

cell bio II

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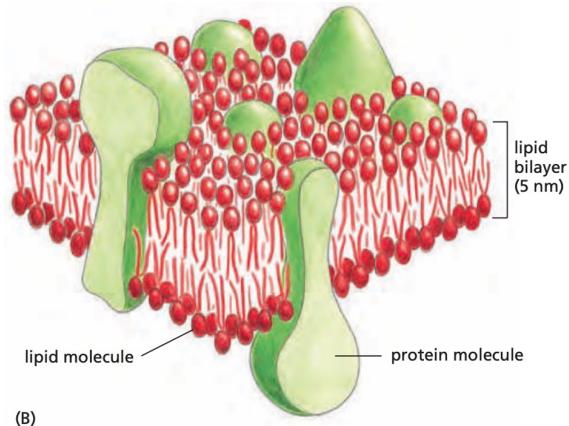
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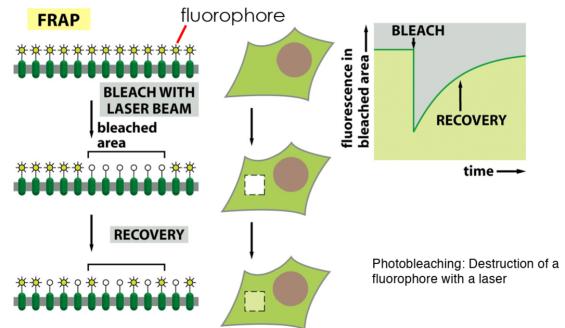
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# 1 Membrane Structure

## 1.1 Introduction to Cell Membranes



(a) membrane structure



(b) sigmoid binding curve

Figure 1: Membrane structure and fluid property

Cell membranes consist of a **lipid bi-layer** and various membrane proteins. This relatively simple structure has very important functions such as **protecting the cytosol** and the chemistry of the cell from the outside. This is very important as it allows the cell to have a different chemical environment than the outside. The cell is also **membrane is a liquid** which can be seen by **FRAP** experiments. The frap experiment works as follows:

- i) tag membrane with GFP
- ii) shoot powerful laser that bleaches the fluorescent protein (stops being green)
- iii) observe how the affected area recovers. How fast the area starts glowing again gives info on the diffusion constant and by extent the mobility of the tagged molecules.

This **liquid property can be used by the cell to deform the membrane at will**.

## 1.2 lipid Composition of Cell Membranes

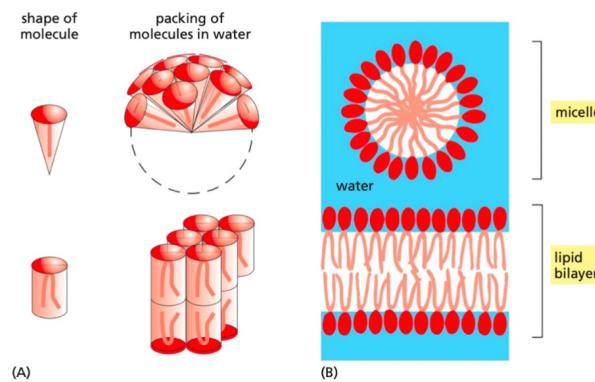


Figure 2: spontaneous formation of lipid bi layer

Phospholipids make up majority of lipids in the membrane. Phospholipids are **amphiphilic**. They have a hydrophilic head and hydrophobic tail. The lipid structure that forms depends to a very large extent on the 3D structure of the lipid. If the lipid only has 1 hydrophobic tail it will form globules called **micelles** however if it has 2 it will form a **bi-layer**. This lipid bi-layer has the unique property that it **will self seal into a closed membrane** as this is the most energetically favorable state.

*Remark 1.1.* The property of the molecule two seal off means that if pierced it will reform giving it the **ability of self repair**

### 1.2.1 types of lipids in membrane

The lipids in a cell membrane are very diverse but can be divided into 3 classes: phospholipids, glycolipids and sterols.

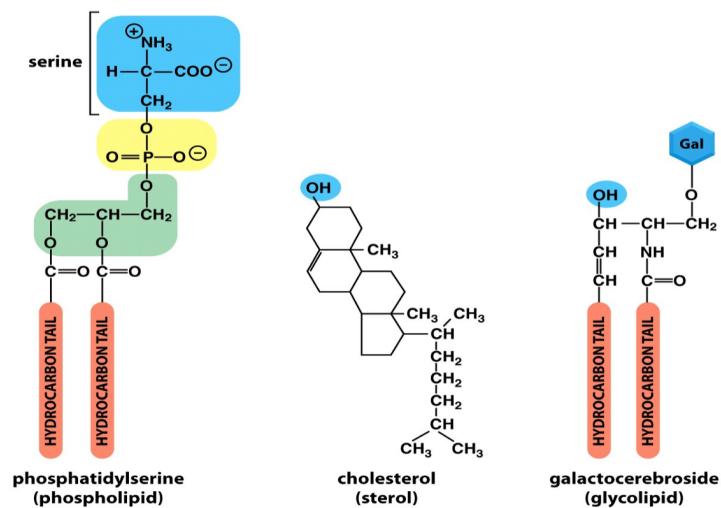
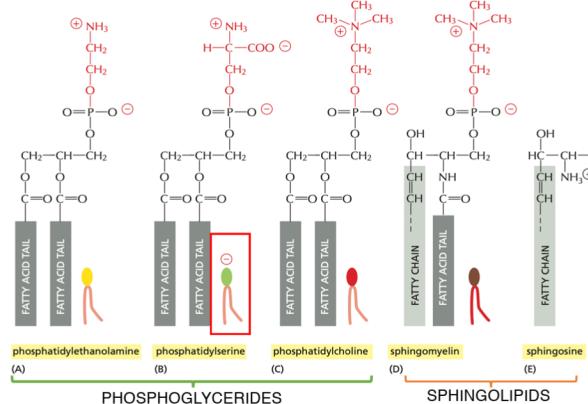
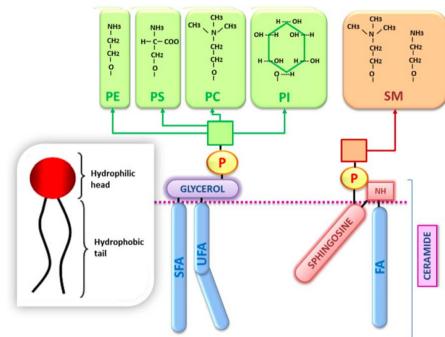


Figure 3: 3 main classes of lipids

### 1.2.1.1 phospholipids



(a) 4 main phospholipids



(b) phospholipids vs sphingolipids

Figure 4: PI can be phosphorylated based on location

the main lipids in a membrane are phospholipids. These consist of a glycerol backbone and a phosphate attached. There are four main phospholipids in our cells are:

- i) phosphatidylethanolamine (PE)
- ii) phosphatidylserine (PS)
- iii) phosphatidylcholine (PC)
- iv) sphingomyelin
- v) sphingosine

These can be further divided into **Phosphoglycerols** and **Sphingolipids**. Each of these have a different backbone where phosphoglycerols have a **glycerol** backbone and sphinolipids have a **sphingosine** backbone plus a **ceramide**. Sphingolipids can be modified in two different ways, they can be modified into **glycosphingolipids** or **phosphosphingolipids**.

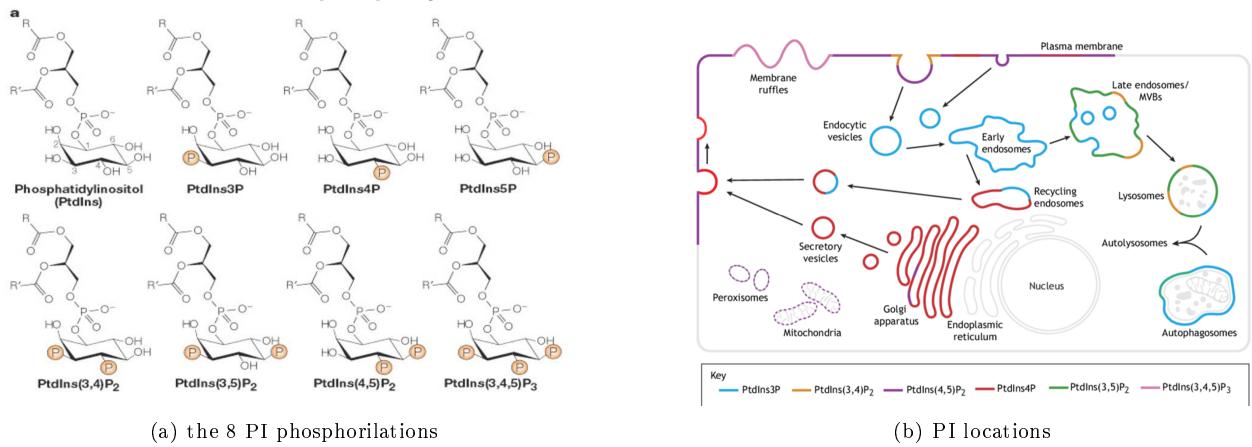


Figure 5: PI can be phosphorylated based on location

**Phosphatidylinosito (PI)** Phosphatidylinositol (PI) is a special phospholipid that is involved in cell signaling it is not one of the main phospholipids but is still important. **These lipids are located on the intracellular leaflet (facing inside the cell)** This can then be used for localization by interaction with various proteins that bind to or phosphorylate PI. (see chapter on cellular localization)

*Remark 1.2. PI are not a sugar but an alcohol*

#### 1.2.1.2 glycolipids

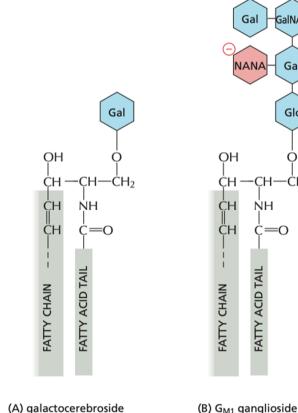


Figure 6: glycolipid structure

Glycolipids are lipids that have been modified by adding a sugar. This modification can be sequential meaning that there can be more than one sugar added to form very complex R-groups. **Glycolipids are on the outside of the cell membrane**

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#### 1.2.1.3 sterols

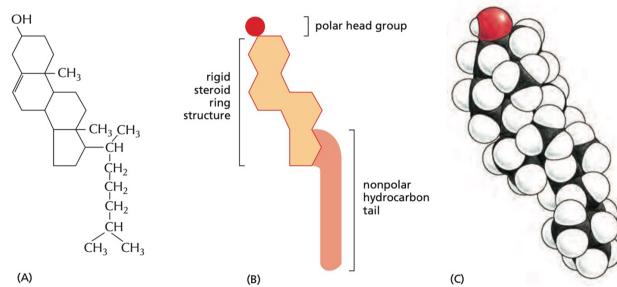


Figure 7: sterols structure

This class of lipids consist of of a **rigid ring structure** and a **polar head group**. The posterboy for this group is **cholesterol**, which incorporates in the membrane to change it's fluidity by incorporating between phospholipids thereby stiffening the membrane.

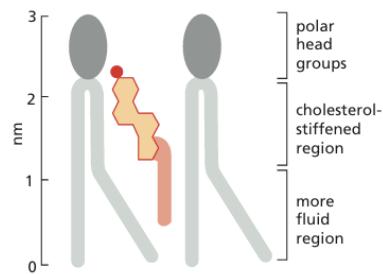


Figure 8: cholesterol stiffens the membrane by incorporating itself between two phospholipids

### 1.2.2 lipid composition of common cells

TABLE 10-1 Approximate Lipid Compositions of Different Cell Membranes

Lipid	Percentage of total lipid by weight					
	Liver cell plasma membrane	Red blood cell plasma membrane	Myelin	Mitochondrion (inner and outer membranes)	Endoplasmic reticulum	<i>E. coli</i> bacterium
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	28	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	44	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	14	8	23	27	30

Figure 9: table of lipid composition and it's cell type variation

### 1.3 Properties of Cell Membranes are dynamic

lipids are incredibly diverse set of molecules that are made in an equally complex process. This diversity gives the cell many mechanisms to influence the membranes properties.

#### 1.3.1 saturated vs unsaturated and tail length

The **amount of cis-double bonds** affects membrane stiffness. The higher the amount of cis Double bonds the harder it is to pack the molecules together making the membrane more fluid.

Another way to affect fluidity is by the length of the fatty acid tails. the longer the tail the stronger the Van der waals forces, thus making the membrane less fluid.

#### 1.3.2 Temperatures effect on the membrane

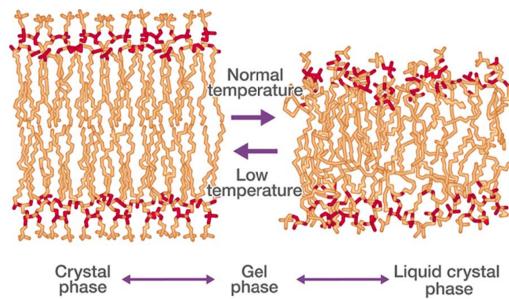


Figure 10: temperature on the membrane rigidity

The temperture an organism is exposed to impacts it's rigidity. Thus the saturation/ length of fatty acids can be used to adapt to the enviroment. **This means that animals living in cold have different membrane compositions**

## 1.4 Movement of Lipids in Cell Membranes

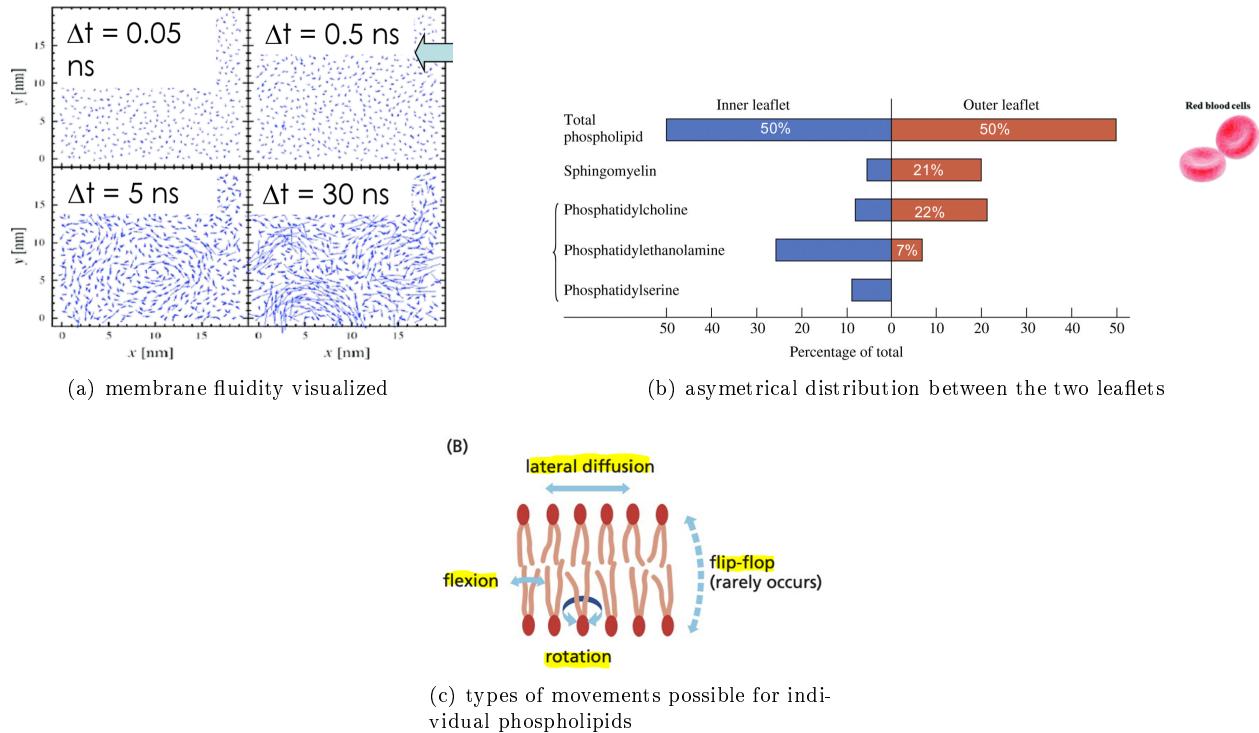


Figure 11: membrane fluidity and asymmetry

Membranes are very mobile. The lipids rapidly diffuse and move around on both of the **leaflets**. They tend to move together and form wave like patterns much like the ocean (albeit a kinda gross and fatty ocean)

The lipids **rarely flip between the leaflets**. This means that there is an **asymmetric distribution of various lipids between the inner and outer leaflet as they are produced at different locations**. The cells has enzymes called **flipases** that move the cells from one leaflet to another. The other type of enzyme are called **scramblases** that indiscriminately move lipids around between the leaflets

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## 1.5 Lipid Rafts

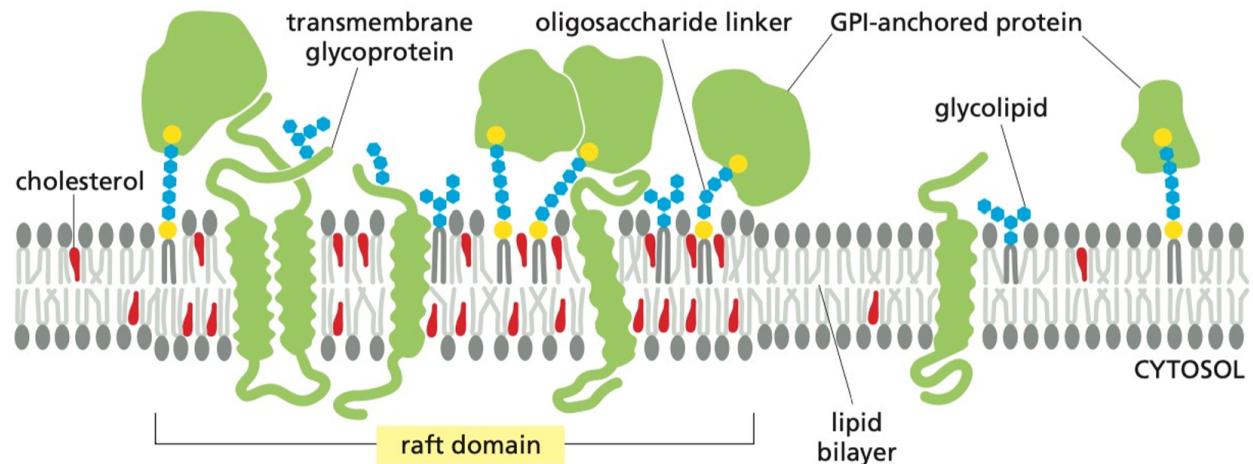


Figure 12: raft domains

**Lateral Phase Separation** is a phenomenon where sphingomyelin and cholesterol seem to group together into larger units called **Lipid rafts**. This phenomenon appears *in vivo* but to a much smaller extent than in artificial membranes. These domains have been theorised to play a role in cell localisation, where the difference in thickness will force larger transmembrane helices to go to these rafts domains.

## 2 membrane proteins

### 2.1 protein basic overview

#### 2.1.1 amino acid structures

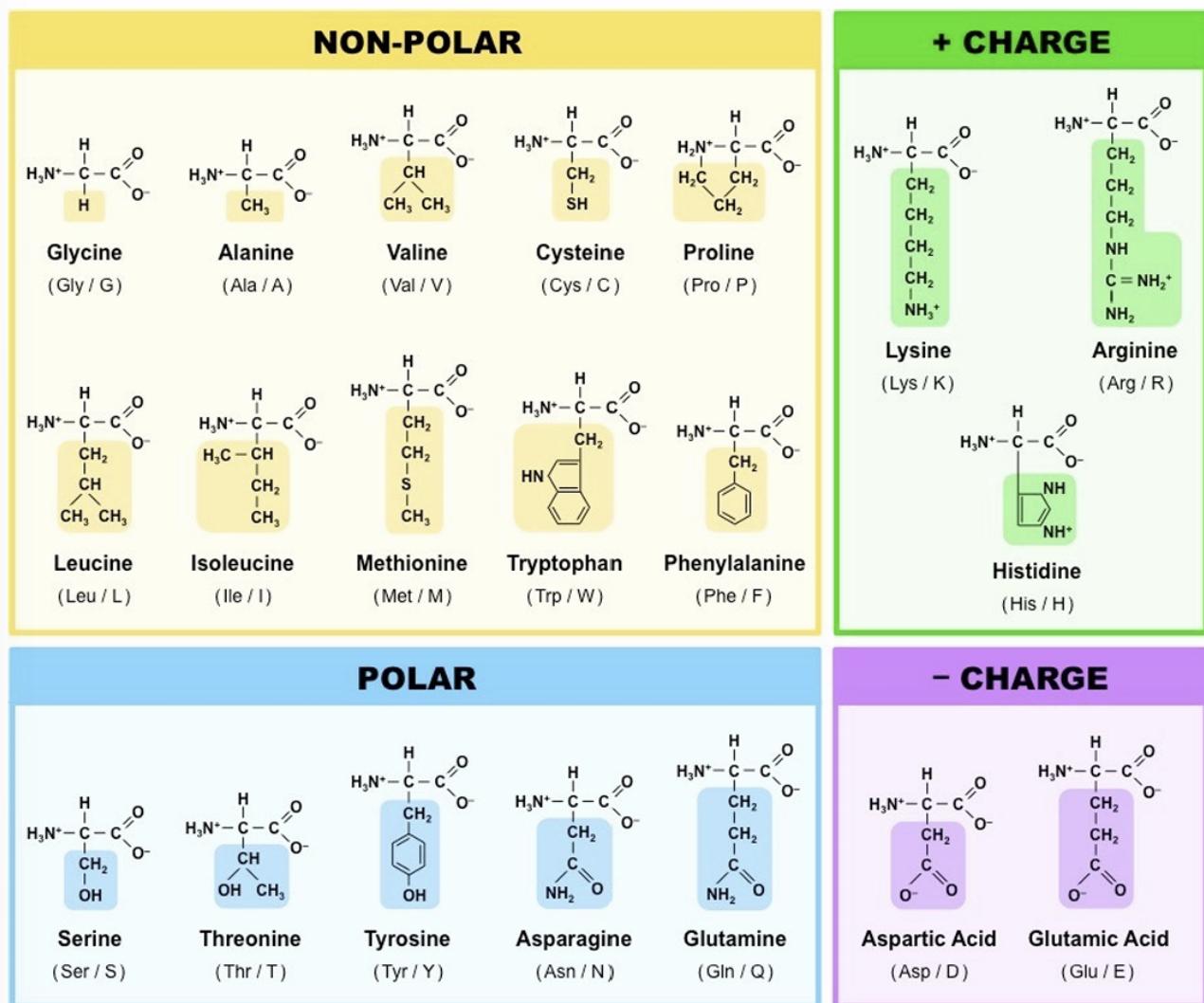


Figure 13: Amino acid structure

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### 2.1.2 amino acid hydrophobicity scores

Amino Acid	3-Letter	1-Letter	Hydrophobicity / Hydropathy Index	Polarity	Acidity (pH)
Alanine	Ala	A	1.8	Nonpolar	Neutral
Arginine	Arg	R	-4.5	Polar	Basic (Strongly)
Asparagine	Asn	N	-3.5	Polar	Neutral
Aspartate (Aspartic acid)	Asp	D	-3.5	Polar	Acidic
Cysteine	Cys	C	2.5	Polar	Neutral
Glutamate (Glutamic acid)	Glu	E	-3.5	Polar	Acidic
Glutamine	Gln	Q	-3.5	Polar	Neutral
Glycine	Gly	G	-0.4	Nonpolar	Neutral
Histidine	His	H	-3.2	Polar	Basic (Weakly)
Isoleucine	Ile	I	4.5	Nonpolar	Neutral
Leucine	Leu	L	3.8	Nonpolar	Neutral
Lysine	Lys	K	-3.9	Polar	Basic
Methionine	Met	M	1.9	Nonpolar	Neutral
Phenylalanine	Phe	F	2.8	Nonpolar	Neutral
Proline	Pro	P	-1.6	Nonpolar	Neutral
Serine	Ser	S	-0.8	Polar	Neutral
Threonine	Thr	T	-0.7	Polar	Neutral
Tryptophan	Trp	W	-0.9	Nonpolar	Neutral
Tyrosine	Tyr	Y	-1.3	Polar	Neutral
Valine	Val	V	4.2	Nonpolar	Neutral

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Table 1: hydrophobicity scores Amino acids

## 2.2 membrane embedding

Membrane proteins can be 1 of many different forms but in general they can be divided into: **Lipid anchors** or **transmembrane proteins**. Membrane proteins face key challenges when folding compared to soluble proteins as they have to **expose hydrophobic residues** as opposed to the usual hydrophilic collapse. This means they often need chaperone proteins to help them fold. (from bio last year fyi)

### 2.2.1 transmembrane proteins

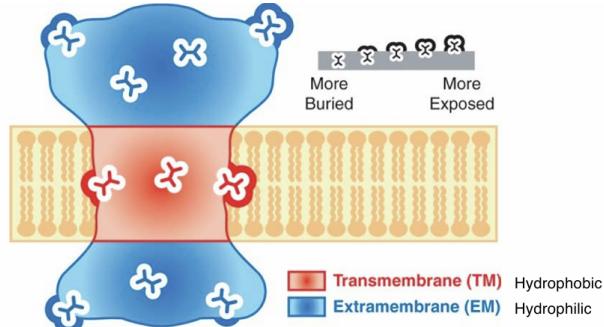


Figure 14: general structural requirements of a membrane protein

Transmembrane proteins need to be **Amphiphilic** in nature. This is needed as the membrane passing domain needs to be hydrophobic, however the domains not embedded in the membrane are exposed to water and need to be hydrophilic. Transmembrane protein will **contain either alpha helices or beta sheets but not both**. This means we can divide them into two classes: transmembrane helix and beta barrels.

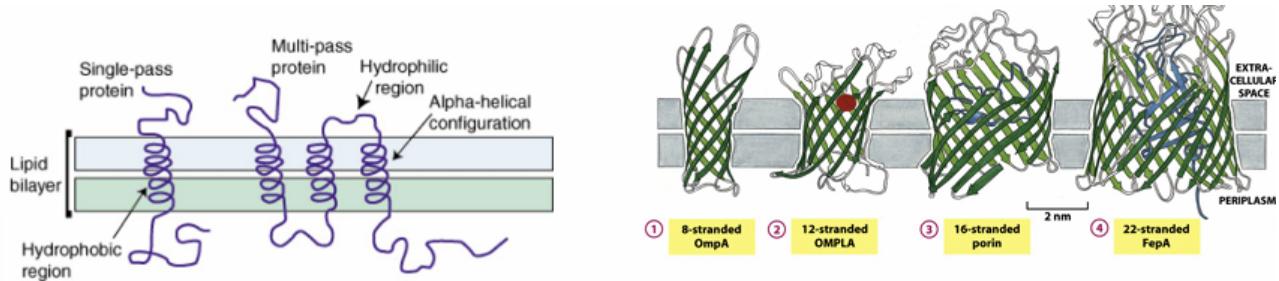


Figure 15: beta barrels vs transmembrane helix

#### 2.2.1.1 transmembrane helix

Transmembrane helices consist of alpha-helices that have **exclusively hydrophobic residues** since all residues in the alpha-helix are exposed to the membrane. This allows them to pass through the membrane. An alpha helix has **3.6 Å per turn** and each **turn is 5.4 Å long**. This means that a helix passing through the membrane which is around 3nm this will take 20 amino acids perpendicularly. However a helix does not have to cross perpendicularly so its size can vary. Also note that the **membrane thickness varies and these fluctuations may have a role in localization**. In general membrane proteins are **asymmetric**. There are always exceptions: It is possible to have a charged a.i. in one transmembrane helix, forming for example an ionic interaction with another charged a.i. of another helix.

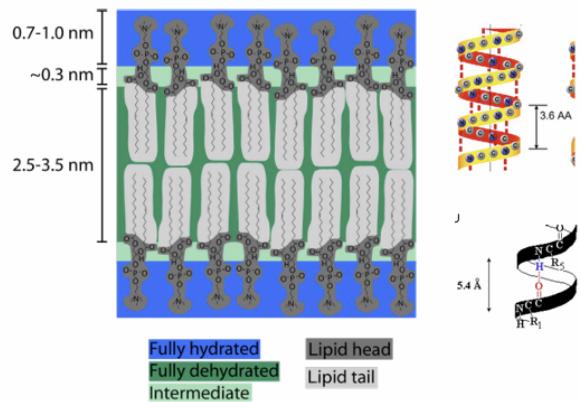


Figure 16: helix stats

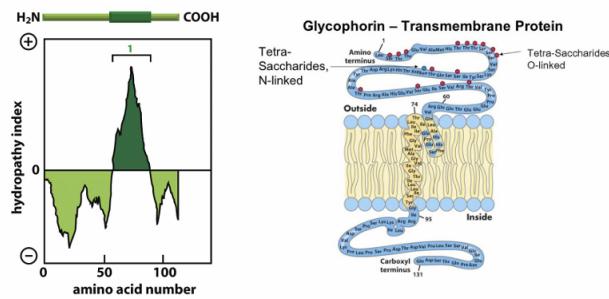


Figure 17: Predicting Transmembrane helices based on hyrophobicity score

**predicting transmembrane helices** It is possible to predict transmembrane helices off of the **Hydrophobicity Score** which is an average of the  $\pm 9$  residues from the one being measured. This is important as it gives an overall estimate of the local hydrophobicity of this part of the protein. the **window is chosen to be 19 as around 20AA is needed to cross the membrane.**

#### 2.2.1.2 beta barrels

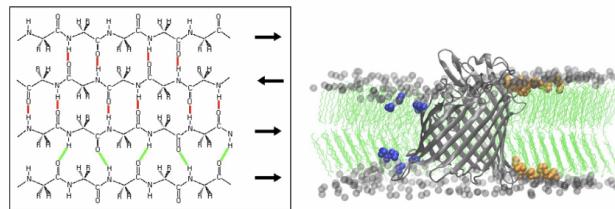


Figure 18: Beta Barrel

B-Barrels are made out of B sheets which are made from B Strands. The number of **B strands vary from 8 to 200**. Recall that these strands can form parallel or antiparallel sheets.

Beta strands are quite different compared to transmembrane helices. Since the beta sheet has two sides **only the residues facing the membrane need to be hydrophobic, making their prediction based off of hydrophobicity index next to impossible**. Transmembrane proteins consisting of beta strands take up a beta barrel shape. where one side of the strand has hydrophobic residues on the outside while the other side of the strand has hydrophilic residues. These then fold to form a barrel hence beta barrel.

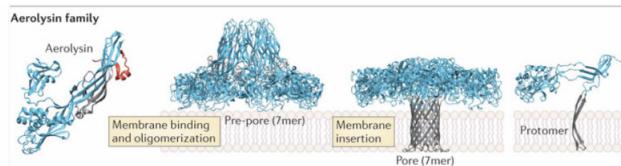


Figure 19: membrane attack complex

**A cool side note: The membrane attack complex** A rather cool protein of this group is the so called membrane attack complex which is giant protein that shoves it'self in the membrane and then assembles into beta barrel thereby making a huge hole. This kills the cell and is used among other things to kill bacteria and tumor cells.

### 2.2.2 lipid anchors

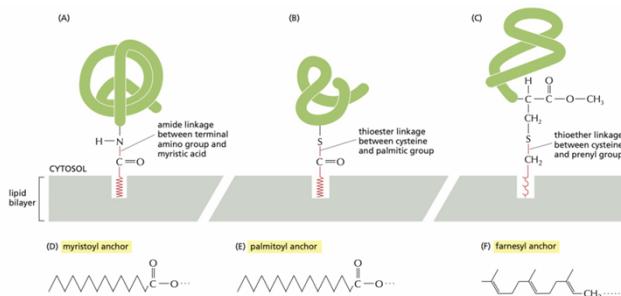


Figure 20: 3 main types of lipid anchors

Lipid anchors are covalent lipid additions to proteins, helping them attach to membranes.

Lipid anchors serve to hold part of a protein in place on the membrane. **Most of the time the anchor is on the inside of the cell** They are also important for membrane localization. There are 3 main types of lipid anchors:

- myristoyl anchor
- palmitoyl anchor (this is the **only reversible lipidic modification**)
- farnesyl anchor

### 2.2.2.1 special case: GPI anchor

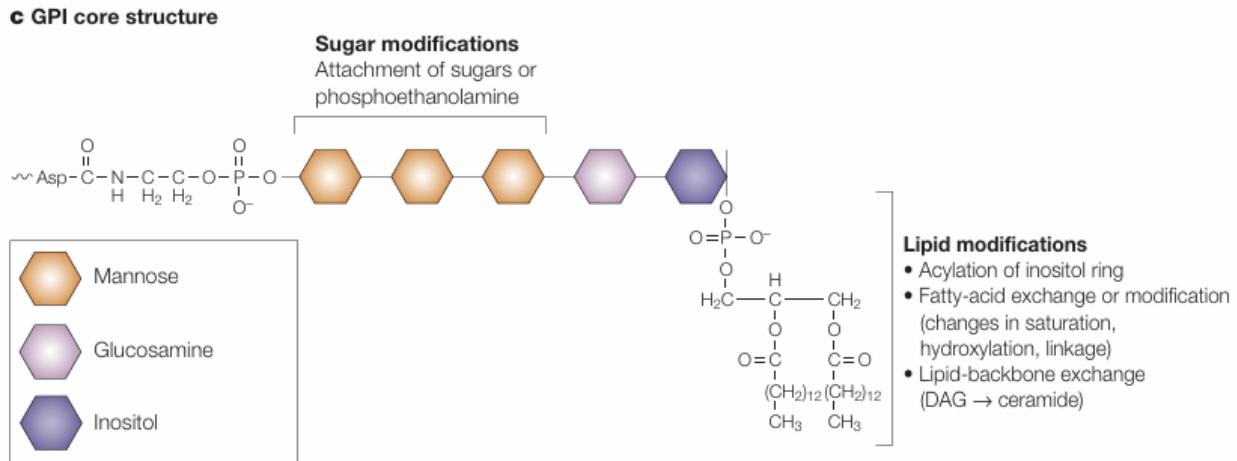


Figure 21: GPI anchor structure

The GPI anchor is special as it is actually on the **outside of the cell** even though PI usually is on the cytosolic side!

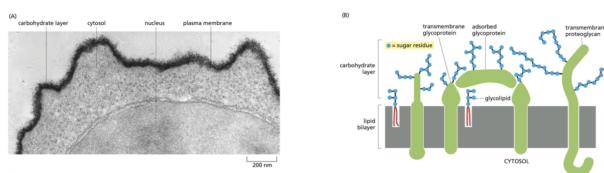


Figure 22: The carbohydrate layer of the cell membrane

**the carbohydrate layer of the cell** The carbohydrate layer of the cell is a critical structure on the outer surface of the plasma membrane. It is a sugar-rich coating on the outer surface of animal cells. It consists of glycoproteins, glycolipids, and proteoglycans, and **plays roles in protection, recognition, and adhesion**.

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## 2.3 membrane protein isolation

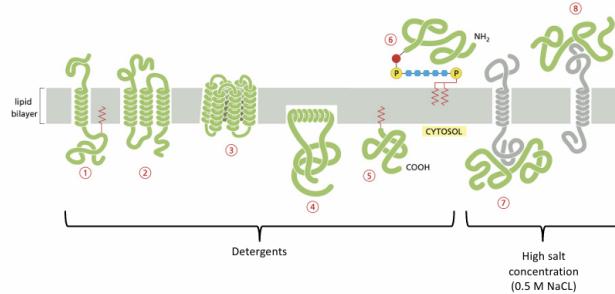


Figure 23: isolation of membrane proteins

The figure shows the following:

- i) single pass alpha helix
- ii) multipass alpha helix
- iii) Beta-barrel
- iv) alpha helix partitioned in the cytosolic monolayer of the lipid
- v) covalently linked to a lipid
- vi) anchored to GPI on the outside
- vii) non covalent binding to another protein
- viii) non covalent binding to another protein

In general detergents are needed to isolate membrane proteins but if they are non covalently bound to a protein in the membrane these can be detached with high salt concentrations.

### 2.3.1 detergents

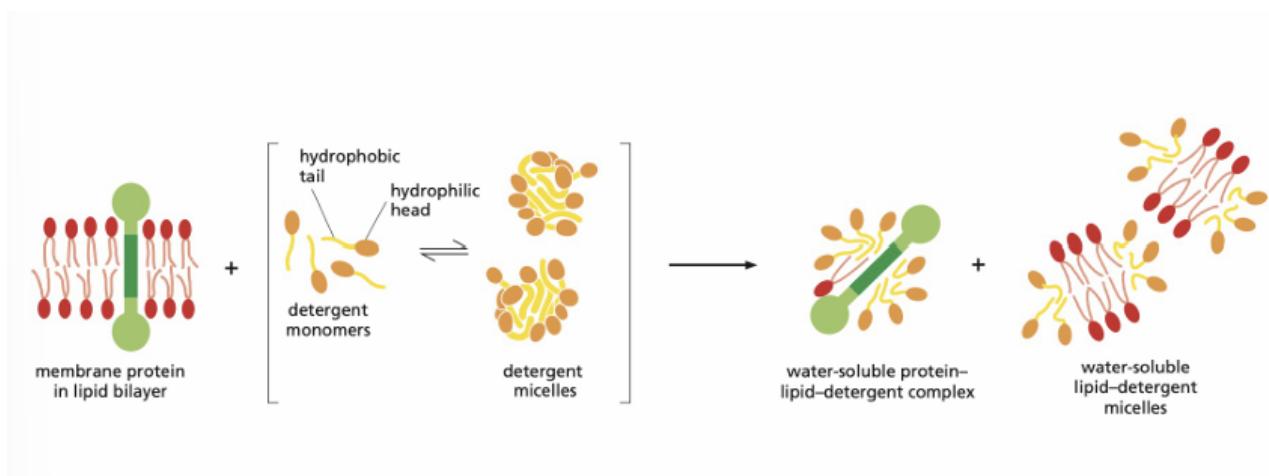


Figure 24: Detergents function

Detergents are aphiphilic molecules that help solubilize membrane proteins, forming micelles. The ones seen in class are:

- SDS (Sodium Dodecyl Sulfate)** The negative charge will denature them though
- Triton X-100** this detergent is less harsh than sds so will not denature the proteins. This is called **Soft Solubilization** and is useful when you want to isolate the protein in functional conformation. It has a smaller nonpolar portion and a polar but uncharged end, which allows it to mimic the solvation effect of lipids.

*Remark 2.1.* Note that the micelles formed are often irregularly shaped due to packing constraints

### 2.3.2 nanodiscs

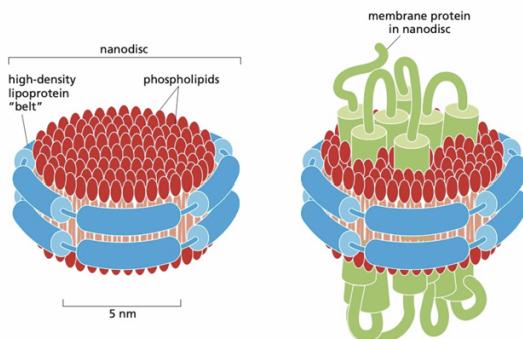


Figure 25: Nanodisks

Another cool method of isolating membrane proteins is to put them on so called **nanodisks**. These are essentially tiny membrane pieces that are held together by a lipoprotein belt.

Proteins are incorporated into nanodisks by mixing them with scaffold proteins and lipids in detergent, then removing the detergent to allow spontaneous assembly of the lipid bilayer around the protein.

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## 2.4 membrane protein localization

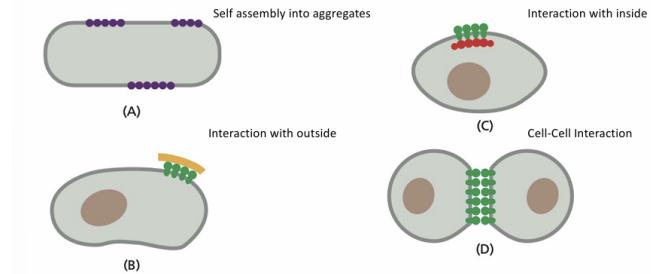


Figure 26: membrane protein localization mechanisms

The cell membrane is very fluid and dynamic however membrane proteins need to be kept at certain places of the cell. This is essential for survival as the cell depends on having the right proteins at the right place. To do this it has 4 methods for restricting lateral mobility of specific membrane proteins:

- i) self assembly into aggregates. These can then form specific domains
- ii) interaction with outside
- iii) interaction with inside
- iv) cell cell interactions

The **membrane proteins can also affect how the membrane bends**

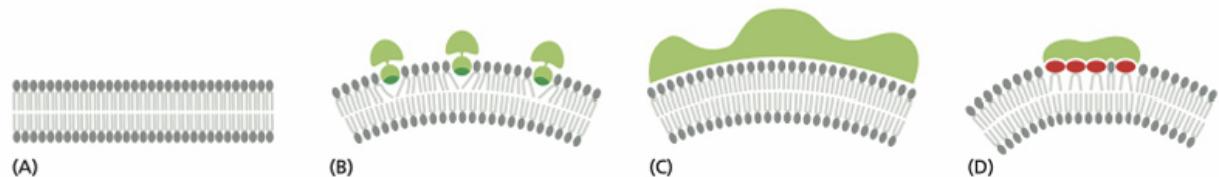


Figure 27: membrane protein bending

this can be achieved by (b)wedging themselves in the membrane, (c)by physically pulling on the membrane, or (d) by binding to lipids with large head groups and stabilizing the curvature of the membrane

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#### 2.4.1 special case: Restriction by the cytoskeleton (spectrin- based)

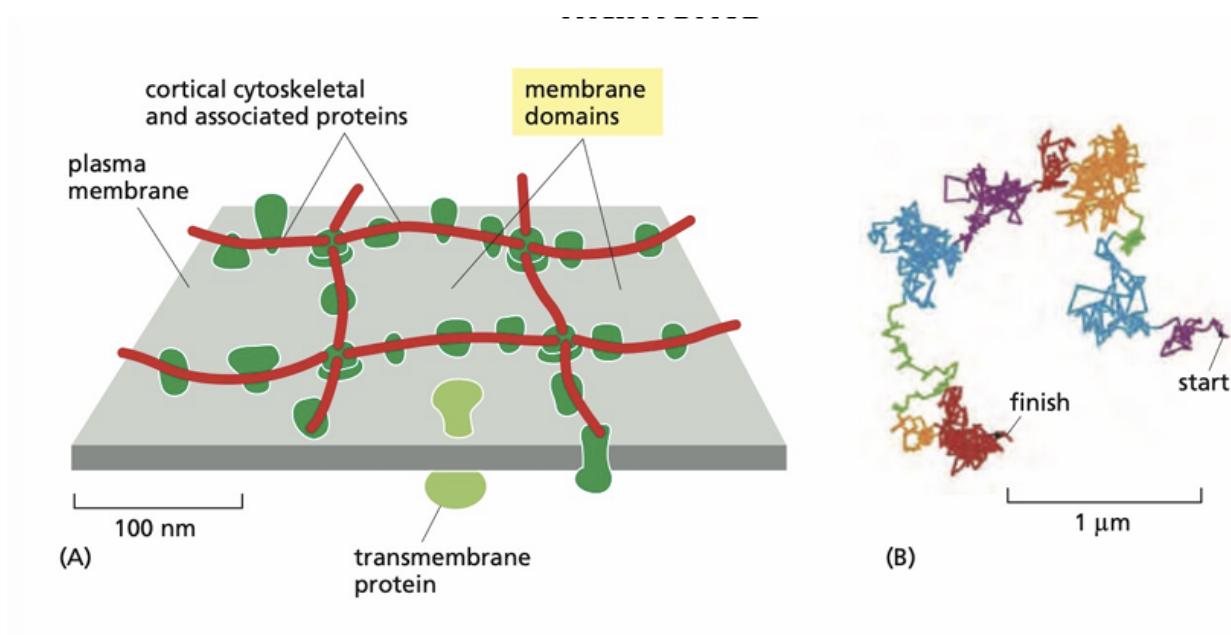


Figure 28: Spectrin corralling plasma membranes

A rather special case of membrane localization is that of **spectrin** which is primarily found in red blood cells. This protein acts like a litteral fence therby corralling off certain domains on the plasma membrane and ensureing that the proteins inside stay in a certain area of the membrane. Kinda like sheep just chilling in a field.

### 3 Membrane Transport

Because of its hydrophobic interior, the lipid bilayer of cell membranes restricts the passage of most polar molecules. This barrier function allows the cell to maintain concentrations of solutes in its cytosol that differ from those in the extracellular fluid.

Note that **given enough time**, virtually any molecule will diffuse across a protein-free lipid bilayer down its concentration gradient. In general there are 2 properties that determine the permeability for a molecule: Its **size and hydrophobicity**.

Nevertheless these rates are pretty shit. Therefore in order to benefit from this barrier cells have had to evolve ways of transferring specific molecules and ions across their membranes in order to ingest essential nutrients, excrete metabolic waste products, and regulate intracellular ion concentrations. Cells use specialized membrane transport proteins to accomplish this goal.

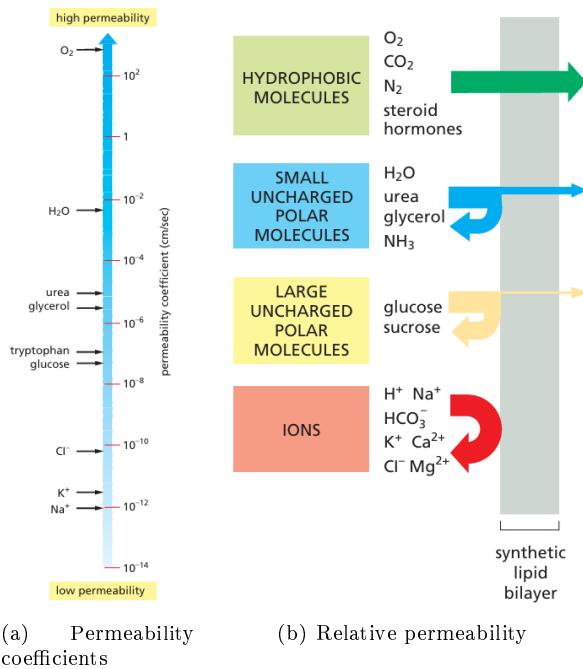


Figure 29: Permeability of the cellular membrane

**Remark 3.1 (Transporters vs Channels).** There are 2 main classes of **membrane transport proteins**: **Transport Proteins** bind the **specific solute** to be transported and undergo a series of conformational changes that alternately expose solute-binding sites on one side of the membrane and then on the other to transfer the solute across it.

**Channels**, by contrast, interact with the solute to be transported much more weakly (no conformational changes). They form continuous pores that extend across the lipid bilayer.

Not surprisingly transport through channels occurs at a **much faster rate** than transport mediated by transporters.

**Remark 3.2 (Active vs Passive Transport).** All channels and many transporters allow solutes to cross the membrane only passively ("downhill"), this is called **passive transport**. In this case of an uncharged molecule the driving force is the concentration gradient while charged molecules are also influenced by the membrane potential (electrochemical gradient).

There are also transporters that transport "uphill", against the electrochemical gradient. Such **active transport** is mediated by transporters whose pumping activity is directional because it is tightly coupled to a source of metabolic energy, such as an ion gradient or ATP hydrolysis.

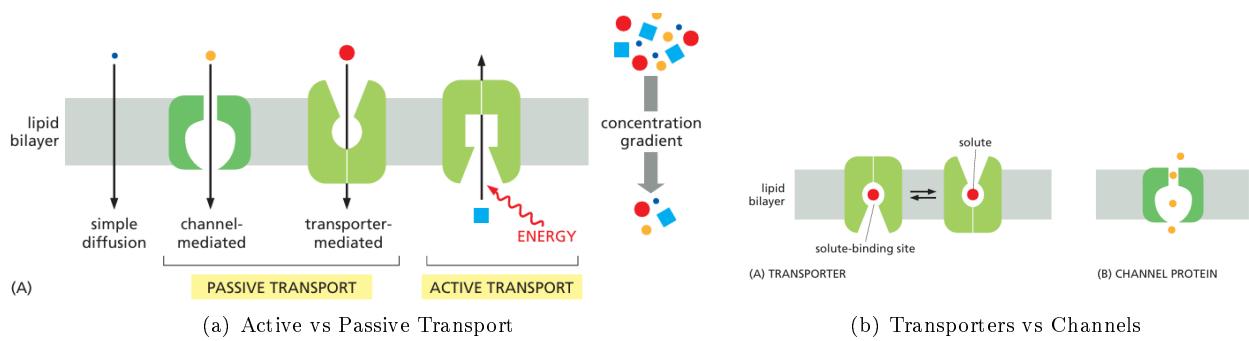


Figure 30: Transport Membrane Proteins

### 3.0.1 Ion concentrations

The barrier function of the cell membrane allows the cell to maintain concentrations of solutes in its cytosol that differ from those in the extracellular fluid. **The cell membrane is particularly impermeable to ions.** Note that a cell must contain equal quantities of positive and negative charges (**neutral**). The cell contains many other anions not listed in table 2 like inorganic phosphate, nucleic acids, etc.

**Definition 3.3 (Electrochemical gradient).** *The concentration gradient and the electrical potential difference across the membrane combine to form a **net driving force** the electrochemical gradient.*

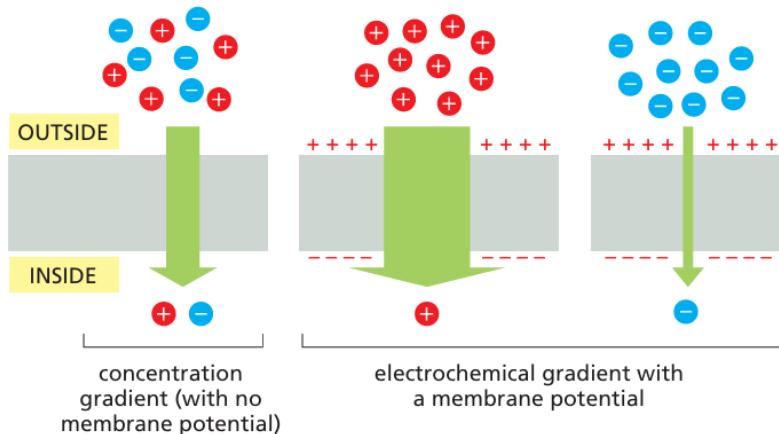


Figure 31: The electrochemical gradient of a charged solute (an ion) affects its transport

The equilibrium potential for ion  $i$  is given by the Nernst equation:

$$E_i = \frac{RT}{zF} \ln \left( \frac{[ion]_{\text{outside}}}{[ion]_{\text{inside}}} \right)$$

Note that the **resting membrane potential is around -70 mV =  $V_m$** . Using this one can calculate the driving force for an **cation** across the membrane (att. for neg charge switch).

$$\Delta E_i = E_i - V_m$$

- $\Delta E_i > 0$ : The ion will move **inward** (from outside to inside the cell),

Ion	Concentration Inside the Cell (mM)	Concentration Outside the Cell (mM)	Equilibrium Potential ( $E_i$ ) (mV)	Direction of Movement
$\text{Na}^+$	5–15	145	+60	Inward (strong chemical and electrical gradients)
$\text{K}^+$	140	5	-90	Outward but opposing forces <b>nearly balanced</b> , near equilibrium
$\text{Ca}^{2+}$	$10^{-4}$	1–2	+120	Inward (very strong chemical and electrical gradients)
$\text{Mg}^{2+}$	0.5	1–2	-10 to -20	Outward or near equilibrium (small gradient)
$\text{H}^+$	$7 \times 10^{-5}$ ( $10^{-7.2}$ M or pH 7.2)	$4 \times 10^{-5}$ ( $10^{-7.4}$ M or pH 7.4)	Varies with pH	Varies (affects pH balance, weak gradient under normal conditions)
$\text{Cl}^-$	5–15	110	-70 to -80	Inward but opposing forces <b>nearly balanced</b> , near equilibrium

Table 2: Ion Concentrations and Equilibrium Potentials

- $\Delta E_i < 0$ : The ion will move **outward** (from inside to outside the cell),
- $\Delta E_i = 0$ : There is no net movement of that ion (the ion is at equilibrium).

Note that the movement of only a minute number of inorganic ions across the plasma membrane through ion channels suffices to set up the membrane potential. Thus, we can think of the membrane potential as arising from movements of charge that leave ion concentrations practically unaffected.

**Example 3.4 (Acetylcholine-gated cation channels do not discriminatie between  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{Ca}^{2+}$ . But when they open mostly  $\text{Na}^+$  enters the cell.).** There is little net movement of  $\text{K}^+$  because it is nearly at equilibrium distribution, by contrast  $\text{Na}^+$  and  $\text{Ca}^{2+}$  are not at equilibrium distribution. However  $\text{Ca}^{2+}$  is present in way lower concentration then  $\text{Na}^+$ . Therefore manly  $\text{Na}^+$  enters the cell.

### 3.1 Transporters

Transporters are typically built from bundles of **10 or more  $\alpha$  helices** that span the membrane. **Solute-and ion-binding sites are located midway through the membrane**, where some helices are broken or distorted and amino acid side chains form ion- and solute-binding sites.

In the inward-open and outward-open conformations, these binding sites are accessible by passageways from one side of the membrane but not the other. The switching between the two conformations. The switching between these two (3) states transfers the solute from one side to the other. See fig. 33(a)

Moreover Transporters are built from inverted repeats. This leads to the fact that the repeats can move relative to each other. Therefore opening one side leads to the closing of the other.

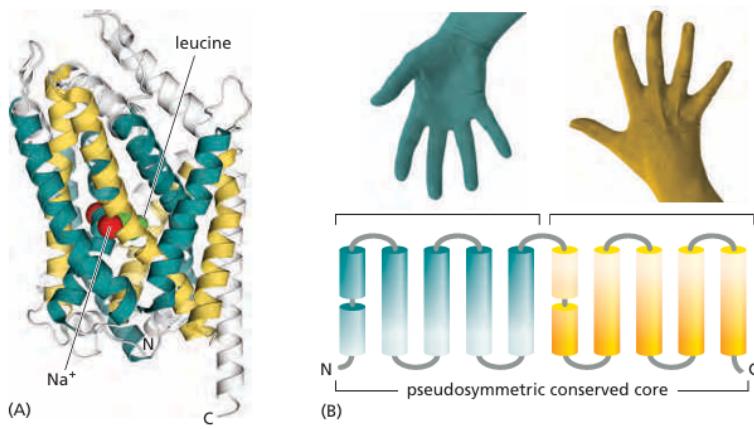


Figure 32: Transporters are built from inverted repeats.

In many ways **Transporters behave like enzymes**. Each type of transporter has one or more **specific binding sites for its solute**. Moreover, when the transporter is saturated, the rate of transport is maximal ( $V_{max}$ ), is characteristic of a specific carrier. In addition, each transporter has a characteristic affinity for its solute, reflected in the  **$K_m$**  of the reaction, which is equal to the concentration of solute when the transport rate is half its maximum value. There can also be an interplay with an inhibitor. See fig. 33(b)

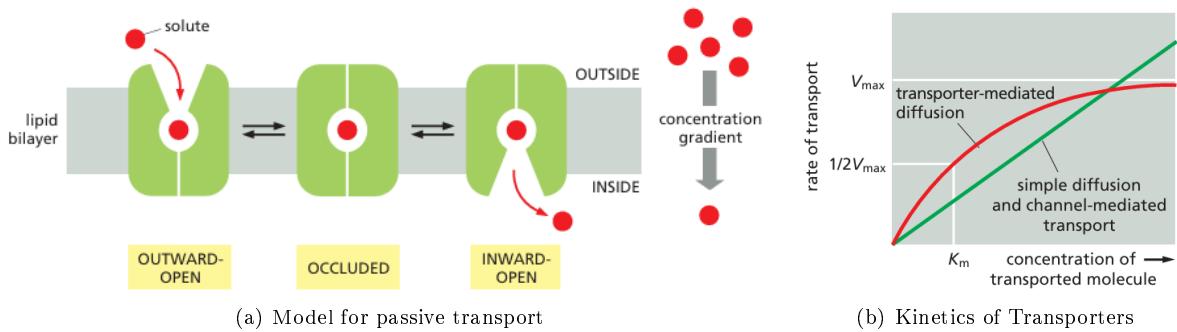


Figure 33:

Apart from passive transport, transporters can also engage in active transport. There are strong similarities in structure between transporters that mediate active transport and those that mediate passive transport. This suggests an evolutionary relationship.

There are 3 main ways of driving active transport:

- **Coupled transporters** harness the energy stored in concentration gradients to couple the uphill transport of one solute across the membrane to the downhill transport of another.
- **ATP-driven pumps** couple uphill transport to the hydrolysis of ATP
- Light- or redox-driven pumps, which are known in bacteria, archaea, mitochondria, and chloroplasts, couple uphill transport to an input of energy from light.

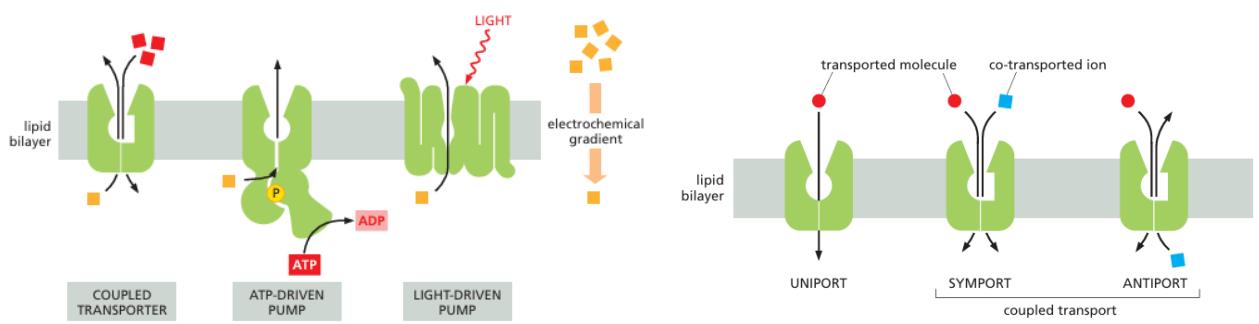


Figure 34:

### 3.1.1 Active Transport driven by Ion-Concentration Gradients

Some Transporters simply **passively** mediate the movement of a single solute from one side of the membrane; they are called **Uniporter**. See. Fig. 38(b)

Others function as **coupled transporters** (type of active transport), in which the transfer of one solute strictly depends on the transport of a second. In some the coupled transport is performed in the same direction (**Symporter**), while in others the transport is performed in oposite directions (**Antiporter**). See. Fig. 38(b)

The tight coupling between the transfer of two solutes allows the coupled transporters to harvest the energy stored in the electrochemical gradient of one solute, typically an inorganic ion, to transport the other.

**Na<sup>+</sup>** is the usual **co-transported ion** because its electrochemical gradient provides a large driving force for the active transport of a second molecule. The Na<sup>+</sup> that enters the cell during coupled transport is **subsequently pumped out by an ATP-driven Na<sup>+</sup>-K<sup>+</sup> pump** in the plasma membrane, which, by maintaining the Na<sup>+</sup> gradient, indirectly drives the coupled transport.

Such ion-driven transport is called **Secondary active transport**, while the ATP-driven pump are said to mediate **Primary active transport**

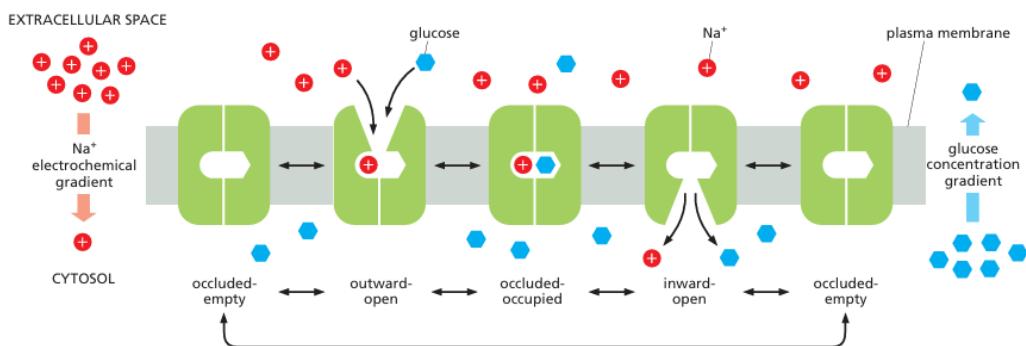


Figure 35: Mechanism of glucose transport fueled by a Na<sup>+</sup> gradient (SGLT family)

#### 3.1.1.1 Transcellular Transport

In epithelial cells, such as those that absorb nutrients from the gut, transporters are **distributed nonuniformly** in the plasma membrane and thereby contribute to the transcellular transport of absorbed solutes.

---

Transporters are evolutionarily placed where it makes sense for the cell.

$\text{Na}^+$ -linked symporters (SGLT1) located in the apical (absorptive) domain of the plasma membrane actively transport nutrients into the cell, building up substantial concentration gradients.

Uniporters in the basal and lateral (basolateral) domains allow the nutrients to leave the cell passively down these concentration gradients. (See fig. 36)

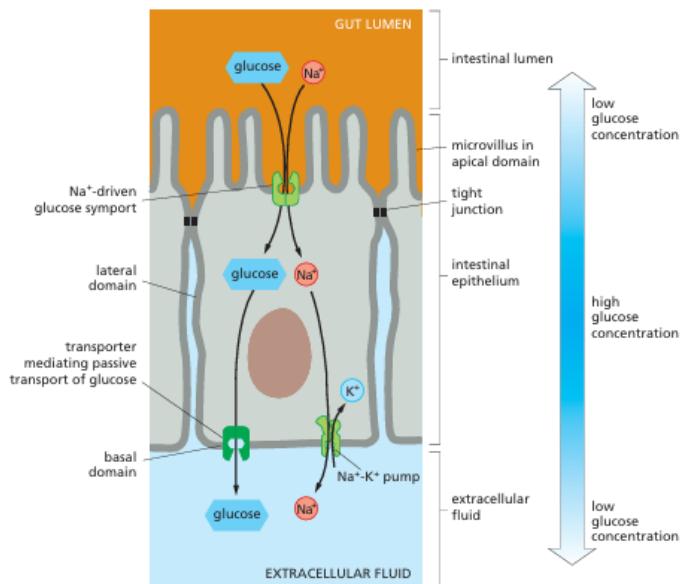


Figure 36: An Asymmetric Distribution of Transporters in Epithelial Cells Underlies the Transcellular Transport of Solutes

### 3.1.2 Active Transport by ATP-Driven Pumps

There are 3 classes of ATP driven pumps (also often called **transport ATPases** because the hydrolyze ATP to ADP).

- **P-type pump** pumps are structurally and functionally related **multipass transmembrane proteins**. They are called “P-type” because they **phosphorylate themselves** during the pumping cycle. This class includes many of the **ion pumps** that are responsible for setting up and maintaining gradients of  **$\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{H}^+$ , and  $\text{Ca}^{2+}$**  across cell membranes.
- **ABC transporter** (ATP-Binding Cassette transporters) differ structurally from P-type ATPases and primarily pump **small molecules** across cell membranes.
- **V-type pump** pumps are turbine-like protein machines, constructed from **multiple different subunits**. The V-type proton pump transfers  **$\text{H}^+$  into organelles**.

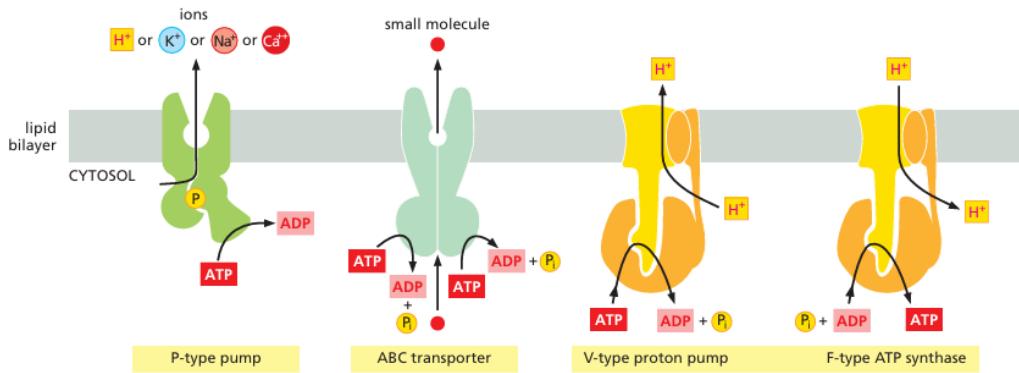


Figure 37: Three types of aTP-driven pumps.

### 3.1.2.1 $\text{Na}^+/\text{K}^+$ pump

The  $\text{Na}^+/\text{K}^+$  ATPase is a **ATP-driven antiporter P-type ATPase**. It maintains the  **$\text{Na}^+$  gradient** important for the transport of ntirens into the cells (**osmotic balance**). The importance is underlined by the fact that 1/3 of the cells energy is devoted to this pump.

Since the  $\text{Na}^+/\text{K}^+$  pump drives three positively charged ions out of the cell for every two it pumps in, it is **electrogenic**: it drives a net electric current across the membrane. This corresponds to about 10 % of the membrane potential.

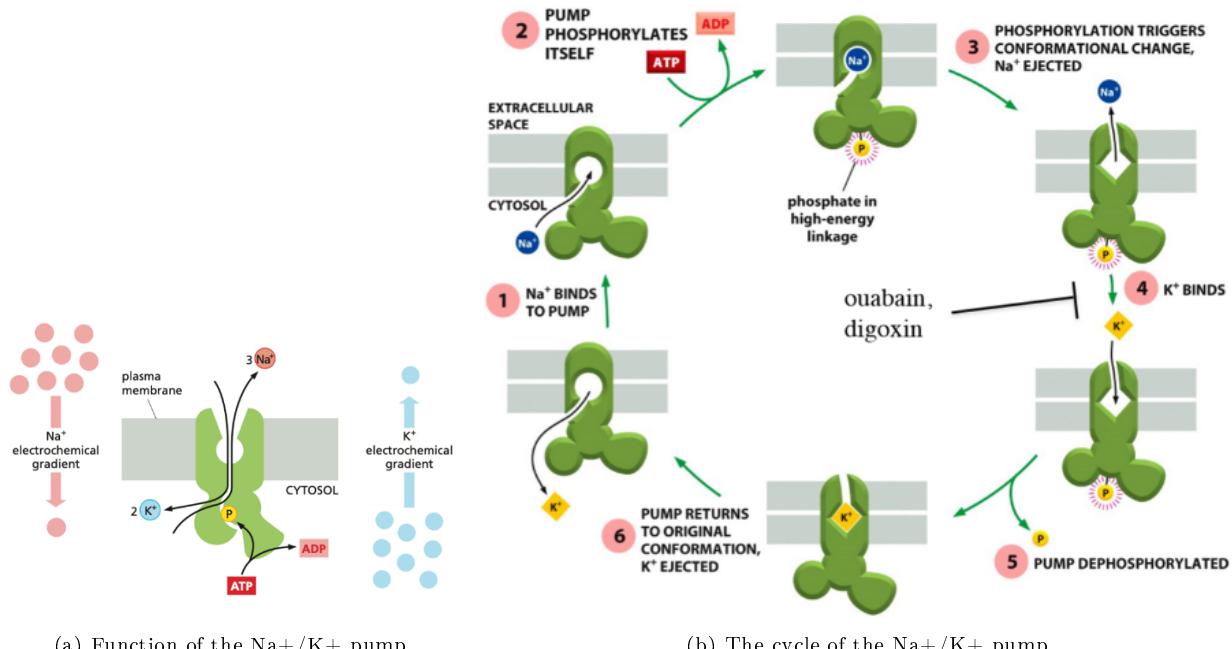


Figure 38:  $\text{Na}^+/\text{K}^+$  pump

**Remark 3.5 (Osmolarity).** **Osmolarity** is a measure of the total concentration of solute particles in a solution. It determines the direction of water movement across membranes: water tends to **move from areas of lower to higher osmolarity**. In cells, the  $\text{Na}^+/\text{K}^+$  pump helps regulate osmolarity by exporting more ions than

it imports, thereby reducing intracellular solute concentration and helping to prevent excessive water entry. This regulation is essential for maintaining cell volume and structure, keeping the cell **Isotonic** rather than **Hypertonic** or **Hypotonic**.

### 3.1.2.2 Ca<sup>2+</sup> pump

The Ca<sup>2+</sup> pump, or Ca<sup>2+</sup> ATPase, in the sarcoplasmic reticulum (SR) membrane of skeletal muscle cells is a well-understood **P-type transport ATPase**.

*Remark 3.6 (sarcoplasmic reticulum (SR)).* The SR is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytoplasm, and it serves as an **intracellular store of Ca<sup>2+</sup>**.

When an action potential depolarizes the muscle cell plasma membrane, Ca<sup>2+</sup> is released into the cytosol from the SR through Ca<sup>2+</sup>-release channels, stimulating the muscle to contract.

The Ca<sup>2+</sup> pump, which accounts for about 90 % of the membrane protein of the SR, moves Ca<sup>2+</sup> from the cytosol back into the SR. The endoplasmic reticulum of nonmuscle cells contains a similar Ca<sup>2+</sup> pump, but in smaller quantities.

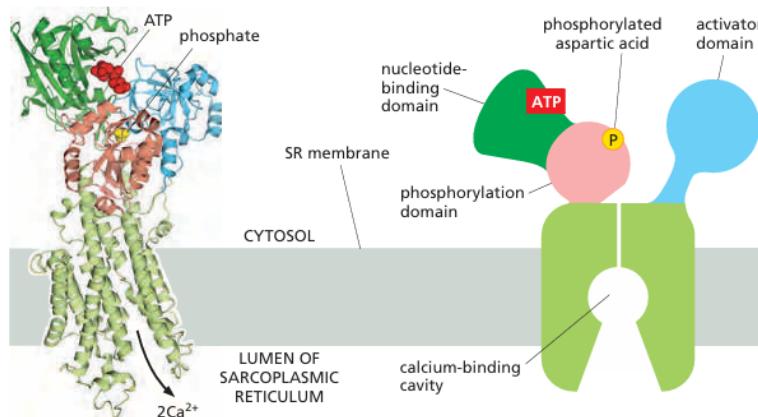


Figure 39: The structure of the sarcoplasmic reticulum Ca<sup>2+</sup> pump.

Ca<sup>2+</sup> binding triggers a series of conformational changes that close the passageway to the cytosol and activate a phosphotransfer reaction in which the terminal phosphate of the **bound ATP** is transferred to an **aspartate**. *This aspartate is highly conserved among all P-type ATPases.* The new ADP then dissociates and is replaced with a fresh ATP, causing another conformational change that opens a passageway to the SR lumen through which the two Ca<sup>2+</sup> ions exit. They are replaced by two H<sup>+</sup> ions and a water molecule that stabilize the empty Ca<sup>2+</sup>-binding sites and close the passageway to the SR lumen. Hydrolysis of the labile phosphoryl-aspartate bond returns the pump to the initial conformation, and the cycle starts again. See fig. 40

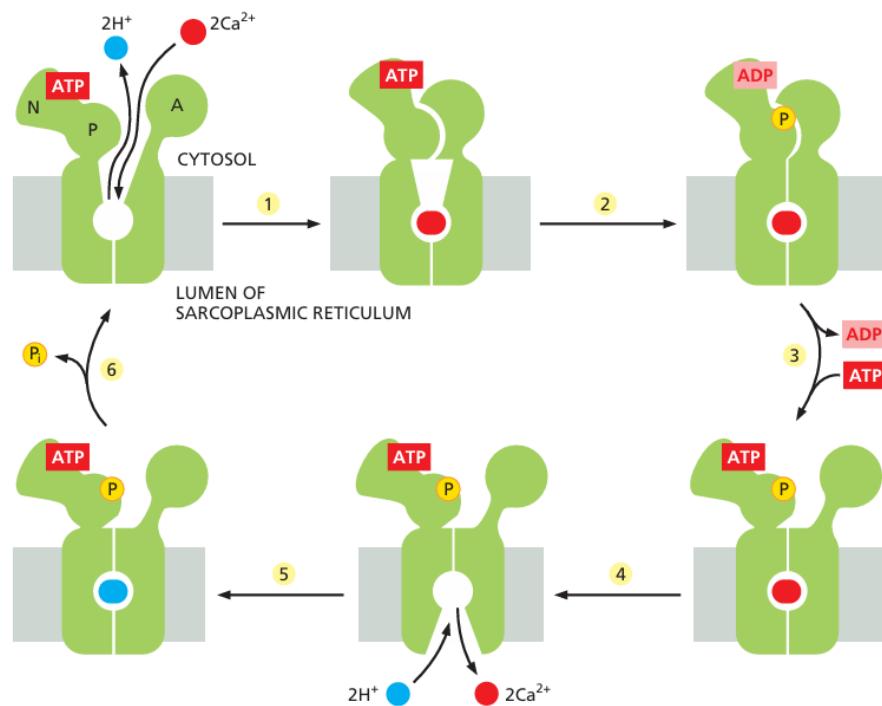


Figure 40: The pumping cycle of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  pump.

### 3.1.2.3 ABC-Transporters

They are a large family of membrane transport proteins (ex: 5% of E.coli genome). There exist 48 proteins in humans.

ABC transporters contain **two highly conserved ATPase domains**, or ATP-Binding “Cassettes,” on the cytosolic side of the membrane. ATP binding brings together the two ATPase domains (dimerization), and ATP hydrolysis leads to their dissociation.

They transport a high variety of substrates: sugar, amino acids, drugs, antibiotics, toxins, lipids, peptides, nucleotides and more.

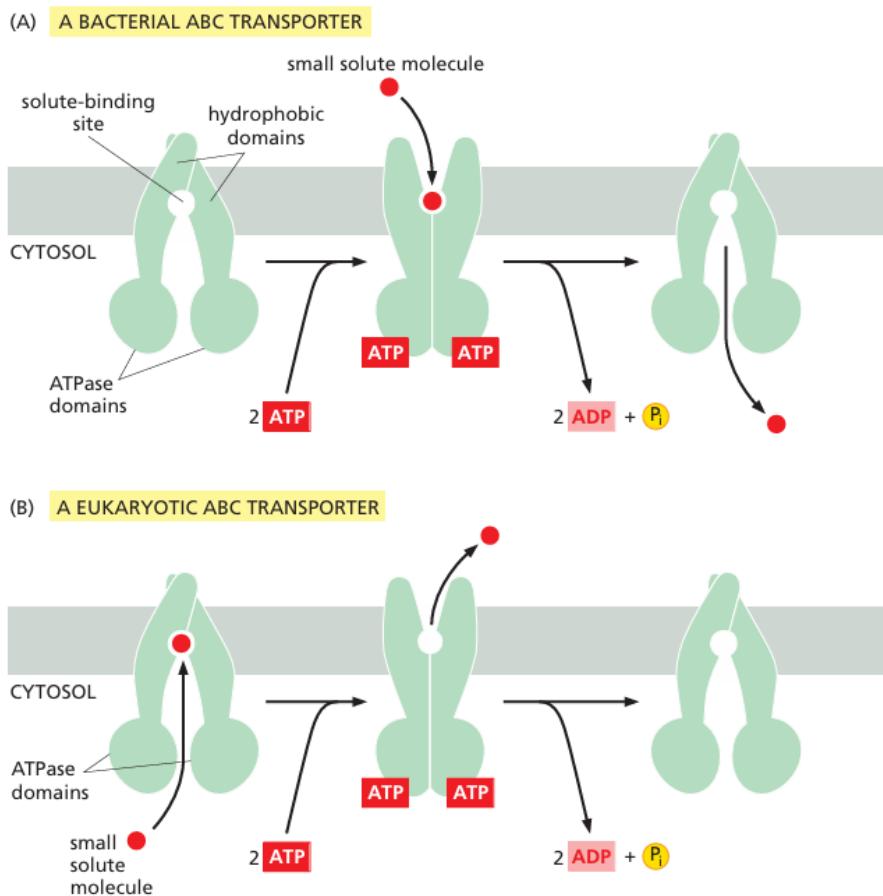


Figure 41: Small-molecule transport by typical ABC transporters.

*Note in eukaryotes, most ABC transporters export substances.*

*Remark 3.7 (Multidrug resistance (MDR)).* A phenomenon where cells become resistant to a wide range of structurally unrelated drugs, often due to the activity of **MDR proteins** (ABC transporters) that actively export toxic substances and therapeutic drugs out of the cell, reducing their intracellular concentrations and effectiveness.

Note these MDR proteins can also promote resistance to chemotherapies.

## 3.2 Channels

Unlike transporters, channels form pores across membranes. One class of channel proteins found in virtually all animals forms **gap junctions** between adjacent cells.

As discussed earlier, however, channels cannot be coupled to an energy source to perform active transport, so the transport they mediate is always passive (downhill).

### 3.2.1 Aquaporins

Aquaporins solve a problem that is opposite to that facing ion channels. To avoid disrupting ion gradients across membranes, they have to allow the **rapid passage of water molecules while completely blocking the passage of ions**. The three-dimensional structure of an aquaporin reveals how it achieves this remarkable selectivity.

The channels have a narrow pore that allows water molecules to traverse the membrane in single file, following the path of carbonyl oxygens that line one side of the pore.

Hydrophobic amino acids line the other side of the pore. The pore is too narrow for any hydrated ion to enter, and **the energy cost of dehydrating an ion would be enormous** because the hydrophobic wall of the pore cannot interact with a dehydrated ion to compensate for the loss of water. Therefore K<sup>+</sup> and other ions can not transfer through aquaporins.

Moreover these channels are also **impermeable to H<sup>+</sup>**. Because aquaporins contain **two strategically placed asparagines**, which bind to the oxygen atom of the central water molecule in the line of water molecules traversing the pore, imposing a bipolarity on the entire column of water molecules. This makes it impossible for the “making and breaking” sequence of hydrogen bonds to get past the central asparagine-bonded water molecule, because both valences of this central oxygen are unavailable.

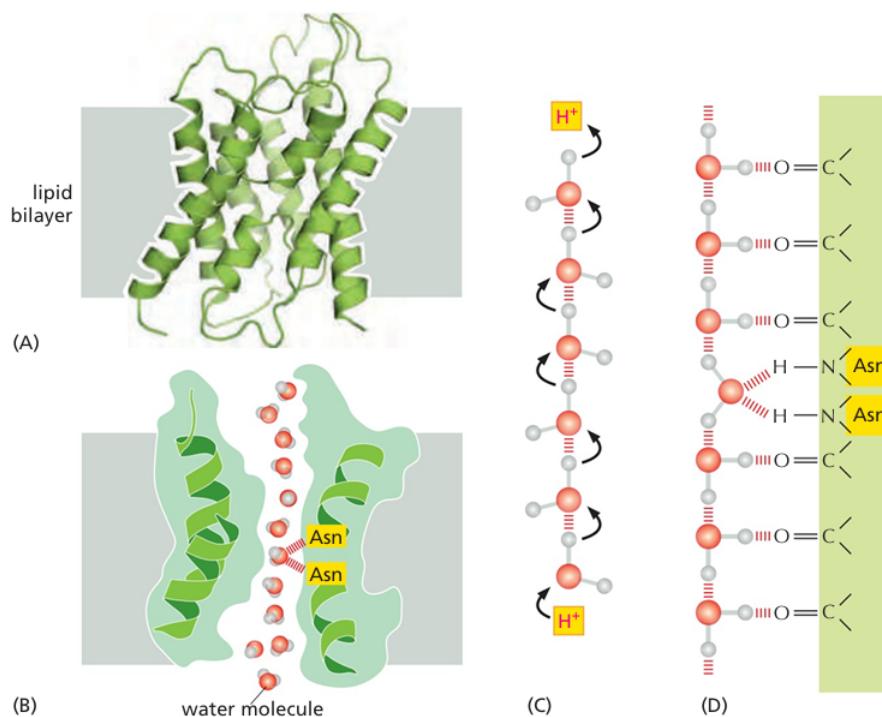


Figure 42: The structure of aquaporins

*Remark 3.8 (The response to dehydration).* **Vasopressin** is a peptide hormone released by the posterior pituitary in response to dehydration or increased plasma osmolarity. It promotes water reabsorption in the kidneys by stimulating the insertion of aquaporin-2 channels in the collecting ducts, thereby reducing urine output and conserving body water

### 3.2.2 Ion channels

Two important properties distinguish ion channels from aqueous pores.

- First, they show **ion selectivity**, permitting some inorganic ions to pass, but not others. The permeating ions have to shed most or all of their associated water molecules to pass, often **in single file**, through the narrowest part of the channel, which is called the **selectivity filter**; this limits their rate of passage

- Second, ion channels are not continuously open. Instead, they are **gated**, which allows them to open briefly and then close again. In most cases, the gate opens in response to a specific stimulus. See fig. 43

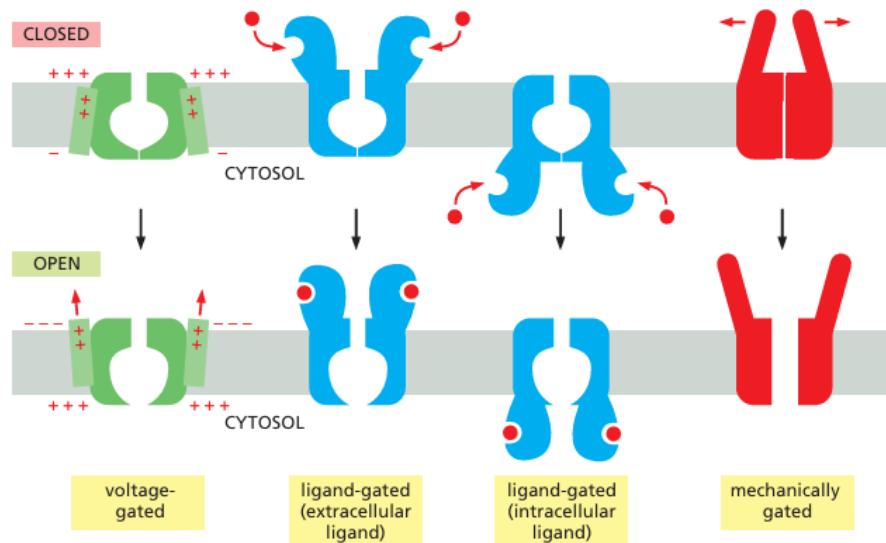


Figure 43: The gating of ion channels

Moreover, protein **phosphorylation** and dephosphorylation regulates the activity of many ion channels; this type of channel regulation is discussed, together with nucleotide-gated ion channels.

