

cell bio II

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spring 2025

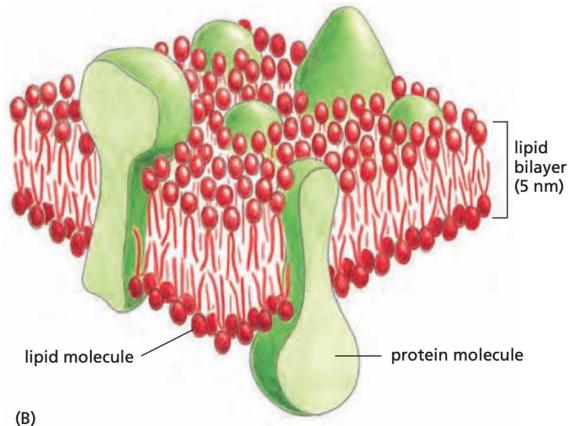
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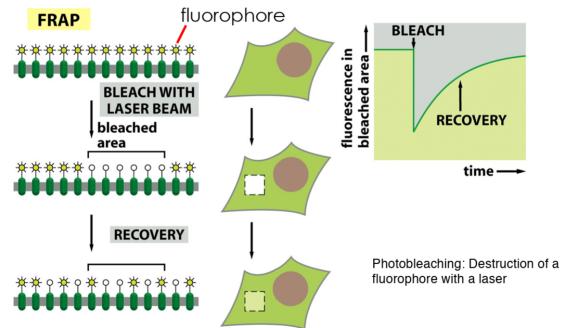
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1 Membrane Structure

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

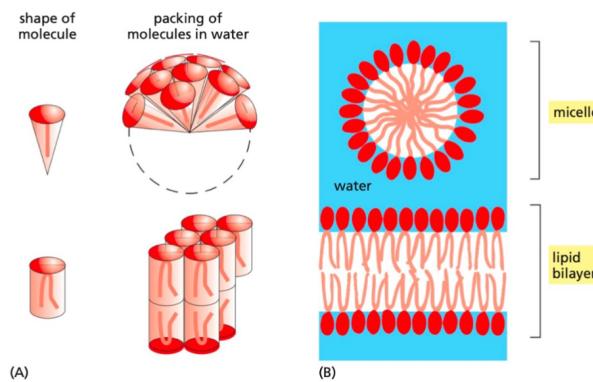


Figure 2: spontaneous formation of lipid bi layer

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.

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.

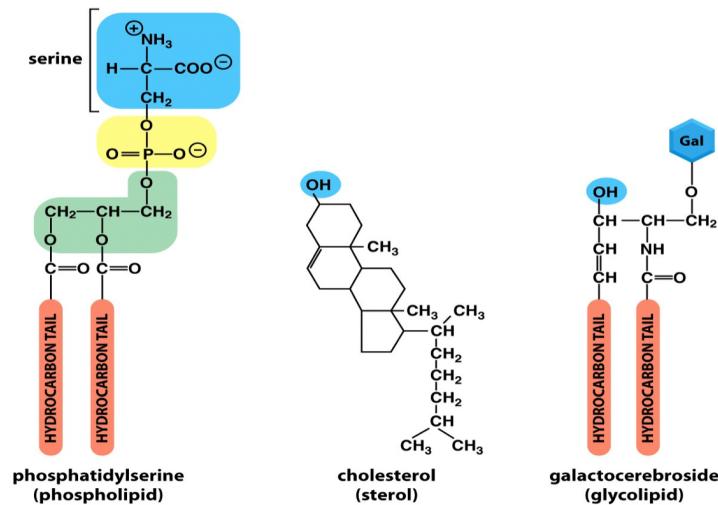
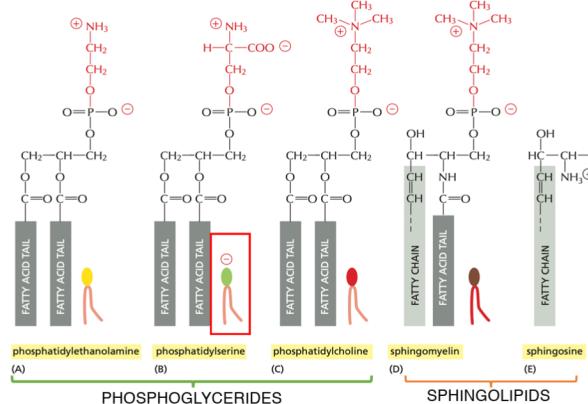
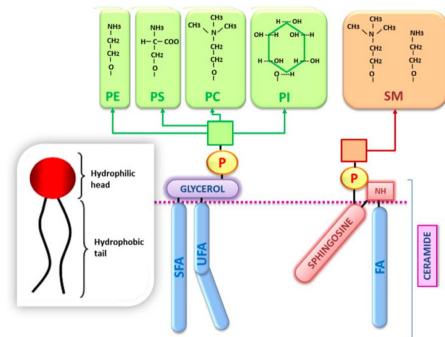


Figure 3: 3 main classes of lipids

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**.

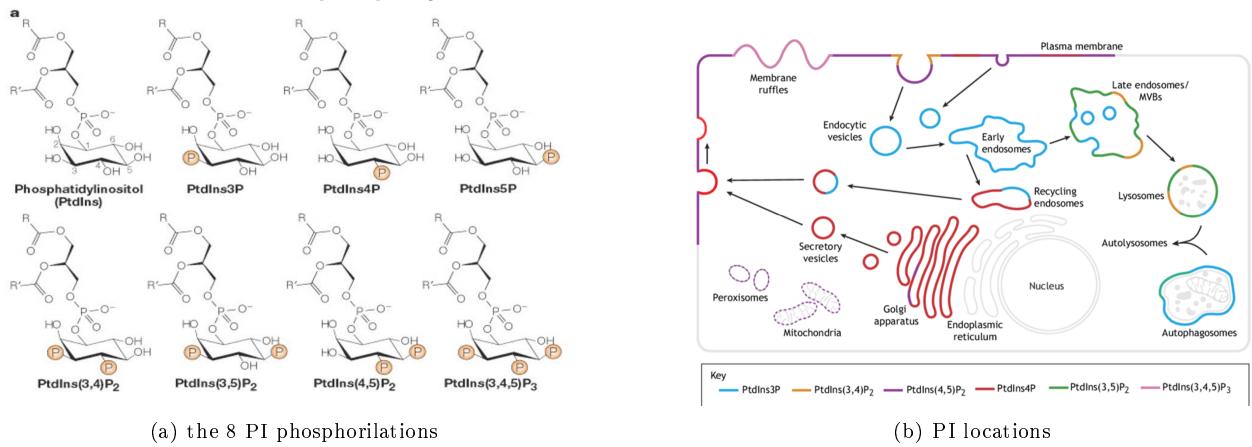


Figure 5: PI can be phosphorylated based on location

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. **They are located inside the cell and can be phosphorylated.** This can then be used for localization of other proteins. (coming soon I guess...)

1.2.1.2 glycolipids

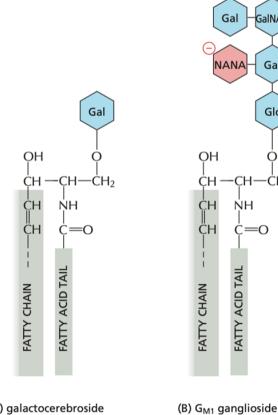


Figure 6: glycolipid structure

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**

1.2.1.3 sterols

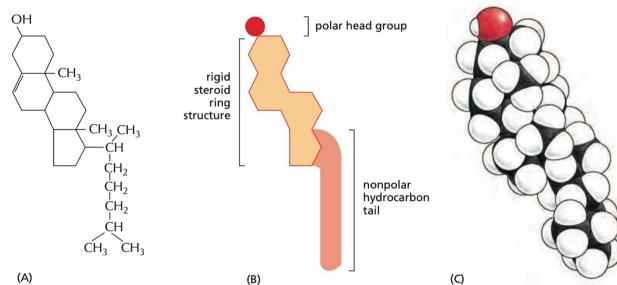


Figure 7: sterols structure

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

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 8: table of lipid composition

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.

1.3.2 Temperatures effect on the membrane

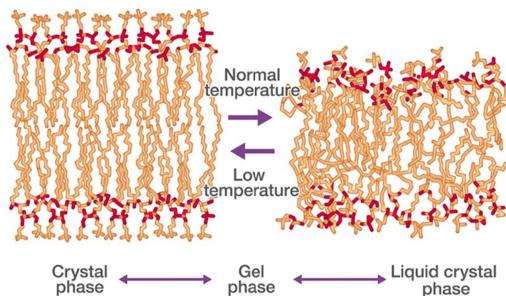


Figure 9: temperature on the membrane rigidity

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

1.4 Movement of Lipids in Cell Membranes

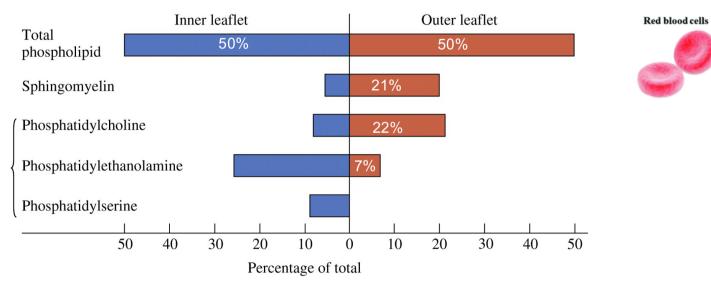
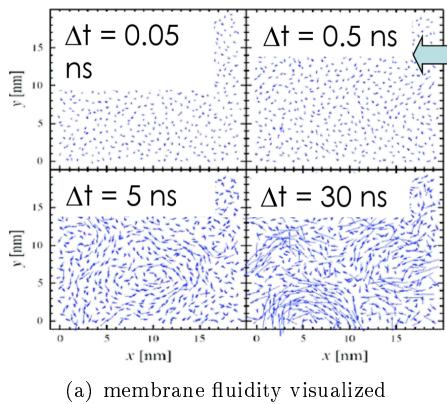


Figure 10: membrane fluidity and asymmetry

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. 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**

1.5 Lipid Rafts

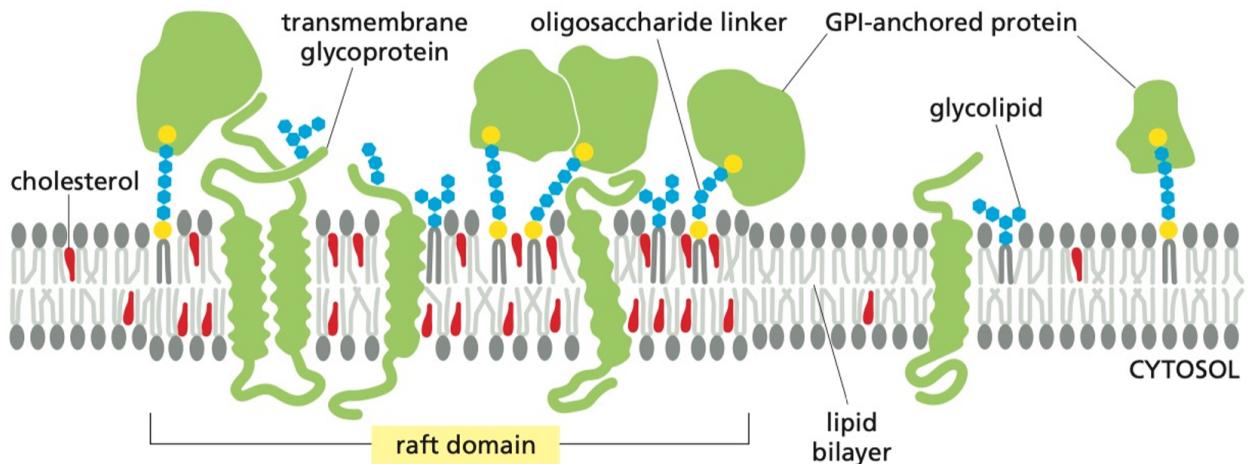


Figure 11: raft domains

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.

Glossary

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

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.. 7

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

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

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

2 membrane proteins

2.1 protein basic overview

2.1.1 amino acid structures

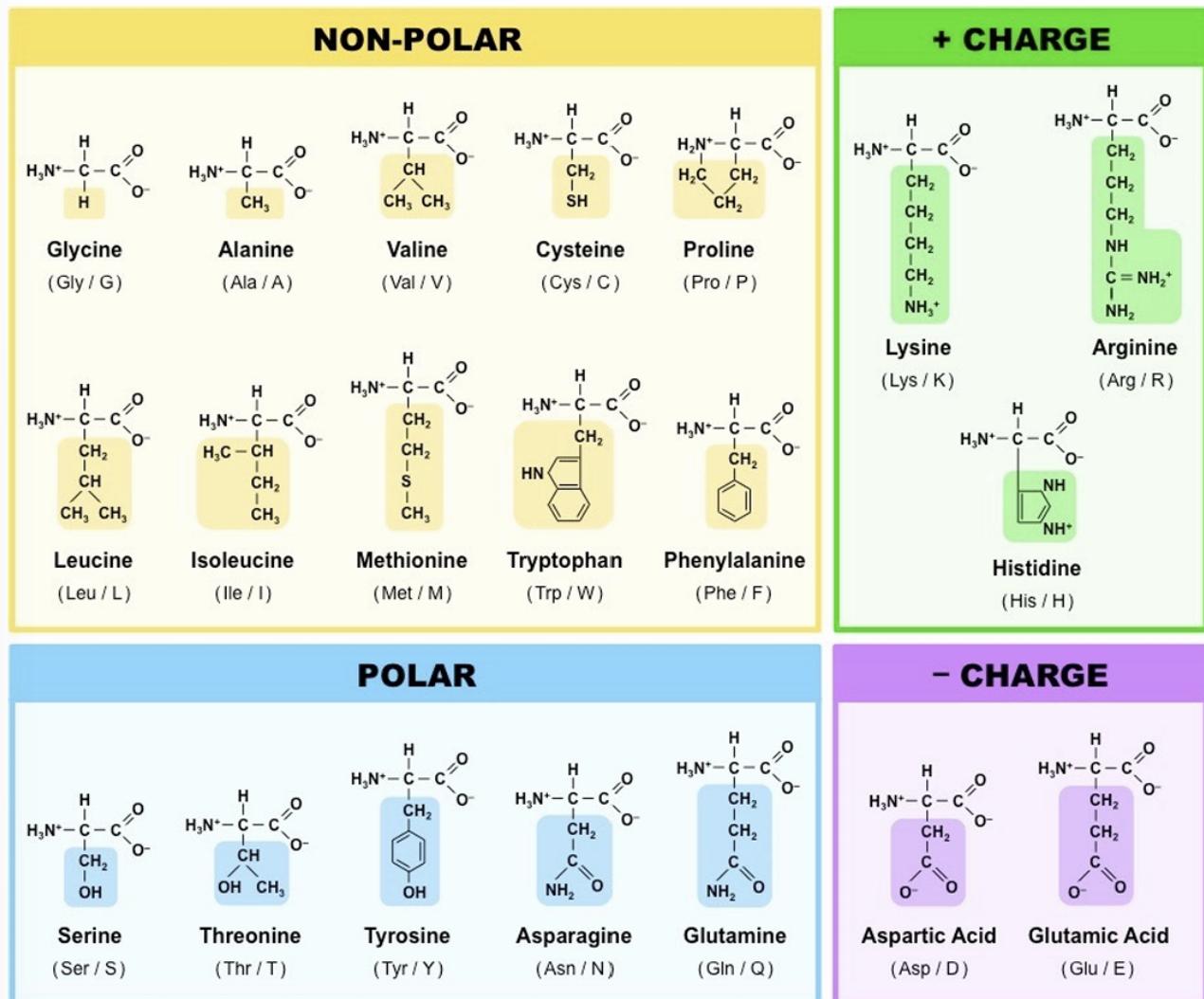


Figure 12: Amino acid structure

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

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

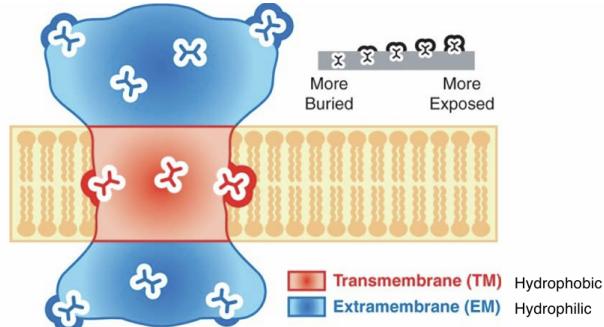


Figure 13: general structural requirements of a membrane protein

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.

