaments in skeletal muscle is what produces contractions. In the picture below we see the different filaments present in a sarcomere. Notice that the minus

end of the actin filament is on the M line. The thick filament in muscles is the myosin filament, which contains the myosin heads.



During muscle contraction the myosin head is bounded to the actin filament, at this point ATP can bind to the myosin head causing a conformational change as well as a decrease of the affinity for actin. So this causes the myosin head to be released from the actin filament. After the ATP binds to the head it can be hydrolyzed, producing ADP and Pi, which remain bounded to the myosin head. A shape change causes the myosin head to be displaced about 5nm along the filament. Then this myosin head binds to a new point in the actin filament, which causes the release of Pi. This event causes a stronger bond between the two; at this point ADP may also leave which causes the myosin head to go back to its original position, causing what we know as a power stroke. What really provides the energy for a power stroke is elastic energy stored in the myosin head, not the energy in ATP.



A few things to know about muscle contraction, not all of it was taught in lecture but we did cover it in the lab. The sarcomere as you probably know is the enclosed space where you find all the filaments that are part in the contraction. When there is a need for the contraction, the sarcomere receives a signal that allows Ca<sup>++</sup> to freely diffuse into the cell which opens and releases more Ca<sup>++</sup> into the sarcomere (Calcium induced calcium release), this is what signals the muscle to contract. What calcium does is expose the

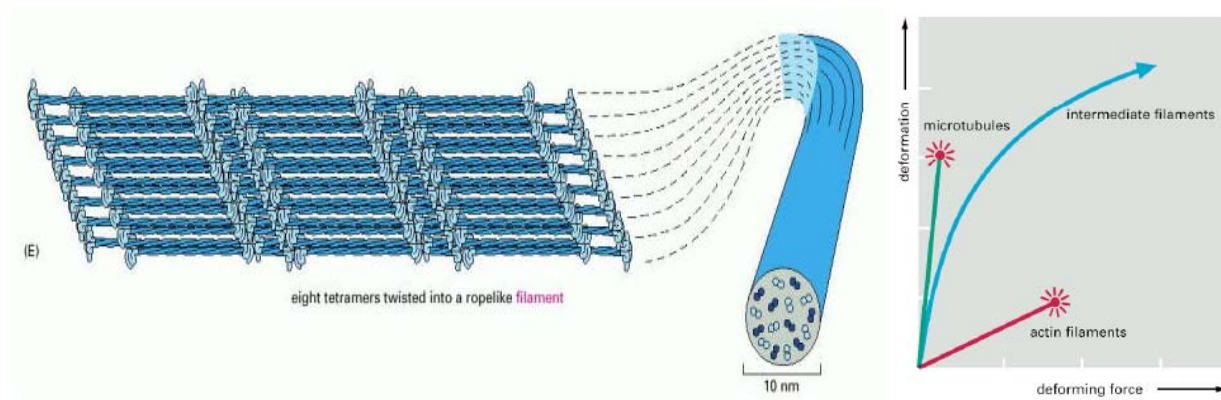


binding sites for the myosin heads. This is what actually allows the whole process with ATP to occur. Otherwise they troponin, which is some other type of protein complex, will be covering the binding sites from the myosin heads. When calcium is present it binds to the troponin and moves it out of the way. Also just to make sure you see the big picture, know that at one time only less of a third of the myosin heads are bound to their binding sites, this makes sense because if all of them were binding and unbinding at the same time the actin filament would just slide back to its origin due to the stored elastic energy in the muscle fiber.

## Intermediate Filaments

Intermediate filaments (IF) are a component of the cytoskeleton, and unlike microfilaments and microtubules, they are not found in all eukariotic cells. Their size is intermediate between that of microfilaments and microtubules. They are rope like fibers with 10nm in diameter, assembled from a large and heterogeneous family of proteins. Their main function in the cell is to provide mechanical strength, maintaining cell integrity. Another major difference from the other two filaments studied, is that they are not polarized. Good examples of different types of IF are the epithelial IF type, which is composed of keratin, and is found in the skin, nails and hair. Nuclear IF is composed of Lamin A and is found in the nuclear lamina. There is also Neuronal (neurofilaments in neurons) and Vimetin-like (Vimetin Desmin in many muscle cells).

Intermediate filaments can be found as the illustration bellow shows. A-helical monomers form dimmers in a coiled-coil structure. These dimmers join with another dimer to form a staggered tetramer of two coiled-coil dimmers, usually identical proteins. This is what we count as an IF subunit. These tetramer subunits then are packed together head to head to form the structure below.



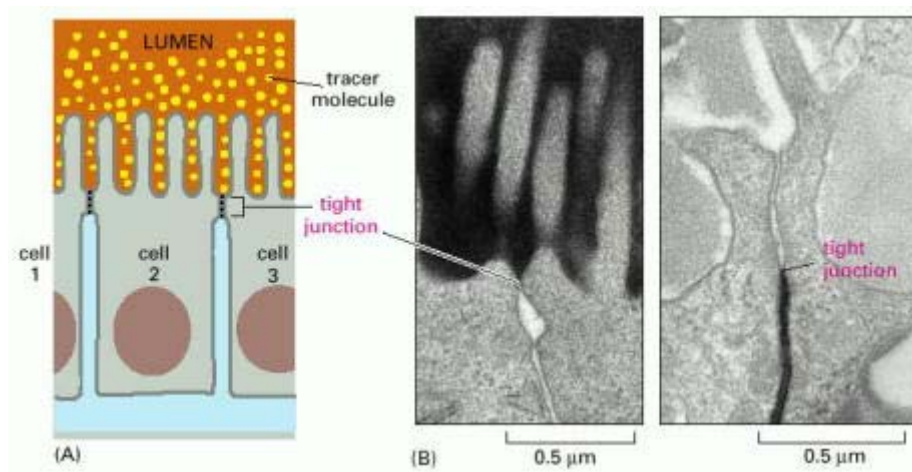
Unlike microtubules and microfilaments, IFs can be easily deformed and they withstand large stresses and strains without rupture. Refer to the graph above.

## Cell Junctions and adhesions

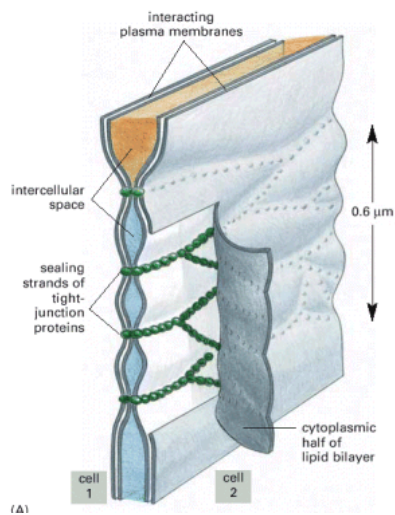
Cell junctions occur at point of contact between a cell with another cell or between a cell and the matrix, in all tissues. There are three functional groups of cell junctions: Occluding Junctions, Anchoring Junctions and Communicating Junctions.

### OCCLUDING JUNCTIONS

It is a selective permeability barrier across epithelia. Vertebrate cells use tight junctions to perform this barrier function. On the picture below tight junctions are shown in a drawing showing both the apical side and the baso-lateral side. Notice how tight junctions are located closer to the apical side (lumen). On the picture B, an experiment was done to show how tight junctions are impermeable to certain substances.



Occluding junctions are composed of sealing strands completely encircling the apical end of each cell in an epithelial sheet. There are two major 4-pass transmembrane proteins in a tight junction:



[Claudin](#) – It is essential for tight junction formation and function. Different claudin proteins found in distinct junctions. It's essential for sealing strands.

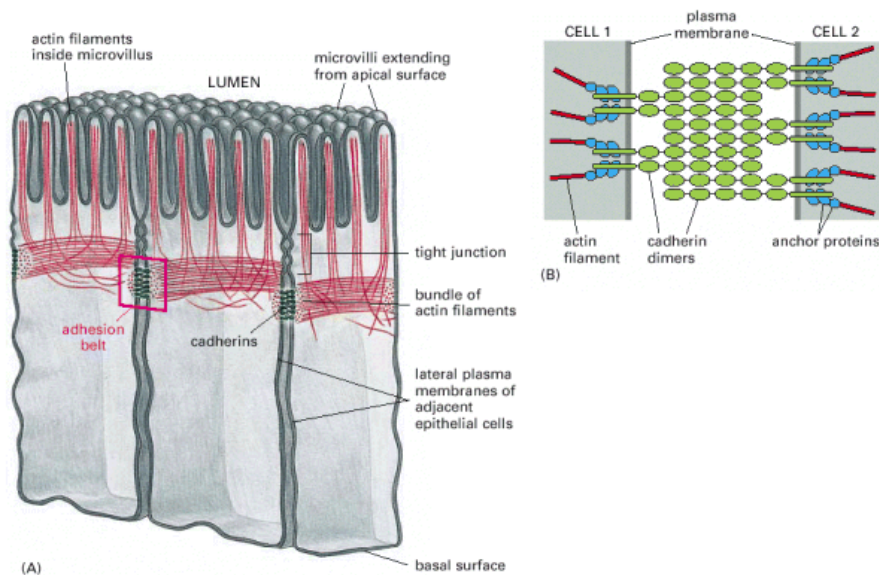
[Occludin](#) – Not involved in maintaining structure of tight junction, but it is important for its barrier function. It is not essential but it is needed for impermeability.

## ANCHORING JUNCTIONS

Anchoring junctions serve to span the membrane and connect to tension-bearing filaments. They attach cells (via cytoskeleton) to their neighbors or to the ECM. They are necessary since plasma membrane is flimsy and cannot transmit large forces. In essence, cytoskeletal systems are joined from cell to cell and cell to matrix creating **strength and stability**.

The actin filament attachment sites are: Adherens junctions (cell-cell) and Focal adhesions (cell-matrix). The intermediate filament attachment sites are: Desmosomes (cell-cell) and Hemidesmosomes (cell-matrix). Let's look at some of these in more detail.

**Adherens junctions** – They form a continuous adhesion belt (zonula adherens) just below tight junctions. They are common in epithelial cells in the small intestine. Zonula adherens are directly apposed in adjacent epithelial cells. Interacting plasma membranes, of these cells, are held together by transmembrane proteins called cadherins. Which are important for structure, polarity and adhesion. **Basically they link actin microfilaments between cells through classical cadherins.**

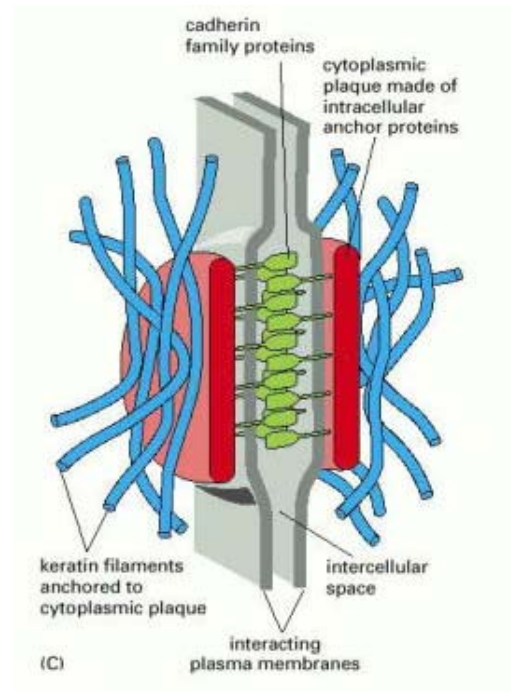


**Focal Adhesions** – They link **actin filaments to the ECM** through **integrins**.

**Desmosomes** – They are points of intercellular contacts that rivet cells together. They are anchoring sites for **intermediate filaments** (through **non-classical cadherins**) and they have a dense cytoplasmic plaque (intracellular anchoring proteins). Intermediate filaments are attached to the surface of each plaque. Transmembrane cadherin proteins interact through extracellular domains to hold cells together.

We will look at cadherins later but for now know that they are proteins on the surface of cells that are important for adhesion, and they are dependent on calcium ions to function, hence their

name.



**Hemidesmosomes** – They basically link **intermediate filaments to the ECM** through integrins.

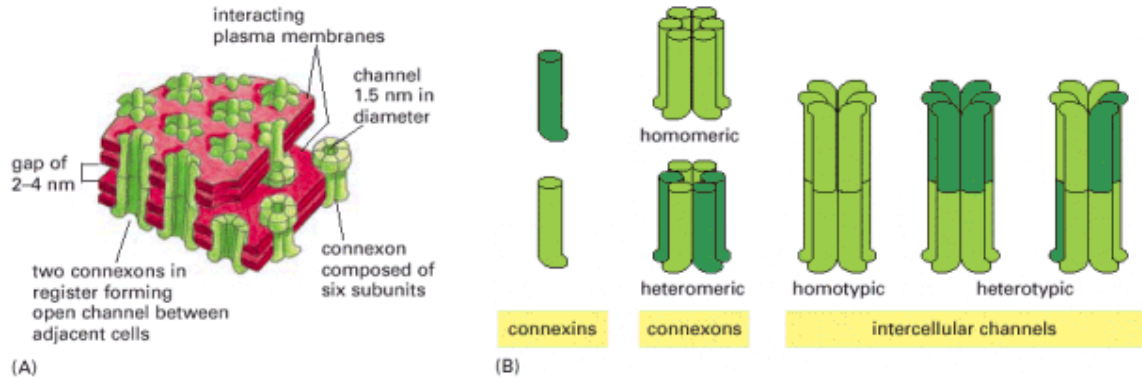
## GAP JUNCTIONS

They are small patches of communication where two adjacent membranes are separated by a tiny gap of 2-4nm. The gap is spanned by channel-forming proteins called connexins and channel complexes are called **connexons** with a pore size of 1.5nm. They are very important in the transport of sugar, inorganic ions, amino acids, nucleotides, vitamins and signaling mediators (such as IP3 and cAMP). Bigger molecules like proteins, nucleic acids and polysaccharides will not pass through gap junctions.

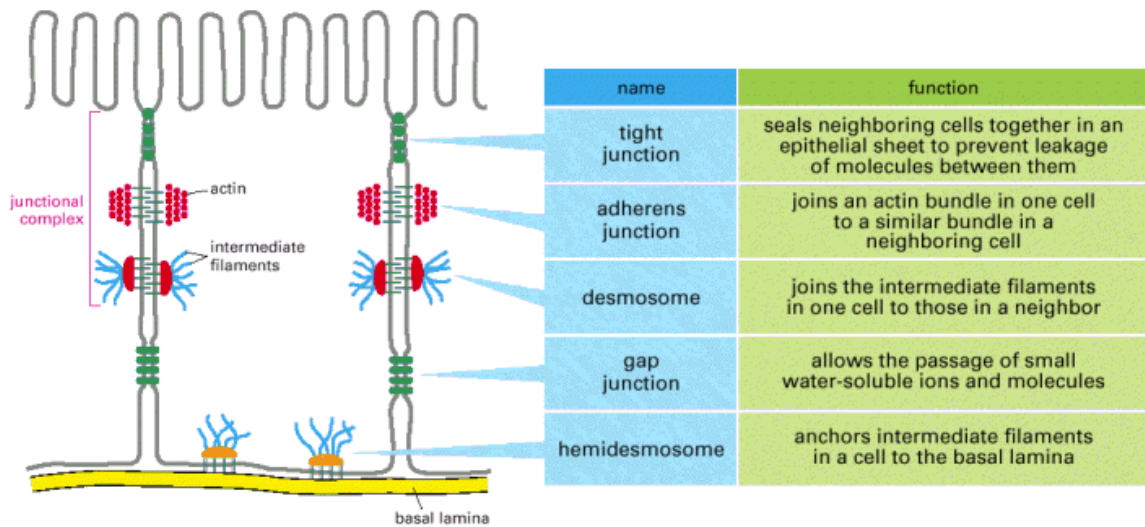
Connexons, in humans, are formed from 6 connexin proteins. There are 14 distinct connexin proteins that can form connexons. They can be homomeric or heteromeric, they can also be homotypic or heterotypic. Basically they can be any combination of 6 connexins and can bind to any other connexon to form a gap junction.

Gap junctions can also be of different sizes, large ones can be formed from many connexons next to each other in a region where the 2 cell membranes are close together. Connexons can be in open or closed conformation in response to extracellular signals. Permeability of these junctions, can be rapidly and reversibly reduced by manipulating the **cytosolic pH, cytosolic free Ca<sup>++</sup> and cytosolic cAMP**.





The picture below summarized the different cell junctions we looked at:



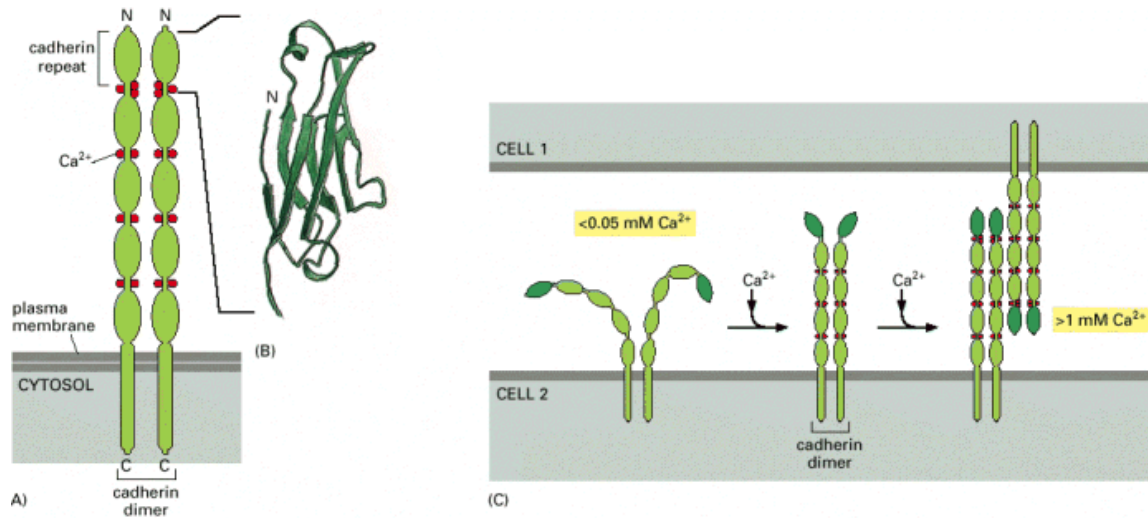
## Cell Adhesion

Before Cell junctions are fully assembled, cells can still adhere to each other using active adhesion mechanisms. This is an extremely important process during embryogenesis because “selective adhesion” forms the basis of tissue formation. CAMs (cell adhesion molecules) are a large group of proteins that can be  $Ca^{++}$  dependent (cadherins) or  $Ca^{++}$  independent.

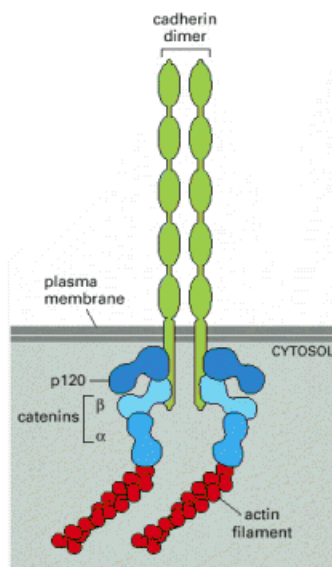
**Cadherins** as we have seen already are Calcium dependent adhesion molecules. These are divided into two groups:

**Classical Cadherins** – **E-cadherin** (epithelial adherens junctions), **N-cadherins** (neuronal adherens junction and synapses, muscle adherens junctions), **P-cadherins** (placenta and skin adherens junctions) and **VE-cadherins** (Vascular endothelial cell – arteries and veins – adherens junctions).

**Non-Classical cadherins** – **Desmocollin** and **Desmoglein** (skin desmosomes). **Protocadherins** (neuronal synapses).