In general, **gating involves movement of the helices** in the membrane so that they either obstruct or open the path for ion movement. Depending on the particular type of channel, helices tilt, rotate, or bend during gating.

Ligand-gated **channels open and close periodically**. The probability to switch from the closed to the open state depends largely on the concentration of the ligand. But they will always close spontaneously. The simplest way is that the ligand just unbinds. But there are also channels that enter desensitized state while their still bound to the ligand, preventing overfiring.

Moreover note that channels always completely open or closed, there is nothing in between.

### 3.2.2.1 K<sup>+</sup> (leak) channels

Ion channels that are permeable mainly to K<sup>+</sup> are found in the plasma membrane of almost all cells. An important subset of K<sup>+</sup> channels opens even in an unstimulated or “resting” cell, and hence these are called K<sup>+</sup> leak channels.

K<sup>+</sup> leak channels conduct K<sup>+</sup> 10,000-fold faster than Na<sup>+</sup>, yet the two ions are both featureless spheres and have similar diameters (0.133 nm and 0.095 nm, respectively).

The polypeptide chain that connects the two transmembrane helices forms a short  $\alpha$  helix (the pore helix) and a crucial loop that protrudes into the wide section of the cone to form the **selectivity filter**. In this filter functions thanks to the **coordination between carbonyl oxygens and the dehydrated K<sup>+</sup>**.

Moreover, they channel attracts cation by negative charged amino acids. See fig. 44

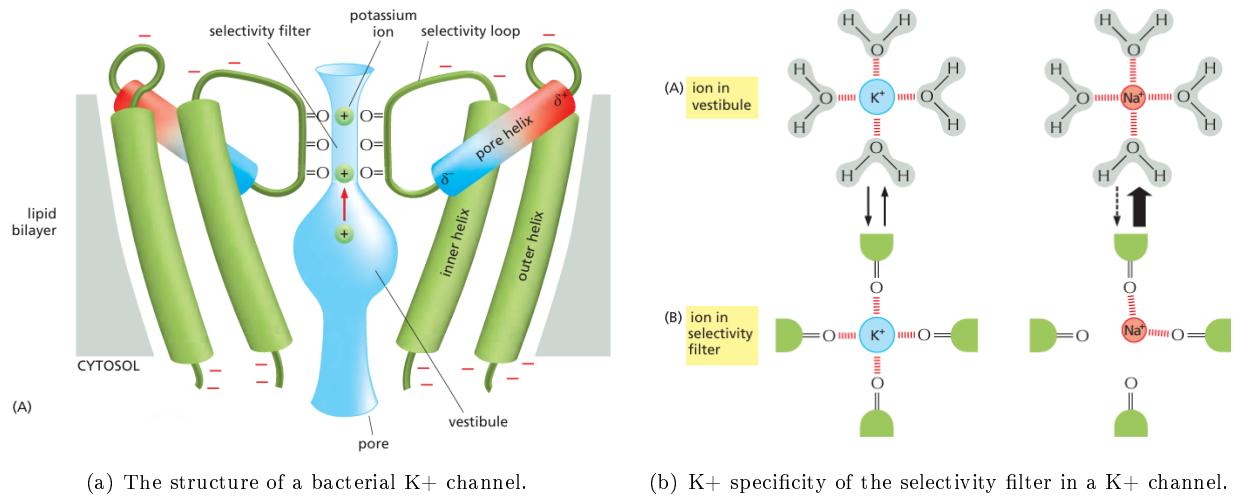


Figure 44:  $K^+$  channel

Many  $K^+$  channels are **voltage gated** and essential for electrical signaling.

*Remark 3.9 (Electrical signaling).* At rest, a neuron keeps more  $K^+$  inside and more  $Na^+$  outside, creating an electrical difference across the membrane. When a signal arrives (action potential starts), voltage-gated  $Na^+$  channels open, and  $Na^+$  rushes in depolarizing the membrane, making the inside more positive (rising phase of the signal). Shortly after,  $K^+$  channels open, and  $K^+$  flows out, repolarizing the cell (back to its resting state). When the action potential reaches the end of the neuron,  $Ca^{2+}$  channels open during depolarization, and  $Ca^{2+}$  enters, triggering processes like neurotransmitter release.

### 3.2.2.2 Patch-clamp

patch-clamp is a technique to record ionic current flow through individual channels while membrane potential is clamped. Because of the extremely tight seal between the micropipette and the membrane, current only enter or leave the micropipette by passing through the ion channel in the patch.

This enables to determine which molecules activate the channel on an which side.

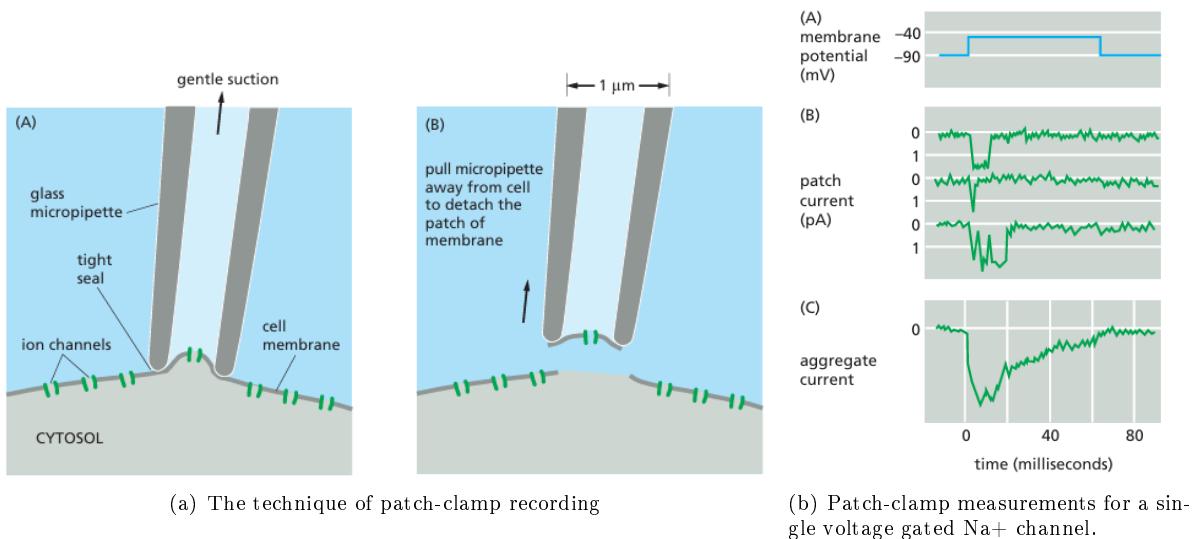


Figure 45: Patch-clamp

Note that the **aggregate current** (the sum of multiple experiments) reflects the probability that any individual channel will be in the open state.

## 4 cellular localization

### 4.1 compartments and organelles overview

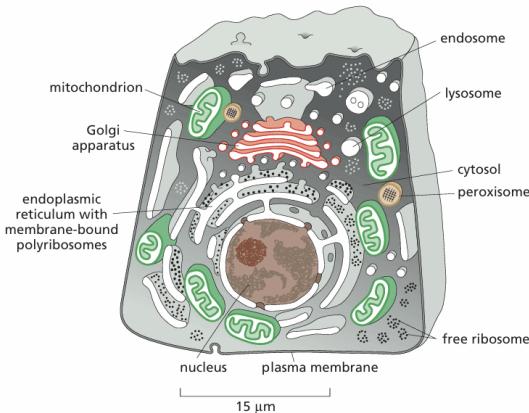


Figure 46: overview of compartments

Compartmentalization in eukaryotic cells is used to improve metabolic process by providing optimal conditions for various reactions (such as very acidic conditions in lysosomes or very high calcium concentrations). In general this is achieved by having organelles.

**Cellular compartments differ in function, form, subcellular location, protein and lipid composition.** Moreover the may differ in ionic composition, e.g: pH can vary from 7.2 in the ER to less than 5 in

lysosomes; Calcium concentration varries from nM in the cytoplasma to uM in the ER; The redox potential differs from reducing in the cytoplasm to oxidizing in the ER

There are two types of organelles: **membrane organelles and membrane-less organelles**

**How many organelles?**

- 1 nucleous
- 1 golgi apparatus
- 1 ER
- hundreds of endosomes/lysosomes
- a mitochondrial network that is not always continous
- memrbaneless organelles

**The number of organelles is not correlated with the size of the cell.**

This is very general and of course there are **exception** such as muscle cells and red blood cells. Muscle fiber are a fusion of many cells so they will have **multiple nucleous**. Whereas red blood cells will lose all internal membrane during differenciation so they will have **no organelles that have a membrane**

#### 4.1.0.1 table on volumes of mebrane and organelles

**TABLE 12-1 Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)**

Intracellular compartment	Percentage of total cell volume
Cytosol	54
Mitochondria	22
Rough ER cisternae	9
Smooth ER cisternae plus Golgi cisternae	6
Nucleus	6
Peroxisomes	1
Lysosomes	1
Endosomes	1

**TABLE 12-2 Relative Amounts of Membrane Types in Two Kinds of Eukaryotic Cells**

Membrane Type	Percentage of total cell membrane	
	Liver hepatocyte*	Pancreatic exocrine cell*
Plasma membrane	2	5
Rough ER membrane	35	60
Smooth ER membrane	16	<1
Golgi apparatus membrane	7	10
Mitochondria		
Outer membrane	7	4
Inner membrane	32	17
Nucleus		
Inner membrane	0.2	0.7
Secretory vesicle membrane	Not determined	3
Lysosome membrane	0.4	Not determined
Peroxisome membrane	0.4	Not determined
Endosome membrane	0.4	Not determined

\*These two cells are of very different sizes: the average hepatocyte has a volume of about  $5000 \mu\text{m}^3$  compared with  $1000 \mu\text{m}^3$  for the pancreatic exocrine cell. Total cell membrane areas are estimated at about  $110,000 \mu\text{m}^2$  and  $13,000 \mu\text{m}^2$ , respectively.

Figure 47: table volumes of membranes

Membrane-less organelles are a rather new discovery but they usually require **liquid–liquid phase separation (LLPS)**. They can consist of protein aggregates or RNA aggregates, a similar principle is responsible for lipid raft formation:

Membrane Organelles	Membrane-less Compartments
Endoplasmic Reticulum	P-bodies
Nucleus	Stress Granules
Golgi System	Nucleolus
Peroxisomes	Many more to be discovered?
Mitochondria	
Lysosome	
Endosome	

Table 3: Examples of membrane and membrane-less compartments in eukaryotic cells

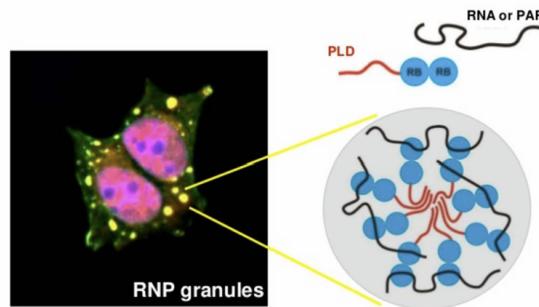


Figure 48: membrane less organelle overview of RNP

#### 4.1.1 Endoplasmatic reticulum

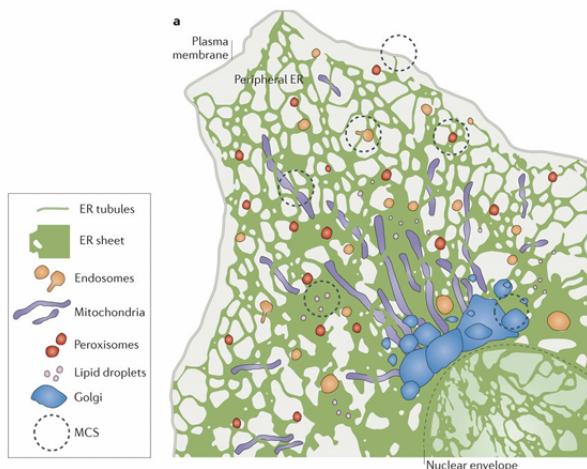


Figure 49: Structure of Endoplasmatic reticulum

The Endoplasmic Reticulum (ER) is the **production site of all transmembrane proteins and lipids** it also serves as a **calcium storage of the cell**. The calcium is imported into the ER by the **SERCA pump** There

are two types of ER **smooth and rough ER**. The difference being that the **rough ER has membrane bound ribosomes** and the smooth one does not. Most cells will have both but the ratio will be different depending on cell type.

#### 4.1.1.1 Smooth and rough ER separation

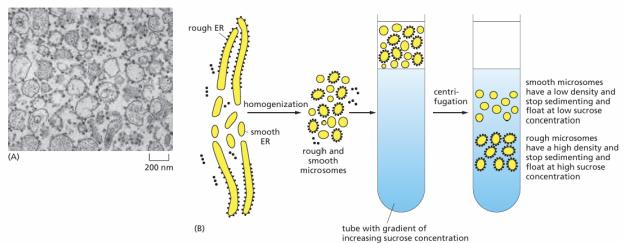


Figure 50: ER separation experiment

Since smooth and rough ER have different densities they can be separated using a sucrose gradient and centrifugation. Here is how the process works:

- i) **Cell Lysis:** Cells are lysed using mechanical disruption (e.g., homogenization) or chemical methods to release cellular contents, including organelles and membrane fragments.
- ii) **Differential Centrifugation:** The lysate is subjected to sequential centrifugation steps to remove nuclei and large organelles. The remaining supernatant is centrifuged at high speed to pellet **microsomes**, which are small vesicles derived from fragmented endoplasmic reticulum membranes.
- iii) **Preparation of Sucrose Gradient:** A discontinuous or continuous sucrose gradient is prepared, typically ranging from 0.25 M to 2.0 M sucrose, to separate membrane vesicles based on density.
- iv) **Loading of Microsomes:** The microsomal pellet is resuspended and carefully layered onto the sucrose gradient.
- v) **Ultracentrifugation:** The sample is centrifuged at high speed (e.g.,  $100,000 \times g$ ) for several hours. Microsomes migrate within the gradient according to their buoyant densities.
- vi) **Fraction Collection:** Distinct layers form in the gradient: **rough ER-derived microsomes** (containing ribosomes) settle at higher density positions than **smooth ER-derived microsomes**, which are lighter. These fractions are collected separately.
- vii) **Analysis:** Isolated ER fractions can be analyzed using protein assays, Western blotting, enzymatic activity tests, or proteomic approaches to study their composition and function.

#### 4.1.2 Golgi system

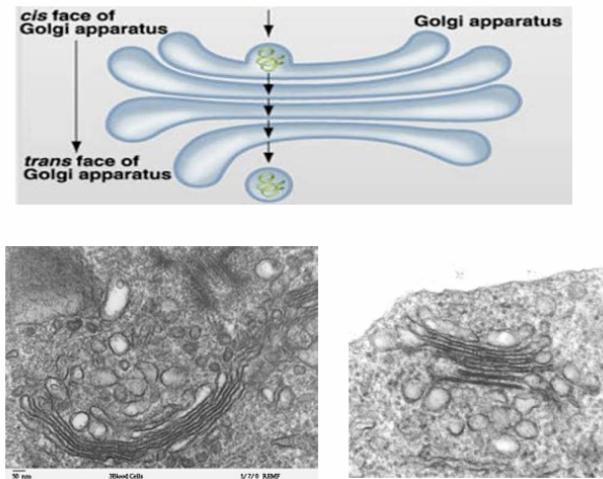


Figure 51: Golgi apparatus overview

The Golgi apparatus acts as a **collection and dispatch center for proteins from the ER**. Proteins are packaged into vesicles, which fuse with the Golgi for further processing, mainly through **post-translational modifications such as glycosylation and phosphorylation**. These modifications help direct proteins to their final destination.

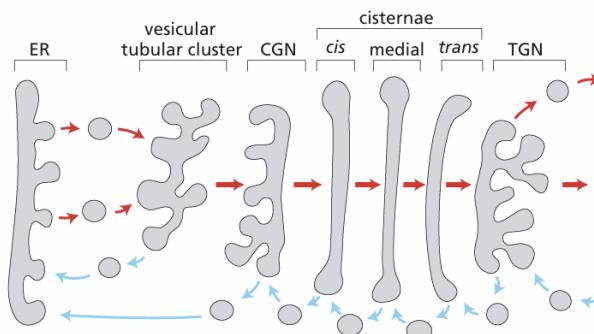


Figure 52: domains of the golgi

The golgi can be further subdivided into domains (brief chaptGPT overview of these):

- **Cis-Golgi Network (CGN):** The entry face of the Golgi apparatus, located closest to the endoplasmic reticulum (ER). It receives proteins and lipids from the ER and is involved in initial processing, such as phosphorylation and sorting for further transport or ER retrieval.
- **Medial-Golgi:** The central region of the Golgi stack. It performs further modification of glycoproteins, including the processing of N-linked oligosaccharides and initiation of O-linked glycosylation.
- **Trans-Golgi:** The portion closer to the exit side of the Golgi. It completes final processing steps like sulfation and complex glycosylation, preparing molecules for sorting.

- 
- **Trans-Golgi Network (TGN):** A dynamic sorting compartment at the trans face of the Golgi. It directs modified proteins and lipids to their final destinations, including the plasma membrane, lysosomes, or secretory vesicles, using different types of transport vesicles.

#### 4.1.3 lysosomes

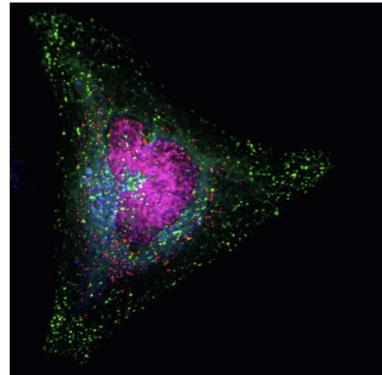


Figure 53: lysosome fluorescent microscopy image

lysosomes are specialized vesicles with very low pH (4.5-5) they house a multitude of enzymes that require these conditions such as enzymes involved in lysis of peptides nucleic acids carbohydrates and lipids. They contain more than 60 enzymes and more than 50 membrane proteins and highly variable in size

#### 4.1.4 Mitochondria

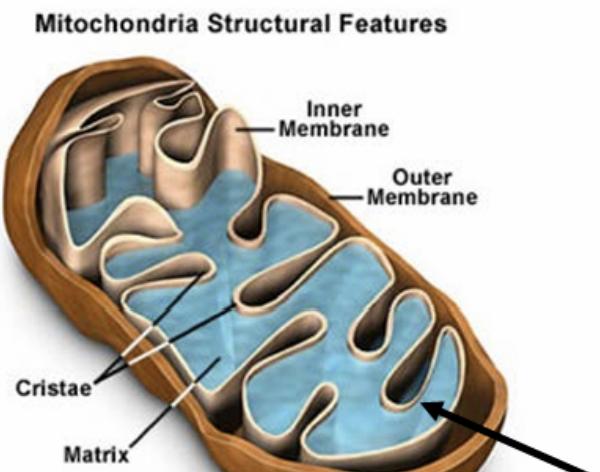


Figure 1

Figure 54: Mitochondria structure

The **mitochondria** is a double membraned organelle. This is due to the fact that it is an ex. bacteria that the cell tried to eat but fucked up. However now it can't survive outside of the cell.

#### 4.1.5 Endosomes

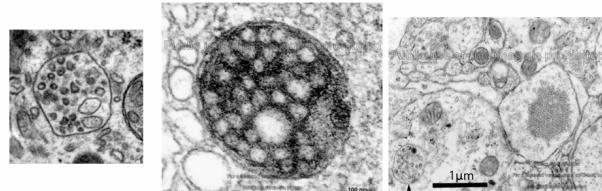


Figure 55: Endosomes

Endosomes are a **collection of intracellular sorting organelles in eukaryotes** (there was literally nothing else here...)

#### 4.1.6 Peroxisomes

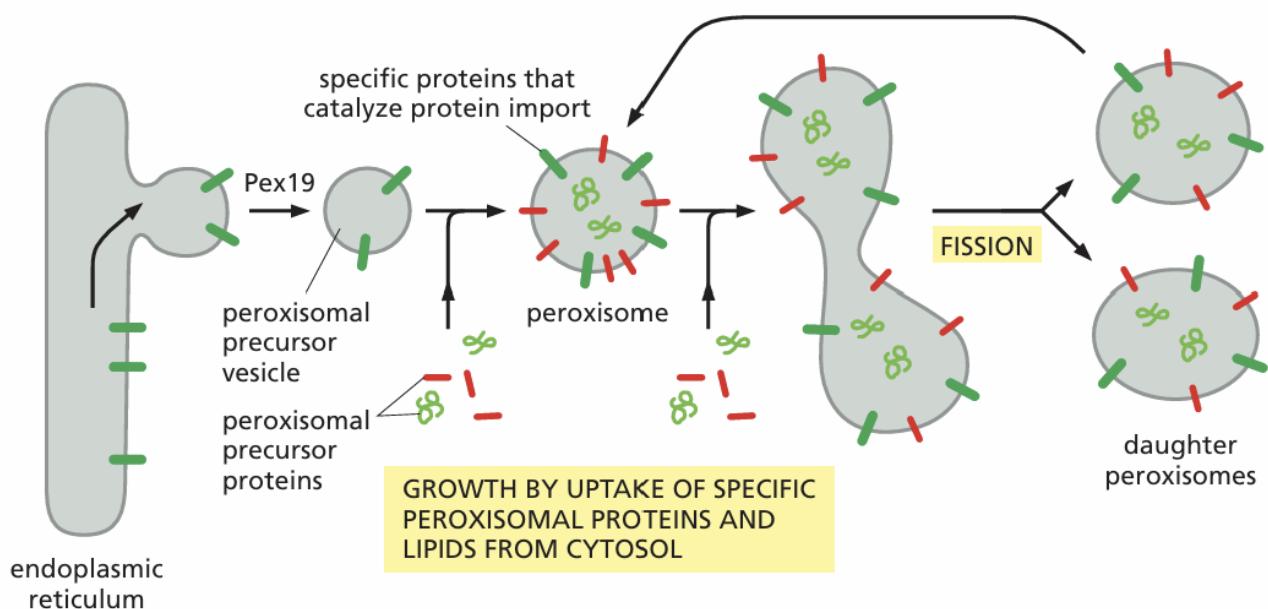


Figure 56: Peroxisomes proliferation

Peroxisomes are specialized vesicle that is used to **peroxidize shit**. (hence the name) Usually it's substrates are toxins that come from the liver or the kidney cells. The reaction is as follows:



---

They are created from the ER first as a **peroxisomal precursor vesicle**. These then fuse with **peroxisomal precursor proteins and lipids**. (these are found in the cytosol) forming the main peroxisome that can then fission to produce more peroxisomes. They are also responsible for the **break down of fatty acids**, however unlike  $\beta$ -oxidation it isn't ATP coupled.

#### 4.1.7 lipid droplets

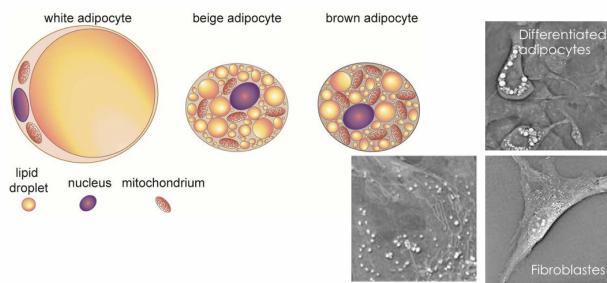


Figure 57: adipocyte overview

These are specialized cells that store lipid droplets. Note that **white adipocytes are mainly used for storage** hence the oversized lipid droplet, whereas **brown adipocytes are used for heat generation**, which is why they have a lot of mitochondria

## 4.2 lipid composition of various organelles

The lipid composition is different across the various organelles.

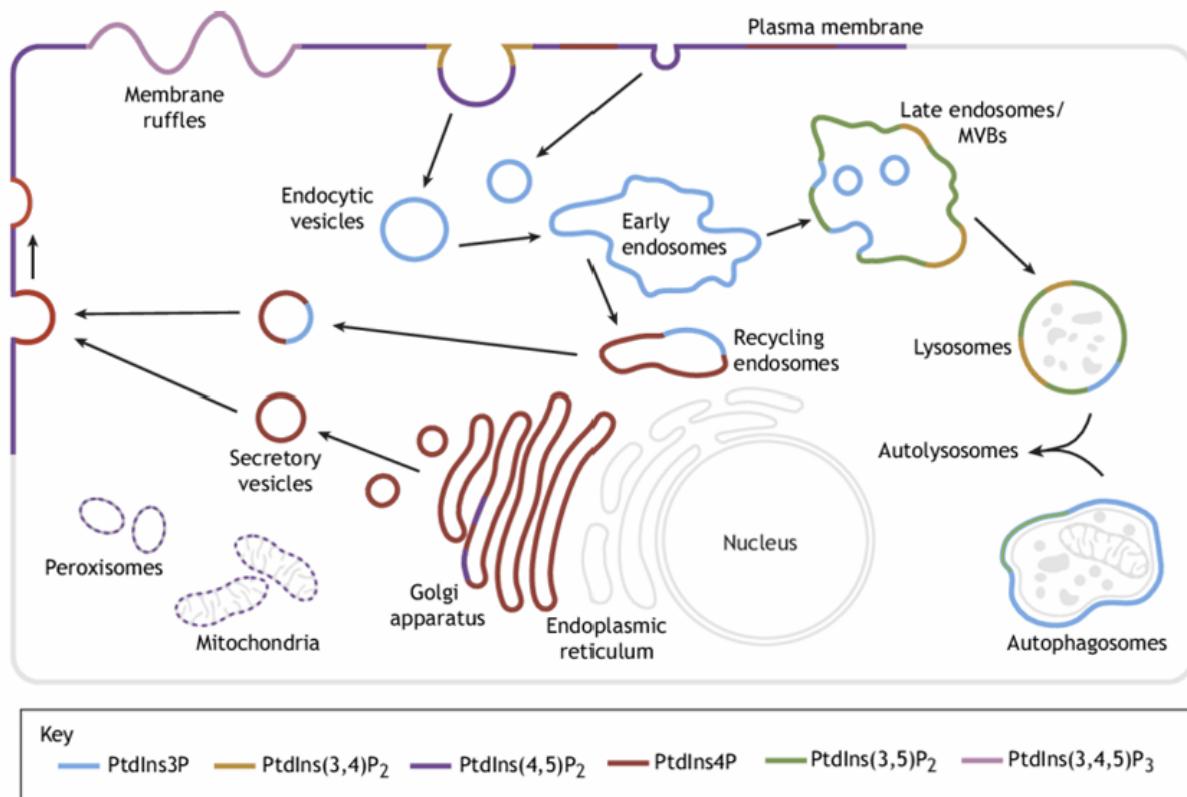


Figure 58: Phosphatidylinositol location and phosphorylation state

An interesting case is that of PI, which depending on the **cellular location will be phosphorylated differently**

The composition of the cell membrane will also vary across cell types. This is illustrated in the table below:

**Table 10–1 Approximate Lipid Compositions of Different Cell Membranes**

LIPID	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM
<b>Cholesterol</b>	17	23	22	3	6
<b>Phosphatidylethanolamine</b>	7	18	15	28	17
<b>Phosphatidylserine</b>	4	7	9	2	5
<b>Phosphatidylcholine</b>	24	17	10	44	40
<b>Sphingomyelin</b>	19	18	8	0	5
<b>Glycolipids</b>	7	3	28	trace	trace
<b>Others</b>	22	13	8	23	27

Figure 59: Membrane composition based on cell type

## 4.3 protein localization

### 4.3.1 types of transport

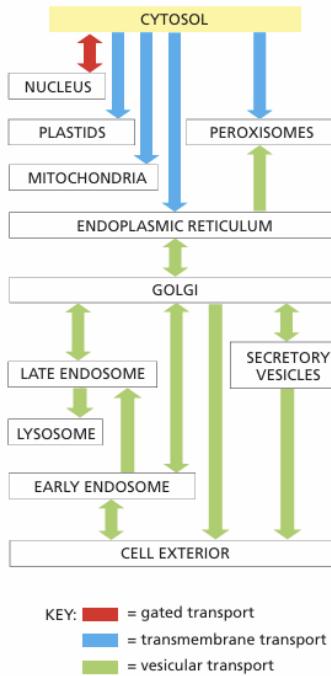


Figure 60: Types of transport overview

There are three types of **transport** **Gated, transmembrane localization, vesicular transport:**

- i) **gated transport:** In gated transport, proteins and RNA molecules move between the cytosol and the nucleus through **nuclear pore complexes** in the nuclear envelope. The nuclear pore complexes function as **selective gates** that support the active transport of specific macromolecules and macromolecular assemblies **between the two topologically equivalent spaces**, although they also allow free diffusion of smaller molecules
- ii) **Transmembrane localisation:** In protein translocation, transmembrane protein translocators directly transport specific proteins across a membrane from the cytosol into a **space that is topologically distinct**. The transported protein molecule usually must unfold to snake through the **translocators**. The initial transport of selected proteins from the cytosol into the ER lumen or mitochondria, for example, occurs in this way. Integral membrane proteins often use the same translocators but translocate only partially across the membrane, so that the protein becomes embedded in the lipid bilayer.
- iii) **vesicular transport** in Vesicular transport, membrane-enclosed transport intermediates—which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments—ferry proteins from **one topologically equivalent compartment to another**. This involves the creation of vesicles where the membrane proteins are loaded onto. These then are ferried around and fuse with another compartment.

#### 4.3.2 important signals

TABLE 12-3 Some Typical Signal Sequences	
Function of signal sequence	Example of signal sequence
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into mitochondria	<sup>+</sup> H <sub>3</sub> N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	<sup>+</sup> H <sub>3</sub> N-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO <sup>-</sup>
Import into ER	<sup>+</sup> H <sub>3</sub> N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO <sup>-</sup>

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in red and negatively charged amino acids are shown in green. Similarly, important hydrophobic amino acids are shown in orange and important hydroxylated amino acids are shown in blue. <sup>+</sup>H<sub>3</sub>N indicates the N-terminus of a protein; COO<sup>-</sup> indicates the C-terminus.

Figure 61: list of important localization signals

Note that the signals can be either N or C terminal. **N-terminal signals are read during translation, whereas C-terminal tags are read post-translationally.** The **nuclear export signals can be in the middle of the CDS.** Note also that most of the localization signals will be cut off after their use, with exceptions such as nuclear localization tag (NLS) and Peroxisomal targeting signal

#### 4.3.3 nuclear import

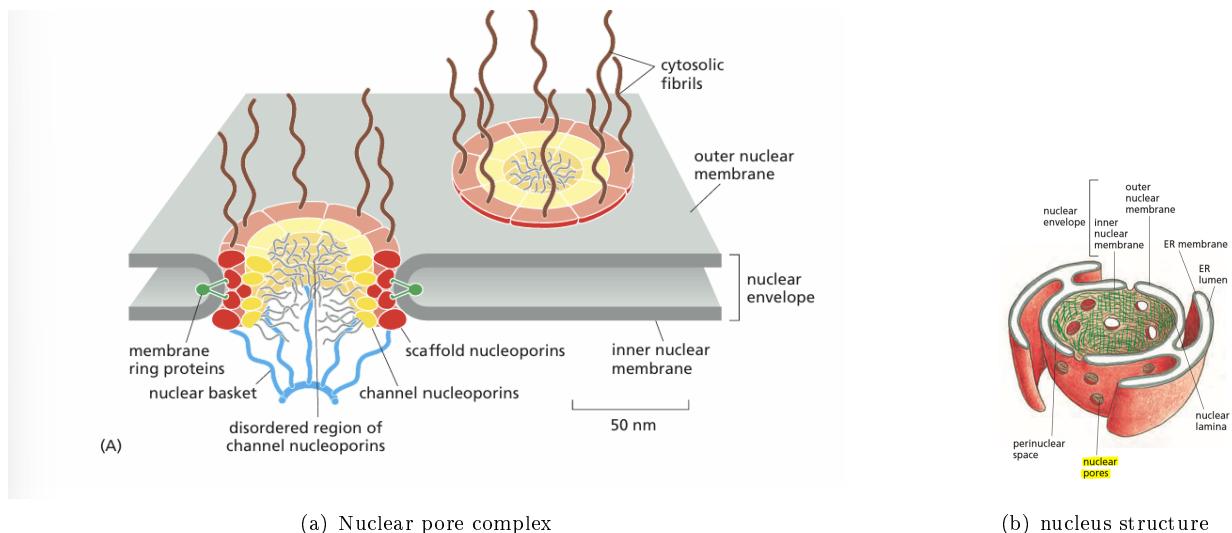


Figure 62: Application of protein design

The nuclear import is special as it requires the molecule to pass through the **nuclear pore complexes (NPC)** which are giant channels in the nuclear membrane that **regulate traffic into the nucleous**. The nuclear pores **allow any protein less than 5kDa and ions to pass freely**. Another thing to note is that the nuclear pore complexes **regulates traffic in both ways, and is unclear how it does this as to avoid crashes**

##### 4.3.3.1 Ribosome production example

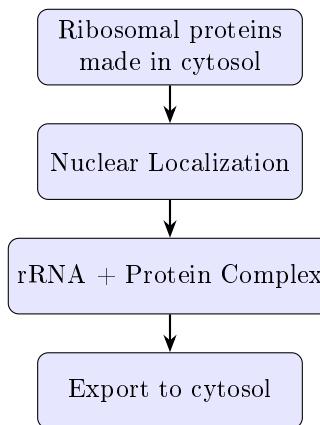


Figure 63: Overview of Ribosome Production and Trafficking

Ribosomes are **made in the cytosol** then **imported into the nucleus** where they complex with rRNA's. Then they are **exported back out** of the nucleous

#### 4.3.3.2 Nuclear import receptors(importins)

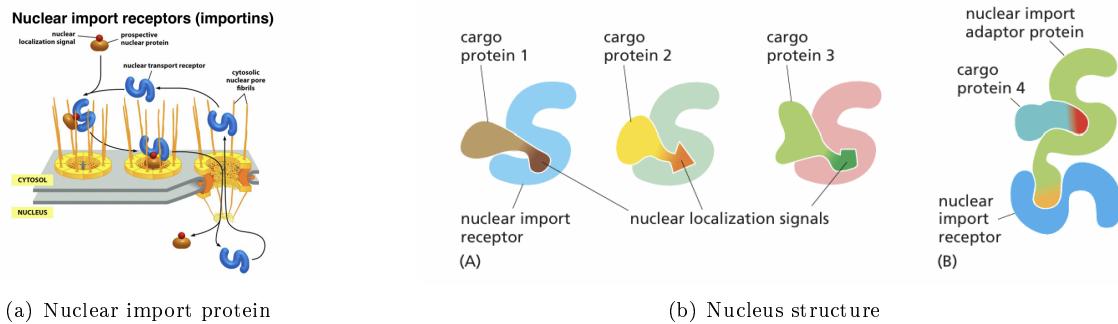


Figure 64: Importins bind to cargo proteins that have NLS

Proteins destined for the nucleus need to be shuttled into it by the **nuclear import receptors (importin)** that bind to the **localization signal** on the **cargo protein**. The localization signals is **not found on the N or C terminus and are not cut off**. This is due to the fact that they need to be **used everytime the nucleus is reformed after cell division**. Nuclear import receptors (importins) are **tightly linked to a GTPase cycle**, specifically involving the small GTPase Ran

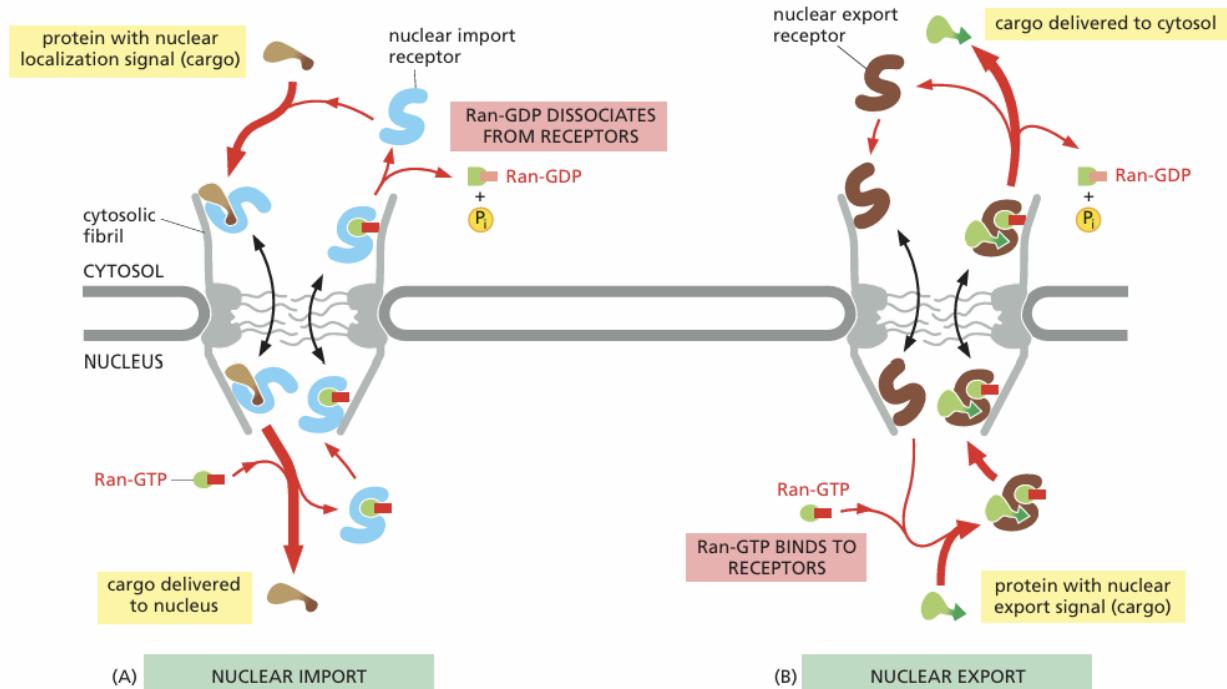


Figure 65: ran-GDP ran-GTP and its role in shuttling cargo across nuclear pore complexes

**ran-GDP Ran-GTP** Ran-GTP is primarily found inside nucleus due to presence of (**Ran-GEF (Guanine nucleotide exchange factor)**). Ran-GDP is found in the cytosol due to presence of **Ran-GAP**

(GTPase-activating protein). The **Ran-GPT/GDP** works as a molecular switch which allows regulation of directionality of nuclear import and export.

- **Nuclear Import**

- i) Cargo with nuclear localization signals binds to import receptors.
- ii) The import receptor-cargo complex enters the nucleus through the nuclear pore.
- iii) Ran-GTP binds to the import receptor, causing cargo release inside the nucleus.
- iv) The Ran-GTP-import receptor complex is exported to the cytosol.
- v) Ran-GAP promotes GTP hydrolysis, converting Ran-GTP to Ran-GDP, leading to receptor release.

- **Nuclear Export**

- i) Cargo with nuclear export signals binds to export receptors (**Exportin**) in the nucleus.
- ii) Ran-GTP binds to the export receptor-cargo complex.
- iii) The complex exits the nucleus through the nuclear pore.
- iv) In the cytosol, Ran-GAP promotes GTP hydrolysis, converting Ran-GTP to Ran-GDP.
- v) Cargo is released into the cytosol, and the export receptor is recycled.

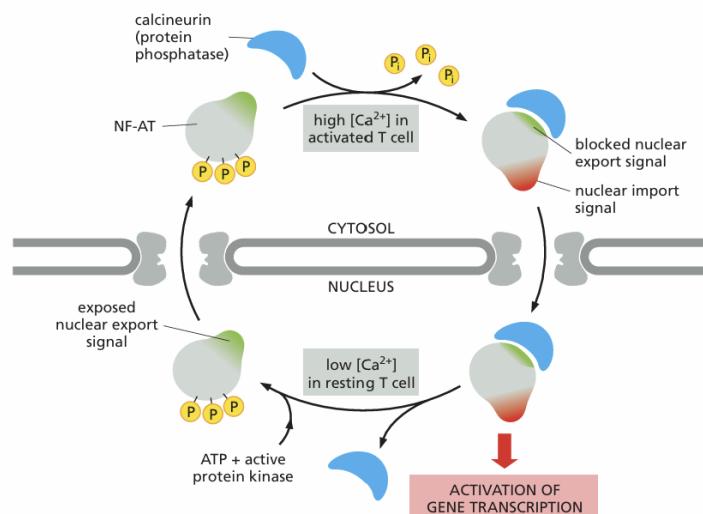


Figure 66: role of nuclear import/ export signals in T cell activation

*Remark 4.1.* Nuclear and import signals play a crucial role in T cell activation. **Upon activation the T-cell's cytosolic Ca<sup>2+</sup> concentration will be very high.** (it usually is low, like all cells). This then triggers Calcineurin to dephosphorylate the nuclear import signal and bind to the nuclear export signal. this the moves the NF-AT (transcription factor inside the nucleus promoting gene expression needed for T-Cell activation.

#### 4.3.3.3 the role of nuclear import in cell division

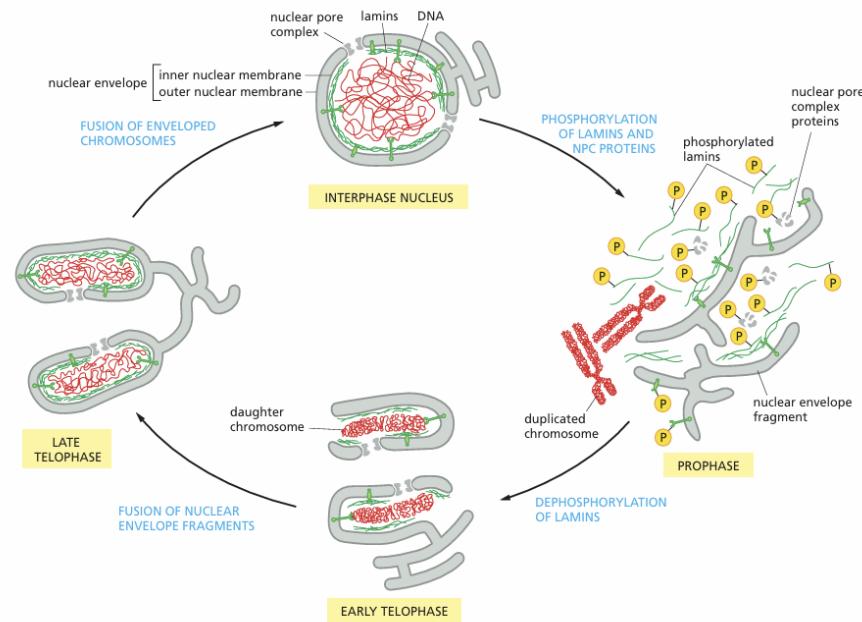


Figure 67: Reformation of the nucleus requires resusable NLS

After the cell divides the Nuclear pore complexes are closed, as they are tightly bound to chromosomes. This means that **only proteins bound to mitotic chromosomes will be found inside the nucleus during cell division** cytosolic proteins are virtually excluded from the reforming the nucleus and will need to be imported once the nuclear envelope is completed

#### 4.3.4 main transport pathways for proteins

There are two transport pathways in the cell that of **biosynthesis** and that of **endocytosis**

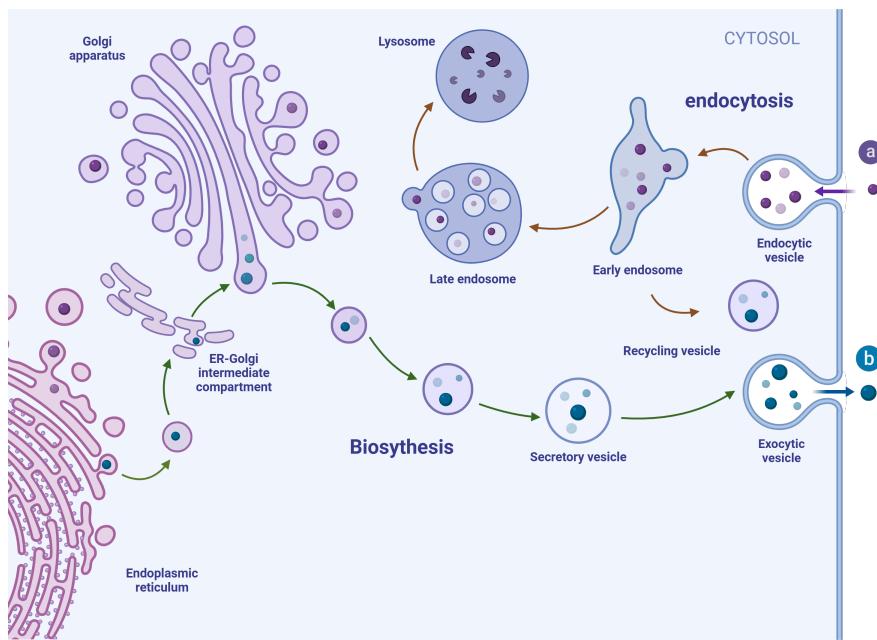


Figure 68: Main Pathways looked at in this chapter

### Biosynthesis

- i) ER
- ii) Golgi apparatus
- iii) Trans Golgi network
- iv) plasma membrane where vesicle fuses and proteins are secreted.

### Endocytosis

- i) uptake of proteins from **plasma membrane**
- ii) **early endosomes**: here the cell has to decide whether to recycle the material taken up (such as receptors) or destroy it. if it recycles it it will become a recycling endosome otherwise it will become a lysosome where the proteins will be digested and broken down into individual AA.
- iii) **recycling endosomes**: used for receptor reuptake. It will fuse with membrane reexposing the receptors in the cell exterior.
- iv) **late endosome** will eventually convert to **lysosome** where the uptaken material will be digested

#### 4.3.5 translocation to ER

##### 4.3.5.1 cotranslational and post translational localization

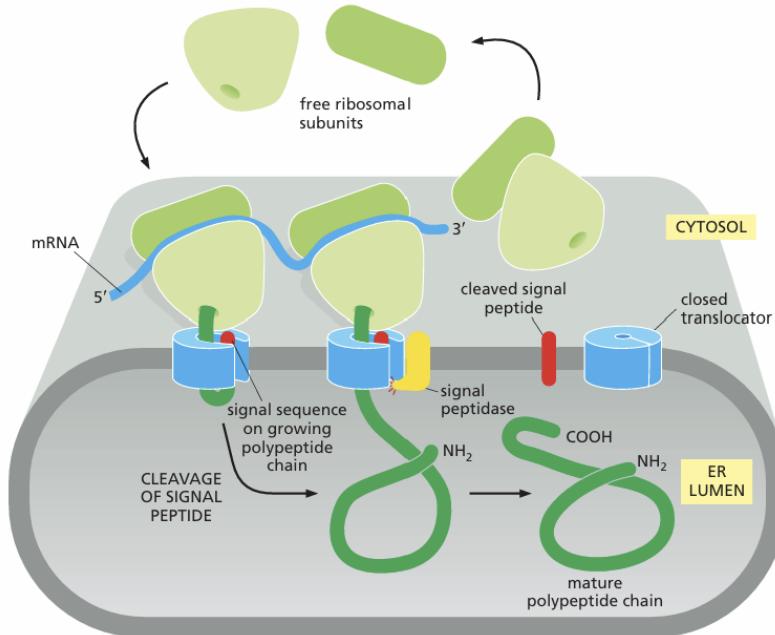


Figure 69: co and post translocation to the ER

**co-translational translocation** involves the ribosome being moved to the ER as soon as the nascent protein's ER localization signal is produced. Then the signal peptide is **cleaved off if the protein is an ER lumen cytosolic protein**. **All membrane proteins are cotranslational** Whereas post-translational translocation involves the protein being localized to ER after it has been fully translated.

##### 4.3.5.2 soluble proteins

**Soluble proteins that are ER bound must have an ER localization signal.** Once in the ER Lumen the localization signal will be **cleaved off**. Note that the ER-Lumen is not a highly reducing environment like the cytosol. Thus ER cytosolic proteins can have disulfide bonds unlike cytosolic proteins.

#### 4.3.5.3 transmembrane proteins

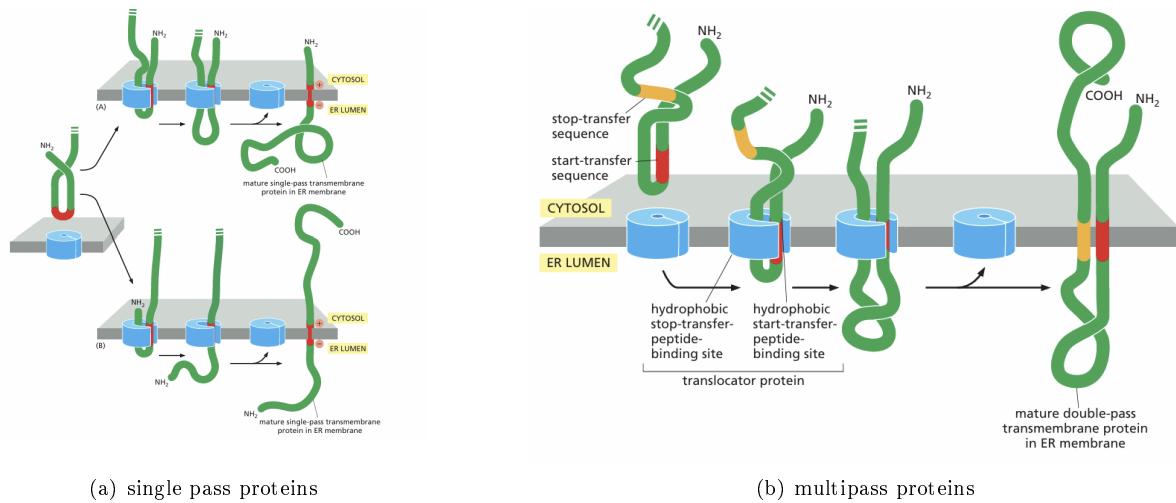


Figure 70: Role of localization in transmembrane protein translation

Transmembrane proteins are special in that they **need cotranslational traslocation into ER membrane** as otherwise they will not be embedded into membrane. As they are produced they will be funneled through translocator until a **start signal** is found which will create the transmembrane domain until the next **stop-transfer sequence**. Unlike soluble proteins they also **don't necessarily need an N-terminal localization signal** when the N-terminus is cytoplasmic they can have an "internal" localization signal that won't be cut off at one of the transmembrane domains.

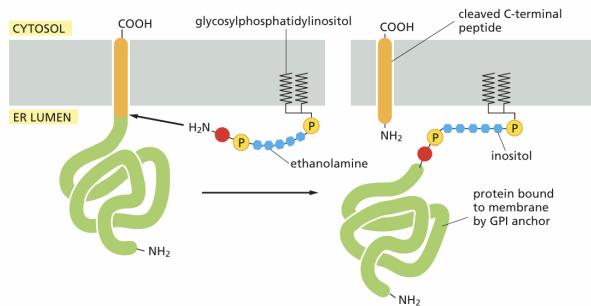


Figure 71: GPI anchor synthesis

**the GPI anchor and tail anchored proteins** Proteins bound to GPI anchor are translocated to the ER by an N-terminal tag that is cleaved off (not shown in figure). The hydrophobic C-terminus remains **bound to the membrane**. This is cleaved off and replaced by the **GPI anchor**, which faces into lumen

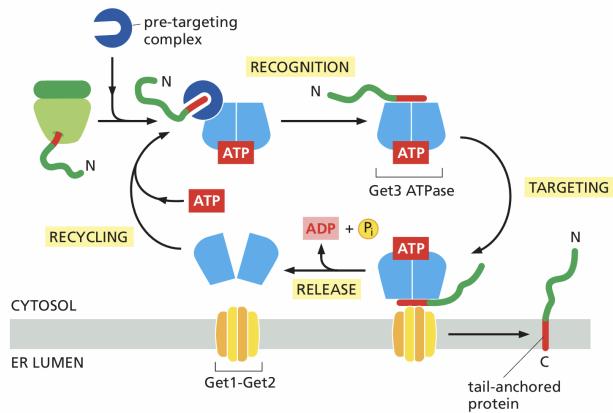


Figure 72: tail anchored proteins

Another method of anchoring the protein is directly via the C-terminus. This works as follows:

- i) tail anchored protein is synthesized in cytosol
- ii) **pre-targeting complex** recognizes the hydrophobic tail of the protein (red segment) and delivers it to **Get3**
- iii) the get3-"cargo" complex is handed to get3-ATPase, which will localize to the ER where it will bind to **get1-get2**
- iv) the get1-get2 complex will release the hydrophobic C tail of the protein into the membrane. **Unlike GPI these face the cytosolic side.**

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#### 4.3.5.4 moving ribosomes to ER: The signal-recognition particle (SRP)

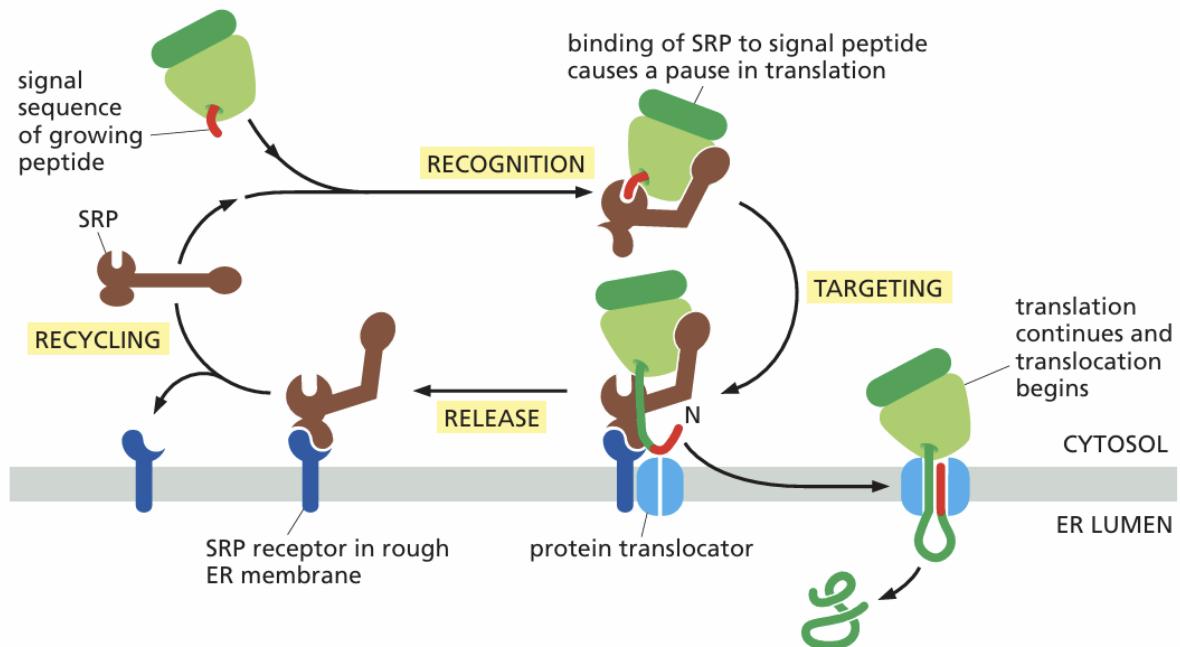


Figure 73: The SRP function in ribosome localization

- i) **Recognition:** The SRP binds to the signal sequence emerging from the ribosome as the protein is being synthesized. This binding pauses translation.
- ii) **Targeting:** The SRP-ribosome complex is directed to the ER membrane, where it binds to the SRP receptor located in the rough ER membrane.
- iii) **Release:** The SRP dissociates from the ribosome upon interaction with the SRP receptor, allowing the ribosome to engage with the protein translocator.
- iv) **Translocation:** Translation resumes, and the growing polypeptide is threaded through the translocator (Sec61 complex) into the ER lumen.
- v) **Recycling:** The SRP is released and recycled for future rounds of protein targeting.

#### 4.3.6 the role of contact patches in protein transport

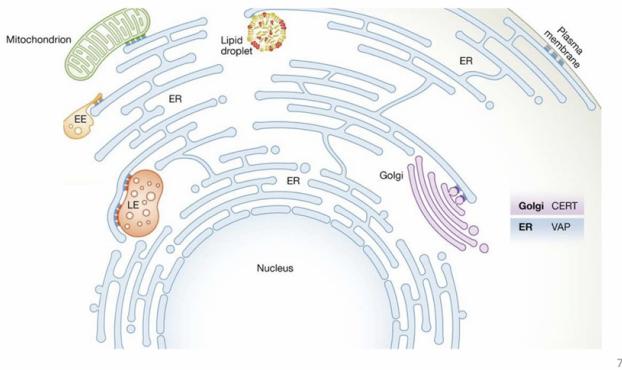


Figure 74: Contact patches role in protein transport

Contact patches are contact points between organelles where certain proteins and lipids can be exchanged. these are not random bumping into each other kinda thing but **highly regulated interactions** that are **new and not well understood**

#### 4.3.7 topological equivalence principle

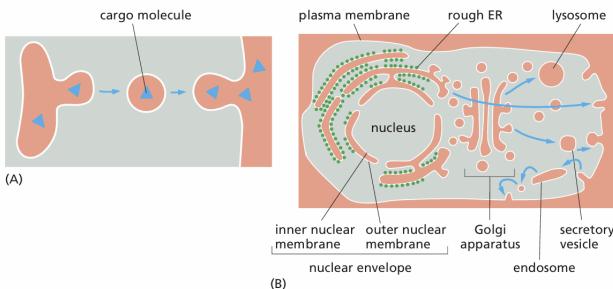


Figure 75: topological equivalence

The idea of topological equivalence is that the leaflets exposed to the cytosol have similar composition and thus can communicate with each other. The **inner compartments of the organelles are equivalent to the extracellular space**. This is a **consequence of vesicle transport**.

##### 4.3.7.1 how do luminal proteins and secreted proteins form if ribosomes are cytoplasmic?

Via the topological equivalence principle, a protein can move via vesicular transport to any other topologically equivalent region. However if a protein is luminal (in the ER lumen etc.) It faces the issue that the ribosomes themselves are cytosolic so it couldn't enter the lumen. This is **solved by cotranslational translocation** where the ribosomes are translocated so they synthesize the protein directly into the ER lumen. **All proteins that are luminal, membrane or secreted are synthesized by ribosomes attached to ER not free ribosomes**

#### 4.3.7.2 membrane directionality

Membrane directionality is also a cause of vesicular transport as for example a luminal leaflet will never point towards the cytosol and vesicle transport only allows transport between topologically equivalent domains.

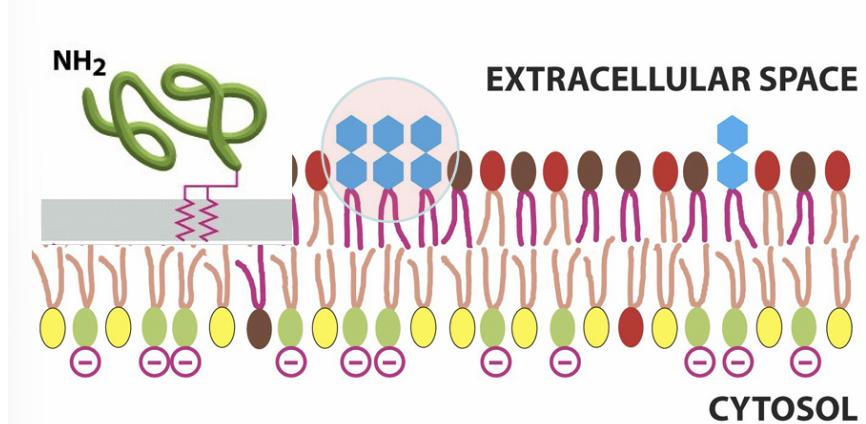


Figure 76: Asymmetry in membrane leaflets

#### 4.4 glycosylation

Glycosylation serves many purposes in the cell some of those being:

- i) helps the protein fold
- ii) their presence on the surface of a protein will protect them from extracellular proteases
- iii) give information on how long a protein has been around and it's fold status (misfolded or not)  
glycosylation occurs in the **sequence: Asn-X-Ser/Th**

#### 4.4.0.1 N linked glycosylation in ER vs O linked in golgi

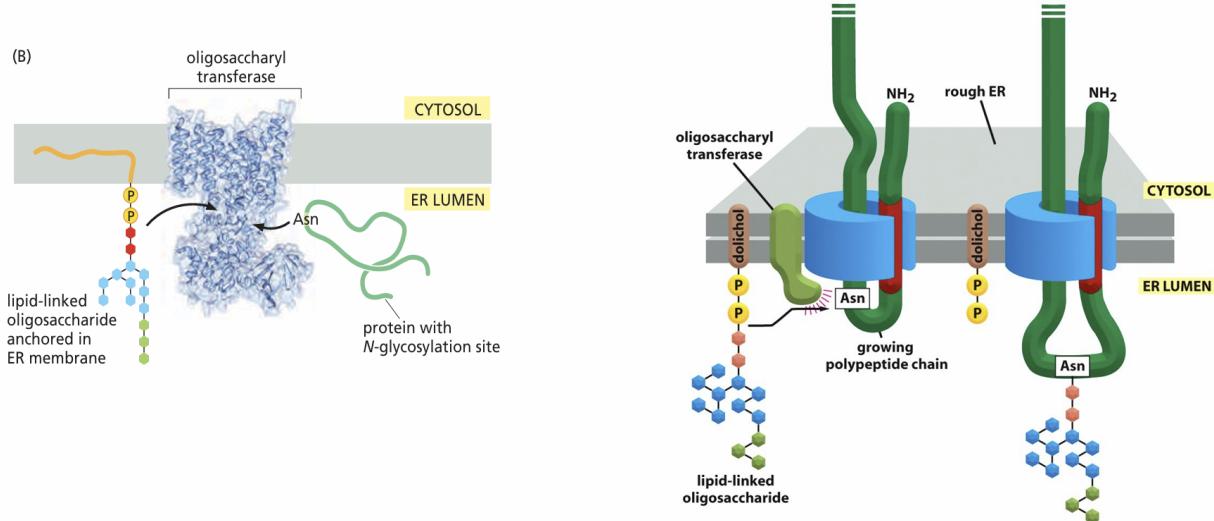


Figure 77: ER linked glycosylation

In the ER glycosylation occurs **all at once**, where the entire "sugar tree" that was built on diolchol is transferred to the protein as it passes through the translocator complex.

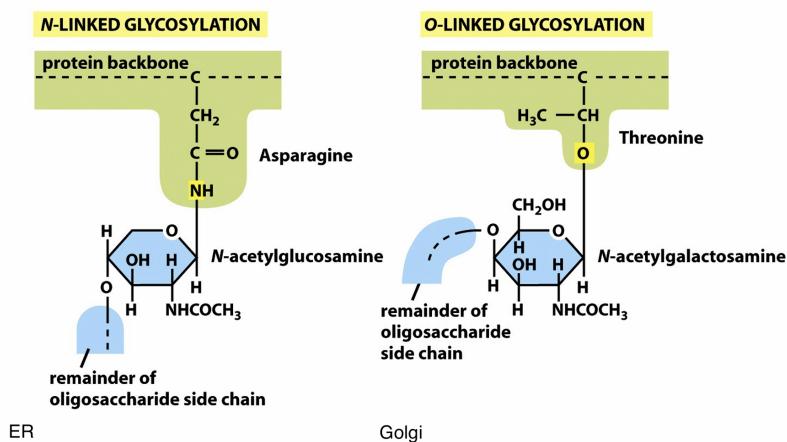


Figure 78: N linked vs O linked glycosylation

**N linked glycosylation vs O-linked glycosylation** There is a slight difference in glycosylation depending on where it is produced. The glycosylation occurring in the **ER** will be **N-linked** (i.e on an Oxygen atom) while the glycosylation occurring in the **Golgi** will be **O-linked** (i.e on a Nitrogen atom)

#### 4.4.0.2 Calnexin/Calreticulin cycle & ER protein folding

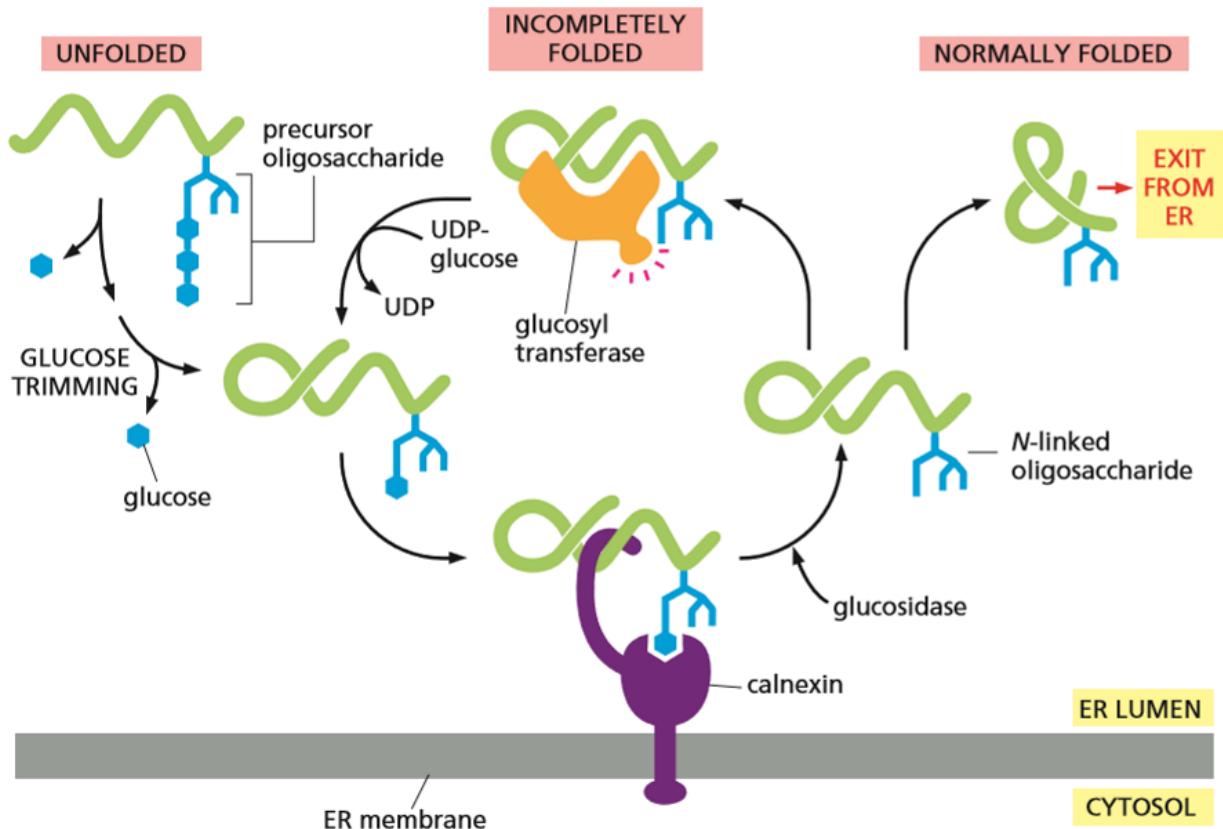


Figure 79: Calnexin/Calreticulin cycle cycle in ER

**Calnexin** and **calreticulin** are two chaperones that will **bind to N-linked sugars**. This binding then prevents the protein from aggregating before it is fully folded.

If the protein has completed folding **Glucosyltransferase** will add a glucose. At the same time **Mannosidase** will **cleave off mannoses from the sugar tree that was added all at once**. The amount of mannose is thus an indicator of how long the protein has been trying to fold. If it takes too long it will be marked for destruction by addition of **polyubiquitin** that signals them to be sent to the **proteosome**.

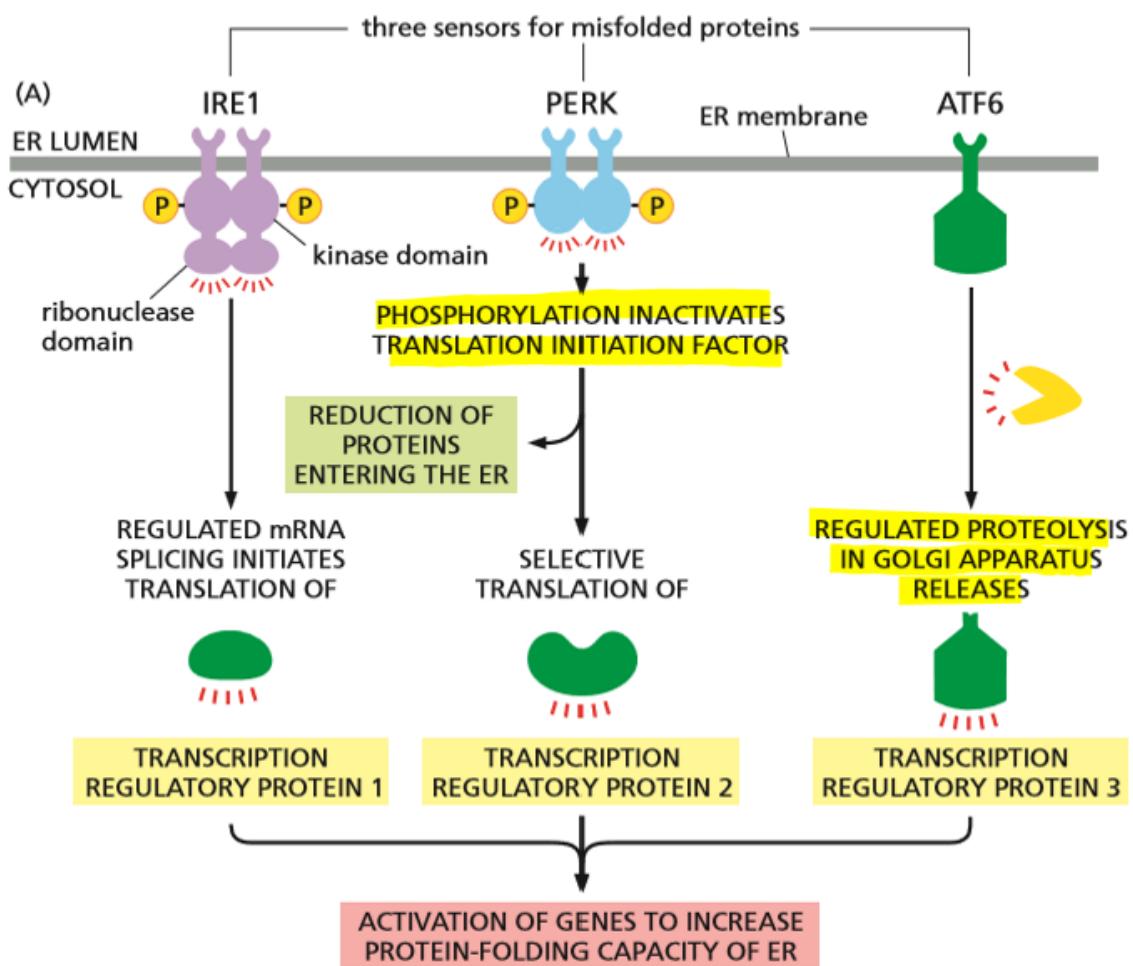


Figure 80: the unfolded protein response

### The unfolded protein response

- Misfolded proteins in ER signal the need for more ER chaperones. They bind to and activate a transmembrane kinase.
- Activated kinase unmasks an endoribonuclease activity (domain that cuts RNA).
- Endoribonuclease cuts specific RNA molecules at two positions, removing an intron.
- Two exons are ligated to form an active mRNA.
- mRNA is translated to make a transcription regulator.
- Transcription regulator enters nucleus and activates genes encoding ER chaperones.
- Chaperones are made in ER, where they help fold proteins.

## 4.5 phosphatydilcholine synthesis in Er

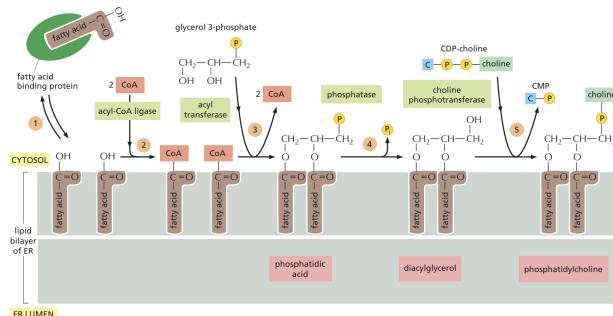


Figure 81: Phosphatydil Choline Sythesis

Phosphatydil choline is synthesized on the **cytosolic leaflet of the ER**. This would lead to assymetries where there no **scrabblases** to shuffle the lipids between the two leaflets.

## 5 Cell Signaling: Principles

### 5.0.1 The Basic Vocabulary of Cell Signaling

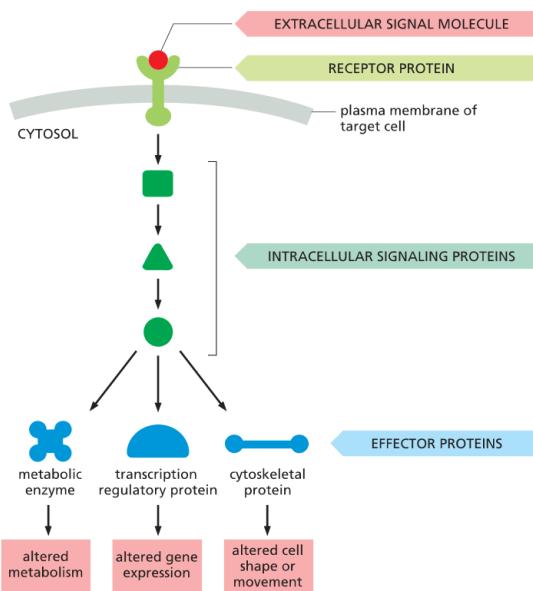


Figure 82: Basic Vocabulary of Cell Signaling

There are some key terms in Cell Signaling. Here's a run down of how they connect in cell signaling:

- An **Extracellular Signaling Molecule** binds to a **signal receiving protein or receptor**.
- That Receptor is generally a transmembrane protein but can also be intracellular. This receptor is activated through the binding (generally some sort of conformational change).

- iii) This causes a **Signaling cascade** through a chain of **intracellular signaling proteins** activating each other, usually branching out.
- iv) The final protein in that cascade will then change the activity of the **Effector proteins**, which launches the cellular response.
- v) These **effector proteins** can be metabolic enzymes, transcription regulators, or cytoskeletal proteins.

### 5.0.2 Cell-surface vs. Intracellular Receptors

There are two main differences between Cell-Surface and Intracellular receptors:

First, the location of the receptors (surprised you with that am I right):

- Cell-Surface Receptor: generally transmembrane protein, where the signaling molecule binds extracellularly.
- Intracellular Receptor: The receptor protein will be close or even inside the nucleus.

Then, accordingly the signaling molecule will also be different, in the case of:

- Cell-Surface, it is generally a **hydrophilic** signaling molecule. This means the molecule can't enter the cell, so we need the receptor to have some extracellular component.
- Intracellular, it is a small **hydrophilic** signaling molecule, which can transfer the cell membrane. This is necessary as it needs to reach the receptor in the nucleus. They are carried through the blood by carrier proteins (hydrophilic).

### 5.0.3 The Four Subtypes of Cell Signaling and Two Variations

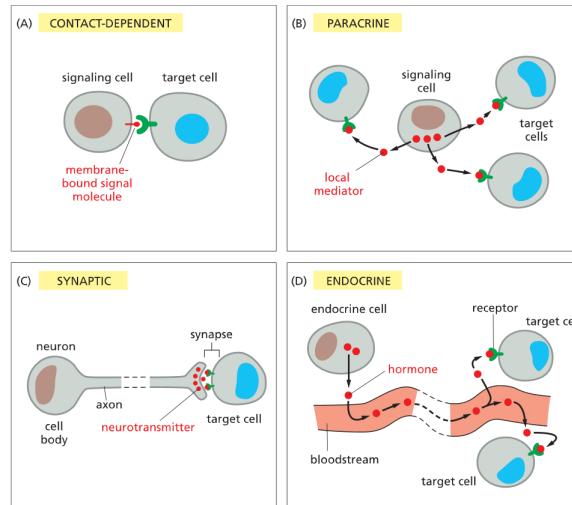


Figure 83: The Four types of Signaling

#### Contact Dependent Signaling:

- Area of signal: Cells are in contact.
- Form of communication: Proteins which are attached to the cells interact. One protein serves as the signal and the other as receptor.

- Variation: the cell can also have contact dependent interactions with the extracellular matrix (e.g., collagen), for more details see section on ECM.

### Paracrine Signaling:

- Area of signal: Cells are not in contact. This is usually a local signal, just a few cells away.
- Form of communication: One protein secreted by a cell, is the signal or ligand and attaches to the receptor of a different cell.

### Synaptic Signaling:

- Area of signal: Cells are not in contact. Small distance between releaser of ligand and receptor, called the synapse. Very local signal.
- Form of communication: Secretion of a ligand or Neurotransmitter. Released by one cell and received by another.

### Endocrine Signaling a.k.a. hormonal signaling:

- Area of signal: Cells are not in contact. Can be long distance and have effects from anywhere to anywhere
- Form of communication: A hormone is produced by cell A and then released into the bloodstream, where it can then leave at some point and serve as a signal to a receptor protein.

**Definition 5.1 (Autocrine Signaling).** If a cell receives its own signal it is called autocrine signaling.

**Definition 5.2 (Constitutively Active).** A protein (usually a receptor or enzyme) that is always active, regardless of whether it has received a signal or a ligand is bound. This can lead to uncontrolled signaling and is often seen in cancer. The reason for this is generally a mutation to the gene of the protein.

#### 5.0.4 The diversity in Signals

The same signal can cause a multitude of signals. This section will have a look of the consequences and opportunities of that.

**Multiple signaling molecules**, better the combination can cause very different signals: Depending on the combination of signals received a cell can kill, proliferate, or differentiate itself. Further the same signaling ligand or protein can have very different consequences depending on the receptor or cell it attaches to. See for example acetylcholine:

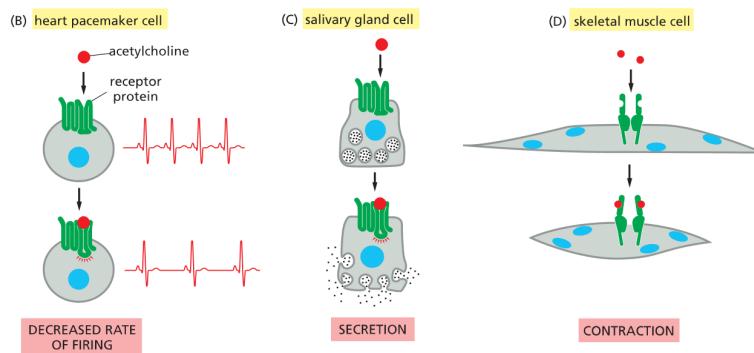


Figure 84: Examples of Acetylcholine having vastly different responses to its signal.

**Speed of the response:** Depending on the response path, the response by the cell can be fast or slow. If the response alters a protein it will take seconds to minutes, while if the gene has to be transcribed it takes minutes

to hours. These two types are called **Protein Response** or **Transcriptional response**. Some receptors also cause both the fast and slow response path.

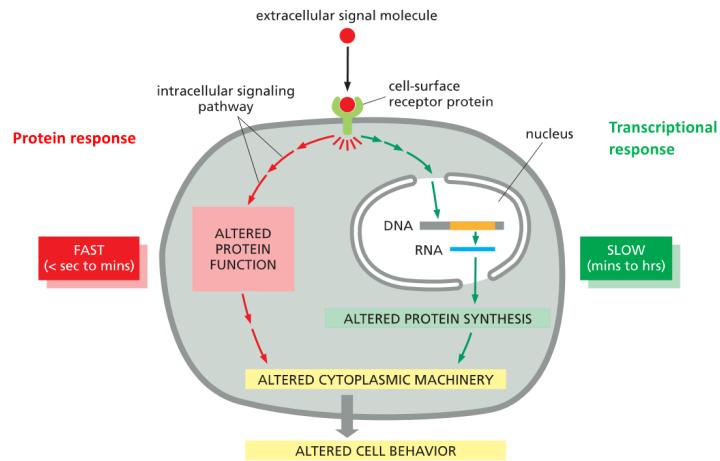


Figure 85: An overview of protein (fast) vs. transcriptional (slow) response.

Examples of a fast response: change in movement, secretion, or metabolism, caused by e.g., phosphorylation. Concretely the recruitment of GLUT transporters from recycling endosomes, has to occur very rapidly once insulin docks onto the receptor.

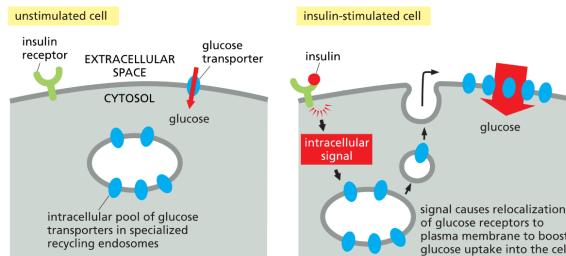


Figure 86: An example of a fast protein response with the GLUT transporter.