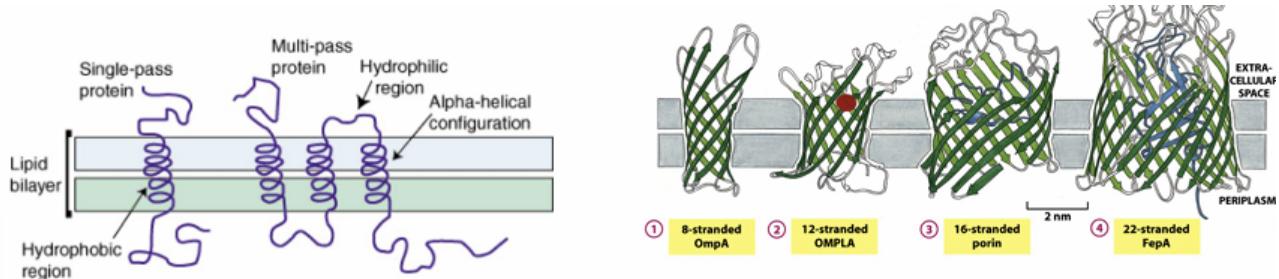


Figure 14: beta barrels vs transmembrane helix

2.2.1.1 transmembrane helix

Transmembrane helices consist of alpha-helices that have hydrophobic residues allowing them to pass through the membrane. An alpha helix has **3.6AA per turn** and each **turn is 5.4A 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.

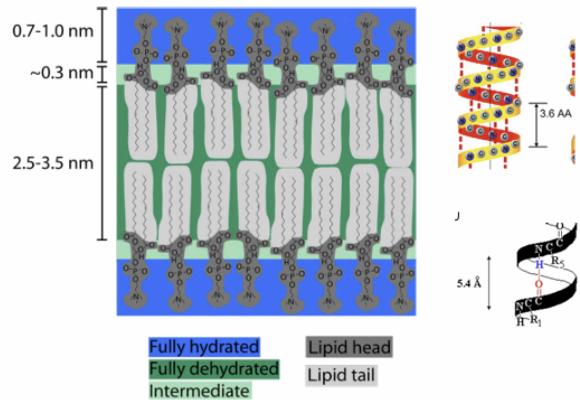


Figure 15: helix stats

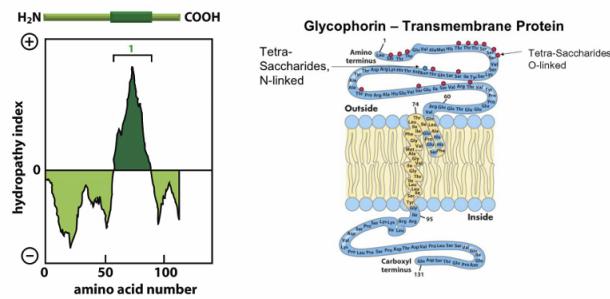


Figure 16: Predicting Transmembrane helices based on hydrophobicity score

predicting transmembrane helices It is possible to predict transmembrane helices off of the **Hydrophobicity Score** which is an average of the ± 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

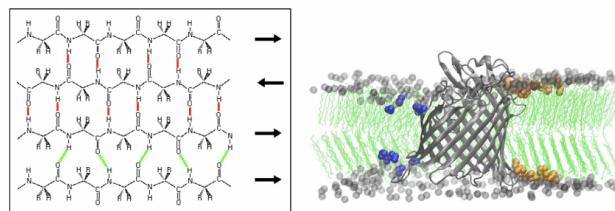


Figure 17: Beta Barrel

Beta strands are quite different compared to transmembrane helices. Since beta strands have two sides, 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.

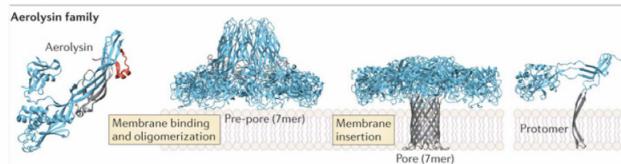


Figure 18: membrane attack complex

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

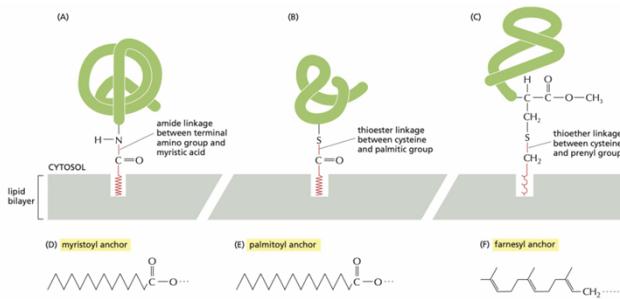


Figure 19: 3 main types of lipid anchors

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

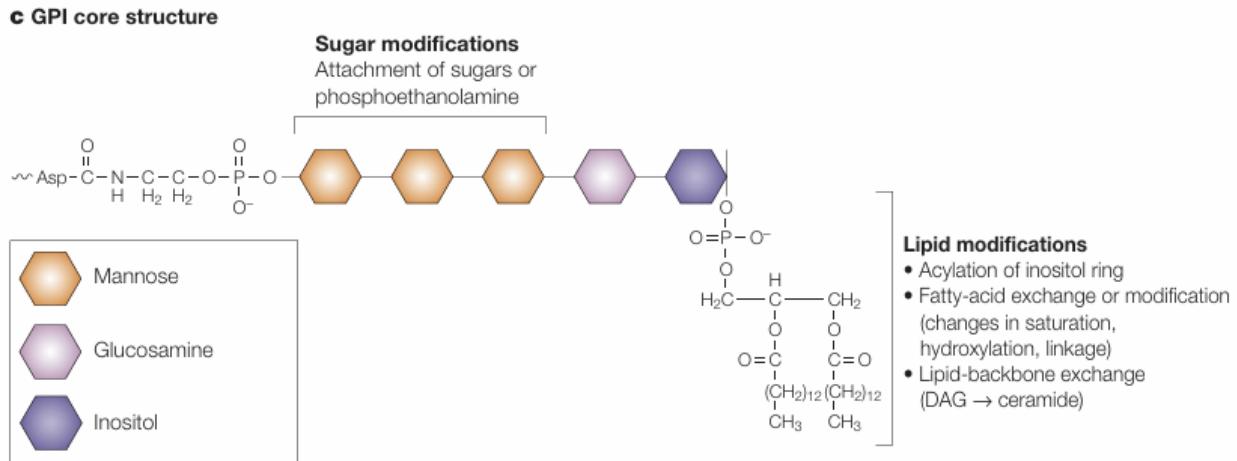


Figure 20: GPI anchor structure

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

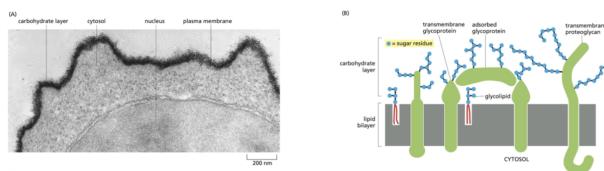


Figure 21: The carbohydrate layer of the cell membrane

the carbohydrate layer of the cell The cell membrane has a lot of glycolipids sticking out. (we will look at later I think..)

2.3 membrane protein isolation

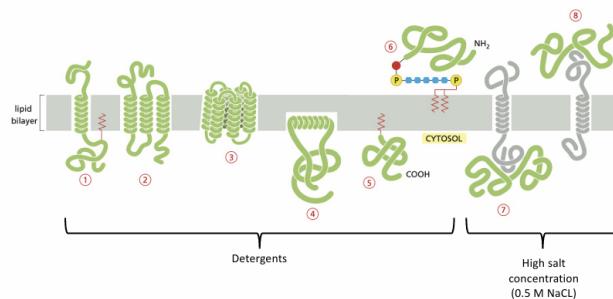


Figure 22: isolation of membrane proteins

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

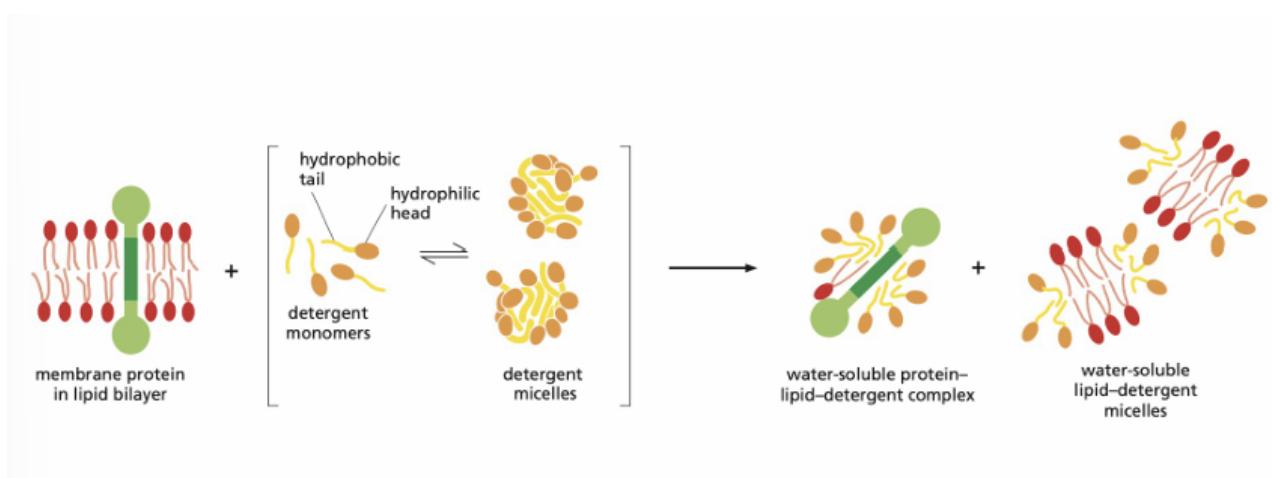


Figure 23: Detergents function

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

- i) **SDS (Sodium Dodecyl Sulfate)** The negative charge will denature them though
- ii) **Triton X-100** this detergent is less harsh than sds so will not denature the proteins. This is called **Soft Solubilization** and is usefull when you want to isolate the protein in functional conformation.

2.3.2 nanodiscs

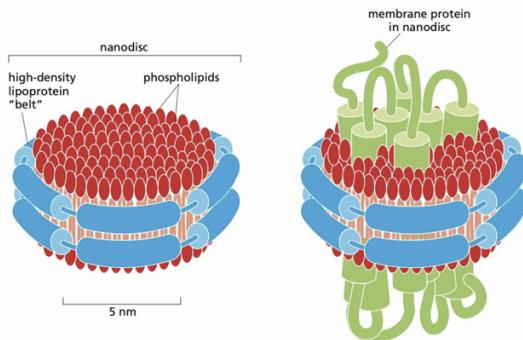


Figure 24: Nanodiscs

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

2.4 membrane protein localization

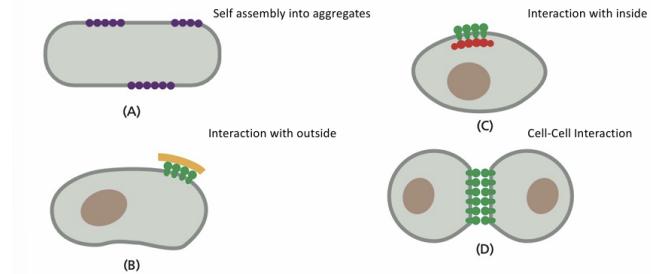


Figure 25: membrane protein localization mechanisms

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**

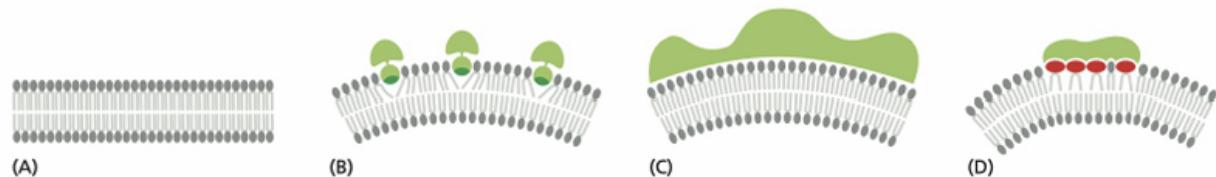


Figure 26: membrane protein bending

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

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

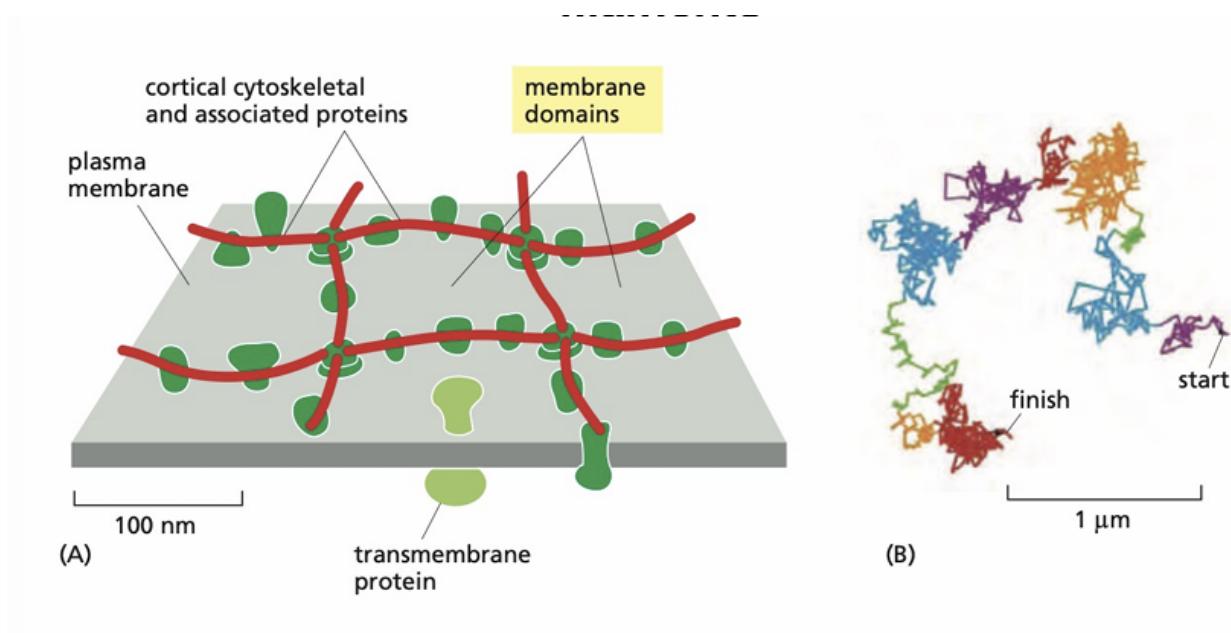


Figure 27: Spectrin corraling plasma membranes

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 corraling 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.

Glossary

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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**.

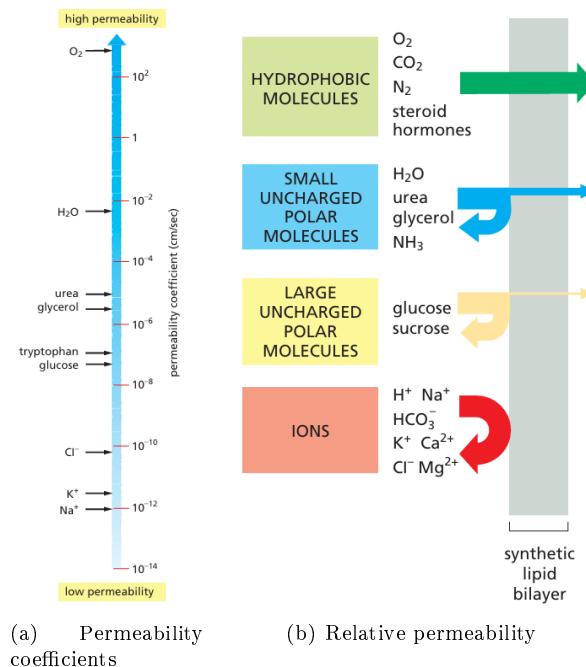


Figure 28: Permeability of the cellular membrane

Nevertheless these rates are pretty shit. Therefore in order to benefit from this barrier cells have had to evolve ways of transferring specific water-soluble 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.