**Figure 19-24.** The structure and function of cadherins. (A) A classical cadherin molecule. The protein is a homodimer, with the extracellular part of each polypeptide folded into five cadherin repeats. There are  $\text{Ca}^{2+}$ -binding sites between each pair of repeats. (B) The crystal structure of a single cadherin repeat, which resembles an immunoglobulin (Ig) domain. (C) The influence of extracellular  $\text{Ca}^{2+}$ . As the amount of  $\text{Ca}^{2+}$  increases, the extracellular parts of the cadherin chains become more rigid. When enough  $\text{Ca}^{2+}$  is bound, the cadherin dimer extends from the surface, where it can bind to a cadherin dimer on a neighboring cell. If  $\text{Ca}^{2+}$  is removed, the extracellular part of the protein becomes floppy and is degraded by proteolytic enzymes.



Classical cadherins link to actin filaments (e.g. adherens junctions). Cadherins and actin filaments are coupled through intracellular anchoring complex containing catenins. As you can see in the picture cadherins are connected to acting filaments through catenins. B-catenin is critical for adhesion function of cadherins, but it also has a second, very important function in intracellular signaling.

Cadherins are important for many reasons, one of them is in the formation of tubes and folding of epithelial sheets of cells. An oriented contraction of bundles of actin filaments can cause epithelial cells to deform and roll up into a tube. The formation of the neural tube in early vertebrate development is an example of this.

(<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookHL&term=19.10+AND+mboc4%5Bbook%5D+AND+374314%5Buid%5D&rid=m>  
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookHL&term=19.10+AND+mboc4%5Bbook%5D+AND+374285%5Buid%5D&rid=m>  
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Homophilic binding among cells, determine how they are sorted. For example in a population of cells, let's say some of them express E-cadherin, and some express N-cadherin. What happens is that every cell expressing the same cadherin will bind together due to what we call **homophilic binding**. This separates the cells expressing different cadherins. Similar phenomenon happens when different cells express different levels of, let's say, E-cadherins. Cells that have higher concentration of E-cad will be sorted out from cells expressing low levels of E-cad.

A diverse expression of cadherins play a fundamental role in region-specific cellular adhesion, which contributes to the distinct anatomical development of each brain region. Alternative promoters and alternative splicing at the protocadherin gene cluster results in potentially thousands of distinct proteins. This is a very complicated gene, with many exons and introns. Some exons are always expressed however many other are variably present resulting in many possible combinations. These proteins can be found at synapses and may be important for maintaining different connections in the brain.

### **Selectins**

They are cell surface carbohydrate binding proteins (lectins). They are prominently used by white blood cells, which must move between blood vessels and tissues constantly. It goes through transient,  $Ca^{++}$  dependent cell adhesion, linked to microfilaments. Transmembrane proteins with conserved lectin domains, bind specific oligosaccharides on other cells. This can slow leukocytes and allow them to leave the blood vessel and enter the site of infections.

There are three main types of selectins:

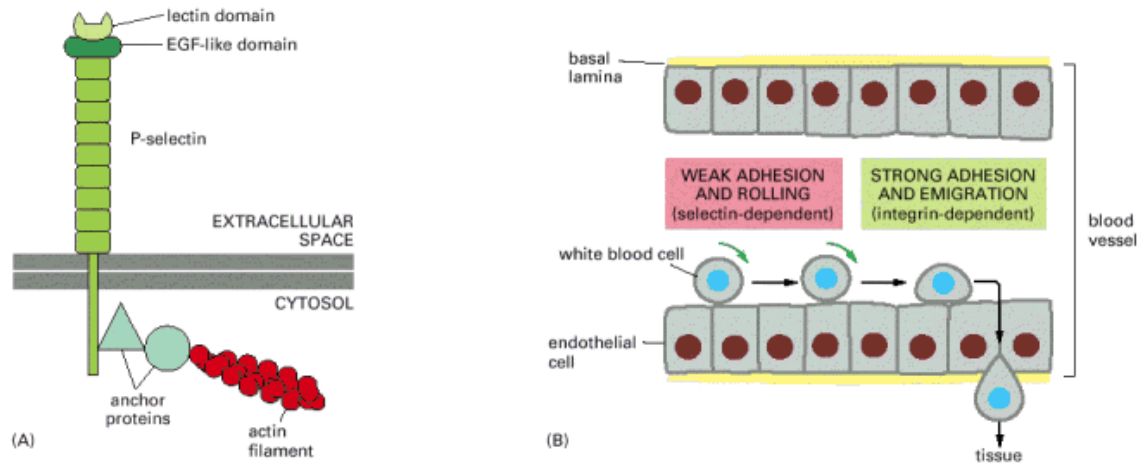
L-selectin found in White blood cells (leukocytes).

P-selectin found in platelets and endothelial cells.

E-selectin found in endothelial cells.

In the picture below we can see how WBC still and roll over the vessel walls, which is a selectively dependent process. Eventually they stop and go into the walls to reach the site of infection.

As for  **$Ca^{++}$  independent cell-cell adhesions**, we only looked at NCAM (neural cell adhesion molecule), which is most abundant in nerve cells, and they work through homophilic adhesion.

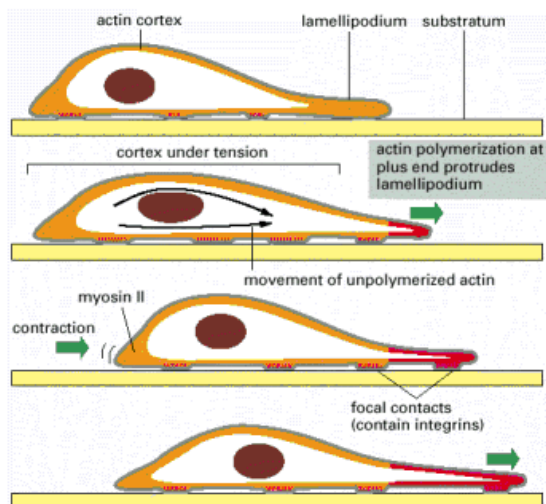


*Figure 19.30.* The structure and function of selectins. (A) The structure of P-selectin. The selectin attaches to the actin cytoskeleton through anchor proteins that are still poorly characterized. (B) How selectins and integrins mediate the cell-cell adhesions required for a white blood cell to migrate out of the bloodstream into a tissue.

## Cell Motility and Extracellular Matrix

There are many examples of cellular cell motility; some of them are actin based events, such as muscle contraction or cell migration. Others are based on microtubules, and good examples of that are cilia movement and swimming of sperms.

During cell migration the **lamellipodium** at the leading edge of the cell moves the edge forward (*green arrows at front*) and stretches the actin cortex. Contraction at the rear of the cell propels the body of the cell forward (*green arrow at back*) to relax some of the tension (traction). New focal contacts are made at the front, and old ones are disassembled at the back as the cell crawls forward. The same cycle can be repeated, moving the cell forward in a stepwise fashion. Alternatively, all steps can be tightly coordinated, moving the cell forward smoothly. The newly polymerized cortical actin is shown in *red*.

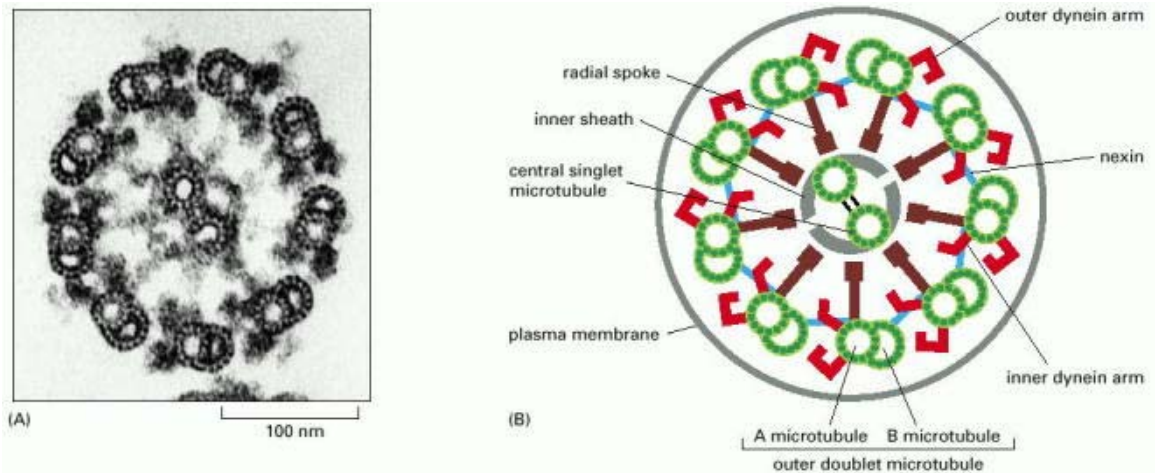




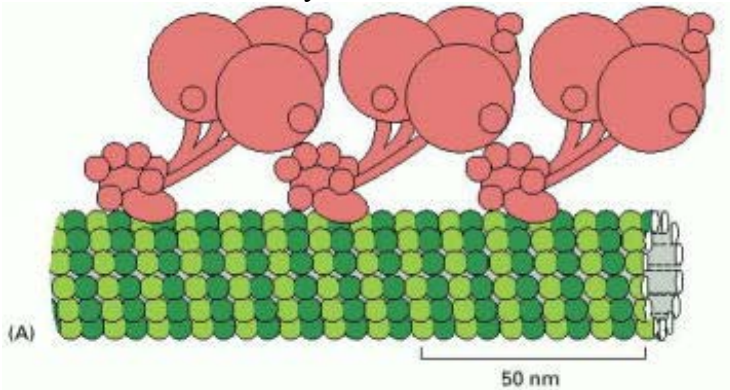
The **lamellipodium** is an actin projection on the mobile edge of the cell and it is essentially a 2D actin mesh that pulls the cell across a substrate.

Now as mentioned before, there are also examples of cell motility linked with microtubules, such as flagella and cilia (shorter in length than the flagella). Using these structures cells are able to move in a certain medium. The motility is a result of the interaction of microtubules and dynein (motor protein) in a structure called an **axonema**. However for an effective movement, all the cilia must move synchronously in a wave like modulating movement.

In the picture below we can see a cross section of a cilia or flagella, and we can see that it is composed of 9 special double MTs (1 complete fused with a partial MT) arranged in a ring surrounding a pair of single MTs. Several associated proteins allow for a stable ring structure. This characteristic structure of the axoneme is conserved in flagella and cilia from protozoans to humans. In the picture we can see that nexin is what bridges the different proteins together in this structure.



The motor protein in this case is a ciliary (axonemal) dynein, which are more complex than kinesins. These are minus-end directed MT motor proteins. They are larger protein assemblies. **Dyneins** are composed of 2-3 heavy chains and a large variable number of light chains. The base of the dynein binds A-MTs in an ATP-independent manner. Their globular heads bind B-MT in an ATP-dependent manner. When they hydrolyze their ATP, they move towards the minus end of the B-MT. Note on the picture below that the base of the heavy chain is bonded to an A-Microtubule.



As mentioned before sperm motility is also done through the activity of dynein, which causes MT bending. The MT bend because nexin in this case is also holding the MT together so that there can be no sliding and the force caused by the contraction creates a bending force.

Ciliary axoneme have in their lower portion, structures called **Basal Bodies**. These are used to firmly root the cilium or flagellum at the cell surface. Basal bodies are composed of 9 sets of triplets MTs (A, B, C). One complete MT (A) fused to 2 incomplete MTs (B and C) and linked together in a ring by associated proteins. Essentially, this is the same arrangement of MTs as in a centriole, but without the inner microtubules.

The cytoskeleton plays an important role in developmental processes as well. Examples of cytoskeletal based motor proteins are:

- Asymmetric localization of mRNA during cell division enables daughter cell to acquire a distinct identity from the mother cell (actin based). Basically a specific mRNA is bound to proteins that are also bound to actin filaments at one region of the cell, this region will be part of one of the daughter cells. This way, the other daughter cell will not have this mRNA, that was specifically selected to be on the other cell.
- Extracellular signals can be propelled in one direction to affect a field of cells in an unequal manner (MT based). For example, the left-right asymmetry in the body, is created using cilia that direct the flow of certain factors towards one side of the embryo, and this allows for different organs to develop one different sides of the body. Proteins produced in certain nodes that are swept to one side activate gene expression, causing this phenomenon. Mutations on some of these genes cause a loss of asymmetry.

## **Extracellular Matrix (ECM) – Lecture 6**

The ECM is an intricate network of macromolecules. It plays an active and complex role in regulating the behavior of cell: Survival, migration, proliferation, shape and function. They are mainly secreted by **fibroblasts**. There are two main classes of ECM macromolecules: Glycosaminoglycans (GAGs) and fibrous proteins.

**Glycosaminoglycans** are unbranched polysaccharide chains made up of disaccharide subunits. Disaccharide subunit:

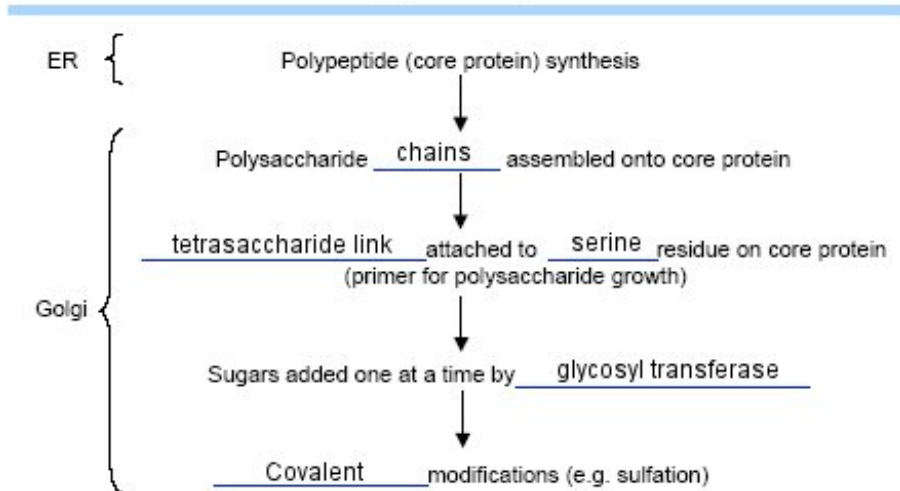
Amino sugar	+	Uronic Acid
N-acetylglucosamine	+	Glucuronic Acid
N-acetylgalactosamine	+	Iduronic Acid

Sulfate and carboxyl groups on most sugars make GAGs highly negatively charged. They are usually found covalently linked to proteins, proteoglycan. There are 4 main groups of GAGs:

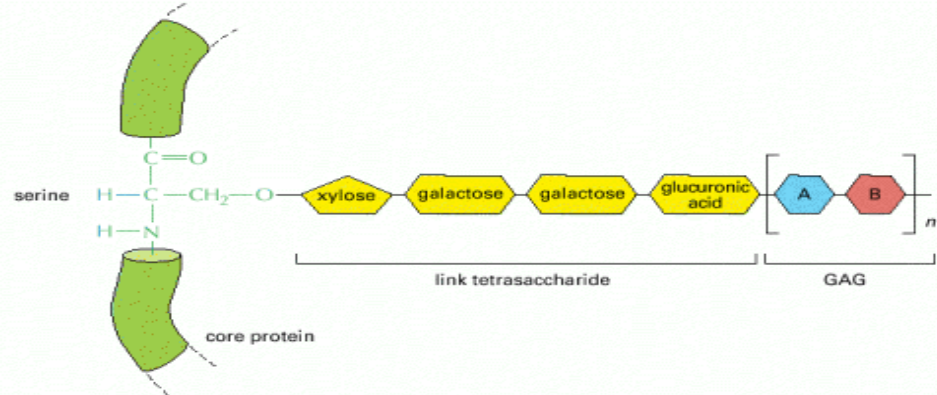
- Hyaluronan – the most important one, contains no sulfate sugars, not linked to proteins, disaccharide units are identical and they are very long sugars.
- Chondroitin sulfate & Dermatan sulfate.
- Heparan sulfate.
- Keratan sulfate.

Proteoglycans must have at least one sugar side chain that is a GAG, they are long and unbranched. They are usually made up of as much as 95% carbohydrate by weight. Single type of core protein can vary in number and type of attached GAG chains. There is a high degree of heterogeneity in this family of macromolecule. GAGs function as sieves to regulate extracellular molecular traffic, and they can also enhance or inhibit signal function of growth factors (for example they can help growth factors bind to their receptors).

### Proteoglycan synthesis

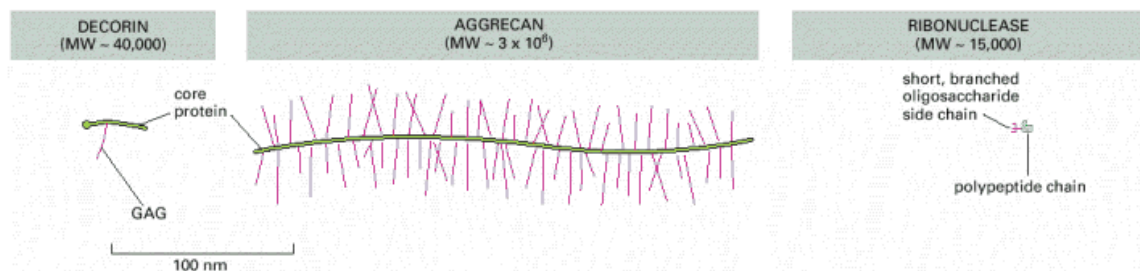


Another important thing to know about proteoglycans is the tetrasaccharide link. These links are first assembled on a serine side chain. The rest of the GAG chain (mainly repeating disaccharide) is synthesized one sugar at a time.



In the review lecture he didn't mention these, but here are some examples of common proteoglycans:

Proteoglycan	GAG chains	Location	Function
Aggrecan	Chondroitin sulfate + keratan sulfate	Cartilage	Mechanical support
Betaglycan	Chondroitin sulfate / dermatan sulfate	Cell surface and matrix	Binds to TGFβ
Perlecan	Heparan sulfate	Basal laminae	Structural and filtering
Syndecan-1	Chondroitin sulfate + heparan sulfate	Epithelial cell surface	Cell adhesion and binds to FGF
Dally (Drosophila)	Heparan sulfate	Cell surface	Co-receptor for wingless and decapentaplegic



Now there are four different types of **fibrous proteins**:

- **Collagen** – Most important one, it is the major component in skin and bones. It is the most abundant protein in mammals, making up 25% of total protein mass. It is a long, stiff, triple helical structure. With 3  $\alpha$ -chains coiled around each other, each one is rich in **proline** (stabilizes conformation) and **glycine** (allows chains to pack tightly together).

**Fibrillar collagen** is a type of collagen molecule, which assembles into rope-like structures. Collagens type I (common in skin), II, III, V and XI are of this type. There are 25 distinct  $\alpha$ -chains that are each encoded by a different gene, however there are only 20 combinations of triple-stranded collagen molecules found.

Another important event is the synthesis of collagen, which starts in the ER/Golgi compartment:



1. ER/Golgi compartment → Pro- $\alpha$ -chain is synthesized
2. Hydroxylation (addition of OH) of selected prolines and lysines
3. Glycosylation of selected hydroxylysines
4. Self-assembly of three pro- $\alpha$ -chains
5. Within cytoplasm, in secretory vesicle → Procollagen triple helix is formed.
6. At extracellular space → Secretion of procollagen molecule.
7. Cleavage of propetides.
8. Self-assembly in collagen fibril.
9. Aggregation of collagen fibrils into collagen fiber, which is around 3 $\mu$ m in diameter, very large structures outside of the cell.
10. Collagen fibers are organized as a network in the extracellular space.

**Fibril-associated collagens** – Organization of collagen in the ECM can vary depending on the type of tissue. This type of collagen (e.g. type IX and XII) facilitates fiber organization. They have a more flexible structure than fibrillar collagen, they can also retain their propeptides after secretion. They do not aggregate to form fibrils in EC space, and they can bind to the surface of fibrils in a periodic manner. Basically they are important to form structures with the fibrillar collagens. In many places mesh like structures can be formed using these collagens.