### 5.0.5 Classes of Cell-Surface Receptors

There are three main classes of cell-surface receptors, which we will all be diving into later on:

- i) Ion-channel-coupled receptors a.k.a. transmitter-gated ion channels;
- ii) G-protein-coupled receptors;
- iii) Enzyme coupled receptors;

Note on the enzyme-coupled one: There are two options here: one where the enzyme is part of the receptor and another where the enzyme is recruited. Ligands activate the receptors by promoting their dimerization though, regardless if the enzyme is directly attached or not.

### 5.0.6 Regulation of Intracellular Signaling Proteins

There are two main **Molecular switches** for intracellular signaling proteins:

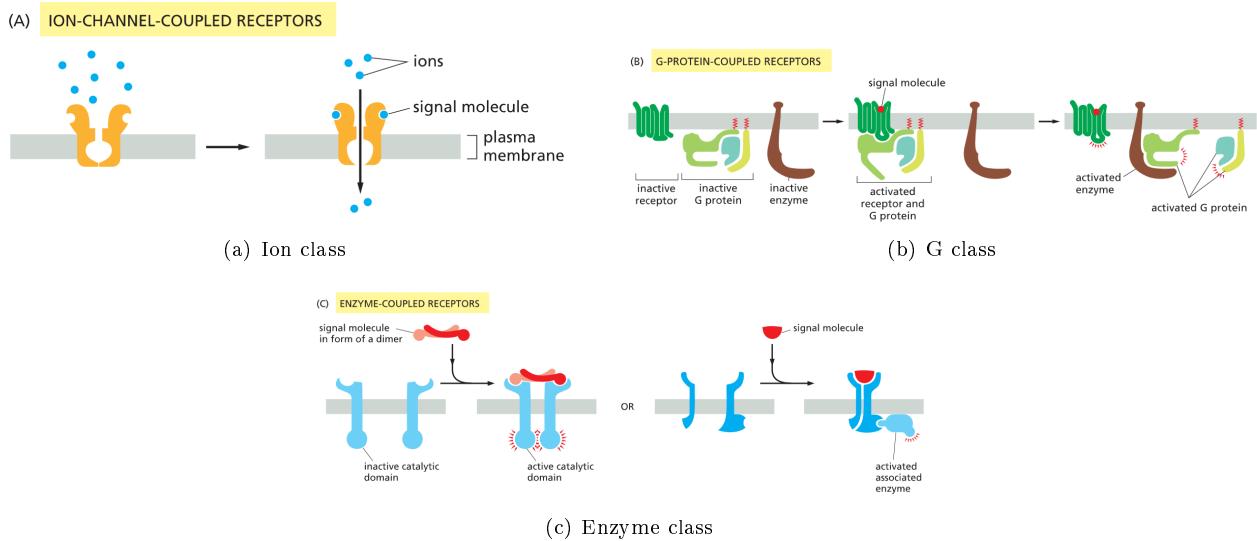


Figure 87: The three classes of cell-surface receptors

### i) Phosphorylation

- based on a phosphate group being attached to the protein (attached means active).
- phosphorylation or dephosphorylation often leads to change in formation and to activation.
- Addition by kinases.
- Removal by phosphatases.
- This group can be added to three amino acids: Tyrosine, Threonine, or Serine. This is because they have an alcohol which works for the attack on the phosphate.

### ii) GTP binding

- **GTPases** are molecular switches that cycle between active (GTP-bound) and inactive (GDP-bound) states. This is also a type of G-Protein, but a different class of monomeric "small" GTPases.
- A phosphate is removed from GTP to make GDP, this deactivates the molecule. GDP stays bound.
- With an incoming signal, this GDP can be exchanged for a GTP.
- **GEF** activate GTPases, by exchanging GDP for GTP.
- **GAPs** inactivate GTPases by hydrolyzing GTP and yes GAP stands for GTPase-activating protein, as it activates the inactivation.
- Since the cell has a ratio of 10:1 for GTP:GDP, the exchange of GDP to GTP is very favorable.

### 5.0.7 Inhibitory Signals as Activators

Signal transduction isn't always a positive signal, sometimes a **Inhibitory signals** can lead to activation. Basically the idea is to **inhibit the inhibitor**.

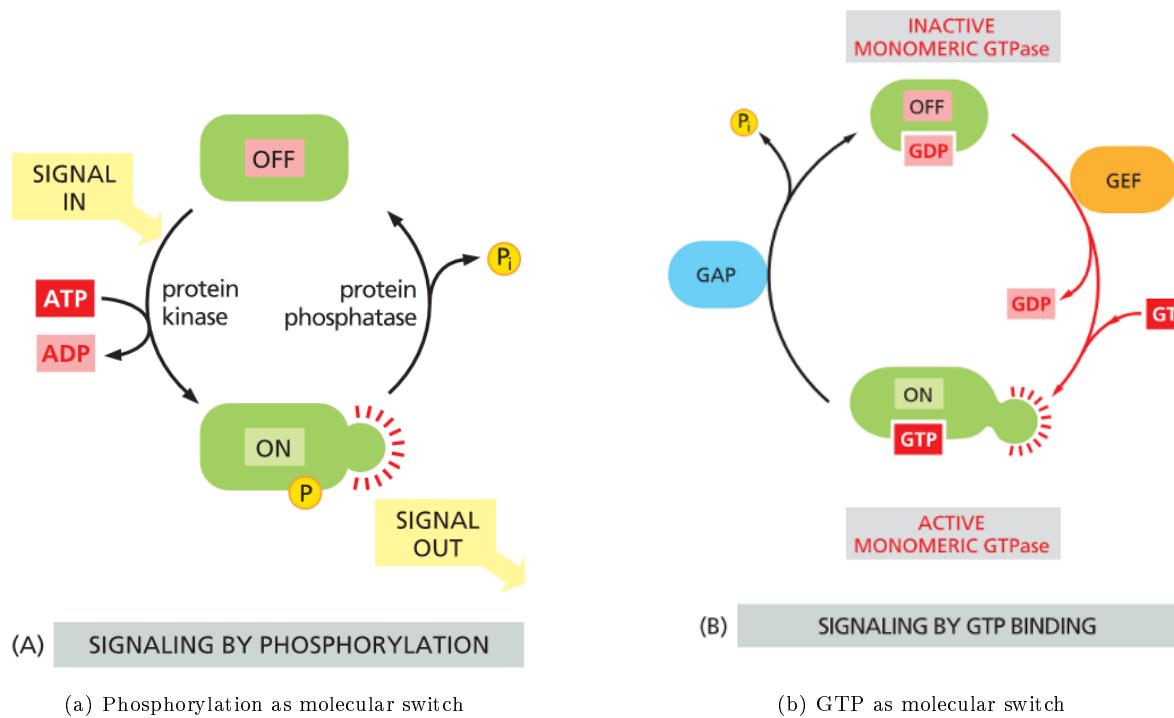


Figure 88: Two types of molecular switches in intracellular signaling

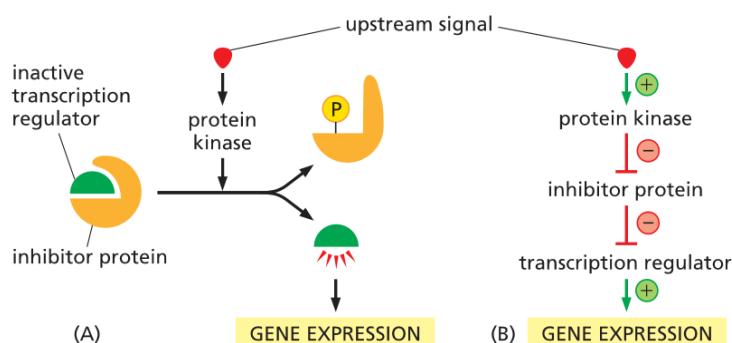


Figure 89: Example pathway of an inhibitory signal leads to activation. Note that the left and right path show the same pathway.

### 5.0.8 Initiating the Signal

A signal starts through a protein being in **close proximity** to the signaling compound. This proximity is key and the minimum for a signal to start, additionally ATP can also be required.

There are three main types of starts to signaling (see figure 90):

#### i) Preassembled signaling complex

- The signal complex is already assembled with all its intracellular signaling proteins, generally in the form of a **Scaffolding protein**.

## ii) Protein Recruitment

- the signaling proteins are in close proximity. Once the signal molecule attaches they attach to the receptor.
- For the signal to be activated, the signaling proteins don't always need to be physically attached, but just being in close proximity is enough.

## iii) Lipid recruitment

- Instead of having the signaling proteins attach to the receptor they attach to a Phosphoinositides (PI, a type of phospholipid). PIs are part of the membrane, in close proximity to the receptor
- These phospholipids can be phosphorylated in the cell, which allows the signaling proteins to attach.

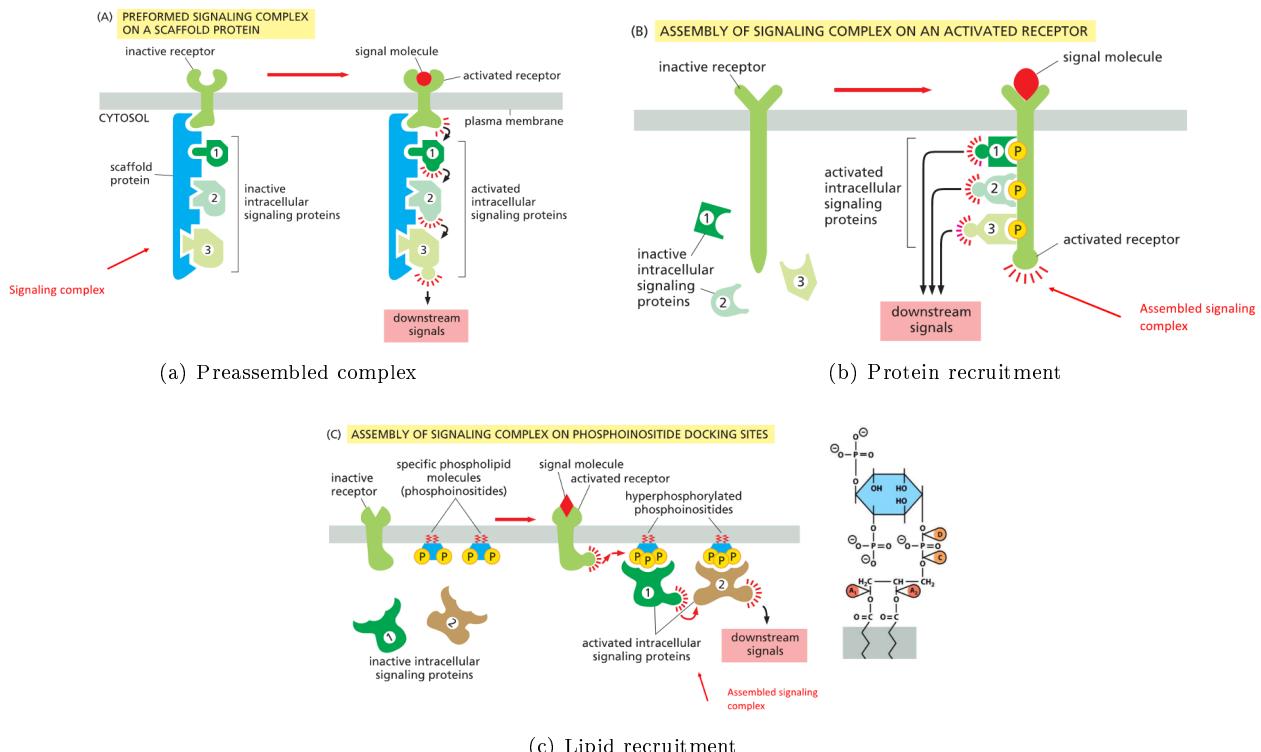


Figure 90: The three classes of cell-surface receptors

These signaling complex's got their name because they can get very complex. They are formed using **Modular Interaction Domain**. Here is an example of an insulin receptor:

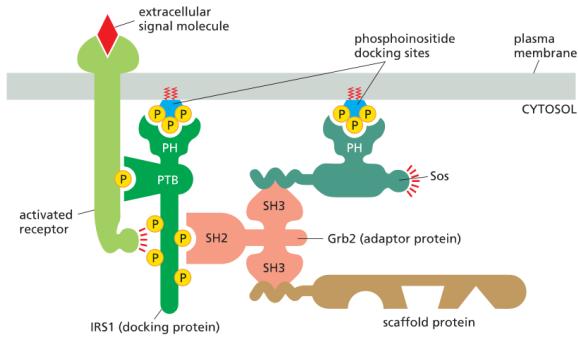


Figure 91: insulin signaling complex as an example for the complexity and modularity of a signaling complex.  
startins

The shortcuts of the molecules in the figure ??:

- PH = **Pleckstrin Homology (PH)** - binds to phosphorylated PI's
- PTB = **Phosphotyrosine Binding (PTB)** - binds phosphotyrosine
- SH = **Src Homology (SH)**, Src is on the first signaling proteins identified in a viral induced chicken sarcoma.
- IRS = **Insulin Receptor Substrate (IRS)**

#### 5.0.9 Regulating and Dampening the Signal

One way a cell can add extra regulation to a pathway is to require multiple independent pathways to integrate for them to signal downstream, called **Signal Integration**.

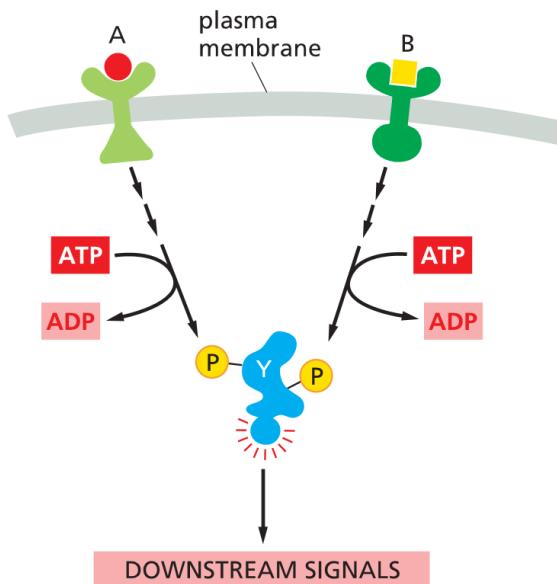


Figure 92: An example pathway showing how multiple streams need to come together to allow downstream signaling.

Now, this need for multiple proteins gives the cell the power to change the response duration and strength depending on how it changes the production and degradation rate of each protein.

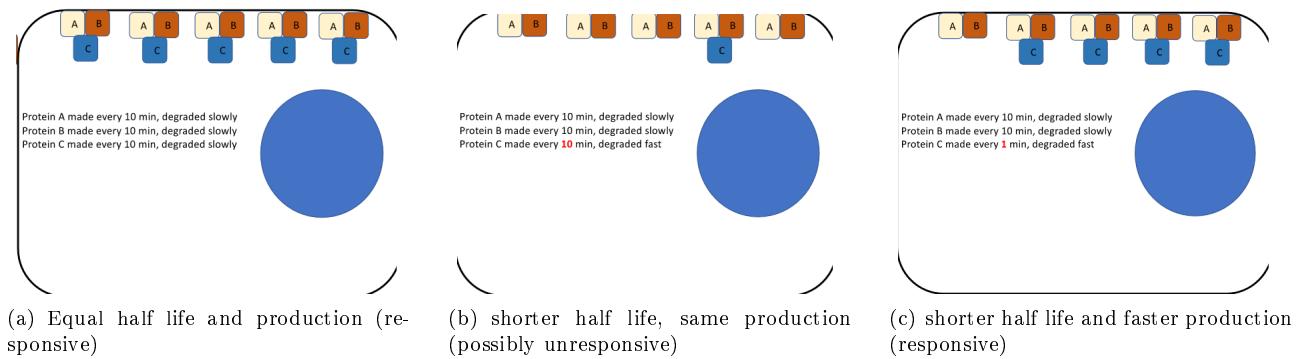


Figure 93: An example of how influencing the half life and production of certain proteins can seriously influence the responsiveness of a protein complex.

)halflife

To further show the role and importance of degradation in protein complexes here are some graphs:

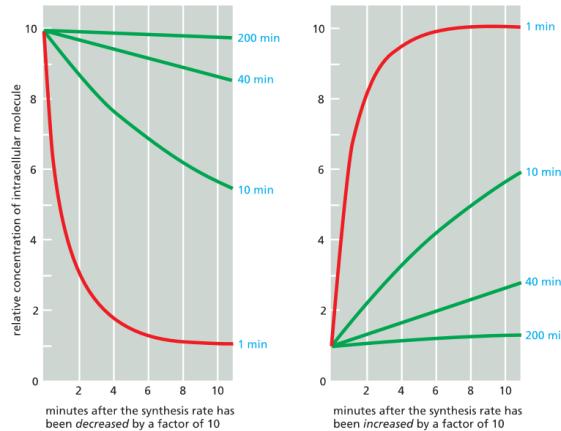
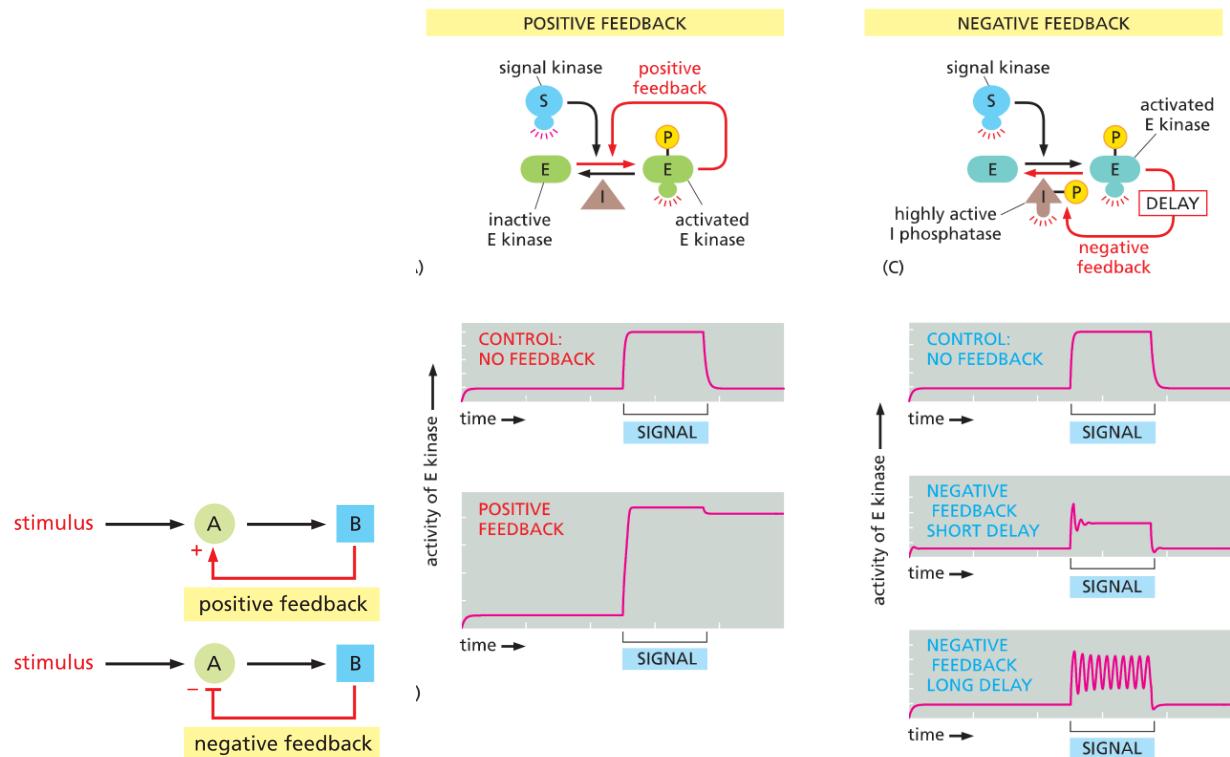


Figure 94: Shows the connection between Half-Life (blue time) and the synthesis rate. The x-axis is the time after the synthesis rate is increased by a factor 10, the y-axis the concentration of the intracellular molecule. A protein with a short half-life will probably also have a high production rate (red line) as otherwise it will be unresponsive (see fig: ??). Hence it will react much more strongly to a signal than a protein with a long half-life and slow production rate (green line).

The next key concept is **Positive Feedback and Negative Feedback**. This is when a downstream molecule will signal upwards in the pathway to either increase or decrease its activity.



(a) Shows both positive and negative

(b) Shows both a positive and negative feedback loop and corresponding time vs. enzyme activity graph.

Discussion of the fig: 5.0.9: in feedback the positive one is pretty straightforward. You add positive feedback the signal gets extended. For negative feedback the type of delay which is in effect plays a major role, as the dampening gets stronger the more is being produced upstream. So, the reaction is stronger there more upstream there is.

- Short feedback delay: in this case after a short pretty strong response it finds a stable damped state pretty quickly.
- Long feedback delay: here the signal becomes strong, meaning we get a strong but delayed feedback reaction, once that feedback hits, it kills the signal too strongly, so the feedback gets turned back really strongly. That again allows the signal to become strong and we start over again.

Next up is **adapting the extracellular signal molecule**. This will lead to the desensitization or sensitization of the signal molecule. This happens mainly by messing around with the receptor protein, its quantity and function. It is often done through phosphorylation or ubiquitylation of the receptor proteins. Some are also cases of feedback:

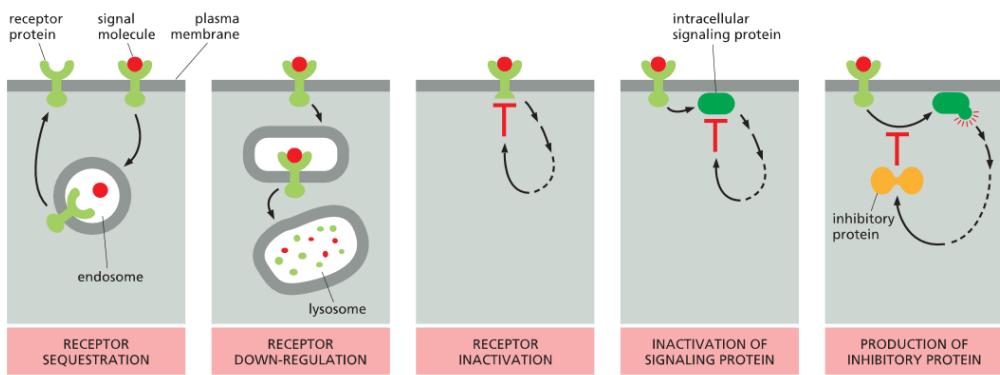


Figure 95: A bunch of ways the extracellular signal molecule's strength on the pathway can be adapted. This happens mainly by messing around with the receptor protein, it's quantity and function.

#### 5.0.10 All or nothing, Hyperbolic, Sigmoidal Signals

There are three main shapes a signal response will take on:

- **Hyperbolic signal:** a gradually increasing cell response to a gradually increasing signal, eventually reaching a plateau.
- **Sigmoidal Signal:** it takes a while for the signal to take effect, but then results in a steeper reaction at some intermediate concentration
- **All or nothing signal:** extreme form; nothing happens until a certain concentration threshold is reached and then we get a full signal.

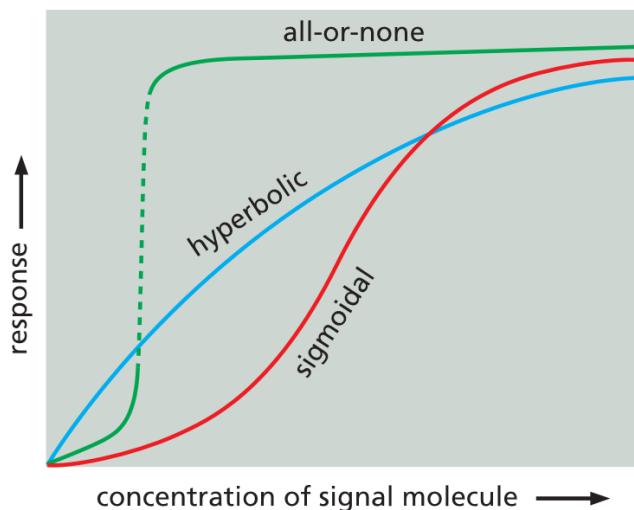


Figure 96: The three shapes a response tends to take in reaction to the signal. This is determined by how it is processed.

When we analyze cells it is important to remember that we are taking an average response of all cells. While a hyperbolic response average is probably hyperbolic in all cells, what appears to be sigmoidal could actually

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be a all or nothing response with some cells firing and others doing nothing. Hence, it is important to analyze the individual cells too. Here is a visualization:

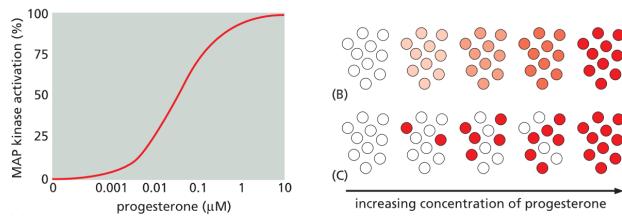


Figure 97: What appears to be sigmoidal may actually be all or nothing.

## 6 Cell Signaling The World of G-proteins

### 6.1 The Components of a G-Protein Pathway

Guanine nucleotide-binding proteins or G-Proteins are a major type of cell-surface receptor. There are many different types of G proteins.

#### 6.1.1 G-Protein-Coupled Receptor or GPCR

the G-protein-coupled-receptor (GPCR) is the place the ligand attaches too. Then the GPCR will activate the G-protein. GPCR uses trimeric G-proteins.

**Structure:** A GPCR has seven transmembrane regions, composed of **7 alpha helices and 6 loops**. It has a N-terminal extracellular region and a C-terminal intracellular region. The alpha helices form a pocket for the ligand to bind. Depending on the size of the ligand GPCR will have a differently sized extracellular domain to accomodate for the ligand, while remaining specific. There are over 700 different GPCR in humans.

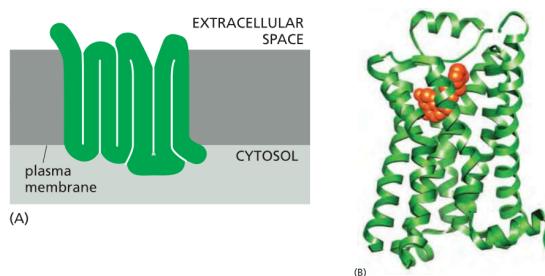


Figure 98: Simplified image of GPCR in membrane and its 3D structure.

### 6.1.2 Heterotrimeric G Protein

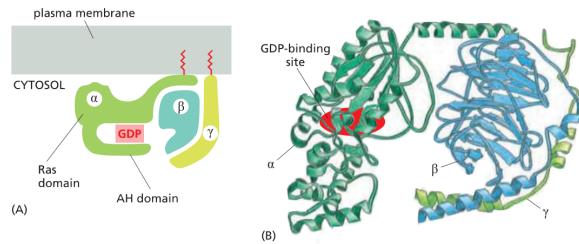


Figure 99: Simplified image of G-Protein in membrane and its 3D structure.

Some quick facts:

- 3 proteins that make up the G-protein
- The G-proteins Alpha Subunit is a GTPases.
- GPCR uses trimeric G-proteins.
- at least 20 different alpha subunits exist.
- there are numerous different Beta Complex and Gamma Complex, meaning we have quite a number of different G-proteins out there.

There are a bunch of different trimeric G-proteins, which are split into four major families, which all have different functions:

TABLE 15-3 Four Major Families of Trimeric G Proteins*			
Family	Some family members	Subunits that mediate action	Some functions
I	G <sub>s</sub>	α	Activates adenylyl cyclase; activates Ca <sup>2+</sup> channels
	G <sub>olf</sub>	α	Activates adenylyl cyclase in olfactory sensory neurons
II	G <sub>i</sub>	α	Inhibits adenylyl cyclase
		βγ	Activates K <sup>+</sup> channels
	G <sub>o</sub>	βγ	Activates K <sup>+</sup> channels; inactivates Ca <sup>2+</sup> channels
		α and βγ	Activates phospholipase C-β
III	G <sub>t</sub> (transducin)	α	Activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
	G <sub>q</sub>	α	Activates phospholipase C-β
IV	G <sub>12/13</sub>	α	Activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

\*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

Figure 100: Shows the four major families of trimeric G-proteins.

**Function:** The Ras Domain is part of the alpha subunit and is related to GTPases and provides a face for GDP/GTP to bind too. The Alpha Helix (AH) domain binds it in place. Activation from a GPCR triggers the release of GDP from the alpha subunit followed by the binding of GTP

### 6.1.3 Activation of G-Protein by GPCR

Here is how GPCR activates a G-protein:

- i) An extracellular signal molecule binds to the GPCR molecule;
- ii) The GPCR molecule changes conformation, which allows it to bind to the Ras domain of the G-protein;
- iii) This alters the conformation of the alpha subunit, specifically the alpha helix subunit, releasing the GDP.
- iv) The binding of GTP then promotes the closing of the subunit
- v) This triggers conformational changes causing the alpha subunit to dissociate from both the GPCR as well as the beta-gamma subunit.
- vi) Both the alpha and the beta-gamma subunit then become active in downstream pathways.
- vii) GPCR stays active as long as the ligand is bound to it, meaning it can activate many G-proteins.

How an activated G-protein starts the Downstream Cascade:

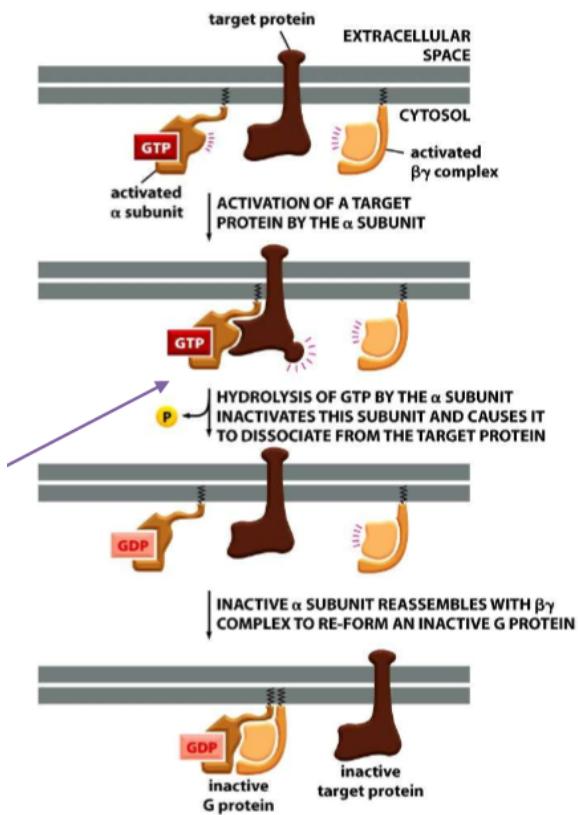


Figure 101: Once we have activated the G-protein and the subunits split, once they are used and the GTP is converted to GDP the inactive subunits merge back together and the cycle can start again.

The main **downstream targets**:

- **Adenylate Cyclase**, which in turn increases or decreases **cAMP** (very common target);

- Channels;
- PLC, which in turn generates IP<sub>3</sub> and diacylglycerol.

#### 6.1.4 Stopping GPCR signaling

The signalling of GPCR can be stopped through **GPCR Kinases (GRKs)** (**GRKs**) and **Arrestins**, as they cause desensitization of the GPCR. The process:

- i) **Negative Feedback:** Activated GPCR stimulates GRKs which phosphorylate the GPCR on multiple sites. Note therefore GRK can only phosphorylate activated receptors.
- ii) This allows the Arrestin to prevent the receptor from binding to its G-protein and directs the receptors endocytosis.

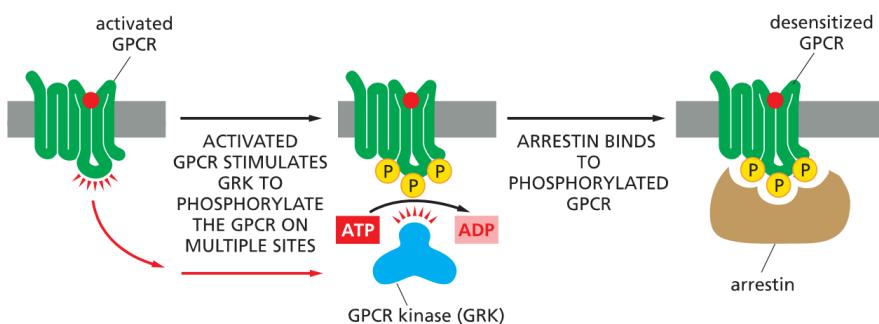


Figure 102: How GRK is a negative regulator, through negative feedback, for GPCR.

## 6.2 GPCR signaling through Cyclic AMP a.k.a. cAMP

### 6.2.1 cAMP

Cyclic AMP a.k.a. cAMP is a derivate of ATP. Two phosphates are replaced by a sugar bond, by enzyme **Adenylate Cyclase**. cAMP is a shortlived molecule which is "uncycled" to 5'-AMP, by enzyme **cAMP Phosphodiesterase**. The fact the molecule is so shortlived makes it great as a signaling molecule.

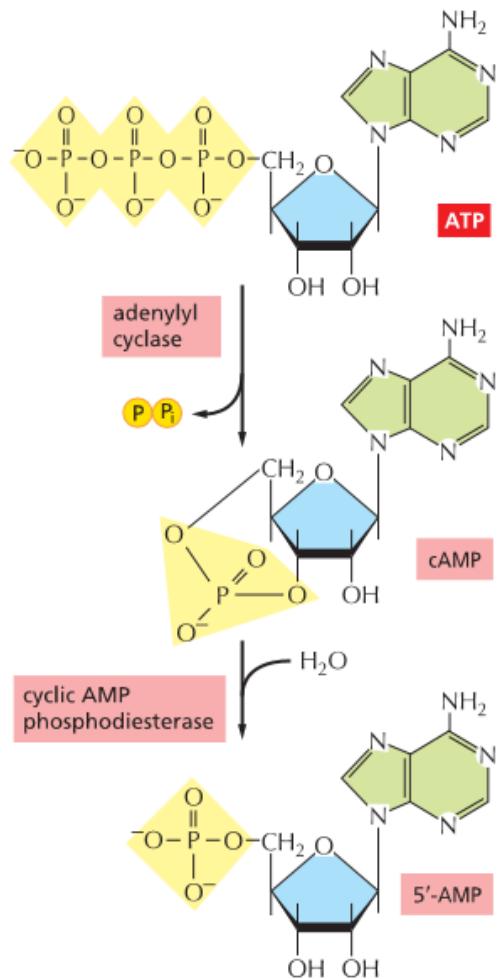


Figure 103: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

*Remark 6.1 (cAMP jumping between cells).* cAMP can be transported to other cells via **GAP Junctions**.

#### 6.2.2 cAMP as a signaling molecule

The pathway is as follows:

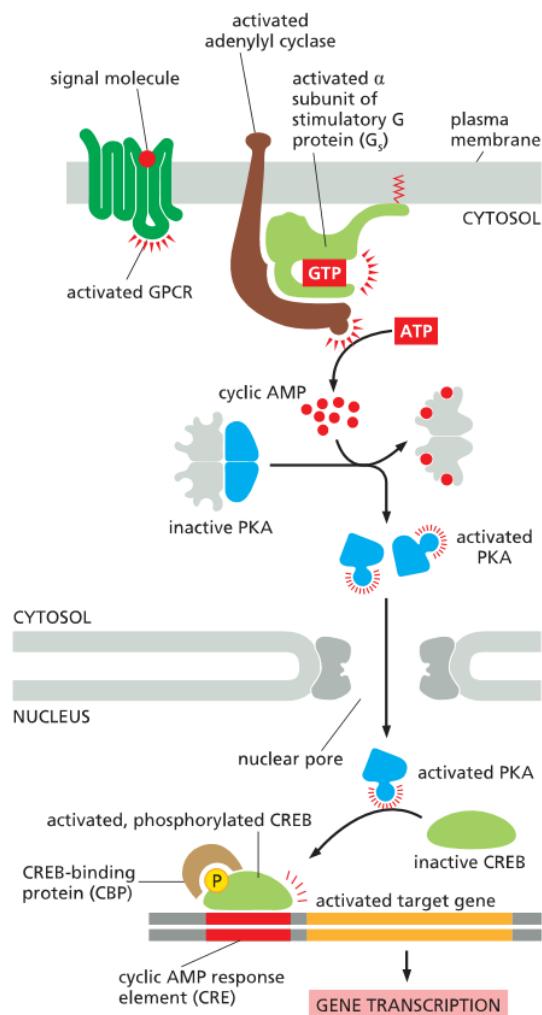


Figure 104: The production of cAMP with the enzymes adenylyl cyclase and cAMP phosphodiesterase.

- Activation of GPCR:** GPCR gets activated, which in turn activates the adenylyl cyclase and the G-protein.
- cAMP produced:** The activated adenylyl cyclase converts ATP into cAMP.
- Activation of PKA:** The main role of cAMP is the activation cAMP-dependent protein kinases (PKAs). By binding to the regulatory subunits of the PKA tetramer induces a conformational change, which makes the regulatory subunits to dissociate from the catalytic subunits activating them. This release requires multiple cAMPs per regulatory unit. This means a lot of cAMP is required, as cAMP quickly decays, so we get a pretty sharp response.

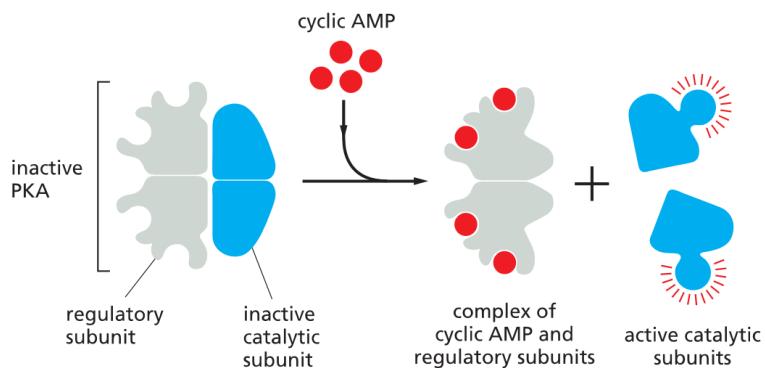


Figure 105: Shows the activation of PKAs by cAMP.

- iv) The active PKA is then translocated to the nucleus, where it activates a transcription factor CREB (cAMP response binding protein) through phosphorylation.
- v) CREB interacts with CREB-binding protein and activates transcription on the cAMP response element.

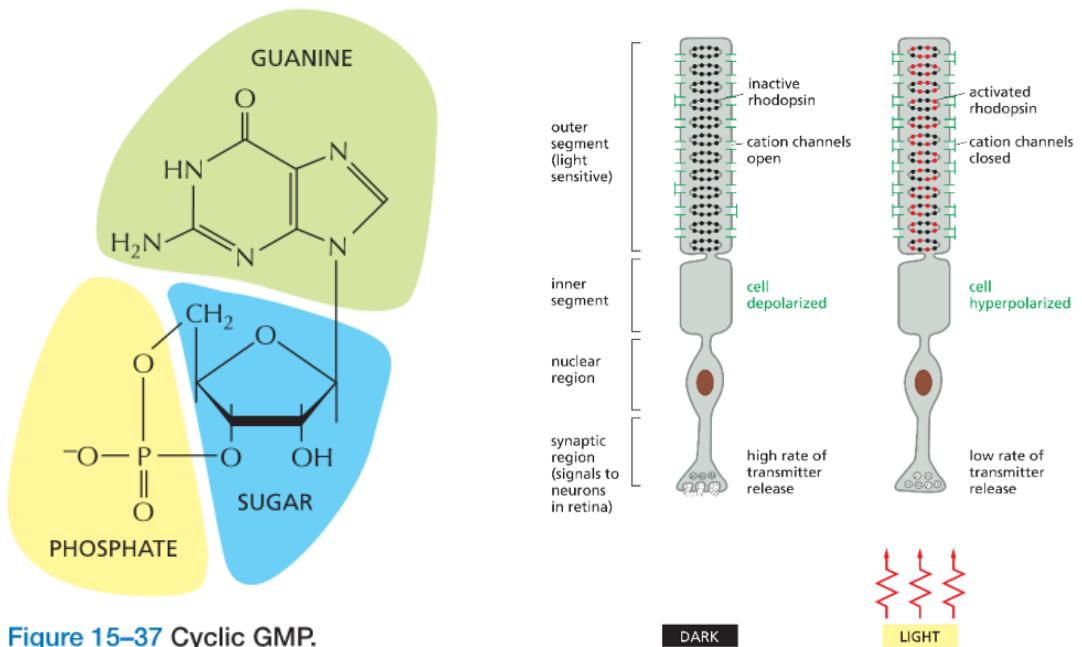
The activation of GPCR by a ligand, say serotonin, increasing the signal strength 20fold in a matter of seconds. Depending on the cell and the ligand we will get very different cell responses. The PKA however will always be the same and it is the substrate of the PKA that changes. Hence we can receive so many different responses. Here are some examples with hormones:

TABLE 15-1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP		
Target tissue	Hormone	Major response
Thyroid gland	Thyroid-stimulating hormone (TSH)	Thyroid hormone synthesis and secretion
Adrenal cortex	Adrenocorticotropic hormone (ACTH)	Cortisol secretion
Ovary	Luteinizing hormone (LH)	Progesterone secretion
Muscle	Adrenaline	Glycogen breakdown
Bone	Parathormone	Bone resorption
Heart	Adrenaline	Increase in heart rate and force of contraction
Liver	Glucagon	Glycogen breakdown
Kidney	Vasopressin	Water resorption
Fat	Adrenaline, ACTH, glucagon, TSH	Triglyceride breakdown

Figure 106: The expression using different hormones in different cells. Vasopressin is also a "love" hormone, meanign that when you are in love GPCR is active

### 6.2.3 cGMP

Cyclic-Guanine-Mono-Phosphate or **cGMP** is an alternative in the cAMP pathway. So, sometimes cGMP is activated by GPCR not cAMP. The only difference is the guanine instead of adenine. The enzyme is called **Guanylate Cyclase**.



**Figure 15-37 Cyclic GMP.**

- (a) The structure of cGMP, where the only difference to (b) How the cell changes when light is received. Note that cAMP is the guanine for adenosine.  
 (b) the signal sent to the brain is inverted of how a normal neuron is fired (channels close instead of open).

Figure 107: cGMP

#### 6.2.4 Case study with response to light

The process of recognition is as follows (see fig:107):

- i) a **Rhodopsin** molecule absorbs a photon;
- ii) 500 G-proteins molecules (Transducin) are activated (signal is amplified);
- iii) 500 cGMP Phosphodiesterase molecules are activated;
- iv)  $10^5$  cGMP are hydrolyzed (signal has been amplified);
- v) They block 250 cation channels;
- vi)  $10^6 - 10^7 Na^+$ -ions per second are prevented from entering the cell for a period of around a second (signal has been amplified);
- vii) The membrane potential is altered by 1mV, which in turn relays a signal to the brain.

#### 6.3 GPCR Signaling through phospholipase C

Here are some example cell responses where GPCRs activate PLC $\beta$

TABLE 15–2 Some Cell Responses in Which GPCRs Activate PLC $\beta$

Target tissue	Signal molecule	Major response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Muscle contraction
Blood platelets	Thrombin	Platelet aggregation

Figure 108: Some cell responses where GPCR activates PLC $\beta$ , shoutout to Vasopressin for all the lovin'.

### 6.3.1 Case Study: GPCRs activating Cytosolic Ca $^{2+}$ and activating protein Kinase C

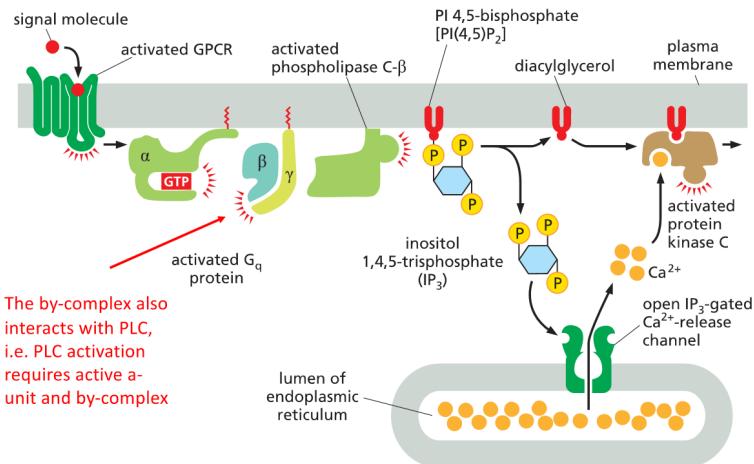


Figure 109: Shows the pathway of GPCR activating Cytosolic Ca $^{2+}$ .

Rundown of the pathway:

- The GPCR activates the PLC $\beta$  via a G-protein called G<sub>q</sub>. The G<sub>q</sub> Beta-gamma complex and the alpha-complex activates the PLC $\beta$ .
- PLC $\beta$  hydrolyzes PI(4,5)P<sub>2</sub>, causing it to split into two messengers
- IP<sub>3</sub> diffuses through the cytosol and releases Ca<sup>2+</sup> from the ER by binding to the IP<sub>3</sub>-gated Ca<sup>2+</sup> channels.
- Then the diacylglycerol, DAG, (other part of PI(4,5)P<sub>2</sub>), remaining in the membrane, together with the Ca<sup>2+</sup> and Phosphatidylserine activate the protein Kinase C (PKC). Of the min. 10 forms of PKC at least 4 are activated by DAG.

### 6.3.2 Ca $^{2+}$ feedback waves and oscillations

The concentration of Ca<sup>2+</sup> plays a big role in activating or inactivating. So, giving itself positive or negative feedback. Here's how:

- Activation:** At low concentrations, Ca<sup>2+</sup> goes to neighboring channels and activates them, causing the release of more Ca<sup>2+</sup> and a wave like reaction across receptors (first couple pics). This means that the channels can stay active even without any IP<sub>3</sub> being present.

- **Inactivation:** When  $\text{Ca}^{2+}$  is present at very high concentrations it inactivates the channels. That means that now we create a wave of inactivation.
- **Oscillation:** In the continued presence of the ligand activator, or even without, this mix of feedback can cause oscillations in  $\text{Ca}^{2+}$  excretion.

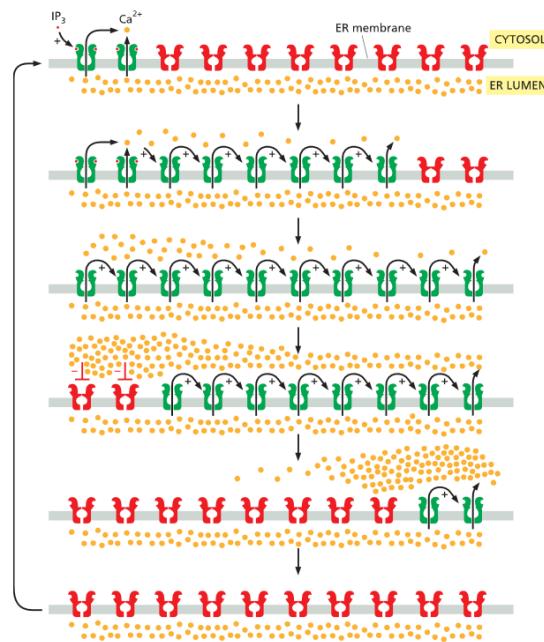


Figure 110: Shows how  $\text{Ca}^{2+}$  influences the activity of its own channels

### 6.3.3 How $\text{Ca}^{2+}$ plays an important role in regulating and relaying signals

**Ca<sup>2+</sup> and Calmodulin:** With the help of  $\text{Ca}^{2+}$ /calmodulin,  $\text{Ca}^{2+}$  is able to bind to target proteins and with that relay the signal. The dumbbell shape of calmodulin and alpha-helix allows it to take on numerous different conformations.

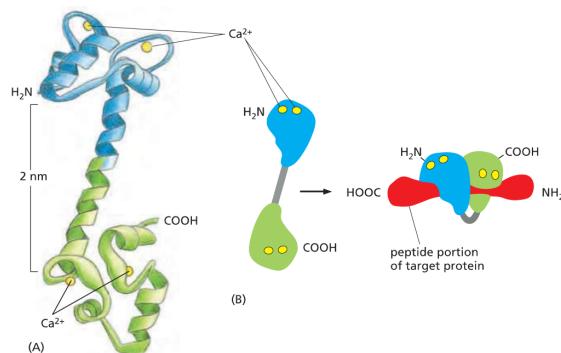


Figure 111: On the left the structure of calmodulin and on the right an example of how it can bind to target proteins (this move is called the jackknife).

**CaM-Kinase II** is regulated by calmodulin. Here's how that runs down:

- i) 6 CaM-Kinase II (green) form a ring.
- ii) The kinase domains pop in and out naturally.
- iii) Calmodulin can bind the popped-out domain in place when it is bound to  $\text{Ca}^{2+}$
- iv) Then that kinase domain gets Autophosphorylation, making it active.
- v) In the continued presence of calmodulin it is even more active.
- vi) Becomes inactive through dephosphorylation
- vii) The more domains are active, the more active the enzyme as a whole.

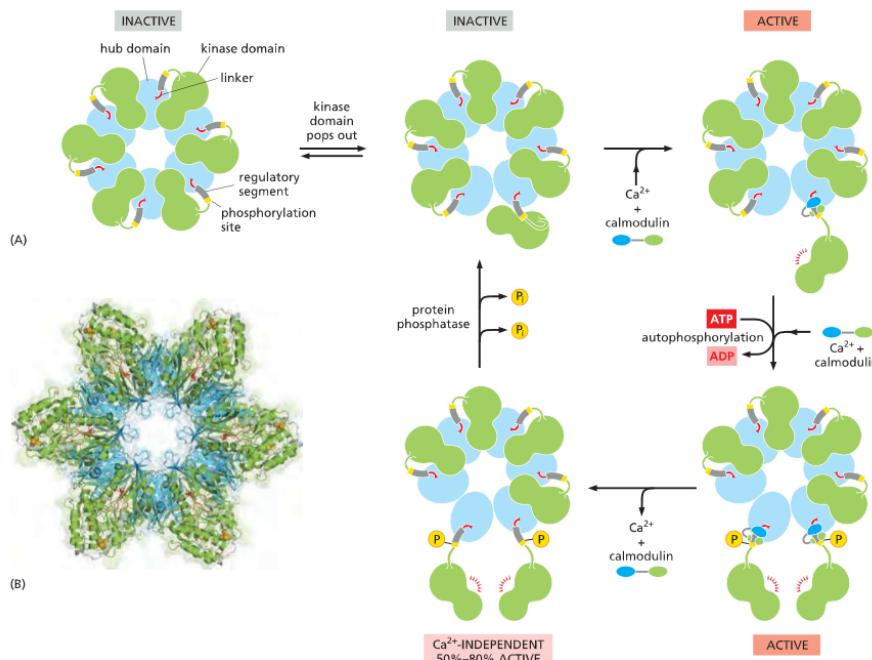


Figure 112: Shows how  $\text{Ca}^{2+}$ /calmodulin activate enzymes, in this case CaM-KII.

Depending on the frequency of the  $\text{Ca}^{2+}$  oscillations, the activity of the enzyme is influenced in major ways. The more frequent the oscillations the more the activity as a whole will rise. For instance at low frequency the autophosphorylation induced by the  $\text{Ca}^{2+}$ /calmodulin binding does not maintain the enzyme's activity long enough for the enzyme to remain active until the next  $\text{Ca}^{2+}$  spike arrives. At a higher spike frequencies, however, the enzyme fails to deactivate completely between the spikes, therefore its activity ratchets up with each spike. Hence CaM-KII is a good mechanism of decoding the frequencies of oscillations in a cell. Here's a figure to visualize:

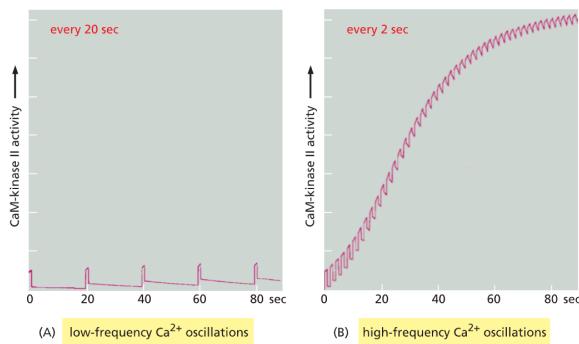


Figure 113: How different frequencies of oscillations cause major differences in enzyme activity.

## 7 Cell Signaling: Receptor Tyrosine Kinase a.k.a. RTKs

Receptor Tyrosine Kinases are a large group, here are some of them:

TABLE 15-4 Some Signal Proteins That Act Via RTKs		
Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (M-CSF)	M-CSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

Figure 114: A bunch of different RTK groups

**RTK** are connected by the fact that they all have an intracellular kinase domain which can phosphorylate a Tyrosine. The extracellular domain on the other hand is completely variable. the kinase region can also have a **Kinase insert region** important for interactions with other proteins.

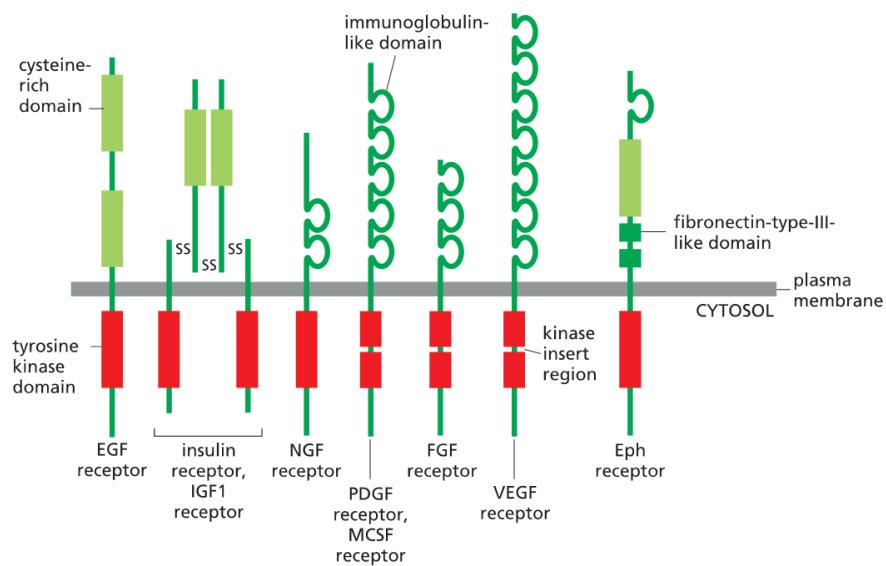


Figure 115: A bunch of different RTK types, with the core features.

## 7.1 Activation of RTKs by Dimerization

- Two RTKs are initially inactive until some type of ligand arrives to bring them together.
- In this proximity the RTKs **dimerize** and make an initial Tyrosine **Autophosphorylation**.
- Once the first phosphorylation has happened that initiates Transphosphorylation of several Tyrosines.
- Phosphotyrosine sites recruit and/or activate downstream signaling proteins.

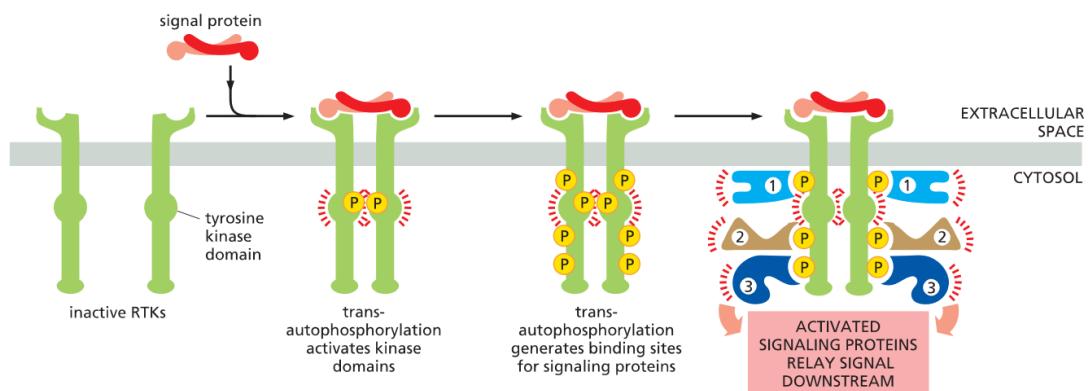


Figure 116: Activation of a RTK dimer

## 7.2 An exception to the rule: Activation of EGF Kinase

Compared to the regular activation the kinase domains are not both auto-phosphorylated to be activated. We still have two identical domains, but one takes on the role of activator, while the other is the receiver.

- Both domains are activated through EGF.

- 
- ii) Then the activator pushes on the receiver, causing a conformational change in the receiver domain, which makes it active.
  - iii) The receiver Kinase domain then phosphorylates the tyrosines on both receptors.

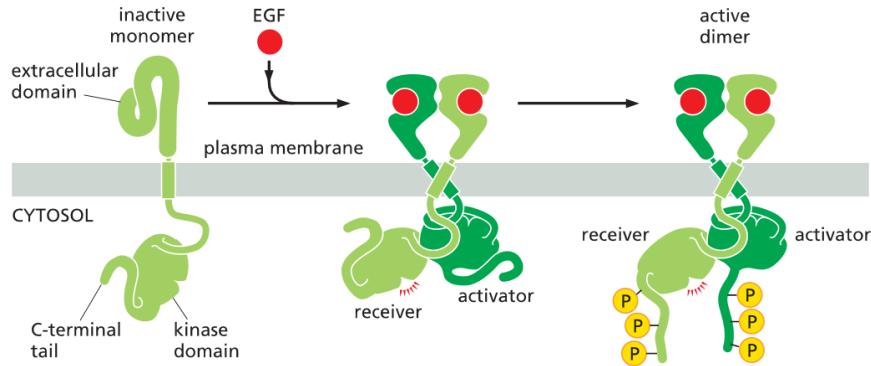


Figure 117: The activation of a EGF receptor kinase.

**Remark 7.1 (Cancerous EGF Kinase).** If the receiver domain mutates in such a way that it is constitutively active, then Ras MPK and PI3K are also always activated resulting in uncontrolled cell growth.

## 7.3 Tyrosine Kinase Associated Signaling

### 7.3.1 JAK-SAT

JAK stands for JANUS Kinases, which are part of **Kinase cytokine receptors**.

They work very similar to a regular RTK, with the JAK-STAT being cytokine receptors:

- i) once the ligand connects the cytokine receptors get together and become active.
- ii) The activation of the JAKs, which are associated with the receptors, happens through cross phosphorylation of each other.
- iii) Then the JAKs phosphorylate the receptors at a Tyrosine, on the receptor.
- iv) Said phospho-Tyrosine can then recruit **STAT** proteins.
- v) Once they have docked they get phosphorylated by the JAKs, making them active.
- vi) Then they enter the cytosol and translocate to the nucleus.
- vii) In the nucleus they associate with a complex and activate transcription.

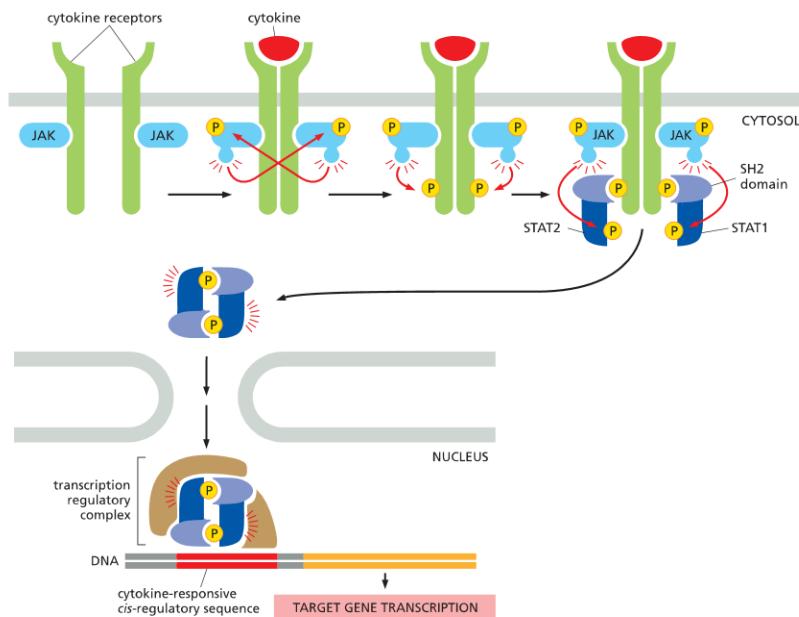


Figure 118: The JAK-SAT pathway. The exact details on the complex for transcription are beyond this course.

There are 4 different JAKs and 7 STATs, which are very important and especially known for immune responses. They are the following:

- JANUS Kinases:
  - i) JAK1
  - ii) JAK2
  - iii) JAK3
  - iv) TYK2 (you thought this was about to say JAK 4 didn't you)
- STATS
  - i) STAT1
  - ii) STAT2
  - iii) STAT3
  - iv) STAT4
  - v) STAT5a
  - vi) STAT5b (gotcha again, not 6 just yet)
  - vii) STAT6

It is a very diversely used pathway, which over 128 extracellular signaling proteins and their receptors using JAK-STAT. Here are some signaling proteins:

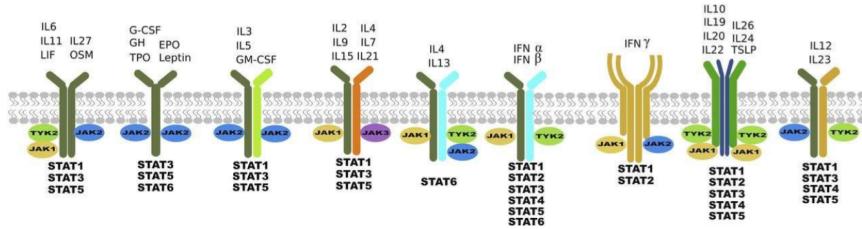


Figure 119: A bunch of signaling proteins which use JAK-STAT.

As per usual here a bunch of different JAK-STATs in the body:

TABLE 15-6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK-STAT Signaling Pathway			
Signal protein	Receptor-associated JAKs	STATs activated	Some responses
Interferon- $\gamma$ (IFN $\gamma$ )	JAK1 and JAK2	STAT1	Activates macrophages
Interferon- $\alpha$ (IFN $\alpha$ )	Tyk2 and JAK2	STAT1 and STAT2	Increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	Stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	Stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	Stimulates growth by inducing IGF1 production
Granulocyte-Macrophage-Colony-Stimulating Factor (GMCSF)	JAK2	STAT5	Stimulates production of granulocytes and macrophages

Figure 120: A bunch of different JAK-STATS in humans

Also JAK-STAT and FGFR signaling inhibition can restore sensitivity to anti-hormonal drugs in prostate cancer [Editor's note: I have no idea how important this is, guessing this is to some degree his research or something.]

### 7.3.2 TGF Beta Signaling

While Karthaus doesn't put it in the tyro-kinase associated box, I feel like it fits, so we putting it here :)

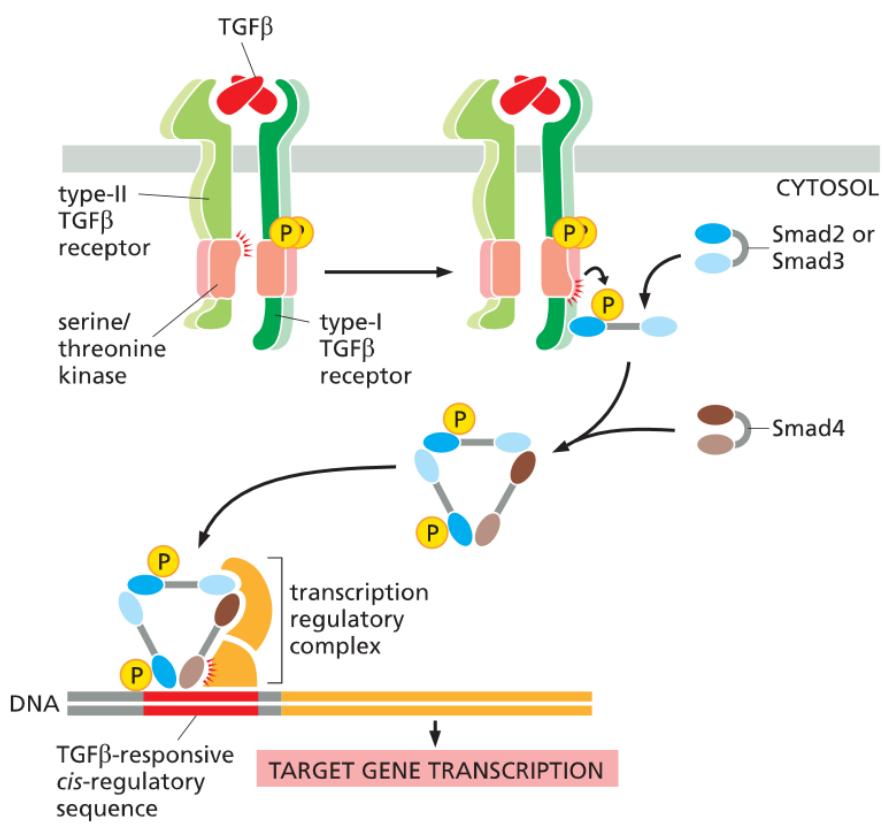


Figure 121: The TGF-beta pathway, using Smad

- i) The TGF beta dimer promotes the assembly of a tetrameric receptor complex of TGF $\beta$ 's containing two copies of the Type I receptor and Type II receptor.
- ii) type-II receptors phosphorylate type-I receptors, which activate their kinase activity.
- iii) type-I receptors then activate R-Smad, such as Smad2 or Smad3.
- iv) This leads p-Smads to open up and be exposed to dimerization of the phosphorylated surface
- v) This leads to trimerization, with the co-Smad, Smad 4.
- vi) This Smad complex then enters the nucleus, and joins an even bigger transcription complex

## 8 RTK and G-Protein: Downstream and Similarities

### 8.1 Checking out how GPCRs and RTKs are intertwined

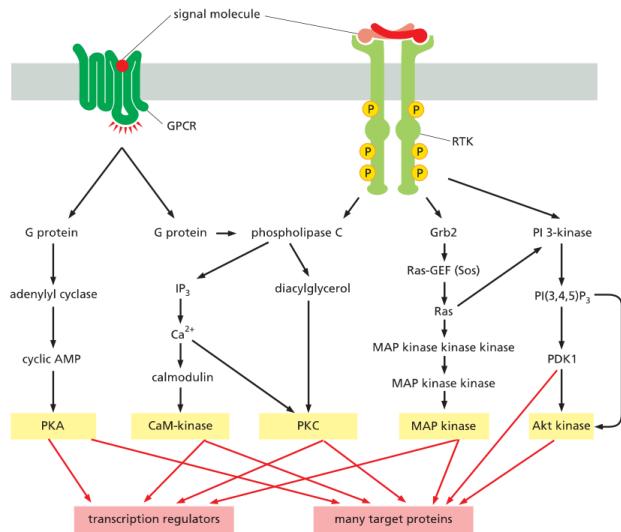


Figure 122: Compares the pathways caused by GPCRs and RTKs, also shows which are shared. All of them end with a Kinase, which then causes a reaction chain downstream

Both GPCR and RTK have a PLC enzyme, called beta and gamma respectively. The effect is very similar.

### 8.2 Binding to the Receptor

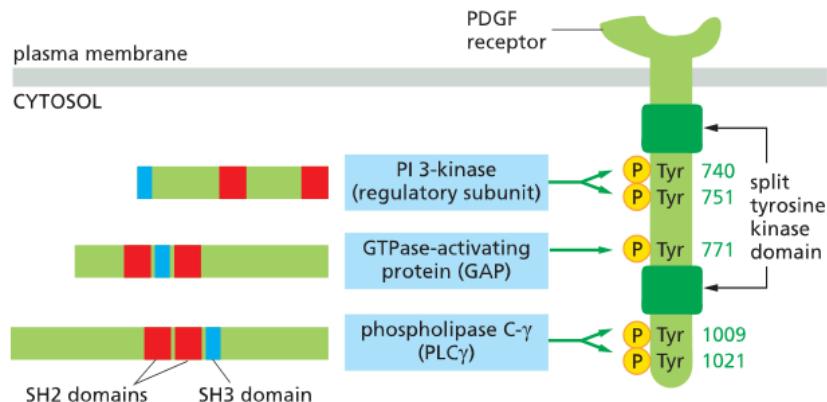


Figure 123: Phospho-tyrosine on PDGF receptors being docking-sites for proteins containing SH2 or PTB domains.

The phospho-tyrosines are docking sites for proteins containing:

- i) Src Homology (SH), Proto-oncogene

- ii) Phosphotyrosine Binding (PTB), Proto-oncogene
- iii) PLC

Because of the multitude of phospho-tyrosines many different proteins, and consequently different pathways, can interact with the receptors.

### 8.2.1 Ras signaling

Ras is essentially a **monomeric GTPase**. Ras is **anchored to the membrane** through a lipid modification. For a refresher on how GTP can be regulated please refer to Section 5.0.6.

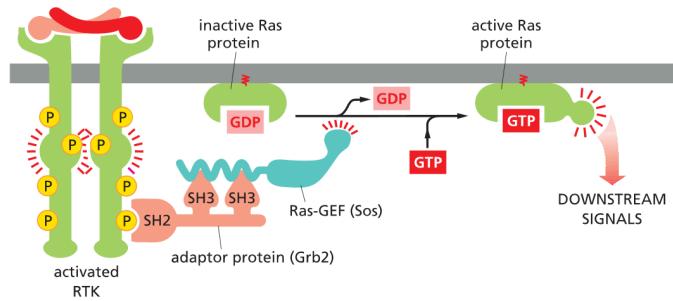
Here are some groups:

TABLE 15–5 The Ras Superfamily of Monomeric GTPases

Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
Rheb		Activates mTOR to stimulate cell growth
Rap1		Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1–ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1–60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

\*The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3–67.

(a) Ras groups in our body.



(b) The pathway for Ras.

#### Activation of Ras by an RTK:

- i) Adaptor protein Grb2 docks to RTK with Src Homology (SH)
- ii) Ras-GEF then interacts with Grb2
- iii) Ras-GEF then exchanges the GDP for a GTP
- iv) Ras is activated.

**Remark 8.1 (Detecting Ras activity).** We use FRET (Fluorescence resonance energy transfer), by attaching a yellow fluorescent protein (YFP) to the gene of Ras. Then we add a red fluorescent dye to GTP. That way when no GTP is there (Ras inactive), it emits yellow light, but when GTP is attached to the Ras (active) red light is emitted.

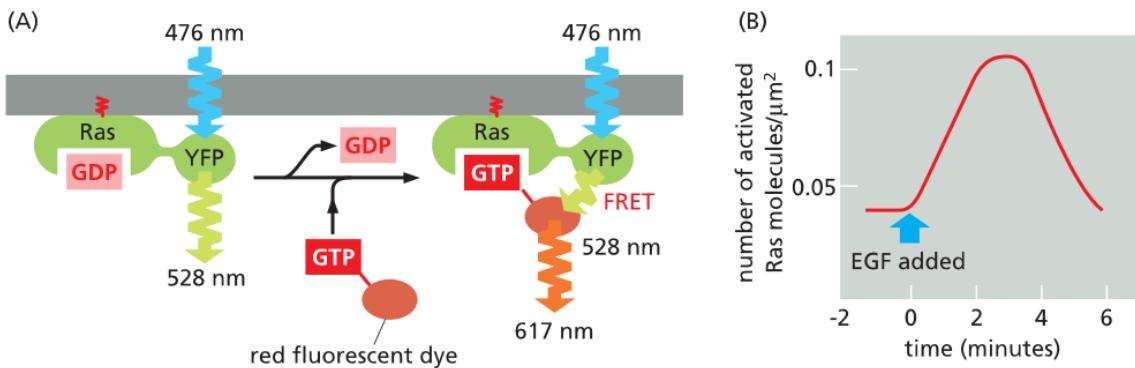


Figure 124: How FRET can be used to detect the activity of Ras.

**Case study: MAP kinase module** is a module activated by Ras. This is done the following way: Ras activates Raf to the membrane, which in turn activates MEK, which in turn activates Erk, which phosphorylates a bunch of downstream proteins, such as further kinases, and transcription regulators. The resulting activations cause complex changes in the cell.

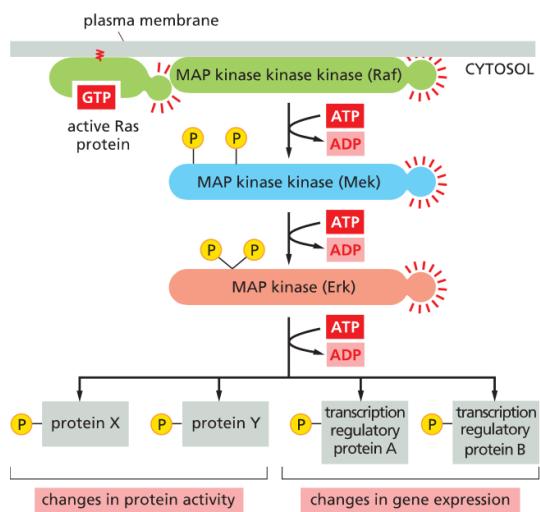


Figure 125: How the MAP kinase module is activated by Ras.

**How cancer changes Ras:** By changing certain amino acids (G12, G13, Q61), the mutants show impaired GTPase activity, leading to a gain-of-function. So, the GAP proteins no longer work as well. Of the three types of famous Ras (K,H,N-Ras) it seems mostly the mutant K-Ras is found in cancers. Mutant Ras probably important in the initiation of tumors. GAP is a Tumor Suppressor, EGF-R a Proto-oncogene.

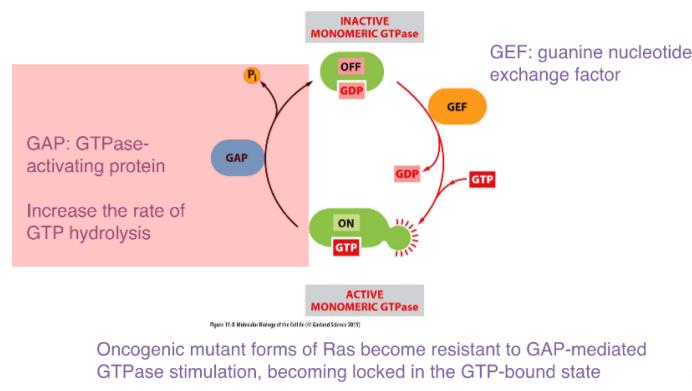


Figure 126: Showing how mutating the GAP messes with the regulation of Ras.

### 8.2.2 PI3K signaling

The PI3-Kinase phosphorylates the 3-carbon of a given PI molecule (can already be phosphorylated at other carbons or not). This then creates docking sites for downstream proteins, prominently AKT. In an alternative pathway the PI can also be activated by PLC causing it to go down the PLC pathway (see section 6.3). However, these are two independent pathways, even if the starting substrate PI is the same!

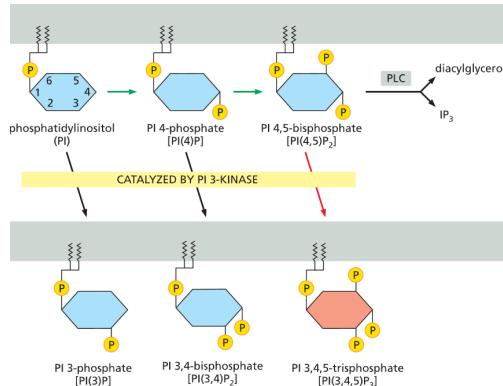


Figure 127: Phosphorylation of the 3-carbon activates the PI.

#### PI 3-Kinase activates AKT:

- i) PI3K is recruited by RTK
- ii) PI3K creates docking sites on the PI where proteins with a PH domain can dock.
- iii) PDK1 and mTORC2 activate AKT a.k.a. Protein Kinase B by phosphorylation at two different sites, allowing p-AKT to disassociate from the membrane.
- iv) p-AKT activates many cellular programs including cell growth and anti-apoptosis (hinting that cancer may be interested here).

**Negative regulation:** Phosphatase PTEN removes phosphate from  $\text{PI}(3,4,5)\text{P}_3$ , making it a negative regulator of the PI3K.

The fig: 128 below shows on the left the entire pathway which promotes cell growth. The one on the left shows how the chain on the left continues leading to cell growth. The MAP kinase can also go down the pathway on the right, meaning bot can promote cell growth.

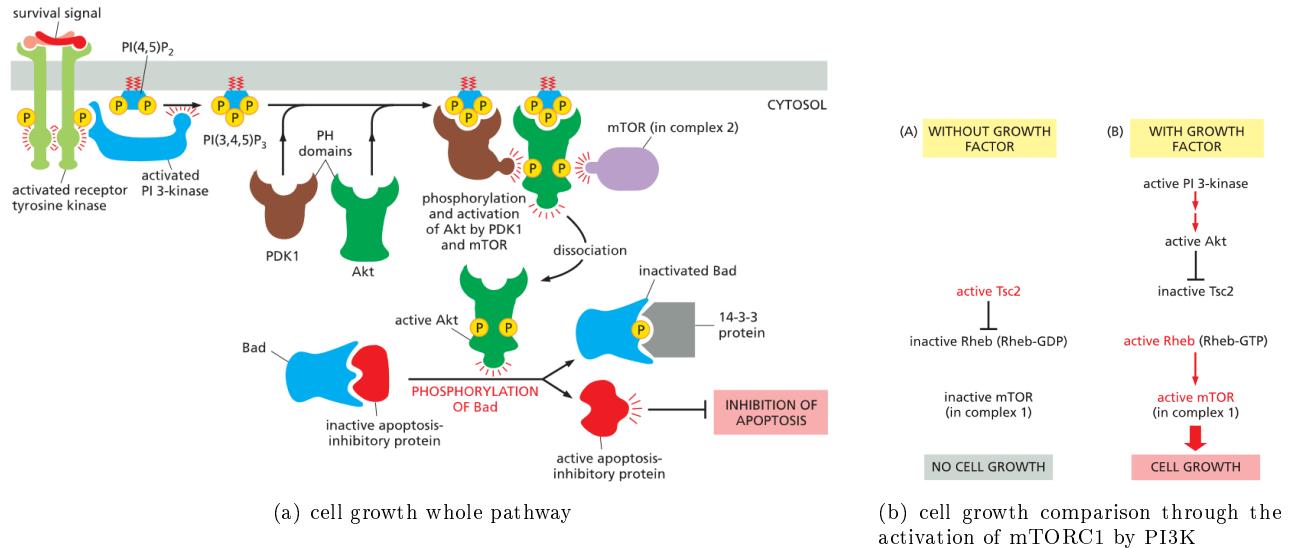


Figure 128: Note: (a) mTORC2 is in action, activating ATK, while in (b) mTORC1 is in action, about controlling protein synthesis, growth, and metabolism (further downstream of ATK).

### 8.3 EGF Receptors in cancer

In cancer EGFR can become over-activated, making it a Proto-oncogene.

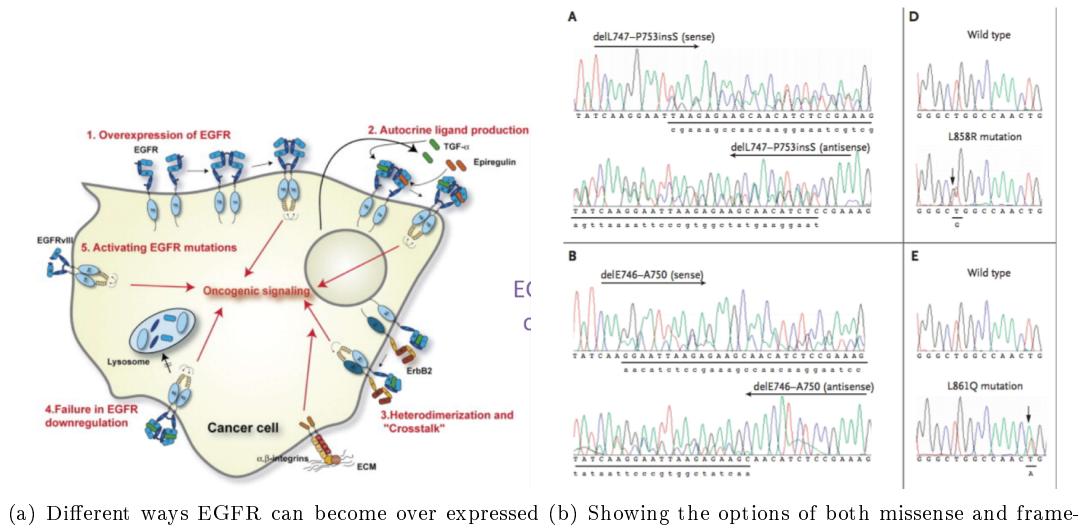


Figure 129:

Mutations in cancer occur at the kinase domain of the receptor, leading to the domain being permanently

---

active. Thus Ras-MAP Kinase and PI3K are always active, leading to uncontrolled cell growth a.k.a. cancer. These mutations will be deletions which keep the reading frame or missense mutations:

Figure 130:

Inhibitors can target always active EGFR. Through this some pathways are now slowed down, leading to more normalized cell growth.

## 9 Cell Signaling: Alternative Signaling

### 9.1 Signaling with Regulated Proteolysis

The namesake for all these beauties comes from how the fruit fly looked when we fucked it up. Details at the start of each section.

#### 9.1.1 Notch

Notch = V-shaped indentation in the wing.

The idea here is that instead of having the receptor cause a phosphorylation or similar, we cleave the receptor so that the intracellular part becomes the downstream signal, entering the nucleus. Here is the detailed process, oriented around the red numbered arrows in the picture:

- i) First Proteolytic Cleavage (red arrow 1): inside the trans Golgi network NOTCH is cut to become a mature version where the two parts are connected noncovalently.
- ii) these parts then migrate to the cell membrane.
- iii) Once the Notch complex binds to the Delta, through its repeating EGF regions on a neighboring cell, the two parts are split by endocytosis (red arrow 3).
- iv) This split then exposes the cleavage site (red arrow 3) to make the cut, allowing the Notch tail (a.k.a. notch intracellular domain a.k.a. NICD) to migrate the nucleus.
- v) the tail binds to Rbpsuh protein, which then converts from a repressor to an activator.