Remark 3.1 (Transporters vs Channels). There are 2 main classes of **membrane transport proteins**: textbf{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 Passiv 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 influenced by the membrane potential (electrochemical gradient).

There also 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.

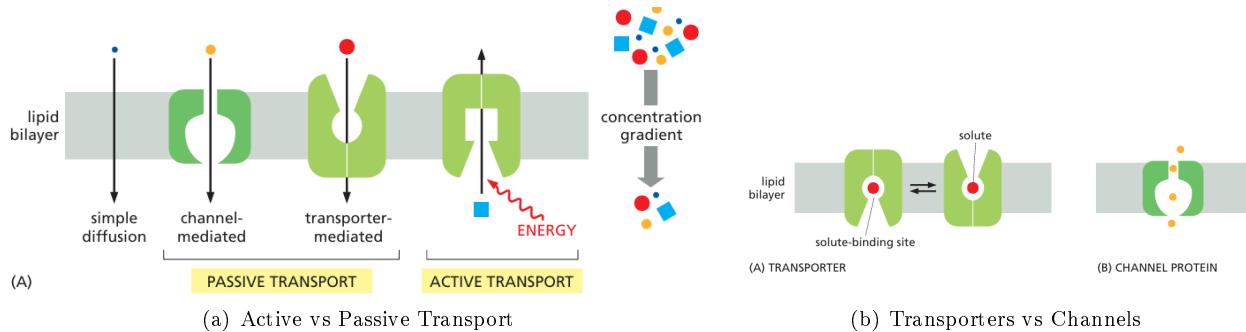


Figure 29: Transport Membrane Proteins

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.*

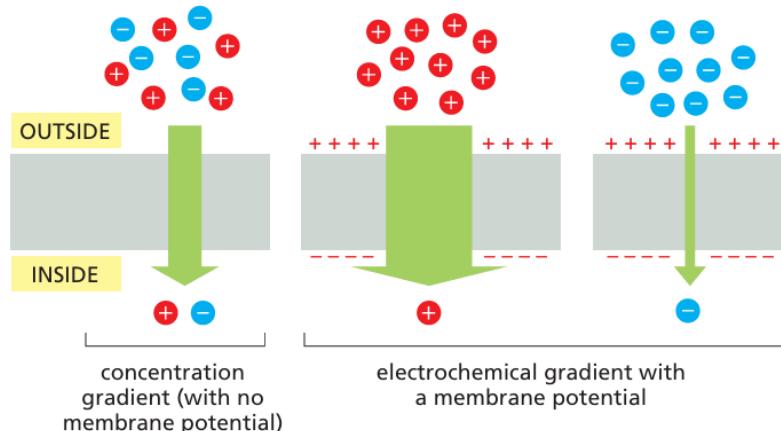


Figure 30: The electrochemical gradient of a charged solute (an ion) affects its transport

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

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

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

Table 2: Ion Concentrations and Equilibrium Potentials

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),
- $\Delta E_i < 0$: 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 Na^+ , K^+ or Ca^{2+} . But when they open mostly Na^+ enters the cell.). There is little net movement of K^+ because it is nearly at equilibrium distribution, by contrast Na^+ and Ca^{2+} are not at equilibrium distribution. However Ca^{2+} is present in way lower concentration than Na^+ . Therefore Na^+ enters the cell.

3.1 Transporters

Transporters are typically built from bundles of **10 or more α 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. 32(a)

Moreover Transporters are build 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.

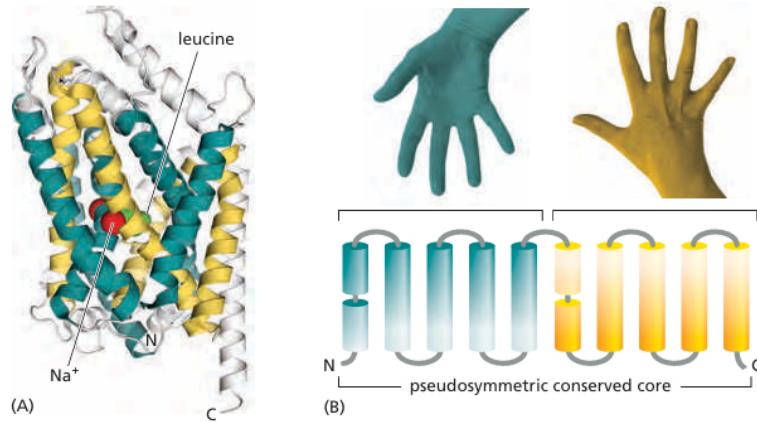


Figure 31: Transporters are built from inverted repeats.

In many ways **Transporters behave like enzymes**. Each type of transporter has one ore 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. 32(b)

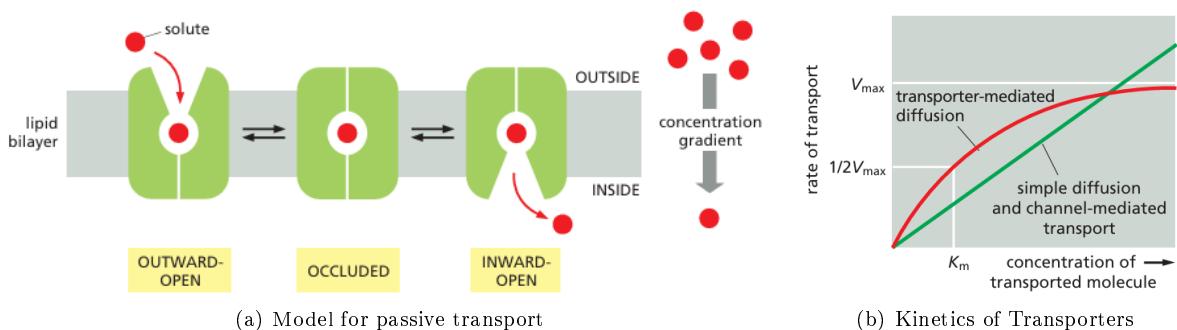


Figure 32:

Apart from passive transport, trasnporters 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.

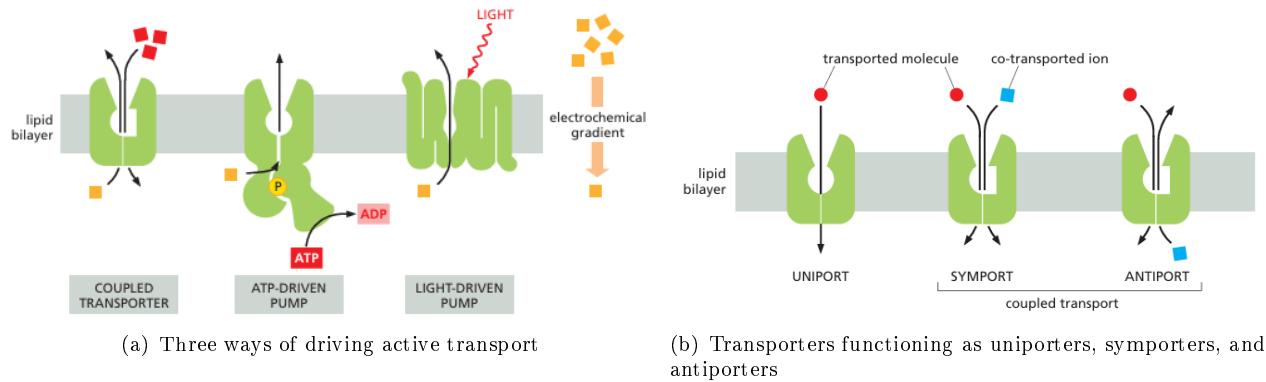


Figure 33:

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.

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 oposit directions (**Antiporter**). See. Fig.

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⁺ 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⁺ that enters the cell during coupled transport is **subsequently pumped out by an ATP-driven Na⁺-K⁺ pump** in the plasma membrane, which, by maintaining the Na⁺ 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**

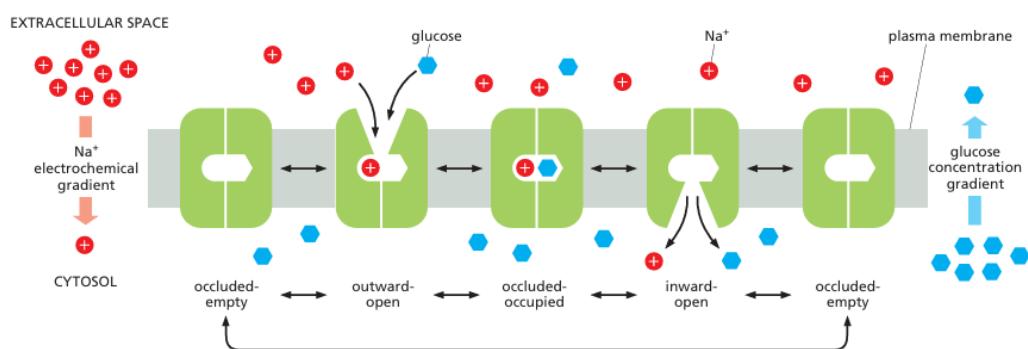


Figure 34: Mechanism of glucose transport fueled by a Na⁺ gradient (SGLT family)

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 evolutionary placed where it makes sense for the cell.**

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. 35)

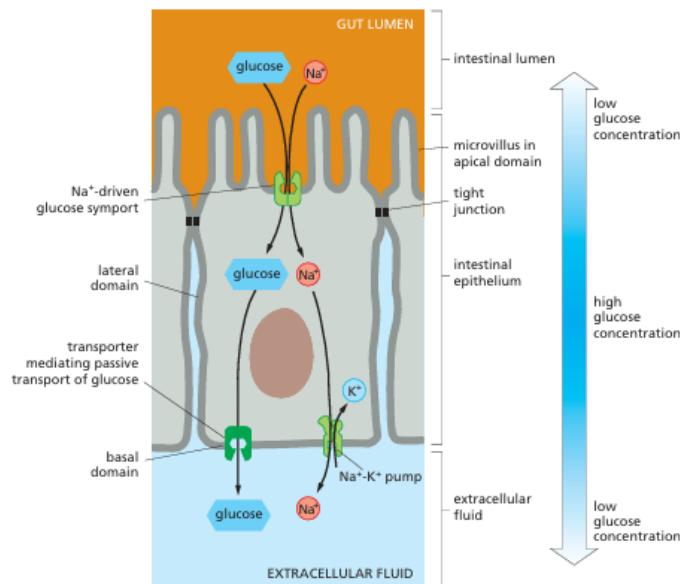


Figure 35: An Asymmetric Distribution of Transporters in Epithelial Cells Underlies the Transcellular Transport of Solutes

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** 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 **Na^+ , K^+ , H^+ , and Ca^{2+}** across cell membranes.
- **ABC transporters** (ATP-Binding Cassette transporters) differ structurally from P-type ATPases and primarily pump **small molecules** across cell membranes.
- **V-type** pumps are turbine-like protein machines, constructed from **multiple different subunits**. The V-type proton pump transfers **H^+ into organelles**.

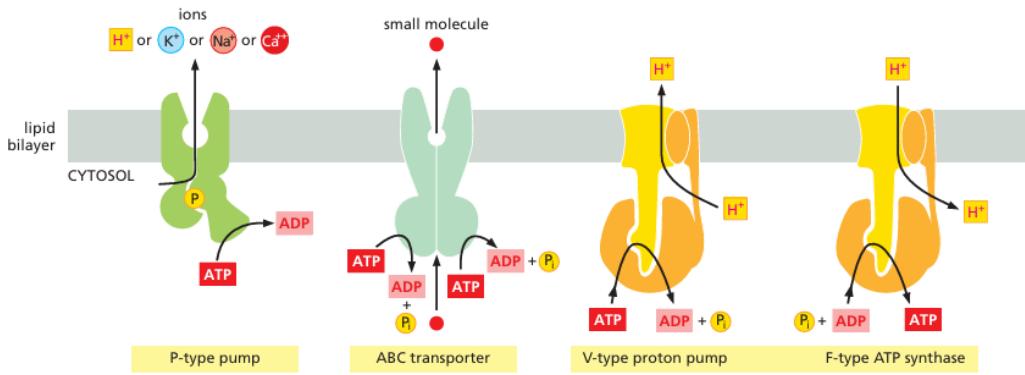


Figure 36: Three types of aTP-driven pumps.

3.1.2.1 Na^+/K^+ pump

The Na^+/K^+ ATPase is a **ATP-driven antiporter P-type ATPase**. It maintains the **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 Na^+/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.

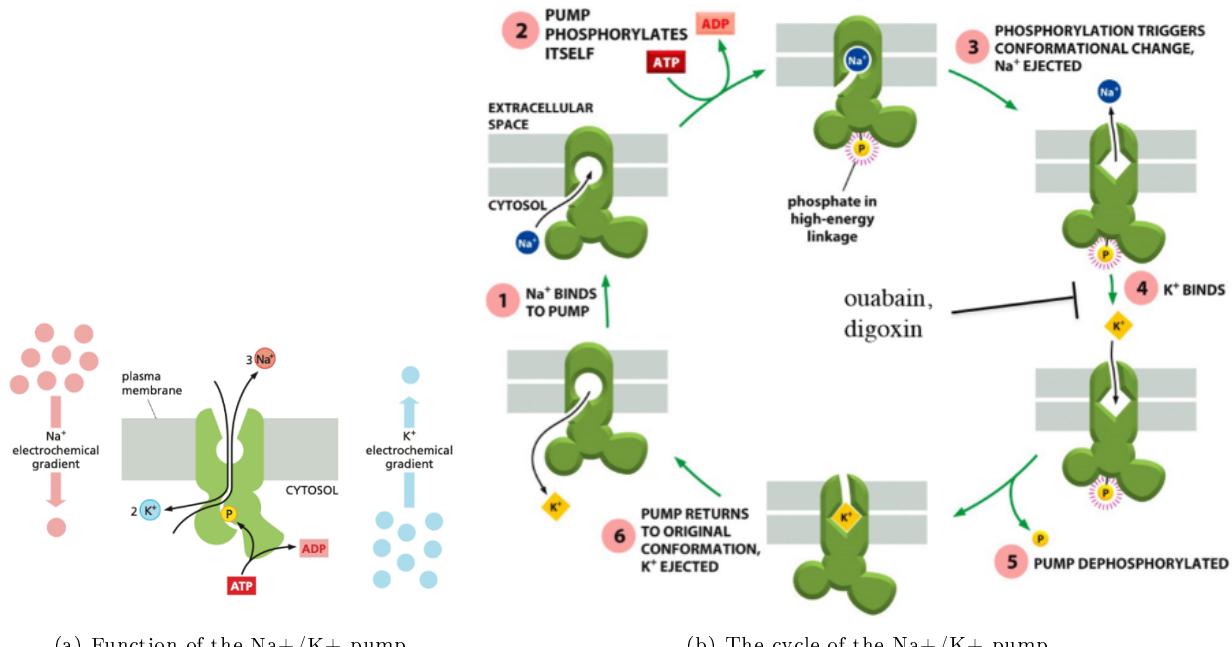


Figure 37: Na^+/K^+ pump

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 Na^+/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 Ca²⁺ pump

The Ca²⁺ pump, or Ca²⁺ 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 Ca²⁺.

When an action potential depolarizes the muscle cell plasma membrane, Ca²⁺ is released into the cytosol from the SR through Ca²⁺-release channels, stimulating the muscle to contract.

The Ca²⁺ pump, which accounts for about 90 % of the membrane protein of the SR, moves Ca²⁺ from the cytosol back into the SR. The endoplasmic reticulum of nonmuscle cells contains a similar Ca²⁺ pump, but in smaller quantities.

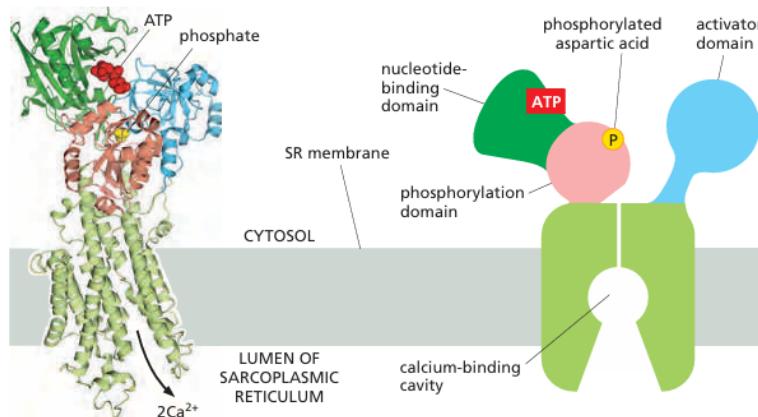


Figure 38: The structure of the sarcoplasmic reticulum Ca²⁺ pump.