These were not mentioned in the review lecture but there are several diseases related to collagen deficiency:

Scurvy (Vitamin C deficiency prevents proline hydroxylation, weakening collagen fibril formation and stability); Osteogenesis imperfecta (mutation that affects type I collagen, causing weak bones); Ehlers-Danlos syndrome (mutation affecting type III collagen, resulting in fragile skin and blood vessels, hyper mobile joints); Alport syndrome (hereditary kidney disorder caused by a mutation in type IV collagen  $\alpha$ -chain genes).

- **Elastin** – Protein abundant in highly flexible tissues, such as blood vessels and the lungs. Elastic fibers are resilient so that tissues recoil after stretching. Elastin is the main component of elastic fibers. Elastin is made by linking many soluble tropoelastin protein molecules that have been secreted into the EC space. It is highly cross-linked at lysine residues. Fibrillin are glycoproteins that bind to elastin and support the integrity of elastic fibers. A person with Marfan's syndrome has a mutation in fibrillin which weakens the connective tissues, so they are prone to ruptures in the aorta. In **elastic fibers** the elastin molecules are joined together by covalent bonds to generate a cross-linked network. Each elastin molecule in the network can expand and contract as a random coil, so that the entire assembly can stretch and recoil like a rubber band.
- **Fibronectin** – They are heterodimeric glycoproteins. The fibronectin gene contains 50 exons, where alternative splicing is possible. Fibronectin binds to integrin via the RGD (Arg-Gly-Asp) motif. Fibronectin also binds to

collagen and other ECM components. One of their main functions is to allow cells to bind to the ECM.

- **Laminin** – It is the major component of the **basal lamina** (specialized ECM – thin and flexible – that underlies epithelial sheets or tubes and surround cells. Most basal laminae contain collagen type IV, perlecan and laminin). Laminin is composed of 3 long polypeptide chains. They can bind to perlecan, nidogen and type IV collagen. Laminin plays a critical role in the development, maintenance and regeneration of the neuro-muscular junction.
- **Integrin** – Integrin receptors bind collagen, fibronectin and laminin. They are composed of transmembrane heterodimers of non-covalently associated glycoprotein subunits. The ligands are usually divalent cations, like  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$ . Integrins can determine shape, orientation or movement of cells. The figure below shows how this works.

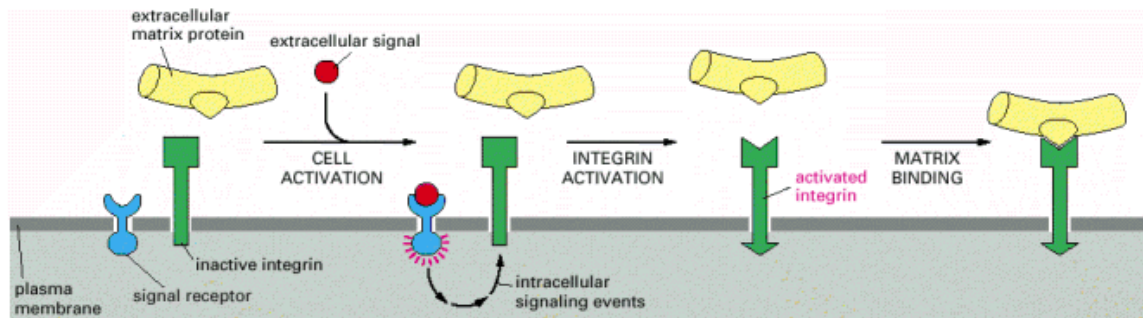


Figure 19.65. The regulation of the extracellular binding activity of a cell's integrins from within. In this example, an extracellular signal activates an intracellular signaling cascade that alters the integrin so that its extracellular binding site can now mediate cell adhesion. The molecular nature of the alteration is still poorly understood.

## The Cell Cycle – lecture 7

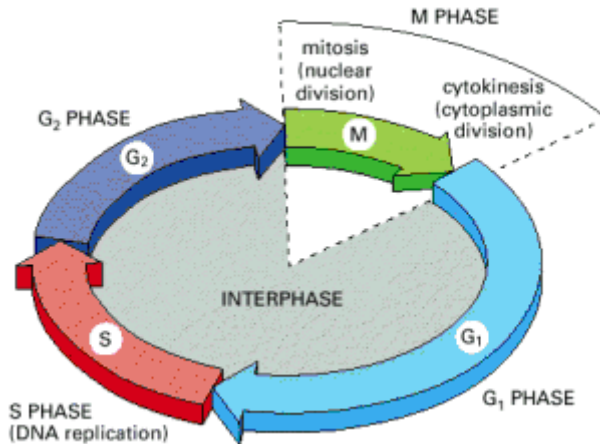
All living organisms are a product of repeated rounds of cell growth and division since the beginning of life on Earth. The most important aspect of cell division is the fidelity with which the cell duplicates and segregates the genome. So by definition, the cell cycle is the orderly sequence of duplication of the cell's contents, division of the cell into two and growth of the daughter cells.

Eukaryotic cell cycle is divided into:

**S-phase** (S for synthesis) where duplication of genomic DNA occurs.

**G<sub>1</sub>- and G<sub>2</sub>-phases** (Gap phases) are where cell growth occurs and the mass of proteins and organelles double.

**M-phase** (mitosis phase) where the chromosome is segregated and the cell divides.



## G-Phases

It is the time where cell grows. Cell uses this time to monitor the internal and external environment to ensure conditions for S-phase and M-phase are suitable.

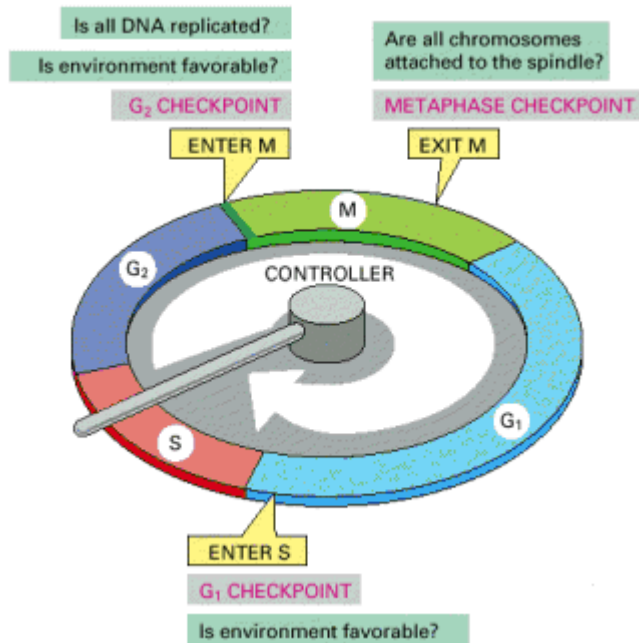
G<sub>1</sub>-phase: if conditions are not favourable, then cell enters a specialized resting state (G<sub>0</sub>). Now if conditions are favourable, the cells in early G<sub>1</sub> or G<sub>0</sub> progress through a commitment point at the end of G<sub>1</sub> called the restriction point. At the restriction point cells are committed to DNA replication, even if conditions become unfavourable. There are examples of cells that develop and go into G<sub>0</sub> state permanently, and there are other examples of cells that maybe go to a G<sub>0</sub> state, but when conditions are right they may go back to normal.

There are several techniques for studying cell cycle. One of them is to expose the studied tissue to thymidine, which allows us to see which cells are dividing or to see if cells are in the S-phase since that is when cells take up thymidine. It is also possible to investigate cell cycle in a cell-free system, where the nuclei of a sperm is mixed with ATP and cytoplasm from activated eggs, this allows the visualization of the division of the DNA. A third method is to use flow cytometry, where you label DNA with a fluorescent marker. A machine can then sort DNA and find the relative amount of DNA per cell. This produces a graph that shows how many cells have the right amount of DNA and how many have more DNA than a normal cell, which indicates that it must be in the G<sub>2</sub> or M phases.

As you can imagine there must be ways to control cell cycle, and one of the ways to control it is to have checkpoints. There are 3 of them. These must be:

- Points in the cell cycle that can be regulated by extracellular signals from other cells. Can promote or inhibit cell proliferation.
- They must be points at which the cell can be arrested if previous events have not been completed (Mitosis entry: blocked if DNA replication is not complete; Chromosome separation: blocked if not all chromosomes are attached to the spindle; Progression through G<sub>1</sub> or G<sub>2</sub>, blocked if DNA is damaged by radiation or chemicals).

- Generally operates through negative intracellular signaling.



Important players in the cell cycle are the **Cdks** (Cyclin-dependent kinases). They are protein kinases, and their activity rises and falls during the cell cycle. Oscillations lead to cyclical changes in phosphorylation of proteins that regulate cell cycle. Cyclical changes are controlled by cyclins, which are synthesized and degraded every cell cycle. So in order to continue the cell cycle, Cdk must be turned off. Notice how Cdks need the bound cyclin in order to be functional.

On figure 17.17 that is further down a little you can see how Cdk is activated. Cyclin alone, changes the conformation and only partially activates Cdk. After this Cdk-activating kinase (CAK) phosphorylates the active site of the Cdk which finally fully activates the protein. This is done by changing the conformation of the T-loop which allows the protein to bind to its protein substrate more easily.

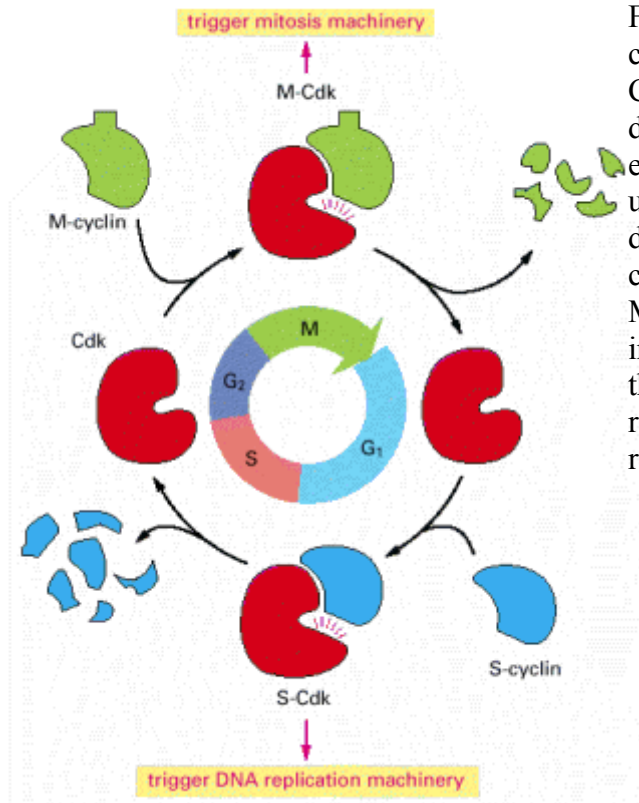
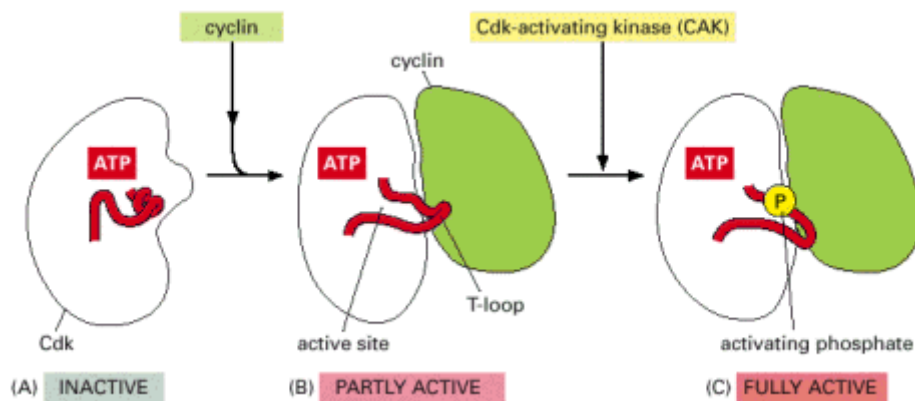


Figure 17.16. A simplified view of the core of the cell-cycle control system. Cdk associates successively with different cyclins to trigger the different events of the cycle. Cdk activity is usually terminated by cyclin degradation. For simplicity, only the cyclins that act in S phase (S-cyclin) and M phase (M-cyclin) are shown, and they interact with a single Cdk; as indicated, the resulting cyclin-Cdk complexes are referred to as S-Cdk and M-Cdk, respectively.



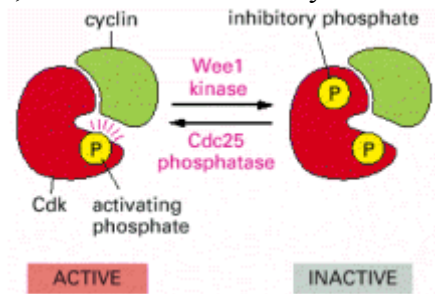
There are a few different classes of cyclins:

- G<sub>1</sub>-cyclins – promote passage through the restriction point G<sub>0</sub>.
- G<sub>1</sub>/S-cyclins – binds Cdks at end of G<sub>1</sub> and commit cell to DNA replication.
- S-cyclins – binds Cdks during S-phase required for initiation of DNA replication.
- M-cyclins – promotes mitosis.

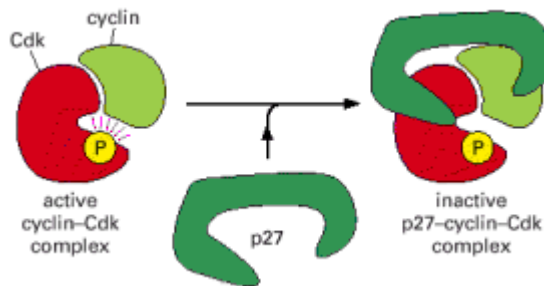
On slide 11 there is a table of the major cyclin-Cdks in mammals, I don't know how important that is but if you want you can take a look at that.



Cdk activity can be regulated in many ways. One way to regulate it is by **phosphorylation**. As the picture below shows, the active cyclin-Cdk complex is turned off when the kinase Wee1 phosphorylates two closely spaced sites above the active site. This masks the active site, and the removal of these phosphates by phosphatase Cdc25, will result in the activation of the complex. The activating phosphate in the picture is added by CAK, which we have already looked at before.

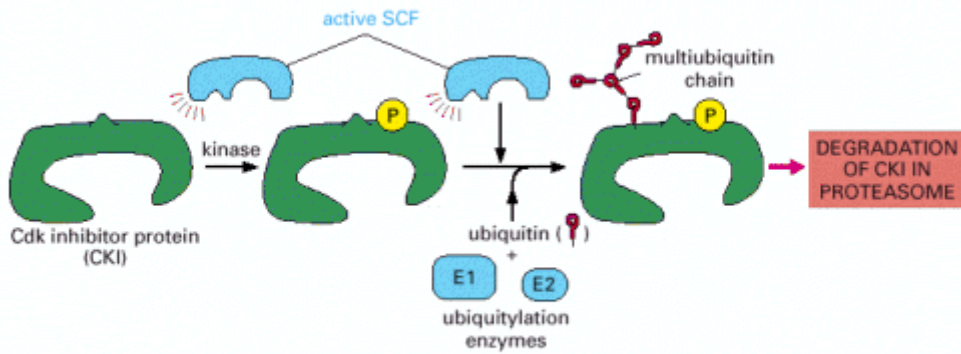


There is another way to regulate Cdk activity, by Cdk inhibitors (CKIs). The figure below shows how the Cdk complex can be deactivated if the CKI p27 is bound to both cyclin and Cdk. This distorts and blocks the active site of Cdk.

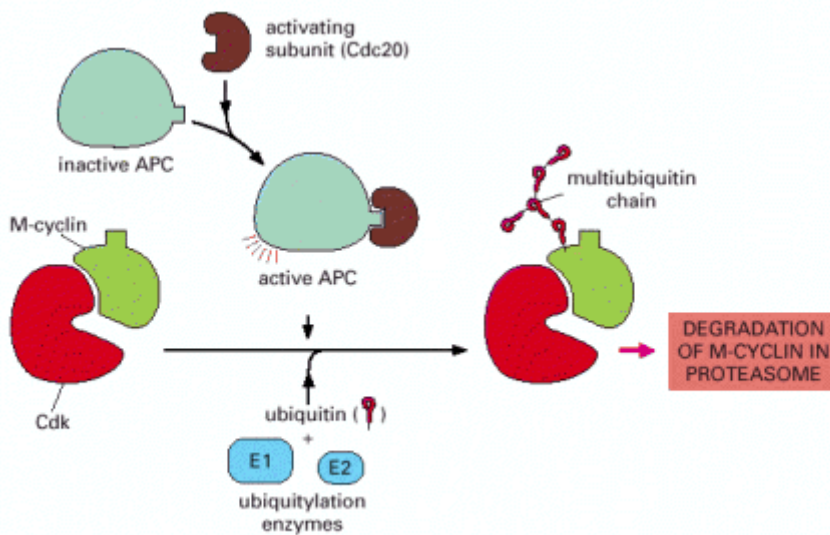


As we have seen before, Cyclin-Cdk complexes are inactivated by regulated proteolysis of cyclins at specific stages. Cyclin degradation occurs by an ubiquitin dependent mechanism. Cyclins tagged with multiple copies of ubiquitin molecules are recognized by proteasomes and are destroyed. During G<sub>1</sub> and S phases, SCF complex is responsible for the degradation of cyclins and certain CKI proteins that control S-phase initiation. This is depicted on **A** on the figure below. Note how E1 and E2 interaction facilitates the insertion of ubiquitin to CKI, and how SCF can only recognize CKI after it has been phosphorylated. On part **B** of the figure, we see how during the M-phase, anaphase promoting complex (APC) is responsible for the degradation of cyclins and other mitosis regulators.