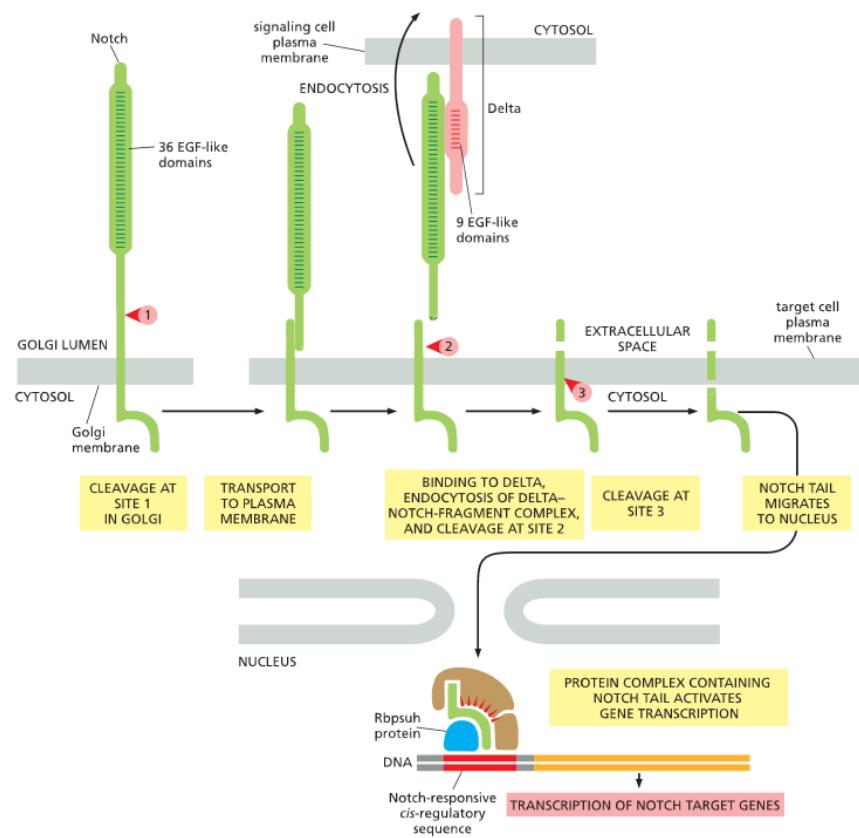


Figure 131: The Notch pathway, note that red arrows are cleavage sites.

**Definition 9.1 (Lateral Inhibition in Notch).** *Notch is contact-dependent and not autocrine. This allows for one cell to become excited and in the process inhibiting the neighboring one, which is called lateral inhibition.*

Looking at the example of neural cell development. Initially all Epithelial cells want to become neural cells, however we don't want that many. So, instead if a **cell expresses Delta than the neighboring ones know not to become a neural cell anymore**. Since, they all want to be neural cells though a competition starts to appear of who can produce the most delta ligands and the winner becomes the neural cell.

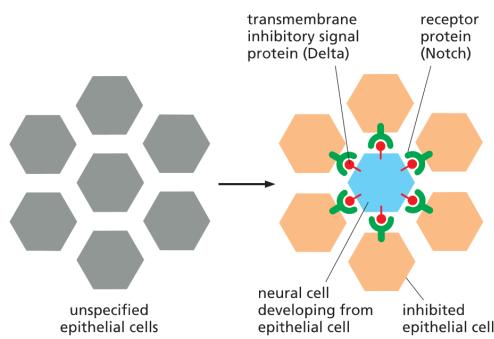


Figure 132: Case study of Notch, showing how Notch and Delta help in cell development.

### 9.1.2 WNT

Wnt = Wingless (WG) + Integration (t).

**Wnt is a special secreted ligand**, that through a bunch of lipid modifications, **associates to the plasma membrane** and doesn't travel very far. That means that the spread of Wnt is very limited to the neighboring cells.

Looking at the Wnt ligand pathway: the broad idea is that we want to stop the degradation of the anti-repressor.

Here are the details, first in the absence of the Wnt ligand:

- i) Beta Catenin interacts with degradation complex.
- ii) In this complex it is phosphorylated first by CK1, then GSK3, triggering its ubiquitylation and then degraded.
- iii) The degraded form prohibits it from binding to LEF1/TCF, meaning it can't kick out the repressor Groucho.

Now, in the presence of the Wnt ligand:

- i) Wnt binds to frizzled and LRP, clustering the two co-receptors together
- ii) The tail of LRP is phosphorylated by GSK3 and then CK1. Further Disheveled is recruited to the Frizzled site. The exact role of disheveled isn't known.
- iii) The Axin, of the degradation complex, is then recruited by the disheveled and then bound to the phosphorylated LRP.
- iv) This results in the disassembly of the degradation complex.
- v) This means the  $\beta$ -catenin stays stable, attaches to the LEF1/TCF and kicks out Groucho.

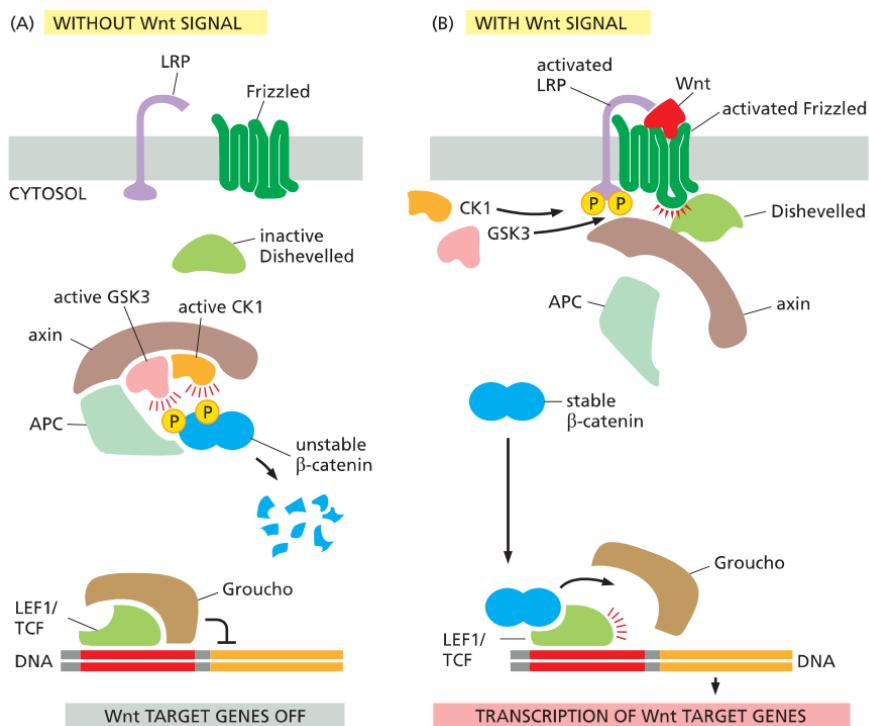


Figure 133: The Wnt pathway, on the left without Wnt, leading to no expression, while on the right it has Wnt, meaning the gene is expressed.

### 9.1.3 Hedgehog

Hedgehog = Larva looked like a hedgehog.

The game is all about Cubitus Interruptus (Ci) (Ci). Without Hedgehog it get cleaved to a repressor, with Hedgehog it is an activator.

Here is what happens when we have no hedgehog:

- Through the absence of Hedgehog, Patched is allowed to exist, which means it inhibits Smoothened.
- The lack of Smoothened which causes Ci to be caught in a degradation complex.
- This degradation complex includes a Fused kinase and a scaffold protein Costal2. Costal2 recruits three other kinases (PKA, GSK3, CK1), which phosphorylate Ci.
- That P-Ci is ubiquitylated and cleaved in proteasomes.
- the cleaved Ci then forms a repressor and moves to the nucleus to do just that.

Now, when we have hedgehog:

- Hedgehog binding to iHog as Patched is removed and degraded, stopping the inhibition of Smoothened.
- Smoothened is then phosphorylated by PKA and CK1 and translocated to the plasma membrane.
- There it recruits Fused, Costal2, which are forced to let go the Ci they were holding on to.
- The Ci enters the nucleus in its complete form and activates the hedgehog genes.

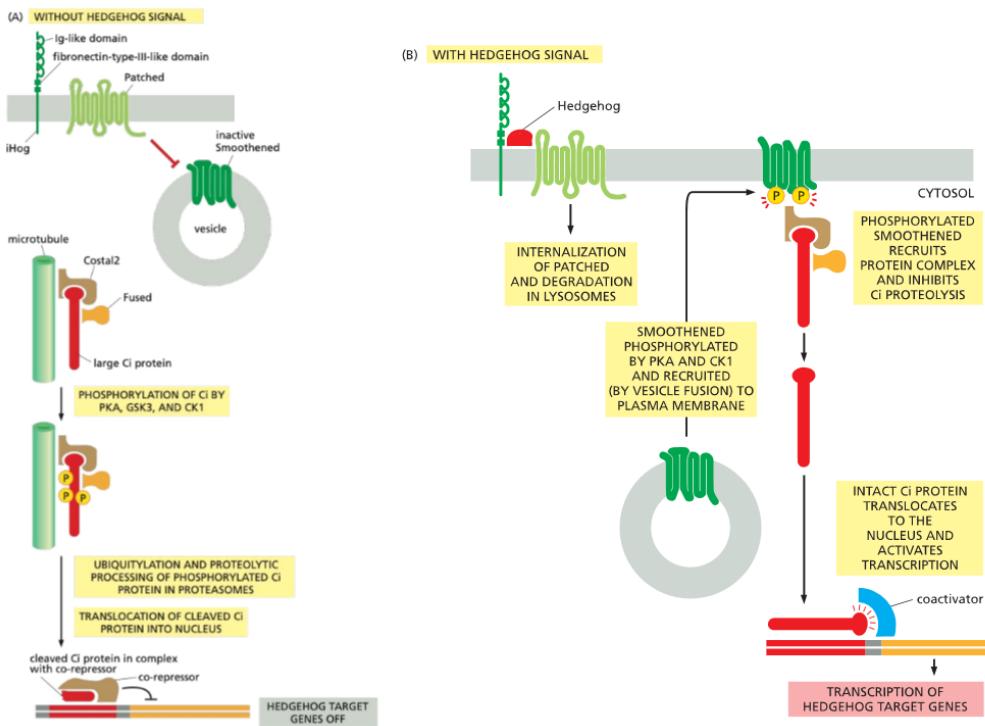


Figure 134: Shows the pathway of Hedgehog; on the left without Hedgehog, meaning gene repression; and on the right with Hedgehog and activation.

#### 9.1.4 NF $\kappa$ B pathway

Core concept - Negative Feedback Loop: What this causes is that the moment a gene is expressed it immediately inhibits its own transcription. That leads to a short expression, which is quickly blocked out.

**Remark 9.2 (Application to NF $\kappa$ b).** Activated NF $\kappa$ B increases expression of the I $\kappa$ B $\alpha$  gene, and I $\kappa$ B $\alpha$  then binds to NF $\kappa$ B and inactivates it, thereby shutting off the response. If the initial activating signal persists, then additional cycles of NF $\kappa$ B act on and inactivation may follow.

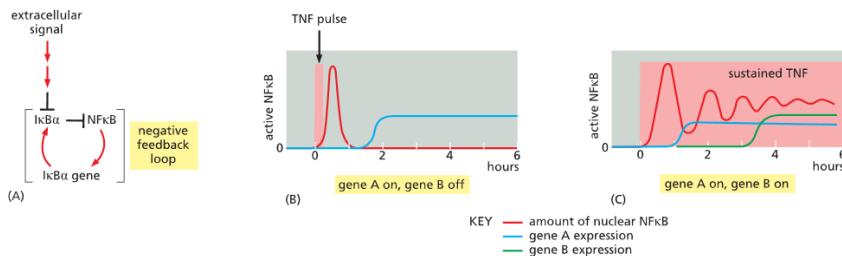


Figure 135: The left shows the idea behind a negative feedback. The middle has a short TNF pulse, while the one on the right has a sustained one.

For the short TNF pulse: Gene A gets its transcription turned on by this while gene B does not. For the prolonged TNF exposure: gene B will also be transcribed. Why gene B needs that prolonged exposure isn't understood. Note that due to the negative feedback loop we get an oscillation of TNF until it finds the right

concentration steady state with the gene.

NF- $\kappa$ B can be activated by many signals, a lot of them being **inflammatory signals** (infections, Bacteria, and immune cells secreting signals).

Now an example of the NF $\kappa$ B pathway, activated by TNF $\alpha$ :

- i) TNF $\alpha$  is a trimer, as are its receptors. The binding of TNF $\alpha$  causes a rearrangement of the clustered tails of the receptors.
- ii) These tails can now recruit a bunch of signaling proteins.
- iii) This in turn activates the protein kinase which activates I $\kappa$ B kinase kinase (IKK). IKK is a heterotrimer composed of three subunits (IKK alpha and IKK beta (catalytic subunits), and NEMO (regulatory subunit)).
- iv) IKK $\beta$  then phosphorylates two serine of I $\kappa$ B, which marks it for ubiquitylation and consequent degradation.
- v) The released NF $\kappa$ B translocates to into the nucleus, where it with co-activators stimulates transcription.

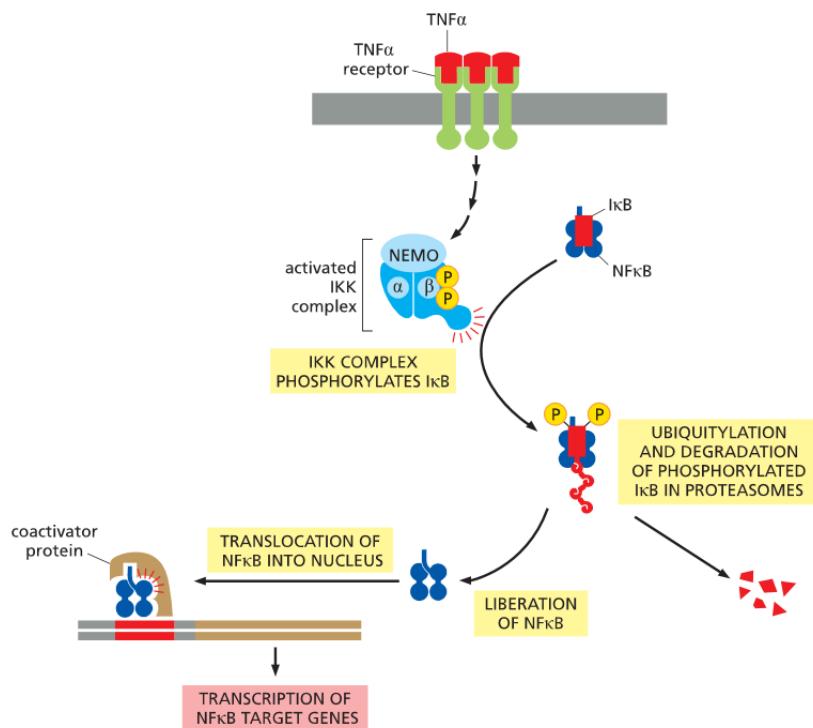


Figure 136: This shows the NF $\kappa$ B pathway.

In cells lacking NEMO, TNF triggers cell death.

## 9.2 Nuclear Receptor Signaling

Some signaling molecules can bind to intracellular molecules. This means they need to get through the membrane, so they got to be lipophilic. **Cholesterol is kinda the God of precursors**, as its already in the

membrane so must be popular. However, most of the signaling molecules will be more hydrophilic than cholesterol making them better for exiting membranes and transport throughout body.

The signaling with these molecules is incredibly simple. A dimer molecule comes in, reaches the nucleus and

- changes the factors to activate gene expression. In the this process the receptors are already on the gene. This binding can also kick out inhibitors and often binds further activators.
- changes the factors to repress gene expression. This really is really just the same thing but opposite: activators are cleared out and repressor recruited.

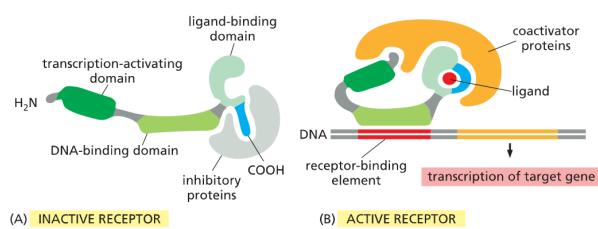


Figure 137: How nuclear receptors attach right to the DNA, at the repressor (not shown) or activator site

Depending on the cell we will get completely different reactions and gene expressions.

*Remark 9.3 (Use study - Prostate Cancer):* In prostate cancer, Androgen activate the Androgen receptor (AR) (AR), which then turns on genes that support tumor growth and survival. In some therapeutic or experimental contexts, researchers can design genes controlled by androgen-responsive promoters so that their expression is turned on in the presence of androgen.

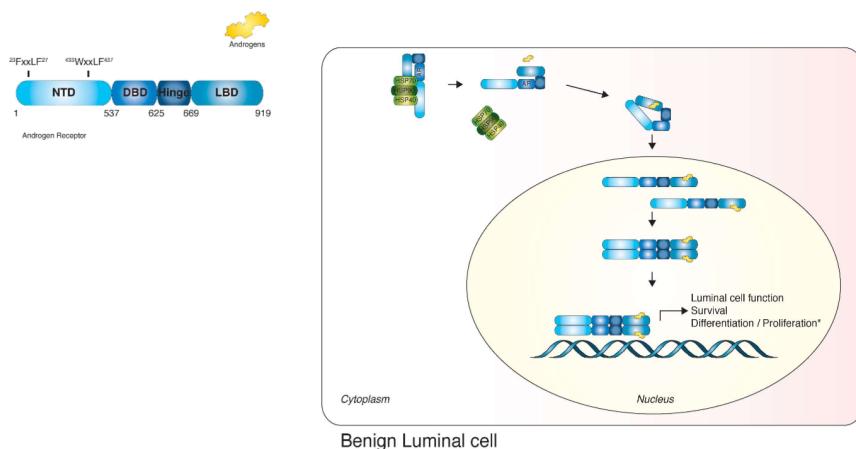


Figure 138: Yh I'm done with this, this is an example of Androgen being used to reprogram prostate cancer.

## 10 Intracellular Membrane Traffic

Every cell must communicate with the world around it, and quickly respond to changes in the environment. To archive this cells add and remove cell-surface proteins, such as receptors, ion channels, and transporters.

Trough the process of exocytosis, the **secretory pathway** delivers newly synthesized proteins, carbohydrates,

and lipids either to the plasma membrane or the extracellular space.

By the **converse process of endocytosis**, cells remove plasma membrane components and deliver them to internal compartments called endosomes, from where they can be recycled to the same or different regions of the plasma membrane or be delivered to lysosomes for degradation.

There are two main intracellular transport pathways:

- Biosynthetic pathway** (also called the **secretory pathway**, which is a subset of it): This pathway directs newly synthesized proteins and lipids outward from the endoplasmic reticulum (ER) to the Golgi apparatus, and then to the plasma membrane or extracellular space. It also includes delivery to lysosomes.
- Endocytic pathway**: This pathway transports materials inward from the plasma membrane. Internalized cargo is first delivered to early endosomes, from which it can be recycled back to the plasma membrane or forwarded to late endosomes and eventually lysosomes for degradation.

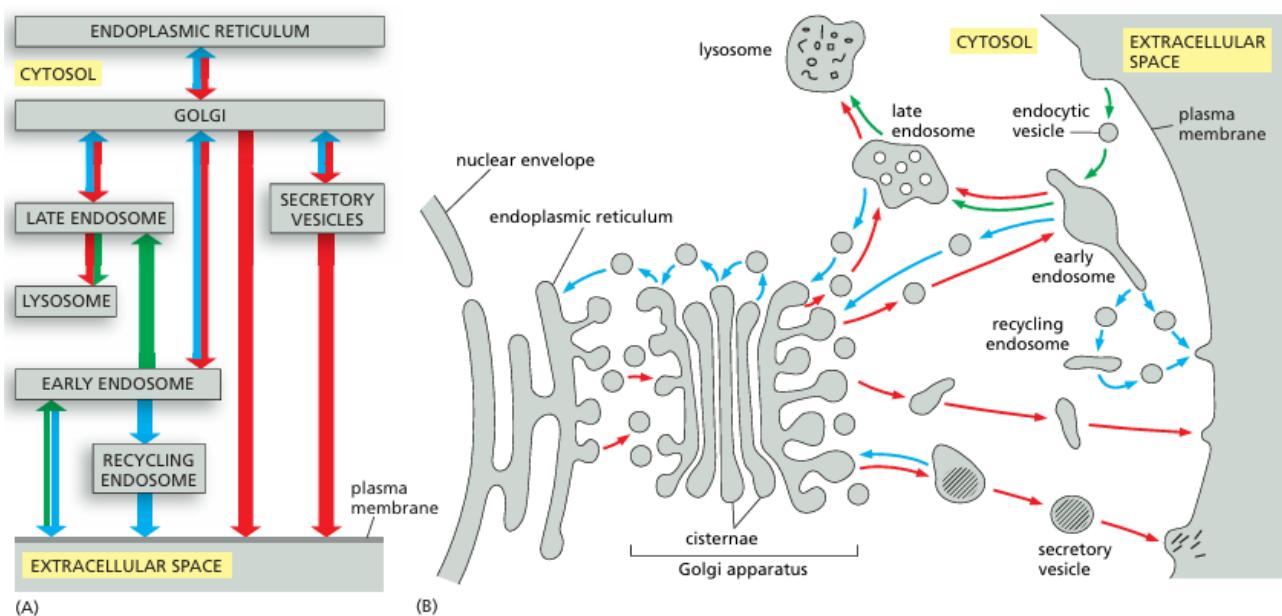


Figure 139: A “road-map” of the secretory and endocytic pathways.

#### 10.0.1 Vesicle Transport

Vesicle transport refers to the movement of cargo within cells via membrane-bound carriers. **Note that vesicles are not always spherical**. The movement of vesicles is often aided by the **cytoskeleton**. For instance vesicles can move along **microtubules** via **motor-proteins**.

**Definition 10.1 (Exocytosis).** *exocytosis is a cellular process in which substances contained in vesicles are released from the cell to the extracellular environment by fusion of the vesicle with the plasma membrane.*

**Definition 10.2 (Endocytosis).** *endocytosis is a cellular process in which the cell membrane folds inward to form a vesicle that encloses extracellular material for internalization into the cell*

As a **consequence of vesicular transport**, exocytosis and endocytosis, compartments that are able to communicate and they will be topologically equivalent.

**Definition 10.3 (Topologically equivalent).** The concept of topological equivalence refers to the idea that membrane leaflets facing the cytosol share a similar composition and can directly communicate with each other. In contrast, the luminal spaces of organelles are topologically equivalent to the extracellular space. This relationship arises as a consequence of vesicular transport, which preserves membrane orientation during budding and fusion.

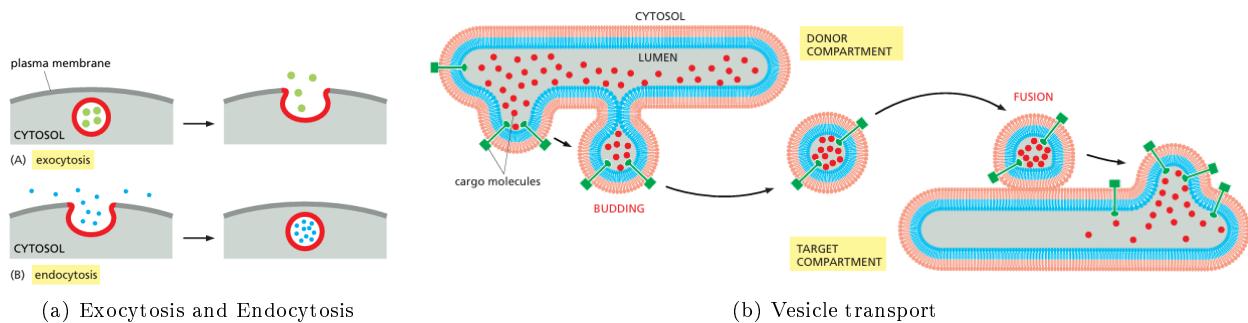


Figure 140: Topologically equivalent compartments, are able to "communicate"

Most transport vesicles form from specialized, coated regions of membranes . There are **various types of coated vesicles**, which have distinctive cage of proteins covering their **cytosolic face**. Before they fuse with the target membrane they **discard their coat**. This is required for the membranes to fuse.

The **coat** performs two main function:

- The inner layer selects the appropriate membrane molecules for transport.
- The outer layer shapes the vesicle.

There are three well-characterized types of coated vesicles, distinguished by their major coat proteins: clathrin-coated, COPI-coated, and COPII-coated. Each type is used for different trasport steps.

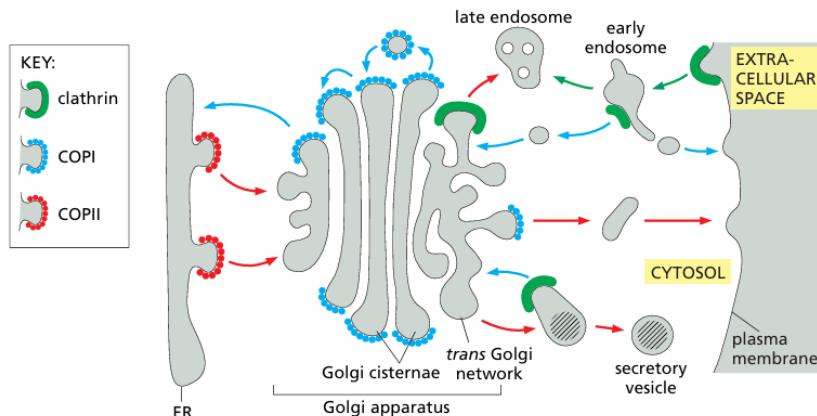


Figure 141: Use of different coats for different steps in vesicle traffic

#### 10.0.1.1 Clathrin-coated vesicle

The major protein component of clathrin-coated vesicles is **clathrin** itself, which forms the outer layer of the coat. Each clathrin subunit consists of **three large and three small polypeptide chains** that together

form a three-legged structure called a **triskelion**. These triskelions assemble into a **basketlike framework** of hexagons and pentagons to form coated pits (**buds**) on the cytosolic surface of membranes.

**The specificity does not come from the coat but from adapter proteins.** They are a major component and bind the clathrin coat to the membrane and trap various transmembrane proteins - the so called **cargo receptors**. There are several adapter proteins each is specific for a different set of cargo proteins.

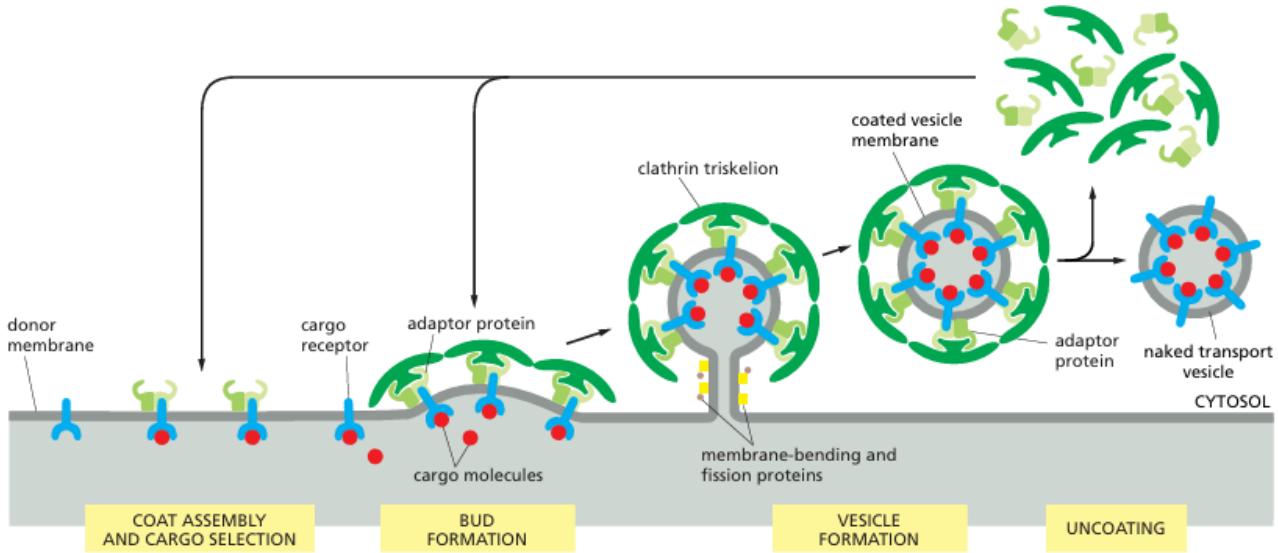


Figure 142: The assembly and disassembly of a clathrin coat

**Example 10.4 (The adaptor protein AP2).** AP2 binds to specific phosphorylated phosphoinositides in the plasma membrane, which triggers a conformational change exposing binding sites for cargo receptors. Acting as a **coincidence detector**, it requires simultaneous interactions with both lipids and cargo to stably associate with the membrane. Upon binding, AP2 induces membrane curvature and promotes clathrin coat assembly, **facilitating vesicle formation**. See fig. 143(a)

*Remark 10.5 (BAR domains, bending membrane).* BAR domain proteins are diverse and enable many membrane-bending processes in the cell. BAR domains are built from coiled coils that dimerize into modules with a positively charged inner surface, which preferentially interacts with negatively charged lipid head groups **to bend membranes**. See fig. 143(b)

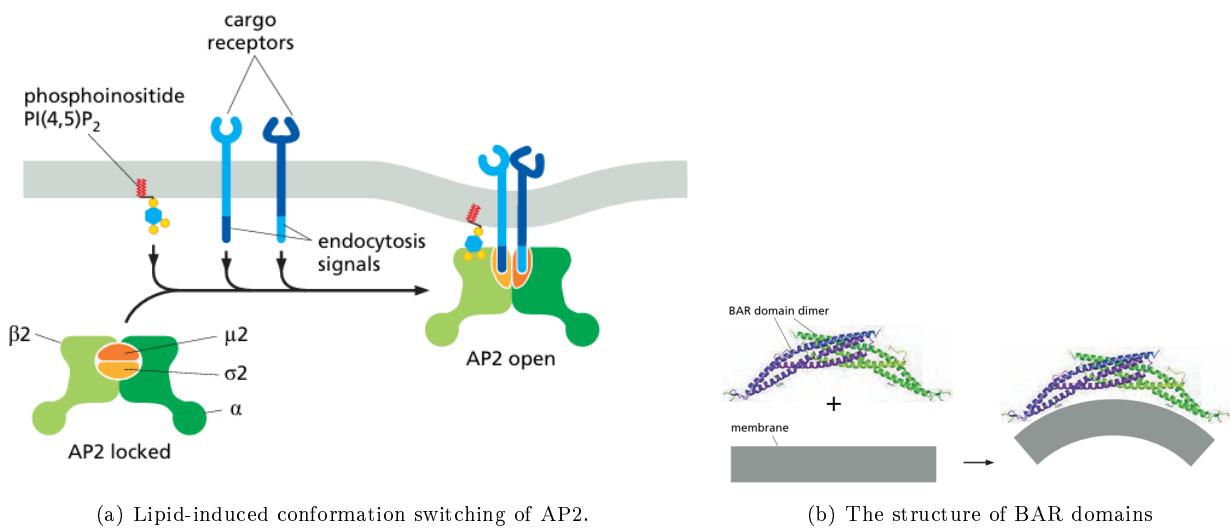


Figure 143:

As the bud grows, cytoplasmic proteins, including **Dynamin**, assemble at the neck. Dynamin contains a PI(4,5)P<sub>2</sub>-binding domain, which tethers the protein to the membrane, and a **GTPase domain**, which regulates the rate at which vesicles **pinch off from the membrane**.

To bring the two noncytosolic leaflets of the membrane into close proximity in order to fuse them dynamin recruits other proteins helping to bend the patch of membrane. See fig. 144

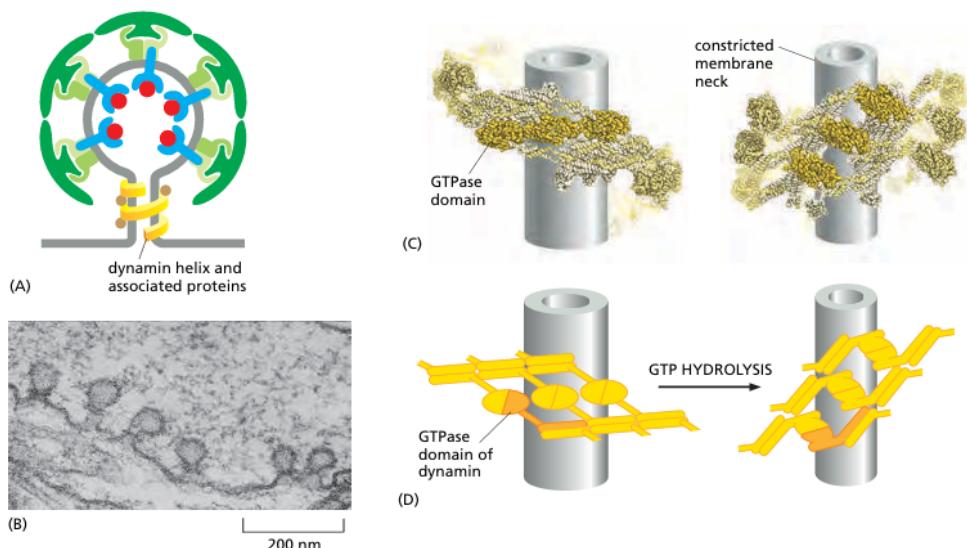


Figure 144: The role of dynamin in pinching off clathrin-coated vesicles.

#### 10.0.1.2 COPII-coated vesicle

There are many ways to regulate coat formation. For example **Coat-recruitment GTPases** control the assembly of clathrin coats on endosomes and the COPI and COPII coats on Golgi and ER membranes.

The **Sar1 protein** responsible for the COPII coats at the ER membrane is part of the Coat-recruitment GTPase family.

**Coat-recruitment GTPases** are usually found in high concentration in the cytosol in an inactive, GDP-bound state. In the formation of a COPII-coated vesicle, **Sar1-GEF** is embedded in the ER membrane and binds to the **cytosolic Sar1**, causing Sar1 to **release GDP and bind GTP**. This leads to the **expression an amphiphilic helix**, which inserts into the cytoplasmic leaflet of the lipid bilayer. Sar1 then **recruits adaptor coat protein** (like **Sec23** and **Sec24**) subunits to **initiate budding**. See fig. 145

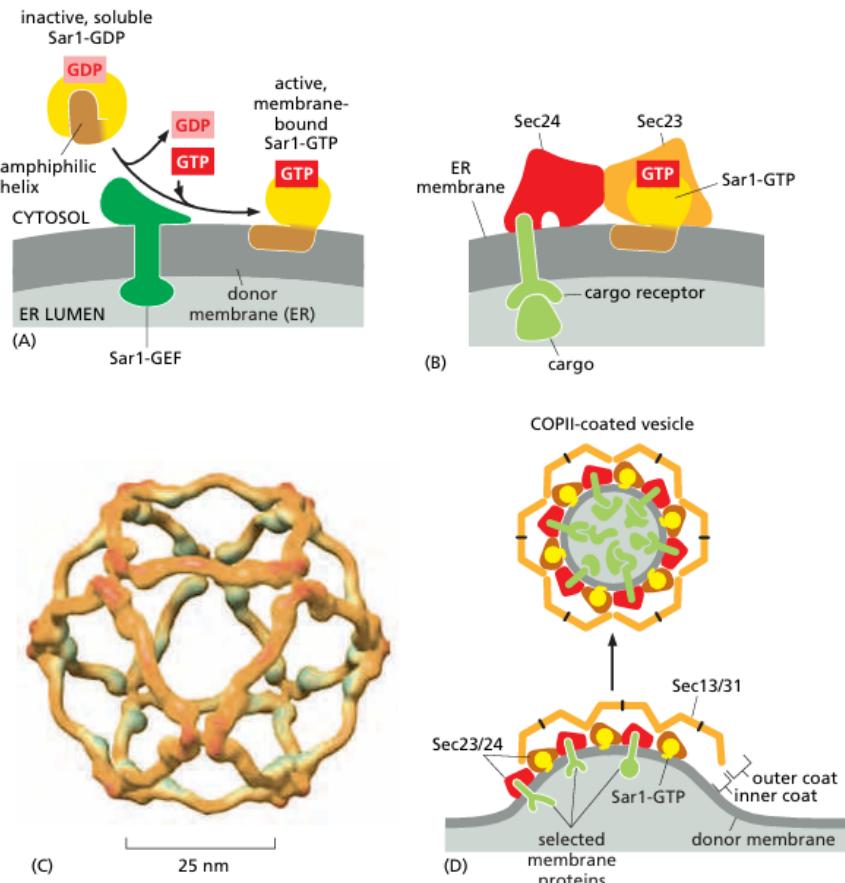


Figure 145: Formation of a COPII coated vesicle.

#### 10.0.2 Recognition of Destination

Specificity in targeting is ensured because all transport vesicles display surface markers that identify them according to their origin and type of cargo. Moreover target membrane displays complementary receptors that recognize this markers.

First, **Rab proteins** and Rab effectors direct vesicles to specific spots on the correct target membrane. In addition, distinct **phosphoinositide compositions** on different membranes help recruit the correct Rab effectors, adaptors, and SNARE regulators to ensure fidelity. Second, **SNARE proteins** and SNARE regulators mediate the fusion of the lipid bilayers.

*Remark 10.6 (PIPs varies from organelle to organelle).* Recall that PIPs varies from organelle to organelle. Many proteins involved in vesicle transport contain domains that bind with high specificity to the head group

of particular PIPs. Therefore local control of the PI and PIP kinases and PIP phosphatases is important for the control of the vesicle traffic. See fig. 149(a)

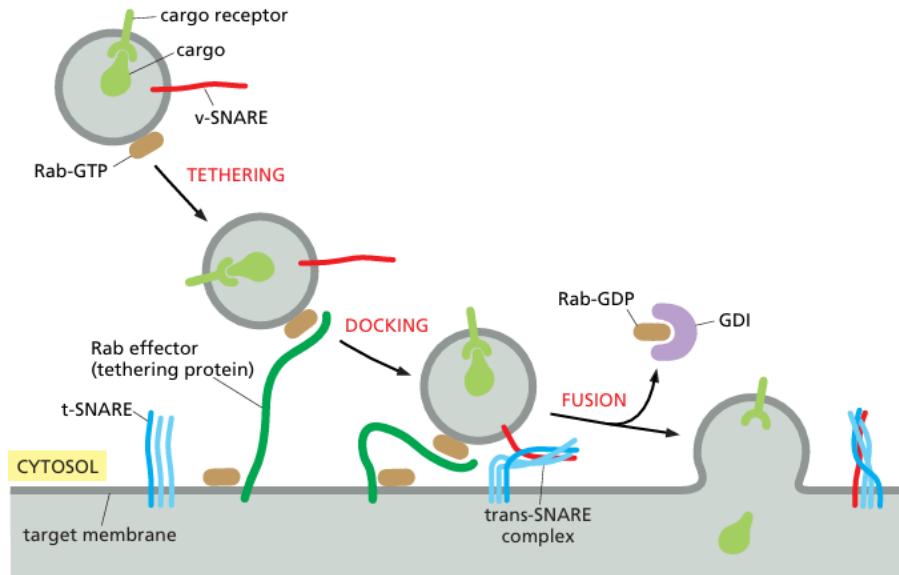


Figure 146: Tethering of a transport vesicle to a target membrane.

#### 10.0.2.1 Rab proteins

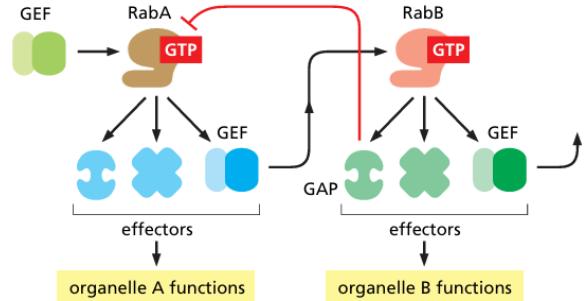
Rab protein is a family of small GTPases that regulate vesicle transport by ensuring specificity in vesicle targeting. Rab proteins recruit effector molecules that help guide vesicles to the correct membrane compartment.

Like **coat-recruitment GTPases**, Rab proteins cycle between a membrane and the cytosol. In their GDP-state they are in the cytosol bound to another protein that keeps them soluble. While in the **GTP-state** they are active and tightly associated with the membrane transport.

**Membrane bound Rab-GEFs** activate Rab proteins on both transport vesicles and target membranes. Once in the **GTP-state** they bind to other proteins called, **Rab effectors**, which are the downstream mediators of vesicle transport.

Protein	Organelle
Rab1	ER and Golgi complex
Rab2	cis Golgi network
Rab3A	Synaptic vesicles, secretory vesicles
Rab4/Rab11	Recycling endosomes
Rab5	Early endosomes, plasma membrane, clathrin-coated vesicles
Rab6	Medial and <i>trans</i> Golgi
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, <i>trans</i> Golgi

(a) Subcellular Locations of Some Rab Proteins



(b) A model for a generic Rab cascade.

Figure 147: Rab

The assembly of Rab proteins and their effectors on a membrane is cooperative and results in the formation of large, **specialized membrane patches**. **Rab5**, for example, assembles on **endosomes** and mediates the **capture of endocytic vesicles** arriving from the plasma membrane.

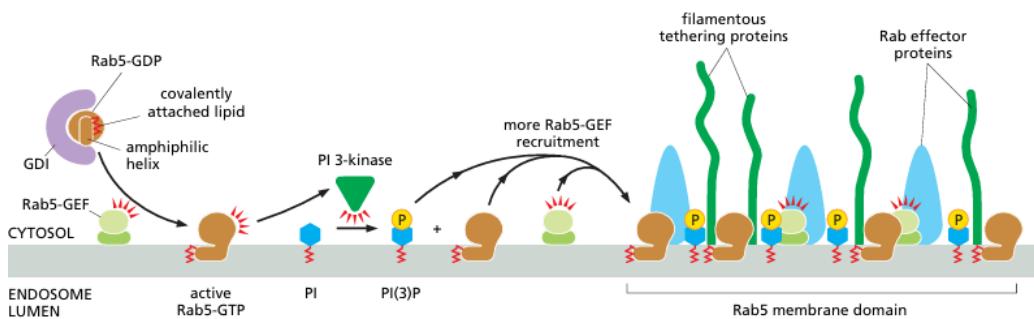


Figure 148: The formation of a Rab5 domain on the endosome membrane.

A Rab domain can be disassembled and replaced by a different **Rab** domain, changing the identity of an organelle. Such ordered recruitment of sequentially acting Rab proteins is called a **Rab cascade**. See fig. 147(b)

Over time, for example, **Rab5 domains are replaced by Rab7 domains** on endosomal membranes. This converts an **early endosome**, marked by Rab5, into a **late endosome**, marked by Rab7.

### 10.0.2.2 SNARE proteins

**Membrane fusion** requires bringing the lipid bilayers of two membranes to within **1.5 nm** of each other so that they can merge. When the membranes are in such close apposition, lipids can flow from one bilayer to the other.

For this close approach, **water must be displaced** from the hydrophilic surface of the membrane—a process that is highly energetically unfavorable and requires **specialized fusion proteins** that overcome this energy barrier.

SNARE protein are a group of membrane-associated proteins that mediate the fusion of vesicle and target membranes. SNAREs on the vesicle (v-SNAREs) and on the target membrane (t-SNAREs) form complexes that bring membranes close enough to fuse.

Note that SNARE complexes can only be made with certain combinations and thus define **specificity**.

In addition to the specificity of t- and v-SNARES, **Rab** proteins can regulate the availability of SNARE proteins.

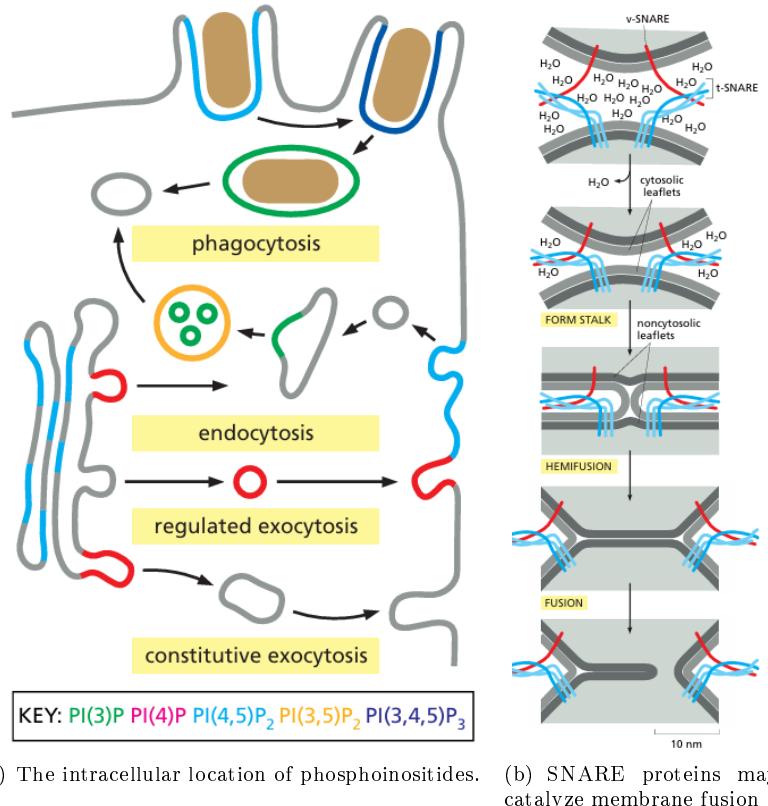


Figure 149:

**Intacting SNAREs need to be pried apart before they can function again.** A crucial protein to archive this is **NSF**, an ATPase that disassembles SNARE complexes after membrane fusion. **NSF uses energy** from ATP hydrolysis to recycle SNARE proteins for further rounds of vesicle fusion. This is because the SNARE Complex is just that damn stable.

Note the requirement for NSF-mediated reactivation of SNAREs by SNARE complex disassembly helps prevent membranes from fusing indiscriminately. NSF can be used to activate the SNARE machinery at the right time.

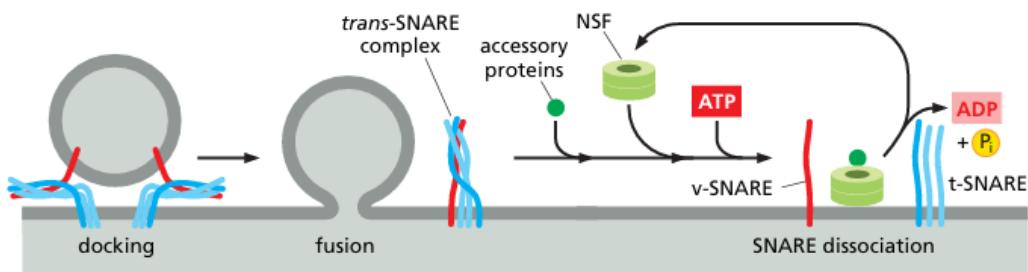


Figure 150: Dissociation of SNARE pairs by NSF after a membrane fusion cycle.

## 10.1 Between ER and Golgi

The newly synthesized proteins cross the **ER** membrane from the **cytosol** to enter the **secretory pathway**. Then they will get transported from the **ER to the Golgi apparatus** and from there to the **cell surface**. The proteins are **successively modified** as they pass through a series of compartments.

Recall that the **Golgi apparatus** is a major site of carbohydrate synthesis, as well as a sorting and dispatching station for products from the ER.

Proteins that have entered the ER and are destined for the Golgi apparatus or beyond are first packed into **COPII-coated** transport vesicles. These vesicles bud from specialized regions of the ER called **ER exit sites**, whose membrane lacks bound ribosomes. The cargo membrane proteins display exit signals on their cytosolic surface that adaptor proteins of the inner COPII coat recognize. *Note some of these cargo proteins will be recycled back to the ER once they have delivered their cargo.*

Moreover the transport from the ER to the Golgi apparatus serves also as a **quality control step**. Then to exit from the ER, proteins must be **properly folded** and, if they are subunits of multiprotein complexes, they need to be **completely assembled**. Those that are misfolded or incompletely assembled transiently remain in the ER, where they are bound to chaperone proteins.

**Definition 10.7 (Homotypic membrane fusion).** *After transport vesicles have budded from ER exit sites and have shed their coat, they begin to fuse with one another. This is called Homotypic membrane fusion, in contrast to heterotypic fusion where compartments from different origin fuse. For Homotypic membrane fusion matching SNAREs are required.*

When ER-derived vesicles fuse with one another we speak of **Vesicular tubular clusters**. These are generated continuously and makes the transport more efficient as: They send transport vesicles on their own (**COP1-coated**) that feed into the **retrieval pathway**; They move quickly along **microtubules** (using **motor proteins**) from the ER to the Golgi.

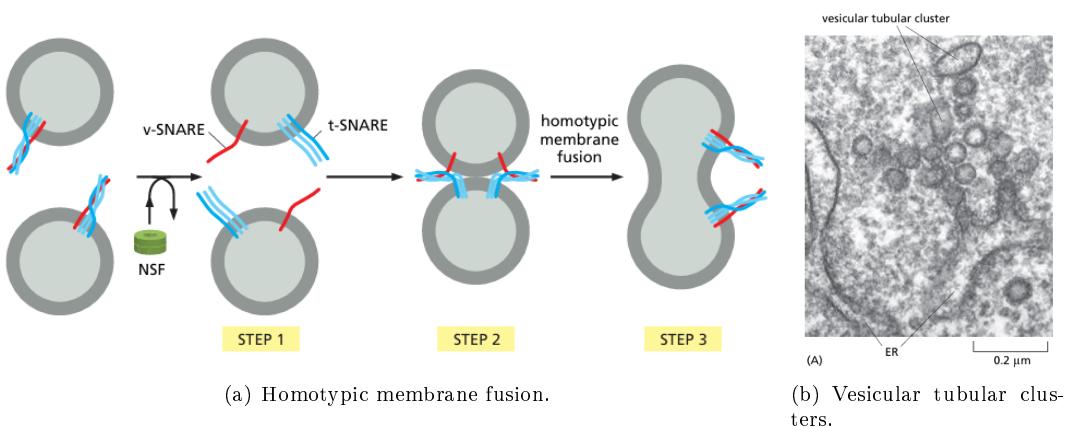


Figure 151: Vesicular Tubular Clusters Mediate Transport from the ER to the Golgi Apparatus

Since **Biology is imperfect** sometimes a soluble ER resident protein is wrongfully imported to the Golgi. This creates the need of a **backward (retrieval) transport**.

This transport is mediated by **COPⅡ-coated** vesicles. Therefore resident ER membrane proteins contain signals that directly bind to COPⅡ coats. The best-characterized retrieval signal of this type is the **KKXX sequence** (Lysine-Lysine-X-X).

However, **soluble ER resident proteins**, such as BiP, also contain a short ER retrieval signal at their C-terminal end: it consists of a Lys-Asp-Glu-Leu or a similar sequence. This signal is called **KDEL sequence**. Note that if this signal is removed from BiP by genetic engineering, the protein is slowly secreted from the cell.

These proteins then bind to the **KDEL receptor** a multipass transmembrane protein, which cycles between the ER and Golgi. Therefore the receptor must have a different affinity for the KDEL sequence depending on its location. This can be explained by the **lower pH in the Golgi compartments**.

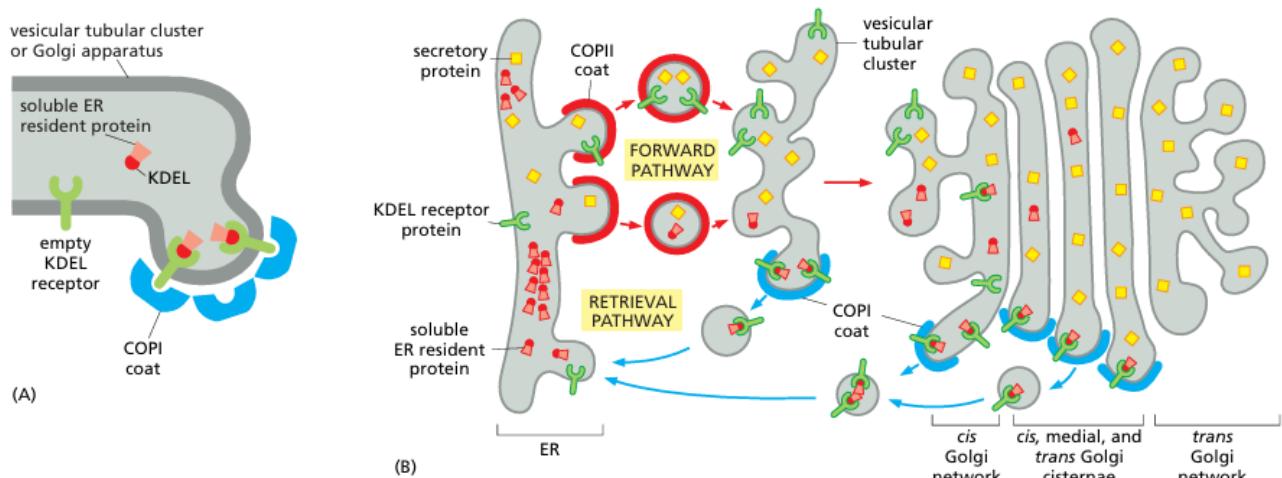


Figure 152: Retrieval of soluble ER resident proteins.

*Remark 10.8 (Kin Recognition).* Not all proteins with a KDEL signal are efficiently retained in the ER. This suggests that, beyond KDEL-based retrieval, *kin recognition* may contribute to ER retention. ER-resident

proteins may interact with one another, slowing their export. Thus, proteins lacking KDEL but interacting with ER residents are still retained longer than typical secreted proteins.

## 10.2 In the Golgi Apparatus

Each Golgi stack typically **consists of four to six cisternae**. Tubular connections between corresponding cisternae link many stacks, thus forming a single complex, which is usually located **near the cell nucleus and close to the centrosome**. Note its localization depends on microtubules. If microtubules are experimentally depolymerized, the Golgi apparatus reorganizes into individual stacks that are found throughout the cytoplasm, adjacent to ER exit sites.

There are **two possible models** explaining the movement through the Golgi. However it is likely that the **truth lies somewhere in between**. A long-lasting cisternae might exist in the center of each Golgi cisterna, while regions at the rim may undergo continuous maturation, perhaps utilizing Rab cascades that change their identity.

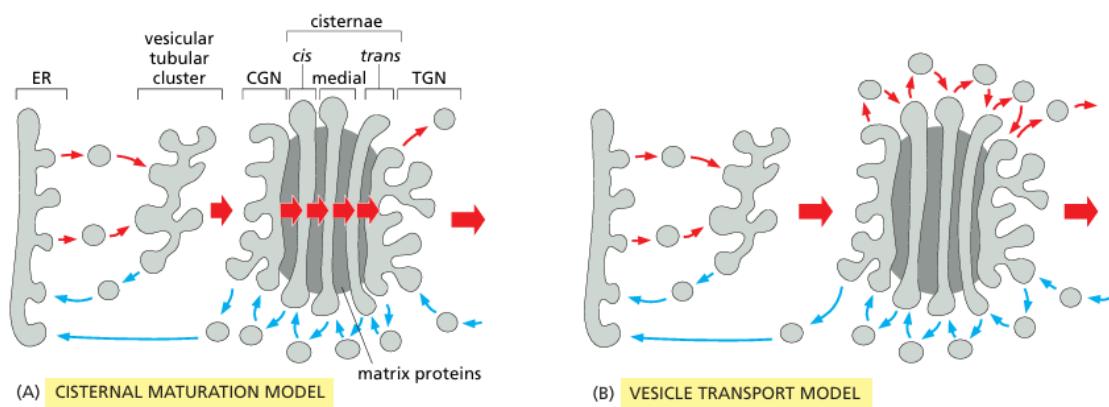


Figure 153: Two possible models explaining the organization of the Golgi and how proteins move through it.

**Remark 10.9 (Golgi Matrix Proteins Help Organize the Stack).** The distinctive structure of the Golgi apparatus relies apart from both the microtubule cytoskeleton also on a **network of Golgi matrix proteins**. These matrix proteins form a **scaffold between adjacent cisternae**, helping maintain the Golgi stack's architecture. Among them, **golgins** play a key role. Golgins are long, coiled-coil proteins with flexible hinge regions that **extend like tentacles**—up to 100–400 nm from the Golgi surface. They help **capture and retain transport vesicles** near the Golgi by **interacting with Rab proteins**, thus supporting efficient vesicle docking and trafficking. See fig. 154(b)

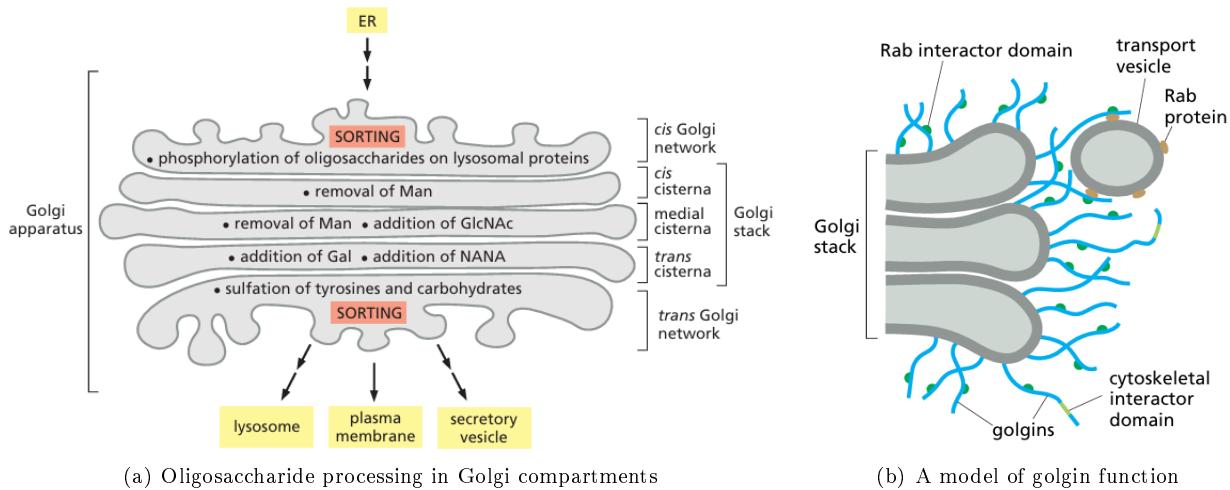


Figure 154: Oligosaccharide Chains Are Processed in the Golgi Apparatus

Note that during their passage through the Golgi (**cis face** (or entry face) to **trans face** (or exit face)), transported molecules undergo an ordered **series of modification**.

### 10.2.1 Glycosylation in the Golgi

Whereas the ER lumen is full of soluble luminal resident proteins and enzymes, the **resident proteins in the Golgi apparatus are all membrane bound**. Therefore the enzymatic reactions occur entirely on the membrane surface. For example Golgi glycosidases and glycosyl transferases are single-pass transmembrane proteins, many of which are organized in multienzyme complexes.

Two broad classes of **N-linked oligosaccharides**, the complex oligosaccharides and the high-mannose oligosaccharides, are attached to mammalian glycoproteins. *Sometimes, both types are attached (in different places) to the same polypeptide chain.*

- **Complex oligosaccharides** are generated when the original N-linked oligosaccharide added in the ER is trimmed and further sugars are added.
- By contrast, **high-mannose oligosaccharides** are trimmed but have no new sugars added to them in the Golgi apparatus.

Whether a given oligosaccharide remains high-mannose or is processed depends largely on its position in the protein. If the oligosaccharide is accessible to the processing enzymes in the Golgi apparatus, it is likely to be converted to a complex form.

*Remark 10.10 (Endo H).* Endo H is short for Endoglycosidase H, an enzyme that cleaves high-mannose and some hybrid N-linked oligosaccharides from glycoproteins (**Endo H-sensitive**). It does not cleave complex oligosaccharides (**Endo H-resistant**), making it a useful tool to distinguish between early (ER) and later (Golgi-processed) stages of glycan maturation.

Note that **sialic acids** is of special relevance as it has a **negative charge**.

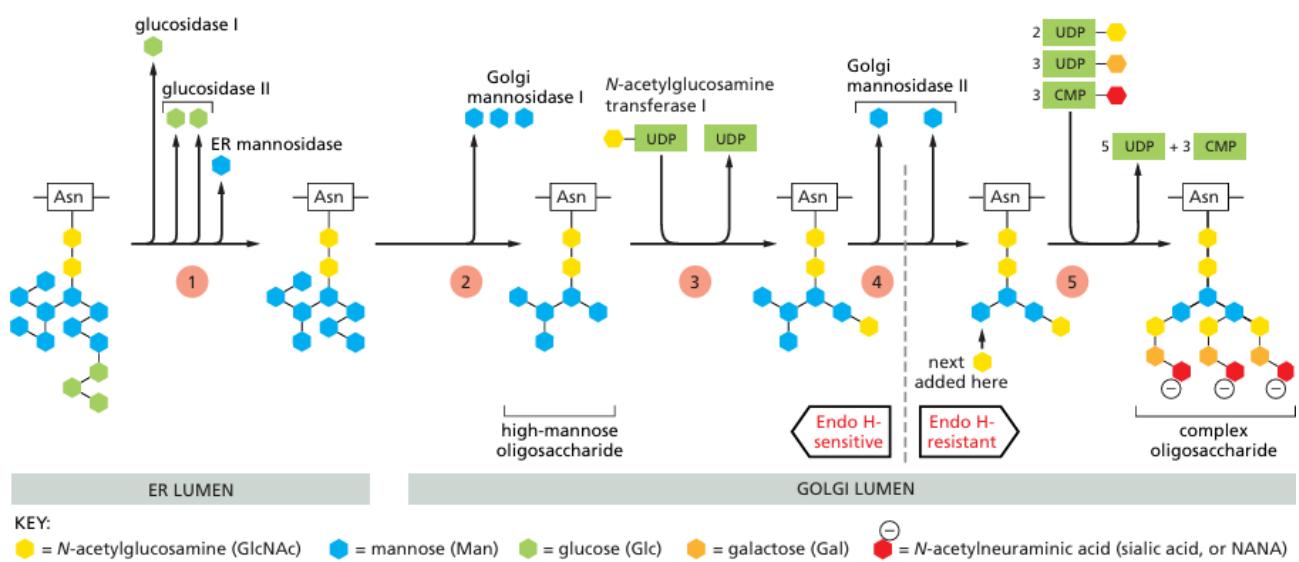


Figure 155: Oligosaccharide processing in the ER and the Golgi apparatus.

In addition there are also some proteins that have sugars added to hydroxyl groups of selected serines or threonines. These are **O-linked glycosylations** and like N-linked they are catalyzed by a series of glycosyl transferase enzymes that use sugar nucleotides in the lumen of the Golgi. Therefore the Golgi produces **mucins** (heavily glycosylated proteins found in mucus) and **Proteoglycans** (proteins with one or more glycosaminoglycan (GAG) chains).

Moreover sugars in **Glycosaminoglycans (GAGs)** are heavily sulfated in the Golgi apparatus right after the polymers are synthesized. This sulfation contributes significantly to their strong negative charge. In addition, some tyrosine residues in proteins are also sulfated just before the proteins leave the Golgi. In both cases, the sulfate groups are donated by **PAPS**.

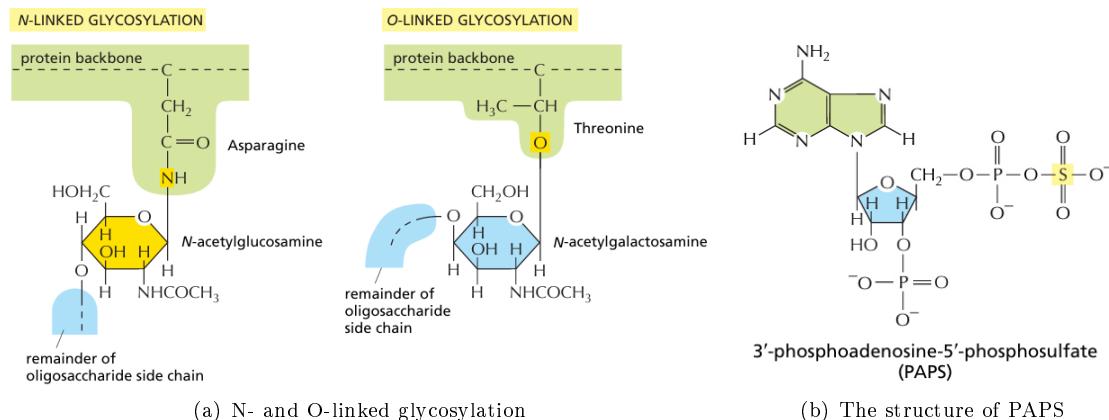


Figure 156: Proteoglycans Are Assembled in the Golgi Apparatus

**Definition 10.11 (Lectins).** *Lectins are a type of protein that specifically binds to carbohydrate structures without modifying them. Therefore, it plays key roles in cell-cell recognition, signaling, and intracellular trafficking by recognizing specific glycan patterns on glycoproteins and glycolipids. In the Golgi apparatus and elsewhere,*

---

*lectins help sort glycoproteins by interacting with their attached sugar chains.*

*Remark 10.12 (Purpose of glycosylation).* The purpose of glycosylation is diverse:

- **N-linked glycosylation promotes protein folding** in two ways. First, it has a direct role in making folding intermediates **more soluble**, thereby preventing their aggregation. Second, the sequential modifications of the N-linked oligosaccharide establish a “**glyco-code**” that **marks the progression** of protein folding and mediates the binding of the protein to chaperones.
- It **protects proteins** protruding the surface, thus limiting the approach of other macromolecules, like proteolytic enzymes.
- The mucus coat of lung and intestinal cells **protects against many pathogens**.
- The recognition of sugar chains by lectins in the extracellular space is important in many developmental processes and in **cell-cell recognition**.
- Glycosylation can also have important **regulatory roles**. For example Notch where glycosylation changes the specificity.

### 10.3 Around Lysosomes

Lysosomes are membrane-enclosed organelles filled with soluble hydrolytic enzymes that digest macromolecules. These enzymes are **acid hydrolases**; that are hydrolases that work at acidic pH. Therefore these enzymes do not work outside of the lysosome, otherwise it would be pretty dangerous if those enzymes would go berserk. To maintain the low pH lysosomes uses a vacuolar H<sup>+</sup> ATPase (V-type ATPase).

**Lysosomes are a meeting place** where several streams of intracellular traffic converge. A route that leads outward from the ER via the Golgi apparatus delivers most of the lysosome's digestive enzymes, while at least **four paths** from different sources feed substances into lysosomes for digestion:

- **Endocytosis** – internalizes molecules from the extracellular fluid into the cell.
- **Phagocytosis** – specialized in engulfing large particles or microorganisms, mainly by immune cells.
- **Macropinocytosis** – non-specifically engulfs extracellular fluid, membrane, and surface-bound particles.
- **Autophagy** – degrades cytosolic components and damaged organelles from within the cell.

#### 10.3.0.1 Lysosome Maturation

Lysosome maturation is a dynamic, multistep process that transforms **late endosomes into fully functional lysosomes**. This maturation involves:

- **Fusion of late endosomes** with existing lysosomes and vesicles from the Golgi, which deliver newly synthesized acid hydrolases.
- **Formation of endolysosomes**, hybrid compartments where active degradation begins.
- As contents are digested, the endolysosome **condenses and matures into a “classical” lysosome** — dense and enzyme-rich.
- **Mature lysosomes can re-enter the cycle**, fusing with other endosomes or endolysosomes to continue degradation.

Because of this continuous remodeling, lysosomes appear **morphologically diverse** and are better understood as *stages of a maturation cycle*, not fixed entities.

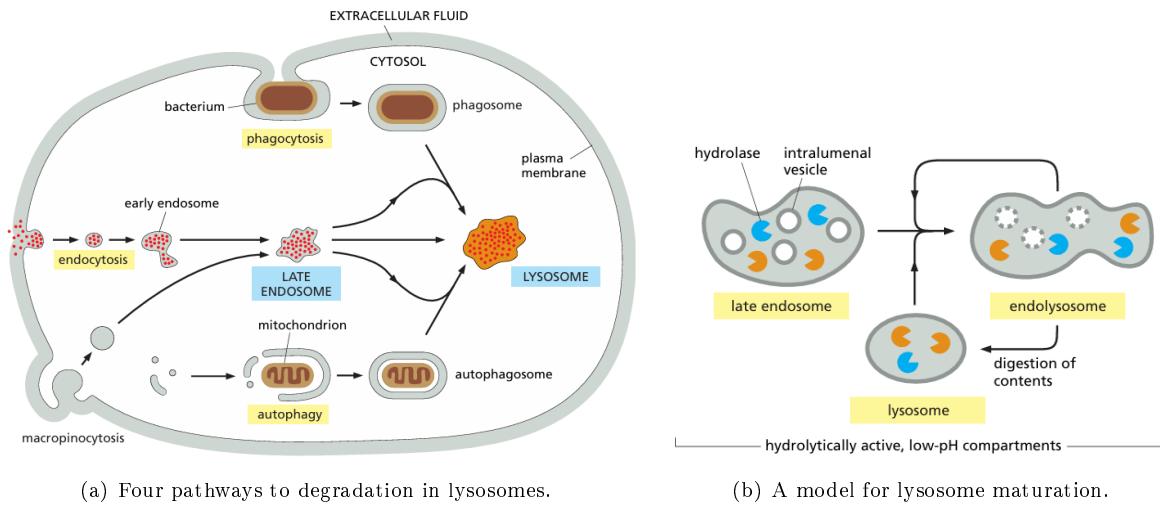


Figure 157: Lysosomes Are Heterogeneous

**Remark 10.13 ( Autophagy Degrades Unwanted Proteins and Organelles).** When a signaling pathway is activated, it triggers the start of autophagosome formation in the cytoplasm. A curved membrane structure begins to grow, formed by the fusion of small vesicles from unknown sources. This eventually closes into a double membrane, creating an autophagosome that surrounds part of the cytoplasm. The autophagosome then fuses with lysosomes, where the enclosed material is broken down by digestive enzymes. During this process, special ubiquitin-like proteins are activated by attaching to lipid anchors, helping to shape the membrane and guide vesicle fusion.

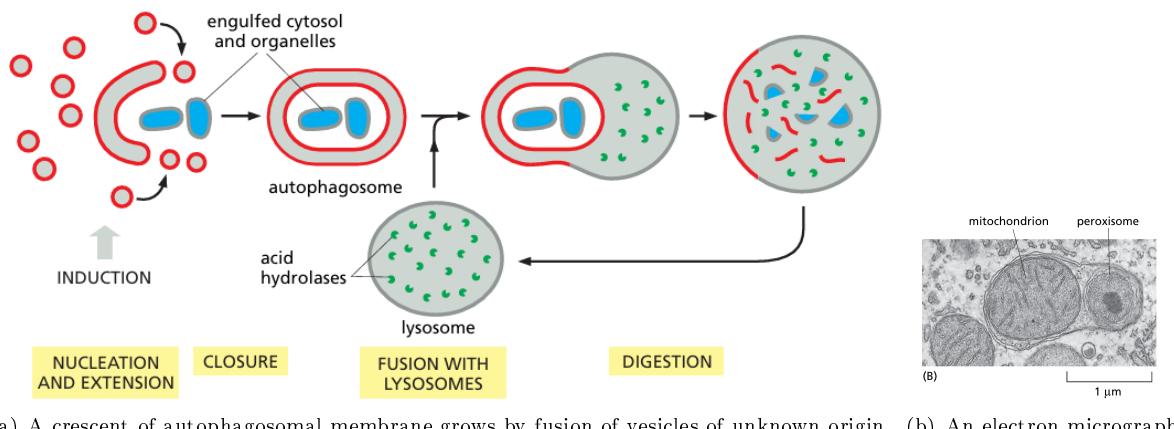


Figure 158: A model of autophagy

#### 10.3.0.2 Delivery of lysosomal hydrolases from the TGN to the lysosome

The enzymes are first delivered to endosomes in transport vesicles that bud from the TGN (trans golgi network), before they move on to endolysosomes and lysosomes (maturation of Lysosomes).

Lysosomal hydrolases are **recognized** and selected in the TGN because of a unique marker. **Mannose 6-phosphate (M6P)** groups are added exclusively to the **N-linked oligosaccharides** of these soluble lysosomal enzymes as they pass through the lumen of the cis Golgi network.

Transmembrane **M6P receptor proteins**, which are present in the TGN, recognize the M6P groups and bind to the lysosomal hydrolases on the luminal side of the membrane and to adaptor proteins in assembling **clathrin coats** on the cytosolic side. In this way, the receptors help package the hydrolases into clathrin-coated vesicles that bud from the TGN and deliver their contents to early endosomes.

The M6P receptor (like many proteins in transport) is an example for a protein that **changes specificity**: The M6P receptor protein binds to M6P at pH 6.5–6.7 in the TGN lumen and releases it at pH 6, which is the pH in the lumen of endosomes.

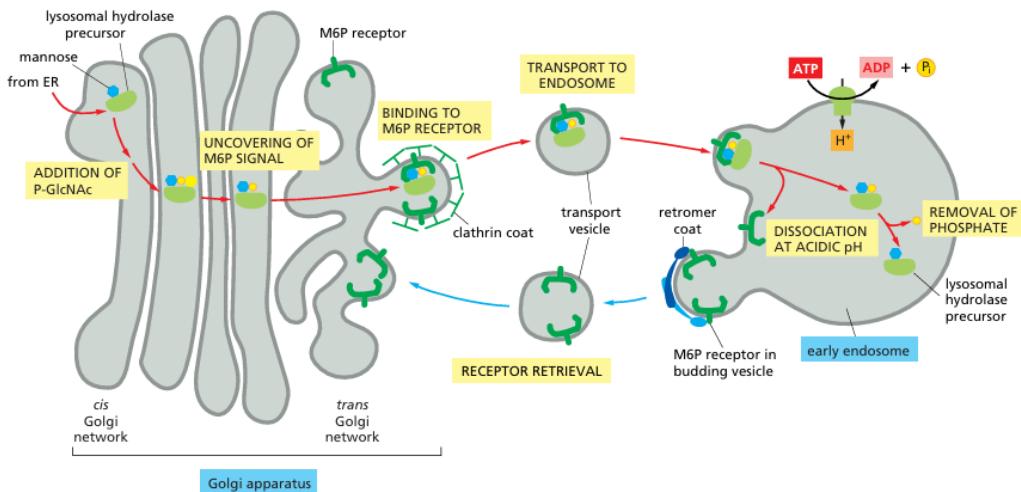


Figure 159: The transport of newly synthesized lysosomal hydrolases to endosomes.

The specialized enzyme called **GlcNAc phosphotransferase** identifies lysosomal hydrolases by recognizing a **unique signal patch** on their surface. This enzyme has two distinct domains: a **recognition site** that binds specifically to the signal patch on lysosomal enzymes, and a **catalytic site** that attaches a GlcNAc-phosphate group from UDP-GlcNAc to a mannose residue on the hydrolase's high-mannose N-linked oligosaccharide. Afterward, a second enzyme removes the GlcNAc, leaving behind an exposed **mannose 6-phosphate (M6P)** the crucial signal for lysosomal targeting.

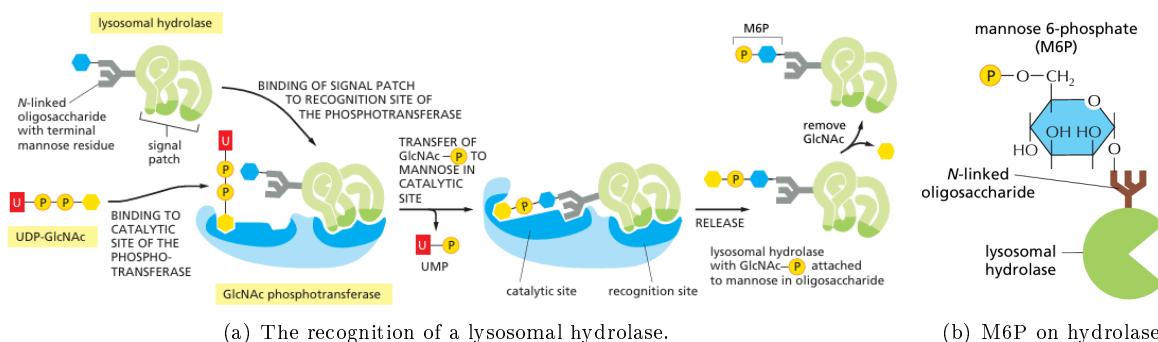


Figure 160: How are lysosomal hydrolases recognized in the trans golgi network?

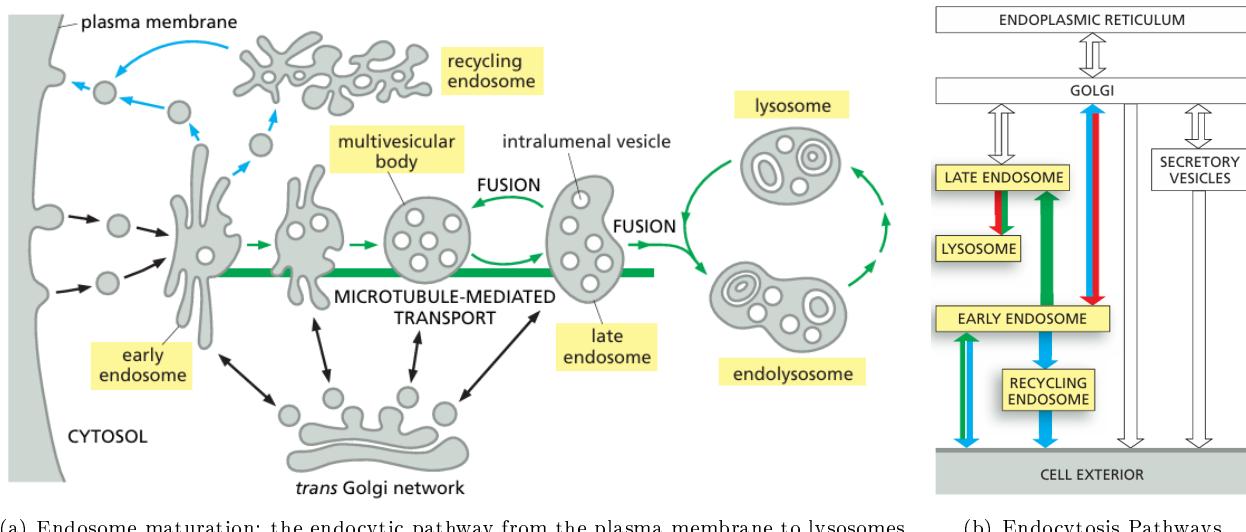
## 10.4 Endocytosis

The inward routes from the cell surface begin with endocytosis, a process by which cells internalize plasma membrane components, fluids, solutes, macromolecules, and particles. This mechanism allows the cell to dynamically regulate the composition of its plasma membrane in response to changing extracellular conditions.

In endocytosis, material is enclosed by an invaginating section of the plasma membrane, which pinches off to form a **endocytic vesicle** containing the ingested substance. This vesicles are formed constantly in a process called **Pinocytosis** ("cell drinking"). Recall that some specialized cells contain dedicated pathways to take up large particles on demand via the process called phagacytosis ("cell eating").

Once generated at the plasma membrane, most endocytic vesicles fuse with a common receiving compartment, the **early endosome**, where **internalized cargo is sorted**: some cargo will be **returned** either directly or via a **recycling endosome**, and others are designated for degradation by inclusion in a **late endosome** (Recall endosome maturation).

Each of the stages of the endosome maturation (from the early endosome to the endolysosome) is connected through **bidirectional vesicle transport to the TGN**. This allows for the **insertion** of newly synthesized materials, such as lysosomal enzymes, and the **retrieval** of components such as the M6P receptors.



(a) Endosome maturation: the endocytic pathway from the plasma membrane to lysosomes. (b) Endocytosis Pathways

Figure 161: Transport into the Cell from the Plasma Membrane: Endocytosis

The endocytic part of the cycle often begins at **clathrin-coated pits**. These specialized regions typically occupy about 2 % of the total plasma membrane area. Note the lifetime of a clathrin-coated pit is short: within a minute or so of being formed, it invaginates into the cell and pinches off to form a clathrin-coated vesicle.

In addition to clathrin-coated pits and vesicles, cells can form other types of pinocytic vesicles, such as **Caveola**. In contrast to clathrin-coated and COPI- or COPII-coated vesicles, **caveolae are usually static structures**. They are invagination that form from **lipid rafts** at the cell surface and bud off to form a **pinocytic vesicle**.

A huge family of structural proteins in caveolae is **Caveolin** which cross the membrane extend multiple hydrophobic loops into the membrane from the cytosolic side, but do not cross the membrane.

Moreover, the **clathrin-coated pits allow for Receptor-mediated endocytosis** which is a highly efficient way for cells to take up specific molecules. These molecules (ligands) bind to matching receptors on the

cell surface, which cluster into clathrin-coated pits. This process allows cells to concentrate and import even rare substances, like **cholesterol**, very effectively.

**Example 10.14 (Cholesterol Receptor-Mediated Endocytosis).** Most cholesterol is transported in the blood as cholestryl esters in the form of lipid–protein particles known as **Low-Density Lipoprotein (LDL)**. Therefore a cell can control the import of cholesterol by expressing more transmembrane receptor proteins for LDL. These receptors are found in clathrin-coated pits and will induce an endocytosis signal.