Ca²⁺ 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 **ATP is transferred to an aspartate that is highly conserved among all P-type ATPases**. The 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 Ca²⁺ ions exit. They are replaced by two H⁺ ions and a water molecule that stabilize the empty Ca²⁺-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. 39

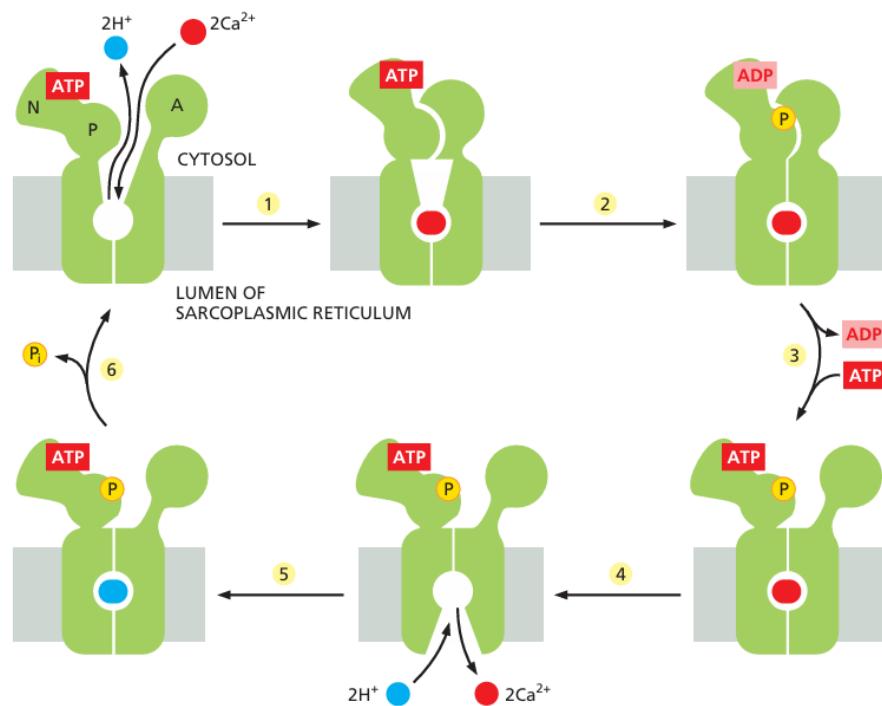


Figure 39: The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump.

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.

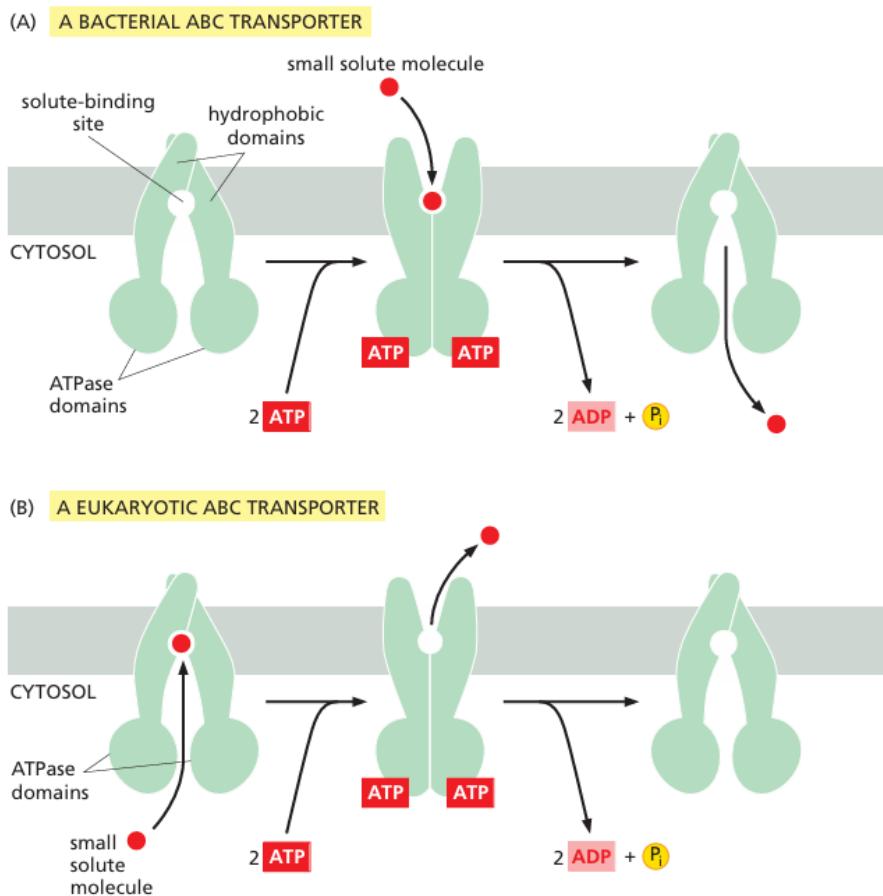


Figure 40: Small-molecule transport by typical ABC transporters.

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⁺ and other ions can not transfer through aquaporins.

Moreover these channels are also impermeable to H⁺. 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.

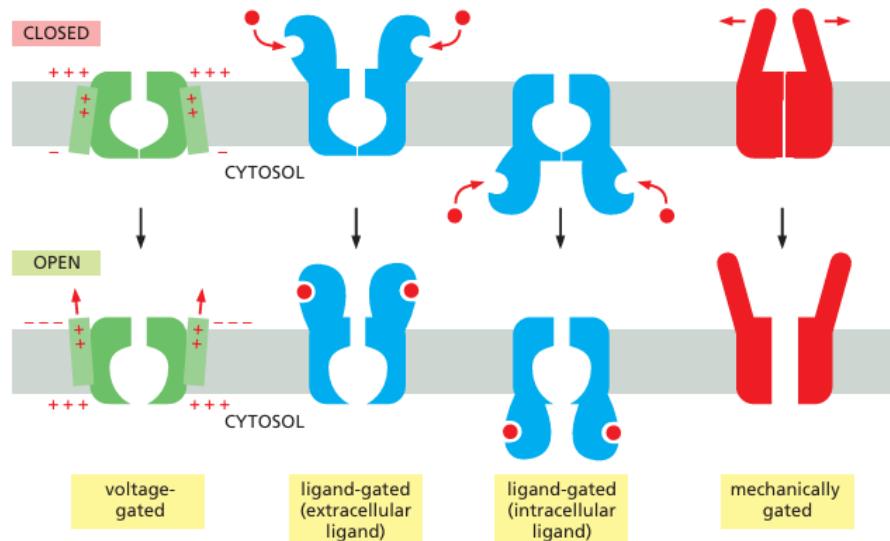


Figure 41: The structure of aquaporins

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. 42

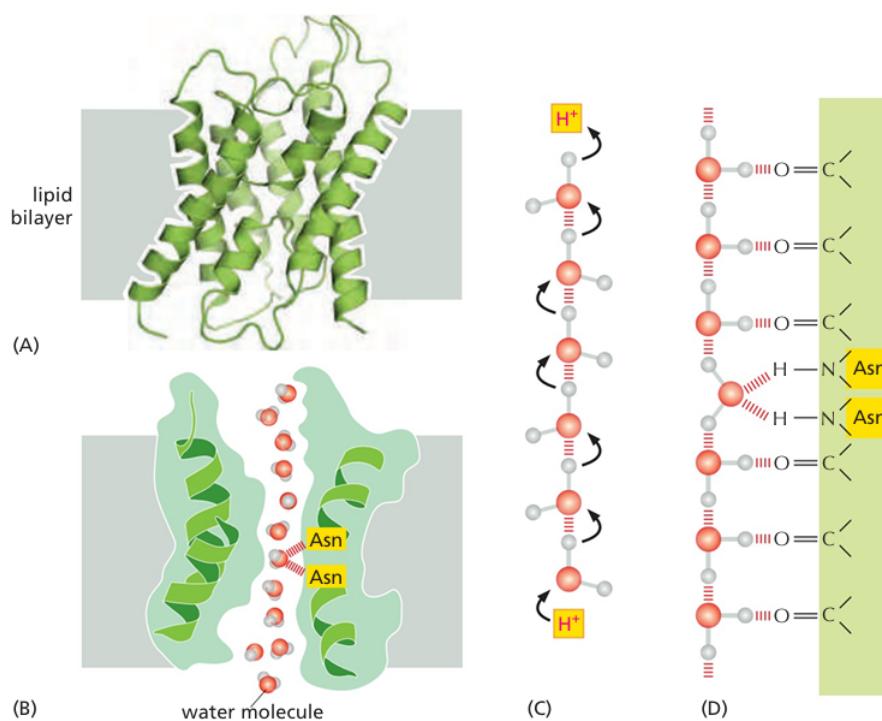


Figure 42: The gating of ion channels

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 they are still bound to the ligand, preventing overfiring.

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

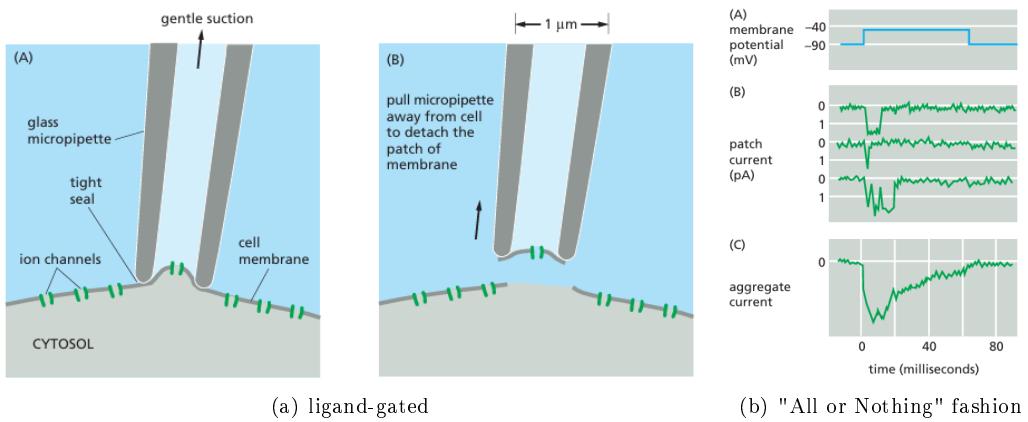


Figure 43:

3.2.2.1 K⁺ (leak) channels

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

K⁺ leak channels conduct K⁺ 10,000-fold faster than Na⁺, 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 α 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⁺**. Moreover, they channel attracts cation by negative charged amino acids. See fig. 44

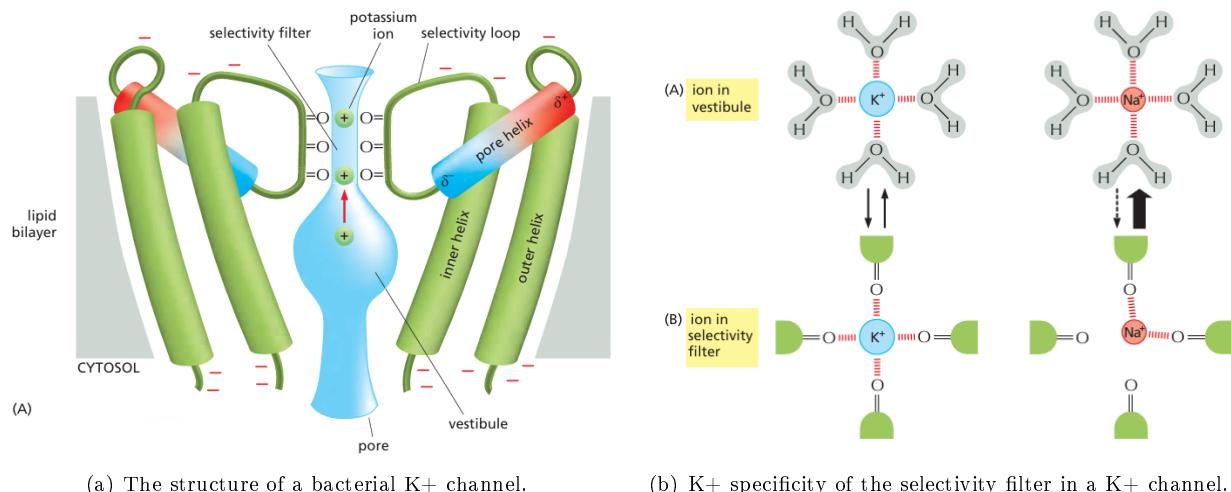


Figure 44: K⁺ channel

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.

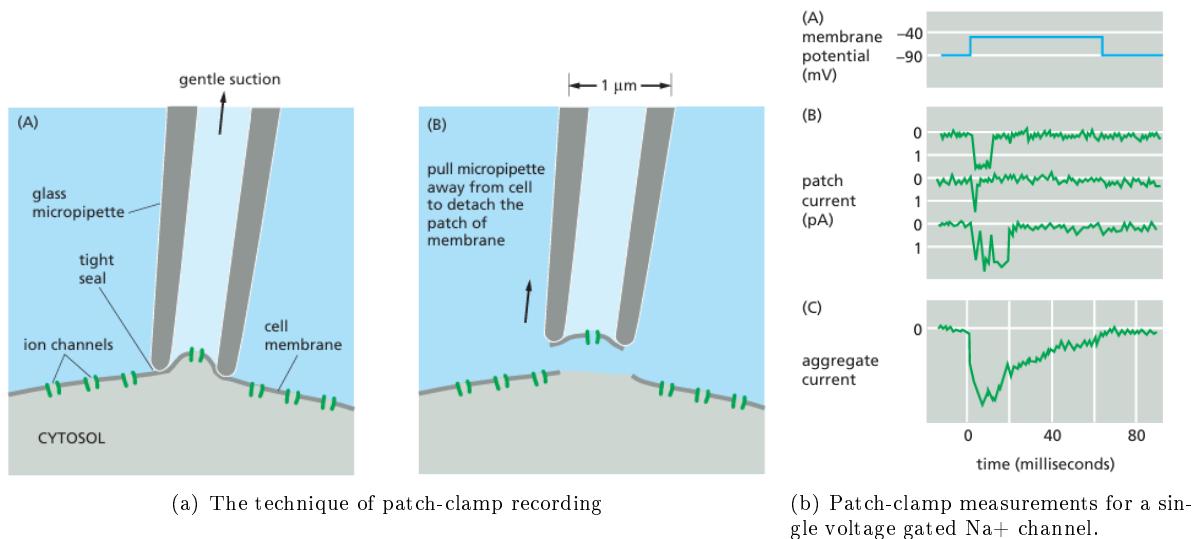


Figure 45: Patch-clamp

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

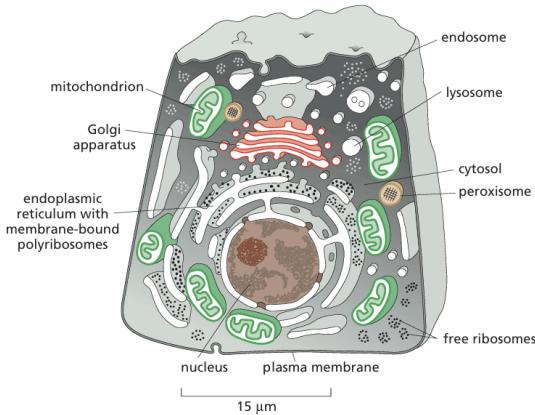


Figure 46: overview of compartments

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. 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 continuous
- membraneless 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 differentiation so they will have **no organelles that have a membrane**

4.1.0.1 table on volumes of membrane 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 μm^3 compared with 1000 μm^3 for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000 μm^2 and 13,000 μm^2 , respectively.