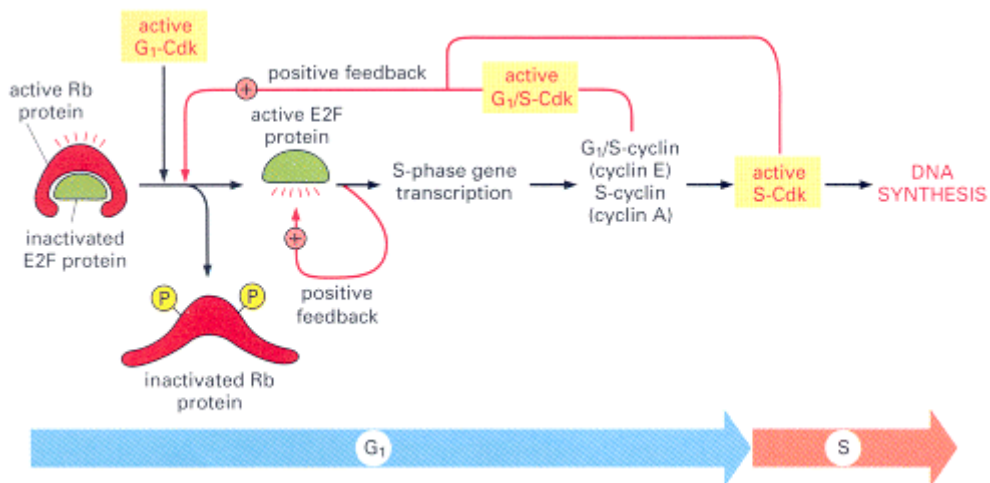
(A) control of proteolysis by SCF



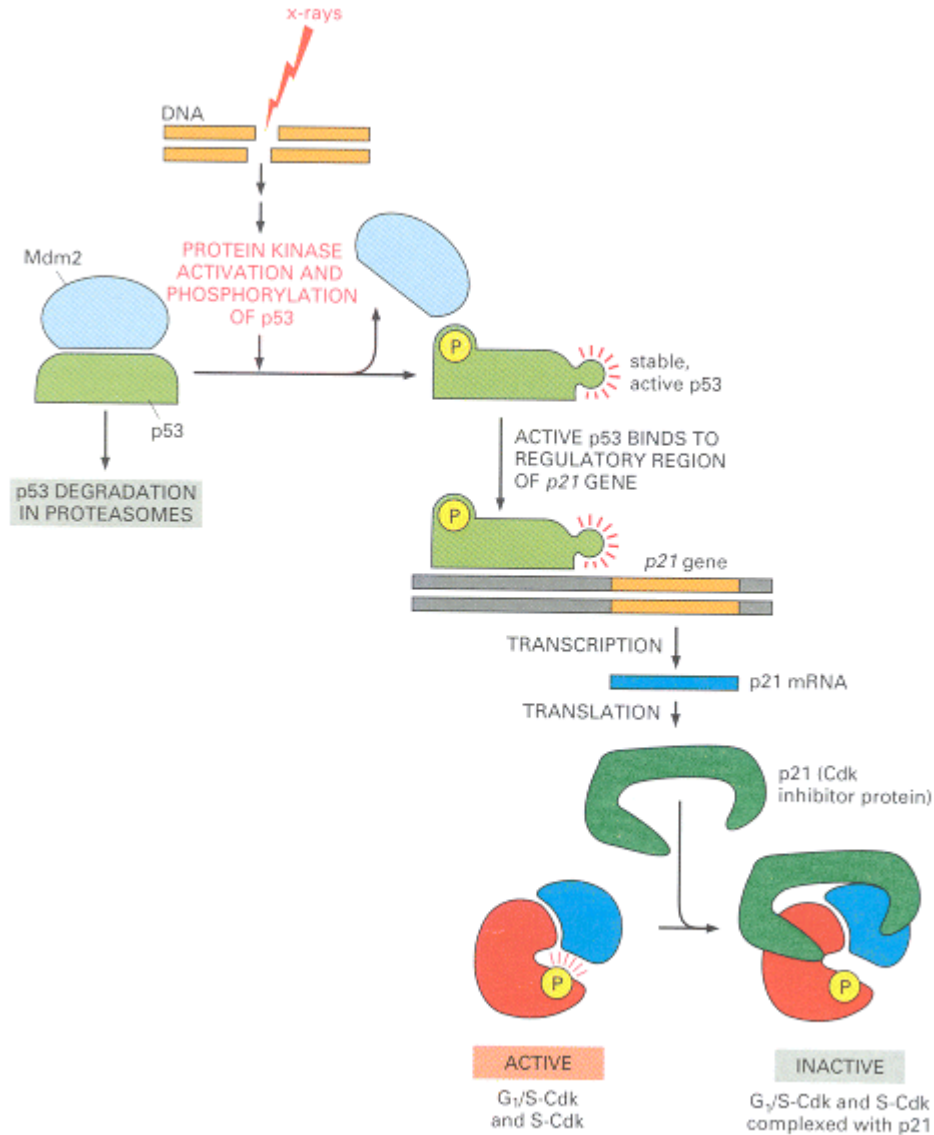
(B) control of proteolysis by APC



Below we see a mechanism for controlling the synthesis of new DNA. Rb acts as a brake in mammalian G<sub>1</sub> cells. Notice how E2F is activated, then activates gene encoding for S-phase proteins.

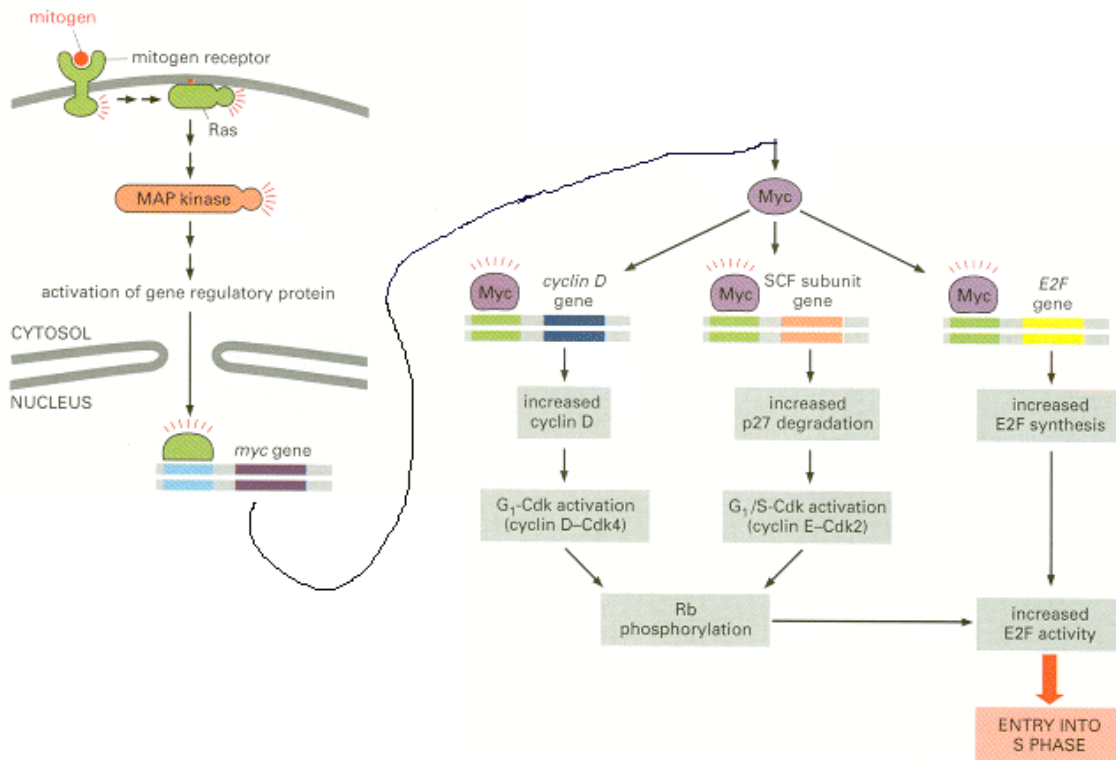


Another way the cell controls its cycle is by activating p53 when there is DNA damage. This is the only way to deal with DNA damage. When this happens the unstable p53 is phosphorylated and becomes activated and stable. p53 then turns on the gene for p21 which is similar to p27 that we have seen above, which binds to Cdk and inactivates it. There are two checkpoints for DNA damage, G<sub>1</sub> and G<sub>2</sub>.



Cell cycle is also controlled by growth factors, which can activate the process. Growth factors that stimulate proliferation are called mitogens (e.g. EGF, FGF2). Most of these act in the G<sub>1</sub>-phase of the cycle in response to the binding of these factors (ligand) with their receptors. They tend to allow entry into the S-phase. EGF binds to the EGF-receptor (a RTK) and activates Myc via a Ras-MAP kinase signaling a cascade. This process is shown on the figure below. However the cell must also protect itself from over activating Myc. Too much Myc activity can cause uncontrolled entry into S-phase. In some cancers, this causes cell to proliferate too much. Therefore when there is an excessive production of Myc, p19<sup>ARF</sup> is activated, which binds and inhibits Mdm2 and

thereby causes increased p53 levels. Depending on the cell type and extracellular conditions, p53 then causes either cell-cycle arrest or apoptosis.



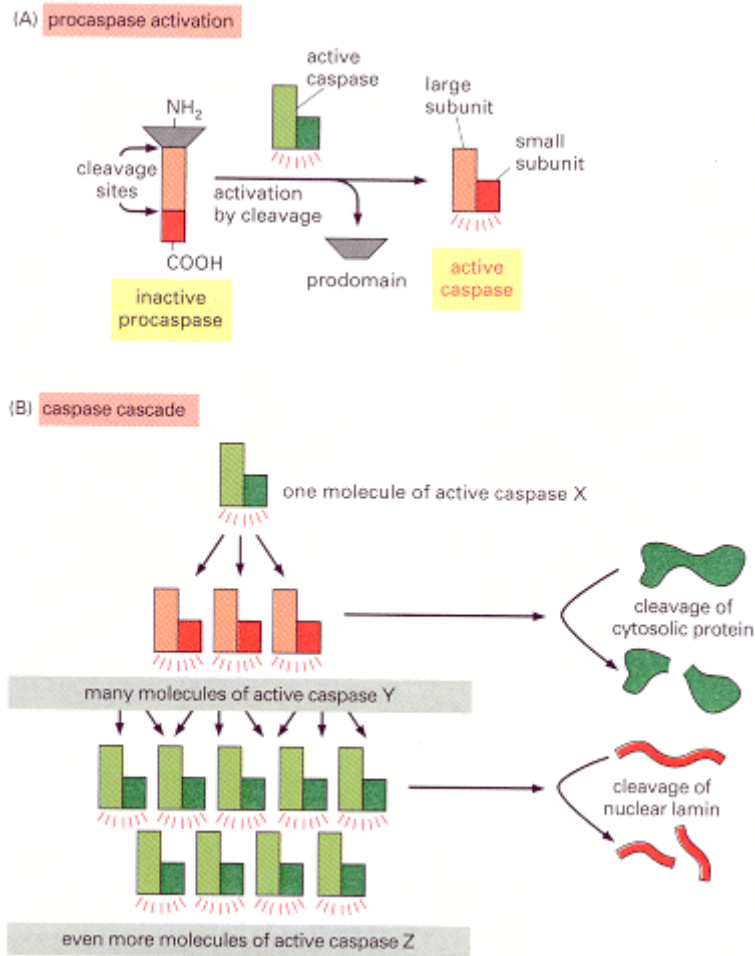
Grow factors are not only used for cellular replication, it can also be used in cases where there is a need for cell growth. In the same manner, a GF is bound to a receptor on the plasma membrane which activates kinases that cause an increase mRNA translation. This stimulates cell growth by making more proteins necessary for growth.

## Programmed Cell Death (APOPTOSIS)

Apoptosis is not like necrosis, when there is an acute injury to cells, causing them to swell and burst, this causes a spill of contents on neighboring cells, this is what we call necrosis. Necrosis leads to a damaging inflammatory reaction. In contrast, apoptosis leads to shrinking of the cells, collapse of the cytoskeleton and DNA fragmentation. Apoptotic cells signal to macrophages that they need to be removed from the scene. Containing the apoptotic cell does not cause an immune response.

The mediators of apoptosis are called caspases (*cysteine-aspartic-acid-proteases*), which are cysteine proteases that control the process. These cysteine proteases cleave target proteins at specific asparic acid residues. Caspases are synthesized as procaspase precursor molecules (which are proteolytically cleaved and activated by other caspases). This leads to an amplified cascade of caspase activity. As indicated on the figure, two of the cleaved fragments associate in order to form the active site of the caspase. Each activated caspase can cleave many other procaspase molecules, which activates them in a cascade. Some caspases, called effector caspases, can cleave a number of key proteins in

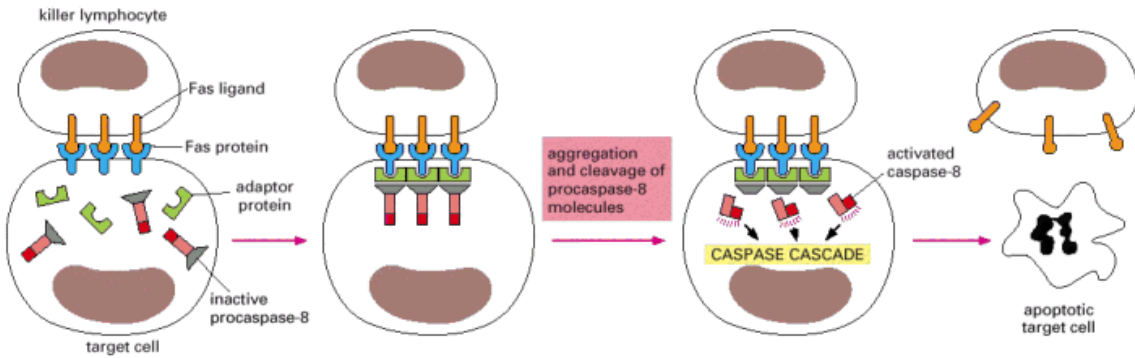
the cell, including specific cytosolic proteins and nuclear lamins, which leads to a controlled cell death.



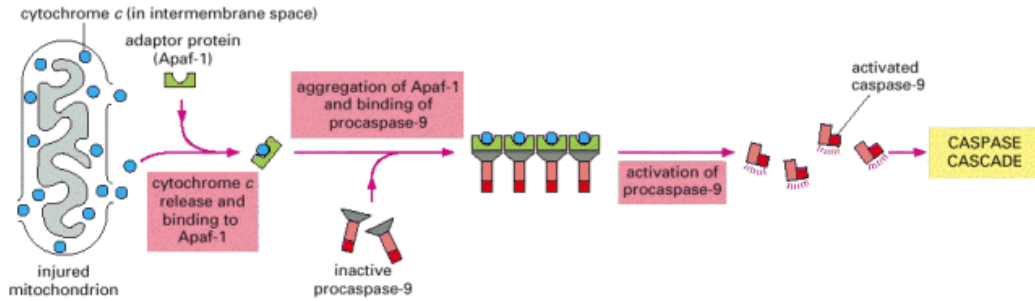
But what triggers apoptosis? It can be triggered from outside the cell (**extrinsic pathway**) by the activation of death receptors, for example the Killer lymphocyte activation of Fas receptors (A). However, cell stress can also trigger death from within (**intrinsic pathway**), for example the release of electron carrier protein cytochrome c by damaged mitochondria (B). Apaf-1 adaptor binds to cytochrome c and activates procaspases. Through this method you can activate caspases that are found further on the caspase cascade, which causes a faster initiation of the process. A third way of initiating apoptosis happens when there is **competition with neighbouring cells for survival factors**. If for example 2 nerve cells are innervating one target cell, and let's imagine the target cell is releasing a survival factor, then only one of the 2 cells will be able to survive while the other cell will go through apoptosis. This may happen to get a 1:1 ratio of neurons per target cell. These survival factors can be growth factors or factors that prevent cell death. They are limiting, and once they are used up, they are gone, so axon has to retract.



(A) **ACTIVATION OF APOPTOSIS FROM OUTSIDE THE CELL (EXTRINSIC PATHWAY)**

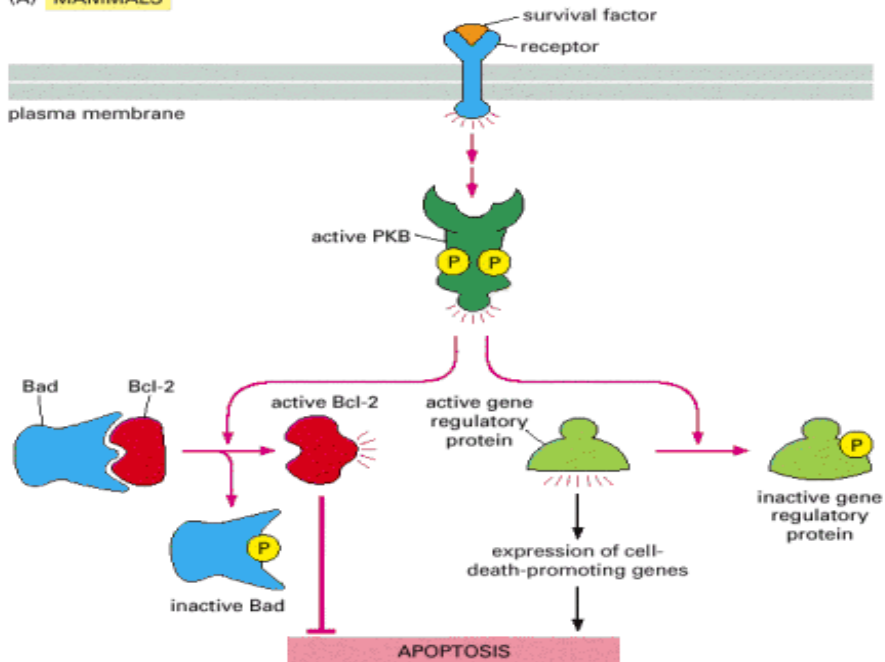


(B) **ACTIVATION OF APOPTOSIS FROM INSIDE THE CELL (INTRINSIC PATHWAY)**



So as mentioned above, some survival factors may inhibit apoptosis, this can happen by the activation of Bcl-2. As it is shown below, PKB is activated which initiates two pathways. One is a direct pathway that inhibits cell death proteins; the other inhibits the expression of genes responsible for cell death. These two together cause a quick response to prevent apoptosis.

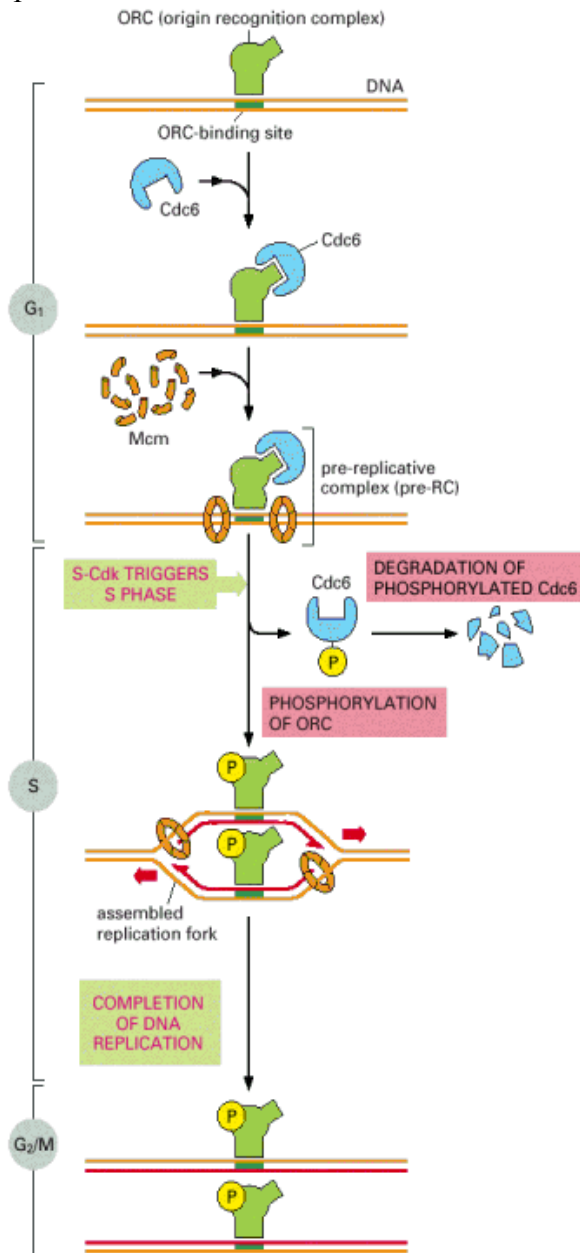
(A) **MAMMALS**



## M-PHASE (Mitosis and cytokinesis) – lecture 9

During a cell cycle, the contents of parent cell are doubled in earlier S-phase and partitioned into two daughter cells in M-phase. Basically the two main goals of the M-phase are: Nuclear division (**mitosis**) and cytoplasmic division (**cytokinesis**).

We have seen before how during the S-Phase DNA is replicated, this is done from multiple origins in the DNA. However no origin is initiated more than once per cell cycle. S-phase continues until replication from all origins along the length of each chromosome is completed in its entirety. Correct gene copy number is maintained as cells proliferate.



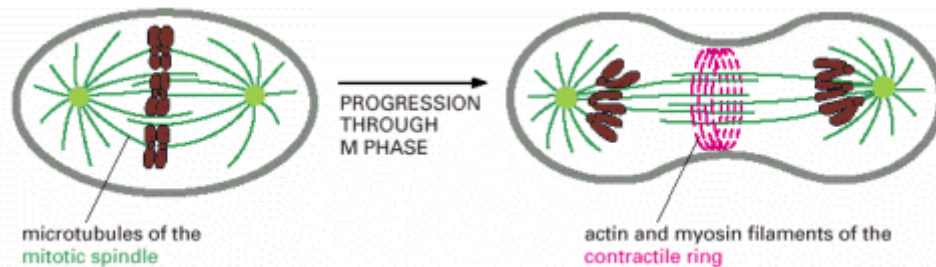
*Figure 17-22.* The initiation of DNA replication once per cell cycle. The ORC remains associated with a replication origin throughout the cell cycle. In early G<sub>1</sub>, Cdc6 associates with ORC. Aided by Cdc6, Mcm ring complexes then assemble on the adjacent DNA, resulting in the formation of the pre-replicative complex. The S-Cdk (with assistance from another protein kinase, not shown) then triggers origin firing, assembling DNA polymerase and other replication proteins and activating the Mcm protein rings to migrate along DNA strands as DNA helicases. The S-Cdk also blocks rereplication by causing the dissociation of Cdc6 from origins, its degradation, and the export of all excess Mcm out of the nucleus. Cdc6 and Mcm cannot return to reset an ORC-containing origin for another round of DNA replication until M-Cdk has been inactivated at the end of mitosis.