Moreover, if you think about what cholesterol does if this uptake does not work and it accumulates in the blood, it is not surprising that, it was a study of humans with a strong genetic predisposition for atherosclerosis that first revealed the mechanism of receptor-mediated endocytosis.

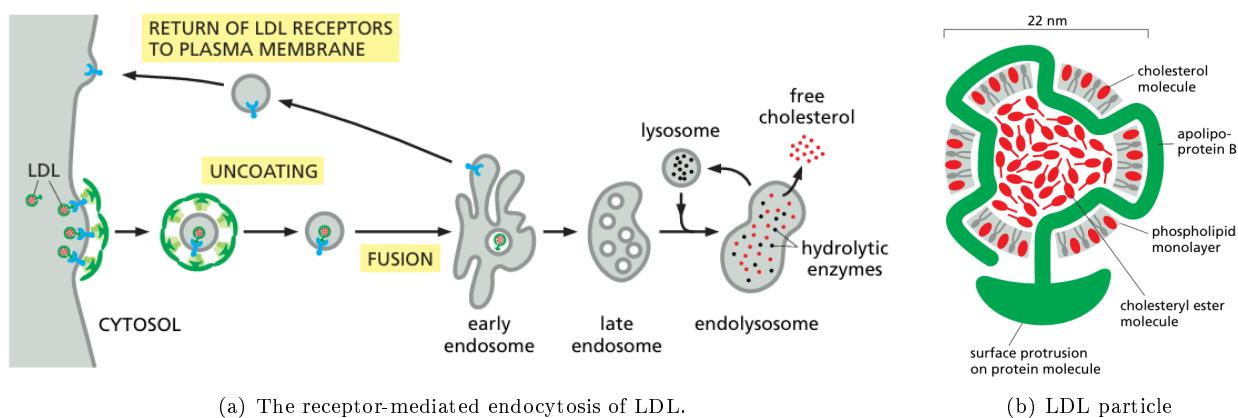


Figure 162: Receptor-Mediated Endocytosis to Import Selected Extracellular Macromolecules

**Remark 10.15 (Macropinocytosis).** Macropinocytosis is a dedicated degradative pathway. It is also a **clathrin-independent** endocytic mechanism that can be activated in practically all animal cells. Mostly it does not operate continuously but is rather **induced for a limited time in response to a cell-surface receptor** activation by a specific cargo (i.e. growth factors). The activation of the receptor leads to a complex signaling pathway, resulting in a **change in actin dynamics** and the **formation of ruffles** (cell-surface protrusions). When the ruffle collapses back onto the cell, the **macropinosome** is created. See fig 163(a)

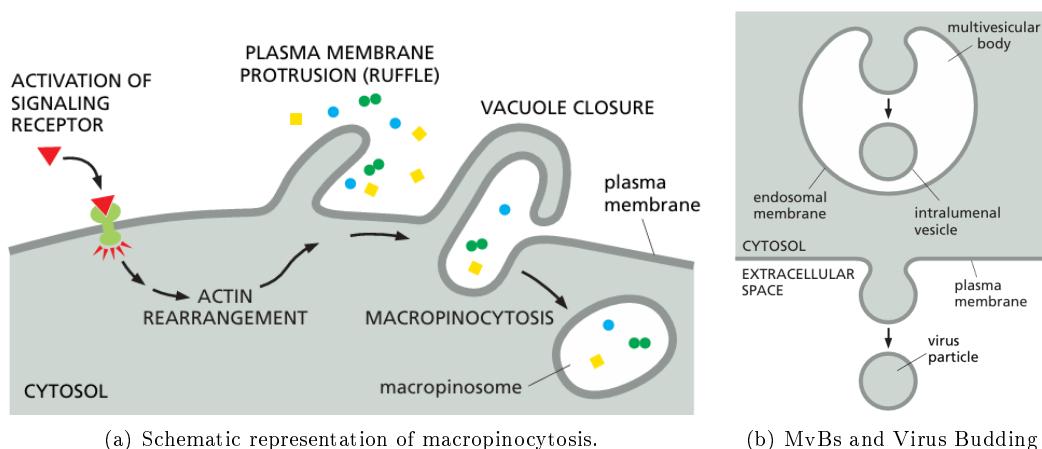


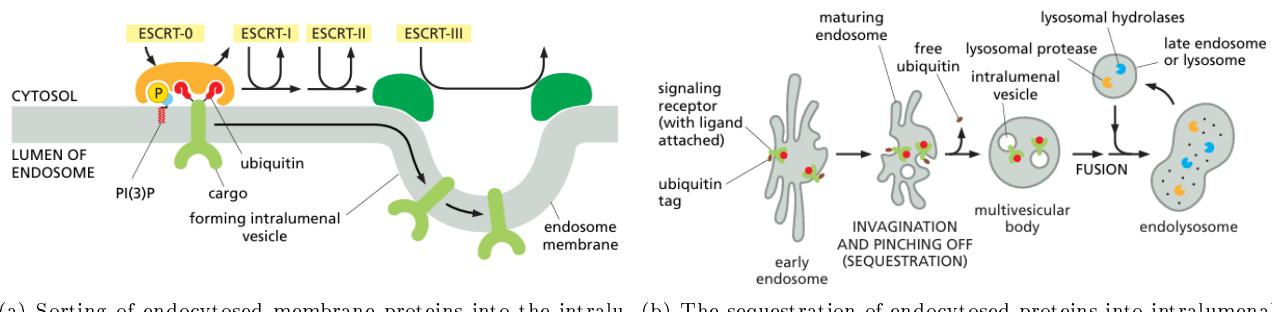
Figure 163: Schematic Mechanism in macropinocytosis and multivesicular body formation

#### 10.4.0.1 Transmembrane receptor proteins get endocytosed

As **endosomes mature**, portions of their membrane invaginate into the lumen and pinch off, forming **internal vesicles**. These maturing endosomes are called **Multivesicular bodies (MVBs)** due to their distinctive appearance. A key role of MVBs is to **hide away ubiquitylated membrane proteins** (like activated EGF receptors) into these intraluminal vesicles. This process ensures that the proteins are isolated from the cytosol, stopping any further signaling and making them fully accessible to lysosomal hydrolases. See fig. 165(b)

Before the vesicles close, **the ubiquitin tag is removed and recycled**. Ultimately, when the multivesicular body fuses with a lysosome, **the enclosed vesicles and their contents are digested**. Without this internal sequestration (hiding away), parts of the membrane proteins exposed to the cytosol would escape degradation, since the lysosomal membrane itself is not broken down.

*Remark 10.16 (ESCORT protein complexes).* ESCRT complex The ESCRT (Endosomal Sorting Complex Required for Transport) protein complexes (ESCRT-0, -I, -II, -III) play a crucial role in **sorting ubiquitylated membrane proteins into the intraluminal vesicles of multivesicular bodies**. Without proper ESCRT function, these receptors may continue to signal inappropriately, potentially contributing to diseases like **cancer**. Note that **ESCRT proteins are recruited from the cytosol to specific domains on the endosome membrane**, where they bind to PI(3)P lipids and ubiquitylated cargo proteins.

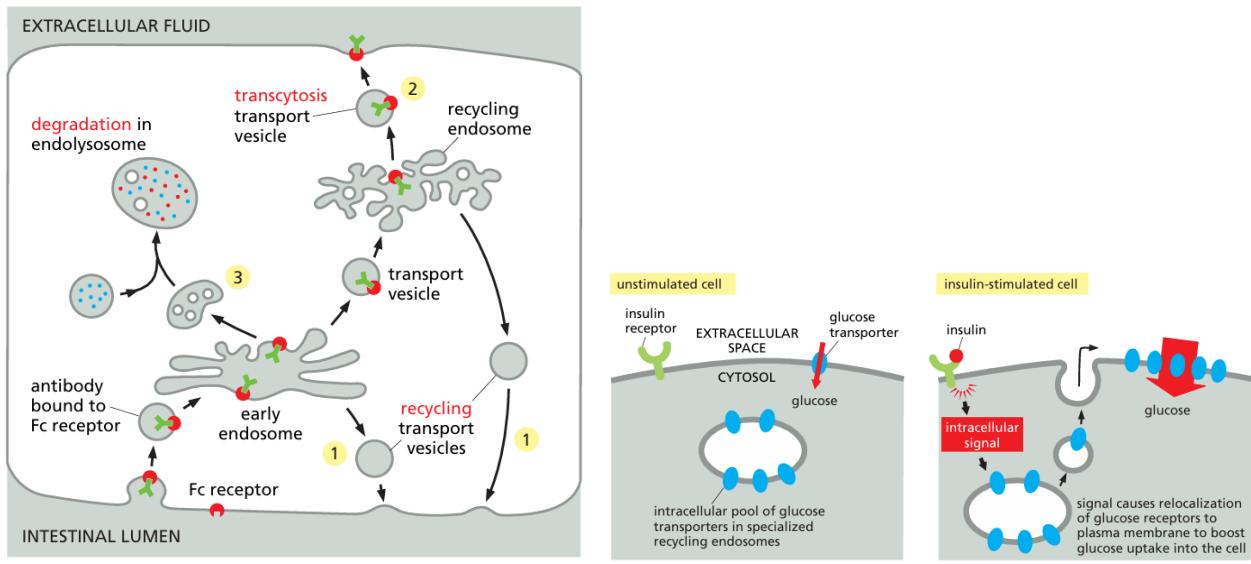


(a) Sortung of endocytosed membrane proteins into the intraluminal vesicles of a multivesicular body. (b) The sequestration of endocytosed proteins into intraluminal vesicles of multivesicular bodies.

Figure 164: ESCRT Protein Complexes Mediate the Formation of Intraluminal Vesicles in Multivesicular Bodies

Receptors behave differently depending on their type. Most are recycled back to their original membrane area, some are sent to a different area through **transcytosis**, and others are degraded in lysosomes. Note that **the transcytotic pathway is not direct**. Therefore the receptors move first to an early endosome and then to a recycling endosome. Many receptors **possess sorting signals** that guide them into the appropriate transport pathway.

**Cells can regulate the release of membrane proteins from recycling endosomes**, thus adjusting the flux of proteins through the transcytotic pathway according to need. For example in response to **insulin**, they can release stored glucose transporters.



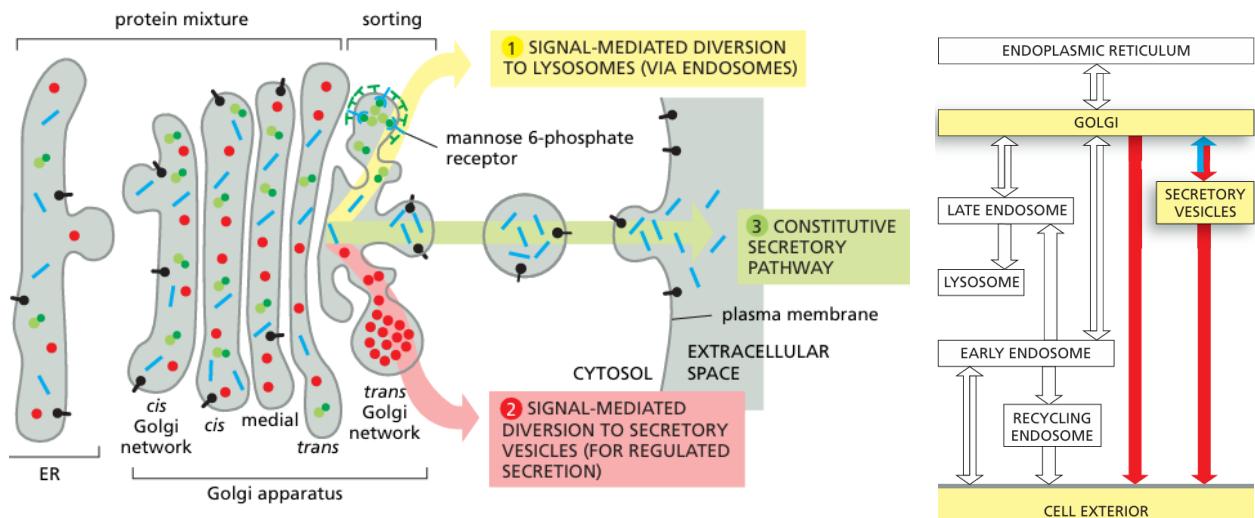
(a) Possible fates for transmembrane receptor proteins that have been endocytosed. (b) Storage of plasma membrane proteins in recycling endosomes.

Figure 165: Recycling Endosomes Regulate Plasma Membrane Composition

## 10.5 Exocytosis

Vesicles from the trans-Golgi network (TGN) deliver membrane and soluble proteins to the cell surface through exocytosis, replenishing the plasma membrane and releasing secreted proteins.

All cells use a **Constitutive secretory pathway** for continuous protein export. **Specialized cells** also have a **Regulated secretory pathway**, where proteins are stored in vesicles and released on demand, such as hormones or neurotransmitters.



(a) The three best-understood pathways of protein sorting in the trans Golgi network.

(b) Exocytosis

Figure 166: Transport from the Trans Golgi Network to the Cell Exterior: Exocytosis

Note that all cell capable of regulated secretion must **separate at least three classes of proteins** before they leave the TGN, those destined for lysosomes (via endosomes, proteins tagged with M6P), those destined for secretory vesicles, and those destined for immediate delivery to the cell surface (**default pathway**: does not require a particular signal).

In an **unpolarized cell**, such as white blood cells or a fibroblast, it seems that particularly any produced protein is carried by the constrictive pathway. While in **polarized cells** the options are more diverse :).

Cells that are specialized to secreting some of their products rapidly on demand concentrate and store these products in **secretory vesicles**. These vesicles are often called **densecore secretory granules** since they have a dense core visible in EM.

These core is dense since the **secretory proteins often aggregate**. It is though **unclear** how the aggregates are packed into secretory vesicles. They uptake may **resemble phagocytosis** quite a bit.

Initially, the **immature secretory vesicles** leaving the trans-Golgi network (TGN) contain loosely packed proteins and **resemble swollen Golgi cisternae**. As they mature, these vesicles **fuse**, their **contents become more concentrated**, and their interiors acidify due to **V-type ATPases**, which pump protons into the lumen.

**Membrane recycling helps return Golgi components** and further concentrates the contents of secretory vesicles. Clathrin-coated buds on immature vesicles mediate this retrieval process. **As a result, mature vesicles are densely packed** and ready to rapidly release their contents upon stimulation.

This increase in concentration happens because:

- **Membrane is continually recycled** from the immature vesicle back to the Golgi, reducing vesicle volume.
- **The vesicle lumen becomes more acidic** due to V-type ATPases, promoting condensation of the protein cargo.

Although the **total amount of protein remains constant** after vesicle formation, the **volume decreases**, leading to a **higher protein concentration**—a key step in preparing for rapid, efficient secretion.

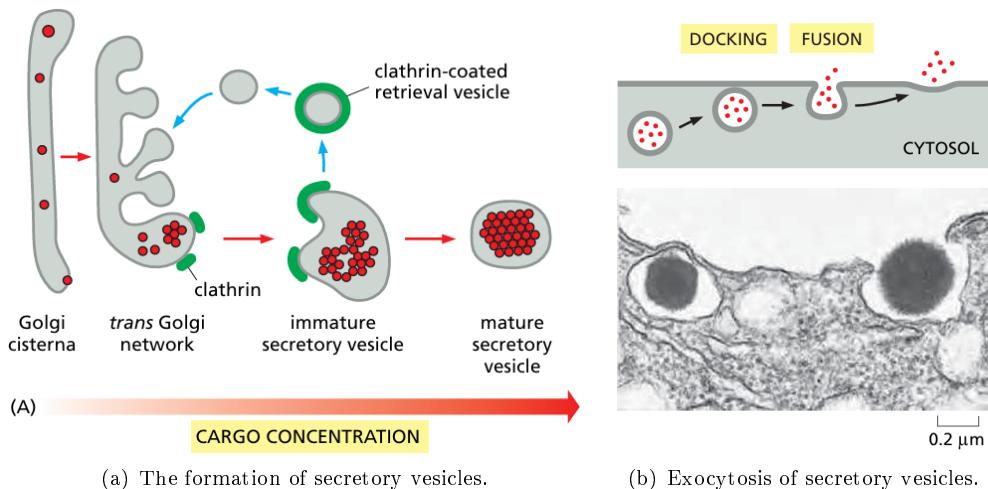


Figure 167: Secretory Vesicles Bud from the Trans Golgi Network

**Remark 10.17 (Proteolytic Processing of Secretory Proteins).** As secretory vesicles mature, many secretory proteins are processed by proteolysis. Hormones, neuropeptides, and hydrolytic enzymes are often

synthesized as inactive precursors that **must be cleaved to become active**.

Proteolytic cleavage occurs in the secretory vesicle or after secretion and helps:

- Activate short signaling peptides that couldn't otherwise be transported or packaged.
- Prevent premature activity of potentially harmful enzymes within the cell.

These precursor proteins are commonly synthesized as *pre-pro-proteins*, or polyproteins, which are processed into one or multiple active peptides, depending on the cell type.

#### 10.5.0.1 Exocytosis of synaptic vesicles

Nerve and some endocrine cells have two types of secretory vesicles. One type, **dense-cored vesicles**, releases proteins and neuropeptides through the regulated secretory pathway. The other type, **synaptic vesicles** (about 50 nm wide), stores small neurotransmitters like acetylcholine, glutamate, glycine, and GABA for fast communication at synapses. When an action potential reaches the nerve terminal, **calcium enters the cell and triggers these vesicles to release their contents**.

This **fast release** is made possible by a **priming step** where **SNARE proteins partially pair**. Complexins hold the SNAREs in this ready (metastable) state. When  $\text{Ca}^{2+}$  levels rise, synaptotagmin binds to the SNAREs and membrane lipids, displacing complexins and allowing full SNARE zippering. This opens a fusion pore, releasing neurotransmitters. Only a few vesicles are primed at a time, allowing rapid and repeated firing as new vesicles continuously dock and prepare for release.

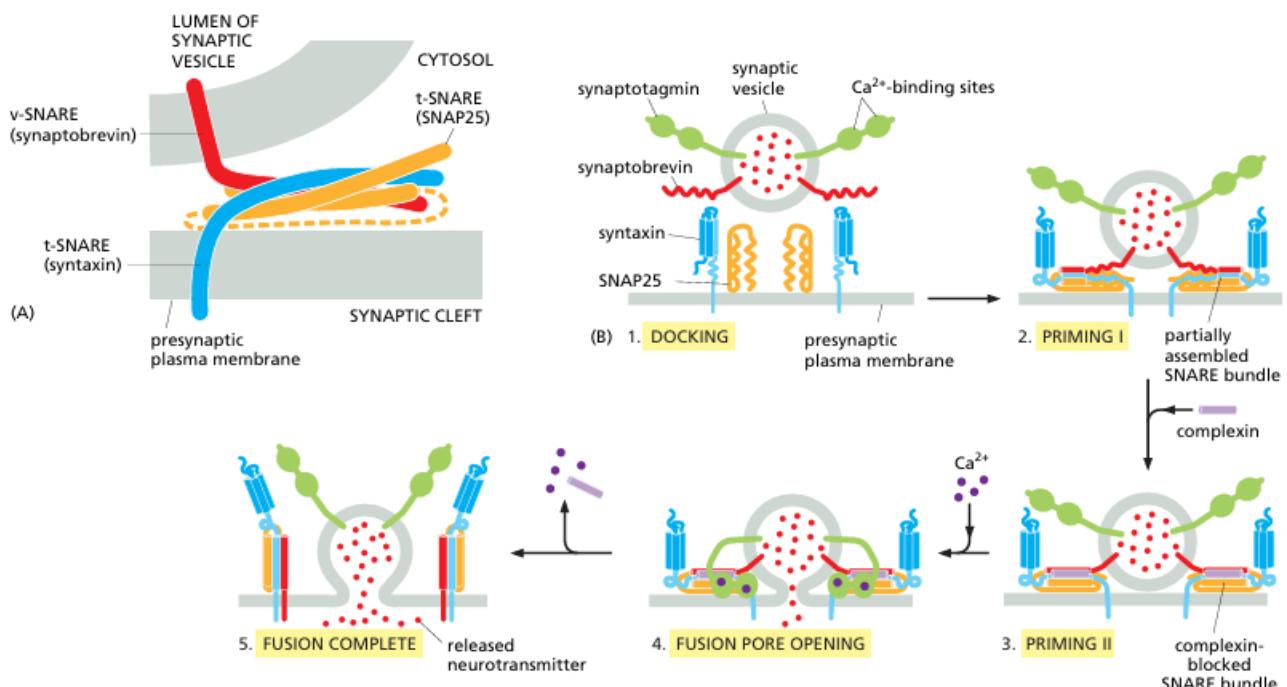


Figure 168: Exocytosis of synaptic vesicles.

To support rapid and repeated firing, **synaptic vesicles are quickly replenished through local recycling at the nerve terminal**, rather than being made in the cell body. New membrane components are first delivered to the plasma membrane, then retrieved by endocytosis. Instead of fusing with endosomes, most endocytic vesicles are quickly refilled with neurotransmitter and reused as synaptic vesicles.

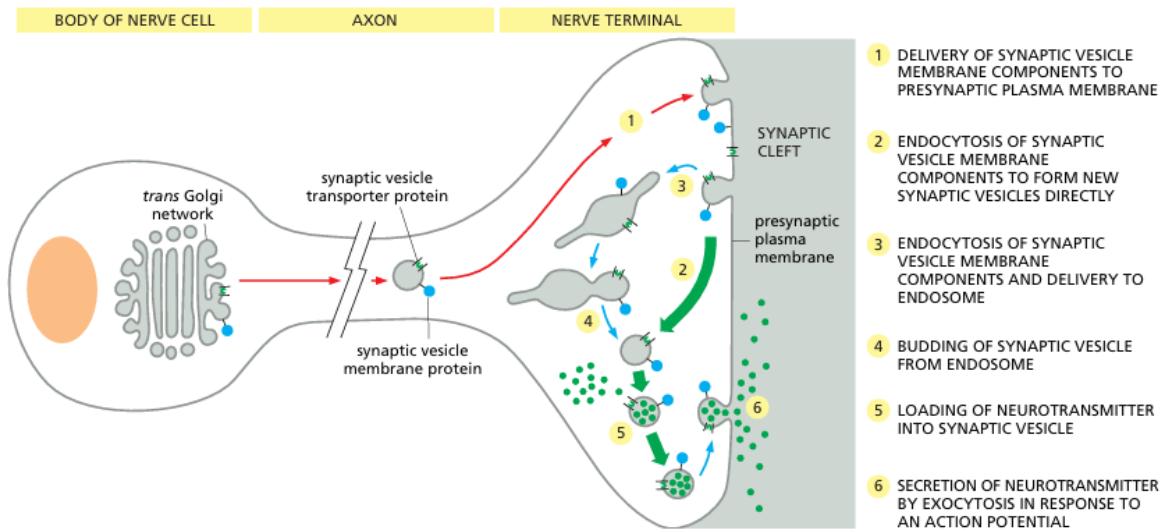


Figure 169: The formation of synaptic vesicles in a nerve cell.

#### 10.5.0.2 Sorting plasma membrane proteins in polarized epithelial cell

In polarized epithelial cells, which have distinct **apical** and **basolateral** membrane domains, plasma membrane proteins are sorted by two main mechanisms:

- i) **Direct Sorting at the Trans-Golgi Network (TGN):**

Proteins are sorted in the TGN and transported directly to either the apical or basolateral membrane. Sorting signals and mechanisms such as lipid rafts (e.g., for GPI-anchored proteins) help target proteins to the correct domain.

- ii) **Indirect Sorting via Transcytosis:**

Some proteins are first delivered to one membrane domain (typically the basolateral side), then internalized and redirected to the correct domain (e.g., apical) through endosomal transport.

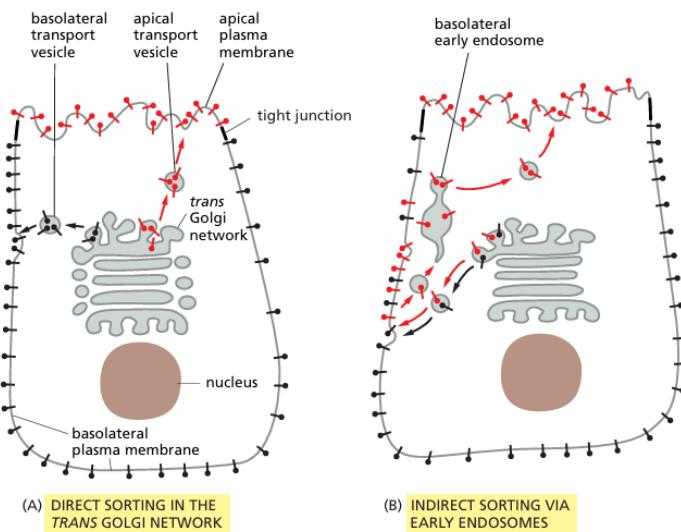


Figure 170: Two ways of sorting plasma membrane proteins in a polarized epithelial cell.

# 11 Cell Junctions and the Extracellular Matrix

The organization of our cell is a pretty complex system. It can be split into two major components: The **epithelial tissue** and the **connective tissue**. The **basal lamina** separates the two, providing stability and also selective permeability between the two tissue types. The **extracellular matrix** with the **collagen fibers**, bears a lot of the mechanical stress on the tissue. This chapter will be looking at the connections between cells, cells and the ecm, and with **cytoskeletal proteins**, focusing also how a cell can sense its surroundings.

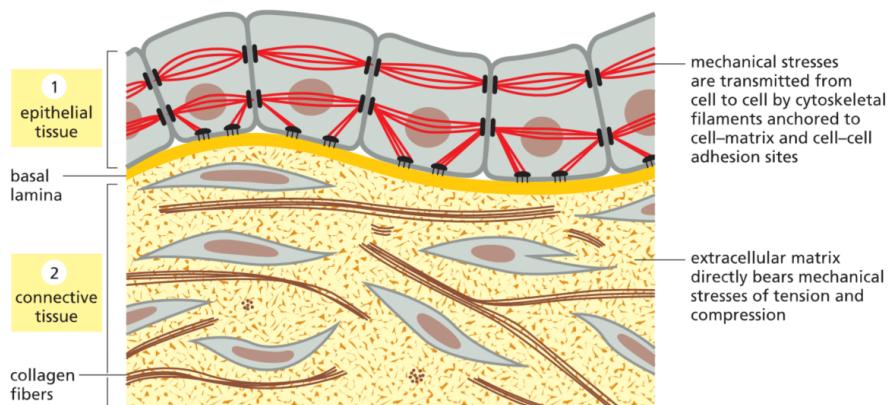


Figure 171: An overview of the tissue structure between the epithelial tissue and the extracellular matrix.

## 11.1 Overview of the Types and Groups of cell junctions

There are four main types of cell junctions, which each have specific functions, as well as subgroups:

- i) **anchoring junctions:** adhesion of cell-cell (c-c) or cell-matrix(c-m) connections.
  - Actin related: adherens junctions for c-c and actin-linked junctions for c-m
  - intermediate filament attachment sites: desmosomes for c-c and hemidesmosomes for c-m.
- ii) **occluding junctions:** Block inter-cellular passage, causing impermeable or selectively permeable barriers
  - tight junctions (vertebrates)
  - septate junctions (in non-vertebrates, not covered in course)
- iii) **channel-forming junctions:** Enable communication between two cell interiors (ex. GAP junctions - electrical conductance between two cells).
  - Gap junctions (animals)
  - plasmodesmata (plants, not covered in course)
- iv) **signal-relaying junctions:** To enable communication between two adjacent cells (ex. neurological synapse).
  - chemical synapses (nervous system)
  - immunological synapses (immune system, not covered in course)
  - transmembrane ligand-receptor cell-cell signaling contacts (Delta-Notch, ephrin-Eph, etc.)
  - Anchoring, occluding, and channel-forming junctions can all have signaling functions in addition to their structural roles.

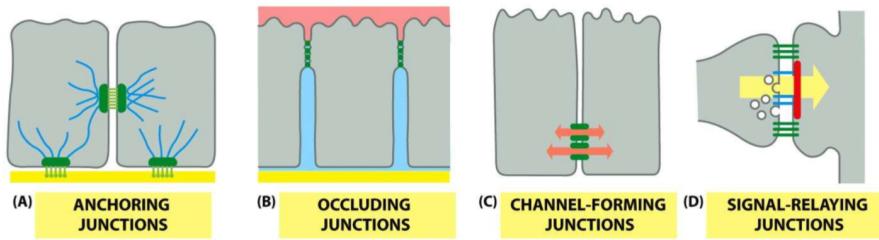


Figure 172: The four main types of junctions between cell-cell and cell-matrix.

The same cell will have many different types of junctions. A **junction complex** is a tight junction, at the most apical position, followed by an adheren junction, followed by a desmosome. These three "glue" a cell together. The figure 173 shows all the different types of junctions, based off of an epithelial cell of the small intestine:

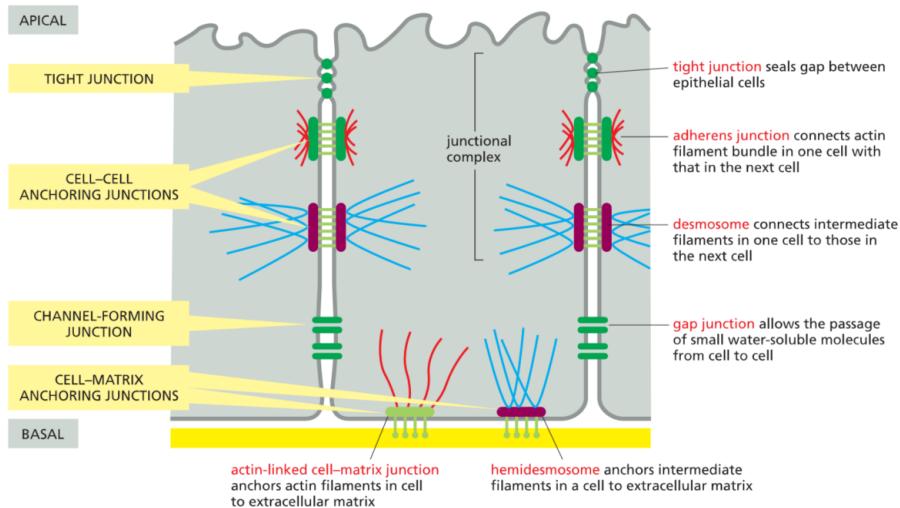


Figure 173: An example of the types of junctions, based off of an epithelial cell in the small intestine.

There are two types cells bind to each other:

- homophilic binding:** both cells have identical extracellular proteins which bind together.
- heterophilic binding:** two different proteins attach to each other. Common for signaling (e.g., Delta-Notch).

## 11.2 Cell-Cell Anchoring Junctions

There are four main different anchoring junctions, two for cell-cell and two for cell-matrix. Here is an overview (fig. 174) of each type and what their main actors are:

TABLE 19-1 Anchoring Junctions				
Junction	Transmembrane adhesion protein	Extracellular ligand	Intracellular cytoskeletal attachment	Intracellular adaptor proteins
<b>Cell–Cell</b>				
Adherens junction	Classical cadherins	Classical cadherin on neighboring cell	Actin filaments	$\alpha$ -Catenin, $\beta$ -catenin, plakoglobin ( $\gamma$ -catenin), p120-catenin, vinculin
Desmosome	Nonclassical cadherins (desmoglein, desmocollin)	Desmoglein and desmocollin on neighboring cell	Intermediate filaments	Plakoglobin ( $\gamma$ -catenin), plakophilin, desmoplakin
<b>Cell–Matrix</b>				
Actin-linked cell–matrix junction	Integrin	Extracellular matrix proteins	Actin filaments	Talin, kindlin, vinculin, paxillin, focal adhesion kinase (FAK), numerous others
Hemidesmosome	$\alpha_6\beta_4$ Integrin, type XVII collagen	Extracellular matrix proteins	Intermediate filaments	Plectin, BP230

Figure 174: An overview of the different types of anchoring junctions.

### 11.2.1 Cadherins and Adherens Junction

There are a lot of different cadherin superfamily members. All of them are structurally related in the ecm domain, as they all have the cadherin domains. The number of cadherin domain varies strongly (5 for classical, 4 or 5 for desmogleins and desmocollins, and at time over 30 for nonclassical cadherins. In addition, their functions, and intracellular proteins vary strongly. Some even lack the transmembrane element (e.g., T-cadherin who is attached through a GPI anchor). All this means a strong variety of cadherins in the superfamily. Here (fig: 175) are some examples:

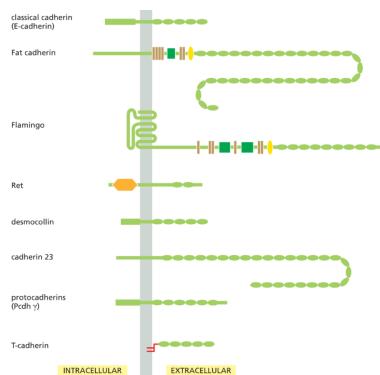


Figure 175: Shows some members of cadherin superfamily.

Cadherins mostly bind **homotypically**. The extracellular of a classical cadherin contain five copies of the cadherin domain, separated by five flexible hinge regions.

(see fig: 177)  $\text{Ca}^{2+}$  ions bind in the neighborhood of each hinge, preventing it from flexing. As a result cadherin forms a rigid, curved structure, which allows it to enter in binding with another rigid cadherin. In the absence of  $\text{Ca}^{2+}$  the cadherin will be more flexible resulting in a floppy molecule that can't interact with a different cadherin. Leading to a failure of adhesion. This means that a **sufficiently high concentration of  $\text{Ca}^{2+}$  is essential for cell adhesion**.

(see fig: 176) To generate the cell-cell adhesion the cadherin domain at the N-terminal tip of one cadherin binds to the domain of the other cadherin.

(see fig: 177) At a typical cell junction, an organized array of cadherin molecules functions like Velcro. Cadherins on the same cell are thought to be coupled by side-to-side interactions between their N-terminals, resulting in a linear array. Each cadherin (green) will bind to a cadherin on the other cell (blue) that is in a perpendicular array to it. This will lead to a tight-knit structure.

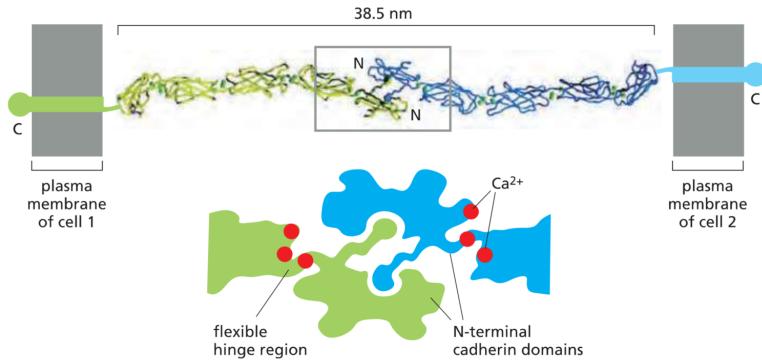
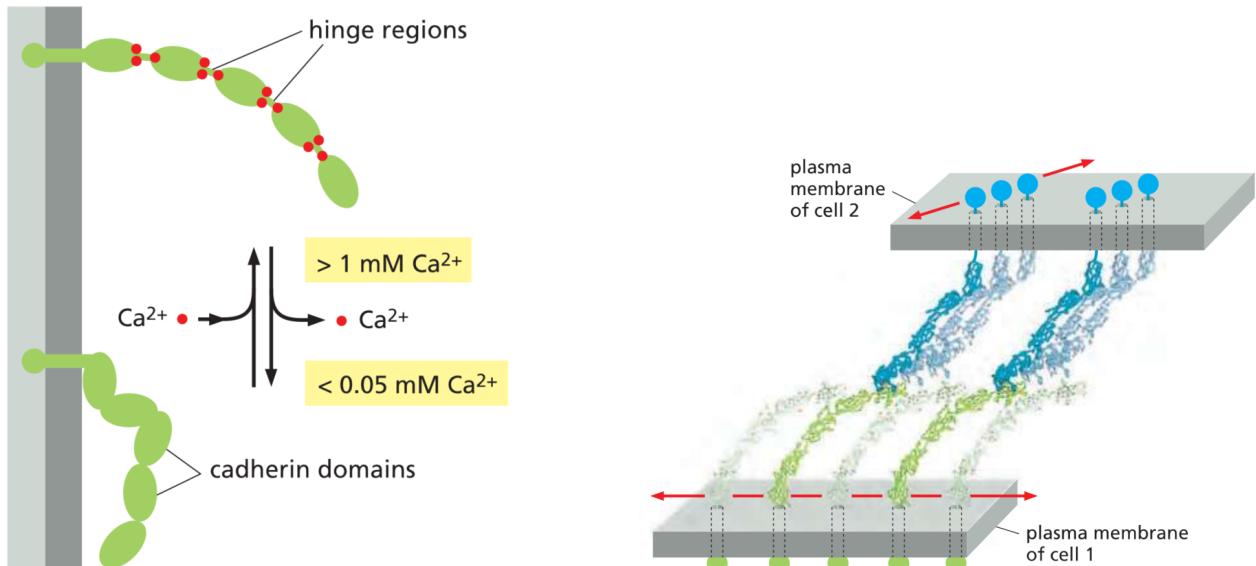


Figure 176: The interaction between two different cadherins at their N-terminals



(a) The interaction between cadherins and  $\text{Ca}^{2+}$  and how it relates to stiffness of the molecule.

(b) How the cadherins chain together like velcro in multiple directions. The arrays of interaction are perpendicular to each other. Having multiple arrays, then gives us a tight knit mat.

Figure 177:

**Classical cadherins interact with the cytoskeleton.** The interaction between cadherin and the **actin** filaments is indirect and mediated by an adaptor complex, which includes **beta-catenin**, which we saw in Wnt signaling. Further it contains **p120-catenin** and **alpha catenin**. Further proteins such as **vinculin** associate with  $\alpha$ -catenin and provide further actin links. This mean that **multiple actins will interact with one cadherin, but through different mediator proteins**.

The adaptor complex will undergo a conformational change when cadherin is attached to another cadherin. The tension can also be increased through a **myosin II**. The **increased tension on cadherin, causes  $\alpha$ -catenin to extend**, which in turn allows proteins like vinculin to attach associate and recruit further actins, strengthening the link between cytoskeleton and the junction. In essence, the **higher the tension, the more the cell strengthens the junction**.

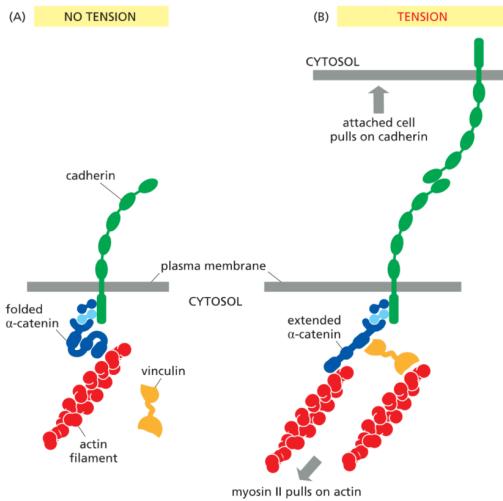


Figure 178: How increased tension causes more recruitment of actin, strengthening the cytoskeleton and junction.

(see fig: 179) This combined strengthening by cytoskeleton and junctions can organize the cells and cytoskeleton. When the first cadherin attaches it becomes easier for the neighboring ones to attach, which leads to the formation of clusters. This leads to the activation of an actin regulator, **GTPase Rac**, which promotes further cadherin binding. This causes a more widespread junction. Eventually it is inhibited and replaced by the related **GTPase Rho**, which moves the actins into a more linear form and promotes myosin II recruitment. That allows contractile move along the cell membrane allows cadherins up and down stream to be activated too. This further expands the junction.

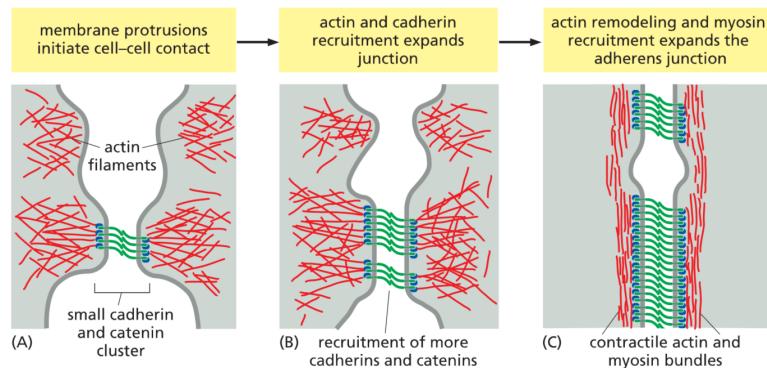


Figure 179: The expansion of a cadherin junction.

**Remark 11.1 (Sorting of cells with the help of cadherins).** Thanks to the number of different cadherins and homophilic nature of cadherins, cells tend to associate better with some cell than others and sort themselves accordingly. In an experiment this was shown, by purposefully mixing up cells and then seeing them re-associate.

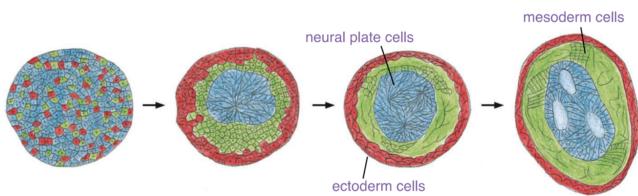


Figure 180: This experiment shows how cells reassociate thanks to having specific cadherin bonds. This allows cells to sort themselves to the correct cell types.

### 11.2.2 Adhesion Belts by Adherens Junctions

The actins in the cell between two cadherins can form a direct line from one adherens junction to one on the other side of the cell. If this line is continued between a number of cells, it forms an **adhesion belt**. This gives a lot of stability and allows for the formation of pretty set structures between epithelial cells.

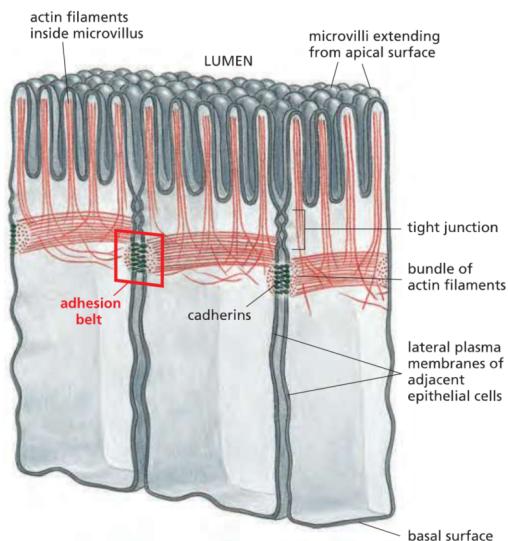


Figure 181: Shows an example of an adhesion belt, in the case of microvilli.

*Remark 11.2 (Microvilli in the small intestine:).* In the small intestine it is important that all the microvilli are tightly packed to maximize surface area. Adhesion belts, keep the cells in place.

*Remark 11.3 (Use Case: Developmental Biology).* The adhesion belt helps in cell development, by providing structure and connectivity between cells. For example, when creating the neural tube in early vertebrate development, it helps the cells to narrow at their apex and roll into a tube.

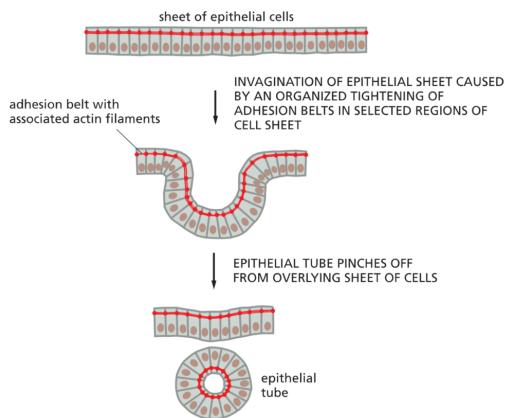


Figure 182: How adhesions belts assist the development of cell groups.

Looking more at the development of the neural tube, we can also observe that at different parts of the adhesion belt, we will have different cadherins. This will have the effect that they will segregate to each other, making it easier to break away from the ectoderm.

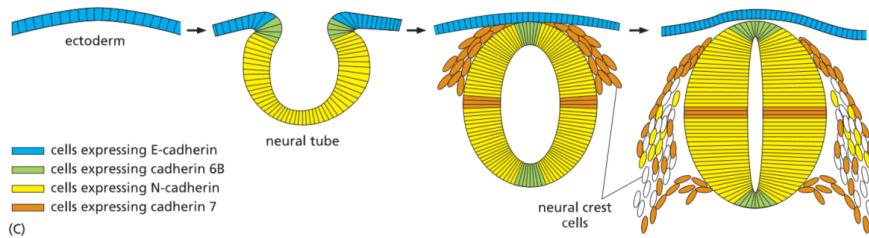


Figure 183: How having different cadherins causes certain shapes to form, looking at the use case of neural development.

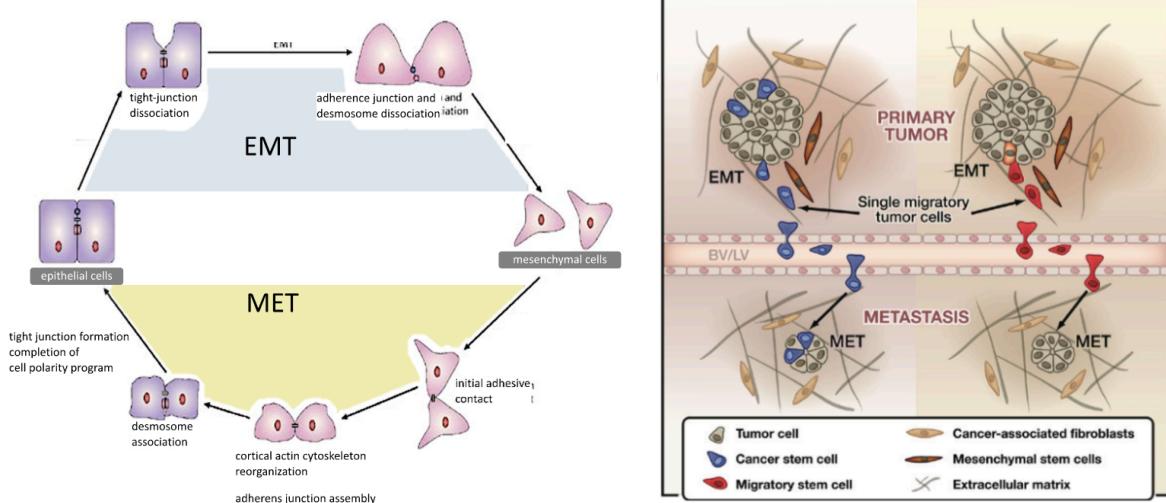
### 11.2.3 EMT and MET

Some key terms and their meaning:

- mesenchymal: multipotent stromal (connective tissue) cells that differentiate into a variety of cells including: fibroblasts, osteoblasts, chondrocytes, adipocytes, myocytes.
- EMT: epithelial-to-mesenchymal transition
- MET: Mesenchymal-to-epithelial transition

Now, looking at the **EMT** first: epithelial cells lose their polarity, as well as their cell adhesion, through the dissolution of tight junctions, adherence junctions (with that E-cadherin and cytoskeletal reorganization), and desmosomes.

Next up **MET**: starting with the initial adhesive contact, then the adherens junction (with its cytoskeletal reorganization), and then the desmosome association. Finally tight junctions will form.



(a) Shows the cycle of met and emt epithelial and mesenchymal cells can go through.

(b) Shows how cancer uses EMT and MET

Figure 184:

*Remark 11.4 (The body applying EMT and MET).* EMT is used in:

- **embryonic development:** helps cell move, adapt to for new cell groups;
- **Wound healing:** helps cells migrate to wound;
- **Cancer metastasis:** allows cells to migrate.

MET is used in:

- **embryonic development:** helps tissue grow together, form, and specialize (less talked about);
- **Wound healing:** crucial in repair of damage (fills the gaps);
- **Cancer metastasis:** allows it to settle into new area.

*Remark 11.5 (Some transcription factors which regulate EMT).* Twist, Snail, Slug, and Zeb are transcription factors that **drive EMT**. They do this by repressing epithelial genes (mainly cadherins) or activating mesenchymal genes (fibronectin and vimentin).

#### 11.2.4 Desmosomes and Hemidesmosomes

The structure of a desmosome is as follows:

- On the cytoplasmic surface is a dense plaque composed of a mix of intracellular adaptor proteins. Some of these components are:
  - **desmogleins** and **desmocollins** are nonclassical cadherins. Their tails bind to **plakoglobin** ( $\gamma$ -catenin) and **plakophilin** (distant relative of p120-catenin). Together they turn into a **desmoplakin**.
  - Desmoplakin binds to the sides of intermediate filaments, tying the desmosomes to the filaments.
- To this plaque a bunch of keratin intermediate filaments are attached.

- On the other side of the plaque a lot of nonclassical cadherins bind to the plaque, whose extracellular domains interact with the cadherin of another molecule.

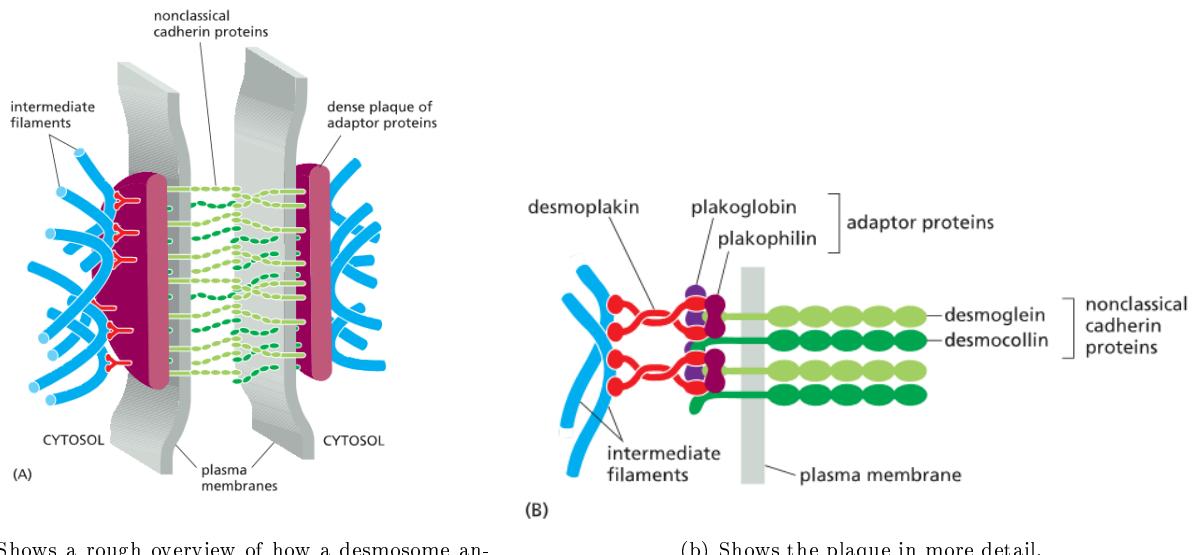


Figure 185:

While a desmosome is cell-cell a hemidesmosome is cell-matrix (hence hemi = half). For the cell side of things the structure is exactly the same. Both types of junctions give rigidity to the cell.

### 11.3 Tight Junctions

tight junctions are everywhere, like tracts in the urinary system.

Tight junctions hold adjacent membranes very close together. The strands are composed of transmembrane proteins that make contact across the intercellular space, creating a seal. They do this multiple times in high numbers, which creates a very large surface area and with that a strong seal. The sealing strand is composed mainly of proteins with four transmembrane elements. The main one is **claudin**, secondary **occludin** have less of an important role in determining **junction permeability**. The two termini for both these proteins are on the cytoplasmic side of the membrane where they interact with scaffolding proteins and link to actin to organize the sealing strands.

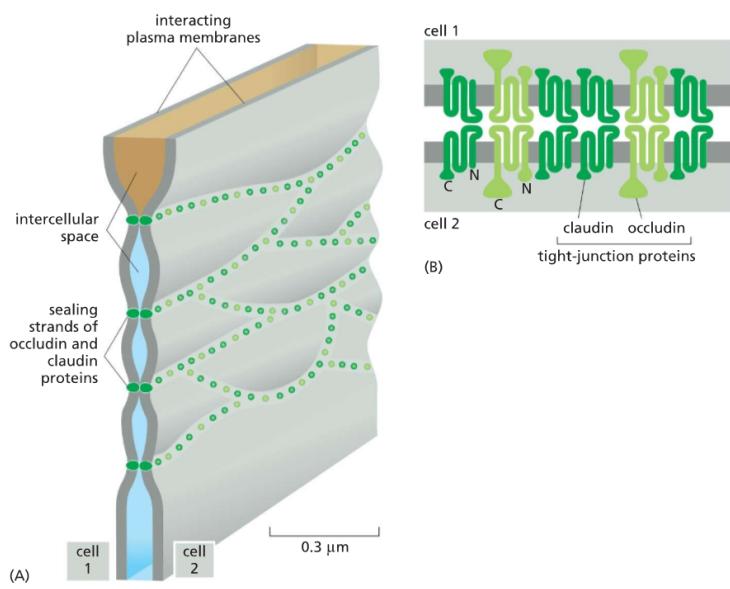


Figure 186: Shows a model of a tight junction. Highlights how sealing strands exist around the molecule. Also shows the main two sealing proteins: claudin and occludin.

*Remark 11.6 (3D-thinking of tight junctions).* In 3D these tight junctions will wrap around the cell forming a band. As otherwise they wouldn't actually block anything if they just existed at selected spots

#### 11.3.1 Tight Junctions in transcellular transport: Intestine

tight junctions **seal off different parts of the tissue**. This allows for the body to create transfers from one part to the other in a more controlled version. Tight junctions also confine transport proteins to their part of the membrane, working as a **fence**, within the lipid bilayer. They also **block the backflow** of unwanted molecules.

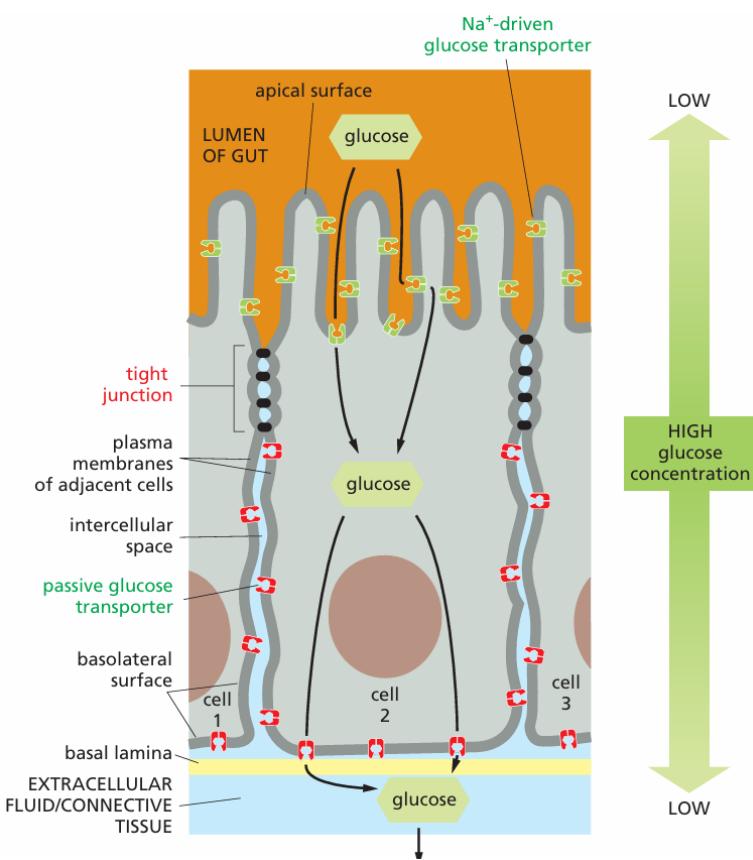


Figure 187: Shows the intake of glucose in the small intestine. For simplicity only tight junctions are shown of the anchor junctions. Glucose is actively transported into the cell through  $\text{Na}^+$ -driven glucose transporters and leaves passively through glucose transporters.

## 11.4 Channel-Forming Junctions

The essence: **Gap junctions decide which molecules are shared between cells**. However, there is a limit at around **1000 Daltons**.

The gap junction is seen as a cluster of homogeneous intramembrane particles. Each intramembrane particle is a protein assembly called a **connexon**, consisting of **6 connexin subunits**, which penetrate the lipid bilayer. Connecting two connexons then creates a channel between two cells. These connexons can be **homotypic** or **heterotypic**, depending on the usage of different connexins. Each connexin consists mainly of  $\alpha$ -helix, with the whole connexon ending up having a pore size of around 1.4nm which matches with the molecule size permitted (around 1000 Daltons).

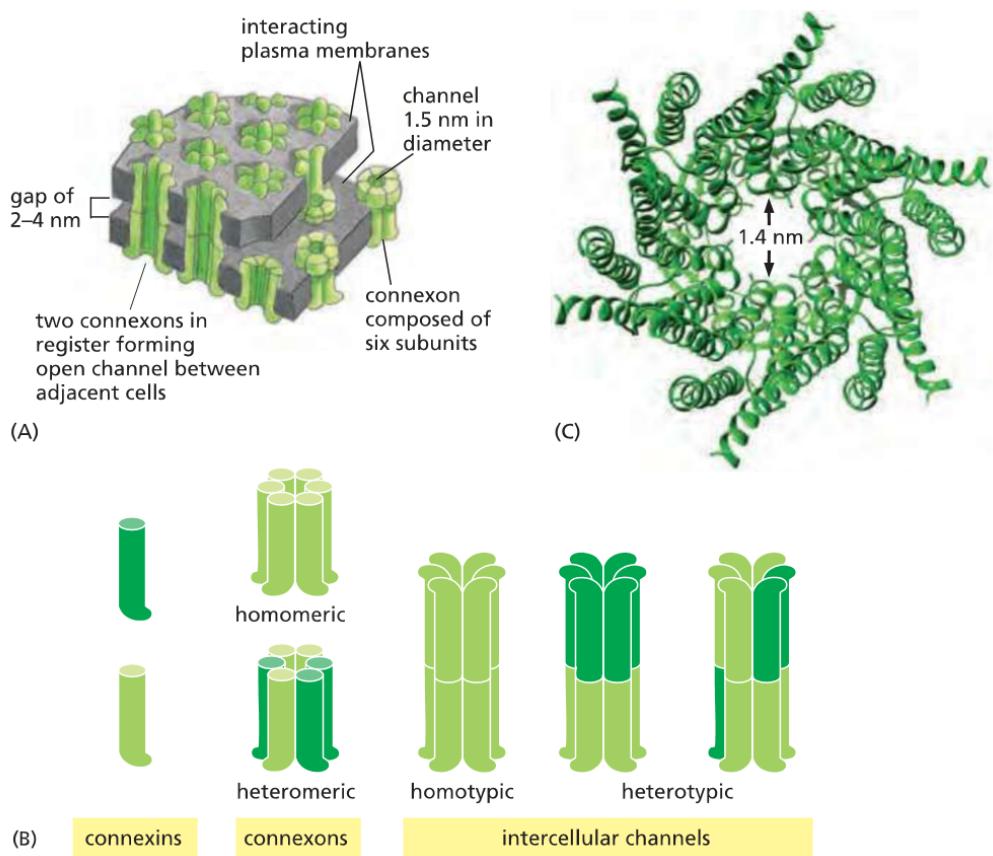


Figure 188: (A) Shows the view on the membrane, (B) the components of a connexon, (C) and the 3D structure of a connexon.

## 11.5 The Extracellular Matrix

The major components of the extracellular matrix (ecm) are the following:

- glycoprotein: laminin, nidogen, and fibronectin
- fibrous: type IV collagen, fibrillar collagen
- proteoglycan and glycosaminoglycan (GAGs): hyaluronan, perlecan, decorin, aggrecan

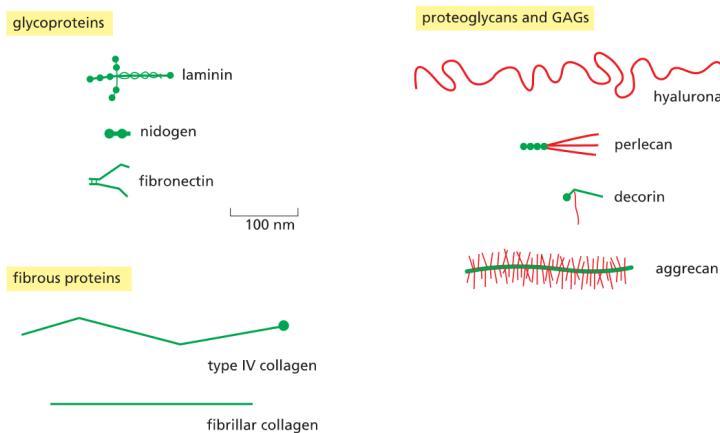


Figure 189: Shows the major components of the ecm. Green is protein and red is GAG.

The size of these molecules also varies very much:

- globular protein (MW 50'000)
- glycogen (MW around 400'000)
- spectrin (MW 460'000)
- collagen (MW 290'000)
- hyaluronan (MW  $8 \times 10^6$  and 300nm in diameter)

### 11.5.1 Glycosylation and GAGs

GAGs are a long chain of **typically sulfated repeating disaccharides**, this means that we will need a bunch of glycosylation bonds. Something about **GAGs is that they are often sulfated**. It will vary from 70% (heparin) to under 50% (heparan) or none at all (hyaluronan). Further the length can vary from 200 pairs of disaccharides to up to 25'000 sugar monomers, again highlighting the high variability. Leading to even higher variability is the fact that the disaccharide chain is also very variable: for **chondroitin sulfate** it is D-glucuronic acid and N-acetyl-D-galactosamine, while for **heparan sulfate** it is N-acetyl-D-glucosamine with either D-glucuronic acid or L-iduronic acid, and finally for **keratan sulfate** it is D-galactose and N-acetyl-D-glucosamine.

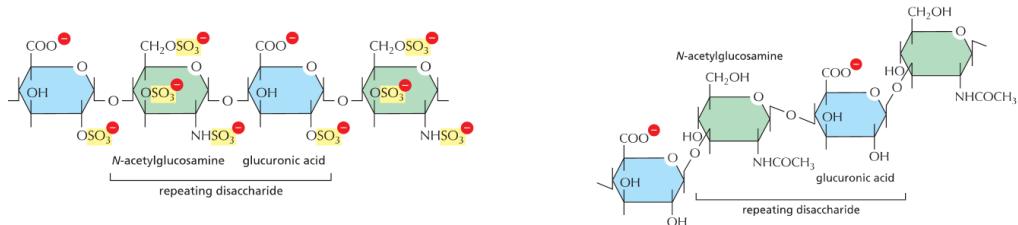


Figure 190: The picture on the left shows a GAG with 100% sulfation (not really a thing). On the right we can see hyaluronan, a rather simple but long GAG (no sulfation).

### 11.5.1.1 Synthesis proteoglycan: adding GAGs to proteins

GAGs are added to their core protein via a special link tetrasaccharide of the GAG and a serine on the protein. Once this linkage has happened the rest of the GAG repeating disaccharide chain can be added one sugar at a time.

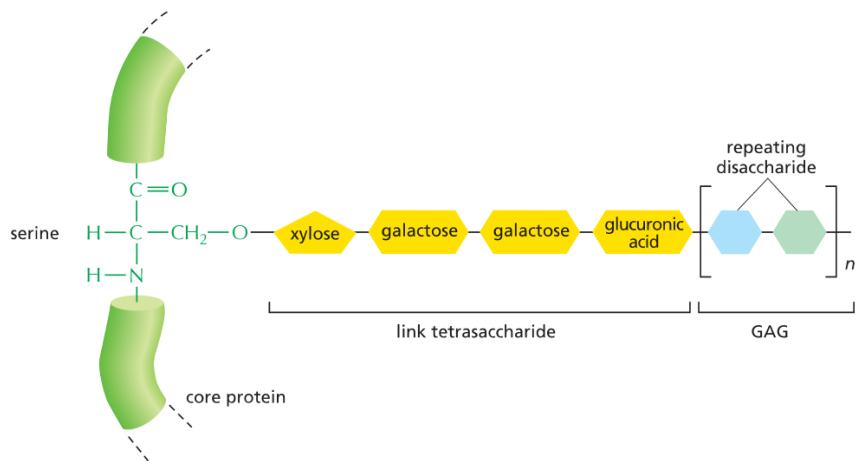


Figure 191: How a proteoglycan bind the GAG and protein.

**Remark 11.7 (Proteoglycans varies a lot).** The Glycosylation degree is very variable in size and absolute number. Aggrecan for example has 300 amino acids in its core protein and 30 keratan sulfate and 100 chondroitin sulfate chains linked to the protein. On the other hand decorin just has one GAG and "decorates" collagen fibrils (so it can't be all too large).

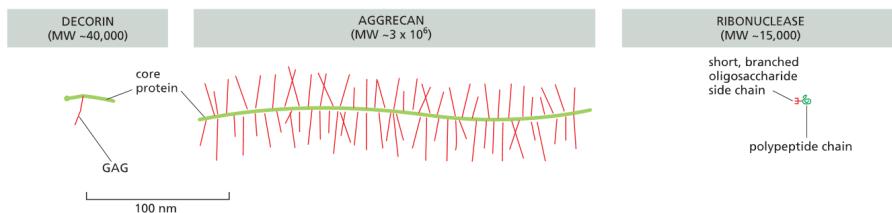


Figure 192: Different proteoglycans and their varying sizes and numbers of glycosylations.

### 11.5.2 Aggrecan aggregation

Aggrecan is the name of the core protein. It has many keratan sulfate glycosylations. The N-terminal of aggrecan then binds noncovalently to a single hyaluronan molecule. A link protein, part of the hyaluronan-binding proteins (can also be cell surface proteins), then binds to both the aggrecan core and the hyaluronan stabilizing the bond. This aggregate can become huge north of  $10^8$  daltons and occupy the volume of a bacterium ( $2 \times 10^{-12} \text{ cm}^3$ ).

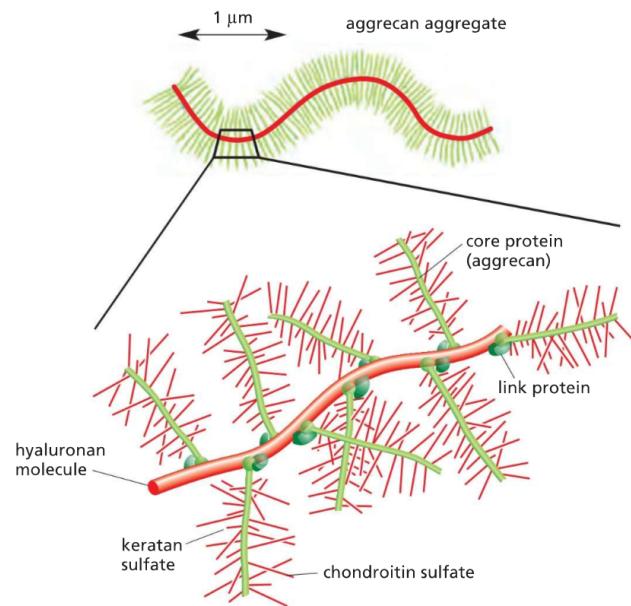


Figure 193: A visualization of the aggrecan aggregate on a hyaluronan molecule

### 11.5.3 Collagen

#### 11.5.3.1 Structure of a typical collagen

Collagen is composed of three  $\alpha$  chains. One  $\alpha$  chain is a long left-handed helix with a set pattern: every third amino acid is a glycine. The other two can be anything but are commonly a Hydroxyproline (Y) (modified during collagen synthesis) and a proline (X). The reason every third amino acid needs to be glycine is because for the three  $\alpha$  chains to wrap into each other, one of the amino acids needs to fit in between and the only amino acid small enough for that is glycine. The entire collage will become up to 300nm long.

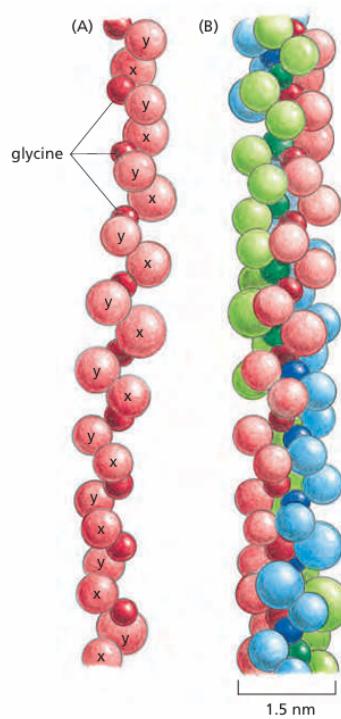


Figure 194: Structure of collagens. X is typically proline and y hydroxyproline

#### 11.5.3.2 The collagen amino acids

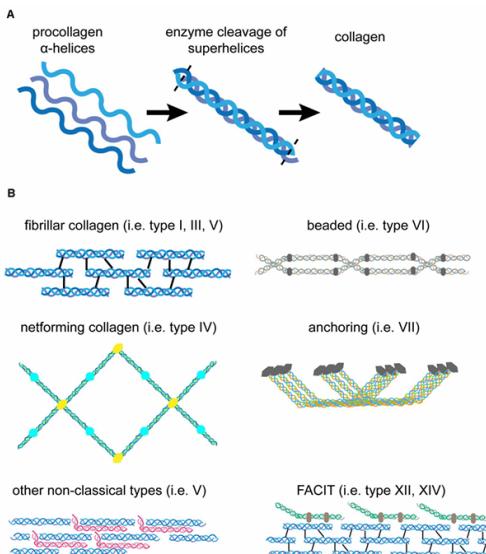
Nearly one third of amino acids in collagen is glycine. 15-30% are Proline and 4-Hydroxyprolyl (Hyp). Then 3-Hydroxyprolyl and 5-Hydroxylysyl (Hyl) (Hyl) residues also occur in collagen, but in smaller amounts. All of these hydroxylation reactions are **Vitamin-C** dependent, as it is a cofactor for the **enzymes lysyl hydroxylase and prolyl hydroxylase**.

*Remark 11.8 (Collagen as connective Tissue: Fibrils).* collagen fibers are organized into bundles which run through the ecm. They are oriented in nearly a right angle, creating a net of fibrils.

### 11.5.3.3 Collagen types

TABLE 19-2 Some Types of Collagen and Their Properties			
	Type	Polymerized form	Tissue distribution
Fibril-forming (fibrillar)	I	Fibril	Bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)
	II	Fibril	Cartilage, intervertebral disc, notochord, vitreous humor of the eye
	III	Fibril	Skin, blood vessels, internal organs
	V	Fibril (with type I)	As for type I
	XI	Fibril (with type II)	As for type II
Fibril-associated	IX	Lateral association with type II fibrils	Cartilage
Network-forming	IV	Sheetlike network	Basal lamina
	VII	Anchoring fibrils	Beneath stratified squamous epithelia
Transmembrane	XVII	Nonfibrillar	Hemidesmosomes
Proteoglycan core protein	XVIII	Nonfibrillar	Basal lamina

Note that types I, IV, V, IX, and XI are each composed of two or three types of  $\alpha$  chains (distinct, nonoverlapping sets in each case), whereas types II, III, VII, XVII, and XVIII are composed of only one type of  $\alpha$  chain each.



(a) A bunch of different types of collagen

(b) (a) shows how collagen forms its trimeric form, and then from it all the diverse forms it can take.

### 11.5.3.4 Synthesis of fibril Collagen I

The synthesis of **collagen I** happens in **fibroblasts**. It happens in three parts: first procollagen is assembled in the ER, then in the cytosol the fibril is assembled. The fiber is then assembled in the ecm. Breaking down the individual parts:

#### Procollagen assembly

- i) **procollagen** assist folding into the left handed  $\alpha$ -helix
- ii) **hydroxylation** of Proline and Lysine
- iii) N-linked glycosylation
- iv) Beginning of quaternary structure through self-assembly of disulfide bonds.
- v) Proline bonds are also forced to be trans so they don't break apart the helical form.
- vi) Formation of the triple helix
- vii) transportation through **Golgi apparatus**
- viii) Modification of N- and O- linked sugars

#### Fibril/fiber assembly

- ix) Cleavage of **propeptides**, which are the parts of the chain which didn't form the tight triple helix
- x) Self assembly of fibril
- xi) Secretion

xii) Fiber assembly

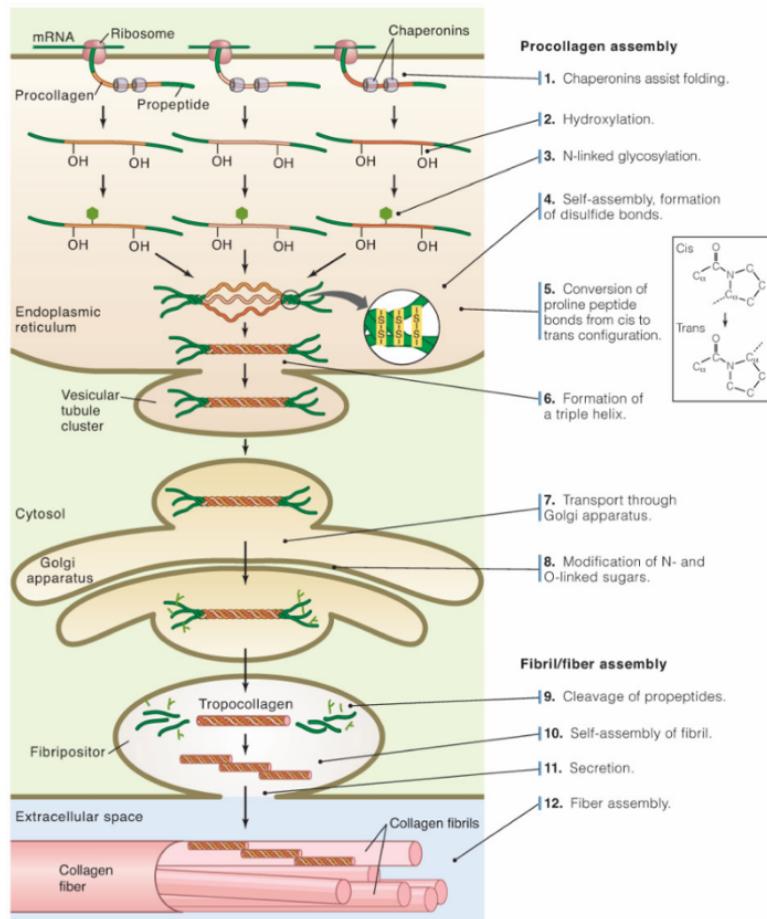


Figure 195: Synthesis of Collagen I a type of fibril.

#### 11.5.3.5 Defective collagen synthesis = bad news

Having a defect in one of the proteins can be really bad really fast, as for the fibril fibers to do their job, we need everything to be packed very tightly in just the right way. For example a mutation in the gene for the **procollagen N-proteinase**, which is responsible for cutting the parts of the gene which didn't fold properly. This will basically just mean that the collagen becomes useless.

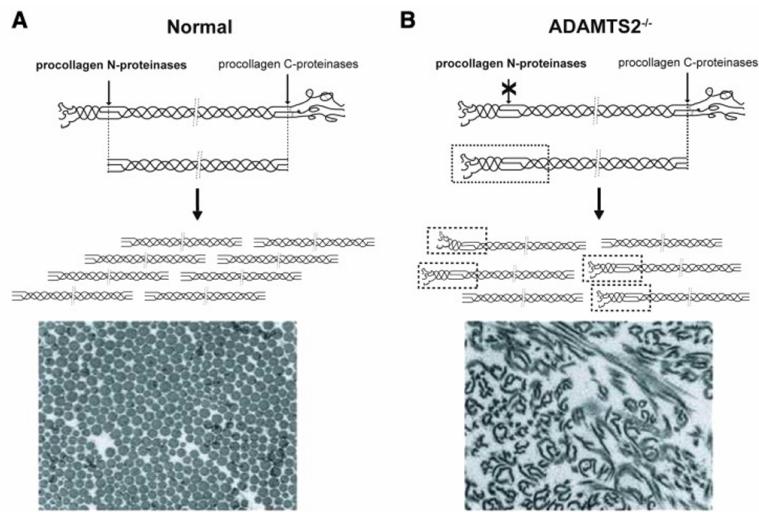


Figure 196: How a defect in collagen synthesis is very very bad.

#### 11.5.4 The ECM's Flexibility

Unlike soccer (a.k.a. football) players the ecm needs to be able to stretch. For this it has a elastin fiber. It is a bunch of elastin molecules bonded covalently to generate a cross-linked network. Each molecule can extend and coil, which allows the fiber as a whole to function as a rubber band. One elastin has a **long half life of around 40 years**. However, elastin is **not really regenerated after puberty**, which lead to Gesichtsfalten.

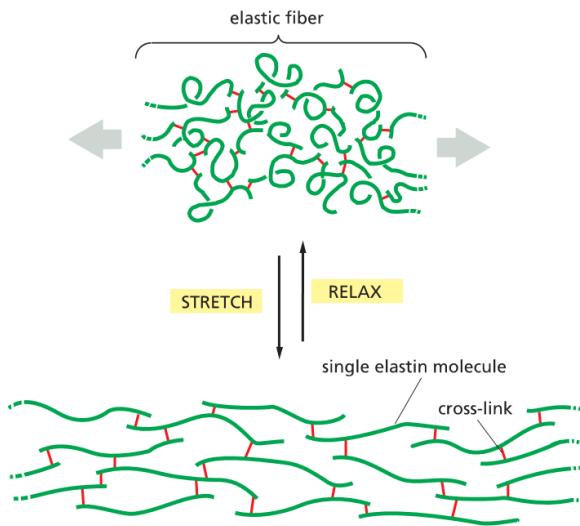


Figure 197: Elastin in its stretched vs. relaxed state.

#### 11.5.5 Complex Glycoproteins

There are over 200 matrix glycoproteins in mammals. Many matrix glycoproteins are large scaffold proteins containing multiple copies of specific protein-interaction domains. Each domain is folded into a discrete globular structure, often having a bead like structure. Each protein contains multiple repeat domains. Example of

Fibronectin which has a numerous copies of different fibronectin repeats: FN1, FN2, and FN3. Two type III at the end are crucial for integrin binding, while other position are important fibrin, collagen, or heparin binding.

Other matrix proteins contain EGF (epidermal growth factor) like sequences, indicating that they might serve a similar signaling purpose. Others on the glycoprotein, like the IGFBP (IGFBP) regulate soluble growth factors. Many of these genes can be spliced, leading to even more diversity among glycoproteins.

Finally some of the domains are responsible for building multimeric forms. For example in fibronectin the C-termini builds dimers, in tenacin and thrombospondin form N-terminally linked hexamers and trimers, respectively.

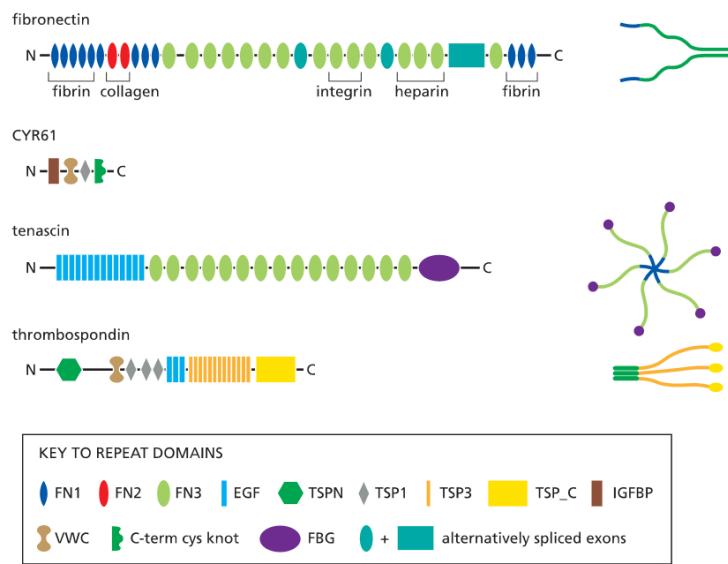


Figure 198: A bunch of complex glycoproteins in the ECM.

### 11.5.6 Fibronectin

fibronectin plays a crucial role in guiding cell structure and behaviors.

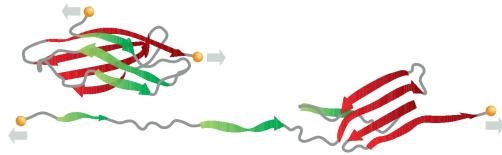


Figure 200: Shows how some domains are exposed when fibronectin is pulled upon.

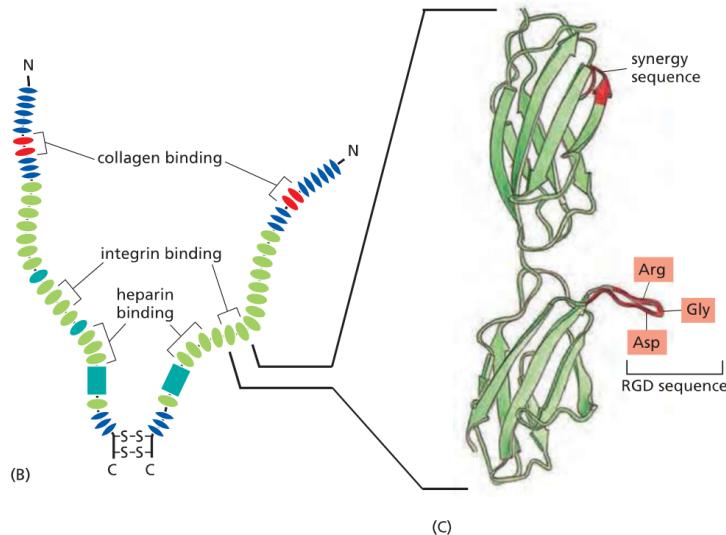


Figure 199: The structure of fibronectin. Note its minor differences between the chains.