Figure 47: table volumes of membranes

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

Membrane-less organelles are a rather new discovery but they usually require **liquid liquid Phase separation**. They can consist of protein aggregates or RNA aggregates:

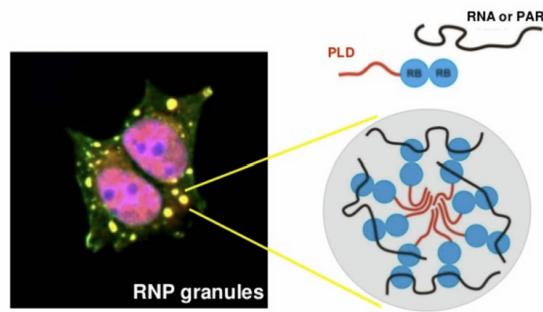


Figure 48: membrane less organelle overview of RNP

4.1.1 Endoplasmatic reticulum

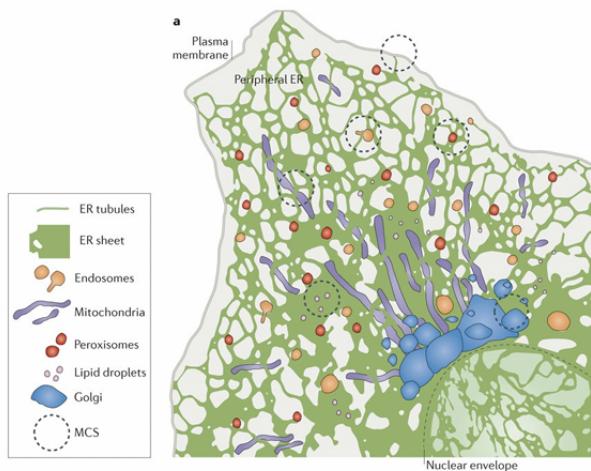


Figure 49: Structure of Endoplasmatic reticulum

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

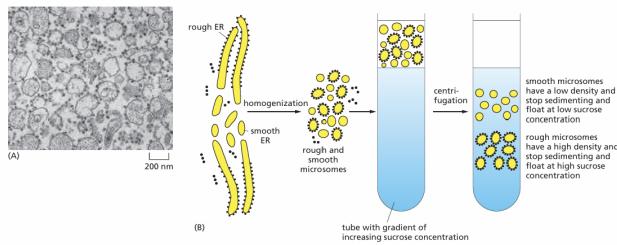


Figure 50: ER separation experiment

Since smooth and rough ER have different densities they can be separated using a sucrose gradient and centrifugation.

4.1.2 Golgi system

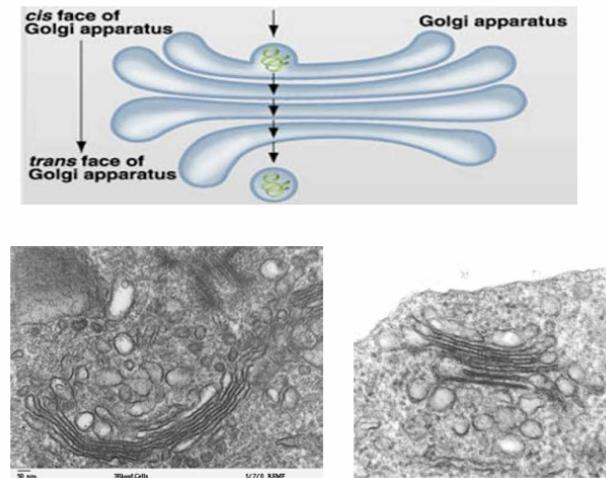


Figure 51: Golgi apparatus overview

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.

4.1.3 lysosomes

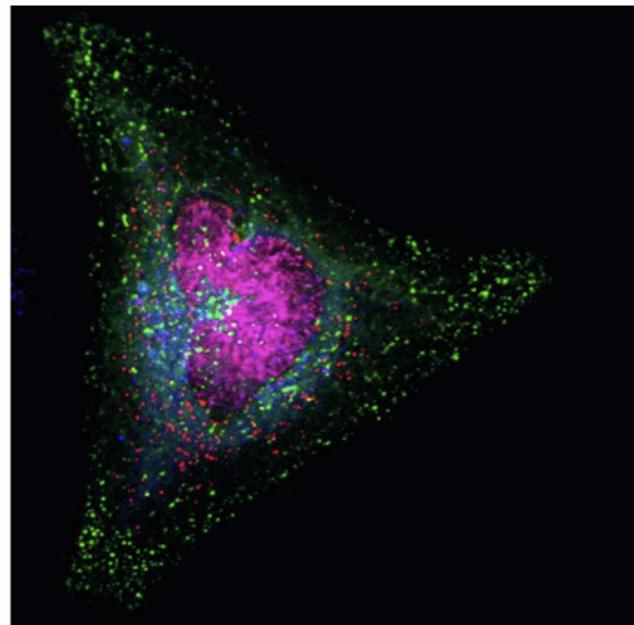


Figure 52: lysosome fluorescent microscopy image

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** (just copied the text from slide cause there wasn't much info on these...)

4.1.4 Mitochondria

Mitochondria Structural Features

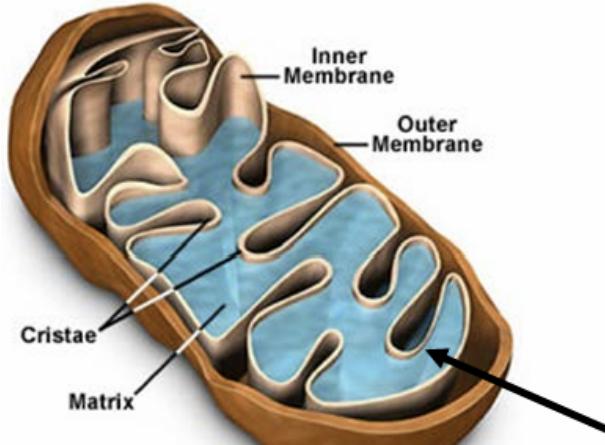


Figure 1

Figure 53: Mitochondra 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

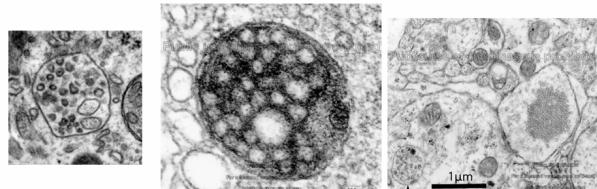


Figure 54: Endosomes

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

4.1.6 Peroxisomes

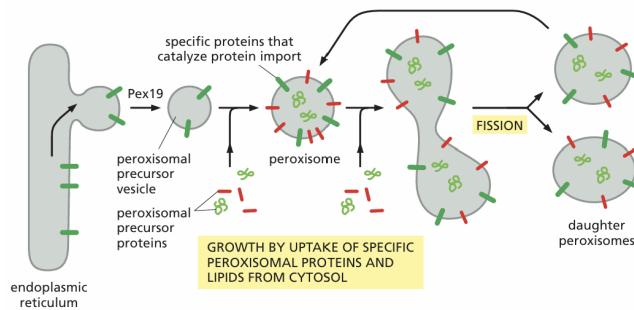


Figure 55: Peroxysomes proliferation

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 peroxysomes. They are also responsible for the **break down of fatty acids** which is β -oxidation.

4.1.7 lipid droplets

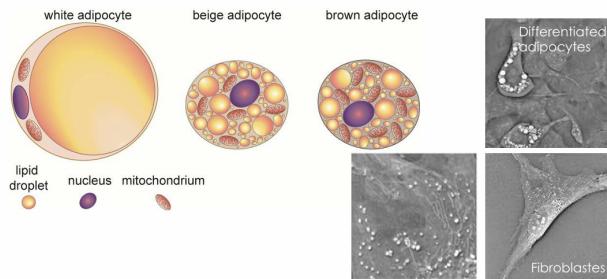


Figure 56: adipocyte overview

These are specialized cells that store lipid droplets. (also nothing about these either)

4.2 lipid composition of various organelles

The lipid composition is different across the various organelles.

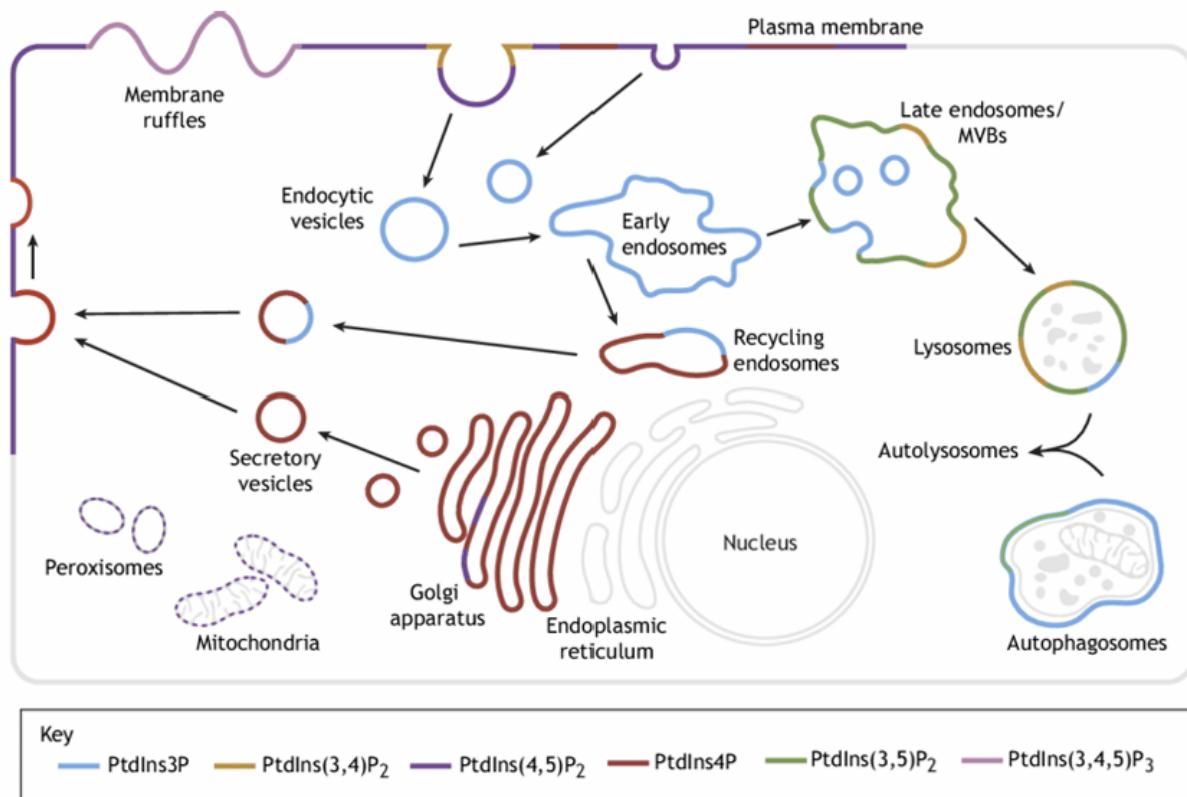


Figure 57: Phosphatidylinositol location and phosphorylation state

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
Cholesterol	17	23	22	3	6
Phosphatidylethanolamine	7	18	15	28	17
Phosphatidylserine	4	7	9	2	5
Phosphatidylcholine	24	17	10	44	40
Sphingomyelin	19	18	8	0	5
Glycolipids	7	3	28	trace	trace
Others	22	13	8	23	27

Figure 58: Membrane composition based on cell type

4.3 protein localization

4.3.1 types of transport

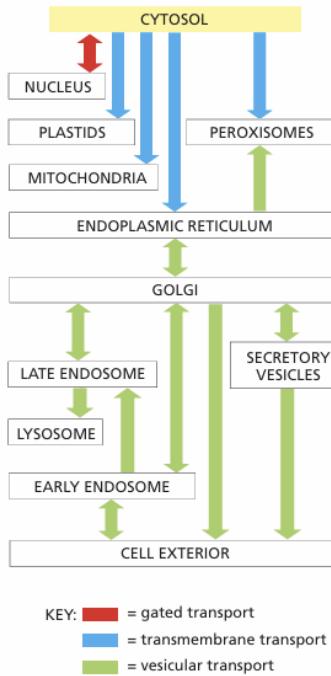


Figure 59: Types of transport overview

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	⁺ H ₃ 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	⁺ H ₃ 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 ⁻
Import into ER	⁺ H ₃ 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 ⁻

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. ⁺H₃N indicates the N-terminus of a protein; COO⁻ indicates the C-terminus.

Figure 60: list of important localization signals

Note that the signals can be either N or C terminal. the **nuclear export signals can be in the middle of the CDS**. Note also that the localization signals will be cut off after their use, with the exception of nuclear localization tag (NLS)

4.3.3 nuclear import

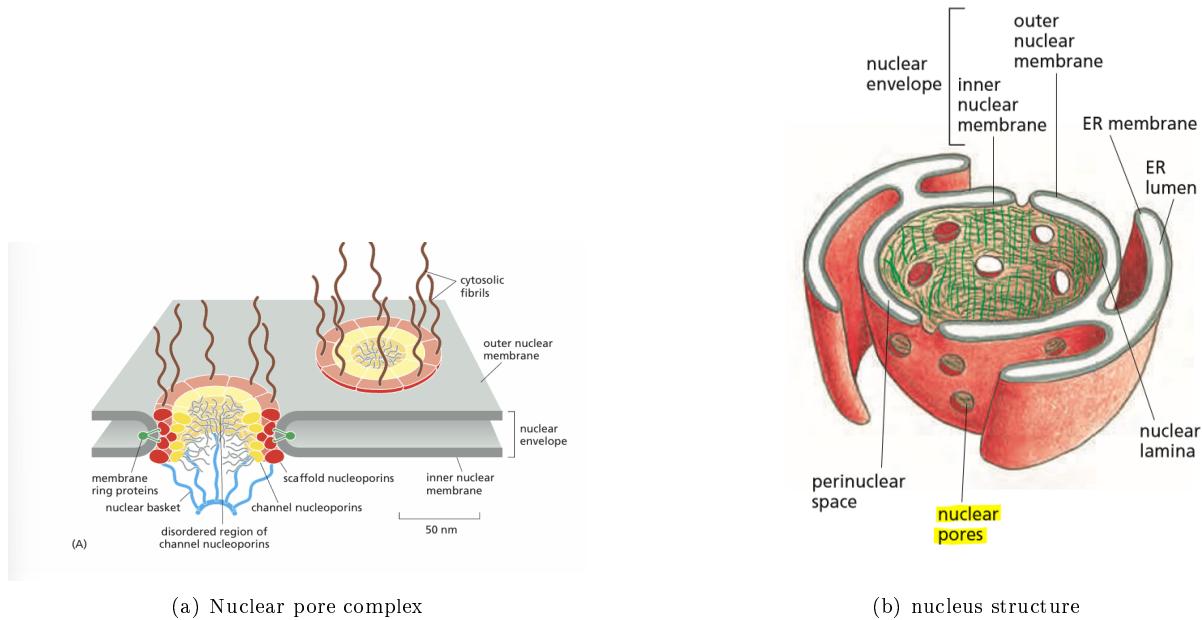


Figure 61: Application of protein design

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 note on ribosome production

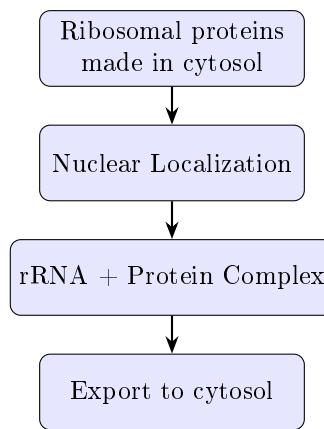


Figure 62: Overview of Ribosome Production and Trafficking

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)

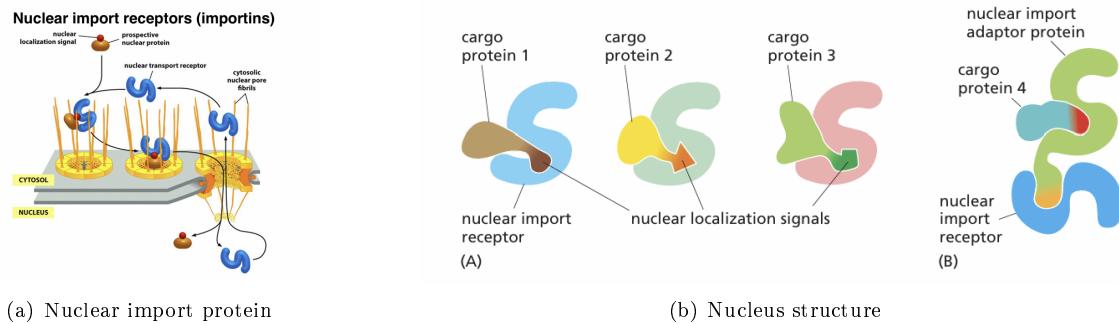


Figure 63: Importins bind to cargo proteins that have NLS

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**.