From lecture → Cdc6 is at low levels except at S-phase. Cdc6 helps assembling Mcm, forming the pre-RC, this stalls until a signal. S-cdk phosphorylates Cdc6 which kicks start synthesis of DNA.

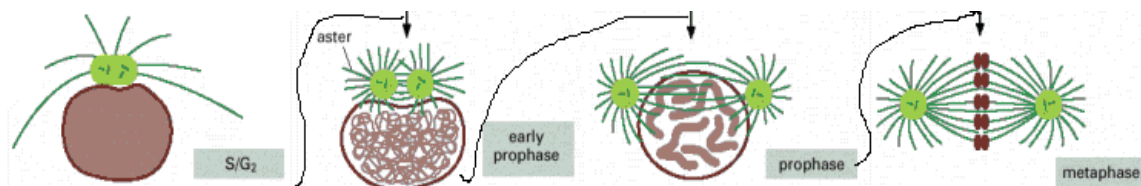
When chromosomes are duplicated in the S-phase, the two copies of each chromosome are tightly bound (sister chromatids). Sister chromatids are glued together by protein complexes called **cohesions** (binds two chromatids together). During chromosome condensation, **condensins** (binding within a single chromatid) compact the chromosomes.

Mitosis always precedes cytokinesis:

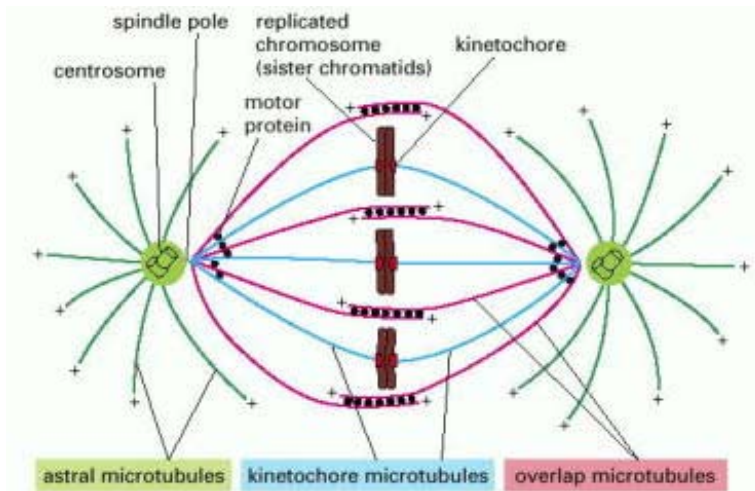
- M-Cdk can inactivate proteins required for cytokinesis.
- The central region of the cell must be cleared of the two sets of chromosome (that get segregated to opposite sides of the cell), this is done by the mitotic spindles, before a stable contractile ring can form.



The centrosome is the main MT organizing center (MTOC), as we saw a way back, and it is composed of a pair of centrioles + matrix. It nucleates fast-growing MTs with plus-ends projecting outward. The matrix contains many proteins (e.g. motor proteins, cross-linking proteins,  $\gamma$ -tubulin ring complex). Centrosomes duplicate before the M-phase and each MTOC nucleates the growth of MTs called an aster. The two asters move to opposite sides of the cell during early M-phase. Astral MTs separate the poles of the cell and correctly position the spindle.

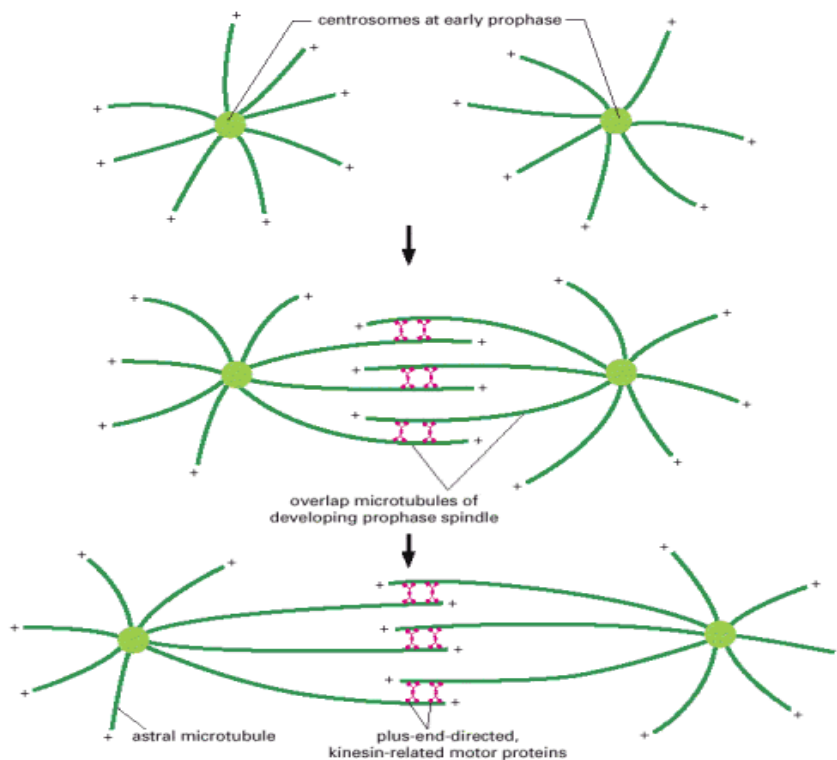


The **mitotic spindle** is a structure involved in mitosis, and it consists of a bundle of MTs joined at the ends but spread out in the middle. In the wide middle portion, antiparallel microtubules are bundled by kinesins. At the pointed ends, known as spindle poles, MTs are nucleated by the centrosomes. Note on the picture below how there are 3 classes of MTs, astral microtubules, kinetochore microtubules (project towards the centre) and overlap microtubules (don't interact with chromosomes). These, with the help of motor proteins can separate the chromatids.



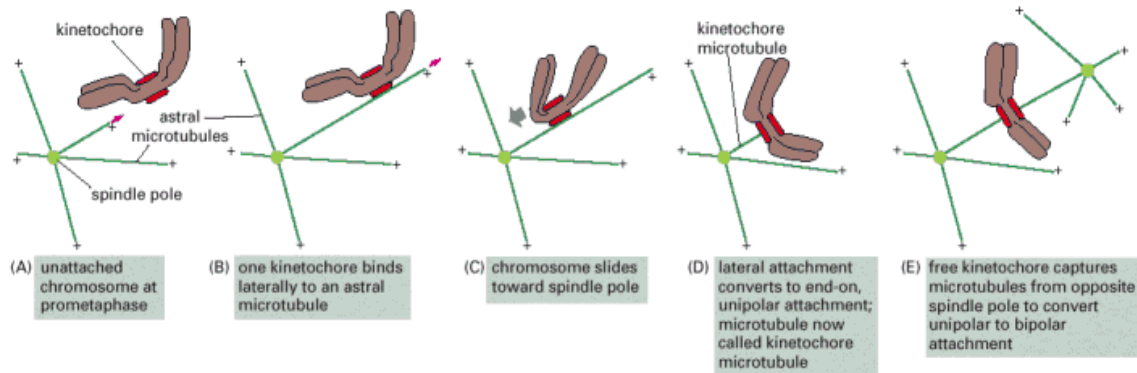
## Phases of Mitosis

**Prophase:** During the Prophase, sister chromatids condense, and replicated centrosomes move apart. The mitotic spindle assembles between two centrosomes. On the picture below we can see how the globular portion of the kinesin is used here to move the MT around, and make sure the centrosomes are split to either end.

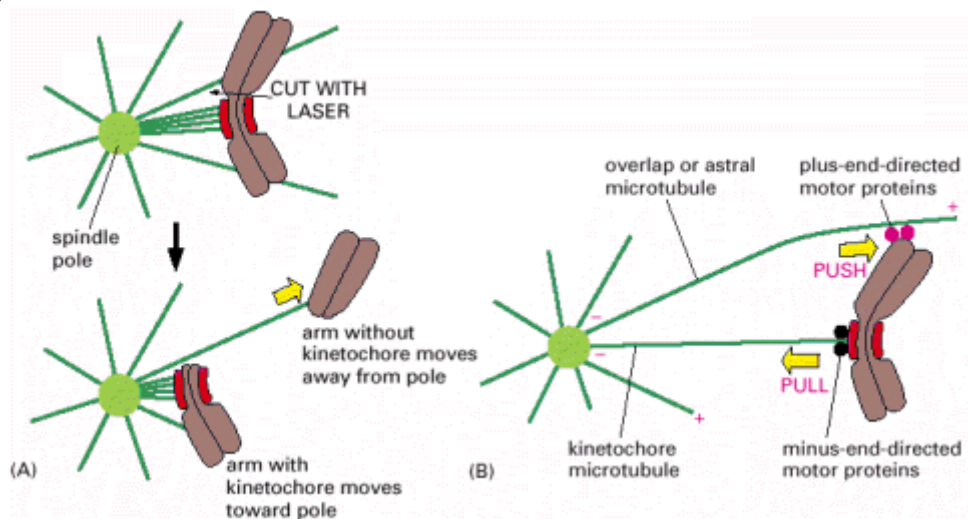


**Prometaphase:** During this phase M-Cdk phosphorylates the nuclear lamina underlying the nuclear envelope. This causes a breakdown of the nuclear envelope.

Chromosomes then attach to spindle MTs via kinetochore and undergo active movement. Centrosomes are positioned at the spindle pole, shown on the pictures above. Each chromosome forms kinetochores at the centromere (dense part of the chromosome), which is a complex protein structure that is used to be picked up by the microtubule hook.



**Metaphase:** At this point chromosomes are aligned at the equator of the spindle, midway between spindle poles (metaphase plate). Kinetochore MTs attach sister chromatids to the opposite poles of the spindle. In lecture there is information about the formation of the metaphase plate, but he didn't mention that in the review but here it is anyway:



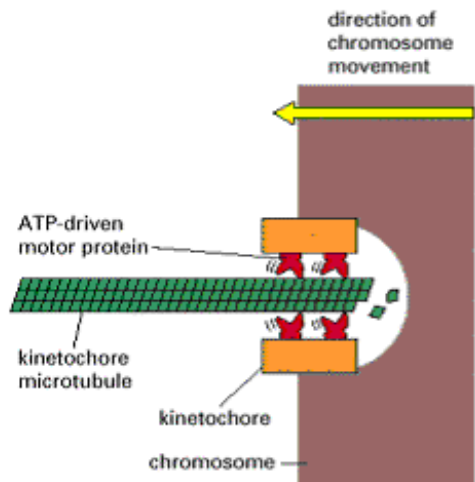
**Figure 18-22.** How opposing forces may drive chromosomes to the metaphase plate. (A) Evidence for an astral ejection force that pushes chromosomes away from the spindle poles toward the spindle equator. In this experiment, a prometaphase chromosome that is temporarily attached to a single pole by kinetochore microtubules is cut in half with a laser beam. The half that is freed from the kinetochore is pushed rapidly away from the pole, whereas the half that remains attached to the kinetochore moves toward the pole, reflecting a decreased repulsion. (B) A model of how two opposing forces may cooperate to move chromosomes to the metaphase plate. Plus-end-directed motor proteins on the chromosome arms are thought to interact with astral microtubules to generate the astral ejection force, which pushes chromosomes toward the spindle



equator. Minus-end-directed motor proteins at the kinetochore are thought to interact with kinetochore microtubules to pull chromosomes toward the pole.

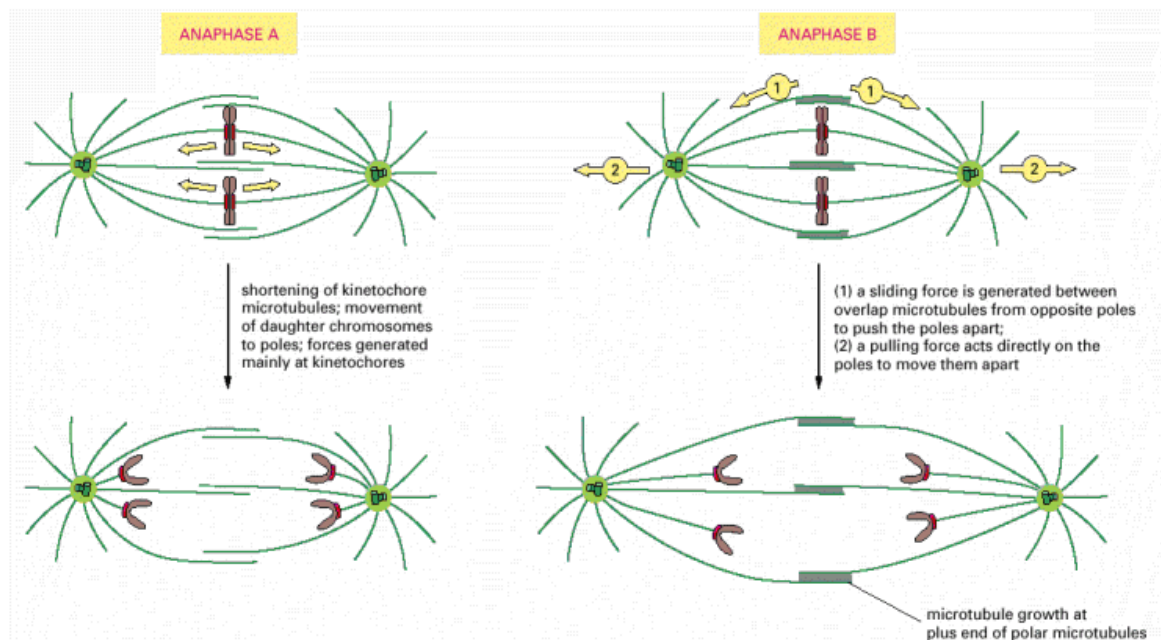
**Anaphase:** Cohesin cross-links are released at this point. APC inactivates *M-Cdk* and it activates *separase*, which inactivates *cohesin*, which if you remember connects the two sister chromatids.

Sister chromatids are synchronously separated to form two daughter chromosomes. Each set of chromosomes is pulled slowly toward each pole it faces. Kinetochore MTs get shorter and spindle poles move apart. At this point is where the pulling is important, this happens synchronously on both sides by depolymerization. Now how do the MTs shrink?



(A) ATP-driven motor protein drives both chromosome movement and microtubule disassembly

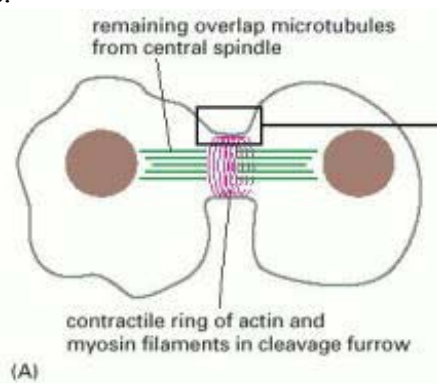
*Figure 18-27.* Two alternative models of how the kinetochore may generate a poleward force on its chromosome during anaphase A. (A) Microtubule motor proteins at the kinetochore use the energy of ATP hydrolysis to pull the chromosome along its bound microtubules. Depolymerization of the kinetochore microtubules follows as a consequence.



In anaphase A we see how it depends on motor proteins that operate together at the kinetochores that pull the daughter chromosomes toward the nearest pole. In anaphase B the two spindle poles move apart. Two separate forces are thought to be responsible for anaphase B. The elongation and sliding of the overlap microtubules past one another in the central spindle push the two poles apart, and outward forces exerted by the astral microtubules at each spindle pole act to pull the poles away from each other, toward the cell surface.

**Telophase:** During this phase, two sets of daughter chromosomes arrive at the poles of the spindle and decondense. New nuclear envelope reassembles around each set of chromosomes. A contractile ring begins to be assembled. The telophase is basically a reversal of the prophase and prometaphase events.

**Cytokinesis:** During this phase, a cleavage furrow around the cell deepens until it divides the cell into two. Cytokinesis depends on the function of a contractile ring that forms just beneath the plasma membrane. The contractile ring is composed of actin filaments, myosin II filaments and many related structural and regulatory proteins. After all this a new membrane is inserted adjacent to the contractile ring by fusion of intracellular vesicles.



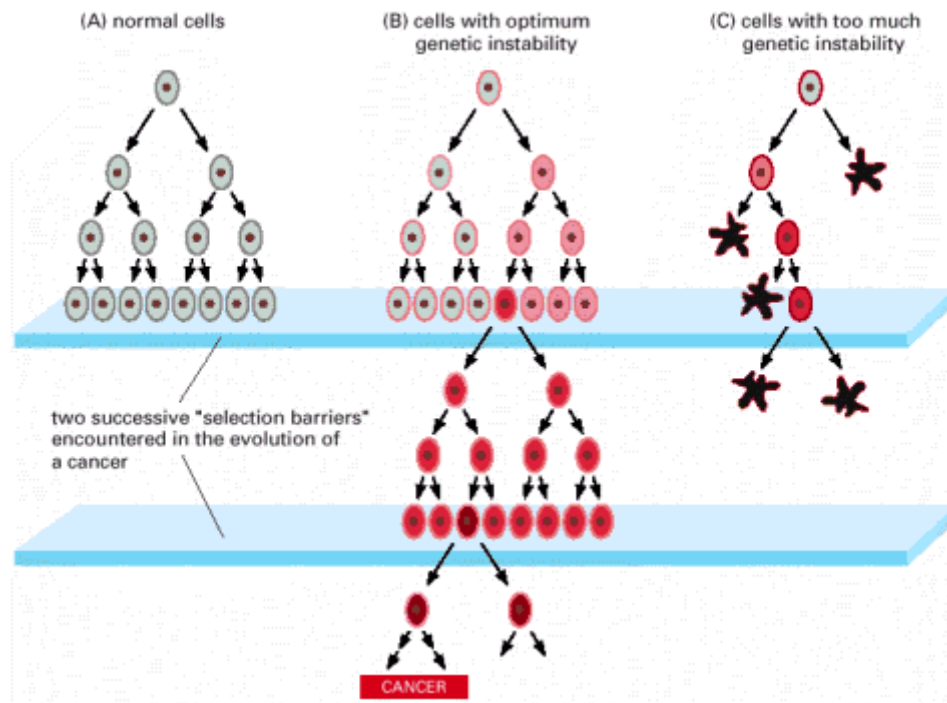
Mitosis however, can happen without cytokinesis, for example in the development of a *Drosophila* embryo. The first 13 nuclear divisions in a *Drosophila* embryo occur synchronously and without cytoplasmic division to create a large syncytium.

## Cancer – lecture 10

There are  $10^{14}$  cells in the human body, therefore probabilistically billions of cells experience mutations everyday. The body usually destroys aberrant cells, but sometimes a mutation may give a cell a selective advantage for growth and survival (microevolution). This cell becomes a founder for a mutant clone of cells. The mutant clone successfully expands at the expense of their neighbors, eventually destroying the tissue and organism.

The great majority of human cancers show signs of a dramatically enhanced mutation rate: the cells are said to be **genetically unstable**. As you can see on the figure below, there must be many gene mutations for tumour progression. In most cases these

mutations are detected and these defective cells are eliminated, but not always. In tumor cell precursors, an increased level of genetic instability makes it likely that at least one cell will contain the requisite genetic alteration to pass the selection barrier and continue the process of tumor progression. This genetic instability is retained in the lineage, and it can be measured in the resulting tumor. This is shown on (B). (C) is an example of really high genetic instability.