Looking at the fig: 199 above we can see on the left that the two chains may be similar but not entirely the same, meaning they were spliced differently (as the same gene). They are joined by two disulfide bonds near the C-termini. Each chain is around 2'500 amino acids long and is folded into a bunch of domains. Some domains are specialized to binding to certain molecules. On the right the sequences in red are important for binding Integrin.

*Remark 11.9 (Fibronectin under tension).* Some type III fibronectin repeats can unfold when fibronectin is put under tension. That unfolding can expose cryptic binding sites resulting in multiple in the formation of multiple fibronectins.

*Remark 11.10 (Fibronectins and the cytoskeleton alinging).* Fibronectin will accumulate at focal adhesions, making the organized in a paralell way to actin filaments. Integrin molecules link the fibronectin outside the cell to the actin filaments inside it (will be covered in more detail in section 11.7). Tension on the fibronectin exposes them exposing sites which promote fibril formation.

## 11.6 Basal Lamina

Depending on where we are in the body, the basal lamina will have a different organization. These differences in composition can also exist between tissues.

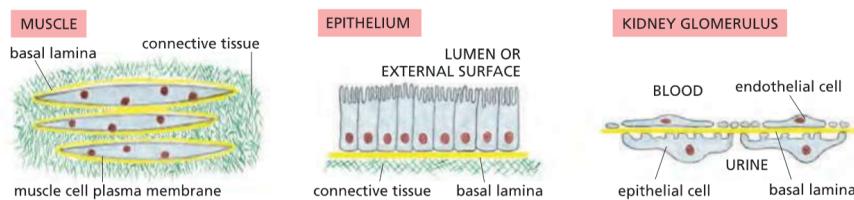


Figure 201: The basal lamina will look very different depending on where in the body it is.

### 11.6.1 Complexity of the basal lamina

The basal lamina is formed by specific interaction between proteins, laminin, type IV collagen, and nidogen, and the proteoglycan perlecan. Transmembrane laminin receptors, Integrin and dystroglycan are thought to organize the assembly of the basal lamina.

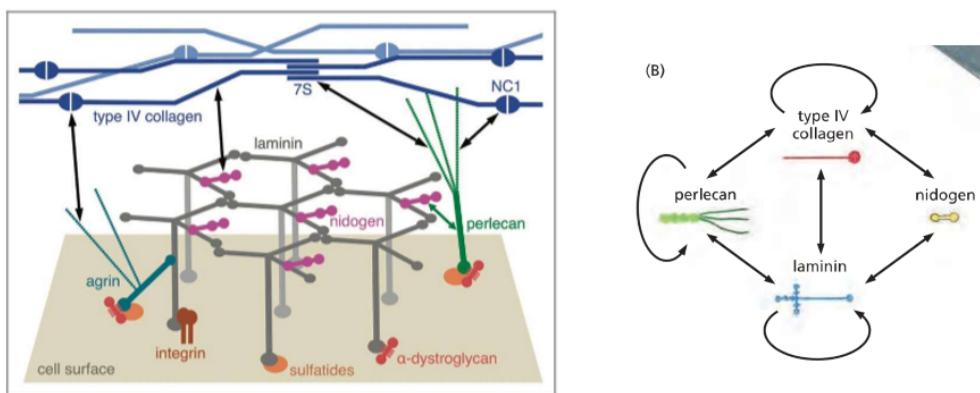


Figure 202: The assembly of the basal lamina is very complex. On the right, one can see the net of interaction. An arrow indicates who can bind to who.

## 11.7 Integrins

Integrins are essential in connecting the two sides of the basal lamina.

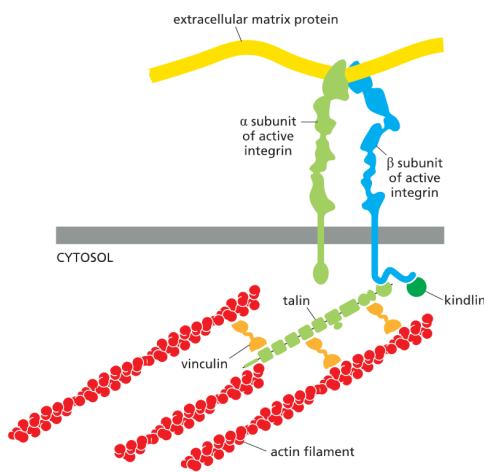


Figure 203: A rough glance at integrins role for the basal lamina

### 11.7.1 The types of integrins

TABLE 19-3 Some Types of Integrins

Integrin	Ligand*	Distribution	Phenotype when $\alpha$ subunit is mutated	Phenotype when $\beta$ subunit is mutated
$\alpha_5\beta_1$	Fibronectin	Ubiquitous	Death of embryo; defects in blood vessels, somites, neural crest	Early death of embryo (at implantation)
$\alpha_6\beta_1$	Laminin	Ubiquitous	Severe skin blistering; defects in other epithelia also	Early death of embryo (at implantation)
$\alpha_7\beta_1$	Laminin	Muscle	Muscular dystrophy; defective myotendinous junctions	Early death of embryo (at implantation)
$\alpha_L\beta_2$ (LFA1)	Ig superfamily counterreceptors (ICAM1)	White blood cells	Impaired recruitment of leucocytes	Leukocyte adhesion deficiency (LAD); impaired inflammatory responses; recurrent life-threatening infections
$\alpha_{IIb}\beta_3$	Fibrinogen	Platelets	Bleeding; no platelet aggregation (Glanzmann's disease)	Bleeding; no platelet aggregation (Glanzmann's disease); mild osteopetrosis
$\alpha_6\beta_4$	Laminin	Hemidesmosomes in epithelia	Severe skin blistering; defects in other epithelia also	Severe skin blistering; defects in other epithelia also

\*Not all ligands are listed.

Figure 204: Some types of integrins

### 11.7.2 Integrin: The Major Activity States

Integrin has two main states: inactive (folded) and active (extended). This switch happens spontaneously.

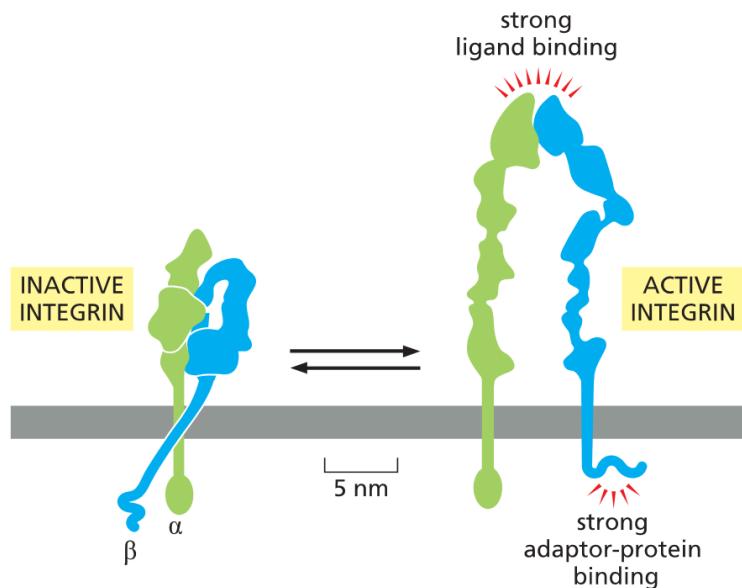


Figure 205: The two different conformations of integrins.

### 11.7.3 Integrins in hemidesmosomes

Hemidesmosomes (see sec:??) glue epithelial cells to the basal lamina. They do this by linking keratin filaments on the inside and out outside of the cell. A specialized integrin( $\alpha 6, \beta 4$ ) attaches to the **keratin** filaments, via adaptor proteins **plectin** and **BP230** and to the laminin extracellularly. The adhesive complex also contain a unusual collagen known as **collagen XVII**, which has a membrane-spanning domain attached to its extracellular collagenous portion.

*Remark 11.11 (Blisters due ot hemisdesmososes).* Defects in any of these proteins may cause blistering of the skin. One such disease **bullous pemphigoid** is an autoimmune disease in which the immune system destroys its own collagen.

### 11.7.4 Talin: tension sensor

Talin is an adaptor protein between integrins and actin filaments. Its long, flexible, C-terminus is divided into a series of folded domains, some of which are vinculin binding-sites, that are hidden when in a relaxed state. Then, once the Talin feels the tension through either the integrin or the actin it unwinds giving way to the vinculin-binding sites. This allows vinculin to attach and recruit more actin stabilizing the complex and relieving of tension.

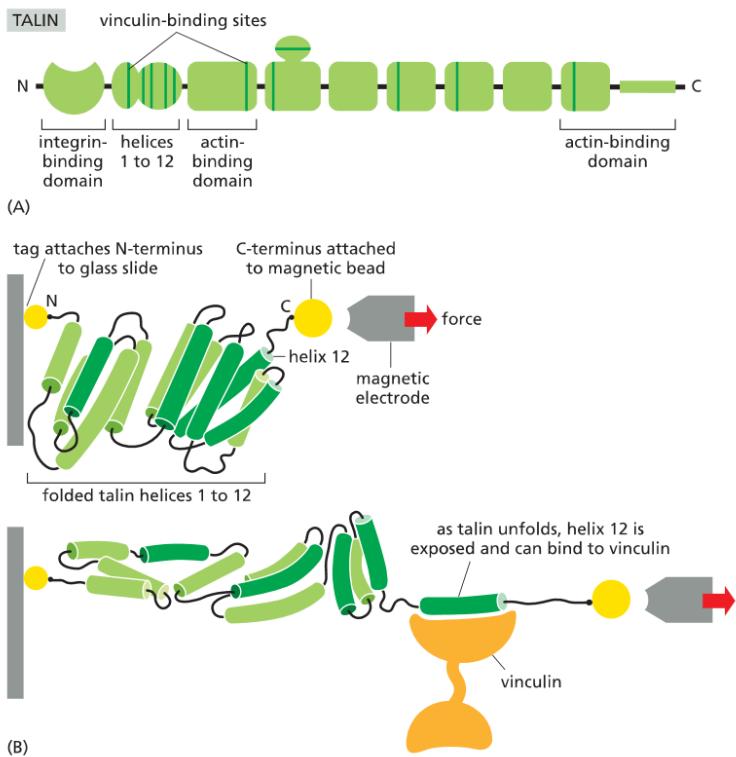


Figure 206: Shows where Talin has different binding domains and how once it gets started to be pulled apart those become uncovered. PSA: this is a pic from an experiment where they used a magnet to pull the molecule apart, we can ignore that.

### 11.7.5 Activation of Integrin Signaling

Signals received from outside the cell can activate integrin. In platelets (thrombocytes):

- i) Thrombin activates a GPCR on the cell surface.
- ii) Which in turn activates Rap1, a member of the GTPases. It should be said that **many other receptors can activate Rap1!**
- iii) Rap1 interacts with RIAM, which then recruits inactive talin and kindlin to the membrane surface.
- iv) Talin and kindlin interact with the integrin beta to trigger integrin activation.
- v) Talin hangs around to interact with vinculin and more.

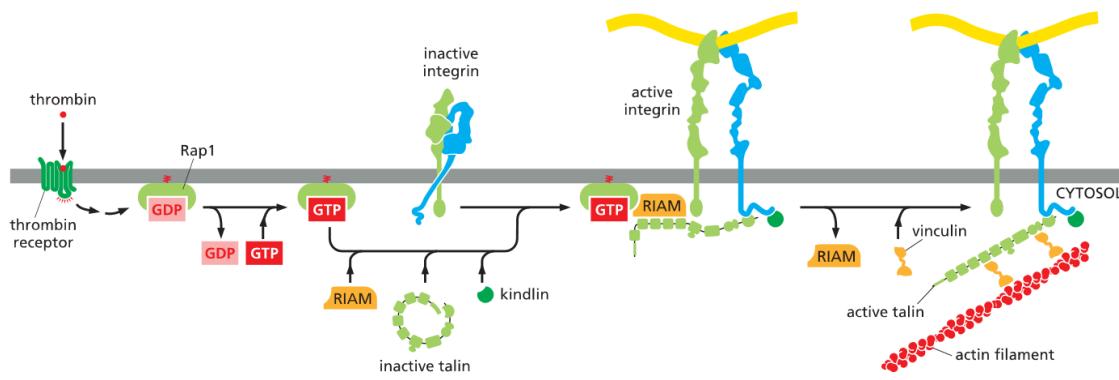


Figure 207: The pathway to activate integrin.

*Remark 11.12 (Talin activation).* Talin is initially inactive due to a rod domain on the C-terminal and N-terminal which would contain the integrin-binding site but is now blocked. However when RIAM recruits Talin to the membrane, it interacts with a Phosphoinositide ( $\text{PI}(4,5)\text{P}_2$ ) resulting in the dissociation of the rod domain. Talin unfolds and binds to integrin.

#### 11.7.6 Integrins interacting with the ECM

Different integrins interact with different components. Arginine-Glycine-Aspartic Acid, RGD are the three amino acids in fibronectin interacting with the integrin  $\alpha 5\beta 1$

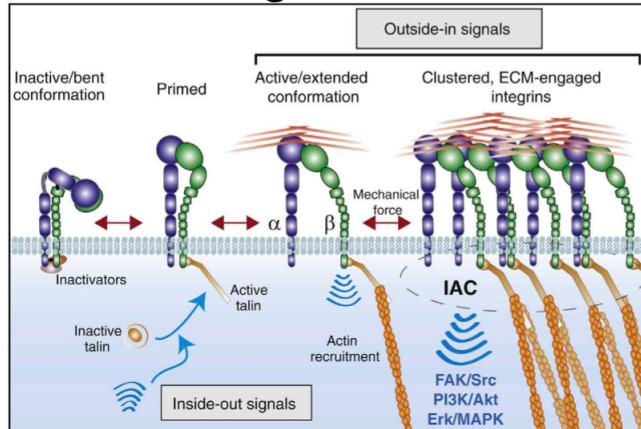


Figure 208: The signal activation and progression from the perspective of integrin.

#### 11.7.7 Laminin

Laminin is very important for the basal lamina. Due to the binding sites with other proteins, laminin plays a central part in organizing and anchoring the basal lamina. Laminins are multidomain glycoproteins composed of three polypeptide ( $\alpha, \beta, \gamma$ ), which are bonded through disulfide bonds, bonding them into an asymmetric crosslike structure. Each chain is over 1500 amino acids long. There are 5  $\alpha$ , 4  $\beta$ , and 3  $\gamma$  different types of chains known to us, which leads to a bunch of different combinations. Laminin-111, the most understood one, has  $\alpha 1, \beta 1$ , and you guessed it  $\gamma 1$  subunits.

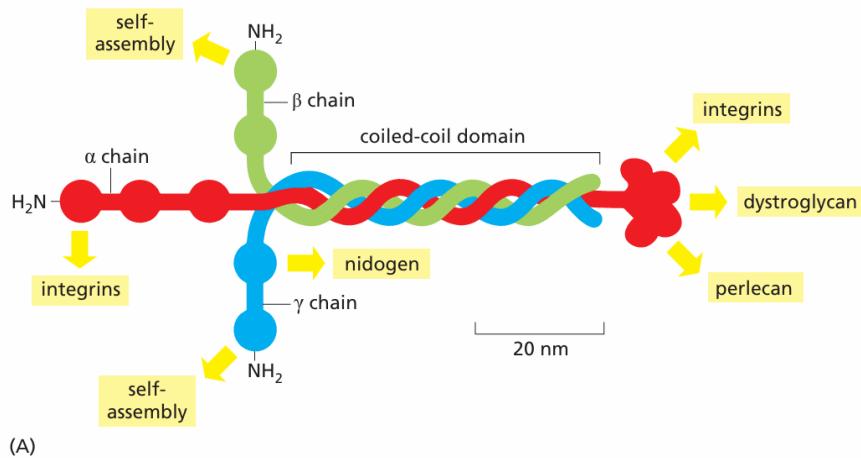


Figure 209: laminin-111, the most understood of laminins, used as example.

#### 11.7.7.1 The types of laminin

The N-terminus is responsible for interactions with other extracellular matrix proteins, making it important for the assembly and stability of basement membranes. The C-terminus is responsible for interactions with cell surface receptors, making it crucial for adhesion vs. migration, survival vs. apoptosis, signaling, differentiation, and gene expression. This also shows that while the C-terminus is essential, some types laminin don't require a N-terminus.

Here are a bunch of laminin and where they are most commonly found:

- 111 mostly in the embryo, rare in adults
- 511 and 521 are the most common isoforms in adults
- 211 and 221 present in skeletal and cardiac muscles.
- 411 and 421 endothelial cells of blood vessels.
- 332 is specific for the basal lamina under the epithelial cells (mainly skin).

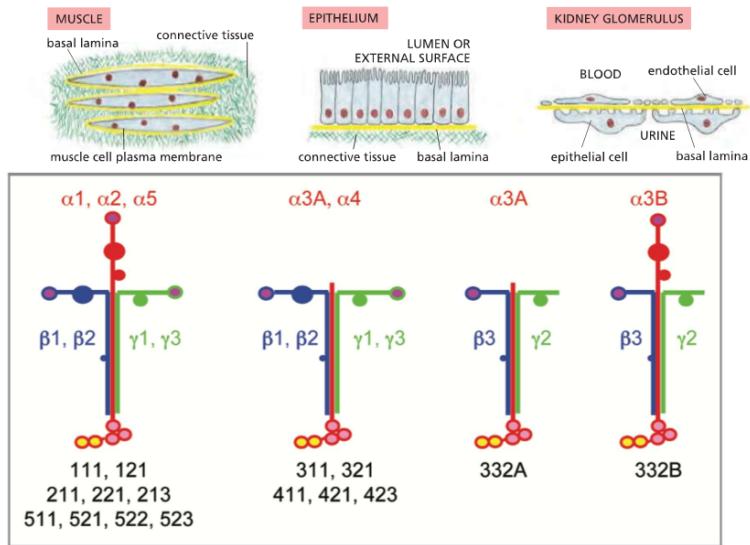


Figure 210: Different groups of laminin and in which types of basal lamina they will mostly be found (laminin looking up is its lamina type).

## 12 The Cytoskeleton

The cytoskeleton is a **dynamic network of protein filaments** that gives cells their **shape, structural strength, and internal organization**. It enables cells to **interact mechanically with their environment, change shape, move, and rearrange internal components** during growth, division, and adaptation. These functions are essential for proper cellular structure and behavior.

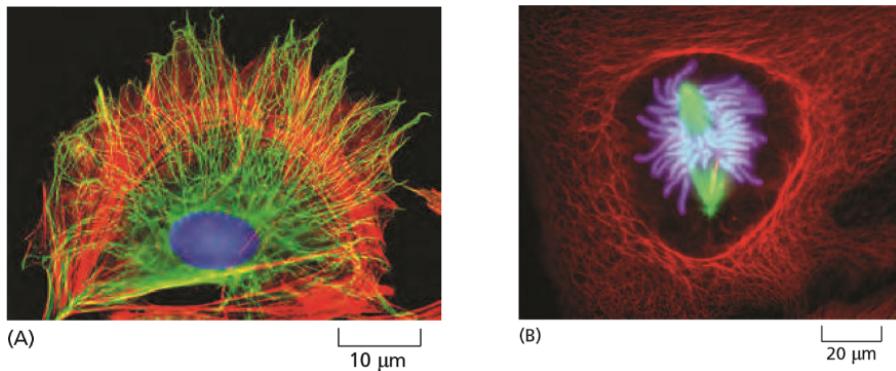


Figure 211: The cytoskeleton. (A) A cell in culture has been fixed and labeled to show its cytoplasmic arrays of **microtubules (green)** and **actin filaments (red)**. (B) This dividing cell has been labeled to show its **spindle microtubules (green)** and surrounding cage of **intermediate filaments (red)**. The DNA in both cells is labeled in blue.

The cytoskeleton's function depends on three families of protein filaments:

- **Actin filaments** - determine the shape of the cell's surface and are necessary for whole-cell locomotion. Moreover they drive the pinching of one cell into two.

- **Microtubules** determine the positioning of membrane-enclosed organelles, direct intracellular transport, and form the mitotic spindle that segregates chromosomes during cell division.
- **Intermediate filaments** provide mechanical strength. There exists many different kinds which are cell specific.

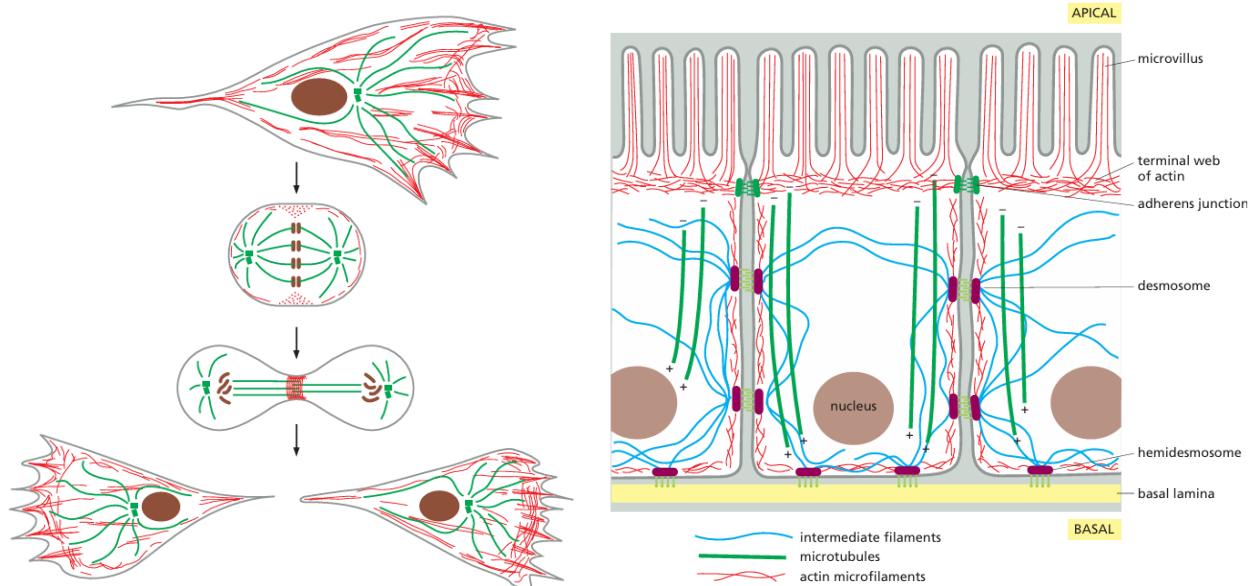
All of these cytoskeletal filaments interact with hundreds of **accessory proteins that regulate and link** the filaments to other cell components, as well as to each other. **motor proteins** are a type of accessory proteins. They convert the energy of ATP hydrolysis into mechanical force that can either **move organelles along the filaments** or **move the filaments themselves**.

In differentiated epithelial cells, the cytoskeleton supports both dynamic and stable structures. It **maintains surface features like microvilli** despite continuous remodeling. Moreover, the cytoskeleton also **establishes cell polarity**, distinguishing the apical and basolateral surfaces to support specialized functions.

As shown in Figure 216(b), **actin filaments (red)** form **microvilli** at the apical surface to enhance nutrient absorption and create a **circumferential belt for cell adhesion**. **Intermediate filaments (blue)** **anchor cells** via desmosomes and hemidesmosomes, while **microtubules (green)** **organize intracellular transport**. These components together maintain the specialized structure and function of epithelial cells.

**Example 12.1 (Cell Division - The Cytoskeleton is dynamic).** An important example of rapid cytoskeletal reorganization occurs during cell division. Figure 212(a) shows a crawling fibroblast with a polarized, dynamic actin cytoskeleton (red), supported by a microtubule cytoskeleton (green). During division, the polarized (interphase) microtubule array reorganizes into a bipolar mitotic spindle, which aligns and segregates the duplicated chromosomes (brown). At the same time, the actin structures that drive fibroblast movement are reorganized, causing the cell to round up and stop migrating.

**Actin and the motor protein myosin then assemble into a contractile ring** around the cell's mid-point. This ring constricts to divide the cell in two. Once division is complete, the cytoskeletons of the daughter cells return to their interphase organization, restoring the flattened, motile shape of the original cell.



(a) Diagram of changes in cytoskeletal organization associated with cell division. (b) Organization of the cytoskeleton in polarized epithelial cells.

Figure 212: Cytoskeleton is dynamic and determines cellular organization and polarization

## 12.1 Actin Filaments

**Actin filaments**, also known as *microfilaments*, are **helical polymers of the protein actin**. These flexible structures, about 8nm in diameter, assemble into **linear bundles, networks, and gels**. They are dispersed throughout the cell but are especially concentrated in the **cell cortex**.

Actin filaments determine the **shape of the cell surface**, are essential for **whole-cell movement**, and play a key role in **cytokinesis**, where they drive the pinching of one cell into two.

Their organization supports diverse actin-based structures, including *stress fibers* that anchor to *focal adhesions*, **cell cortex** (dense, gel-like network of actin filaments), the *lamellipodium* for broad, sheet-like protrusions, the *filopodium* for slender, finger-like extensions, and the contractile apparatus of striated muscle. See fig. 213(b)

As shown in Figure 213(a) the actin monomer has a nucleotide (**either ATP or ADP**). Note that *newly synthesized actin filaments still contain ATP as hydrolysis to ADP is slower than the assembly of a new filament*.

These monomers then arrange into a filament consisting of two protofilaments, that are held together by lateral contacts. These **2 protofilaments** wind around each other as two parallel strands of a **helix** with a twist repeating every 37 nm. Note that every subunits within the filament have the **same orientation**, thus forming an minus and a plus end.

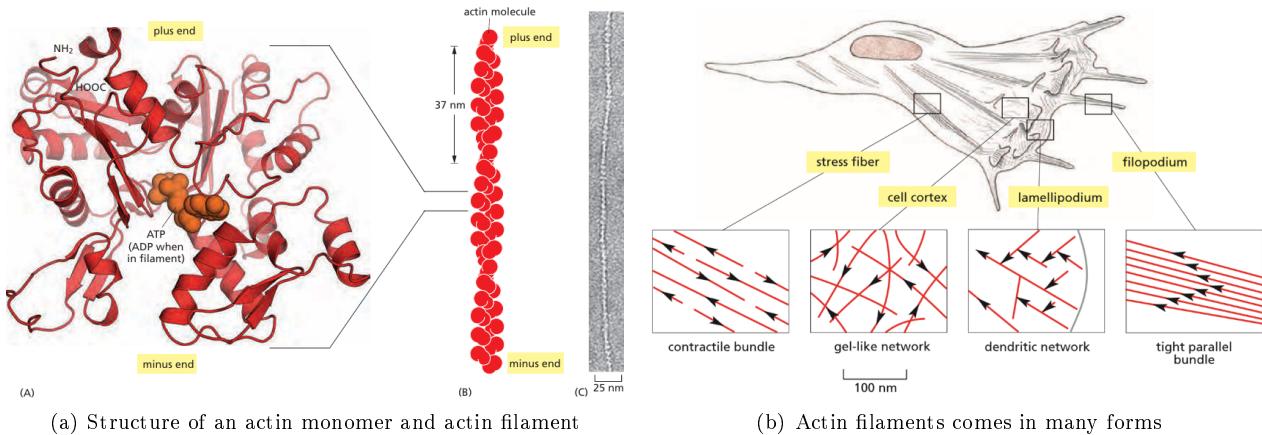


Figure 213: Actin Filaments

### 12.1.1 Actin and Actin-Binding Proteins

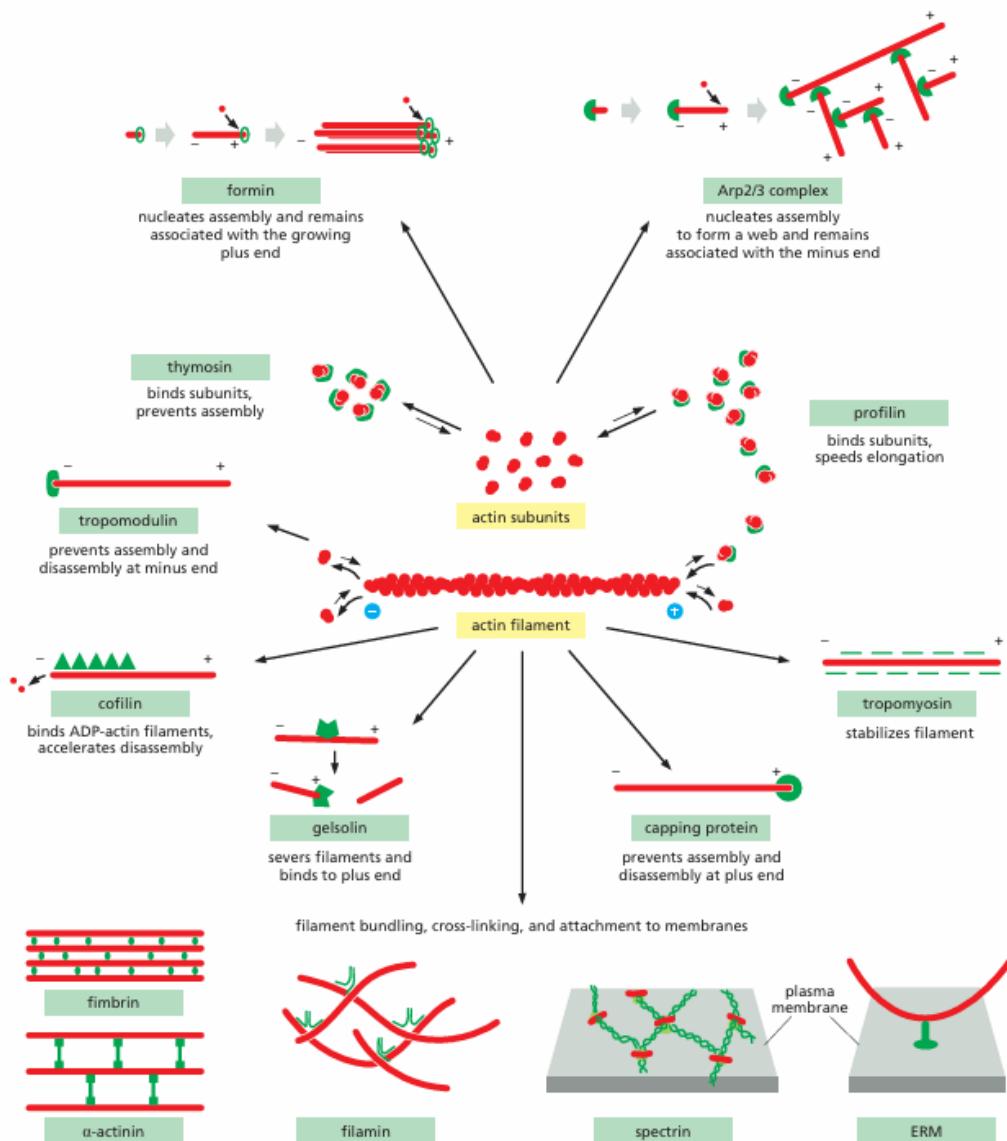


Figure 214: Some of the major accessory proteins of the actin cytoskeleton. (Except Myosin and Motor Proteins)

#### 12.1.1.1 Regulation of Actin Filament Formation

The **regulation** of actin filament formation is an important mechanism by which cells control their shape and movement. This is largely achieved by exploring the following effects:

**Nucleation is the Rate-Limiting Step** When polymerization is initiated, **kinetic barrier to nucleation** results in a **lag phase**, followed by a phase of **rapid elongation**. Finally, as the concentration of actin declines, the system approaches a **steady state**. This point is called the **critical concentration  $C_c$** .

**Remark 12.2 (Barrier to Nucleation).** A helical polymer is stabilized by multiple contacts between adjacent subunits. In the case of actin, two actin molecules bind relatively weakly to each other and thus disassemble

quickly. But the **addition of a third actin monomer** forming a trimer makes the entire group more stable. This creates a barrier to nucleation.

*Remark 12.3 (Critical Concentration  $C_c$ )*.  $C_c$  is the concentration of free subunits at which the rate of subunit addition to a filament equals the rate of subunit loss, resulting in a steady-state polymer length. It is defined by the ratio of kinetic rate constants:

$$C_c = \frac{k_{\text{off}}}{k_{\text{on}}} = K_d = \frac{1}{K_{\text{eq}}}$$

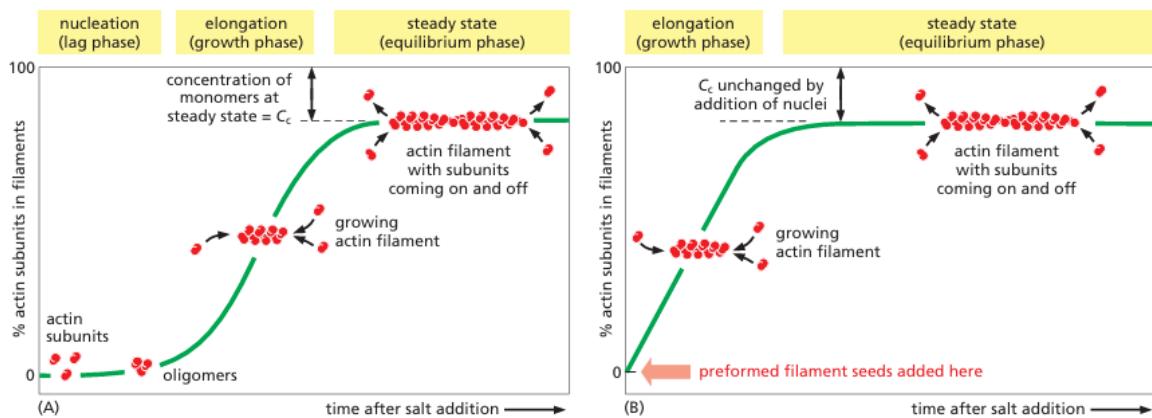


Figure 215: The time course of actin polymerization in a test tube.

**ATP Hydrolysis Within Actin Filaments Leads to Treadmilling at Steady State - Thymosin and Profilin** Actin filaments are **polar structures**, meaning their two ends are structurally and functionally distinct: the **plus (barbed) end grows faster** than the **minus (pointed) end**. This polarity arises from the uniform orientation of actin monomers within the filament.

When ATP-bound actin monomers add to a filament, they preferentially bind to the plus end due to faster kinetics. Once incorporated, the **ATP is hydrolyzed to ADP**, a process that occurs more efficiently within the filament. As a result, a **nucleotide gradient** forms: ATP-actin (T-form) at the plus end and ADP-actin (D-form) toward the minus end.

This hydrolysis does not cause immediate disassembly, but it **weakens the interactions** between subunits. As a result, D-form actin has a **higher critical concentration ( $C_c$ )** than T-form, meaning it dissociates more readily.

At intermediate monomer concentrations, this leads to **treadmilling**:

- The plus end remains in T-form and grows (since monomer concentration  $> C_c$  for T-form).
- The minus end, being in D-form, shrinks (monomer concentration  $< C_c$  for D-form).

Thus, the filament maintains a constant length while subunits cycle through it—**powered by ATP hydrolysis and enabled by filament polarity**. This dynamic turnover is essential for many cellular processes, such as cell movement and shape changes.

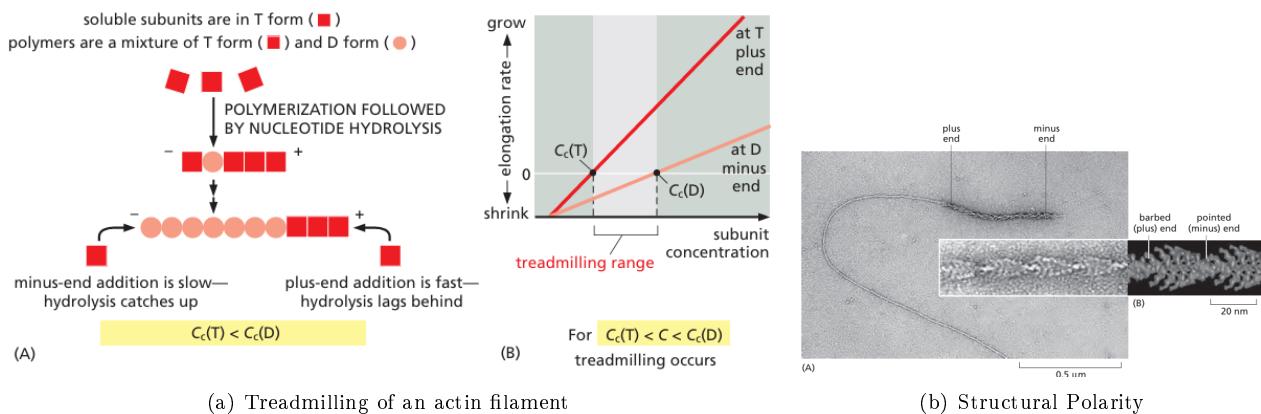


Figure 216: Cytoskeleton is dynamic and determines cellular organization and polarization

**Monomer Availability Controls Actin Filament Assembly** Only about half of the actin in non-muscle vertebrate cells is polymerized into filaments. This is largely due to the action of monomer-binding proteins that sequester actin and inhibit spontaneous polymerization. The most abundant of these is **thymosin**, which binds actin monomers and holds them in an inactive state: they cannot polymerize, hydrolyze ATP, or exchange their nucleotide.

In contrast **profilin** is used to make actin monomers available for filament assembly. Profilin which binds the opposite side of the actin monomer from the ATP-binding cleft. Note that profilin **blocks association with the minus end of filaments**. Upon encountering a filament plus end, the profilin–actin complex adds a monomer, and profilin is released and recycled.

Since **thymosin and profilin compete** for binding to actin monomers, the cell can regulate actin assembly by locally modulating profilin activity.

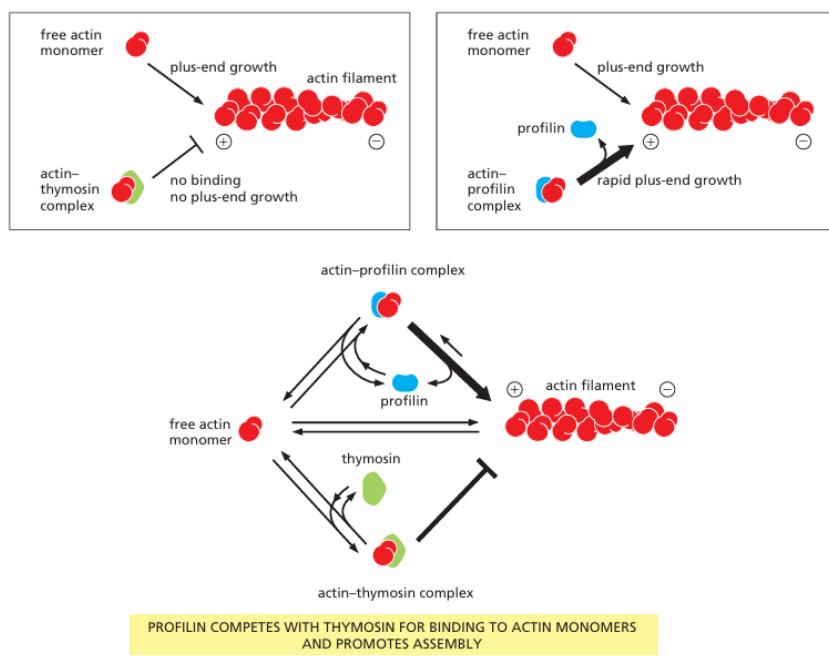


Figure 217: Effects of thymosin and profilin on actin polymerization.

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**Profilin activity is spatially and temporally controlled** through phosphorylation and interactions with acidic membrane phospholipids. This targeting is especially important at the plasma membrane, where extracellular signals can recruit profilin to promote localized filament growth, enabling the extension of actin-based structures like lamellipodia and filopodia.

**Formin-mediated actin filament elongation** formin proteins are dimeric actin nucleators that **promote the growth of long, unbranched actin filaments**. Each subunit in the formin dimer contains a binding site for monomeric actin, and together, they can nucleate new filament formation by capturing and stabilizing two actin monomers. Formins remain attached to the rapidly growing plus end of the filament, allowing continued subunit addition during elongation.

Formin-mediated actin nucleation occurs primarily at the **plasma membrane and contributes to the assembly of parallel actin bundles**. There **rapid remodeling** is essential for cell movement and response to external stimuli.

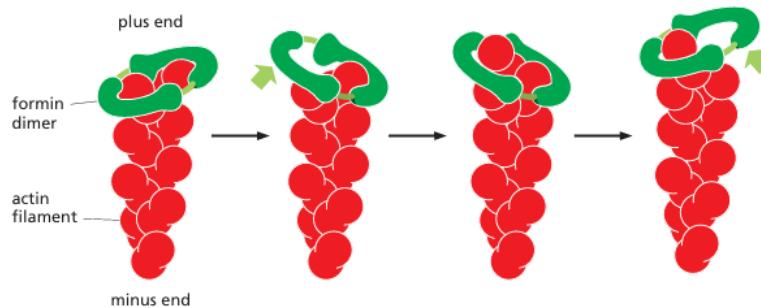


Figure 218: Actin elongation mediated by formins.

Some formins have unstructured domains "whiskers" that contain binding sites for profilin. This enhances the growth rate drastically.

**Nucleation of Actin Filaments by the Arp2/3 Complex** A key requirement for actin polymerization in cells, beyond the availability of actin monomers, is the **nucleation of new filaments**. This process is **energetically unfavorable and thus requires specialized proteins to initiate it**. One of the major actin nucleation mechanisms involves the **Arp2/3 complex**, which contains two actin-related proteins (Arp2 and Arp3) that are structurally similar to actin.

In its inactive state, the Arp2/3 complex is held in a conformation that prevents nucleation. Upon activation by nucleation-promoting factors, **Arp2 and Arp3 are repositioned to mimic the plus end of an actin filament**, enabling actin subunits to assemble and bypass the rate-limiting nucleation step. Importantly, the Arp2/3 complex preferentially nucleates filaments while attached to the side of preexisting actin filaments, forming branches at a 70° angle.

This branching results in the formation of a dense, treelike actin network that is essential for cell motility and structural organization (see Figure 219).

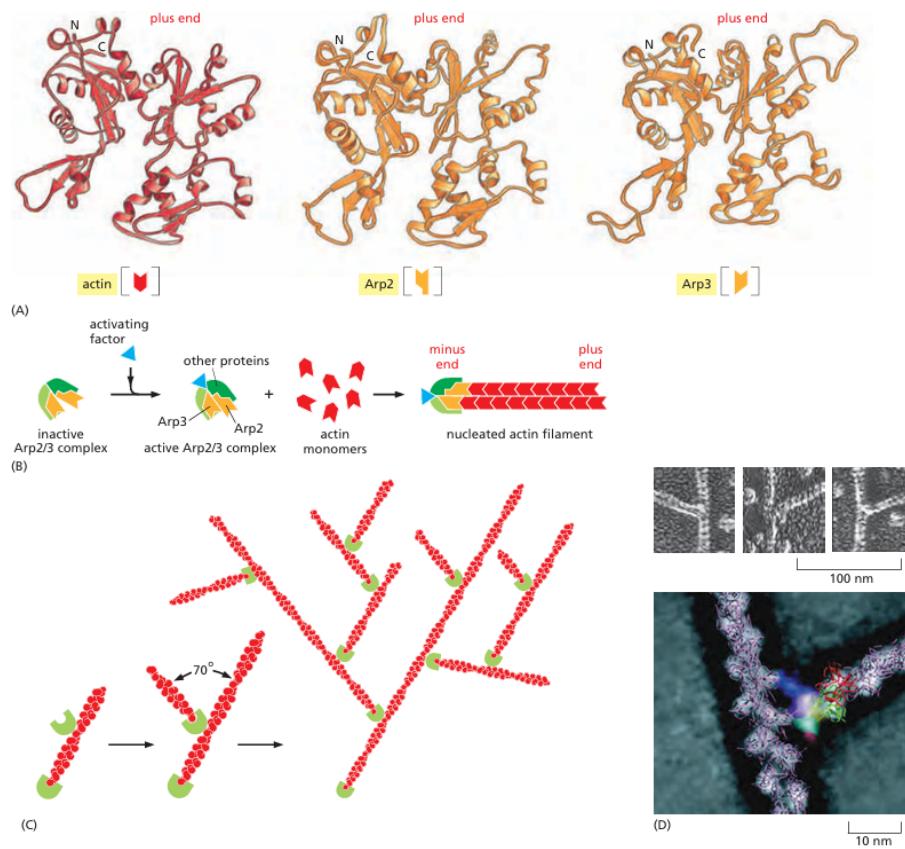


Figure 219: Nucleation and actin web formation by the Arp 2/3 complex.

**Actin-Filament-Binding Proteins Alter Filament Dynamics** Actin filaments are regulated by proteins that bind either **along their sides or at their ends**. Figure 220(a) show the effect of the presence on the filament dynamics. Note that *Side-binding proteins require high concentrations to coat filaments fully, but end-binding proteins act effectively at low levels, controlling filament dynamics by capping filament ends.*

- **Tropomyosin** binds along filament grooves, stabilizing and **stiffening filaments** while **blocking interactions** with other proteins.
- **Plus ends** may be capped by **CapZ** caps **preventing assembly or disassembly**.
- **Minus ends** may be capped by the **Arp 2/3 complex** or **Tropomodulin**, preventing assembly or disassembly

**Severing Proteins Promote Actin Turnover and Cytoskeletal Remodeling** The dynamic reorganization of actin filaments also depends on proteins that **sever existing filaments**. Severing creates numerous new filament ends, whose fate depends on local conditions and regulatory proteins. This can dramatically accelerate filament turnover and fluidize the cytoskeleton.

Two key severing factors are Gelsolin and Cofilin.

- i) **Gelsolin** is activated by elevated cytosolic  $\text{Ca}^{2+}$ . It binds the side of an actin filament and inserts itself between subunits when transient thermal fluctuations create small gaps. This results in filament severing. After cleavage, **gelsolin remains attached to the new plus end**, acting as a **cap and preventing further polymerization**.

- ii) **Cofilin**, also known as *actin depolymerizing factor*, binds to ADP-actin filaments twisting them more tightly and weakening subunit interactions. This mechanical strain increases the likelihood of spontaneous severing and **accelerates filament disassembly**. Since ATP hydrolysis lags behind filament growth, cofilin spares newer, ATP-rich filaments and selectively dismantles older ones, supporting the polarized actin turnover required for processes like cell migration.

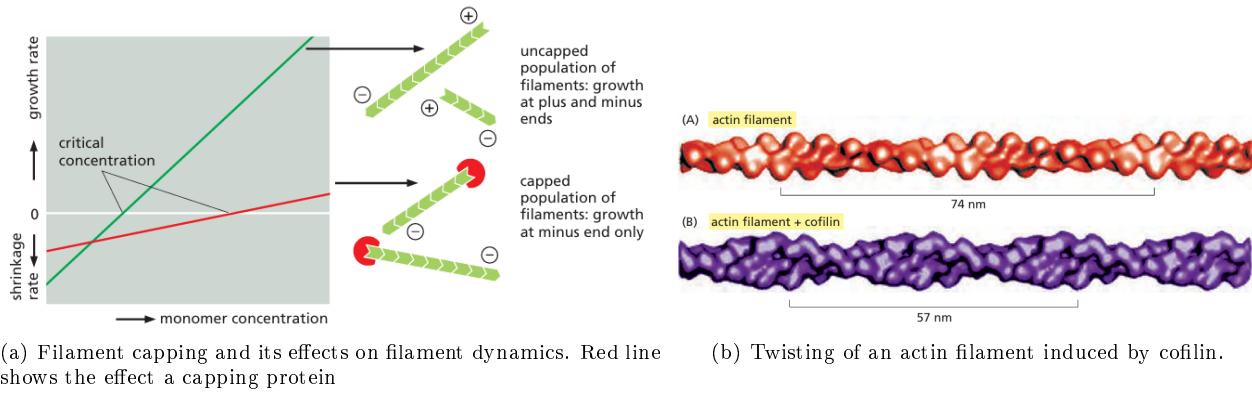


Figure 220: Cytoskeleton is dynamic and determines cellular organization and polarization

### 12.1.1.2 Structural Organization of Actin Filaments

As shown in Figure 213(b) there are different forms of structural organization between actin filaments. This depends on specialized **accessory proteins**.

There are 2 main types **bundling proteins**, which cross-link actin filaments into a parallel array, and **gel-forming proteins**, which hold two actin filaments together in a large angle.

Note that each type of bundling proteins also **determines which other molecules can interact** with the cross-linked actin filaments.

For example, **Myosin II** is the motor protein that enables stress fibers to contract. Thus very close packeding actin filaments caused by the binding protein **fibrin** exclude myosin and are thus not contractile. But on the other hand  **$\alpha$ -actinin** cross-linked oppositely polarized actin filament are more loose bundles, that allow the binding of myosin, creating contractile actin bundles. See fig. 221

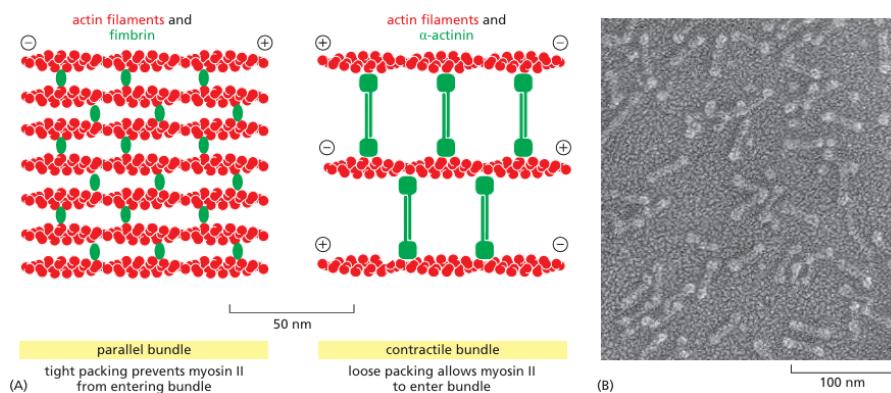


Figure 221: The formation of two types of actin filament bundles.

### 12.1.2 Myosin Motor Proteins

Actin-based motor proteins are members of the **myosin superfamily**. The first motor protein to be identified was skeletal muscle myosin, which generates the force for muscle contraction (*myosin II*).

All myosins share a motor domain dark green (see fig 222(a)), but their C-terminal tails (light green) and N-terminal extensions (light blue) are diverse.

Many myosins form dimers, with two motor domains per molecule. **Myosin VI is unique in moving towards the minus end** (instead of the plus end) of the actin filament.

Motor proteins use structural changes in their ATP-binding sites to produce **cyclic interactions with a cytoskeletal filament**. Each cycle of ATP binding, hydrolysis, and release propels them forward **in a single direction** to a new binding site along the filament. *Note these changes are asymmetric and designed to move in one direction, based on the polarity, for example myosin II moves towards the plus end.*

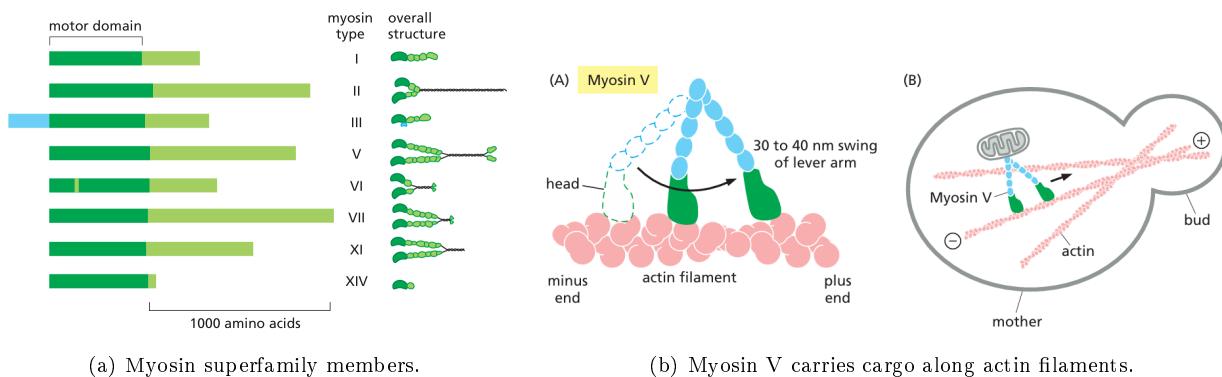


Figure 222: Myosin Motor Proteins

**Myosin V** (see fig 222(b)) is a two-headed myosin with a large step size and is involved in **organelle transport** along actin filaments. In contrast to myosin II motors, which work in ensembles and are attached only transiently to actin filaments, myosin V moves continuously along actin filaments without letting go. Myosin V motors carry a wide range of cargoes including mRNA, ER, and secretory vesicles. *Note that Myosin V takes larger steps than Myosin II.*

Most non-muscle cells contain small amounts of contractile actin myosin II bundles that form transiently. These are regulated by **phosphorylation** (rather than the troponin mechanism in muscle cells). These contractile bundles provide mechanical support to cells, for example, by assembling into cortical **stress fibres** that connect the cell to the ECM through focal adhesion.

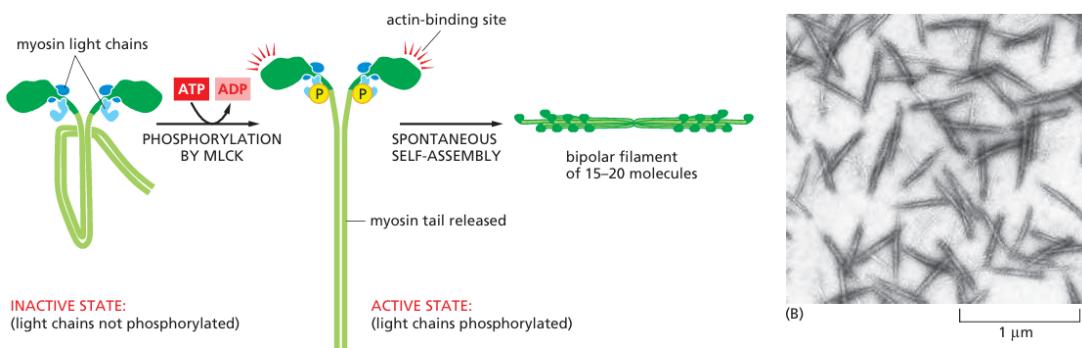


Figure 223: Light-chain phosphorylation and the regulation of the assembly of myosin II into thick filaments.

#### 12.1.3 Cell Migration via Actin Dynamics

Cell movement is driven by coordinated forces generated in the actin-rich cortex. At the front of a migrating cell, **actin polymerization pushes the plasma membrane forward to form a lamellipodium**, which attaches firmly to the substrate via **focal adhesions**. These adhesions are mediated by **integrin proteins**, which connect the actin cytoskeleton to the extracellular matrix. As new adhesions form at the leading edge, older ones at the rear are disassembled.

Simultaneously, **myosin-driven contraction at the back of the cell pulls the cell body forward**, relieving tension and generating traction.

Actin filaments at the leading edge assemble at their barbed ends, and if not anchored to adhesions, they move rearward due to continued polymerization and myosin activity. When linked to integrins via adaptor proteins, contractile forces are transmitted through the adhesions to produce forward motion. **This cycle of protrusion, adhesion, contraction, and release allows the cell to move in a stepwise or smooth fashion.**

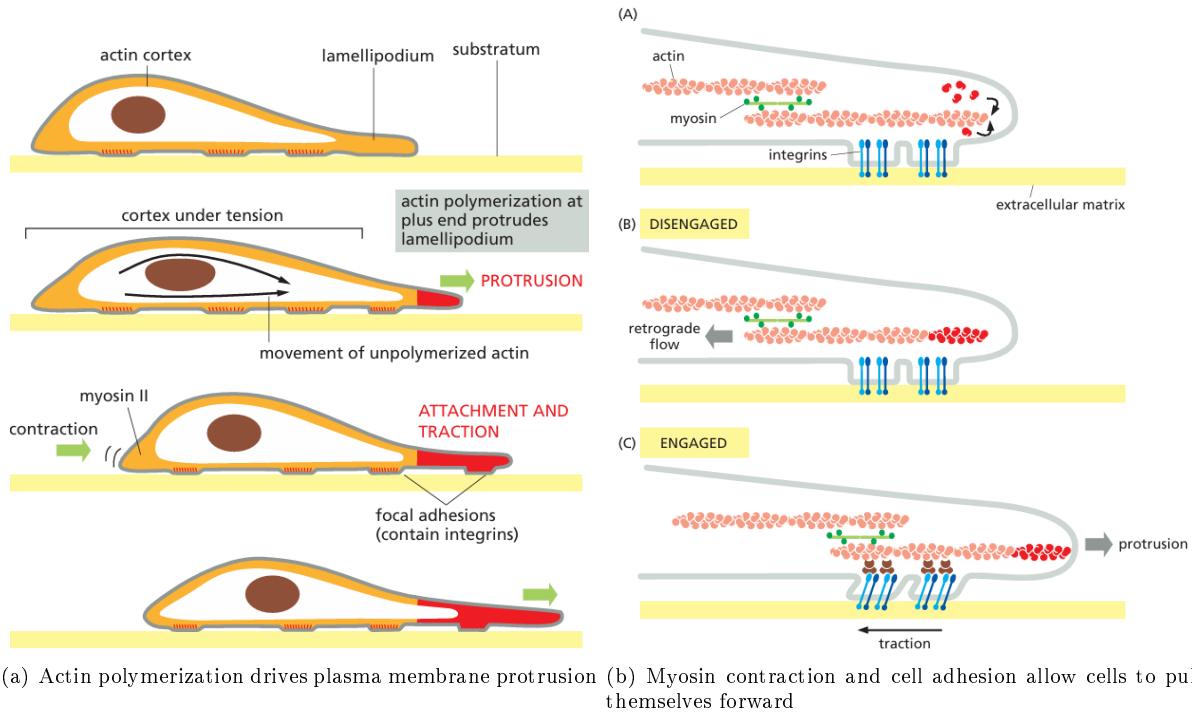


Figure 224: Cell Migration via Actin Dynamics

**Remark 12.4 (Membranes "breathe" thermally, allowing polymerization).** Once the filament contacts the membrane, there would be no room for a new subunit to it onto the end of the growing chain. It is thought that random thermal motions briefly expose the plus end of the filament, allowing a new subunit to be added. By taking advantage of these small windows of opportunity, actin polymerization acts as a ratchet to capture random thermal motions.

#### 12.1.3.1 The Rho-family

The small GTPases of the Rho-family Cdc42, Rac, and Rho play distinct roles in organizing the actin cytoskeleton of fibroblasts.

- The activation of **Cdc42** promotes the formation of **filopodia** (thin, finger-like protrusions) at the cell surface important for sensing and migration.
- The activation of **Rac** leads to the formation of lamellipodia—broad, sheet-like membrane protrusions—by dense branched actin networks (Arp2/3 complex).
- The activated **Rho** stimulates the formation of thick **stress fibers**—contractile actin bundles.

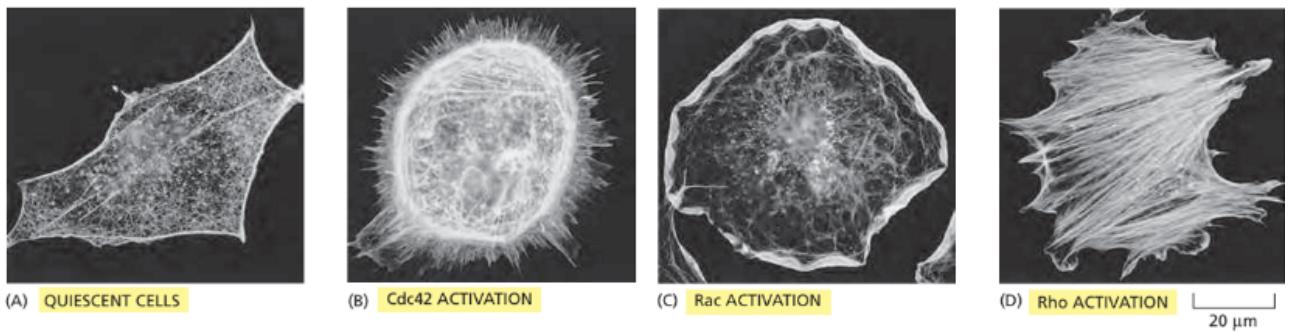


Figure 225: The dramatic effects of Cdc42, Rac, and Rho on actin organization in fibroblasts (visualized through actin staining)

Mechanistically, **Rac-GTP promotes actin branching through activation of the WASp family and the Arp2/3 complex**, while also **inhibiting myosin II activity via the PAK kinase pathway**, reducing contractility and promoting network formation.

Meanwhile, **Rho-GTP activates formins for actin nucleation and enhances myosin II contractility via the Rho-associated kinase (Rock), which maintains myosin activation and stabilizes actin bundles by inhibiting cofilin**.

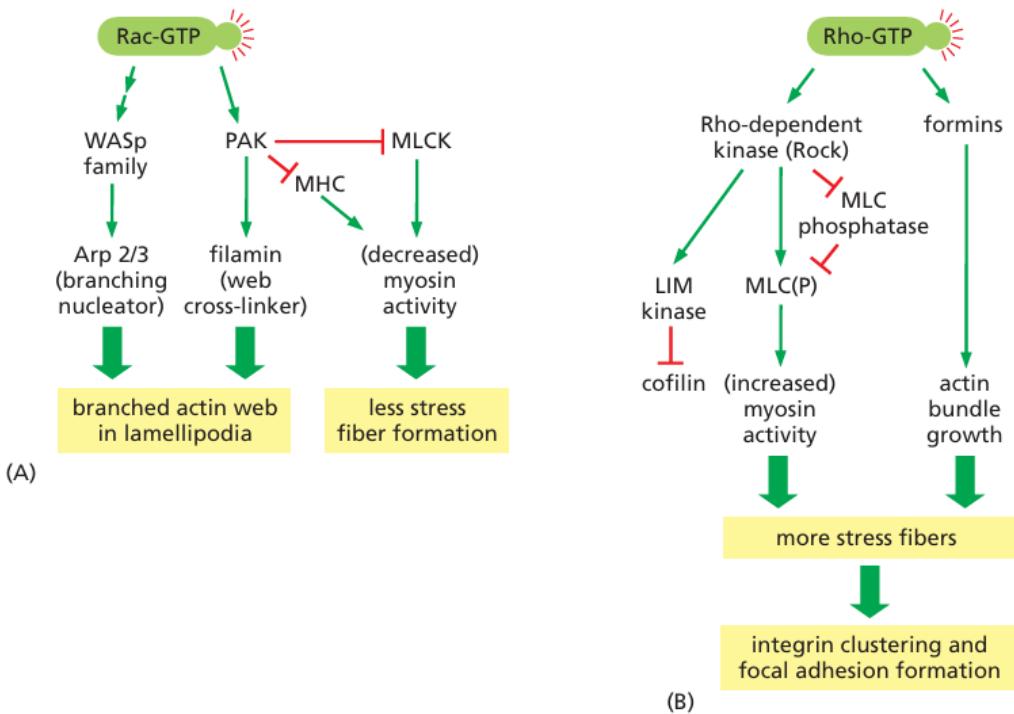


Figure 226: The contrasting effects of Rac and Rho activation on actin organization.

## 12.2 Microtubules

**Microtubules** determine the positions of membrane-enclosed organelles, direct intracellular transport, and form the mitotic spindle that segregates chromosomes during cell division.

Microtubules are structurally more complex than actin filaments, but they are also highly **dynamic** and play comparably diverse and important roles in the cell. Microtubules are polymers of the protein **tubulin**.

The tubulin subunit is itself a heterodimer formed from two closely related globular proteins called  $\alpha$ -tubulin and  $\beta$ -tubulin, which are held tightly together by noncovalent bonds. Each monomer has a binding site for **GTP**. While the GTP is trapped in the  $\alpha$ -monomer, the nucleotide on the  $\beta$ -subunit can be either in the GTP or in GDP form.

**A microtubule is hollow tube made from 13 parallel protofilaments.** This creates multiple contacts among subunits make microtubules stiff and difficult to bend. Microtubules can be several millimeters before it starts to bend because of thermal motion (persistence length), making microtubules the **stiffest** and straightest structural elements found in most animal cells.

The subunits in each protofilament point in the same direction, and the protofilaments themselves are aligned parallel. Therefore, the microtubule lattice itself has a distinct structural **polarity**, which  $\alpha$ -tubulins exposed at the **minus end** and  $\beta$ -tubulins exposed at the **plus end**.

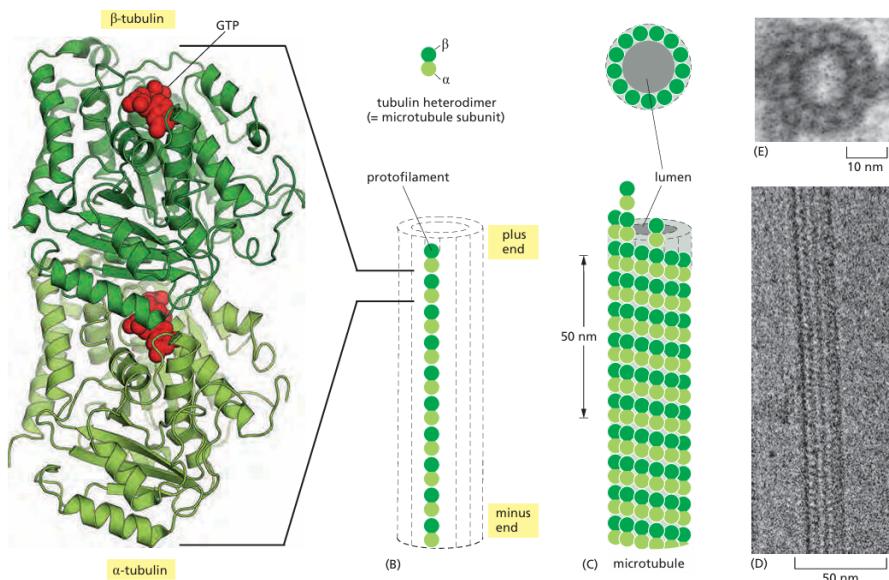


Figure 227: The structure of a microtubule and its subunit.

Microtubules undergo dynamic instability with the **plus end ( $\beta$ ) shrinking and growing more rapidly**. These dynamics, like those of actin filaments, are influenced by the binding and hydrolysis of nucleotide - GTP in this case.

GTP hydrolysis occurs only within the  $\beta$ -tubulin subunit, plus end of dimer. It proceeds very slowly in free tubulin subunits but is accelerated when they are incorporated into microtubules. Following GTP hydrolysis, the free phosphate group is released and the GDP remains bound to  $\beta$ -tubulin within the microtubule lattice. Therefore it exists the **D-form** and the **T-form**. Because of the differences in structure, the T-form tends to polymerize and the D-form to depolymerize.

A growing strand has GTP cap (multiple in T forms at the end). Now if the rate of subunit addition is low, hydrolysis may occur before the next subunit is added, and the tip of the filament will then be in the D form. This would induce depolymerization. This process is called **dynamic instability** and is dependent on the free concentration. See fig. 228

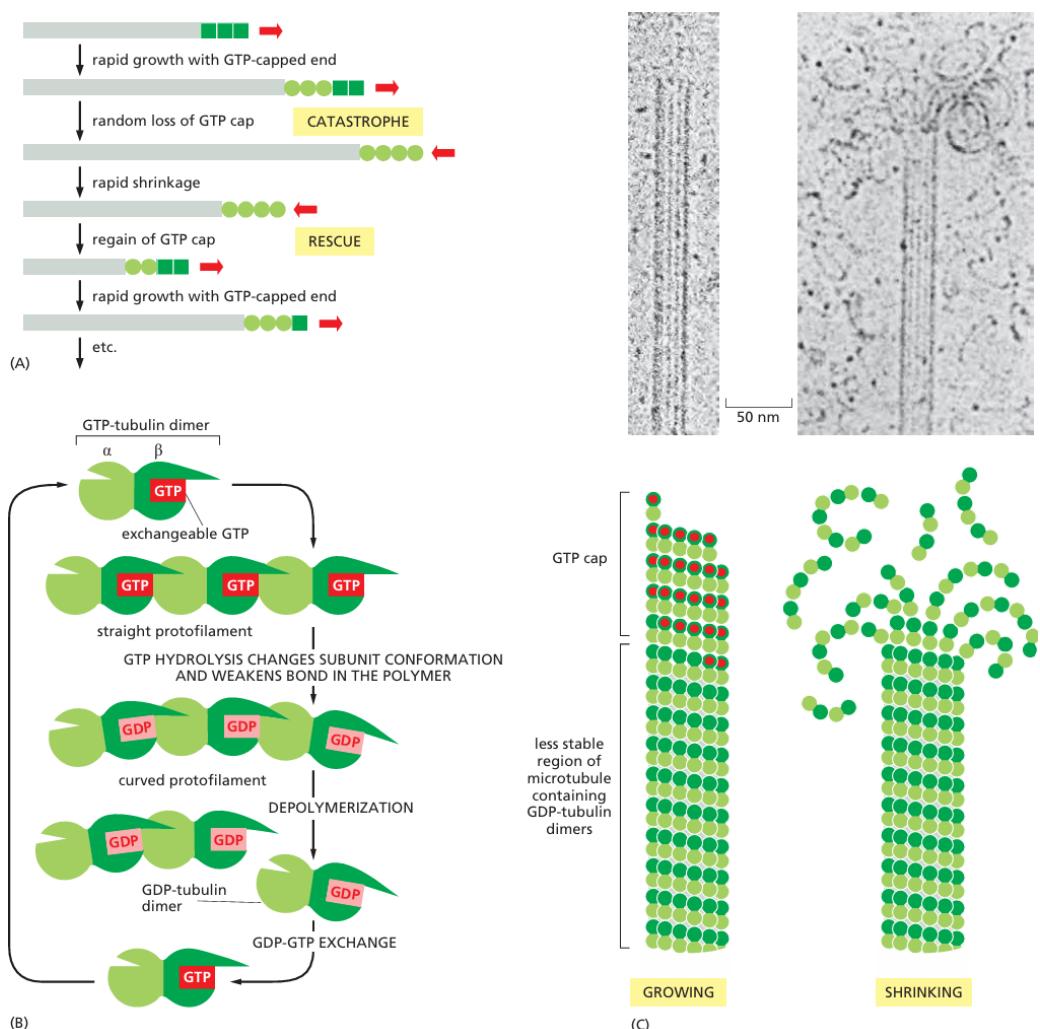


Figure 228: Dynamic instability due to the structural differences between a growing and a shrinking microtubule end.

### 12.2.1 Tubuli binding proteins

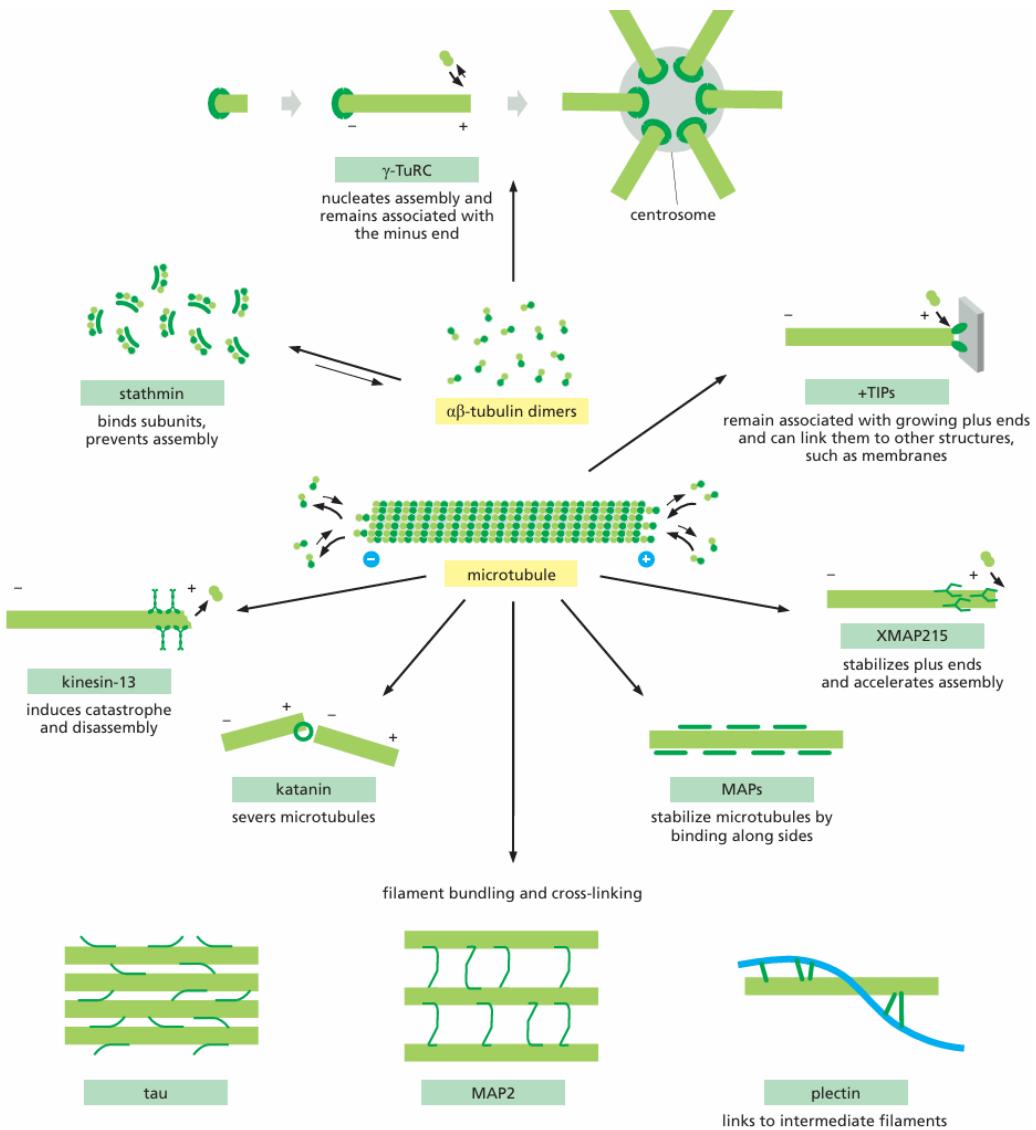


Figure 229: Some of the major accessory proteins of the microtubule cytoskeleton.

#### 12.2.1.1 Regulation of Microtubule Formation

Microtubule formation requires the assembly of many tubulin heterodimers, so spontaneous nucleation demands a very high concentration of subunits. To overcome this, cells use specialized factors to facilitate nucleation. **γ-tubulin** is essential for initiating microtubule growth.

γ-Tubulin is concentrated at the **microtubule-organizing center (MTOC)**, the site where nucleation typically occurs. There, it forms part of the **γ-tubulin ring complex**, which acts as a template for microtubule assembly. The γ-TuRC includes accessory proteins that arrange γ-tubulin into a spiral ring structure, matching the 13-protofilament architecture of a growing microtubule. *Side note, γ-tubulin are one of the most conserved proteins out there*

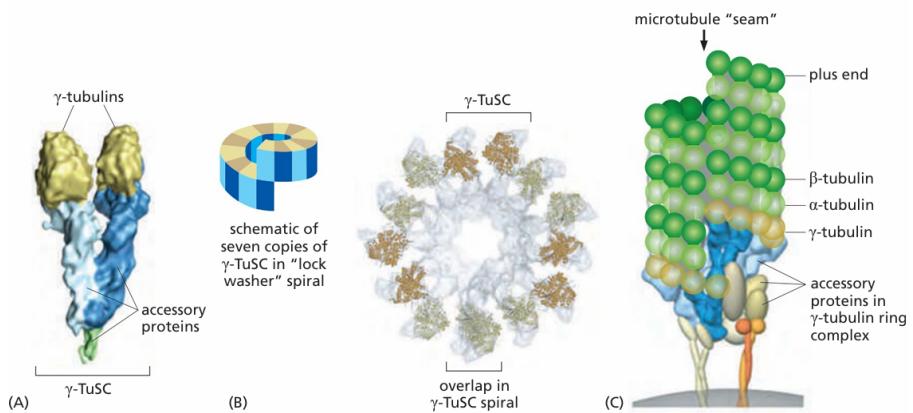
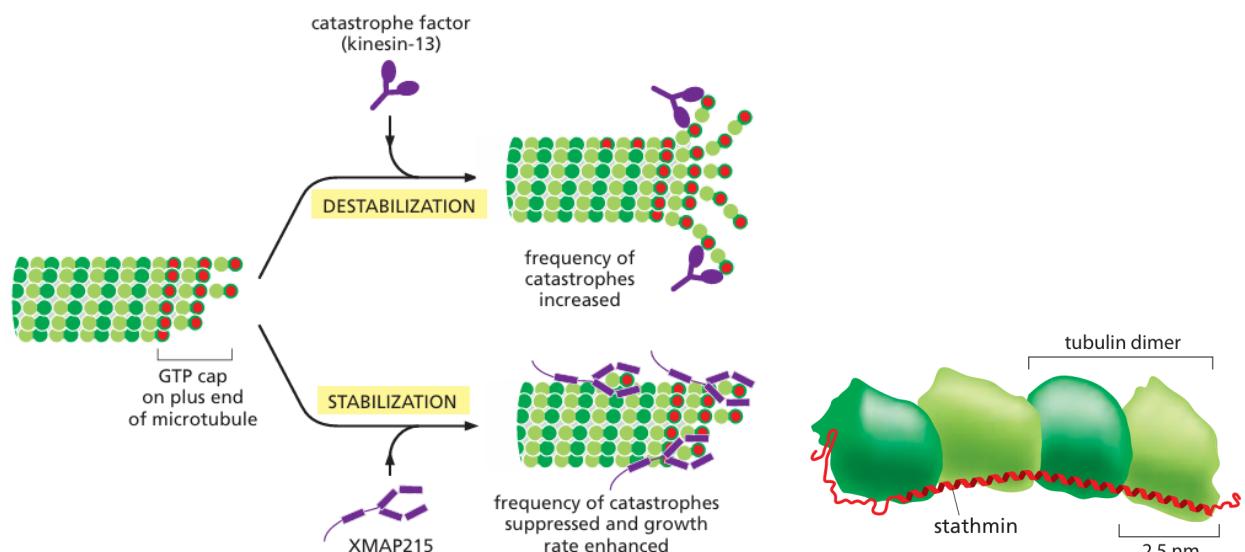


Figure 230: Microtubule nucleation by the  $\gamma$ -tubulin ring complex.

There are other proteins that are involved in regulating the transition between microtubule growth and shrinkage. A few of them bind to the end of the microtubule promoting (de-)polymerization. See fig. 231(a)

- **catastrophe factors** like kinesin-13 destabilize and promote depolymerization. **Kinesin 13** is a member of the kinesin superfamily of motor proteins, but it does not transport cargo. Instead, it binds to the ends of microtubules and promotes depolymerization, increasing microtubule catastrophe frequency. It plays key roles in mitosis, especially in spindle dynamics and chromosome segregation.
- **Microtubule-Associated Proteins (MAPs)** such as **XMAP215** stabilize the end of growing microtubule and promote polymerization. XMAP215 binds to tubulin dimers and delivers them to the growing plus end of microtubules, thereby promoting microtubule growth. XMAP215 is crucial for regulating microtubule dynamics during interphase and mitosis.



(a) The effects of proteins that bind to microtubule ends.

(b) Sequestration of tubulin by stathmin.

Figure 231: Kinesin 13, XMAP215, and Stathmin regulate the formation of microtubule.

An other possibility to regulate the formation of microtubule is to **mess with the concentration of tubulin** like it was done for actin filaments. The cell sequesters unpolymerized tubulin subunits to maintain a pool of active subunits at a level near the critical concentration (like for actin).

One molecule of the small protein **stathmin** (see fig. 231(b)) binds to two tubulin heterodimers and prevents their addition to the ends of microtubules, thus **reducing the effective concentration of tubulin**. Therefore it enhances the likelihood that a growing microtubule will switch to the shrinking state. Its activity is controlled by phosphorylation.

#### 12.2.1.2 Microtubules Originate from the Centrosome in Animal Cells

The **Centrosome** is the major microtubule-organizing center (MTOC) in most animal cells. It is located near the nucleus and consists of a pair of orthogonally arranged Centriole surrounded by **Pericentriolar Material (PCM)**. This protein-rich matrix anchors  $\gamma$ -tubulin ring complexes ( $\gamma$ -TuRCs), which nucleate microtubules.

The minus ends of microtubules are embedded in the centrosome,  $\gamma$ -TuRC, while plus ends extend outward into the cytoplasm, forming a dense array.

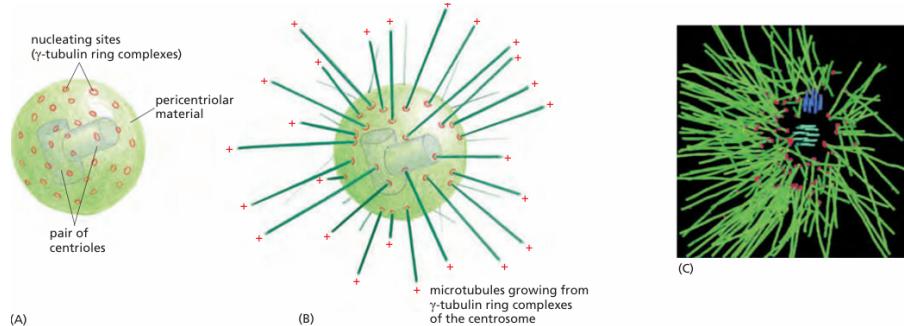


Figure 232: The centrosome

The centrioles are cylindrical cellular structures composed of nine triplet microtubules arranged with ninefold symmetry. his structural symmetry is guided by the **SAS-6** protein, which assembles into a ring at the core of the cartwheel structure. See fig. 233 (d)

Centrioles are found in pairs (**the mother and daughter centrioles**) within the centrosome. During the cell cycle, centrioles **duplicate** and play a key role in spindle formation during mitosis. They can also act as basal bodies to initiate the formation of **cilia and flagella**.