4.3.3.3 ran-GDP Ran-GTP

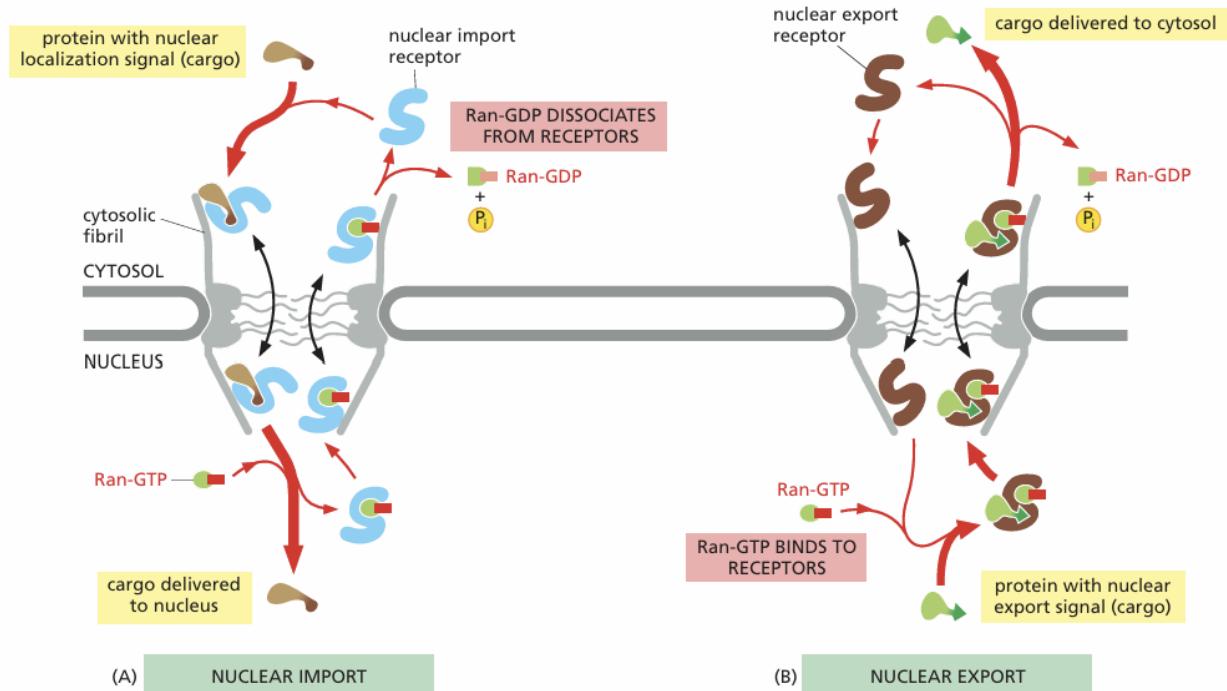


Figure 64: ran-GDP ran-GTP and it's role in shuttling cargo across nuclear pore complexes

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-GTP/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.

4.3.3.4 nuclear import control during T cell activation

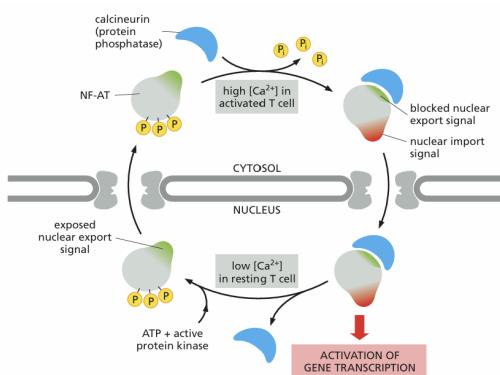


Figure 65: role of nuclear import/ export signals in T cell activation

Nuclear and import signals play a crucial role in T cell activation. **Upon activation the T-cell's cytosolic Ca²⁺ 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).

—————> some stuff about an androgen receptor don't know if to add it or not
(need to discuss that !!)

4.3.3.5 the role of nuclear import in cell division

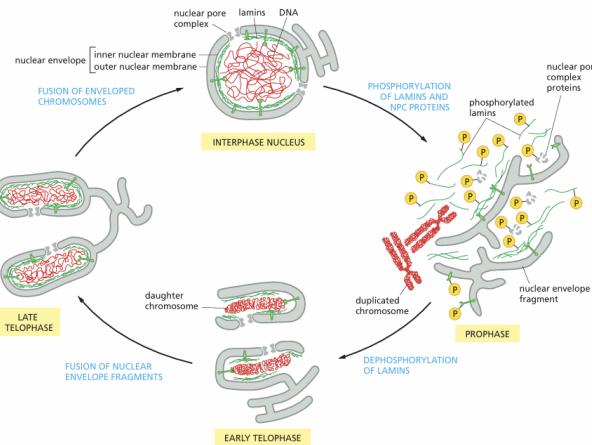


Figure 66: Reformation of the nucleus requires resusable NLS

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 this time** 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**

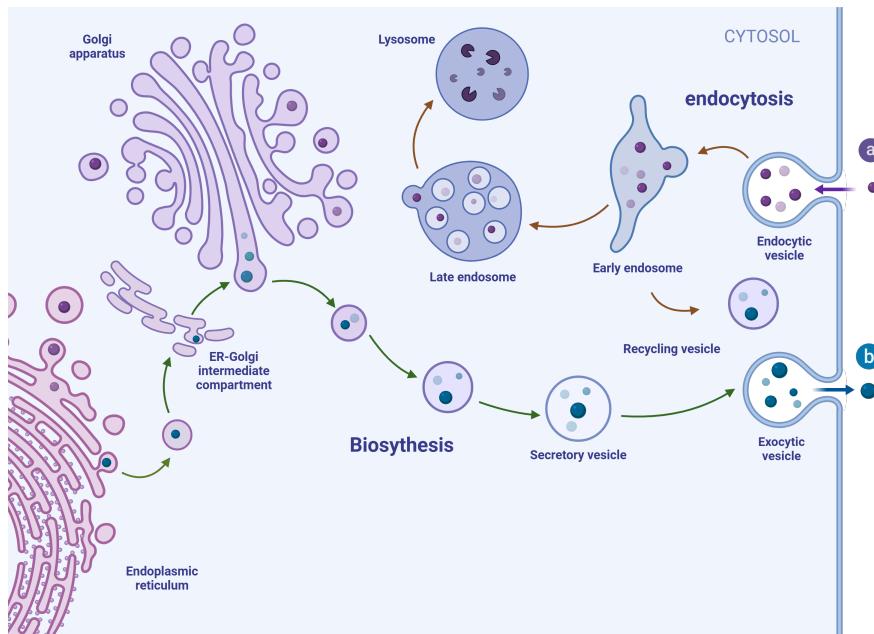


Figure 67: Main Pathways looked at in this chapter

The Biosynthesis or secretory pathways goes:

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

the Endocytosis pathways goes:

- 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

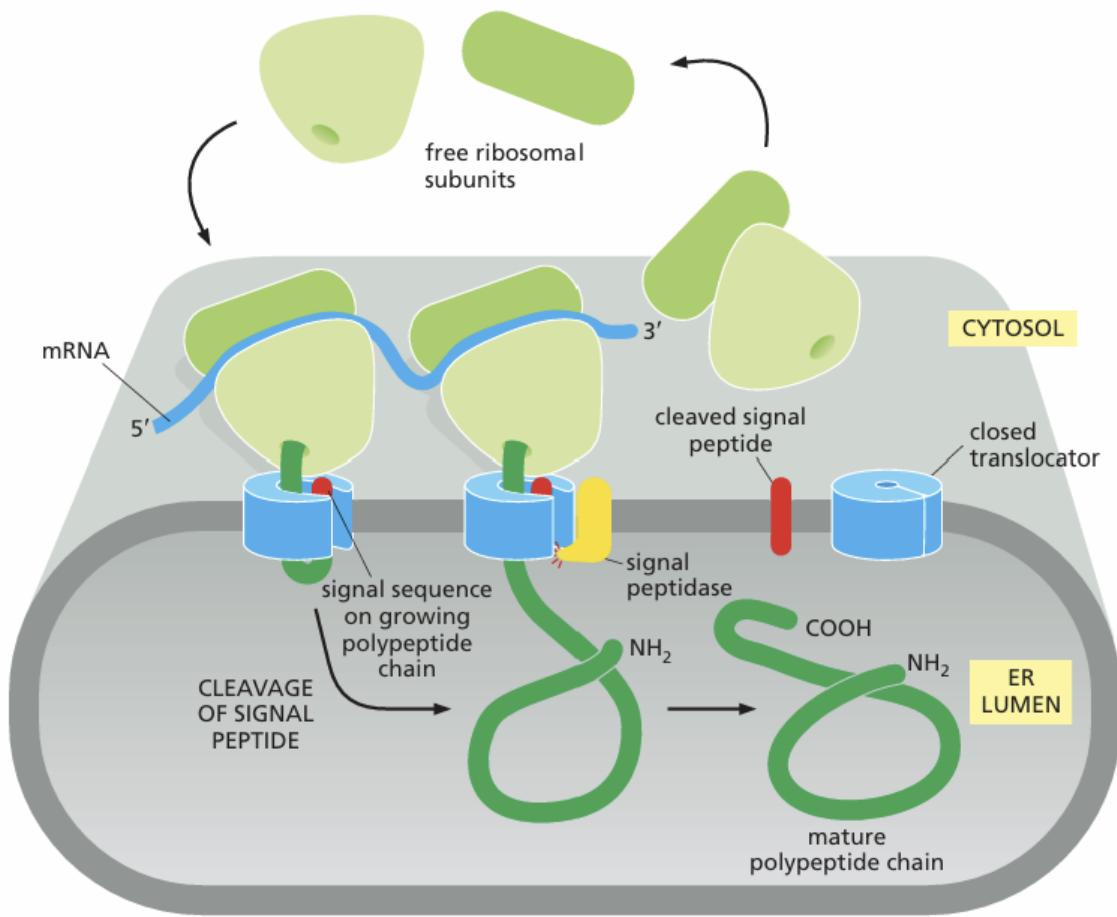


Figure 68: co and post translocation to the ER

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.

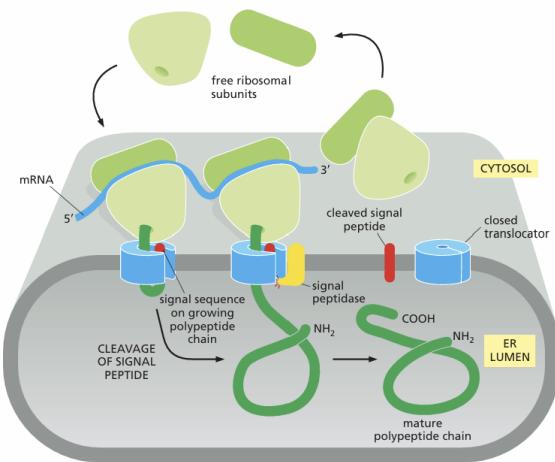


Figure 69: Co translational translocation

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 highly reducing environment like the cytosol. Thus ER cytosolic proteins can have disulfide bonds unlike cytosolic proteins.

4.3.5.3 transmembrane proteins

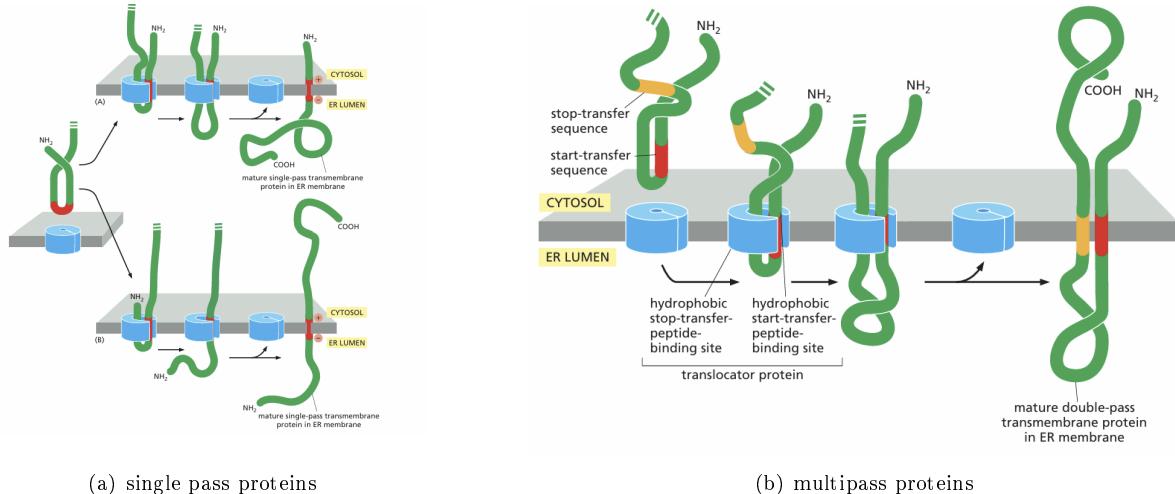


Figure 70: Role of localization in transmembrane protein translation

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.

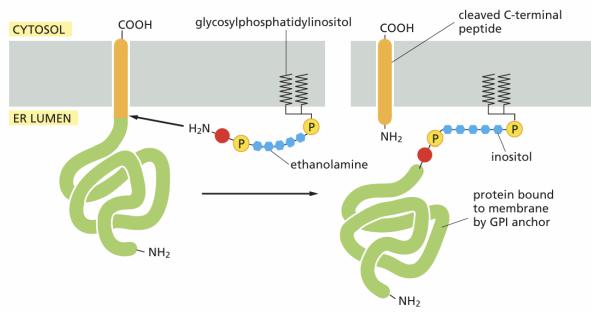


Figure 71: GPI anchor synthesis

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

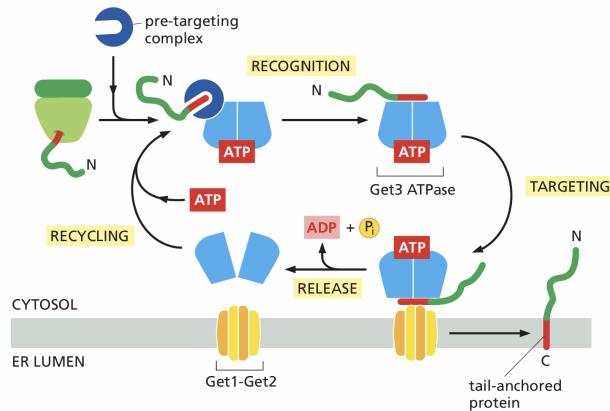


Figure 72: tail anchored proteins

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.**

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

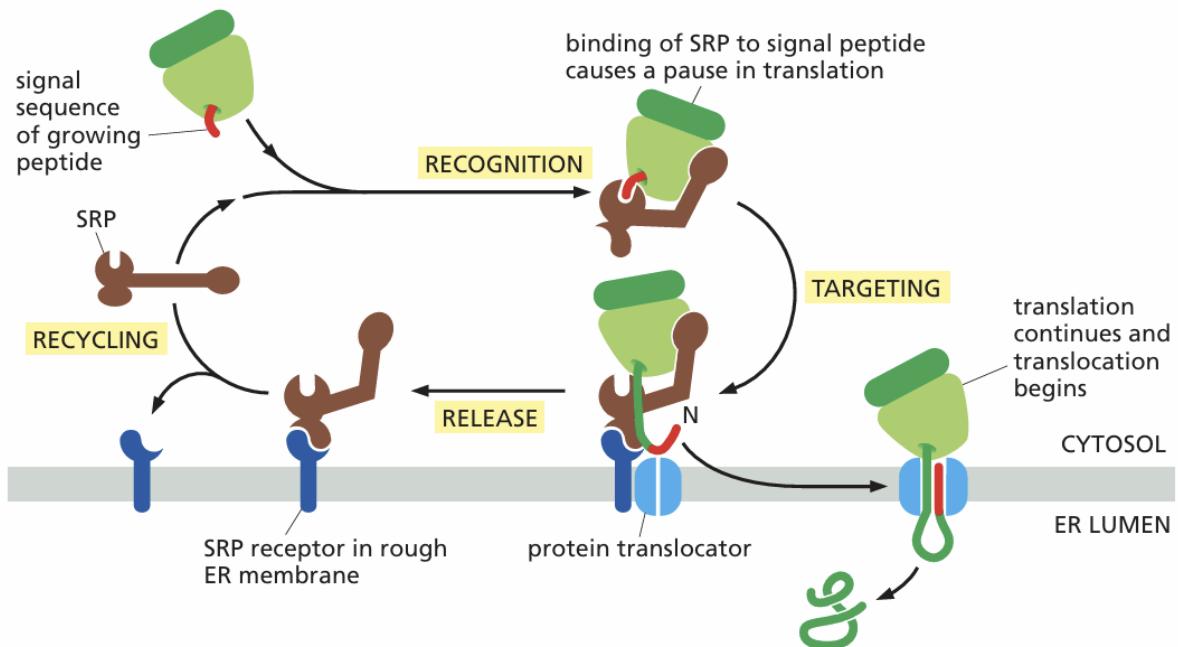


Figure 73: The SRP function in ribosome localization

- 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

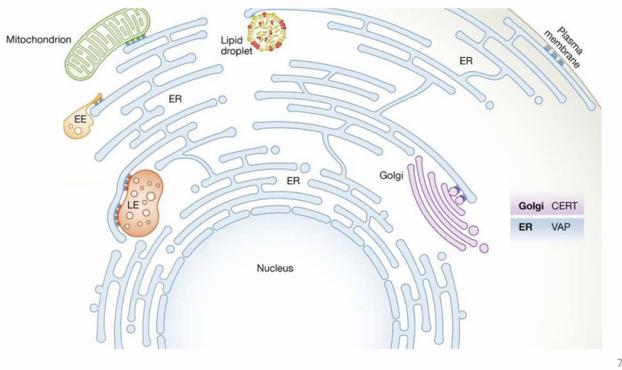


Figure 74: Contact patches role in protein transport

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

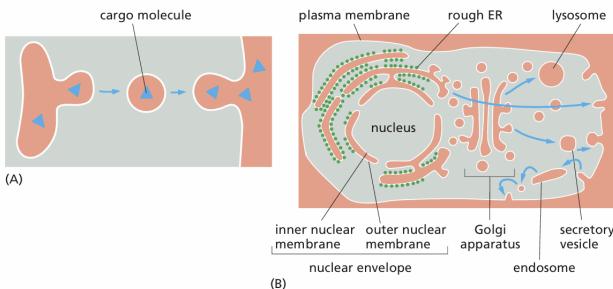


Figure 75: topological equivalence

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.