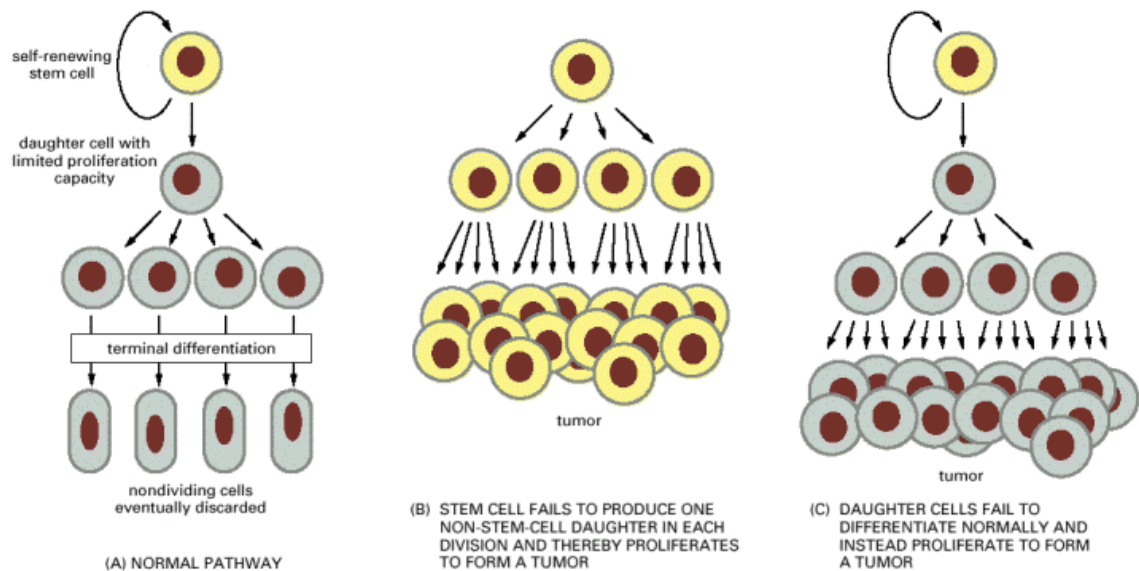
Many experiments have shown that [most cancers originate from a single aberrant cell](#). For example, a single X chromosome is inactivated in female cells (dosage compensation), and every normal tissue of a woman's body contains a mixture of cells with different X-chromosomes heritably inactivated. Cells in a tumor can be tested to determine which X-chromosome is inactivated. All cells in a tumor have the same X inactivated, implying that tumor is derived from a single founder cell.

There are several categories of the cancerous state:

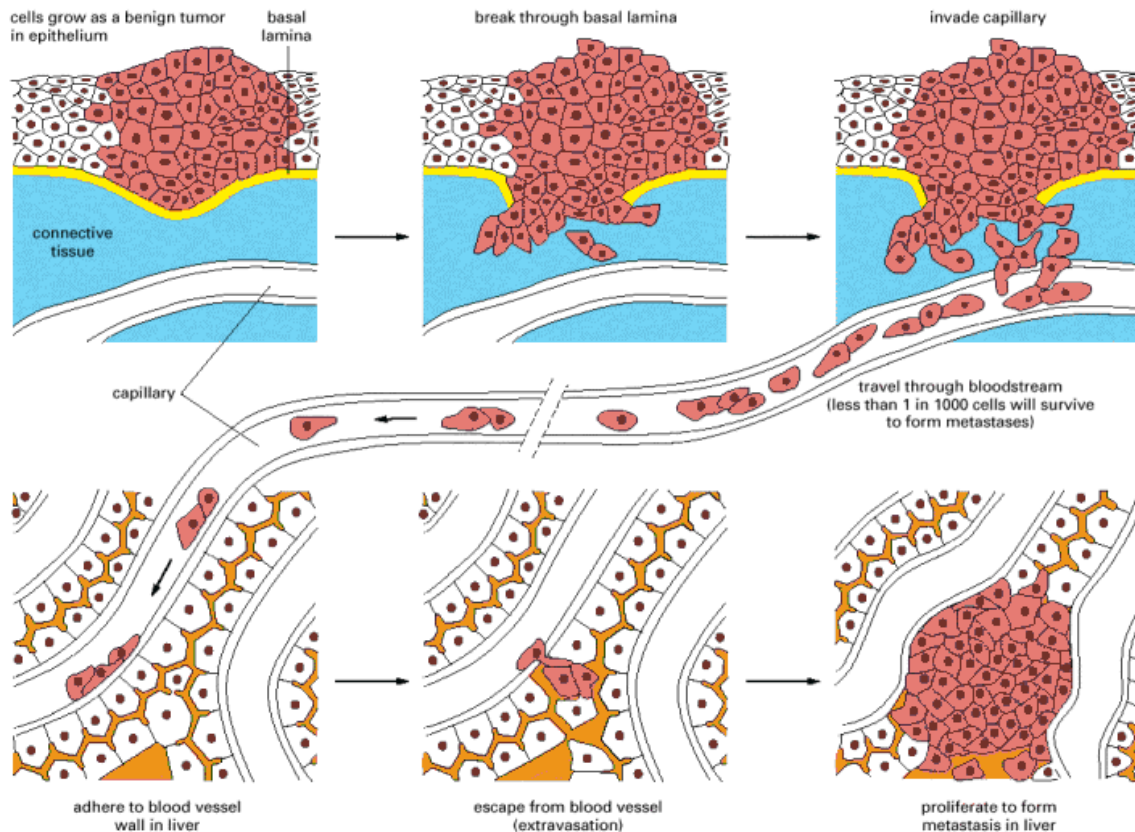
- **Tumor (neoplasm)** – uncontrolled growing mass of abnormal cells
- **Benign tumor** – growing mass is self-contained as a “single” tumor and can usually be cured with surgery.
- **Malignant tumor (cancer)** – Aggressive tumor that has acquired the ability to break free and invade surrounding tissue.
- **Metastases** – Cancerous tumor that can invade other tissues, usually by entering blood stream of lymphatic vessels. Usually either blood or interstitial fluid are the carriers.
- **Carcinoma** – epithelial cell cancers.
- **Sarcoma** – Connective tissue or muscle cell cancer.
- **Leukemia** – Blood cell cancers.

When testing for cancer, for example a pap test (papanicolaou), a sign that an invasive carcinoma is present is the fact that the cells will be undifferentiated and will all look mitotically active. Normal cells are usually large with small nuclei.

But how do mutations cause cancer? This can happen due to **abnormal cell division** (the larger the clone of mutated cells, the greater the chance of acquiring additional mutations), **abnormal cell death** (apoptosis normally counteracts the increase in cell number, but defects in this process can advance tumor progression), and **abnormal cell differentiation** (differentiated cells do not divide, and increased cell number should result in an increase in the number of differentiated cells. Defects in this process could advance tumor progression). The picture below shows the example of defective control of differentiation. “A” shows a normal scenario where the mother stem cell produces daughter cells that will divide only to a point and then are discarded. B shows an example of the stem cell producing daughter cells that are identical to the mother cell, this is a problem since the daughter cells will continue to reproduce without a limit. On case C, the mother cell produces a mutated daughter cell that will form a tumor.

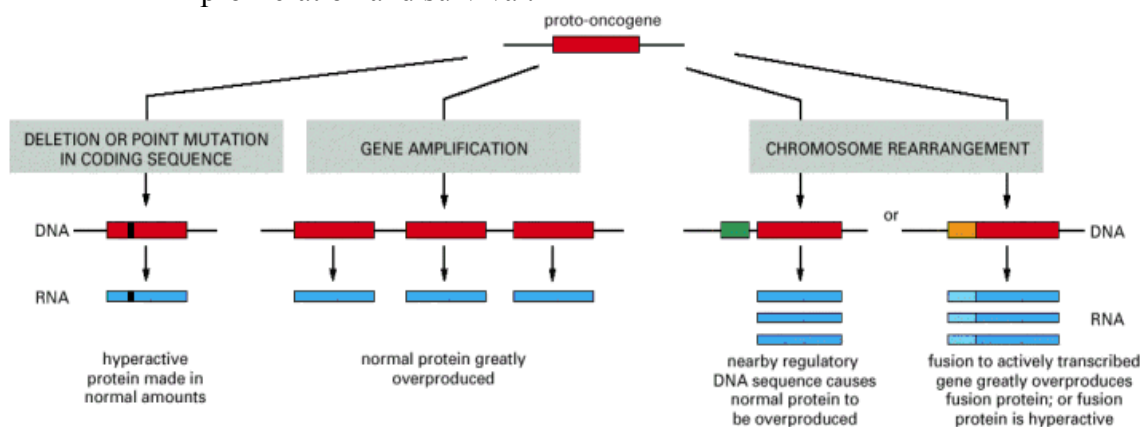


As we defined above, **metastasis** is a spread of some cancer from one tissue to other tissues. For this to happen, malignant cells must thrive in a new environment. The example below illustrates the spread of a tumor from an organ such as the lung or bladder to the liver. Tumor cells may enter the bloodstream directly by crossing the wall of a blood vessel (figure), or, more commonly perhaps, by crossing the wall of a lymphatic vessel that ultimately discharges its contents (lymph) into the bloodstream. Tumor cells that have entered a lymphatic vessel often become trapped in lymph nodes along the way, giving rise to lymph-node metastases. Studies in animals show that typically far less than one in every thousand malignant tumor cells that enter the bloodstream will survive to produce a tumor at a new site. They must also have the correct cell adhesion to stick and crawl on the lumen of blood vessels for this to happen.



There are some genes in which we refer to as **cancer genes**, which are divided into:

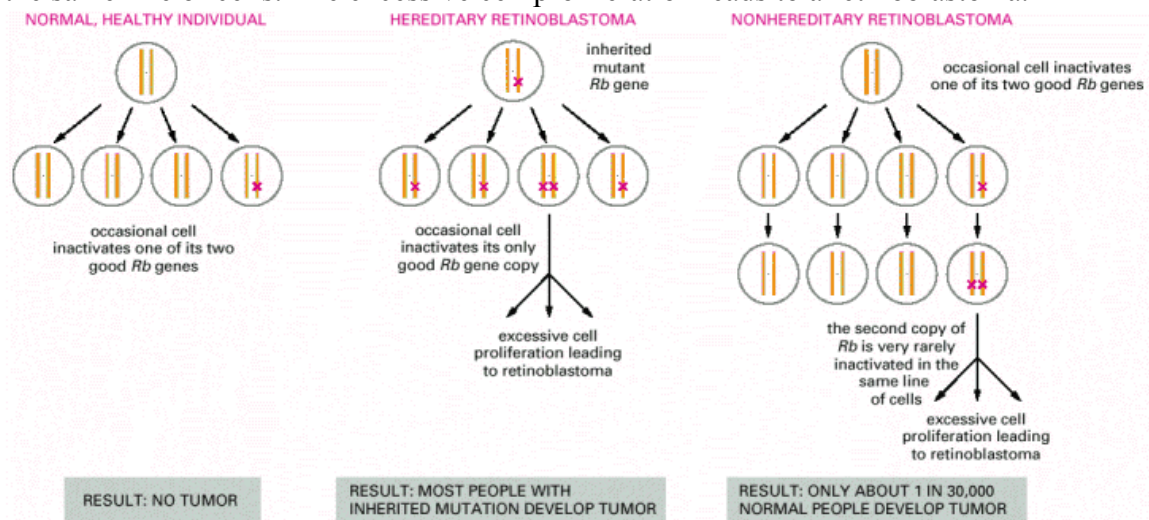
- Proto-oncogenes – Cancer arises from a gain of function mutation. Mutant, overactive form of the gene is called an oncogene (e.g. Ras, Myc). On the picture below we can see three ways in which a proto-oncogene can be made overactive to convert it into an oncogene.
- Tumor suppressor genes – Cancer arises from a loss of function mutation (e.g. p53, Rb). This would happen if the gene mutated use to put breaks on cell cycle.
- Mutations in both types of genes have similar effects on enhancing cell proliferation and survival.





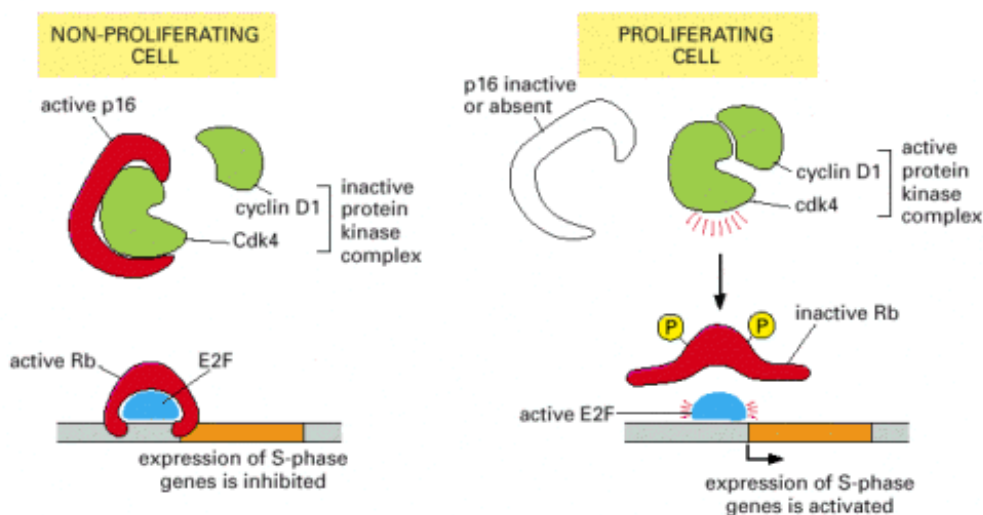
What the picture shows is in the first case where the new kind of protein made after the mutation is hyperactive. On the second case the protein is the same, however, the gene has many copies of itself, which produces more proteins. On the third case, a mutation on the promoter can cause an overproduction of the protein, or it can cause the protein to contain new amino acid sequence bound, which enhances activity.

In the case of **tumor suppressing genes**, we can look at an example of Rb (retinoblastoma). There are three scenarios, one where a normal healthy individual has one of its two good Rb genes inactivated. Since there is another activated copy of the gene, there will be no tumor formed. In some cases however, one of the genes might be inherited mutated. Even though there is one good gene available, an occasional cell inactivates its only good Rb gene copy; this will consequently cause an excessive cell proliferation, leading to retinoblastoma. The third case, which is more rare, is when a normal individual has an cell inactivation of both Rb genes, at different points in time, in the same line of cells. The excessive cell proliferation leads to a retinoblastoma.



Below is a picture showing the disruptive molecular pathways leading to cancer. Notice how the inactivation of Rb causes the cell to go into the S-phase.

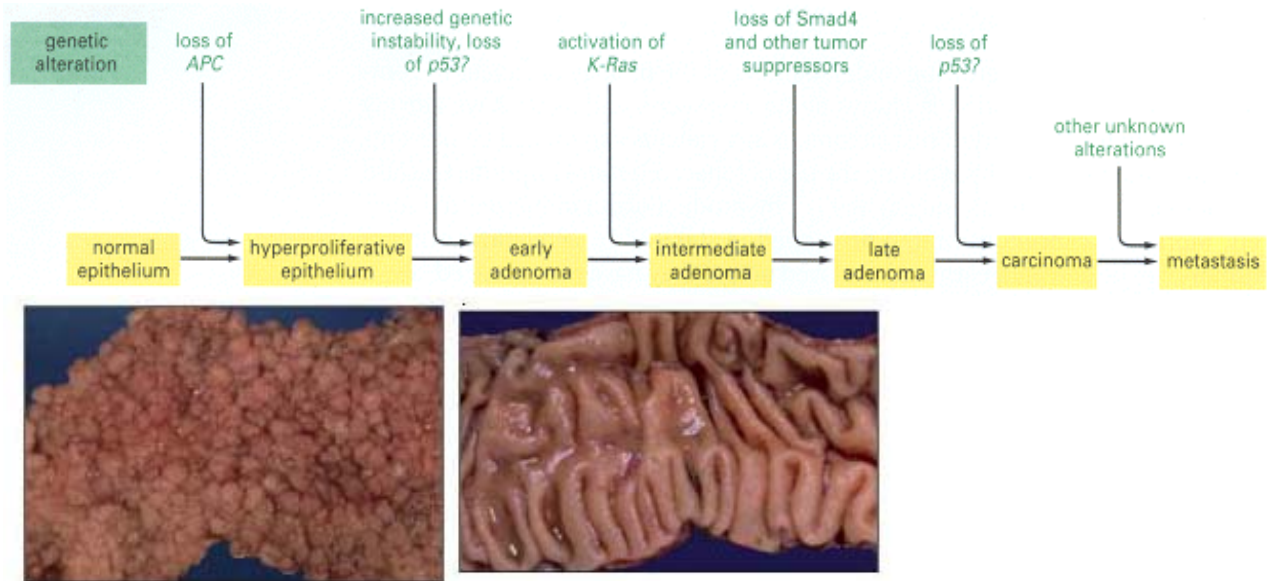
(B)



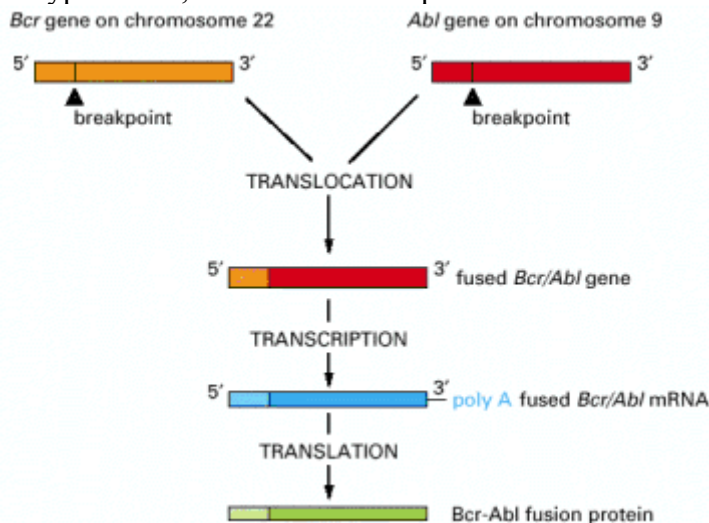
Viral infections can also cause cancer. In some cases there may be an accidental integration of the viral DNA fragment in the host chromosome. This alters the environment of the viral genes. This (or possibly some other cause) disrupts the control of viral gene expression. The unregulated production of viral replication proteins interferes with the control of cell division, thereby helping to generate a cancer.

In a different case, there are DNA tumor viruses that activate the cell's replication machinery. These viruses can interact with proteins that inhibit cellular proliferation, such as Rb or p53. The interaction of these proteins with the viral proteins, cause the cell to proliferate in an uncontrolled fashion. This can cause a tumor.

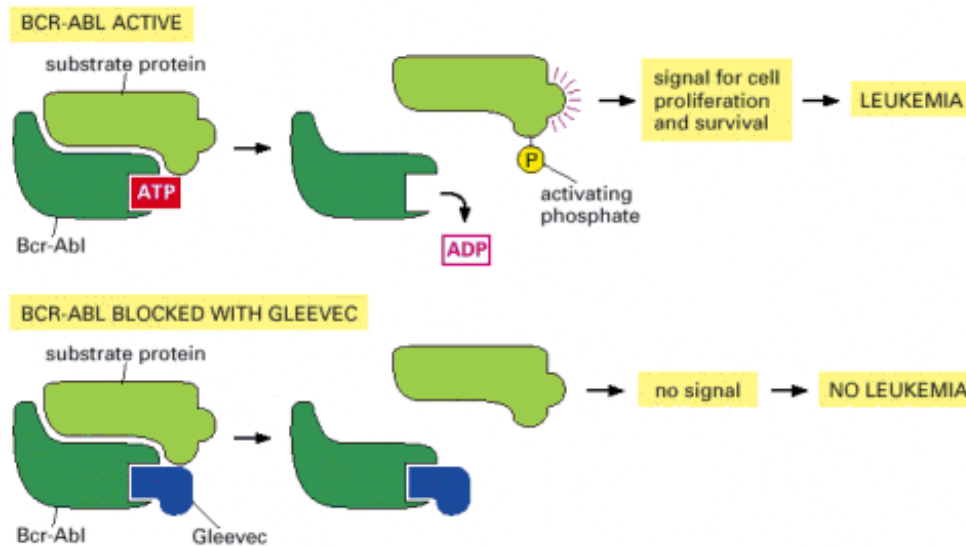
Below is an example of a carcinoma, more specifically the development of colorectal carcinoma.