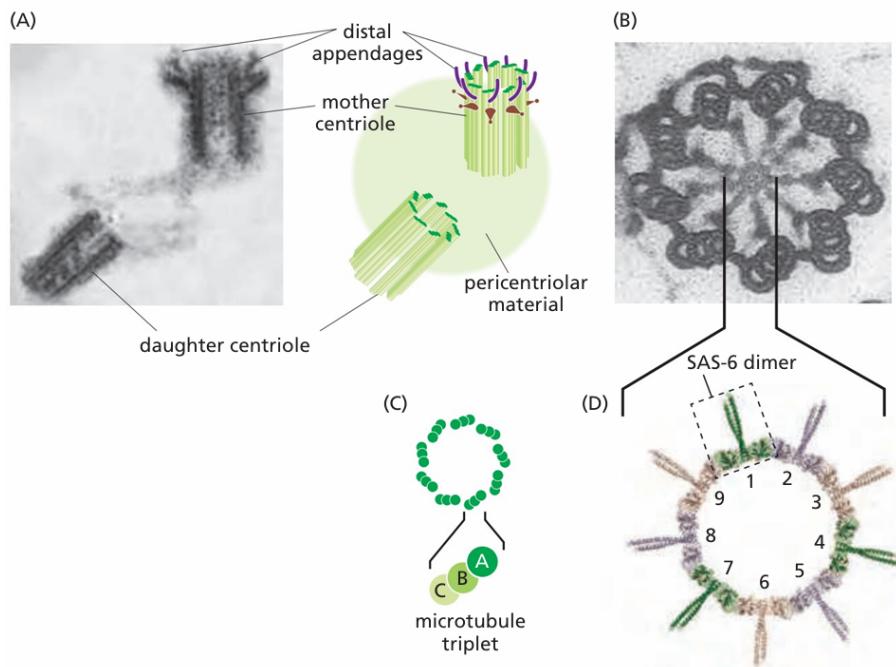


Figure 233: A pair of centrioles in the centrosome.

Remarkably, even without a centrosome, microtubules can self-organize. After physically severing part of a fish pigment cell, the microtubules in the detached fragment reorganize, **clustering their minus ends at the center, forming a new MTOC** (see fig. 234). This shows that microtubules can establish cellular polarity and coordinate internal architecture independently, highlighting their self-organizing capability.

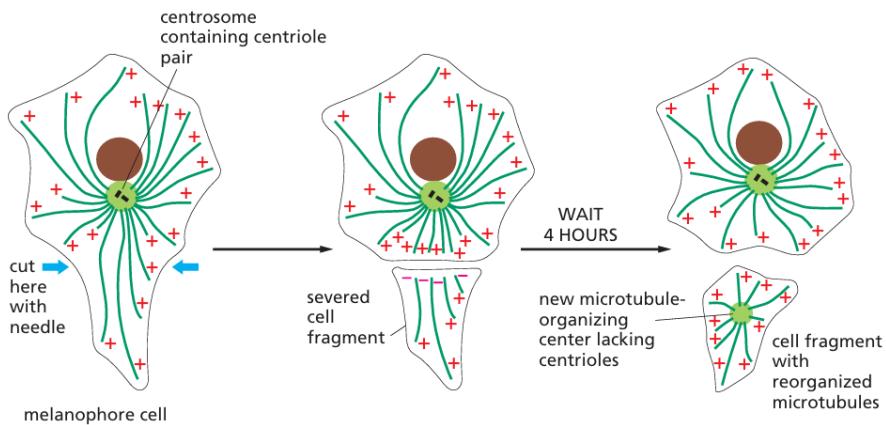


Figure 234: A microtubule array can find the center of a cell.

#### 12.2.1.3 Organization of Microtubules

**Microtubule-associated proteins (MAPs)** stabilize microtubules and mediate their interactions with other cellular structures. These proteins have one domain that binds to the microtubule surface and another that projects outward, affecting microtubule spacing.

Figure 235 illustrates how MAPs organize microtubule bundles.

- **MAP2** has a **long projecting domain**, allowing it to cross-link microtubules at wider distances, resulting in **widely spaced bundles**.
- **Tau**, with a shorter projecting domain, creates more closely packed bundles.

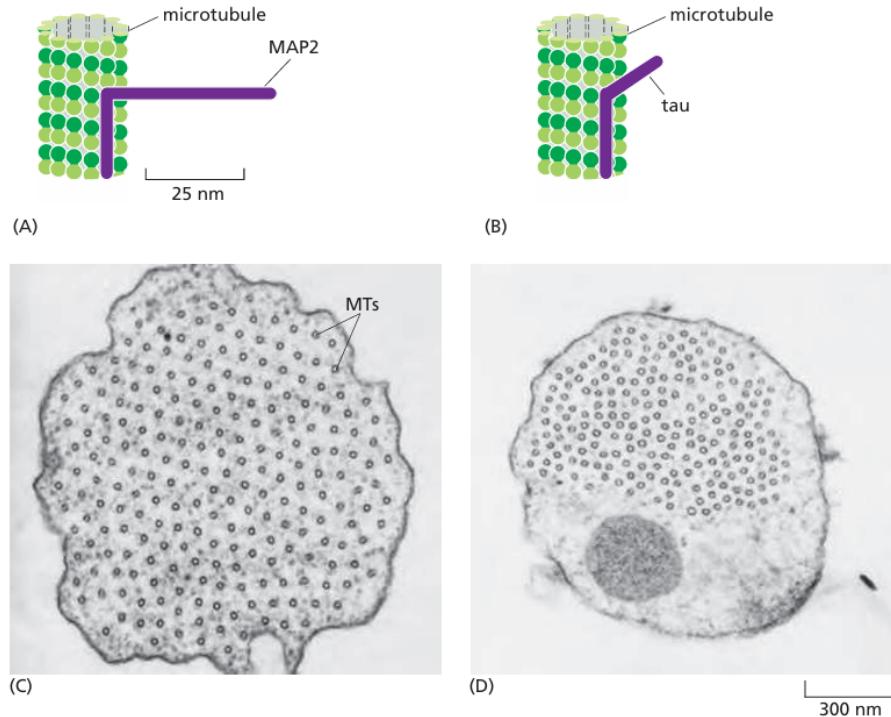


Figure 235: Organization of microtubule bundles by MAPs.

*Remark 12.5 (Microtubule organization in a neuron).* In a neuron, microtubule organization is complex. In the **axon**, all microtubules share the same polarity with the plus end pointing towards the axon terminus. But no single microtubules stretches the entire length of the axon: instead, short overlapping segments of parallel microtubules make the tracks for fast axonal transport.

In the **dendrites**, the microtubules are of mixed polarity. Vesicles can associate with both kinesin and dynein and move in either direction along the microtubules in axons and dendrites.

*Note in neurons, abnormal tau aggregation is associated with neurodegenerative diseases such as Alzheimer's.*

### 12.2.2 Motor Proteins associated with Tubuli

Like actin filaments, microtubules also use motor proteins to transport cargo and perform a variety of other functions within the cell. There are two major classes of microtubule-based motors, **kinesins** and **dyneins**.

#### 12.2.2.1 Kinesins

Kinesin is a class of motor proteins that move generally **along microtubules towards the plus end, but not all**.

Like myosin, kinesin is a member of a large protein superfamily, for which the motor domain is the common element. Despite this conserved region, kinesins differ significantly in the position of the motor domain and the function of their tail domains, leading to a range of specialized roles within the cell.

**Kinesin-5** forms a bipolar tetramer that slides two microtubule past each other, much like the bipolar

filaments of myosin II. **Kinesin-13**, with a central motor domain, lacks conventional motility and instead promotes microtubule depolymerization at the ends. **Kinesin-14** contains a C-terminal motor and moves toward the minus end of microtubules, opposite to most kinesins.

**Kinesin-1**, is one of the best understood members. It is a dimeric motor protein composed of two motor domains (heads) connected via a coiled-coil tail. It walks along microtubules using a coordinated “hand-over-hand” stepping mechanism that allows for processive movement, meaning it can take many steps without detaching from the microtubule. See fig. 237(b)

At the beginning of a step, the **lagging head** is tightly bound to the microtubule and carries **ATP**, while the **front head** is loosely attached with **ADP** in its binding site. ATP binding to the leading head triggers a conformational change in a flexible region called the **neck linker**, which shifts forward. This movement propels the lagging head past the leading head.

Once the lagging head detaches (following ATP hydrolysis and phosphate release), it swings forward and rebinds to a new tubulin site as the new leading head. Meanwhile, the previous leading head becomes the new rear head, preparing for another cycle. This alternating cycle ensures continuous, directional transport of cargo along microtubules.

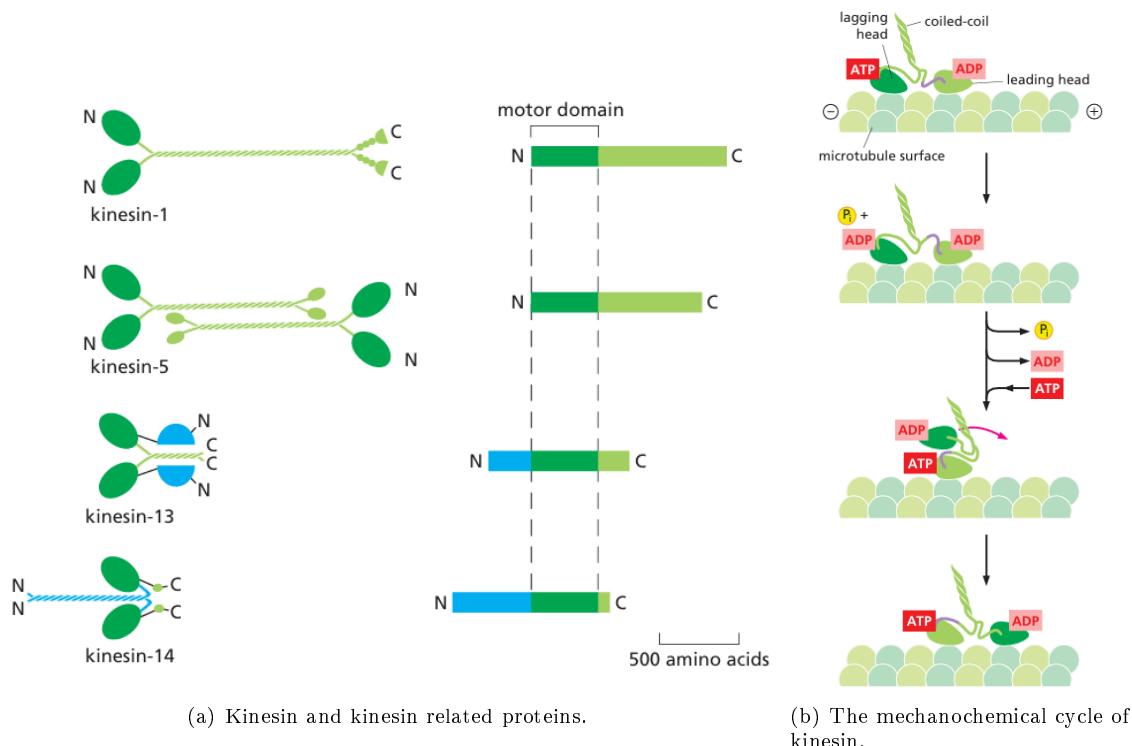


Figure 236: Kinesin 13, XMAP215, and Stathmin regulate the formation of microtubule.

### 12.2.2.2 Dyneins

dynein is a class of motor proteins that move **along microtubules towards the minus end**. They are structurally different from kinesin or myosin.

**cytoplasmic dynein** is a two-headed molecule, with each head formed by a separate heavy chain. Notably, the dynein motor head is **considerably larger** than those of myosin or kinesin. See fig. 237(a)

Each dynein heavy chain contains domains responsible for ATP hydrolysis and microtubule binding, orga-

nized around a large motor domain composed of a ring of **six AAA** (ATPases Associated with diverse cellular Activities) domains. Of these, **only one retains full ATPase activity** (dark red). From this motor ring, a coiled-coil stalk projects to bind microtubules, while a tail domain connects dynein to its cargo or, *in the case of axonemal dyneins, to an adjacent microtubule*.

**Dynein's movement is powered by ATP hydrolysis.** In the ATP-bound state, the stalk detaches from the microtubule. Upon hydrolysis, the stalk reattaches, and release of ADP and phosphate triggers a large conformational change (involving the **rotation of the head and the stalk relative to the tail**) —the **power stroke**— which moves the cargo or slides adjacent microtubules.

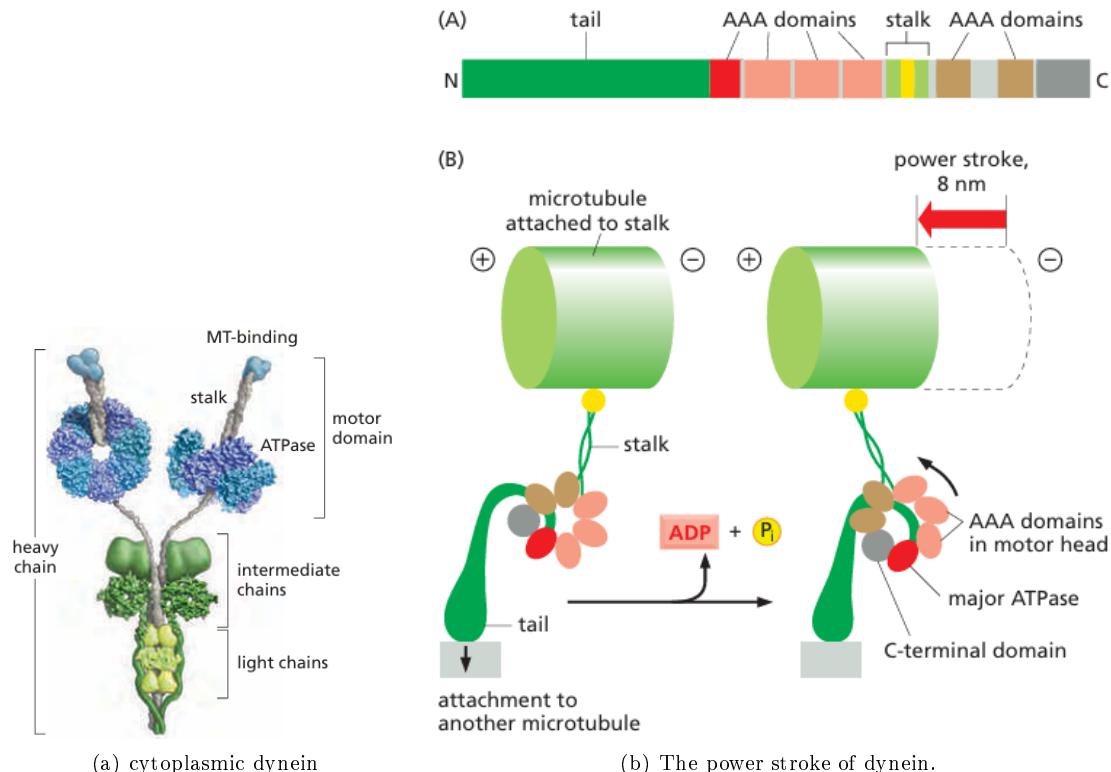


Figure 237: Dynein

**dynactin** mediates the attachment of dynein to a membrane enclosed organelle (See fig. 238(a)). Dynein requires the presence of a large number of **accessory proteins to associate with membrane-enclosed organelles**. Dynactin is a large complex that includes components that bind weakly to microtubules, components that bind to dynein itself, and components that form a small, actin-like filament made of the actin-related protein Arp1.

*Remark 12.6 (Regulated melanosome movements in fish pigment cells.).* In certain fish species, pigment cells regulate skin coloration by actively transporting large pigment granules called melanosomes. These movements are triggered by hormonal or neuronal signals that alter intracellular cyclic AMP (cAMP) levels.

The **melanosomes are moved along microtubules using motor proteins**. This dynamic transport system allows rapid and reversible changes in cell appearance, contributing to color adaptation. See fig 238(b)

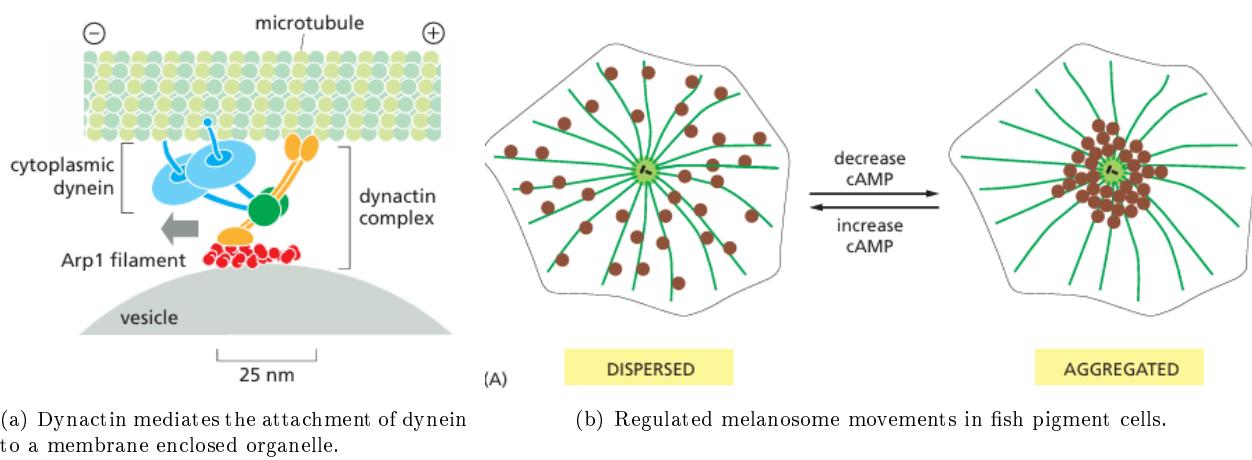


Figure 238:

### 12.2.3 Cilia and Flagella

Cilia and flagella are highly specialized and efficient **motility structures built from microtubules and dynein**. Both cilia and flagella are hairlike cell appendages that have a bundle of microtubules at their core. **Flagella**, found on sperm and protozoa, move with an **undulating motion** (like a snake) to propel cells through liquid. **Cilia** beat in a **whip-like**, breaststroke motion to move cells or fluids across tissue surfaces, such as mucus in the respiratory tract or eggs in the oviduct.

Inside a cilium and a flagellum is a **microtubule-based cytoskeleton called the axoneme**. This consists of nine outer doublet microtubules arranged in a ring around two central singlet microtubules (**9 + 2**). Along the length of the axoneme, linking projections between microtubules occur at regular intervals to maintain structural integrity and enable coordinated bending. See fig. 239

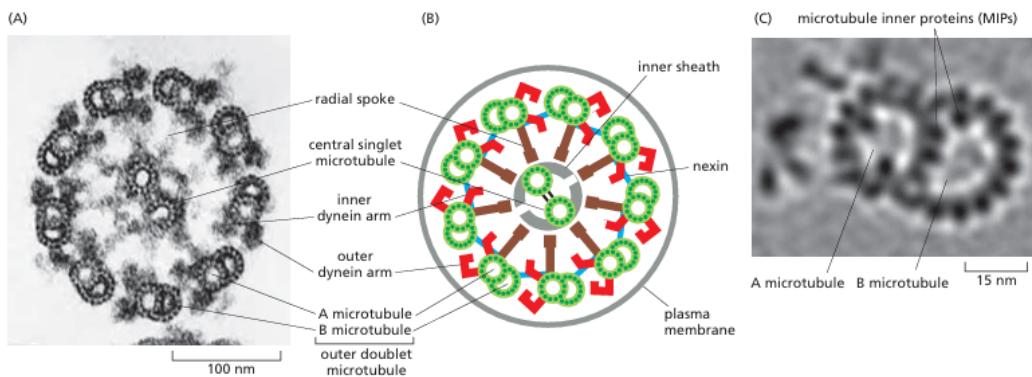


Figure 239: The arrangement of microtubules in a flagellum or cilium.

**Axonemal dynein** forms bridges between neighboring microtubule doublets in the axoneme. When activated, dynein motors attempt to "walk" along adjacent doublets, **generating a sliding force**. However, if linking proteins between the doublets prevent sliding, the force will be converted into a **bending motion**—the basis for ciliary and flagellar beating.

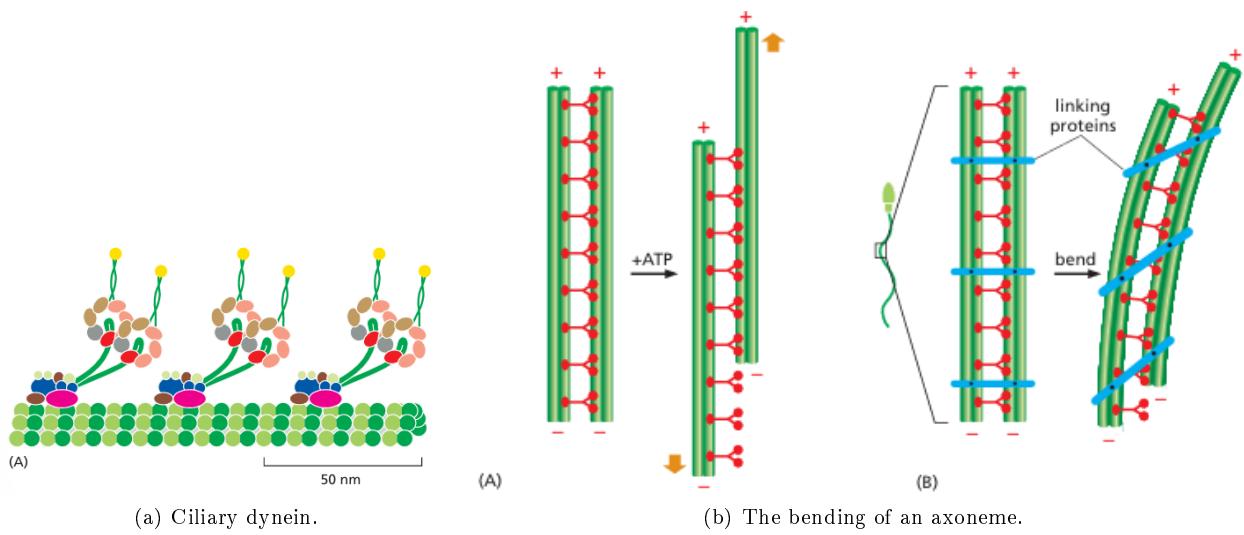


Figure 240: Movement of cilia and flagella

The **Primary cilium** is a non-motile, antenna-like structure present on nearly all vertebrate cells. Though structurally similar to motile cilia, it serves primarily as a **sensory organelle**, detecting signals from the external environment. Built from a **basal body derived from the mother centriole**, the primary cilium **grows during interphase** and is **resorbed before cell division**.

Its internal structure—the axoneme—is assembled and maintained through **intraflagellar transport (IFT)**, using kinesin-2 and cytoplasmic dynein 2 to move proteins and lipids in and out. Primary cilia are essential for functions such as smell and vision, therefore the structure is very **rich in signaling molecules** (like hedgehog signaling components).

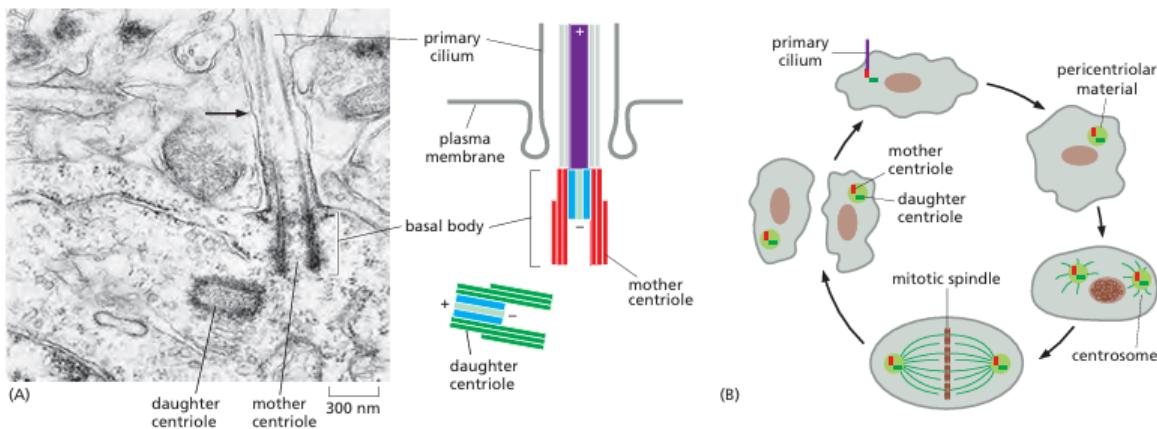


Figure 241: Primary cilia.

### 12.3 Intermediate filaments

Intermediate filaments are a type of *cytoskeletal protein*. They provide **mechanical strength** to cells, particularly those under stress. These filaments evolved from **nuclear lamins**, which form a supportive mesh under the nuclear envelope and are present in most eukaryotes. Unlike actin and tubulin, which are highly conserved, **intermediate filaments are diverse**, encoded by around 70 different genes in humans with cell-type-specific

roles.

Intermediate filament (IF) structure is built from elongated proteins with a central  $\alpha$ -helical domain that forms coiled-coil dimers. Two dimers align antiparallel to form tetramers, the building blocks of IFs. Unlike actin or tubulin, IF subunits **lack nucleotide-binding sites and have no structural polarity**. Tetramers pack laterally into eight-protofilament bundles, resulting in a **strong, flexible, ropelike filament**. While IFs are very stable, some types (like vimentin) are dynamic in cells, with phosphorylation regulating their disassembly.

These filaments are crucial for cellular processes that involve shape changes, like division, migration, and differentiation

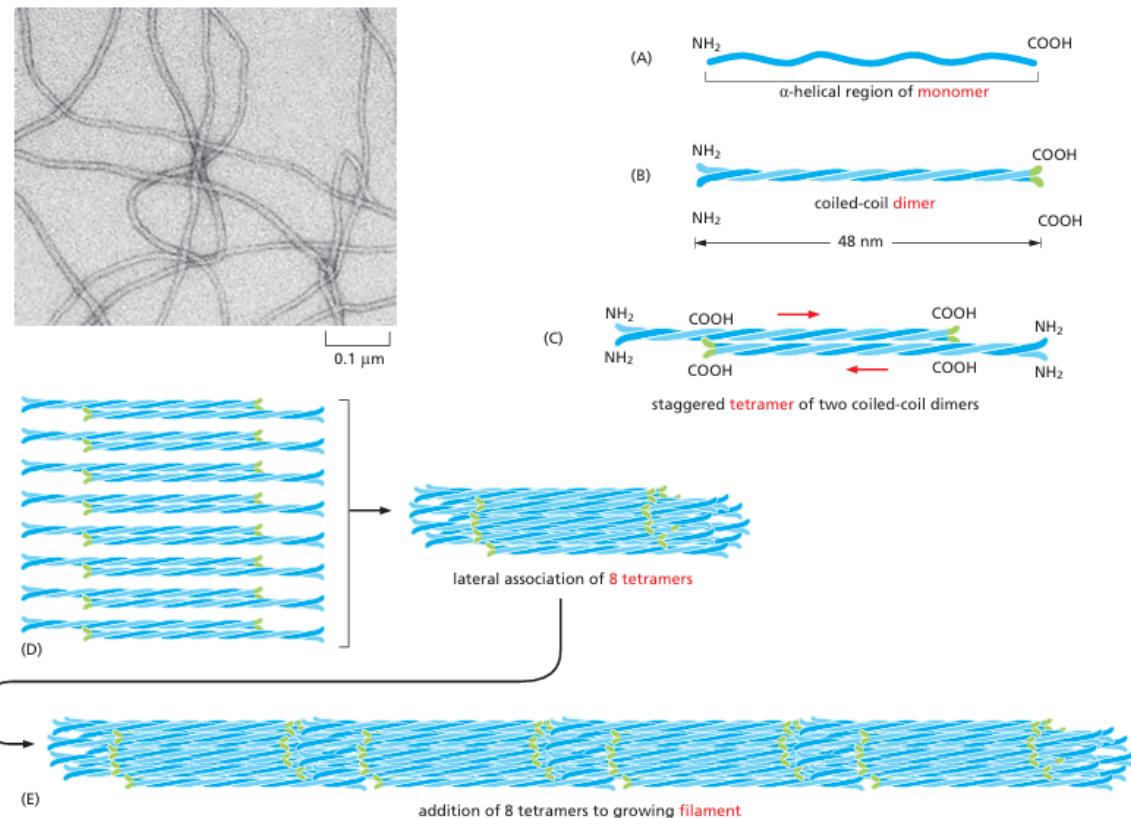


Figure 242: A model of intermediate filament construction.

### 12.3.1 Keratins (not the gym bro stuff)

Keratins are the most diverse family of intermediate filaments, with 54 types identified in humans. They are primarily found in **epithelial cells, hair, and nails**. These filaments provide mechanical strength to epithelial tissues and **persist even after cell death, forming structures like skin, hair, and nails**.

Keratin expression patterns are clinically important in diagnosing and treating epithelial cancers. In cells, keratin filaments anchor at desmosomes and hemidesmosomes to reinforce tissue structure, and proteins like filaggrin help bundle them, contributing to skin toughness. Mutations in filaggrin are linked to dry skin diseases such as eczema.

*Remark 12.7 (Blistering of the skin caused by a mutant keratin gene).* Mutations in keratin genes cause several genetic diseases characterized by fragile skin and other tissues. For example, *epidermolysis bullosa simplex* results from **defective keratins in the basal layer of the epidermis**, causing skin to blister even under minor mechanical stress due to cell rupture.

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*These mutations disrupt keratin filament assembly, particularly when they alter the ends of the protein's central rod domain, leading to cytoskeletal disorganization and increased cell fragility.*

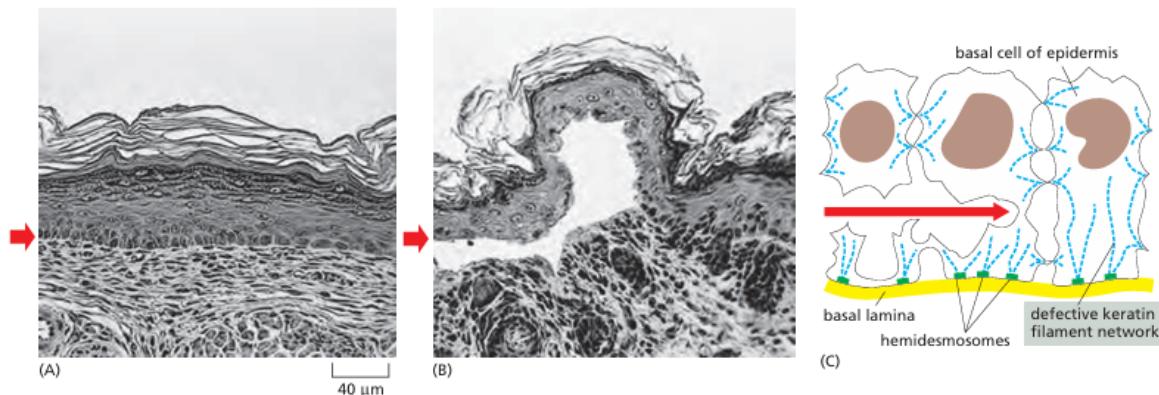


Figure 243: Blistering of the skin caused by a mutant keratin gene.

## 13 Cell Death

### 13.1 Apoptosis - Programmed cell death

The growth, development, and maintenance of multicellular organisms depend not only on the production of cells but also on mechanisms to destroy them. The maintenance of tissue size, for example, requires that cells die at the same rate as they are produced. Moreover cells have to die when they become damaged or infected, ensuring that they are removed before they threaten the health of the organism.

In most cases this is not random but rather precisely controlled, we speak of **programmed cell death**. The most prominent process is called **apoptosis**, which can be intrinsic regulated through the mitochondria or extrinsic regulated through cell signaling. There are also other processes, that won't be looked at, but just for completion: Phagocytosis, Ferroptosis, Necroptosis (similar to necrosis but controlled), Pyroptosis (during infection), and Cornification (skin keratinization).

Nevertheless the cell death can also occur unprogrammed, we then speak of **unprogrammed cell death**, a process is **necrosis** (accidental cell death). In most cases necrosis is caused by **energy depletion**. This often happens to **tumor cells**, as a tumor grows randomly and cells can get cut off from supplies, which leads to accidental cell death.

Cells **dying by necrosis** swell and **burst**, **spilling** their contents over their neighbors and eliciting an inflammatory response. Therefore cells that have died by necrosis look like they **exploded**. See fig. 244

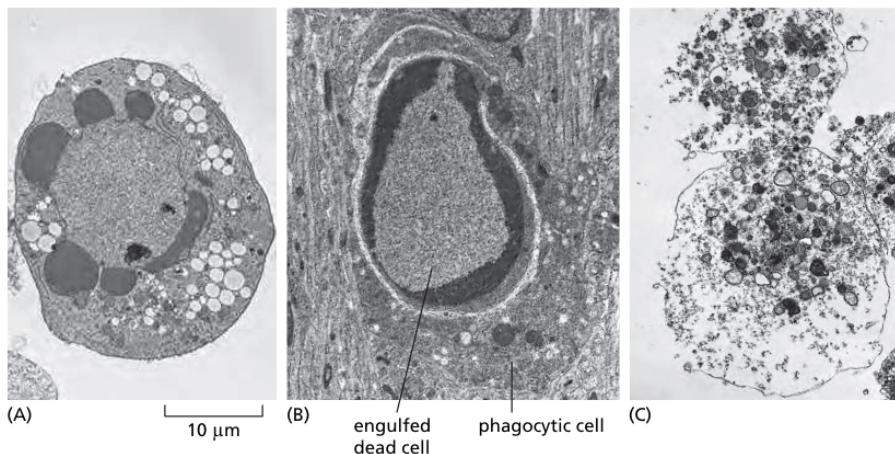


Figure 244: Two distinct forms of cell death. Apoptosis (A & B), Necrosis (C)

In contrast, Cells **dying by apoptosis** undergo characteristic morphological changes (**See fig 244**). They **shrink and condense**, the cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear chromatin condenses and breaks up into fragments. The cell surface often bulges outward and, if the cell is large, it breaks up into membrane-enclosed fragments called **apoptotic bodies**. The surface of the cell or apoptotic bodies becomes chemically altered, so that a neighboring cell or a macrophage rapidly engulfs them, before they can spill their contents, through **phagocytosis**. (**See fig. 245**) In this way, the cell dies neatly and is rapidly cleared away, without causing a damaging inflammatory response.

Basically a **cell needs to be convinced not to die**. Therefore for cell death to happen we deactivate a regulator of cell death, leading to cell death. When apoptosis is triggered (**initiator caspases (9 or 8)**) the commitment to die is still **reversible** (for example DNA repair occurs). The intrinsic and extrinsic pathway then converge to the **executioner caspases (3, 7)**. Once they are activated the cell is committed to apoptosis. (**See fig. 245**) Note the role of **XIAP** and **FLIP** that block caspases and are essential to ensure tight **all-or-nothing regulation**.

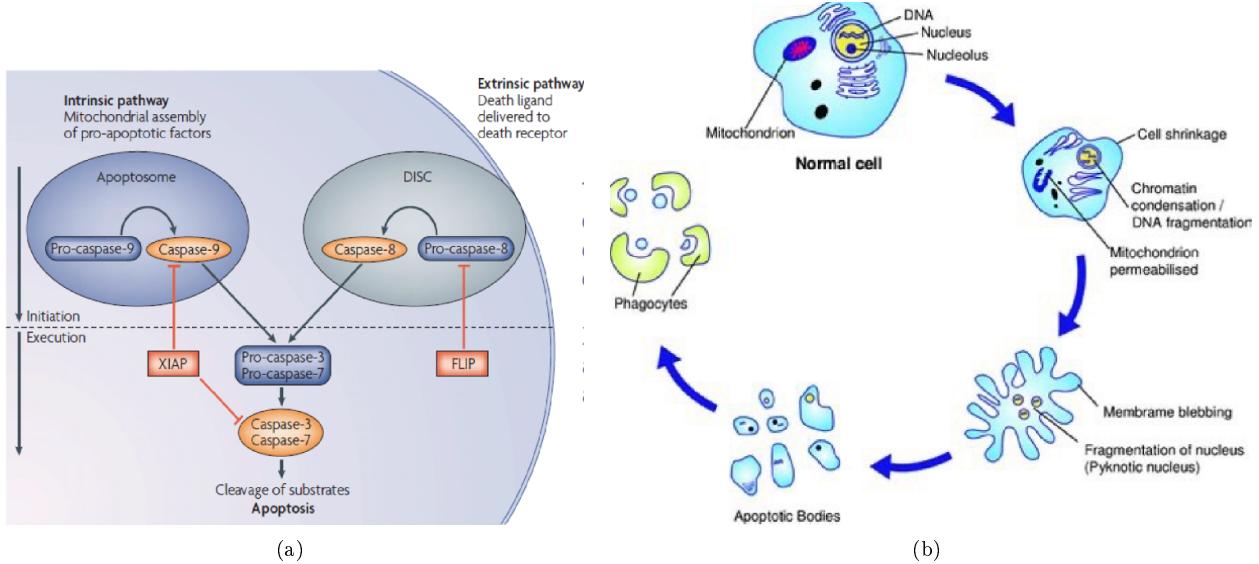


Figure 245: Steps of Apoptosis

**Remark 13.1 (DNA fragmentation during apoptosis.).** During apoptosis, the DNA of a dying cell is systematically fragmented by a nuclease called CAD (Caspase-Activated DNase). Under normal conditions, CAD is kept inactive through binding to its inhibitor, iCAD. However, when executioner caspases are activated during apoptosis, they cleave iCAD, releasing CAD to cut DNA between nucleosomes. This results in DNA fragments that appear as a distinctive "ladder" pattern when separated by gel electrophoresis. The presence of these DNA fragments can also be visualized in tissues using the TUNEL assay, which labels exposed DNA ends with fluorescent markers, highlighting apoptotic cells. See fig. 246

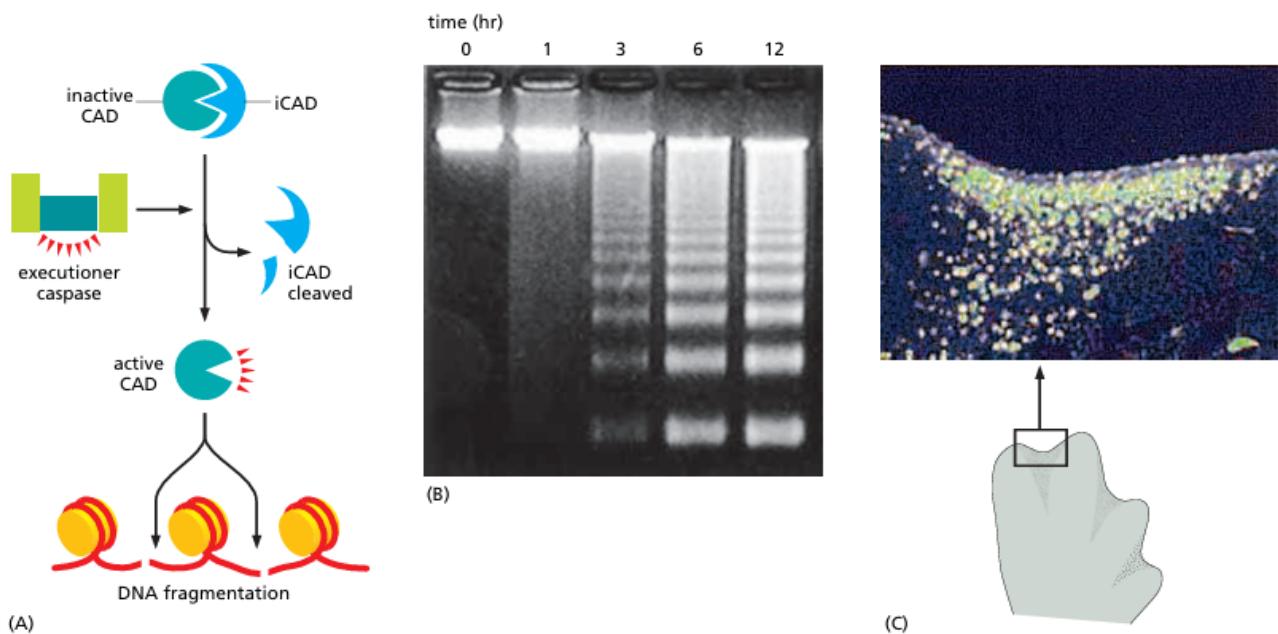


Figure 246: DNA fragmentation during apoptosis.

**Remark 13.2 (Dead cell removal by Efferocytosis).** Elimination of dead cell bodies is important to prevent the release of intracellular content that may function as damage-associated molecular patterns to activate an inflammatory response and possibly lead to autoimmunity.

**Remark 13.3 (Apoptosis plays an important role during development of many tissues.).** Cell death helps sculpt hands and feet during embryonic development: they start out as spade-like structures, and the individual digits separate only as the cells between them die. See fig. 246(c)

Moreover, apoptosis also functions as a **quality-control** process in development, eliminating cells that are abnormal, misplaced, nonfunctional, or potentially dangerous to the animal. Striking examples occur in the vertebrate adaptive immune system, where apoptosis eliminates **developing T and B lymphocytes** that either fail to produce potentially useful antigen-specific receptors or produce self-reactive receptors that make the cells potentially dangerous.

**Caspases** caspases are a family of proteases that use a cysteine residue in their active site and specifically cleave peptide bonds after aspartic acid residues in substrate proteins. They play essential roles in apoptosis, inflammation, and cell differentiation.

An **initiator caspase** contains a protease domain in its carboxy-terminal region and a small protein interaction domain near its amino terminus. It is **initially made in an inactive**, monomeric form, sometimes called **procaspase**. **Apoptotic signals trigger the assembly** of adaptor proteins carrying multiple binding sites for the caspase amino-terminal domain. Upon binding to the adaptor proteins, the **initiator caspases dimerize and are thereby activated**, leading to cleavage of a specific site in their protease domains.

Each protease domain is then rearranged into a large and small subunit. In some cases (not shown in fig. 247), the adaptor-binding domain of the initiator caspase is also cleaved (see. fig fig. 247).

**executioner caspases are initially formed as inactive dimers.** Upon **cleavage at a site in the protease domain by an initiator caspase - this is the point of no return -**, the executioner caspase dimer undergoes an activating conformational change. The executioner caspases then cleave a variety of key proteins, leading to the controlled death of the cell.

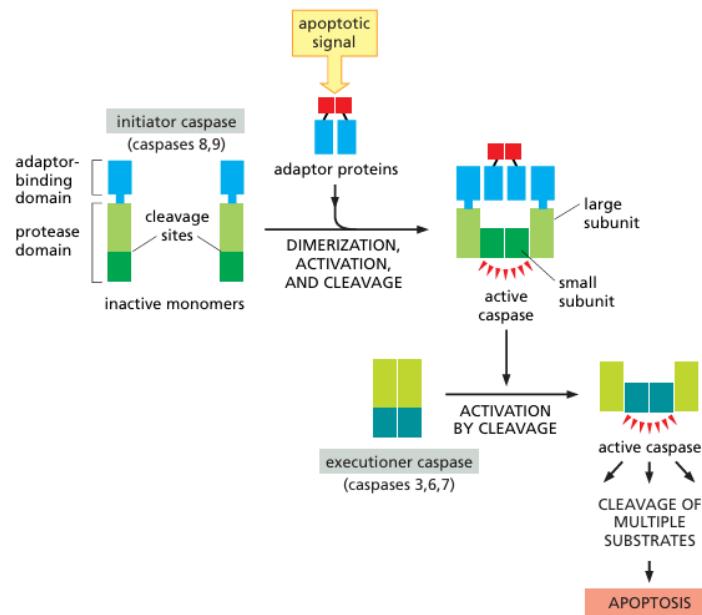


Figure 247: Caspase activation during apoptosis.

### 13.1.0.1 Extrinsic Pathway of Apoptosis

In the extrinsic pathway the cell death is triggered by an other cell. Extracellular signal proteins binding to **cell-surface death receptors** trigger apoptosis. Death receptors are transmembrane proteins containing an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular death domain, which is required for the receptors to activate the apoptotic program. The receptors are homotrimers and belong to the **tumor necrosis factor (TNF) receptor family**, which includes a receptor for TNF itself and the **Fas receptor**.

A well understood example is the **FAS receptor**. Trimeric Fas ligands on the surface of a killer lymphocyte interact with trimeric Fas receptors on the surface of the target cell, leading to clustering of several ligand-bound receptor trimers (only one trimer is shown in fig. 248). **Receptor clustering activates death domains** on the receptor tails, which **interact with similar domains on the adaptor protein FADD** (FADD stands for Fas-associated death domain). Each FADD protein then recruits an initiator caspase (caspase-8) via a death effector domain on both FADD and the caspase, forming a **death-inducing signaling complex (DISC)**.

Within the **DISC**, two adjacent initiator caspases interact and cleave one another to form an activated protease dimer, which then cleaves itself in the region linking the protease to the death effector domain. This **stabilizes and releases the active caspase dimer into the cytosol**, where it activates executioner caspases by cleaving them.

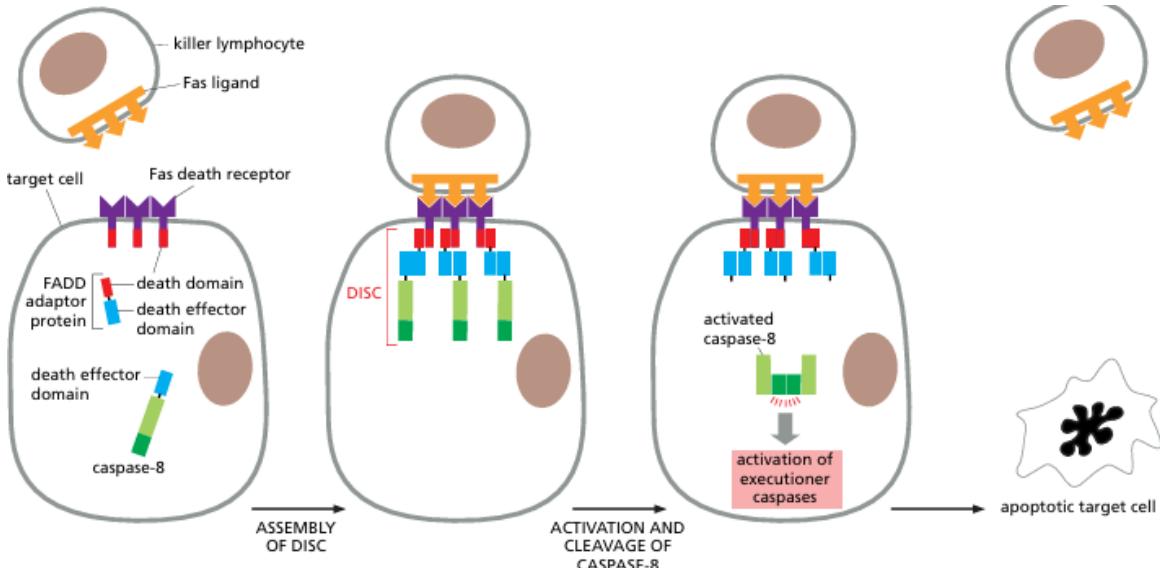


Figure 248: The extrinsic pathway of apoptosis activated through Fas death receptors.

### 13.1.0.2 Intrinsic Pathway of Apoptosis

Cells can also activate their apoptosis program from inside the cell, often in response to stresses, such as DNA damage, or in response to developmental signals (ex. Withdrawal of growth factors). *Some of these signals may technically be on the basis of environmental factors (UV). But the intrinsic property of the cell induces apoptosis and not an other cell.*

The intrinsic pathway is also called the **mitochondrial pathway** because it is triggered by the **release of cytochrome c from the intermembrane space into the cytosol**.

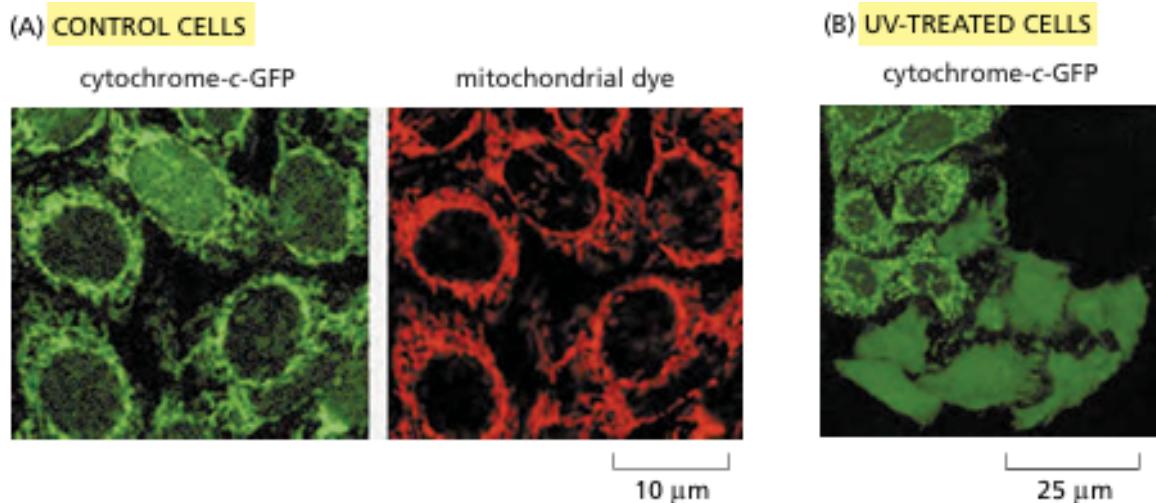


Figure 249: Release of cytochrome c from mitochondria in the intrinsic pathway of apoptosis.

Once in the cytosol, cytochrome c binds to **Apafl** (apoptotic protease activating factor-1), triggering it to assemble into a large, heptameric complex called the **Apoptosome**. This structure recruits and activates caspase-9 via its **CARD domains** (caspase recruitment domains). Activated caspase-9 then initiates a cascade by activating downstream executioner caspases, leading to controlled cell death. See fig. 250

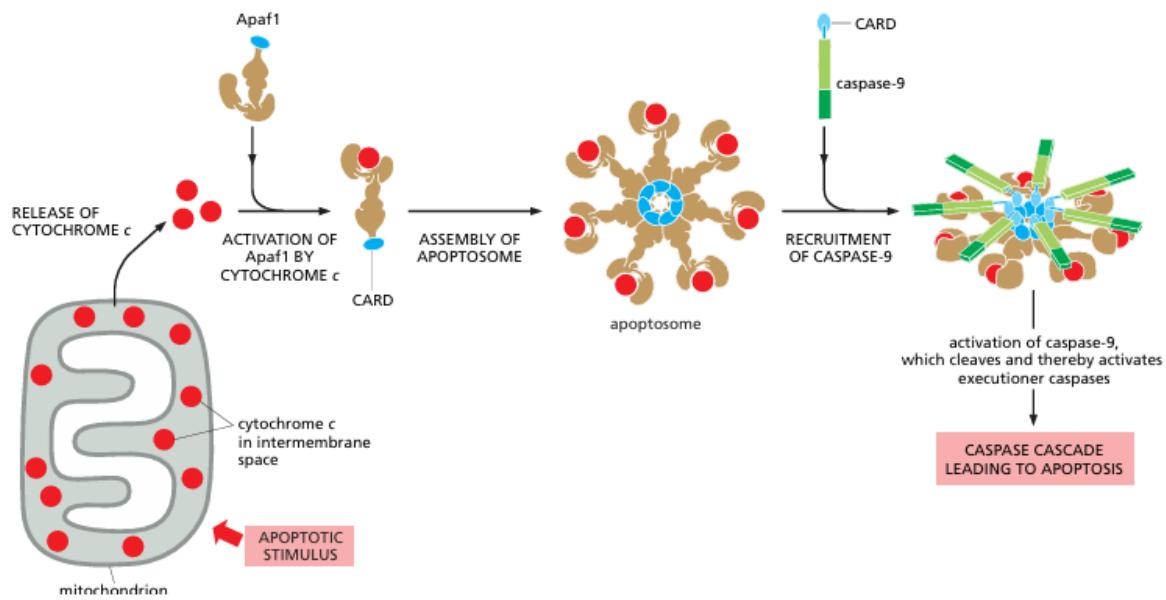


Figure 250: The intrinsic pathway of apoptosis.

*Remark 13.4 (Cytochrome c is important for cell life and also cell death. That fucker!). It is all about its location:*

- For the cell's life: cytochrome c, inside the **mitochondrial interspace**, is an indispensable **electron carrier protein** for the mitochondrial electron-transport chain (transports electrons between III and IV)
- For the cell's death: if **released in the cytosol**, cytochrome c is converted into a pro-apoptotic molecule.

It will bind **Apf-1** to trigger the **activation of procaspase- 9**, altogether forming the “**apoptosome**” complex, which will then **activate caspase-3 and cause apoptosis**.

**The Bcl-2 family** The intrinsic pathway of apoptosis is tightly regulated by the **Bcl-2 family of proteins**, which control the release of **cytochrome c** and other mitochondrial proteins that trigger cell death. These proteins fall into three groups (See fig. 251):

- **Anti-apoptotic proteins** (e.g., Bcl2, BclXL): prevent apoptosis by inhibiting pro-apoptotic proteins.
- **Pro-apoptotic effector proteins** (e.g., **Bax**, **Bak**): promote apoptosis by forming oligomers in the mitochondrial membrane to release cytochrome c.
- **BH3-only proteins** (e.g., Puma, Noxa, Bid): act as sensors of apoptotic stimuli, inhibiting anti-apoptotic proteins and/or activating Bax and Bak.

Note that the **BH3 domain** is the only BH domain shared by all Bcl2 family members. It mediates the direct interactions between pro-apoptotic and anti-apoptotic family members.

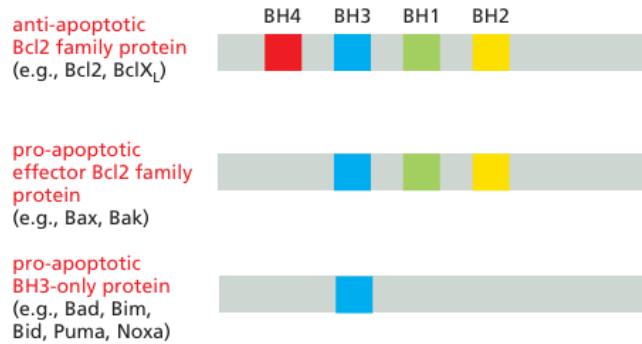


Figure 251: The three classes of Bcl2 family proteins.

The balance between pro- and anti-apoptotic Bcl2 family members determines whether a cell undergoes apoptosis. In response to stress (e.g., DNA damage), BH3-only proteins are activated—often through p53—and **inactivated anti-apoptotic proteins**, enabling **Bax and Bak to permeabilize the mitochondria**.

Furthermore, the extrinsic pathway of apoptosis can activate the intrinsic pathway via the BH3-only protein Bid, which is cleaved by caspase-8, amplifying the apoptotic response.

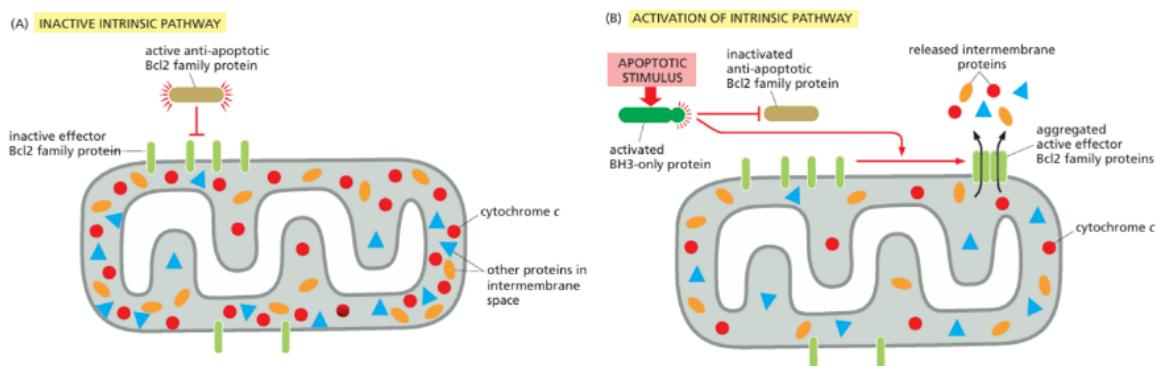


Figure 252: How pro-apoptotic BH3 only and anti-apoptotic Bcl2 family proteins regulate the intrinsic pathway of apoptosis.

### 13.1.0.3 Overview Intrinsic and Extrinsic

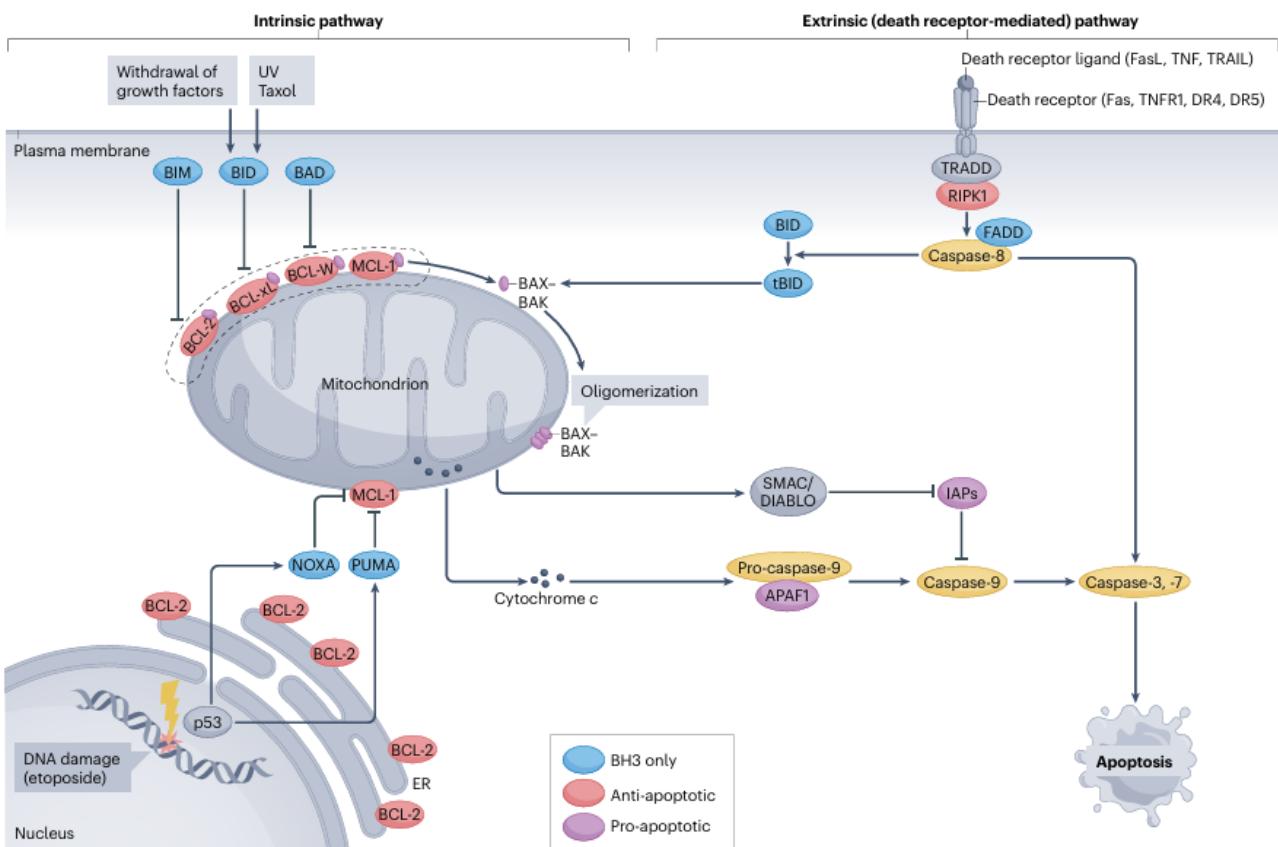


Figure 253: Intrinsic and extrinsic apoptosis.

#### 13.1.1 Inhibiting Apoptosis

Cells strictly regulate apoptosis, especially the caspase cascade, to prevent accidental cell death. One layer of control is provided by **IAPs (Inhibitors of Apoptosis Proteins)**, which inhibit active caspases using their BIR (*baculovirus IAP repeat*) domains.

Some IAPs also tag caspases for destruction via ubiquitylation. In insects like Drosophila, apoptosis is tightly controlled by a **balance between IAPs and anti-IAPs** (e.g. Reaper, Grim, and Hid), which block IAPs and promote cell death. In mammals, mitochondrial release of anti-IAPs (like Smac/Diablo and Omi) helps relieve IAP inhibition during intrinsic apoptosis, but their role appears less essential than in flies.

Additionally, **extracellular survival factor** are crucial in preventing apoptosis. These signals, such as growth factors or neurotrophins, bind to cell-surface receptors and inhibit the apoptotic machinery. They are **3 main ways how they act (see. fig 254):**

- Upregulating anti-apoptotic Bcl2 family proteins (e.g. Bcl2, BclXL),
- Inhibiting pro-apoptotic BH3-only proteins (e.g. Bad),
- Inactivating anti-IAPs through phosphorylation (in Drosophila).

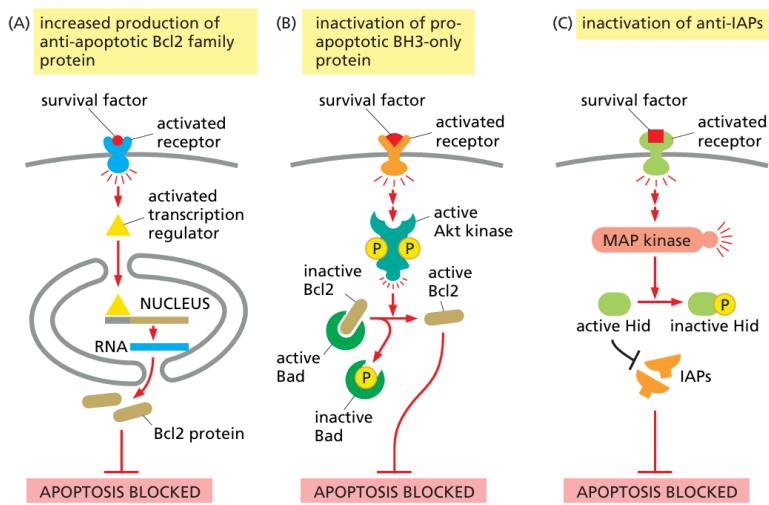


Figure 254: Three ways that extracellular survival factors can inhibit apoptosis.

Cells, especially **during development**, rely on limited survival factors from neighboring cells. For instance, neurons that fail to receive enough survival signals undergo apoptosis, which helps match neuron numbers to their target cells. See fig. 255

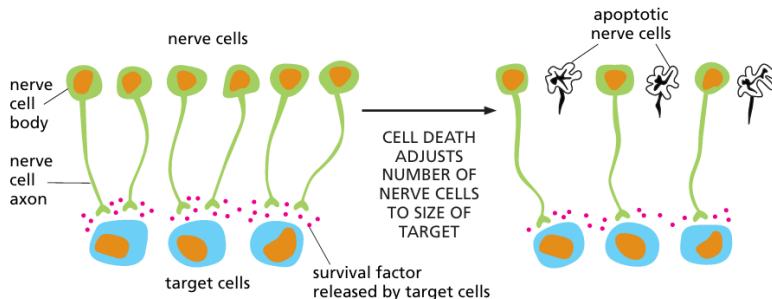


Figure 255: The role of survival factors and cell death in adjusting the number of developing nerve cells to the amount of target tissue.

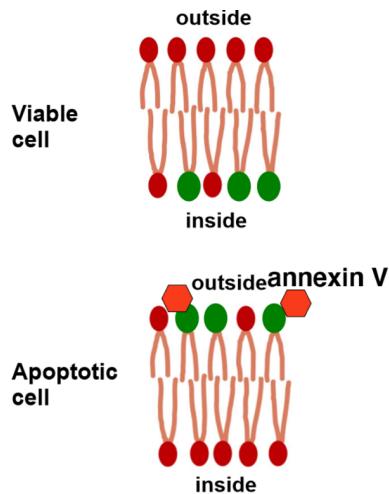
*Remark 13.5 (Control over apoptosis).* Chemical inducers of dimerization (CIDs) such as AP1903 or AP20187 can control apoptosis by activating engineered proteins like inducible caspase-9. When the CID is added, it binds to and dimerizes caspase-9, triggering its activation and initiating the apoptotic cascade. This system allows precise control over cell death in experimental or therapeutic settings.

### 13.1.2 Identifying Apoptotic Cells

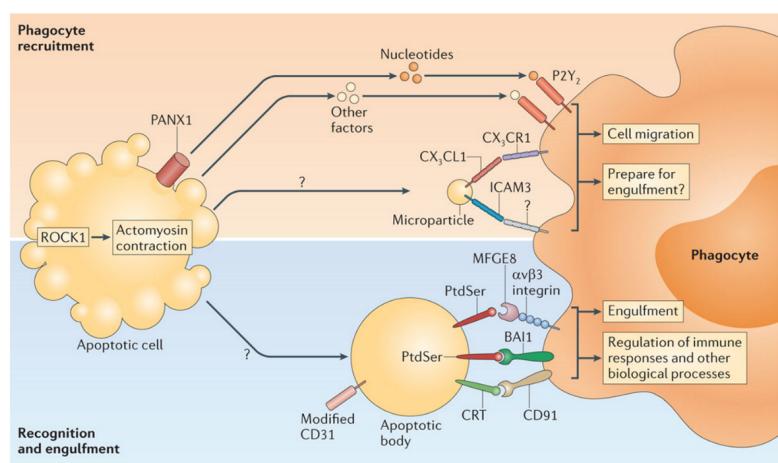
Identifying apoptotic cells involves several complementary methods:

- **Transmission electron microscopy** (TEM) allows visualization of characteristic morphological changes, such as cell shrinkage, chromatin condensation, and membrane blebbing.
- At the molecular level, **DNA fragmentation** into nucleosome-sized fragments produces a distinctive "ladder" pattern detectable by gel electrophoresis.
- Apoptosis is also confirmed by measuring **caspase activity**, often through **colorimetric assays** that detect cleavage of specific caspase substrates, indicating activation of these proteases.

- Additionally, release of **cytochrome c** from mitochondria into the cytosol is a hallmark of the intrinsic apoptotic pathway.
- Finally, apoptotic cells expose **phosphatidylserine (PS)** on their outer membrane leaflet, which acts as an **Eat-me signal** and can be detected using **annexin V staining**, aiding in the recognition and clearance of dying cells. *Recall that PS is normally located on the inner leaflet of the plasma membrane but externalized during apoptosis to promote clearance of dying cells.*



(a) PS translocated to the outer membrane during apoptosis



(b) External PS (PtdSer) "eat-me" signal

Figure 256: Detecting Apoptosis by marking external PS with Annexin V

## 14 Mitochondria

Mitochondria Structural Features

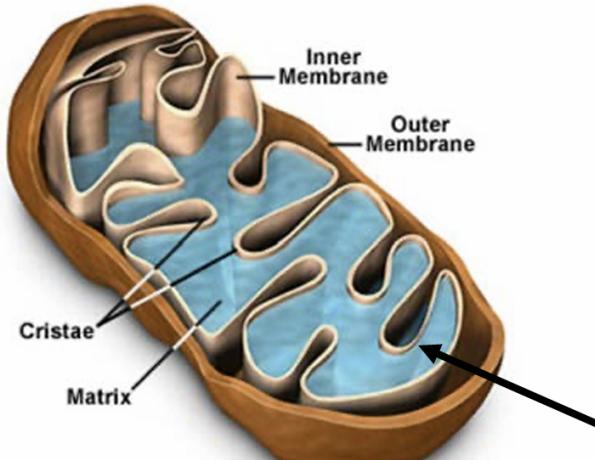


Figure 1

Figure 257: structure of the mitochondria

Mitochondria are one of the only organelles that have a **double membrane**. This gives them two compartments with vastly different chemical properties: **the intermembrane space** including its protrusions called **cristae** and the mitochondrial **matrix**. They originally were self sufficient organisms but now they have evolved to be fully dependent on the host cell, losing all ability to replicate on their own and needing to import a vast majority of proteins from the cytosol.

### 14.1 recap: ATP generation

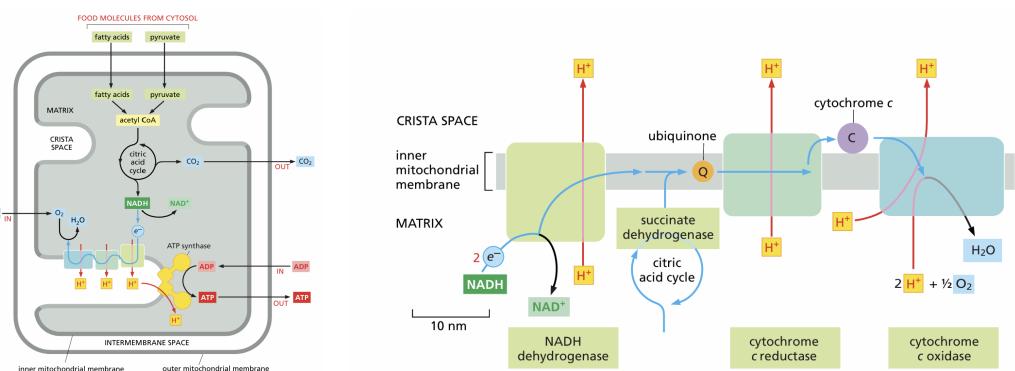


Figure 258: Mitochondria and ATP generation

During the transfer of electrons from NADH to oxygen (blue arrows), **ubiquinone and cytochrome c serve as mobile carriers that ferry electrons from one complex to the next**. During the electron-transfer reactions, protons are pumped across the membrane by each of the respiratory enzyme complexes, as indicated (red arrows).

For historical reasons, the three proton pumps in the respiratory chain are sometimes denoted as Complex I, Complex III, and Complex IV, according to the order in which electrons pass through them from NADH. Electrons from the oxidation of succinate by succinate dehydrogenase (designated as Complex II) are fed into the electron-transport chain in the form of reduced ubiquinone. Although embedded in the crista membrane, **succinate dehydrogenase does not pump protons** and thus does not contribute to the proton-motive force; it is therefore not considered to be an integral part of the respiratory chain.

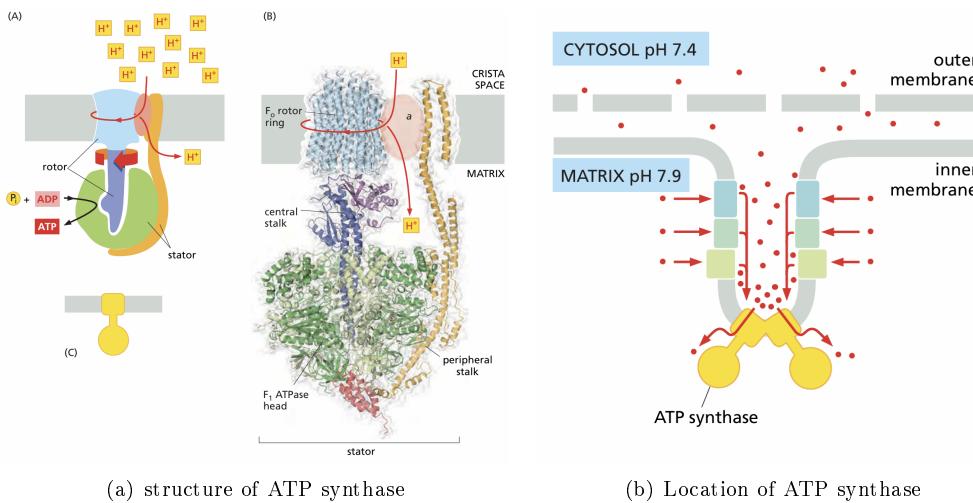


Figure 259: ATP synthase structure and location

**At the crista ridges, the ATP synthases(yellow), which is a F-type pump, form a sink for protons (red).** The proton pumps of the electron-transport chain (green) are located in the membrane regions on either side of the crista. As illustrated, **protons tend to diffuse along the membrane from their source to the proton sink created by the ATP synthase.** This allows efficient ATP production despite the small H<sup>+</sup> gradient between the cytosol and matrix. Red arrows show the direction of the proton flow. **The combined effect of electrochemical gradient and H<sup>+</sup> gradient is called proton motive force**

## 14.2 ATP transport across the double membrane

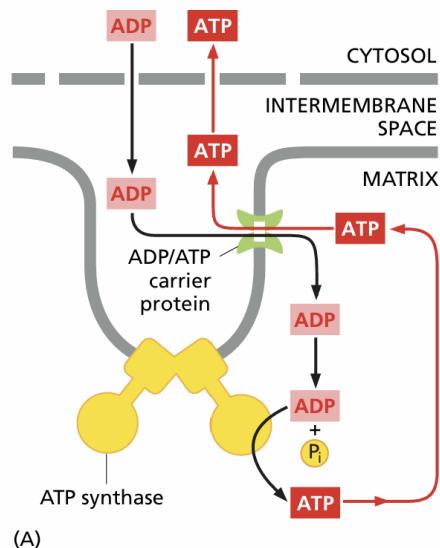


Figure 260: ATP carrier proteins

ATP is produced in the mitochondria but is needed everywhere, this means the cell needs to be able to transport it in and out of the mitochondria. **ATP can diffuse freely across the outer membrane. However it requires special ATP/ADP carrier proteins to pass through the inner membrane into the matrix**

## 14.3 the mitochondrial genome

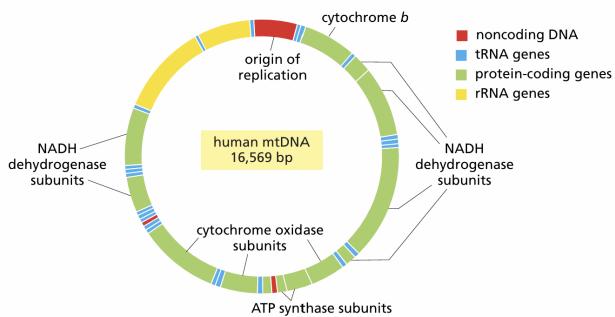


Figure 261: Human mitochondrial genome

Mitochondrial DNA is the **only naturally occurring circular DNA in human cells** and is **inherited maternally** meaning that violations for Mendelian rule are possible and likely to affect organelle genes.

DNA replication occurs **throughout the cell cycle** (not in phase with nuclear DNA replication (S phase)) and that it has its own replication mechanism which is not completely independent as it needs proteins encoded in the nuclear DNA.

The mitochondrial genome is extremely limited and varies greatly from species to species: **Only five genes are invariably found in mitochondrial DNA: ribosomal RNA, cytochrome b (cob), cytochrome oxidase (cox).** The presence of **introns** is also surprising since they originate from bacteria, however this is species dependent. **Human mitochondria don't have introns**

The human mitochondrial genome of 16,600 nucleotide pairs contains 2 rRNA genes, 22 tRNA genes, and 13 protein-coding sequences. There are two transcriptional promoters, one for each strand of the mitochondrial DNA. The protein coding genes include among other things:

- **NADH dehydrogenase:** Complex I
- **Cytochrome b:** Complex III
- **Cytochrome oxidase:** Complex IV
- **ATP synthase**

→ all these genes are related to **oxidative phosphorylation**

#### 14.3.0.1 reading mtDNA

Mitochondrial DNA does not follow the universal code here are a few examples:

**TABLE 14–3 Some Differences Between the “Universal” Code and Mitochondrial Genetic Codes\***

Codon	“Universal” code	Mitochondrial codes			
		Mammals	Invertebrates	Yeast	Plants
UGA	STOP	<i>Trp</i>	<i>Trp</i>	<i>Trp</i>	STOP
AUA	Ile	<i>Met</i>	<i>Met</i>	<i>Met</i>	Ile
CUA	Leu	Leu	Leu	<i>Thr</i>	Leu
AGA AGG	Arg	<i>STOP</i>	<i>Ser</i>	Arg	Arg

\*Red italics indicate that the code differs from the “Universal” code.

Figure 262: reading mtDNA

Since **Mitochondria have their own tRNA and ribosomes** their code can differ from the universal code

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## 14.4 protein localisation to the mitochondria

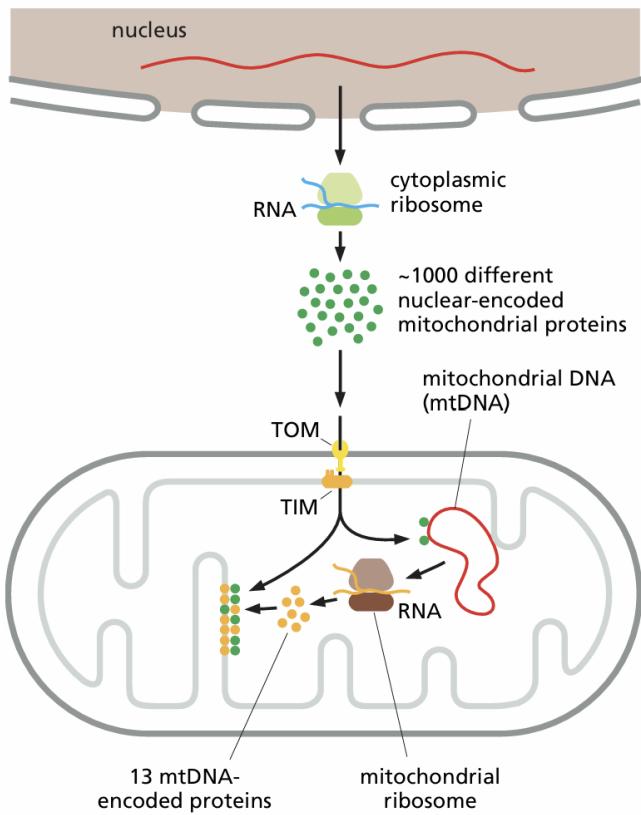


Figure 263: localization to the mitochondria

Protein import into mitochondria is a specialized process due to the presence of a double membrane, which poses a challenge for transporting proteins synthesized in the cytosol. This problem is solved by dedicated translocases: **TOM: Translocase of the Outer Membrane** and **TIM: Translocase of the Inner Membrane**. Since the mitochondria only have 13 proteins that they can encode they need to import most of them from the cytosol (**produced by cytosolic ribosomes**). Proteins destined to the mitochondrial matrix usually have an **N-terminal localization Tag**, that is **removed by a signal peptidase after import**. Proteins that are destined for the intermembrane space or the inner/outer membrane have an **internal signal sequence that is usually not removed**.

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#### 14.4.1 importing into the outer mitochondrial membrane

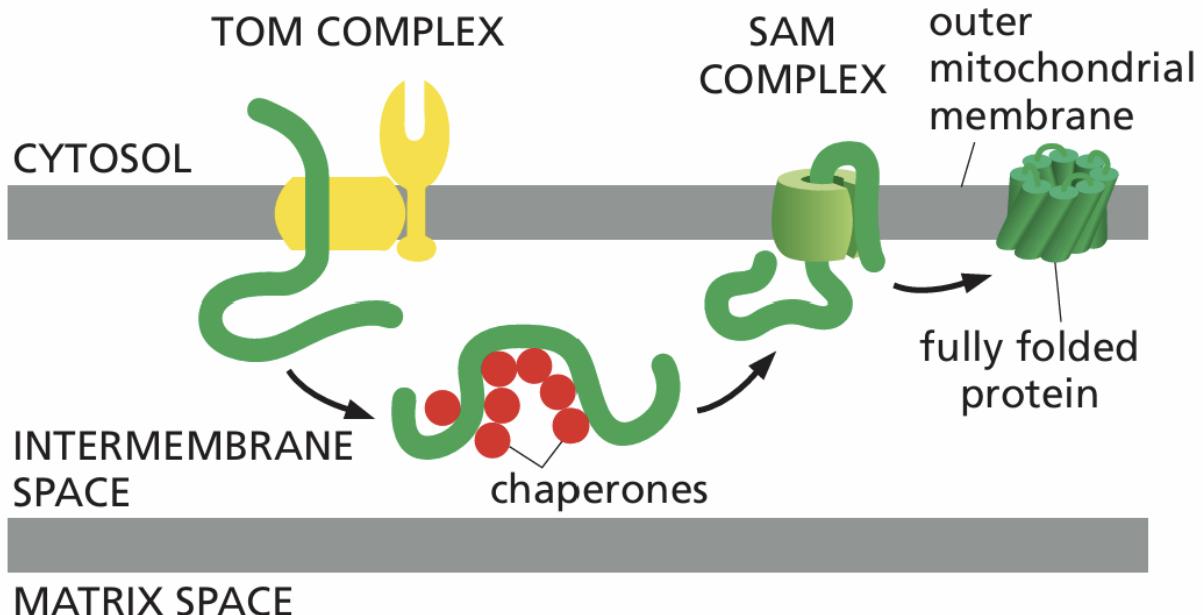


Figure 264: Enter Caption

- proteins enter via the **TOM complex** into the intermembrane space
- they refold with the help of intermembrane chaperones
- the **SAM complex** helps them integrate into the membrane.