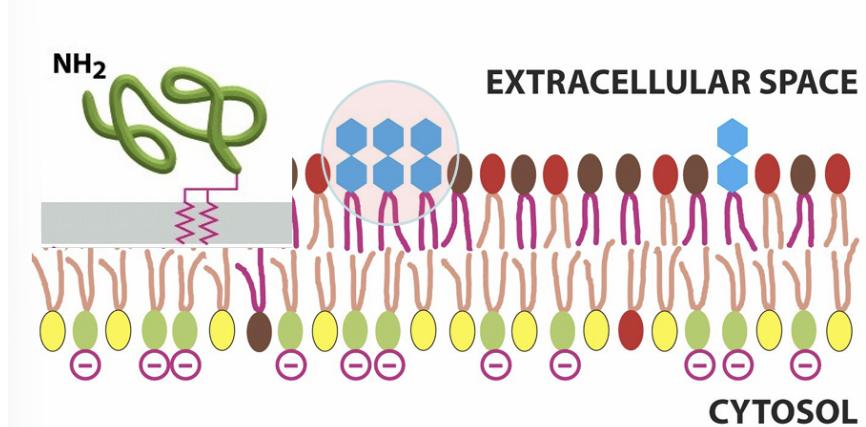


Figure 76: Asymmetry in membrane leaflets

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

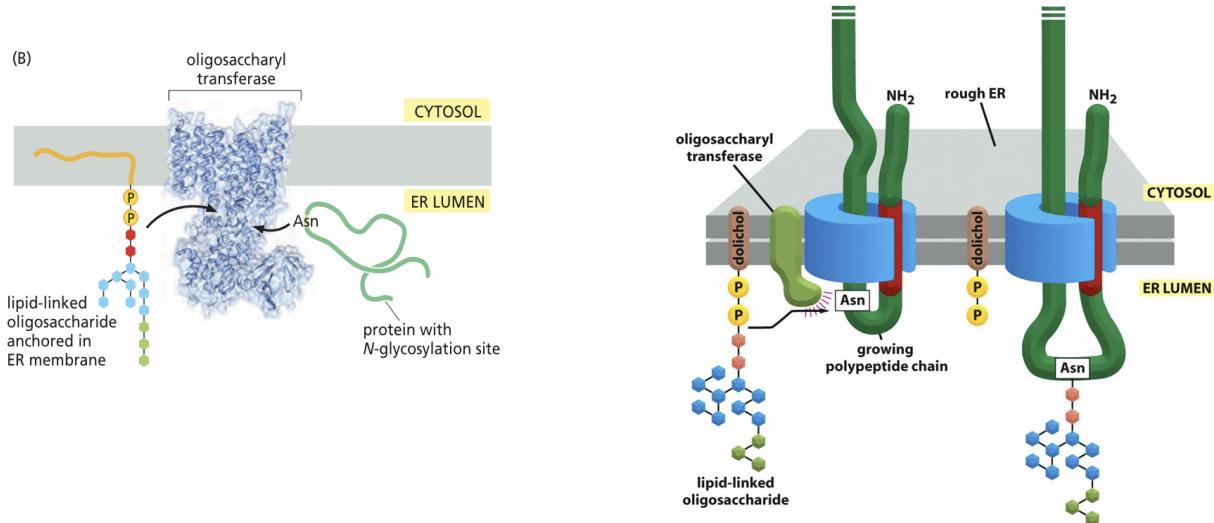


Figure 12-51 Molecular Biology of the Cell (© Garland Science 2008)

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.

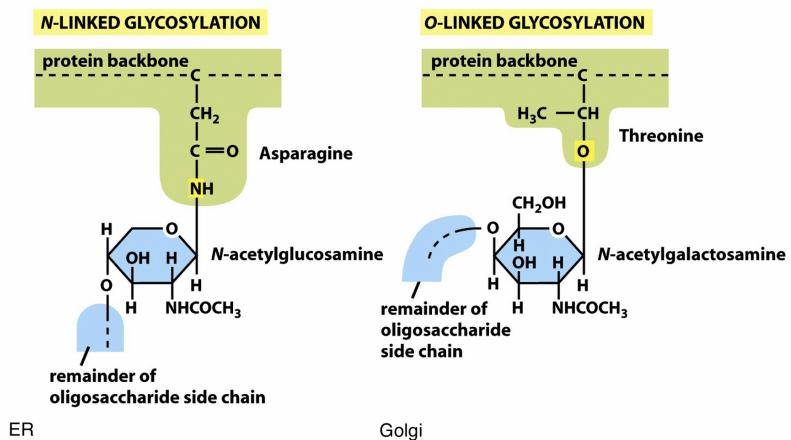


Figure 78: N linked vs O linked glycosylation

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

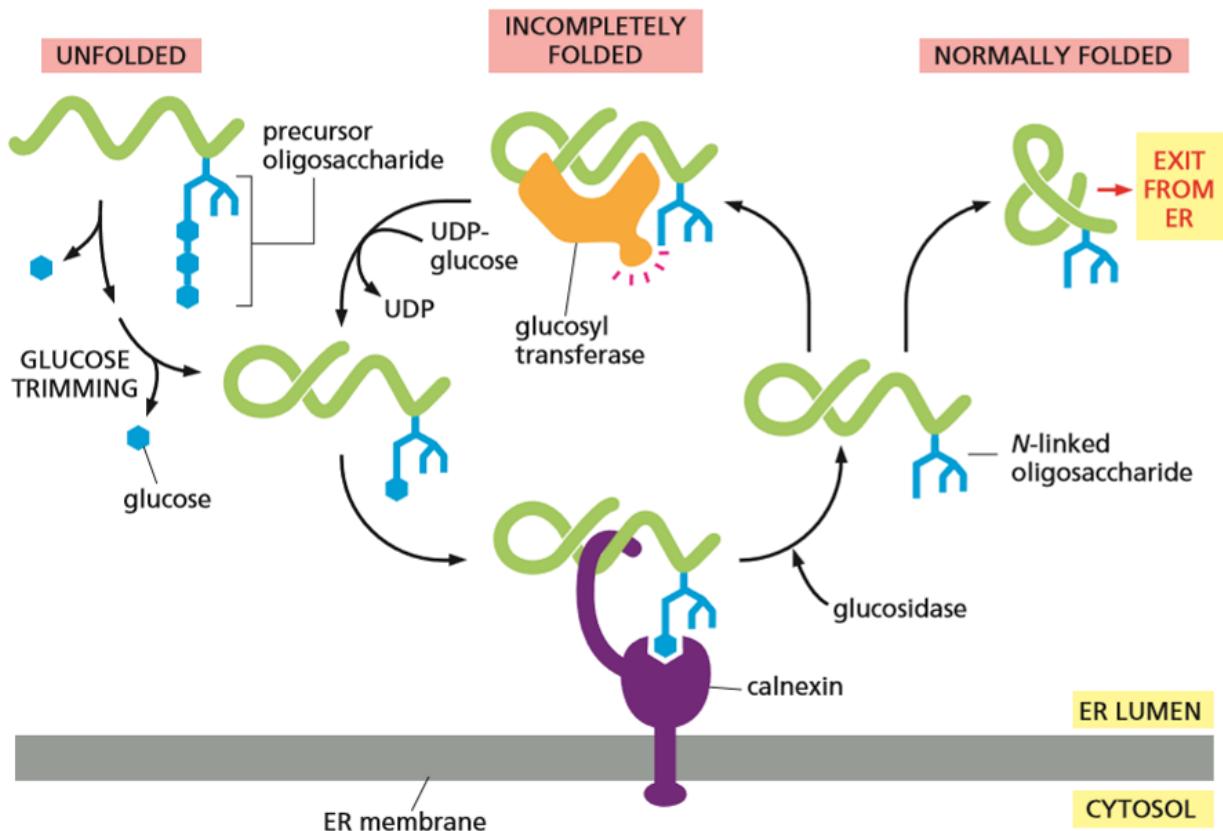


Figure 79: Calnexin/Calreticulin cycle cycle in ER

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.

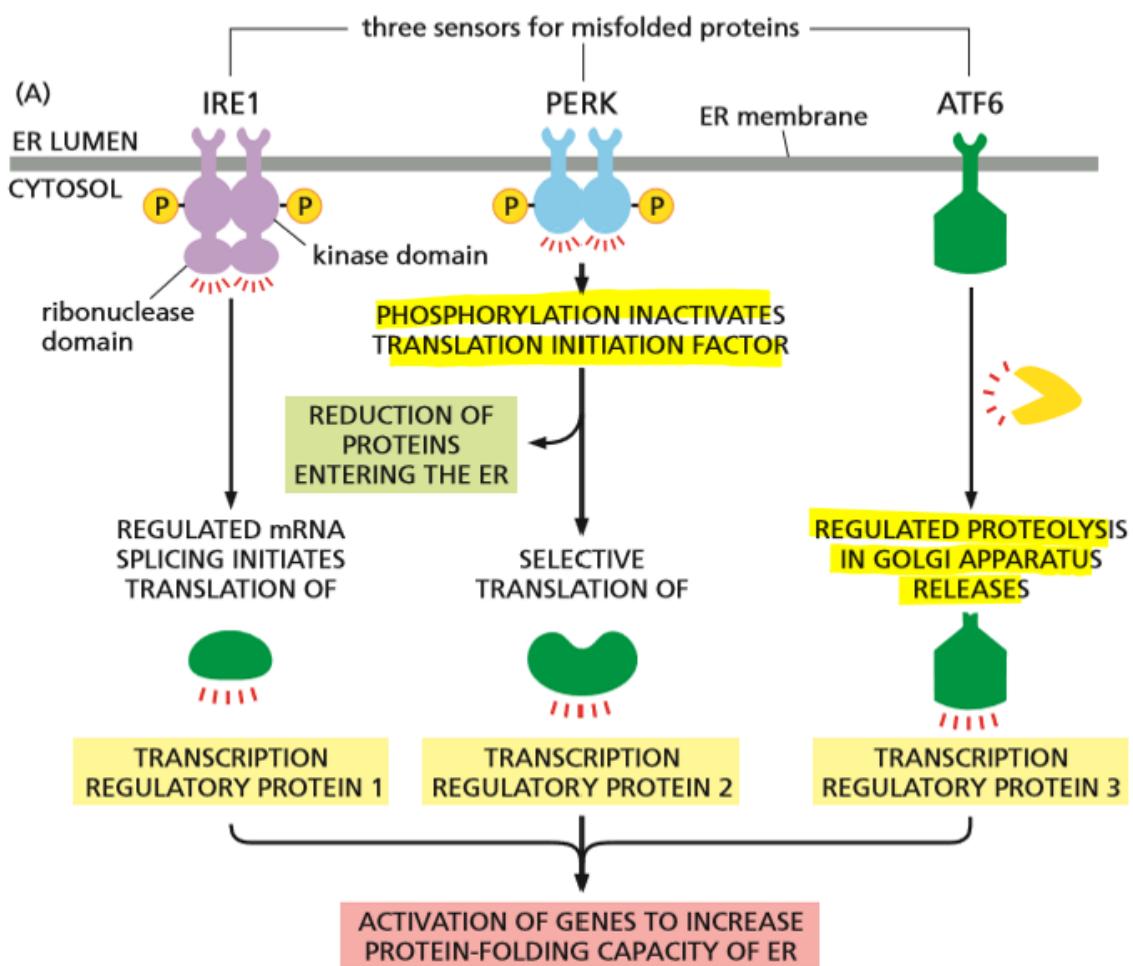


Figure 80: the unfolded protein response

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 sythesis in Er

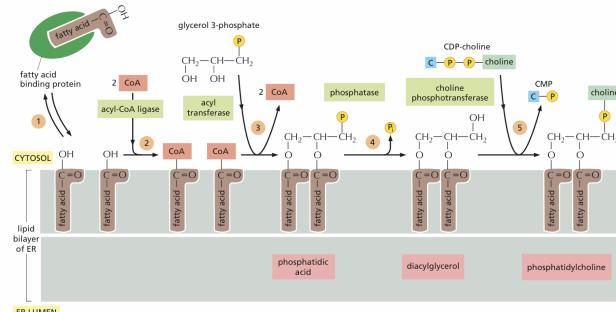


Figure 81: Phosphatydil Choline Sythesis

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.

Glossary

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

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.. 7

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

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

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

5 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.

Through 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.

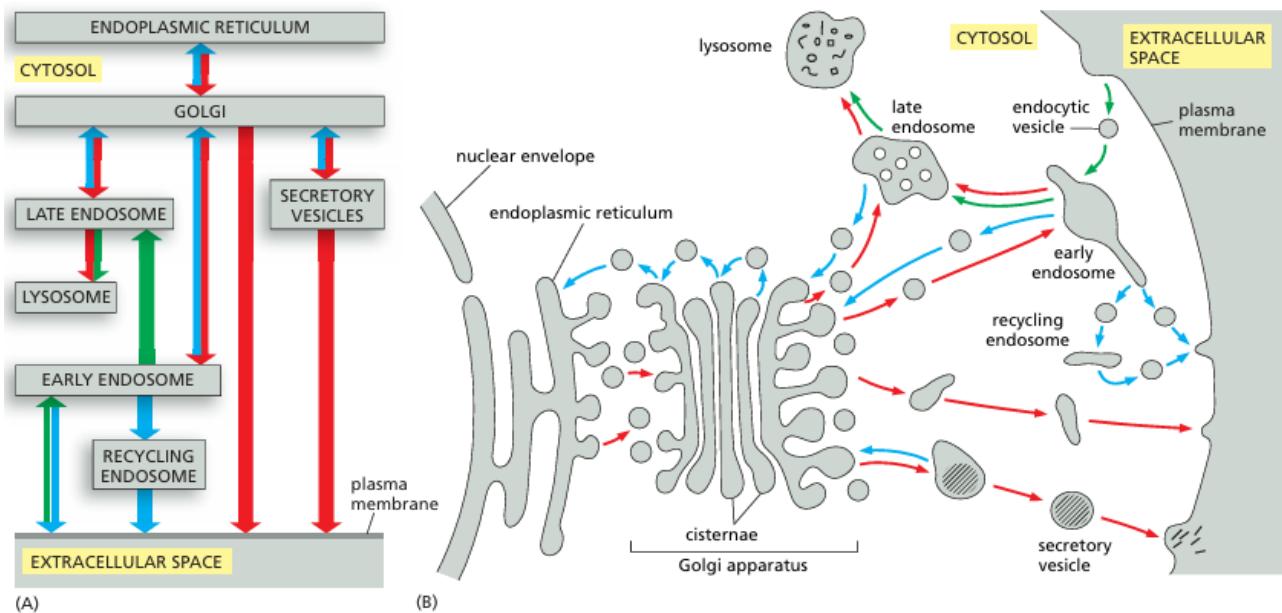


Figure 82: A “road-map” of the secretory and endocytic pathways.