Philadelphia chromosome is a specific chromosomal abnormality that is related to chronic myelogenous leukemia. The picture below shows how there is a translocation between chr9 and chr22 in order for this to occur. This mutation occurs and the resultant protein is hyperactive, which stimulates proliferation and inhibits apoptosis.



This type of leukemia is treated with a drug called Gleevec. The way this drug works is by binding to the activated Bcr-Abl protein at the site of ATP binding. This stops the protein from sending signals that enhances cell proliferation.

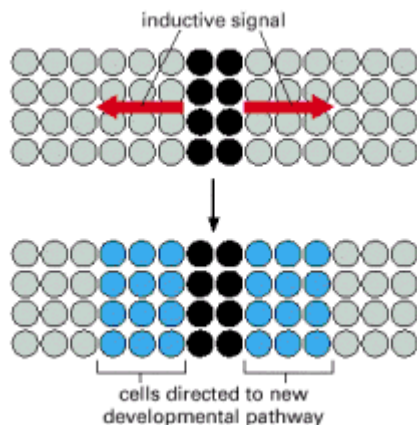


## Development of multicellular organisms – lecture 11 & 12

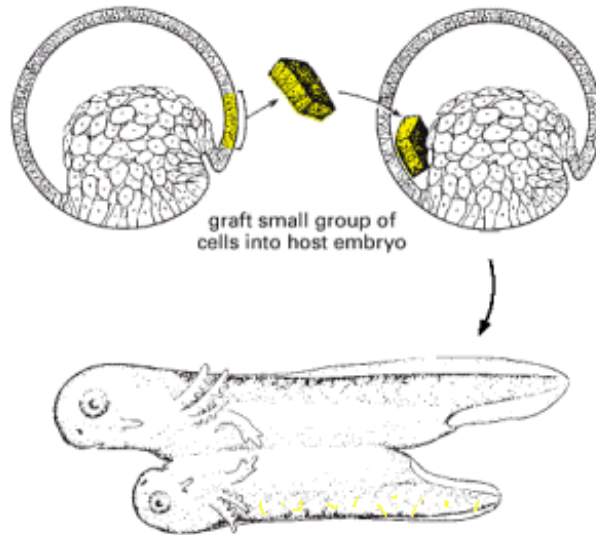
What happens when you coordinate cell proliferation, cell specialization, cell interaction and cell movement and run the program? This is what we'll see in these two final lectures, thank God.

We'll start by looking at the development of zebrafish. We should also get familiar with the fundamental principles in developmental biology which are: Induction, morphogen gradients, pattern formation, cell fate determination and morphogenesis.

**Induction:** A group of cells start off with equal potential, a diffuse signal from neighboring cells drives one or more cells of the group into a distinct developmental pathway. Signal is limited in duration and space, so that only cells closest to the source are affected.



A good example of this is an experiment done on an amphibian embryo. Spemann and Mangold in 1924, transplanted tissue from the “dorsal lip” (back side) of one developing amphibian embryo to the belly side of another developing embryo. There was a formation of a duplicate embryo. The inducing tissue was called the “organizer”, and the donor organizer tissue itself contributed to the formation of the second embryo. Also, donor tissue induced the host tissue to generate structures it normally wouldn’t make, for example a new brain.

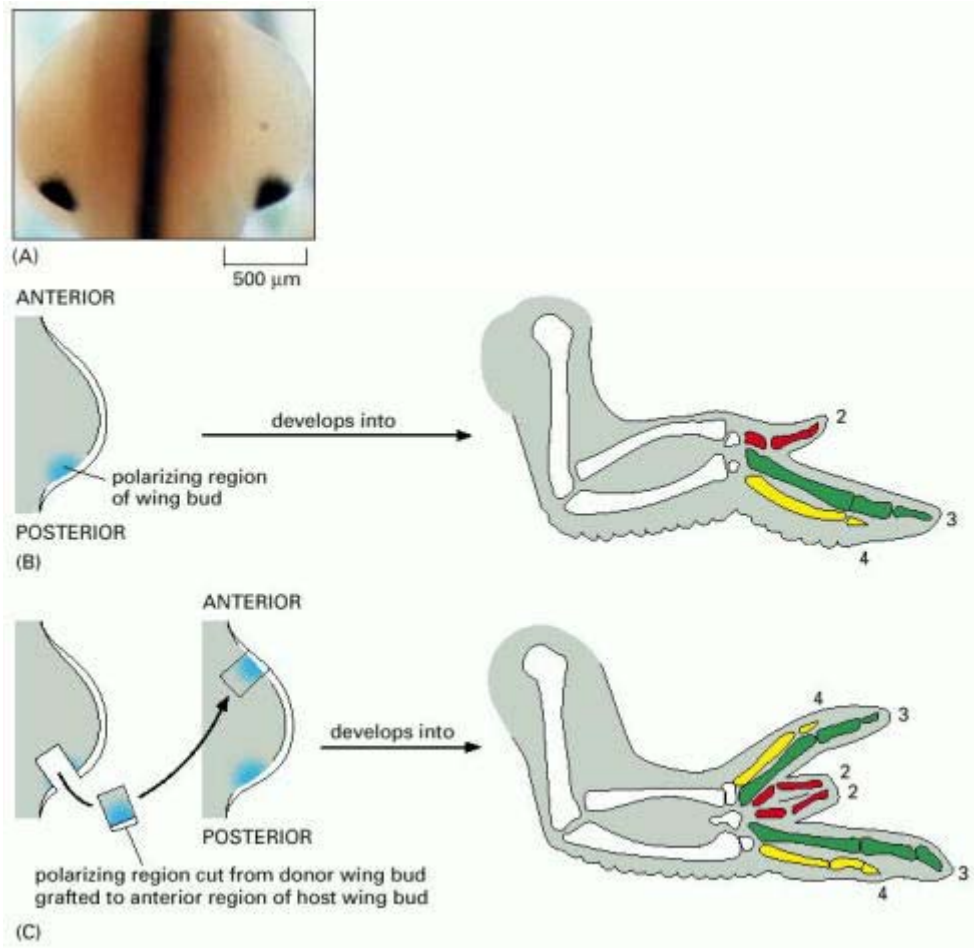


**Morphogen Gradients:** (concentration of certain molecules) During developments some secreted molecules have a gradient pattern of diffusion (ie. High to low concentrations). Cells receiving a signal don’t always respond in a yes or no fashion. The concentration of these molecules (morphogen) often are key in determining its affects on cell fate. High, intermediate and low concentrations of only one signaling molecule can set off different developmental programs. A good example of this is the vertebrate limb formation.

The node in the picture is the source of sonic hedgehog (Shh) signaling in the post limb bud. Protein spreads from this source toward the anterior end. This morphogen gradient controls the formation of distinct digits. As you can see a mirror image duplication of digits happens, when source of Shh is transplanted to the anterior end of the limb bud.

*Figure 21-13.* Sonic hedgehog as a morphogen in chick limb development. (A) Expression of the *Sonic hedgehog* gene in a 4-day chick embryo, shown by *in situ* hybridization (dorsal view of the trunk at the level of the wing buds). The gene is expressed in the midline of the body and at the posterior border (the polarizing region) of each of the two wing buds. Sonic hedgehog protein spreads out from these sources. (B) Normal wing development. (C) A graft of tissue from the polarizing region causes a mirror-image duplication of the pattern of the host wing. The type of digit that develops is thought to be dictated by the local concentration of Sonic hedgehog protein; different

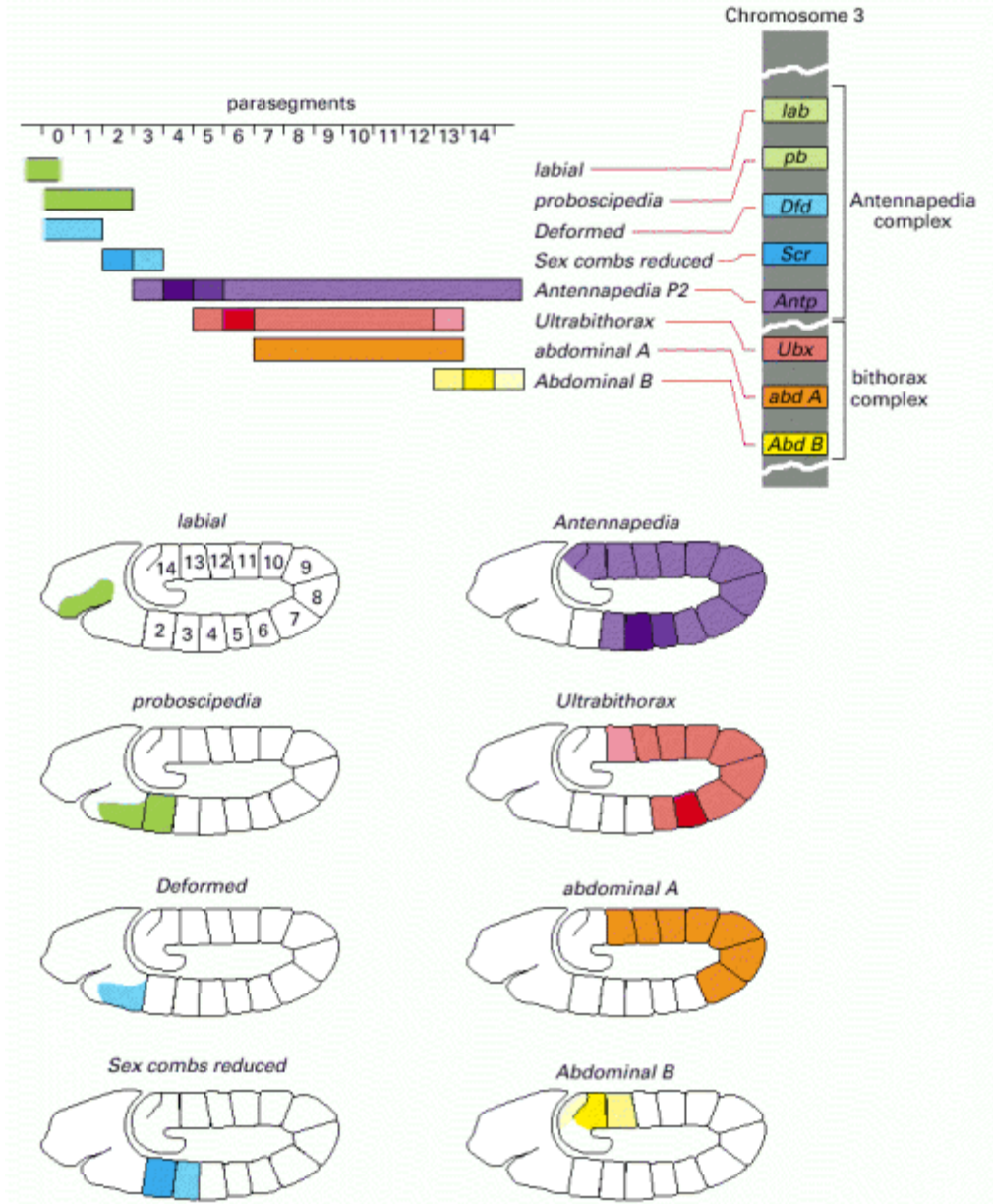
types of digit (labeled 2, 3, and 4) therefore form according to their distance from a source of Sonic hedgehog. (A, courtesy of Randall S. Johnson and Robert D. Riddle.)



**Patterning formation:** The organization of the anterior-posterior axis of the body can be understood as a series of segments from head to tail. The formation of each segment involves the function of specific genes that work together. The discovery of the homeotic selector genes (hox genes) gave us the insight into the segmented nature of A-P development.

In Hox genes, or homeotic selector genes, we can notice that the order of genes on the chromosome corresponds to the order of expression in the embryo. Note that most of the genes are expressed at a high level throughout one parasegment (dark color) and at a lower level at adjacent parasegments. In regions where the expression domains overlap, it is usually the most posterior of the locally active genes that determines the local phenotype.

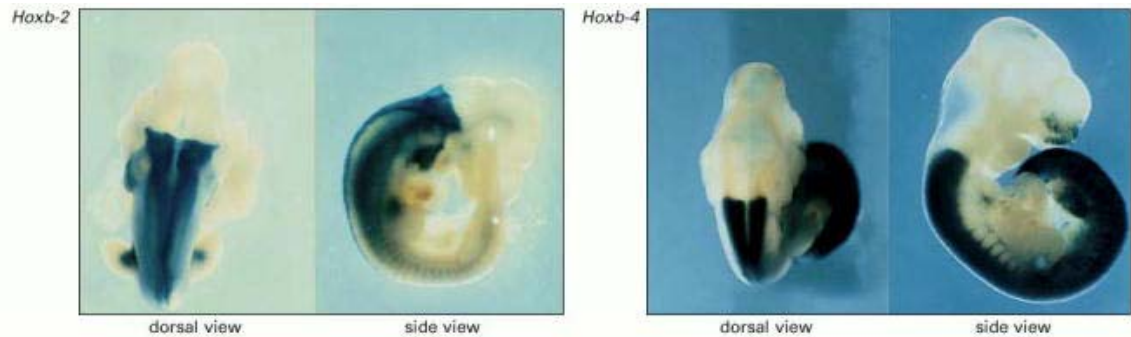




A mutation in *Drosophila* can reveal how each segment should normally form due to the Hox genes. The mutant gene *antennapedia* (*AntP*) has legs sprouting from the head instead of the antennae. A similar principle of segmented gene expression occurs in all animals. An example of this is the Hox expression in anterior-posterior axis of the mammalian embryo (e.g. CNS).

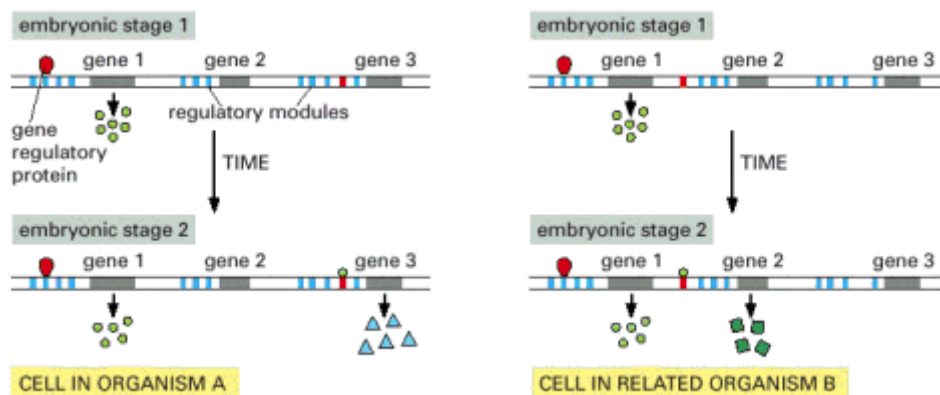
In many cases, genes expressed in anterior domains extend all the way to the posterior end. It is the starting point for the anterior expression domain that differs in each of the genes. The picture below shows whole embryos displaying the expression domains of two Hox genes, stained in blue. Each gene is expressed in a long expanse of tissue with a sharply defined anterior limit. The earlier the position of the gene in its chromosomal complex, the more anterior the anatomical limit of its expression. Thus, with minor exceptions, the anatomical domains of the successive genes form a nested set,

ordered according to the ordering of the genes in the chromosomal complex. (Courtesy of Robb Krumlauf.)

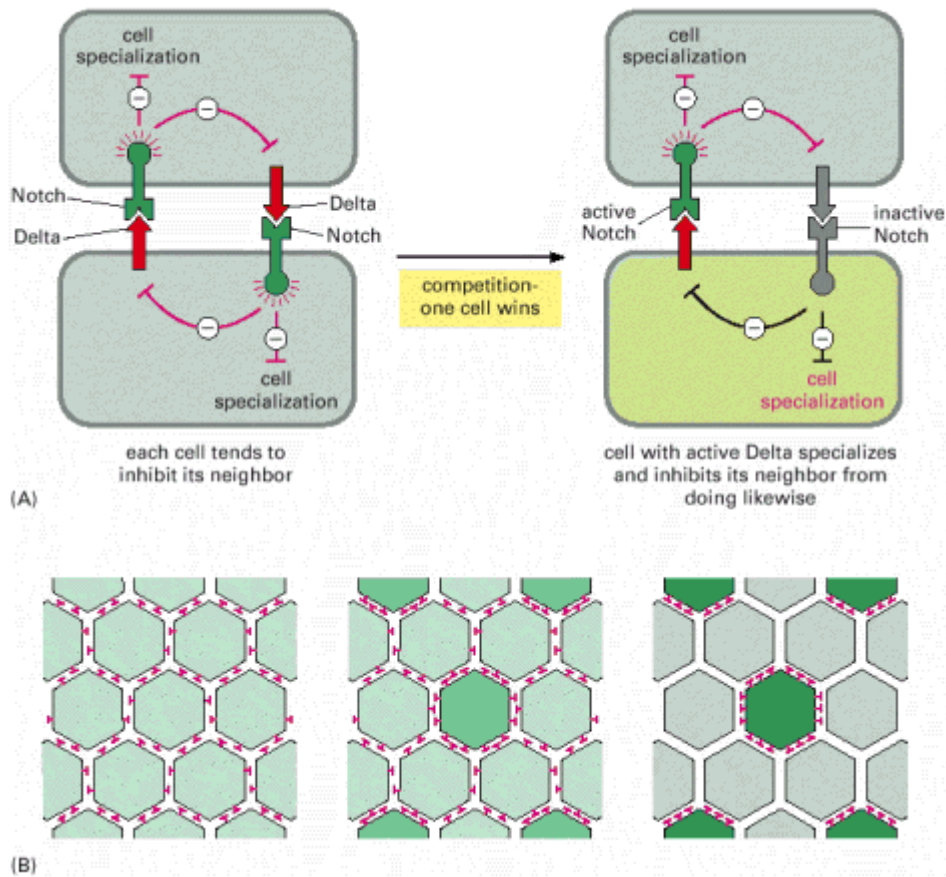


There are many conserved mechanisms in animal development. Homologous proteins can be functionally interchangeable between very different species. For example if you compare a normal mouse brain with a mouse lacking a certain important gene for brain development. There is a major difference, however if the normal mouse is compared with a mouse that has a gene from a fruit fly for the same purpose, the brain is formed normally. Conserved molecular mechanisms allow scientists to make important discoveries about human biology by using “model systems” like these.

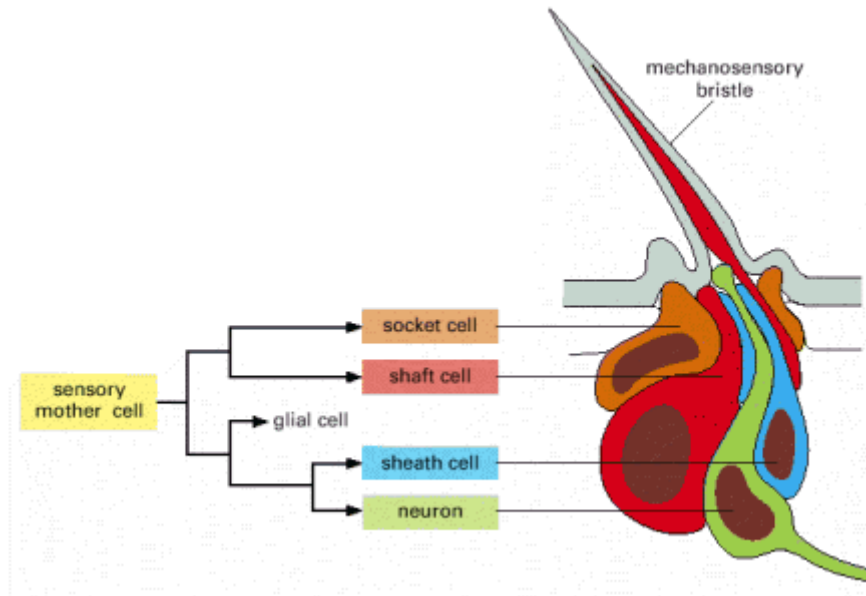
Regulatory DNA helps define the program of development. There are fundamental similarities in the protein coding genes of different animals. Almost all animals share a basic set of cell types as well as tissues, such as a mouth, a gut, a nervous system and skin. So how can animals be so different in their anatomy and behavior? **Most of the instruction used to create a multicellular organism, are encoded within regulatory DNA** (eg. Enhancers) associated with a gene. In a given cell type, genes are expressed based on whether protein complexes recognize the enhancer causing either of activation or repression of transcription. Regulatory sequences are fairly conserved in animals with similar body plans (e.g. vertebrates). However, animals with different body plans usually have poor conservation in regulatory DNA (with some exceptions).