#### 14.4.2 importing into the inner membrane (anchored version)

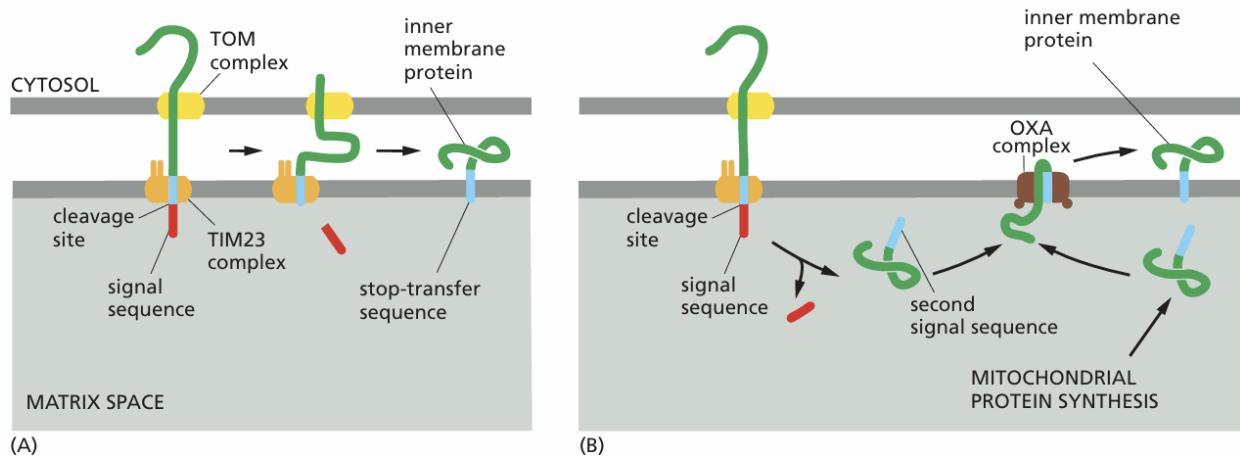


Figure 265: inner membrane localization

There are two ways in which a protein can be localized to the inner membrane (**in all cases the protein needs to unfold to pass through the translocator complexes**):

- passes the outer membrane via the **TOM complex complex**, and then starts to pass through the inner membrane via the **TIM23 complex complex**, until a stop transfer sequence is reached, in which case it leaves the TIM23 complex laterally and becomes embedded into the membrane.
- pass through both TOM and TIM23, upon import the localization signal is cleaved off, revealing a second signal sequence which is used by **OXA complex** to localize it to the inner membrane. A part of the protein itself can be cleaved off, resulting in a intermembrane free floating protein (not memrbane bound)

#### 14.4.3 importing to inner memrbane (transmembrane proteins)

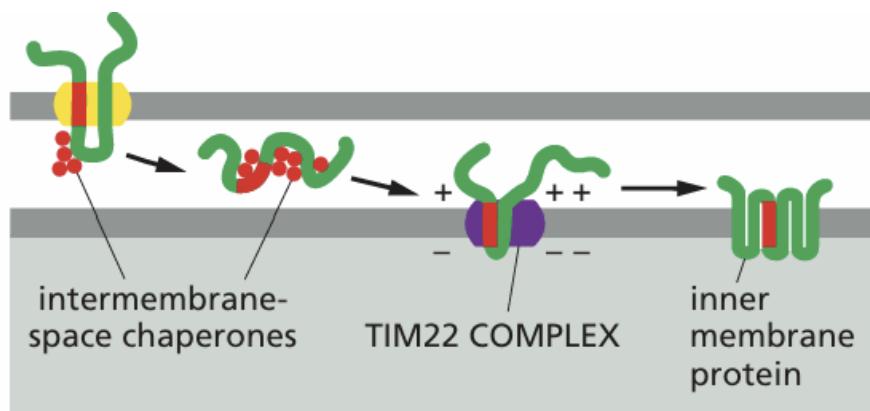


Figure 266: Transmembrane protein localization to inner mebrane

transmembrane proteins are imported via different system than proteins that are just anchored to the membrane. First they pass through the **TOM complex complex**, for which they need to be unfolded, then since they

are hydrophobic they **require intermembrane chaperon proteins** to refold. Then they use the **TIM22 complex** to imbed into the membrane

#### 14.4.4 the role of energy (and chaperone proteins) in localization to the mitochondrial matrix

It is important to note that mitochondrial protein import is a highly complex process, and many import pathways require energy. The following example illustrates one such pathway, highlighting the energy-dependent steps involved in guiding proteins into the mitochondria.

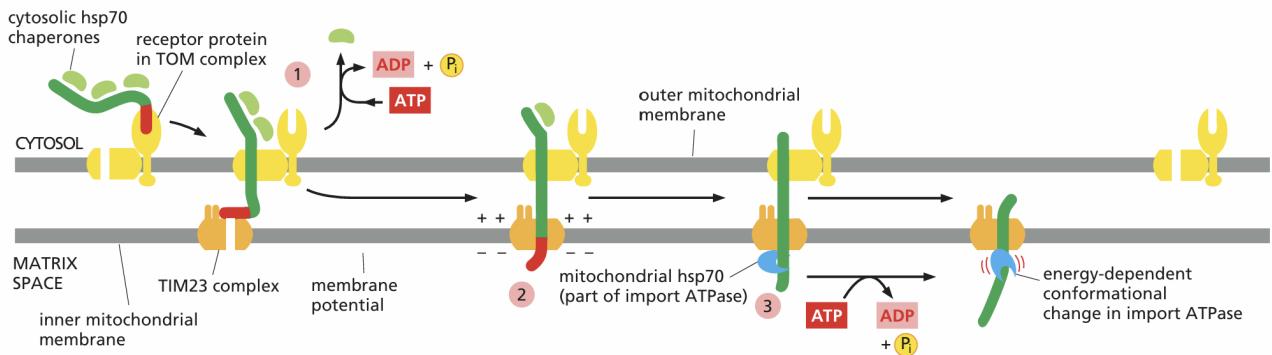


Figure 267: Energy use in mitochondrial localization

- Recognition by TOM complex:** A nuclear-encoded mitochondrial precursor protein in the cytosol is maintained in an unfolded state by cytosolic **hsp70** chaperones. The precursor is recognized by receptor proteins of the TOM (Translocase of the Outer Membrane) complex.
- Translocation through TOM and TIM23:** The protein passes through the TOM complex into the intermembrane space, and then enters the TIM23 complex in the inner mitochondrial membrane. This step is facilitated by the membrane potential ( $\Delta\psi$ ) across the inner membrane.
- Import motor activity:** Inside the matrix, **mitochondrial hsp70** (part of import ATPase) binds to the translocating polypeptide. ATP hydrolysis causes a conformational change in the import ATPase, pulling the protein into the matrix space.

#### 14.5 Fission (splitting) and fusion (joining)

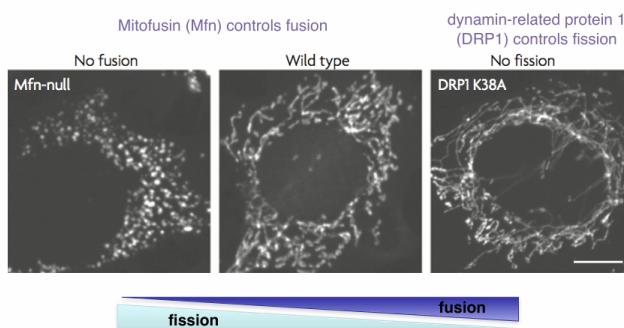


Figure 268: phenotypes resulting from unbalanced fission and fusion

Mitochondria arise from the **division of pre-existing mitochondria**, and their proper distribution within the cell relies on active mitochondrial transport mechanisms. This transport is especially important in cells like neurons, where **mitochondria are recruited to regions with high energy demands**. The morphology of mitochondria—including their length, shape, size, and number—is dynamically regulated by the processes of **mitochondrial fission and fusion**. These processes are inherently complex due to the presence of the double membrane structure and the need to preserve the integrity of distinct mitochondrial compartments during remodeling.

#### 14.5.1 repair and degradation

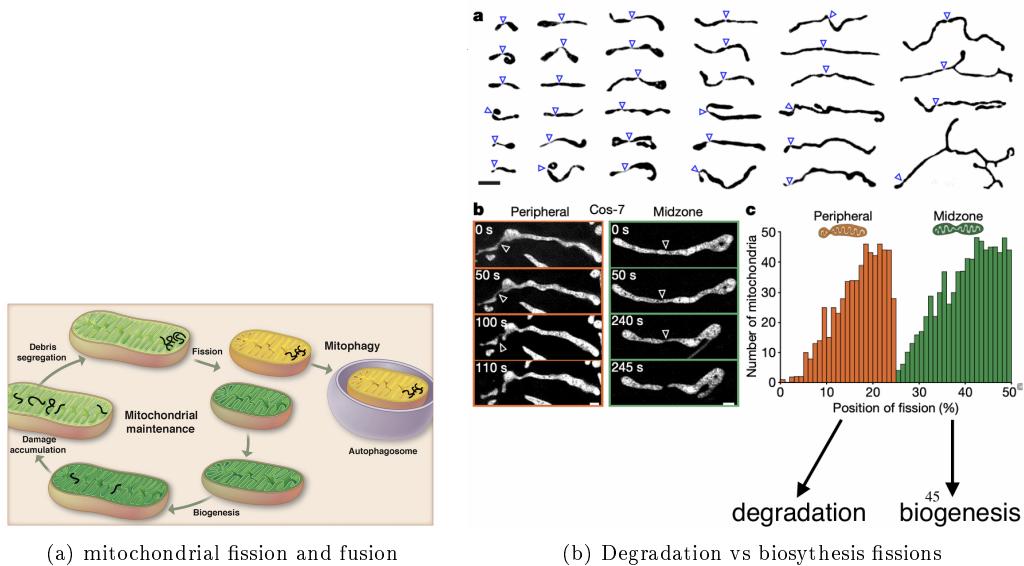


Figure 269: Fission followed by autophagy gets rid of damaged mitochondria

Mitochondrial fission is important not only for its replication but also for damage repair. If a fission is related to replication it will take place towards the middle of the mitochondrion, where as if its purpose is repair it will be towards the extremities of the mitochondrion removing only the damaged part.

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#### 14.5.2 fission

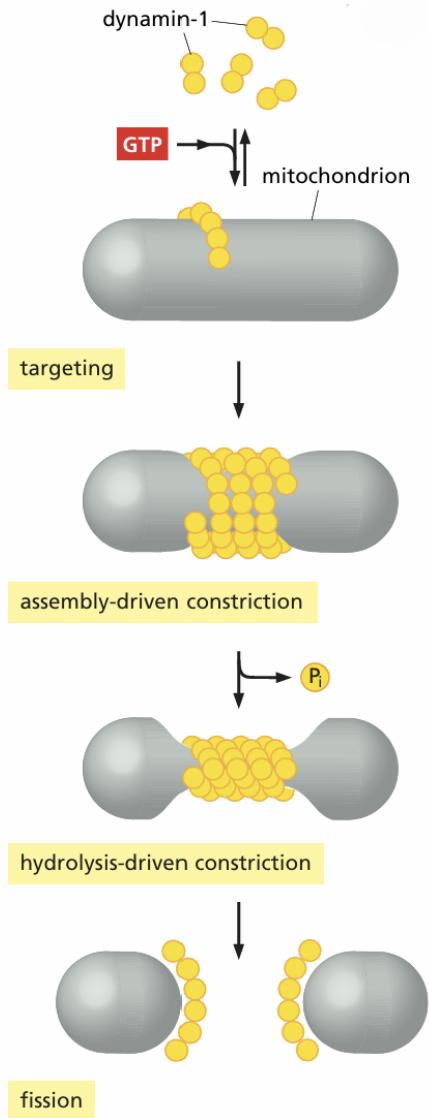
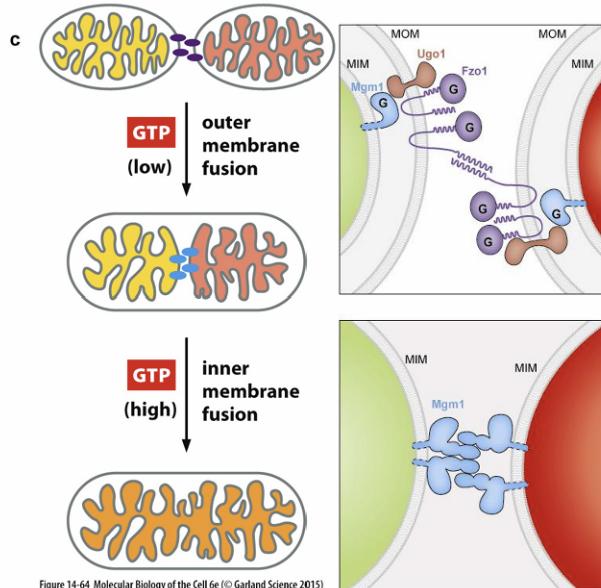


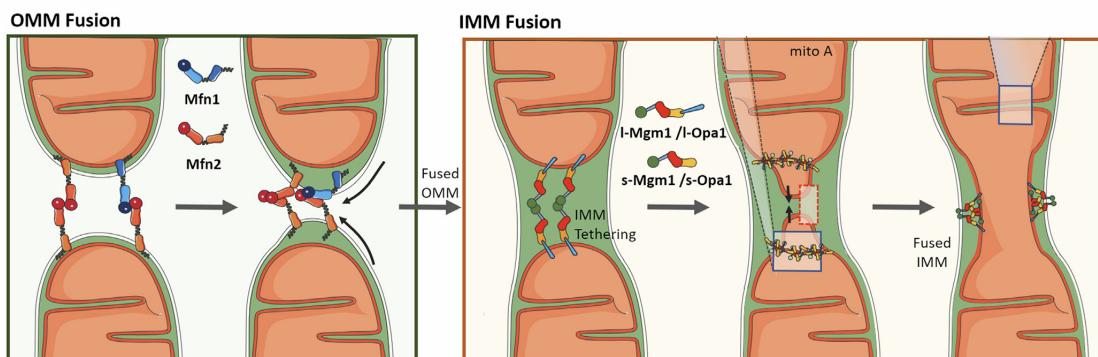
Figure 270: mitochondrial fission

Fission is driven by **Dynamin-1** (more specifically **DRP1** (**Dynamin-related protein 1**)), a GTPase recruited from the cytosol to the outer membrane that will oligomerize into ring- and spiral-like structures, which wrap **around the scission site** – this process is **GTP- hydrolysis dependent**. Once assembled will constrict the mitochondrion until it splits into two. Fission of other organelles like peroxisomes is similar.

### 14.5.3 fusion



(a) fusion is GTP dependent



(b) fusion mechanism

Figure 271: Mitochondrial fusion

Mitochondrial fusion works in two steps: first the outer mitochondrial membrane (OMM) needs to be fused. Then the Inner mitochondrial membrane (IMM) is fused.

- **OMM:** the outer mitochondrial membrane fusion is controlled by mitofusins (**MFN1 (Mitofusin 1), MFN2 (Mitofusin 2)**). These are **GTP dependent**. Thus if they lose GTPase activity they will not work. Apart from **GTP** this process also needs a **H<sup>+</sup> gradient** across the inner membrane. They initiate fusion by oligomerizing.
- **IMM:** Inner mitochondrial membrane fusion is controlled by **OPA1 (Optic Atrophy 1)** and **Mgm1 (Mitochondrial genome maintenance 1)**. These have two isoforms the long form and the short form. (this is why l-opa1, s-opa1 in the image). This process also requires GTP and the electrical potential across the inner membrane.

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## Glossary

- $\beta$ -oxidation** A metabolic process that breaks down fatty acids in the mitochondria (and peroxisomes) to generate acetyl-CoA, NADH, and FADH<sub>2</sub>, which are used in energy production via the citric acid cycle and oxidative phosphorylation.. 85
- ABC transporter** Short for ATP-Binding Cassette transporter, this protein family uses the energy from ATP hydrolysis to transport small molecules across membranes. Structurally distinct from P-type ATPases.. 70
- actin** A cytoskeletal protein that forms filaments involved in maintaining cell shape and enabling junction formation and contractility.. 217
- actin filaments** Thin protein filaments that shape the cell surface, enable cell movement, and drive cell division.. 243
- actin-linked junctions** Also known as actin filament attachment sites, these junctions connect actin filaments to cadherins or integrins, supporting adhesion.. 214
- Adenylate Cyclase** Also called adenylyl cyclase, this membrane-bound enzyme converts ATP to cAMP in response to stimulation by G-proteins. It is a key player in many GPCR-mediated pathways.. 140, 141
- adherens junctions** Junctions that connect actin filaments between cells via cadherins and adaptor proteins like  $\beta$ -catenin and  $\alpha$ -catenin.. 214
- adhesion belt** A continuous band of adherens junctions linked to actin filaments, providing mechanical integrity and shaping epithelial sheets.. 219
- aggrecan** A large proteoglycan with multiple GAG chains that binds hyaluronan to form large ECM aggregates, especially in cartilage.. 225
- AKT** A serine/threonine-specific protein kinase also known as Protein Kinase B, involved in promoting cell survival and growth through downstream effects of PI3K signaling.. 158
- All or nothing signal** A type of cellular response that occurs only after a threshold level of signal is reached, resulting in a binary, digital-like outcome.. 136
- alpha catenin** An adaptor protein connecting cadherin-bound  $\beta$ -catenin to actin filaments, enabling dynamic junction regulation.. 217
- Alpha Helix (AH)** A common structural motif in proteins, including in G-proteins and GPCRs, where it can play a role in conformational change upon activation.. 139
- Alpha Subunit** The component of a heterotrimeric G-protein that binds GDP/GTP and dissociates upon activation to regulate downstream effectors such as adenylate cyclase or phospholipase C.. 138
- Amphiphilic** Refers to a molecule or material that possesses both hydrophilic (water-attracting) and hydrophobic (water-repelling) components, enabling it to interact with both aqueous and oily environments. This property is essential in applications such as emulsions, detergents, and biological membranes. 34
- anchoring junctions** Junctions that attach cells to each other or to the ECM, including adherens junctions, desmosomes, hemidesmosomes, and actin-linked adhesions.. 214
- Androgen** A group of steroid hormones like testosterone that regulate male traits and reproductive activity.. 166

- 
- Androgen receptor (AR)** A type of intracellular receptor that binds androgens, then translocates to the nucleus to regulate target gene transcription.. 166
- Antipporter** A cotransporter that exchanges two (or more) substances across a membrane in opposite directions. Movement of one substance down its electrochemical gradient powers the transport of another substance against its gradient. 69
- AP2** An adaptor protein complex involved in clathrin-mediated endocytosis. AP2 binds to specific phospho-rylated phosphoinositides in the plasma membrane. 169
- Apaf1** Apoptotic protease-activating factor 1; binds cytochrome c and forms the apoptosome to activate caspase-9. 294
- apoptosis** A regulated, energy-dependent form of programmed cell death characterized by cell shrinkage, DNA fragmentation, membrane blebbing, and the absence of inflammation. 290
- Apoptosome** A multiprotein complex formed by Apaf1 and cytochrome c that activates initiator caspase-9. 294
- Arp2/3 complex** A protein complex that includes two actin-related proteins (Arp2 and Arp3). It nucleates new actin filaments by mimicking the plus end of actin, thus it promotes the formation of branched actin networks. 247
- Arrestins** Proteins that bind phosphorylated GPCRs, blocking further G-protein activation and targeting receptors for internalization or alternate signaling.. 140
- Autocrine Signaling** A form of signaling in which a cell secretes signaling molecules that bind to receptors on its own surface, allowing it to regulate itself.. 128
- Autophosphorylation** A process in which a kinase adds a phosphate group to itself, often leading to sustained activation independent of the original signal, as seen in CaM-Kinase II.. 148, 150
- Axin** A scaffold protein that forms part of the destruction complex in Wnt signaling. It promotes degradation of beta-catenin in the absence of Wnt signals.. 162
- axoneme** Inside a cilium and a flagellum is a microtubule-based cytoskeleton called the axoneme. 263
- Bak** A pro-apoptotic Bcl-2 family member that cooperates with Bax to permeabilize the mitochondrial membrane. 295
- BAR domain** A structural domain found in proteins that bind to and stabilize curved membranes. BAR domains can sense or induce membrane curvature and are involved in various trafficking pathways, including clathrin-mediated endocytosis. 169
- basal lamina** A specialized form of extracellular matrix separating epithelial and connective tissue. Composed of laminin, type IV collagen, nidogen, and perlecan.. 213, 235
- Bax** A pro-apoptotic Bcl-2 family protein that promotes cytochrome c release from mitochondria. 295
- Bcl-2 family** A group of proteins that regulate mitochondrial outer membrane permeabilization and apoptosis. 295
- Beta Barrel** A structural motif in proteins consisting of a large beta-sheet that twists and coils to form a closed, cylindrical shape. It is commonly found in porins, lipocalins, and other membrane-spanning or binding proteins. 35

- 
- Beta Catenin** A multifunctional protein involved in the Wnt signaling pathway and in cell adhesion. In Wnt signaling, its stabilization leads to nuclear translocation and activation of Wnt target genes.. 162
- Beta Complex** Part of the G-protein beta-gamma dimer, it remains membrane-associated and contributes to the regulation of ion channels and other signaling proteins.. 138
- beta-catenin** A cytoplasmic adaptor protein linking cadherins to actin and also involved in Wnt signaling when stabilized.. 217
- BH3 domain** A short conserved sequence found in Bcl-2 family proteins. It enables pro-apoptotic proteins to bind to and inhibit anti-apoptotic Bcl-2 proteins, promoting apoptosis. 295
- BP230** A hemidesmosomal protein that connects plectin and keratin filaments to integrins in epithelial cells.. 237
- bullous pemphigoid** An autoimmune skin disease where antibodies target hemidesmosomal proteins, causing blistering.. 237
- Ca<sup>2+</sup>** Calcium ions essential for cadherin-mediated adhesion by stabilizing cadherin domains and preventing molecular flexibility.. 216
- Ca<sup>2+</sup>** A ubiquitous intracellular second messenger that regulates a wide range of cellular processes including muscle contraction, secretion, metabolism, and gene expression. Its release is often triggered by IP<sub>3</sub> in response to upstream signaling events.. 146
- CAD** Caspase-Activated DNase; an endonuclease that, upon release from its inhibitor iCAD by executioner caspases, cleaves chromosomal DNA during apoptosis, producing characteristic nucleosome-sized fragments. 291
- cadherin** A calcium-dependent adhesion molecule mediating homophilic binding between cells. Crucial in adherens junctions and desmosomes.. 215, 222
- cadherin domain** Repeating extracellular domains of cadherins that bind calcium ions and mediate adhesion via N-terminal interactions.. 215
- Calcineurin** A calcium/calmodulin-dependent serine/threonine phosphatase that plays a key role in signal transduction. It dephosphorylates nuclear factor of activated T cells (NFAT), enabling its nuclear translocation and regulating immune responses, as well as other cellular processes such as nuclear transport and synaptic plasticity.. 91
- Calmodulin** A small calcium-binding protein that undergoes conformational change upon Ca<sup>2+</sup> binding, enabling it to activate target enzymes such as CaM-Kinase II.. 147
- Calnexin/Calreticulin cycle** A quality control mechanism in the endoplasmic reticulum (ER) that ensures proper folding of glycoproteins. Calnexin and calreticulin are lectin chaperones that bind to monoglycosylated N-linked oligosaccharides on newly synthesized proteins, facilitating their correct folding. Misfolded proteins are retained in the ER for further processing or degradation. 101
- CaM-Kinase II** Short for calcium/calmodulin-dependent protein kinase II, an important serine/threonine kinase that decodes calcium oscillations via autophosphorylation and regulates memory, gene expression, and metabolism.. 148
- cAMP** Short for cyclic adenosine monophosphate, a second messenger synthesized by adenylate cyclase that activates downstream targets like PKA and regulates cellular responses.. 140, 141
- cAMP Phosphodiesterase** An enzyme that degrades cAMP into AMP, thereby terminating the cAMP signaling pathway. It is a key modulator of signal duration.. 141

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**CapZ** Plus-end capping protein that stabilizes actin filaments by blocking subunit exchange. 248

**CARD** caspase recruitment domain, a protein interaction module that mediates the binding of caspase-9 to the apoptosome. 294

**caspases** Caspases are a family of proteases that use a cysteine residue in their active site and specifically cleave peptide bonds after aspartic acid residues in substrate proteins. 292

**Caveola** Invagination that forms from lipid rafts at the cell surface and buds off internally to form a pinocytic vesicle. 183

**Caveolin** family of structural proteins in caveolae that are unusual because they extend multiple hydrophobic loops into the membrane from the cytosolic side, but do not cross the membrane. 183

**Cdc42** A small GTPase of the Rho family involved in actin cytoskeleton regulation. Its activation promotes the formation of filopodia (thin, finger-like protrusions.). 252

**cell junctions** Structures that link adjacent cells or cells to the ECM. Include anchoring, occluding, channel-forming, and signal-relaying junctions.. 214, IV

**Cell Signaling** A fundamental process by which cells detect, interpret, and respond to external or internal cues through molecular signals. It involves extracellular signaling molecules binding to specific receptors, triggering intracellular signaling cascades that regulate cellular functions such as gene expression, metabolism, division, or apoptosis. Cell signaling enables coordination in multicellular organisms and is essential for development, immune response, and homeostasis.. 126

**cell-surface death receptors** Transmembrane proteins that belong to the TNF (tumor necrosis factor) receptor family and can initiate apoptosis when bound by their ligands. Examples include Fas receptor and TNF receptor. 293

**Cell-Surface Receptor** A transmembrane protein located on the cell membrane that binds extracellular signaling molecules (ligands), such as hormones or neurotransmitters. Upon ligand binding, it initiates an intracellular signaling cascade without the ligand entering the cell. Major classes include ion-channel-coupled receptors, G-protein-coupled receptors, and enzyme-coupled receptors.. 127

**Centriole** A cylindrical cellular structure composed of nine triplet microtubules arranged with ninefold symmetry. They are found in pairs within the centrosome (mother and daughter). 258

**Centrosome** The major microtubule-organizing center (MTOC) in most animal cells. 258

**cGMP** Short for cyclic guanosine monophosphate, a second messenger similar to cAMP that is produced by guanylate cyclase and regulates processes like phototransduction and vasodilation.. 144, 145

**cGMP Phosphodiesterase** An enzyme activated in visual transduction that hydrolyzes cGMP to GMP, leading to the closing of ion channels in photoreceptor cells.. 145

**channel-forming junctions** Junctions that create pores between adjacent cells, allowing the exchange of ions and small molecules. Gap junctions are the primary example in animal cells.. 214

**chemical synapses** Junctions in the nervous system where neurotransmitters are released to relay signals between neurons or to target cells.. 214

**Cholesterol** A lipid molecule essential for membrane structure and function; it also serves as a precursor for steroid hormones and plays a role in modulating signaling pathways such as Hedgehog.. 165

**chondroitin sulfate** A sulfated GAG composed of repeating disaccharides that provides compressive strength to cartilage and connective tissues.. 226

- 
- CK1** Short for Casein Kinase 1, a serine/threonine kinase that phosphorylates signaling components in the Wnt and Hedgehog pathways.. 162
- clathrin** A cytoplasmic coat protein that assembles into a polyhedral lattice on the cytosolic side of membranes, forming clathrin-coated vesicles involved in endocytosis and protein trafficking. 168
- claudin** A four-pass transmembrane protein forming the backbone of tight junction strands and regulating paracellular permeability.. 222
- co-translational translocation** A process in which a nascent protein is simultaneously synthesized and translocated into the endoplasmic reticulum (ER) through the Sec61 translocon complex. This occurs co-translationally, meaning the translation of the protein occurs in parallel with its translocation into the ER.. 94
- Cofilin** A small actin-binding protein that promotes disassembly by twisting ADP-actin filaments, making them more prone to severing. 248
- collagen fibers** Fibrous components of the ECM providing tensile strength. Composed of tightly packed collagen triple helices forming fibrils and bundles.. 213, 229
- collagen I** The most abundant collagen type in the body, forming fibrils and fibers in skin, bone, tendons, and other tissues.. 230
- collagen XVII** A transmembrane collagen found in hemidesmosomes, anchoring epithelial cells to the basal lamina.. 237
- connective tissue** Tissue type that supports and anchors other tissues. It contains extracellular matrix rich in fibers like collagen and is separated from epithelium by the basal lamina.. 213
- connexin** A family of proteins that assemble into connexons, enabling the formation of gap junction channels between cells.. 224
- connexon** A hexameric protein assembly forming half of a gap junction channel, made up of six connexin subunits.. 224
- Constitutive secretory pathway** A continuous secretory route used by all cells, in which vesicles deliver proteins and lipids from the Golgi apparatus to the plasma membrane for immediate secretion or membrane insertion. 186
- Constitutively Active** Describes a receptor or signaling protein that is active without the need for ligand binding, often due to mutations or abnormal expression.. 128
- Contact Dependent Signaling** A signaling mechanism that requires direct membrane-to-membrane contact between cells, typically involving membrane-bound ligands and receptors.. 128
- Costal2** A kinesin-like protein in the Hedgehog pathway that forms a complex with Smoothened and regulates the processing of Cubitus Interruptus.. 163
- CREB** Short for cAMP response element-binding protein, a transcription factor phosphorylated by PKA that regulates genes involved in memory, survival, and metabolism.. 144
- cristae** The folds of the inner mitochondrial membrane that increase surface area for chemical reactions such as ATP synthesis. 322
- Cubitus Interruptus (Ci)** A transcription factor regulated by the Hedgehog pathway in Drosophila; acts as a repressor or activator depending on Hedgehog signal presence.. 163

- 
- Cytochrome c** A mitochondrial protein that, when released into the cytosol, helps activate apoptosis by binding Apaf1.. 294
- cytoskeletal proteins** Proteins like actin and intermediate filaments that provide structural integrity and connect to cell junctions for force transmission.. 213
- DAG** Short for diacylglycerol, a lipid-derived second messenger produced by PLC that activates protein kinase C and regulates membrane-associated signaling.. 146
- decorin** A small proteoglycan with a single GAG chain, known to bind collagen fibrils and regulate ECM assembly.. 225
- Delta** A membrane-bound ligand for the Notch receptor that plays a critical role in lateral inhibition during development.. 160, 161
- desmocollins** Nonclassical cadherins that function similarly to desmogleins in forming stable desmosomal junctions.. 216, 221
- desmogleins** Nonclassical cadherins found in desmosomes that mediate cell-cell adhesion through intermediate filaments.. 216, 221
- desmosomes** Cell-cell anchoring junctions linking intermediate filaments via nonclassical cadherins such as desmogleins and desmocollins.. 214, 221
- Dimerization** The process by which two receptor molecules associate, often as a prerequisite for activation, especially in receptor tyrosine kinases (RTKs).. 150, III
- diochol** A reduced form of dolichol phosphate, specifically dolichol with two hydroxyl groups instead of a phosphate group. Diochol is involved in the endoplasmic reticulum (ER) glycosylation pathway, where dolichol derivatives serve as lipid carriers for sugar residues during the assembly of glycan chains that are transferred to nascent proteins. 100
- DISC** Death-Inducing Signaling Complex; a multiprotein complex formed upon activation of cell-surface death receptors (such as Fas or TNF receptors). It includes adaptor proteins like FADD and initiator caspases (e.g., caspase-8), and initiates the extrinsic apoptosis pathway. 293
- Disheveled** A cytoplasmic protein activated by Frizzled in the Wnt pathway; it inhibits the degradation complex to stabilize  $\beta$ -catenin.. 162
- Downstream Cascade** A sequence of biochemical events triggered by receptor activation, involving multiple intermediates and amplifying the original signal to produce a cellular response.. 139
- DRP1 (Dynamin-related protein 1)** A GTPase enzyme that plays a critical role in mitochondrial fission by assembling on the mitochondrial outer membrane to constrict and sever mitochondria, facilitating mitochondrial division and maintenance of cellular homeostasis. 331
- dynactin** Dynactin links dynein to its cargo and enhances dynein's processivity and binding to microtubules.. 262
- Dynamin** A large GTPase involved in clathrin-mediated endocytosis. Dynamin assembles around the neck of budding vesicles and, through GTP hydrolysis, facilitates membrane scission to release the vesicle into the cytosol. 170
- Dynamin-1** A GTPase enzyme involved in mitochondrial fission, where it assembles at constriction sites on the mitochondrial outer membrane to mediate membrane scission, thereby facilitating mitochondrial division and quality control. 331

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**dynein** A class of motor proteins that move along microtubules towards the minus end.. 261

**dystroglycan** A transmembrane receptor in muscle and epithelial cells that connects the cytoskeleton to the basal lamina via laminin.. 235

**Eat-me signal** A molecular marker exposed on the surface of apoptotic cells that signals phagocytes to recognize and engulf them. A common eat-me signal is phosphatidylserine (PS), a phospholipid normally located on the inner leaflet of the plasma membrane but externalized during apoptosis to promote clearance of dying cells.. 298

**ectoderm** The outermost embryonic germ layer that gives rise to the epidermis, nervous system, and related structures.. 220

**Effector proteins** Proteins that execute the final cellular response to a signal, such as changes in gene expression, metabolism, or cytoskeletal structure.. 126

**EGF** A cell biology concept related to EGF (epidermal growth factor), requiring further specification.. 233

**EGF Kinase** A kinase domain found in the Epidermal Growth Factor Receptor (EGFR), involved in autophosphorylation upon ligand binding and dimerization.. 150, III

**EGF-R** Epidermal Growth Factor Receptor, a receptor tyrosine kinase (RTK) that activates downstream pathways like Ras/MAPK upon EGF binding.. 159

**elastin** A protein that forms elastic fibers in the ECM, allowing tissues like lungs and skin to stretch and recoil.. 232

**EMT** Epithelial-to-mesenchymal transition, a process where epithelial cells lose adhesion and gain migratory, invasive properties.. 220

**Endo H** Short for Endoglycosidase H, an enzyme that cleaves high-mannose and some hybrid N-linked oligosaccharides from glycoproteins. 178

**Endocrine Signaling** A.k.a. hormonal signaling, Long-range signaling in which hormones are secreted into the bloodstream and act on distant target cells.. 128

**endocytosis** A cellular process in which the cell membrane folds inward to form a vesicle that encloses extracellular material for internalization into the cell. Or it can also describe the pathway by which material is transported inwards from the plamsa membrane. 167

**Endoplasmic Reticulum (ER)** A membrane-bound organelle in eukaryotic cells involved in protein and lipid synthesis, calcium storage, and detoxification. It exists in two forms: rough ER (with ribosomes, involved in protein synthesis) and smooth ER (lacking ribosomes, involved in lipid metabolism and detoxification). 80

**Enzyme coupled receptors** Transmembrane receptors that have intrinsic enzymatic activity or are associated with enzymes activated by ligand binding.. 130

**Epithelial cells** Cells that line surfaces and cavities of organs, involved in barrier function, absorption, and signaling processes like lateral inhibition.. 161

**epithelial tissue** A tissue type that forms tightly connected cell layers, covering body surfaces and lining cavities. It plays a role in protection, secretion, and selective permeability.. 213

**Erk** Extracellular signal-regulated kinase, part of the MAPK signaling pathway downstream of Ras, involved in cell proliferation and differentiation.. 157

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**ESCRT complex** A set of cytosolic protein complexes (ESCRT-0, -I, -II, -III) involved in sorting ubiquitylated membrane proteins into intraluminal vesicles of multivesicular bodies. They recognize ubiquitin tags and phosphoinositide signals, enabling membrane invagination and vesicle formation for lysosomal degradation.. 185

**executioner caspases** A class of caspases (e.g., caspase-3 and caspase-7) that carry out apoptosis by cleaving a wide range of cellular substrates, leading to controlled cellular dismantling. 290, 292

**exocytosis** A cellular process in which substances contained in vesicles are released from the cell to the extracellular environment by fusion of the vesicle with the plasma membrane. 167

**Exportin** A transport receptor that binds cargo proteins with nuclear export signals (NES) and facilitates their export from the nucleus to the cytosol in a Ran-GTP-dependent manner.. 91

**extracellular matrix** A complex network of proteins and polysaccharides outside cells. It includes collagen fibers, proteoglycans, and glycoproteins and provides mechanical support.. 213

**Extracellular Signaling Molecule** A molecule, such as a hormone or neurotransmitter, that is released from one cell to bind receptors on another and initiate signaling.. 126

**FADD** Fas-Associated protein with Death Domain; an adaptor protein that binds to death receptors such as Fas. It recruits and activates initiator caspases (e.g., caspase-8), playing a key role in the extrinsic apoptotic pathway. 293

**Fas receptor** A cell-surface death receptor also known as CD95 or APO-1, part of the TNF receptor family. Upon binding its ligand (FasL), it triggers the extrinsic apoptotic signaling pathway. 293

**FGFR** Fibroblast Growth Factor Receptor, a type of RTK that triggers signaling cascades involved in development and cell differentiation.. 153

**fibrillar collagen** Collagen types that assemble into rope-like fibers in connective tissues, including type I, providing tensile strength.. 225

**fibroblasts** Connective tissue cells that synthesize ECM components, especially fibrillar collagens such as collagen I.. 230

**fibronectin** A cell biology concept related to fibronectin, requiring further specification.. 221, 225, 233

**fibrous** Refers to the structural proteins in the ECM, such as collagen, that form fiber-like assemblies to provide tensile strength and mechanical support.. 225

**FLIP** FLICE-like inhibitory protein; a regulator that inhibits caspase-8 activation at the death-inducing signaling complex (DISC), thereby preventing the initiation of extrinsic apoptosis. 290

**FN1** One of the repeating domains in fibronectin responsible for collagen and fibrin binding.. 233

**FN2** A fibronectin domain that contributes to binding interactions within the ECM.. 233

**FN3** A fibronectin domain that contains the RGD motif for integrin binding, crucial for cell adhesion.. 233

**formin** An actin-nucleating protein that promotes the formation of straight, unbranched actin filaments. Formins remain attached to the plus end of the filament during elongation, enabling continuous subunit addition.. 247

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**FRAP** Fluorescence Recovery After Photobleaching. A microscopy technique used to study the dynamics of molecular diffusion and protein mobility within cells. The method involves selectively bleaching a fluorescently labeled region using a high-intensity laser and monitoring fluorescence recovery over time as unbleached molecules move into the area. The recovery rate provides insights into molecular diffusion, binding interactions, and membrane fluidity.. 1

**FRET** Short for Förster Resonance Energy Transfer, a technique used to study molecular interactions based on energy transfer between two fluorescent molecules.. 156

**Frizzled** A family of G-protein-coupled receptors that bind Wnt proteins and initiate the Wnt/ $\beta$ -catenin signaling pathway.. 162

**G-protein-coupled receptors** A large family of membrane receptors (GPCRs) that activate intracellular G-proteins upon ligand binding to transmit signals.. 130

**G-Proteins** Short for guanine nucleotide-binding proteins, these molecular switches relay signals from receptors (like GPCRs) to intracellular effectors by cycling between GDP-bound (inactive) and GTP-bound (active) states.. 137

**Gamma Complex** Forms a functional dimer with the beta subunit in heterotrimeric G-proteins, anchoring the complex to membranes and participating in downstream signaling.. 138

**$\gamma$ -tubulin ring complex** A multi-protein complex that acts as a nucleation template for microtubule polymerization. Located primarily at the centrosome, the  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC) mimics the microtubule minus end, allowing the rapid and spatially controlled nucleation of microtubules. 256

**GAP** GTPase-activating proteins that enhance the intrinsic GTPase activity of G-proteins, leading to signal termination.. 131

**GAP Junctions** Specialized intercellular connections that allow direct chemical communication between adjacent cells via diffusion of small molecules and ions.. 142

**Gap junctions** Specialized intercellular connections composed of connexons that allow direct cytoplasmic exchange of small molecules and ions between neighboring cells.. 214

**GEF** Guanine nucleotide exchange factors, proteins that activate GTP-binding proteins by promoting the exchange of GDP for GTP.. 131

**Gelsolin** A  $\text{Ca}^{2+}$ -activated actin-severing protein that binds the side of filaments and caps the newly formed plus ends after cleavage. 248

**Get3** An ATPase involved in the Guided Entry of Tail-anchored proteins (GET) pathway. Get3 binds tail-anchored proteins in the cytosol using ATP, protects their hydrophobic C-terminal tail, and targets them to the endoplasmic reticulum (ER) membrane. Upon ATP hydrolysis, it releases the protein for membrane insertion and is then recycled. 96

**Glucosyltransferase** An enzyme involved in the Calnexin/Calreticulin cycle that reglucosylates misfolded glycoproteins, allowing them to re-enter the folding cycle. Specifically, it adds a glucose residue to improperly folded glycoproteins, enabling their rebinding to calnexin or calreticulin for further folding attempts. 101

**glycine** The smallest amino acid, appearing every third residue in collagen helices to fit into the tightly packed structure.. 228

**glycoprotein** Proteins with covalently attached carbohydrate chains that play structural and signaling roles in the ECM. Many act as scaffold proteins with multiple interaction domains.. 225

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- glycosaminoglycan (GAGs)** Long unbranched polysaccharides composed of repeating disaccharide units, found in proteoglycans and contributing to ECM viscosity and charge.. 225
- Glycosaminoglycans (GAGs)** Long, unbranched polysaccharides composed of repeating disaccharide units, typically including an amino sugar and a uronic acid. Often sulfated, they are highly negatively charged and play key roles in the extracellular matrix, providing structural support, hydration, and participating in signaling processes. 179
- Golgi apparatus** A cell organelle where glycosylation and processing of ECM proteins like procollagen occur before secretion.. 230
- GPCR** A family of seven-pass transmembrane receptors that activate intracellular G-proteins in response to extracellular ligands. They are among the most abundant and versatile signaling receptors in eukaryotic cells, triggering signaling cascades including those that lead to integrin activation.. 137, 144
- GPCR Kinases (GRKs)** A family of kinases that phosphorylate activated GPCRs, initiating their desensitization by promoting arrestin binding.. 140
- Gq** A subclass of heterotrimeric G-proteins that activates phospholipase C, leading to intracellular calcium release and activation of protein kinase C.. 146
- Grb2** An adaptor protein that links RTKs like EGFR to Ras activation via interaction with SOS, part of the Ras/MAPK pathway.. 156
- Groucho** A transcriptional co-repressor that inhibits Wnt target gene expression by binding LEF1 in the absence of  $\beta$ -catenin.. 162
- GSK3** Short for Glycogen Synthase Kinase 3 (often GSK3), involved in phosphorylating  $\beta$ -catenin to promote its degradation in the Wnt pathway.. 162
- GTP binding** A regulatory mechanism by which proteins, especially G-proteins, toggle between active and inactive states depending on GTP or GDP binding.. 131
- GTPase Rac** A small GTPase that promotes actin polymerization and junction expansion during early cell-cell adhesion.. 218
- GTPase Rho** A GTPase that promotes actomyosin contractility and maturation of adherens junctions into linear actin bundles.. 218
- GTPases** Enzymes that hydrolyze GTP to GDP and phosphate, acting as molecular switches in signaling pathways.. 131, 138
- Guanylate Cyclase** An enzyme that converts GTP to cGMP upon activation by nitric oxide or natriuretic peptides, initiating cGMP-mediated signaling pathways.. 144
- Half-Life** The time required for the concentration of a substance—such as a signaling molecule, mRNA, or protein—to decrease to half of its initial value.. 135
- Hedgehog** A secreted signaling molecule that regulates cell growth and patterning during development. Binding of Hedgehog to the Patched receptor activates Smoothened, initiating downstream signaling through proteins like Cubitus interruptus (Ci).. 163
- hemidesmosomes** Cell-ECM anchoring junctions connecting intermediate filaments to the basal lamina through integrins and collagen XVII.. 214
- heparan sulfate** A variably sulfated GAG attached to proteoglycans, involved in binding growth factors and regulating signaling.. 226

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<b>heparin</b>	A highly sulfated GAG known for its anticoagulant properties, structurally related to heparan sulfate..
	226
<b>heterophilic binding</b>	Binding interaction between different adhesion molecules on adjacent cells, often seen in signal-relaying junctions like Notch-Delta..
	215
<b>Heterotrimeric G Protein</b>	A type of G-protein composed of three distinct subunits—alpha, beta, and gamma—that relay signals from GPCRs to downstream effectors..
	138, II
<b>heterotypic</b>	Refers to interactions or gap junctions involving different types of proteins or connexins on adjacent cells..
	224
<b>homophilic binding</b>	A form of cell adhesion where identical molecules on adjacent cells bind to each other, common in cadherin-mediated adhesion..
	215
<b>homotypic</b>	Describes gap junctions or adhesion interactions where the same type of protein is present on both sides of the junction..
	224
<b>Homotypic membrane fusion</b>	A type of membrane fusion in which two membranes of the same type or origin (e.g., two endosomes) fuse together. This process is important for organelle maturation and requires matching sets of SNARE proteins on both membranes.
	175
<b>hsp70</b>	A family of <b>heat shock proteins</b> (70 kDa) that act as molecular chaperones. In the cytosol, hsp70 binds to newly synthesized or unfolded proteins to prevent aggregation and assist in proper folding.
	22, 55, 117, 203, 280, 312, 329, 346
<b>hyaluronan</b>	A large, non-sulfated GAG that forms a backbone for proteoglycan aggregates, important for tissue hydration and resilience..
	225, 226
<b>Hydrophobicity Score</b>	The measure of how hydrophobic (water-repelling) an amino acid is, based on a specific scale such as the Kyte-Doolittle hydrophobicity scale. It is a moving average of the 19 contiguous ( $\pm 9$ ) residues.
	35
<b>hydroxylation</b>	A post-translational modification essential for stabilizing collagen helices, involving hydroxylation of proline and lysine..
	230
<b>Hydroxylsyl (Hyl)</b>	A hydroxylated derivative of lysine found in collagen. It plays a critical role in forming covalent cross-links between collagen molecules, enhancing tensile strength of the extracellular matrix..
	229
<b>Hydroxyproline</b>	A post-translationally modified derivative of proline, formed by hydroxylation in collagen. It stabilizes the collagen triple helix via hydrogen bonding and requires vitamin C for synthesis..
	228
<b>Hyperbolic signal</b>	A graded signal response that increases steadily with ligand concentration and eventually plateaus, resembling Michaelis-Menten kinetics..
	136
<b>Hypertonic</b>	Describes a solution with higher osmolarity than the cell interior, causing water to leave the cell and leading to cell shrinkage.
	72
<b>Hypotonic</b>	Describes a solution with lower osmolarity than the cell interior, causing water to enter the cell and potentially leading to cell swelling or lysis.
	72
<b>IAP</b>	Short for <i>Inhibitor of Apoptosis Protein</i> . A family of proteins that inhibit caspase activity and thereby block apoptosis. IAPs typically contain BIR (baculovirus IAP repeat) domains and may also promote the degradation of caspases via ubiquitylation.
	296

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- IGFBP** Insulin growth binding factor, a protein domain found in ECM components that binds insulin-like growth factors, modulating their availability and activity.. 233
- iHog** Short for Interference Hedgehog, a co-receptor in the Hedgehog signaling pathway that facilitates ligand reception and signaling.. 163
- IKK alpha** A kinase that is part of the I $\kappa$ B kinase complex; phosphorylates I $\kappa$ B, promoting its degradation and thereby activating NF $\kappa$ B signaling.. 165
- IKK beta** A key catalytic subunit of the IKK complex, required for the canonical NF $\kappa$ B pathway activation through phosphorylation of I $\kappa$ B.. 165
- immunological synapses** Specialized junctions between immune cells that facilitate communication, antigen presentation, and activation. Not covered in this course.. 214
- importin** A transport protein that binds to nuclear localization signals (NLS) on cargo proteins and mediates their transport into the nucleus through the nuclear pore complex (NPC).. 90
- Inhibitory signals** Signals that suppress or diminish cellular responses, often balancing excitatory pathways for proper cell regulation.. 131
- initiator caspases** A class of caspases (e.g., caspase-8 and caspase-9) that are activated early in the apoptotic signaling cascade and initiate apoptosis by activating executioner caspases. 290, 292
- Insulin Receptor Substrate (IRS)** A docking protein phosphorylated by the insulin receptor, serving as a scaffold for downstream signaling molecules.. 134
- Integrin** A family of transmembrane receptors that mediate cell adhesion to the extracellular matrix and other cells. They link the cytoskeleton to the ECM and transmit bidirectional signals to regulate cell shape, motility, and survival.. 234, 235
- integrin beta** The  $\beta$ -subunit of integrins that interacts with Talin and Kindlin to mediate inside-out signaling.. 238
- intermediate filament attachment sites** Sites where intermediate filaments anchor to junctions like desmosomes and hemidesmosomes, stabilizing tissue architecture.. 214
- intermediate filaments** Rope-like fibers that provide mechanical strength and structural support to the cell. 264
- Intracellular Receptor** A receptor located within the cytoplasm or nucleus that binds small, hydrophobic signaling molecules (e.g., steroid hormones) that cross the plasma membrane. Upon activation, many intracellular receptors function as transcription factors that directly modulate gene expression.. 127
- Ion-channel-coupled receptors** Receptors that open or close ion channels in response to ligand binding, converting chemical signals into electrical ones.. 130
- Isotonic** Describes a solution that has the same osmolarity as the inside of a cell, resulting in no net movement of water across the cell membrane. 72
- JAK** Janus Kinase, a family of non-receptor tyrosine kinases associated with cytokine receptors, activating STAT proteins upon phosphorylation.. 151
- junction complex** A cluster of multiple junction types, typically including tight junctions, adherens junctions, and desmosomes, found in epithelial tissues.. 214

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- KDEL sequence** A C-terminal amino acid sequence (Lys-Asp-Glu-Leu) that serves as a retrieval signal for soluble proteins that reside in the endoplasmic reticulum (ER). Proteins with a KDEL sequence are recognized by KDEL receptors in the Golgi and returned to the ER via retrograde transport. 176
- keratan sulfate** A GAG with a different disaccharide composition, found in cartilage and cornea, contributing to ECM structure.. 226
- Keratins** A large and diverse family of intermediate filament proteins found mainly in epithelial cells, hair, and nails. They provide structural support and mechanical strength, especially in skin and other tissues under stress. 265
- kin recognition** A proposed mechanism in which for example ER resident proteins exhibit mutual affinity, helping to retain each other in the ER even without canonical retention signals like KDEL. 176
- Kinase cytokine receptors** Receptors that lack intrinsic kinase activity but associate with tyrosine kinases like JAK to transduce signals from extracellular cytokines.. 151
- Kinase insert region** A flexible loop within the kinase domain of some RTKs involved in substrate specificity or regulation of kinase activity.. 149
- kindlin** A protein that cooperates with Talin to activate integrins by binding to their cytoplasmic tail.. 238
- kinesin** A class of motor proteins that move along microtubules towards the plus end. 260
- kinesin-13** It binds to the ends of microtubules and promotes depolymerization (Catastrophe factor), increasing microtubule catastrophe frequency. 257
- KKXX sequence** A C-terminal amino acid motif (two lysines followed by any two residues) that serves as a retrieval signal for ER membrane proteins via COPI-mediated transport from the Golgi. 176
- Laminin** A cell biology concept related to Laminin, requiring further specification.. 239
- laminin** A cell biology concept related to laminin, requiring further specification.. 225, 235
- Laminin-111** A trimeric laminin isoform found mainly in embryonic tissues, composed of  $\alpha 1$ ,  $\beta 1$ , and  $\gamma 1$  chains, crucial for basement membrane structure.. 239
- Lateral Inhibition** A process during development where a cell inhibits its neighbors from adopting the same fate, often mediated by Notch-Delta signaling.. 161
- Lateral Phase Separation** A phenomenon in biological membranes where different lipid and protein components segregate into distinct coexisting phases within the same membrane plane. This separation can lead to the formation of specialized microdomains, such as lipid rafts, which influence membrane fluidity, signaling, and protein localization. Lateral phase separation is driven by differences in lipid composition, temperature, and molecular interactions.. 8
- Lectin** Proteins that interact with sugar groups . 179
- LEF1** Lymphoid enhancer-binding factor 1, a transcription factor activated by  $\beta$ -catenin in the Wnt signaling pathway.. 162
- Lipid recruitment** The process of signaling molecules being recruited to specific membrane lipids, such as PIP3, for spatial activation.. 132
- liquid–liquid phase separation (LLPS)** A biophysical process in which a homogeneous solution of biomolecules (such as proteins and nucleic acids) spontaneously demixes into two distinct liquid phases: a dense, concentrated phase (often droplet-like) and a dilute phase. Driven by multivalent interactions among the molecules, LLPS results in the formation of dynamic, membrane-less compartments within cells. 79

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- Long feedback delay** A feedback loop that acts over a longer time scale, potentially leading to oscillations or long-term regulation.. 136
- Low-Density Lipoprotein (LDL)** A cholesterol-carrying particle in the bloodstream. LDL delivers cholesterol to cells via receptor-mediated endocytosis and is often referred to as "bad cholesterol" due to its association with atherosclerosis.. 184
- lysyl hydroxylase** An enzyme that hydroxylates lysine residues in collagen, enabling crosslinking and stability of collagen fibrils.. 229
- M6P** Short for mannose-6-phosphate, a carbohydrate modification added to lysosomal enzymes in the Golgi. It serves as a sorting signal, recognized by M6P receptors that direct the enzymes to lysosomes. 182
- Macropinocytosis** A form of endocytosis involving the nonspecific uptake of extracellular fluid and membrane through large vesicles called macropinosomes. It is often triggered by growth factors and involves actin-driven plasma membrane ruffling. 184
- Mannosidase** An enzyme that trims mannose residues from N-linked oligosaccharides on glycoproteins in the endoplasmic reticulum. This trimming serves as a signal for targeting misfolded proteins to ER-associated degradation (ERAD) when they fail to achieve proper folding despite multiple attempts. 101
- MAP** Short for Mitogen-Activated Protein; involved in cascades such as Ras-MAPK that control gene expression, cell division, and survival.. 157, 159
- MAP2** MAP with a long projecting domain, resulting in widely spaced microtubule bundles. 260
- MEK** Mitogen-activated protein kinase kinase (MAPKK), a dual-specificity kinase that phosphorylates and activates ERK in the MAPK signaling cascade. MEK acts downstream of Raf and plays a key role in transmitting growth signals from the cell membrane to the nucleus.. 157
- mesenchymal** Refers to loosely organized, migratory connective tissue cells capable of differentiating into various cell types.. 220
- MET** Mesenchymal-to-epithelial transition, where mesenchymal cells adopt epithelial characteristics and form structured tissues.. 220
- MFN1 (Mitofusin 1)** A GTPase located on the outer mitochondrial membrane that mediates mitochondrial outer membrane fusion by tethering adjacent mitochondria, playing a role in mitochondrial morphology and function. 332
- MFN2 (Mitofusin 2)** A GTPase on the outer mitochondrial membrane that, in addition to promoting mitochondrial outer membrane fusion like MFN1, is also involved in endoplasmic reticulum-mitochondria tethering and cellular metabolism. 332
- Mgm1 (Mitochondrial genome maintenance 1)** ortholog of OPA1, a dynamin-related GTPase that mediates mitochondrial inner membrane fusion and morphology maintenance. Like OPA1, Mgm1 exists in long and short isoforms critical for its function. 332
- microtubule** Hollow tubes that position organelles, guide intracellular transport, and form the mitotic spindle for chromosome segregation. 254
- microvilli** Finger-like cell surface projections supported by actin filaments that increase surface area for absorption. 219, 242
- mitochondrial hsp70** A mitochondrial form of hsp70 located in the matrix, essential for protein import. It binds to precursor proteins as they emerge from the TIM23 complex and uses ATP hydrolysis to pull them into the matrix. 329

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- Modular Interaction Domain** Protein domains that mediate specific interactions with phosphorylated or lipid-modified partners in signaling complexes.. 133
- Molecular switches** Molecules, often proteins, that toggle between 'on' and 'off' states to propagate or terminate signals.. 130
- motor proteins** ATP-powered proteins that move organelles along cytoskeletal filaments or shift the filaments themselves. 242
- mTORC1** Mechanistic target of rapamycin complex 1, regulates protein synthesis, metabolism, and cell growth; activated downstream of AKT.. 159
- mTORC2** A signaling complex that phosphorylates AKT and regulates the cytoskeleton and survival pathways.. 158
- Multidrug resistance (MDR)** A phenomenon where cells become resistant to a wide range of structurally unrelated drugs, often due to the activity of ATP-binding cassette transporters that actively export toxic substances and therapeutic drugs out of the cell, reducing their intracellular concentrations and effectiveness. 74
- Multivesicular bodies (MVBs)** Complex vesicle with invaginating buds and internal vesicles involved in the maturation of early endosomes into late endosomes. 185
- myosin II** A motor protein that generates contractile force at junctions, influencing cadherin tension and cytoskeletal rearrangement.. 217
- necrosis** A form of uncontrolled cell death resulting from acute cellular injury, leading to the rupture of the plasma membrane and inflammation of the surrounding tissue. Cell looks like it exploded. 289
- Negative Feedback** A regulatory mechanism in which a signaling output inhibits an earlier step, stabilizing the pathway.. 135, 140, 164
- NEMO** NF $\kappa$ B essential modulator, a regulatory subunit of the IKK complex that is crucial for NF $\kappa$ B activation.. 165
- Neurotransmitter** A chemical messenger that transmits signals across synapses from one neuron to another.. 128
- NF $\kappa$ B** A transcription factor that regulates immune and inflammatory responses, activated upon degradation of its inhibitor I $\kappa$ B.. 164, 165, III
- nidogen** A glycoprotein in the basal lamina that connects laminin networks to type IV collagen, aiding in the structural integrity of the ECM.. 225, 235
- NLS** Nuclear Localization Signal, a short amino acid sequence that directs the transport of a protein into the nucleus of a cell. 88
- nonclassical cadherins** Variants of cadherins with diverse structures and roles, such as desmogleins and desmocollins, often involved in stronger cell adhesion.. 216, 221, 222
- Notch** A membrane-bound receptor that, upon binding Delta, undergoes proteolytic cleavage releasing the Notch intracellular domain (NICD) to regulate gene transcription.. 160
- Notch tail** Also known as NICD (Notch Intracellular Domain), it translocates to the nucleus and associates with Rbpsuh to influence transcription.. 160

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- NPC** Nuclear Pore Complex, a large protein assembly embedded in the nuclear envelope that regulates the transport of molecules between the nucleus and cytoplasm. 89
- NSF** N-ethylmaleimide-sensitive factor; an ATPase that disassembles SNARE complexes after membrane fusion. NSF uses energy from ATP hydrolysis to recycle SNARE proteins for further rounds of vesicle fusion. 174
- occludin** A transmembrane protein that supports tight junction integrity, working alongside claudins to create a selective barrier.. 222
- occluding junctions** Junctions that seal the space between epithelial cells to regulate permeability. Tight junctions are the main type in vertebrates.. 214
- OPA1 (Optic Atrophy 1)** A dynamin-related GTPase located in the inner mitochondrial membrane, essential for mitochondrial inner membrane fusion. Exists in long and short isoforms that coordinate mitochondrial dynamics. 332
- Oscillation** In cell signaling, a periodic fluctuation in the concentration or activity of signaling molecules (such as  $\text{Ca}^{2+}$ ) that conveys dynamic information to control gene expression or cellular responses.. 147
- Osmolarity** The total concentration of solute particles in a solution, measured in osmoles per liter (Osm/L). It governs water movement across membranes and is regulated in cells in part by ion pumps like the  $\text{Na}^+/\text{K}^+$  pump. 71
- OXA complex Oxidase Assembly (OXA) complex.** A protein translocase located in the inner mitochondrial membrane, responsible for inserting proteins synthesized within the mitochondria, as well as some imported proteins, into the inner membrane from the matrix side. 328
- P-bodies** Processing bodies; cytoplasmic, membrane-less ribonucleoprotein (RNP) granules involved in mRNA degradation, storage, and translational repression. 80
- P-type pump** A class of ATP-powered multipass transmembrane proteins that autophosphorylate during their transport cycle. They are responsible for maintaining ion gradients (e.g.,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{H}^+$ ,  $\text{Ca}^{2+}$ ) across cellular membranes.. 70
- p120-catenin** A component of the adherens junction adaptor complex that stabilizes cadherins and regulates their endocytosis.. 217
- PAPS** Short for 3'-Phosphoadenosine-5'-phosphosulfate, a universal sulfate donor in biological sulfation reactions. It is synthesized in the cytosol and used by sulfotransferases in the Golgi apparatus to add sulfate groups to proteins, lipids, and carbohydrates. 179
- Paracrine Signaling** Short-range signaling where secreted molecules affect nearby target cells without entering the bloodstream.. 128
- patch-clamp** A laboratory technique in electrophysiology used to study ionic currents in individual isolated living cells, tissues, or patches of cell membrane by measuring the current through a small patch of membrane. 77
- Patched** A receptor in the Hedgehog pathway that inhibits Smoothened in the absence of Hedgehog ligand.. 163
- PDK1** Phosphoinositide-dependent kinase-1, activates AKT by phosphorylation following PI3K signaling.. 158
- Pericentriolar Material (PCM)** A dense, protein-rich matrix that surrounds the centrioles in the centrosome. The PCM anchors  $\gamma$ -tubulin ring complexes ( $\gamma$ -TuRCs), which nucleate microtubules. 258

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- perlecan** A large proteoglycan in the basal lamina involved in filtration, ECM organization, and signaling.. 225, 235
- Phosphatidylinositol (PI)** A phospholipid that plays a key role in cell signaling and membrane dynamics. It consists of a glycerol backbone linked to two fatty acid chains and a phosphate group attached to an inositol ring. Phosphatidylinositol and its phosphorylated derivatives (phosphoinositides) are involved in intracellular signaling pathways, membrane trafficking, and cytoskeletal organization.. 4
- Phosphatidylserine** A negatively charged phospholipid found on the inner leaflet of the plasma membrane that helps localize signaling proteins like PKC through electrostatic interactions.. 146
- Phosphoglycerols** A class of phospholipids derived from glycerol-3-phosphate. They form a major component of biological membranes and typically consist of a glycerol backbone, two fatty acid chains, and a phosphate group attached to a polar head. Examples include phosphatidylcholine and phosphatidylserine.. 3
- Phosphoinositide** A lipid component of the membrane that binds Talin and other proteins to regulate integrin activation.. 239
- Phosphorylation** The addition of a phosphate group to a protein or other molecule, often regulating activity or interactions.. 130
- Phosphotyrosine** A phosphorylated tyrosine residue that serves as a binding site for SH2 domain-containing proteins in signaling pathways.. 150
- Phosphotyrosine Binding (PTB)** A domain that binds phosphorylated tyrosines on target proteins, mediating recruitment in signaling pathways.. 133, 156
- PI** Short for phosphatidylinositol, a membrane phospholipid that can be phosphorylated to form various signaling lipids like PI(4,5)P<sub>2</sub>, which are substrates for PLC and involved in many signaling pathways.. 146, 158
- PI3K** Phosphoinositide 3-kinase, an enzyme activated by RTKs that produces PIP3, leading to AKT activation.. 151, 158
- Pinocytosis** A form of endocytosis in which cells nonspecifically engulf extracellular fluid and solutes through small vesicles, often referred to as “cell drinking”. 183
- PKA** Short for protein kinase A, a serine/threonine kinase activated by cAMP that phosphorylates various substrates to regulate metabolism, gene expression, and other cellular processes.. 143
- PKC** Short for protein kinase C, a family of serine/threonine kinases activated by DAG and calcium that phosphorylate a variety of cellular proteins involved in growth, metabolism, and differentiation.. 146
- plakoglobin** Also known as  $\gamma$ -catenin, a component of desmosomes that links cadherins to intermediate filaments.. 221
- plasmodesmata** Plant cell structures analogous to gap junctions, forming channels that traverse cell walls. Not covered in this course.. 214
- platelets** A.k.a. thrombocytes, Small blood cells involved in clotting, where integrin activation plays a role in adhesion and aggregation.. 238
- PLC** Short for phospholipase C, a key enzyme in Gq-mediated signaling that generates second messengers DAG and IP<sub>3</sub> from membrane phospholipids. See also Phospholipase C.. 140, 145, 155, 156, 158
- Pleckstrin Homology (PH)** A protein domain that binds phosphoinositides in membranes, targeting proteins to specific locations.. 133

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- plectin** An adaptor protein linking intermediate filaments to hemidesmosomal components like integrins and BP230.. 237
- Positive Feedback** A mechanism in which a signaling output enhances an earlier step, amplifying the signal.. 135
- post-translational translocation** The process where a protein is synthesized and fully translated in the cytoplasm before being translocated into the endoplasmic reticulum (ER). Unlike co-translocation, post-translocation involves the completion of translation before the protein enters the ER.. 94
- Primary active transport** A form of active transport that directly uses energy, usually from the hydrolysis of ATP, to move molecules or ions against their electrochemical gradient across a membrane. Example: the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump. 69
- Primary cilium** A non-motile, microtubule-based organelle present on most vertebrate cells. It functions as a sensory antenna, detecting environmental signals.. 264
- procollagen** A soluble precursor of collagen with propeptides at both ends that prevent premature fibril formation inside the cell.. 230
- procollagen N-proteinase** An enzyme that cleaves the N-terminal propeptides of procollagen, allowing fibril formation in the extracellular space.. 231
- profilin** An actin monomer-binding protein that promotes filament assembly by facilitating the addition of actin to the plus end and recycling itself afterward. 246
- proline** A cyclic amino acid that contributes to the rigidity of collagen helices. Often hydroxylated in collagen to hydroxyproline.. 228
- prolyl hydroxylase** An enzyme that hydroxylates proline residues, stabilizing the collagen helix through hydrogen bonding.. 229
- propeptides** Terminal extensions on procollagen molecules that prevent premature fibril assembly inside the cell and are cleaved after secretion.. 230
- Protein Recruitment** The assembly of signaling complexes at specific membrane sites or proteins through binding domains.. 132
- Protein Response** The cellular outcome of a signaling event, typically involving activation or repression of specific proteins.. 129
- proteoglycan** A protein core heavily glycosylated with glycosaminoglycan (GAG) chains, contributing to ECM hydration and compression resistance.. 225, 235
- Proteolytic Cleavage** A biochemical process where specific peptide bonds in a protein are broken by proteases, activating or deactivating signaling molecules or receptors, such as in Notch or Hedgehog signaling pathways.. 160
- Proto-oncogene** A normal gene that can become an oncogene through mutation or overexpression, promoting cell proliferation or survival.. 155–157, 159
- PTEN** A phosphatase that antagonizes PI3K signaling by dephosphorylating PIP3 to PIP2, acting as a tumor suppressor.. 158
- Rab protein** A family of small GTPases that regulate vesicle transport by ensuring specificity in vesicle targeting. Rab proteins recruit effector molecules that help guide vesicles to the correct membrane compartment. 172

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- Rac** A small Rho-family GTPase that regulates actin dynamics. Activation of Rac leads to the formation of lamellipodia—broad, sheet-like membrane protrusions.. 252
- Raf** A serine/threonine-specific protein kinase (MAPKKK) that is activated by Ras in the MAPK pathway. Raf phosphorylates and activates MEK, initiating a kinase cascade involved in cell division and differentiation.. 157
- Ran-GAP (GTPase-activating protein)** A cytosolic protein that stimulates the GTP hydrolysis activity of Ran, converting Ran-GTP to Ran-GDP. This process ensures a high concentration of Ran-GDP in the cytosol, maintaining the Ran-GTP gradient necessary for nuclear transport.. 90
- Ran-GDP** The inactive, GDP-bound form of the Ran protein, primarily found in the cytosol, which results from the hydrolysis of Ran-GTP and is necessary for recycling import/export receptors.. 91
- Ran-GEF (Guanine nucleotide exchange factor)** A nuclear protein that facilitates the exchange of GDP for GTP on Ran, maintaining the high concentration of Ran-GTP in the nucleus.. 90
- Ran-GTP** The active, GTP-bound form of the Ran protein, primarily found in the nucleus, which facilitates nuclear export by binding to export receptors and releasing import receptors.. 91
- Rap1** A small GTPase involved in inside-out signaling to activate integrins during processes like platelet adhesion.. 238
- Ras** A small GTPase that transmits signals from RTKs to MAPK cascades, promoting proliferation and differentiation.. 156, 157
- Ras Domain** A conserved GTP-binding domain found in small GTPases like Ras, involved in signal transduction and downstream activation of pathways such as MAPK.. 139
- Ras MPK** Refers to the Ras-MAPK pathway, a cascade where Ras activates RAF, MEK, and ERK, leading to gene regulation and cell proliferation.. 151
- Rbpsuh** Recombination signal-binding protein for immunoglobulin kappa J region, a transcription factor that partners with NICD in Notch signaling.. 160
- Receptor** A protein, usually on the cell surface or in the cytoplasm, that binds a specific signaling molecule and initiates a response.. 126
- Receptor-mediated endocytosis** Process by which macromolecules bind to complementary transmembrane receptor proteins, accumulate in coated pits, and then enter the cells as receptor-macromolecule complexes in clathrin-coated vesicles. 183
- Regulated secretory pathway** A secretion route in specialized cells where proteins are stored in secretory vesicles and released by exocytosis only in response to specific signals, such as hormones or neurotransmitters. 186
- Rho** A Rho-family GTPase that promotes the formation of stress fibers and focal adhesions. Activated Rho stimulates the formation of thick stress fibers (contractile actin bundles). 252
- Rhodopsin** A light-sensitive GPCR found in photoreceptor cells of the retina that activates the visual transduction pathway via transducin and cGMP breakdown.. 145
- RIAM** An adaptor protein that helps recruit Talin to the plasma membrane during integrin activation.. 238
- RNP** Ribonucleoprotein complex; a molecular complex composed of RNA and proteins that plays key roles in various biological processes such as RNA processing, transport, stability, and translation regulation. 80

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- rod domain** A segment of Talin's structure that blocks its binding sites in the inactive state and unfolds under tension.. 239
- RTK** Short for Receptor Tyrosine Kinase, a class of cell-surface receptors that activate intracellular signaling via tyrosine phosphorylation.. 149
- SAM complex Sorting and Assembly Machinery complex**; a protein complex in the mitochondrial outer membrane that facilitates the insertion and assembly of  $\beta$ -barrel proteins into the membrane. It includes key subunits such as Sam50, Sam35, and Sam37. 327
- Sar1** A small GTPase that initiates COPII coat assembly at the ER membrane, playing a key role in vesicle formation for ER-to-Golgi transport. 170
- sarcoplasmic reticulum (SR)** The SR is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytoplasm, and it serves as an intracellular store of  $\text{Ca}^{2+}$ .. 72
- SAS-6** Forms coiled-coil dimers that self-assemble into a ninefold symmetrical ring, providing the scaffold for centriole cartwheel formation. 258
- scaffold proteins** Large, multidomain glycoproteins that organize other ECM components and cell-surface receptors into structured assemblies.. 232
- Scaffolding protein** A protein that binds multiple signaling components, organizing them into functional complexes to enhance efficiency and specificity.. 132
- SDS (Sodium Dodecyl Sulfate)** An anionic detergent widely used in protein denaturation and electrophoresis, known for its ability to disrupt non-covalent bonds in proteins. 39
- Sec23** A core component of the COPII coat complex that mediates vesicle budding from the endoplasmic reticulum (ER). Sec23 forms a heterodimer with Sec24 and acts as a GTPase-activating protein (GAP) for Sar1, helping regulate coat assembly and cargo selection. 171
- Sec24** An adaptor protein that partners with Sec23 in the COPII coat complex. Sec24 is responsible for recognizing and binding ER export signals on cargo proteins or cargo receptors, thereby facilitating their packaging into COPII vesicles for transport to the Golgi. 171
- Secondary active transport** A type of active transport that does not use ATP directly. Instead, it relies on the electrochemical gradient established by primary active transport to drive the movement of other substances against their gradients. Can be in the form of symport or antiport. 69
- septate junctions** Occluding junctions found in invertebrates, functionally similar to tight junctions. Not covered in this course.. 214
- SERCA pump** Short for Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase, a membrane-bound P-type ATPase that transports  $\text{Ca}^{2+}$  from the cytosol into the sarcoplasmic or endoplasmic reticulum using energy from ATP hydrolysis. It plays a critical role in muscle relaxation, calcium signaling, and intracellular calcium homeostasis. 80
- SGLT family** Sodium-Glucose Linked Transporters are secondary active transporters that couple the uptake of glucose with the inward movement of  $\text{Na}^+$  ions, enabling glucose absorption against its concentration gradient. Prominent in intestinal and renal epithelial cells. 69
- Short feedback delay** A feedback loop that acts rapidly after signal initiation, often stabilizing or fine-tuning the signal.. 136

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- Sigmoidal Signal** A type of cellular response curve characterized by a slow initiation, followed by a steep increase, and then saturation—forming an “S” shape. It often reflects cooperative binding or multi-step signaling cascades, allowing cells to respond sensitively to threshold changes in stimulus concentration.. 136
- Signal Integration** The cellular process of combining inputs from multiple signaling pathways to generate a unified response.. 134
- signal-relaying junctions** Cell junctions specialized for transmitting signals, such as neural synapses and receptor-ligand interactions like Notch-Delta.. 214
- Signaling cascade** A series of biochemical events, often involving sequential activation of enzymes, leading to a cellular response.. 126
- Slug** Slug is a transcription factor that regulates EMT by repressing epithelial genes and promoting mesenchymal traits.. 221
- Smad** Intracellular proteins that transmit signals from TGF-beta receptors to the nucleus to regulate transcription.. 154
- Smoothened** A transmembrane protein activated in the Hedgehog pathway upon relief of Patched inhibition, triggering downstream signaling.. 163
- Snail** Snail is a transcription factor that regulates EMT by repressing epithelial genes and promoting mesenchymal traits.. 221
- SNARE protein** A group of membrane-associated proteins that mediate the fusion of vesicle and target membranes. SNAREs on the vesicle (v-SNAREs) and on the target membrane (t-SNAREs) form complexes that bring membranes close enough to fuse. 173
- Soft Solubilization** A gentle method of solubilizing membrane proteins or other biomolecules using mild detergents to preserve their native structure and function. 39
- Spectrin** A cytoskeletal protein that forms a lattice structure beneath the plasma membrane of cells, providing mechanical support and maintaining cell shape, especially in erythrocytes. 41
- Sphingolipids** A class of lipids that contain a sphingosine backbone instead of glycerol. They play crucial roles in cell membrane structure and signaling. Key sphingolipids include ceramides, sphingomyelins, and glycosphingolipids, which are involved in cellular communication and recognition processes.. 3
- Src Homology (SH)** A family of protein domains (e.g., SH2, SH3) involved in recognizing phosphorylated tyrosines or proline-rich motifs.. 133, 155, 156
- STAT** Signal Transducer and Activator of Transcription; phosphorylated by JAKs and then dimerizes to regulate gene expression.. 151
- stathmin** A microtubule-destabilizing protein that binds to tubulin dimers and prevents their polymerization into microtubules. Stathmin sequesters free  $\alpha/\beta$ -tubulin heterodimers, reducing the available pool for microtubule assembly. It plays a crucial role in regulating microtubule dynamics, particularly during cell division and migration. Its activity is controlled by phosphorylation.. 258
- Stress Granules** Cytoplasmic, membrane-less ribonucleoprotein (RNP) granules that form in response to cellular stress; they store untranslated mRNAs and associated proteins, helping regulate translation and protect RNA during stress conditions. 80
- survival factor** An extracellular signaling molecule that promotes cell survival by inhibiting apoptosis. Survival factors typically act by activating cell-surface receptors, which trigger intracellular pathways that suppress pro-apoptotic proteins. 296

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- Symporter** A cotransporter that moves two (or more) different substances across a membrane in the same direction. One substance typically moves down its electrochemical gradient, providing the energy to transport the other substance against its gradient.. 69
- Synaptic Signaling** A specialized form of signaling in neurons where neurotransmitters are released at synapses to stimulate adjacent cells.. 128
- Talin** A cytoskeletal adaptor protein that connects integrins to actin and unfolds under tension to recruit vinculin.. 237
- Tau** MAP with a short projecting domain that cross-links microtubules closely together.. 260
- tenacin** An ECM glycoprotein involved in modulating cell adhesion and migration during development and repair.. 233
- TGF beta** Transforming Growth Factor beta, a family of cytokines involved in cell proliferation, differentiation, and immune regulation via Smad signaling.. 154
- Thrombin** A serine protease involved in blood coagulation. It converts fibrinogen to fibrin, leading to clot formation, and also activates platelets and integrin signaling via protease-activated receptors.. 238
- thrombospondin** A multifunctional ECM protein involved in cell-to-matrix communication, wound healing, and angiogenesis.. 233
- thymosin** A small, abundant actin monomer-binding protein that sequesters actin subunits in an inactive form, preventing filament assembly. 246
- tight junctions** Junctions that form impermeable seals using claudins and occludins, helping compartmentalize tissues and maintain cell polarity.. 214, 222
- TIM22 complex** A subcomplex of the system located in the inner mitochondrial membrane, specialized in importing and inserting multi-pass transmembrane proteins—particularly carrier proteins—into the inner membrane. 329
- TIM23 complex** A subcomplex of the TIM system in the inner mitochondrial membrane, responsible for translocating presequence-containing proteins from the intermembrane space into the mitochondrial matrix or integrating them into the inner membrane. 328
- TNF** Tumor Necrosis Factor, a cytokine involved in systemic inflammation, apoptosis, and immune system signaling through receptors like TNFR.. 165
- TNF** Tumor Necrosis Factor; a cytokine involved in systemic inflammation. It can bind to TNF receptors, including death receptors, and promote cell death or survival depending on cellular context. 293
- TOM complex Translocase of the Outer Membrane.** A protein complex located in the outer mitochondrial membrane responsible for recognizing and importing nuclear-encoded precursor proteins into the mitochondrion. 328
- Transcriptional response** Changes in gene expression triggered by signaling pathways reaching the nucleus.. 129
- transcytosis** A process where molecules are transported across a cell by endocytosis on one side and exocytosis on the other. Common in epithelial cells, e.g., transport of antibodies in newborns. 185
- Transducin** A heterotrimeric G-protein specifically involved in visual signaling, activated by rhodopsin to stimulate cGMP phosphodiesterase.. 145

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**translocator (Sec61 complex)** A protein translocator complex embedded in the membrane of the endoplasmic reticulum (ER). It forms a channel through which nascent polypeptides are co-translationally or post-translationally translocated into the ER lumen or integrated into the membrane. The complex consists of three core subunits: Sec61 $\alpha$ , Sec61 $\beta$ , and Sec61 $\gamma$ . 97

**translocators** Proteins or protein complexes that transport molecules across biological membranes, such as the cell membrane or organelle membranes. They play a critical role in processes like nutrient uptake, waste export, and protein trafficking. 87

**transmembrane ligand-receptor cell-cell signaling contacts** Junctions where ligand-bound receptors such as Notch and Delta span adjacent membranes to transmit signals directly. 214

**Transphosphorylation** A process in which one kinase phosphorylates another kinase, often occurring during receptor activation, such as with receptor tyrosine kinases (RTKs) where two adjacent receptors phosphorylate each other upon dimerization. 150

**Triton X-100** A non-ionic surfactant commonly used in laboratories for solubilizing membrane proteins and disrupting lipid bilayers. 39

**Tropomodulin** Minus-end capping protein that regulates actin filament length and stability. 248

**Tropomyosin** Protein that binds along actin filaments, stabilizing and blocking interactions with other proteins. 248

**tubulin** A globular protein that polymerizes to form microtubules. 254

**Tumor Suppressor** A gene or protein that prevents uncontrolled cell growth by regulating the cell cycle, promoting apoptosis, or repairing DNA. Loss-of-function mutations in tumor suppressors can contribute to cancer. 157

**Twist** Twist is a transcription factor that regulates EMT by repressing epithelial genes and promoting mesenchymal traits. 221

**Type I receptor** A component of the TGF-beta receptor complex that is phosphorylated by type II receptors to propagate Smad signaling. 154

**Type II receptor** A receptor that binds TGF-beta ligand and phosphorylates the associated type I receptor to initiate downstream signaling. 154

**type IV collagen** A network-forming collagen found primarily in the basal lamina, forming flexible, sheet-like structures. 225, 235

**Uniporter** Transporter that facilitates the movement of a single type of molecule or ion across the membrane down its concentration gradient (passive transport), without coupling to the movement of any other substance. 69

**V-type pump** A multisubunit, turbine-like protein complex that uses ATP hydrolysis to pump protons ( $H^+$ ) into intracellular organelles, contributing to their acidification. Commonly found in vacuoles, endosomes, and lysosomes. 70

**Vasopressin** Also known as antidiuretic hormone (ADH); a peptide hormone released by the posterior pituitary in response to dehydration or increased plasma osmolarity. It promotes water reabsorption in the kidneys by stimulating the insertion of aquaporin-2 channels in the collecting ducts, thereby reducing urine output and conserving body water. 75

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- Vesicular tubular clusters** Homotypic membrane fusion of ER-derived vesicles. They function as intermediate sorting stations that mediate cargo transport from the endoplasmic reticulum to the Golgi apparatus. 175
- vimentin** An intermediate filament protein typically expressed in mesenchymal cells and upregulated during EMT.. 221
- vinculin** A cytoskeletal protein that binds  $\alpha$ -catenin under tension and promotes actin recruitment to strengthen adherens junctions.. 217
- Vitamin-C** A cofactor required for the hydroxylation of proline and lysine during collagen synthesis. Its deficiency impairs collagen stability.. 229
- Wnt** A family of secreted glycoproteins that activate Frizzled receptors and regulate  $\beta$ -catenin stabilization, crucial for development and cell fate.. 162
- XIAP** X-linked inhibitor of apoptosis protein; a member of the IAP family that binds to and inhibits caspases, particularly caspase-3, -7, and -9, thereby blocking apoptosis. 290
- XMAP215** A MAP (Xenopus Microtubule-Associated Protein 215) that functions as a microtubule polymerase. It binds to tubulin dimers and delivers them to the growing plus end of microtubules, thereby promoting microtubule growth. 257
- Zeb** Zeb is a transcription factor that regulates EMT by repressing epithelial genes and promoting mesenchymal traits.. 221