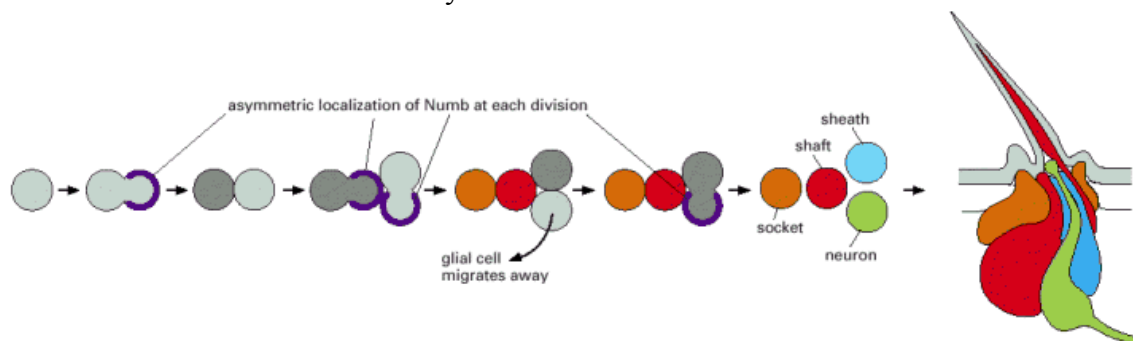
**Cell Fate:** Lets now look at cell fate determination by lateral inhibition. Only cells in immediate contact are influenced by lateral inhibition. This is distinct from morphogen gradients, which can act at a distance. Let's compare membrane-bound signals (in lateral inhibition) versus secreted signals (in morphogen gradients) by looking at the notch-delta signaling control lateral inhibition. In this example we look first at two interacting cells. The bottom cell in this case wins the competition, making so that only this cell sends the inhibitory signal to its neighbor, and it does not receive the signal anymore, this becomes the mother cell. On the second part of the picture we see the same situation however now looking at a larger population of cells. Where at first all cells are equivalent, expressing the receptor Notch and the ligand Delta. All cells have a tendency to specialize to a mother cell, so they send inhibitory signals to their neighbors, to discourage them from specializing in that way. This competitive situation ends up with winners and losers. Where winner become the mother cells and the losers specialize in a different way.



Now lets look at sensory bristle formation in the fly by lateral inhibition. This development follows the same mechanism outlined above where you have a sensory mother cell that gives rise to the bristle, and skin cells around them that will form the surrounding area. This mother sensory cell gives rise to a lineage of the four cells of the bristle, all descendants of a single sensory mother cell.



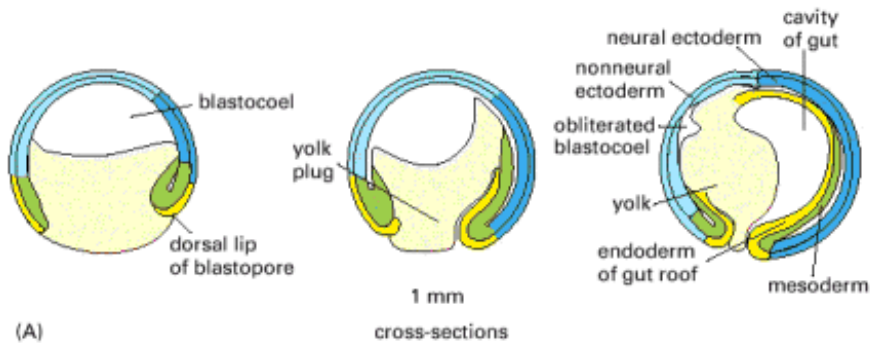
The Numb protein is asymmetrically localized at each cell division starting with the sensory mother cell. Numb interacts with Notch, blocking its activity. Numb-containing cells are insensitive to inhibitory signal from neighbor. The cell that ultimately receives the Numb at the end of the lineage becomes the neuron. Remember that upon the loss of Notch there is an abundance of Delta.



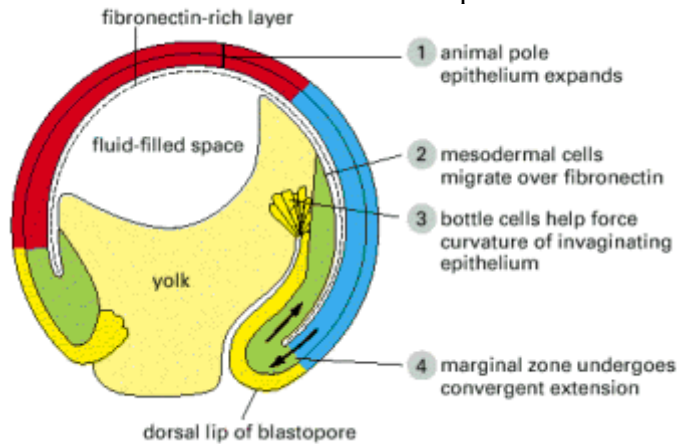
**Morphogenesis:** Lineage tracing analyses help us to understand the pattern of cell behaviour in the developing embryo. Proliferation, differentiation and migration. Cells know their identity because of their history (where they came from) and their neighborhood. Therefore, there is intrinsic (genetic, history) and extrinsic (environment) regulation of cell fate during morphogenesis.

An important concept to understand here is [Gastrulation](#), which is a phase early in the development of animal embryos, during which the morphology of the embryo is dramatically restructured by cell migration.

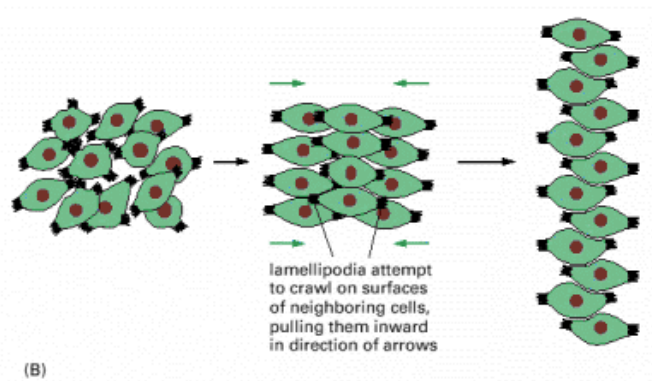
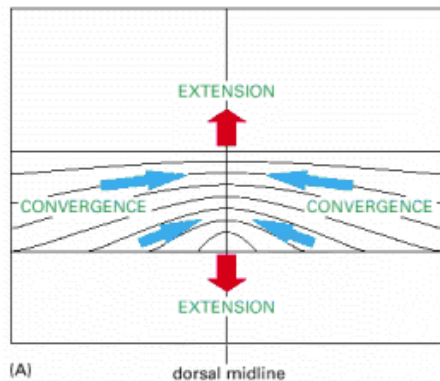
The process of gastrulation generates three germ layers: endoderm, mesoderm and ectoderm. Complex rearrangements of cell position brings cells into contact with new neighbors causing them to signal each other, which also helps define cell fate (induction).



A fate map for the early *Xenopus* embryo (viewed from the side) as it begins gastrulation, showing the origins of the cells that will come to form the three germ layers as a result of the movements of gastrulation. The various parts of the mesoderm (lateral plate, somites, and notochord) derive from deep-lying cells that segregate from the epithelium in the cross-hatched region. The other cells, including the more superficial cells in the cross-hatched region, will give rise to ectoderm (*blue, above*) or endoderm (*yellow, below*). Roughly speaking, the first cells to turn into the interior, or *involute*, will move forward inside the embryo to form the most anterior endodermal and mesodermal structures, while the last to involute will form the most posterior structures.



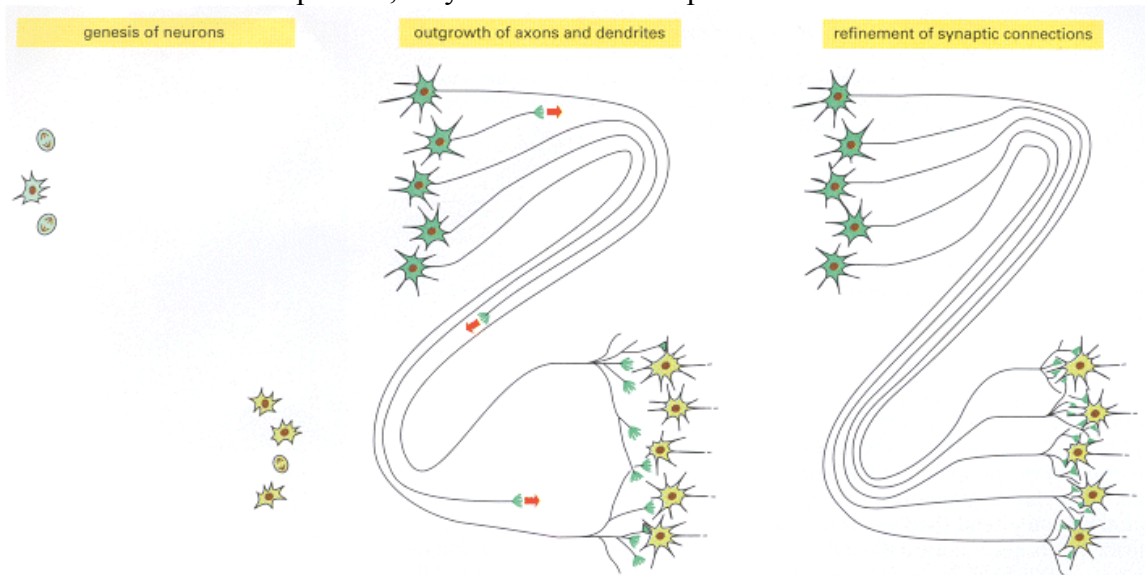
Conversion-extension movements define the AP (Anterior-posterior) axis. See diagram below.





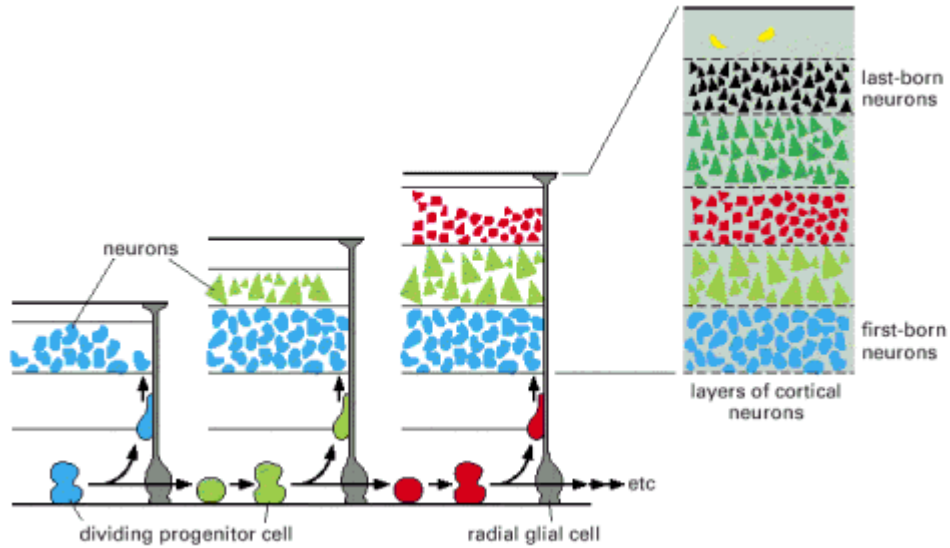
*Figure 21-74.* Convergent extension and its cellular basis. (A) The pattern of convergent extension in the marginal zone of a gastrula as viewed from the dorsal aspect. *Blue arrows* represent convergence toward the dorsal midline, *red arrows* represent extension of the anteroposterior axis. The simplified diagram does not attempt to show the accompanying movement of involution, whereby the cells are tucking into the interior of the embryo. (B) Schematic diagram of the cell behavior that underlies convergent extension. The cells form lamellipodia, with which they attempt to crawl over one another. Alignment of the lamellipodial movements along a common axis leads to convergent extension. The process depends on the Frizzled/Dishevelled polarity-signaling pathway and is presumably cooperative because cells that are already aligned exert forces that tend to align their neighbors in the same way. (B, after J. Shih and R. Keller, *Development* 116:901–914, 1992.)

Now let's look at the **construction of the vertebrate CNS**. There are three main events for CNS developments, they are shown in the picture below.



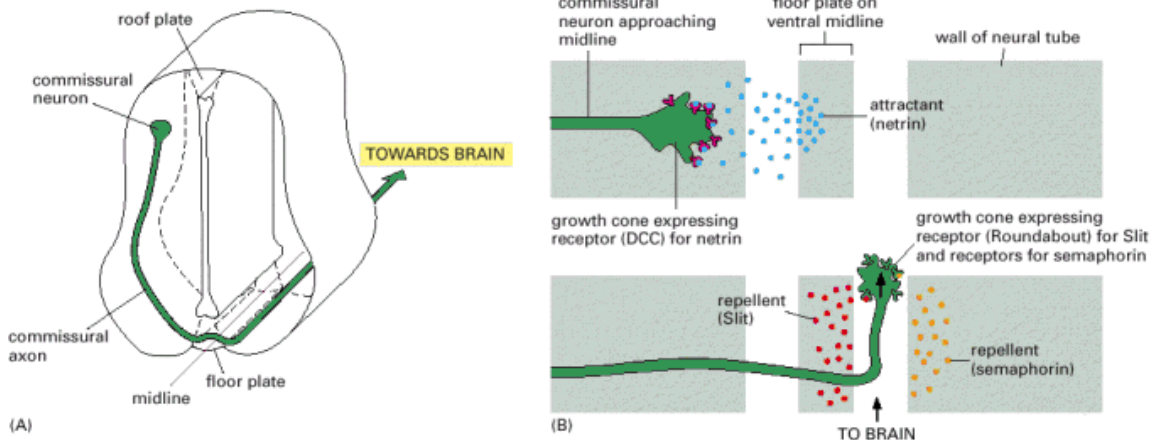
Before sending out axons and dendrites, newborn neurons often migrate from their birthplace and settle in some other location. The neurons go through their final cell division close to the inner, luminal face of the neural tube and then migrate outward by crawling along *radial glial cells*. Each of these cells extends from the inner to the outer surface of the tube, a distance that may be as much as 2 cm in the cerebral cortex of the developing brain of a primate. The radial glial cells can be considered as persisting cells of the original columnar epithelium of the neural tube that become extraordinarily stretched as the wall of the tube thickens.

Cell migration happens in the developing cerebral cortex. Know that the later born neurons migrate past earlier born neurons. On the picture below note how there are layers of neurons in the cerebral cortex, and how newer neurons occupy more superficial layers.



**Figure 21-95. Programmed production of different types of neurons at different times from dividing progenitors in the cerebral cortex of the brain of a mammal.** Close to one face of the cortical neuroepithelium, progenitor cells divide repeatedly, in stem-cell fashion, to produce neurons. The neurons migrate out toward the opposite face of the epithelium by crawling along the surfaces of radial glial cells, as shown in [Figure 21-93](#). The first-born neurons settle closest to their birthplace, while neurons born later crawl past them to settle farther out. Successive generations of neurons thus occupy different layers in the cortex and have different intrinsic characters according to their birth dates.

Another interesting part of the CNS development is axon guidance. As we know, in some parts of the nervous system, the part of our brain that controls these innervations are opposite to them. That is the left side of your brain control the movement of some muscles on the right side of your body. But how do these axons cross over the midline?



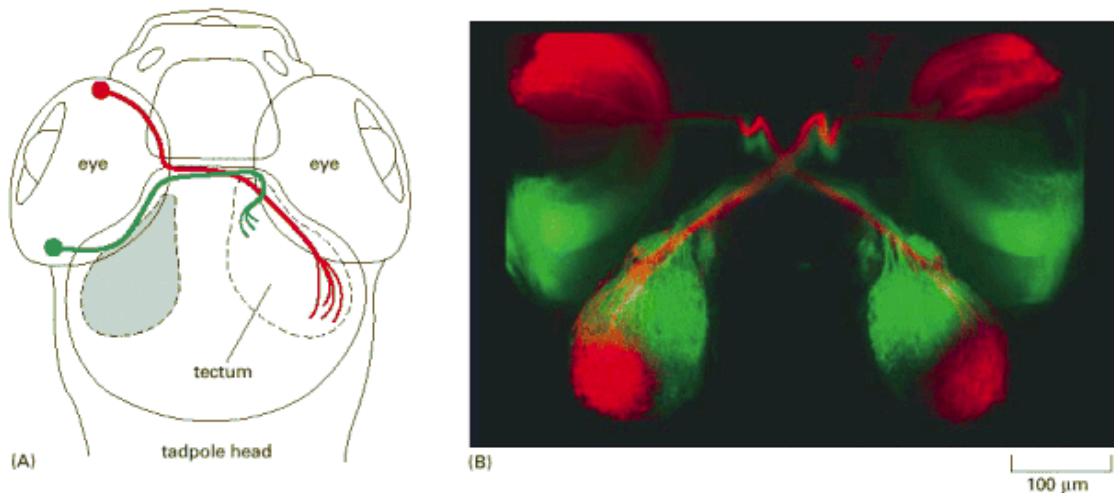
gc = growth cone  
fp = floorplate

1. Toward the midline: netrin (fp) – DCC – growth cone (gc) interaction attracts growth cone to the midline.

2. At the midline: slit (fp) – roundabout (gc) interaction prevents growth cone from backtracking.
3. Beyond the midline: Semaphorin (NT) interaction with its receptor (gc) repels growth cone.
4. Axon heads toward the brain.

*Figure 21-98.* The guidance of commissural axons in the embryonic spinal cord of a vertebrate. (A) The pathway taken by commissural axons in the embryonic spinal cord of a vertebrate. (B) The signals that guide them. The growth cones are first attracted to the floorplate by netrin, which is secreted by the floor-plate cells and acts on the receptor DCC in the axonal membrane. As they cross the floor plate, the growth cones upregulate their expression of Roundabout, the receptor for a repellent protein, Slit, that is also secreted by the floor plate. Slit, binding to Roundabout, not only acts as a repellent to keep the cells from re-entering the floor plate, but also blocks responsiveness to the attractant netrin. At the same time, the growth cones switch on expression of receptors for another repellent protein, semaphorin, that is secreted by the cells in the side walls of the neural tube. Trapped between two repellent territories, the growth cones, having crossed the midline, travel in a tight fascicle up toward the brain.

At last we look at **retinotectal pathway in zebrafish.**



The nasal retina projects to contralateral posterior tectum. The temporal retina projects to contralateral anterior tectum.

*Figure 21-100.* The neural map from eye to brain in a young zebrafish. (A) Diagrammatic view, looking down on the top of the head. (B) Fluorescence micrograph. Fluorescent tracer dyes have been injected into each eye—*red* into the anterior part, *green* into the posterior part. The tracer molecules have been taken up by the neurons in the retina and carried along their axons, revealing the paths they take to the optic tectum in the brain and the map that they form there. (Courtesy of Chi-Bin Chien, from D.H.

Sanes, T.A. Reh, and W.A. Harris, Development of the Nervous System. San Diego, CA: Academic Press, 2000.)

$G_1 \rightarrow$  FIMBRIM – imp in actin fil...+++ ver depois se apareceu nos meus notes senao coloque isso na sessao de actin .