5.0.1 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 5.1 (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 5.2 (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 5.3 (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.

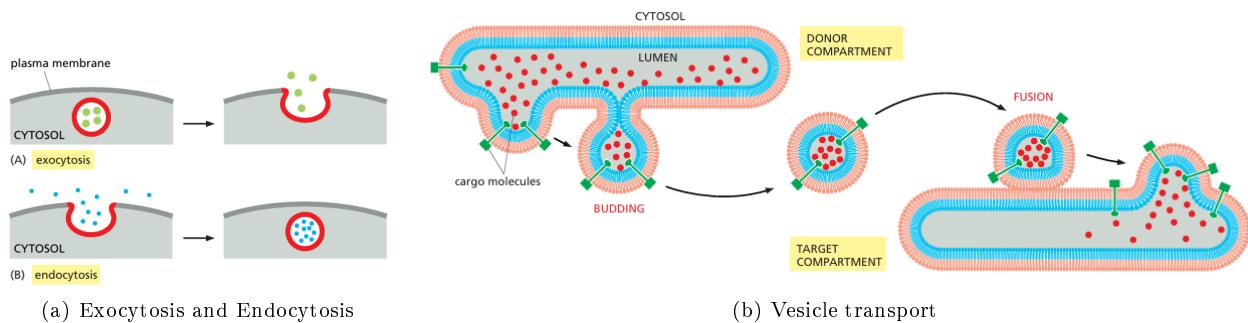


Figure 83: Topologically equivalent compartments, are able to "communicate"

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 **cote** 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 transport steps.

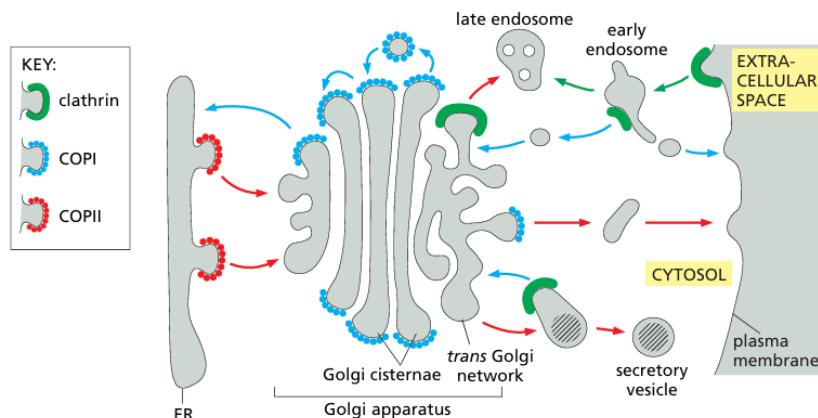


Figure 84: Use of different coats for different steps in vesicle traffic

5.0.1.1 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 **adaptor 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 adaptor proteins each is specific for a different set of cargo proteins.

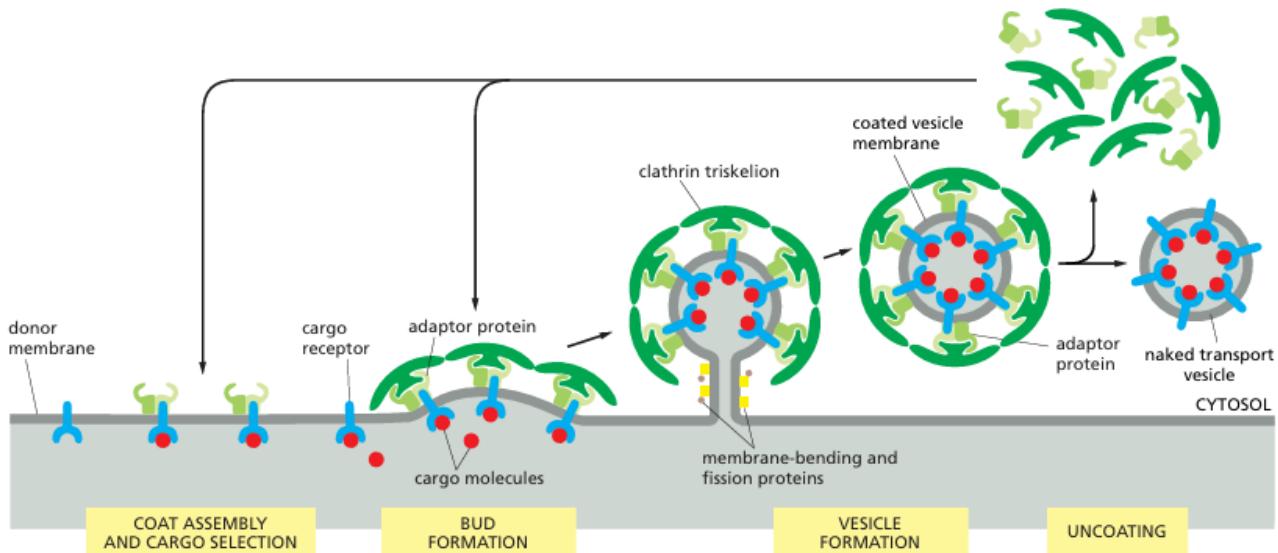


Figure 85: The assembly and disassembly of a clathrin coat

Example 5.4 (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. ??

Remark 5.5 (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. ??

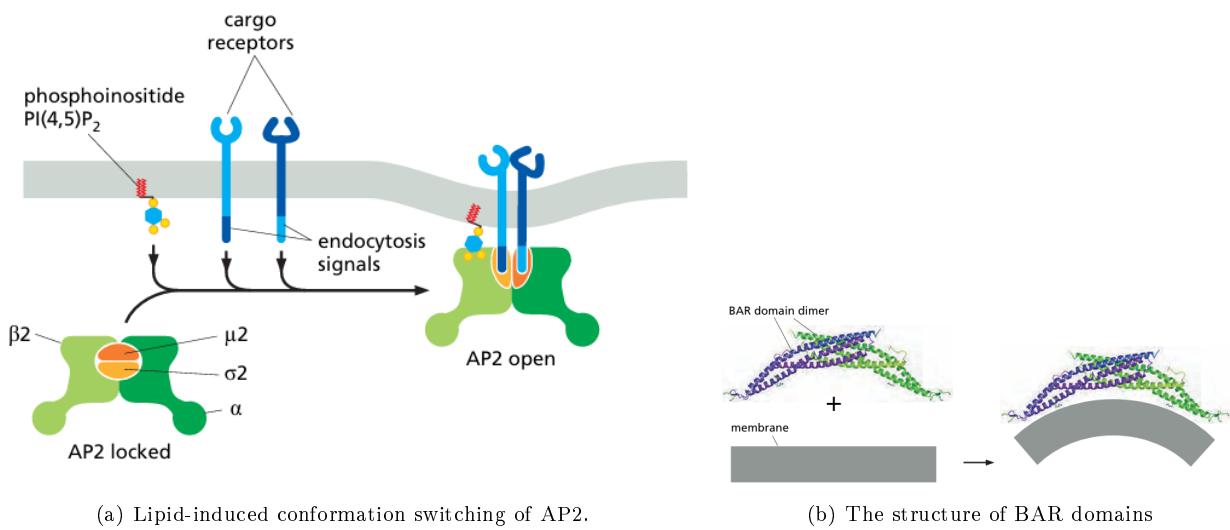


Figure 86:

As the bud grows, cytoplasmic proteins, including **Dynamin**, assemble at the neck. Dynamin contains a PI(4,5)P₂-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. ??

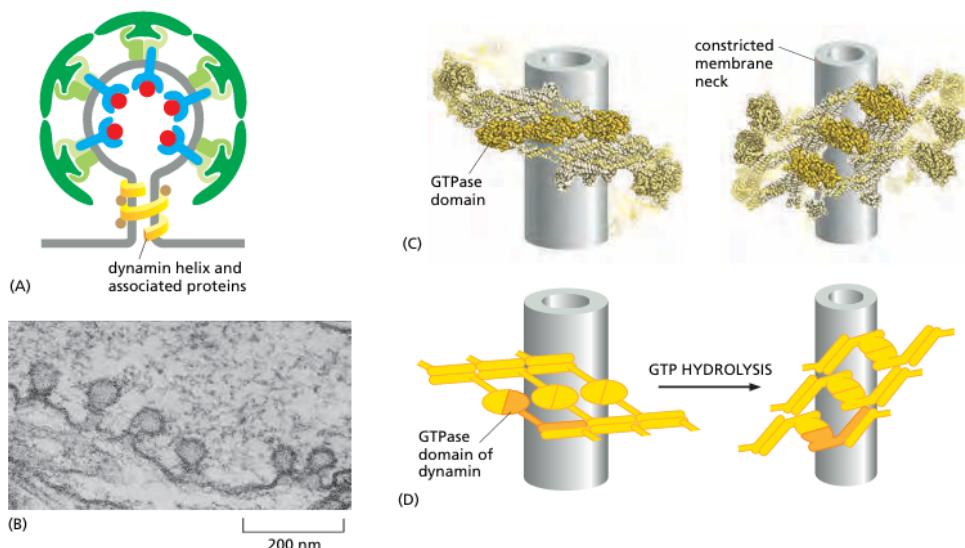


Figure 87: The role of dynamin in pinching off clathrin-coated vesicles.

5.0.1.2 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** is 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 subunits to initiate budding**. See fig. ??

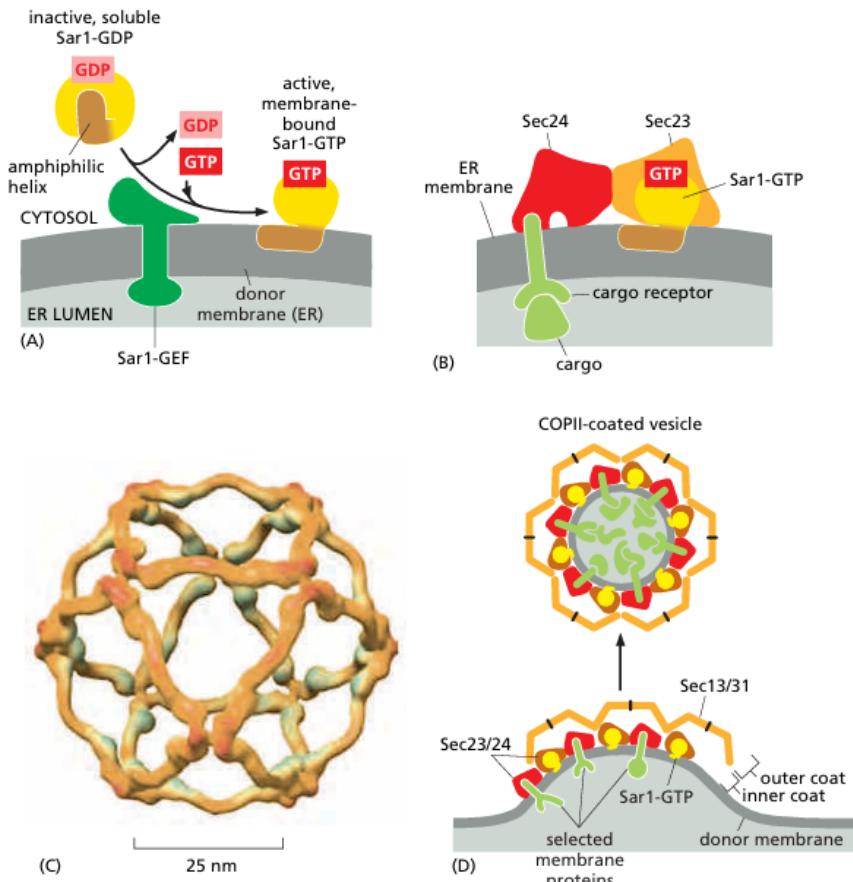


Figure 88: Formation of a COPII coated vesicle.

5.0.2 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 these 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 5.6 (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. ??

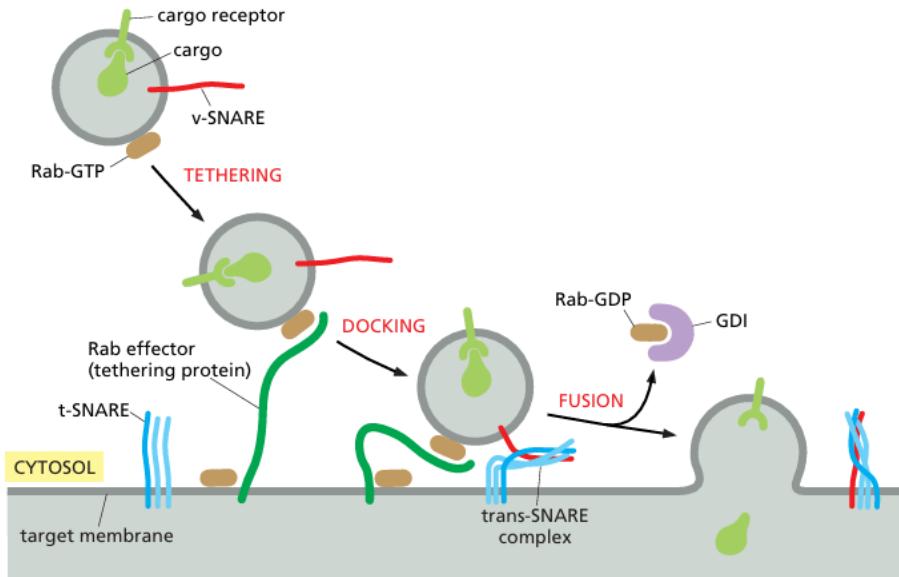


Figure 89: Tethering of a transport vesicle to a target membrane.

5.0.2.1 Rab proteins

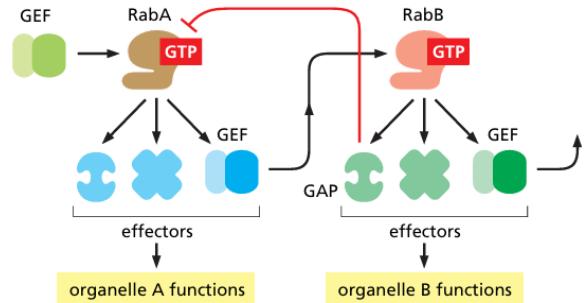
Rab protein is a family of small GTPases that regulate vesicle transport by ensuring specificity in vesicle targeting. Rab proteins recruit effector molecules that help guide vesicles to the correct membrane compartment.

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 90: 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.

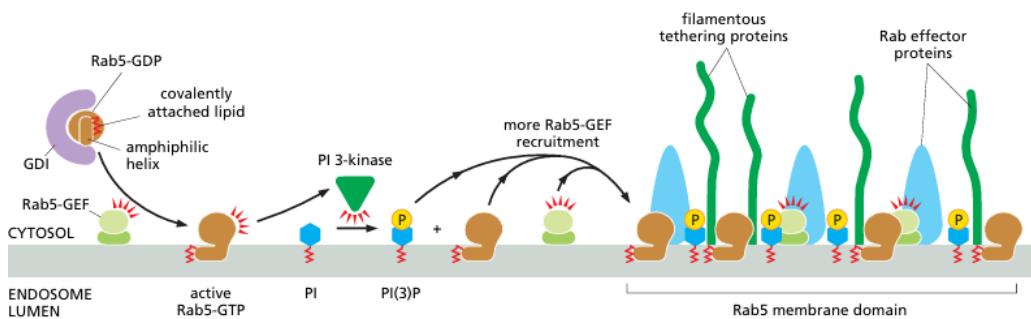


Figure 91: The formation of a Rab5 domain on the endosome membrane.

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. ?? 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.

5.0.2.2 SNARE proteins

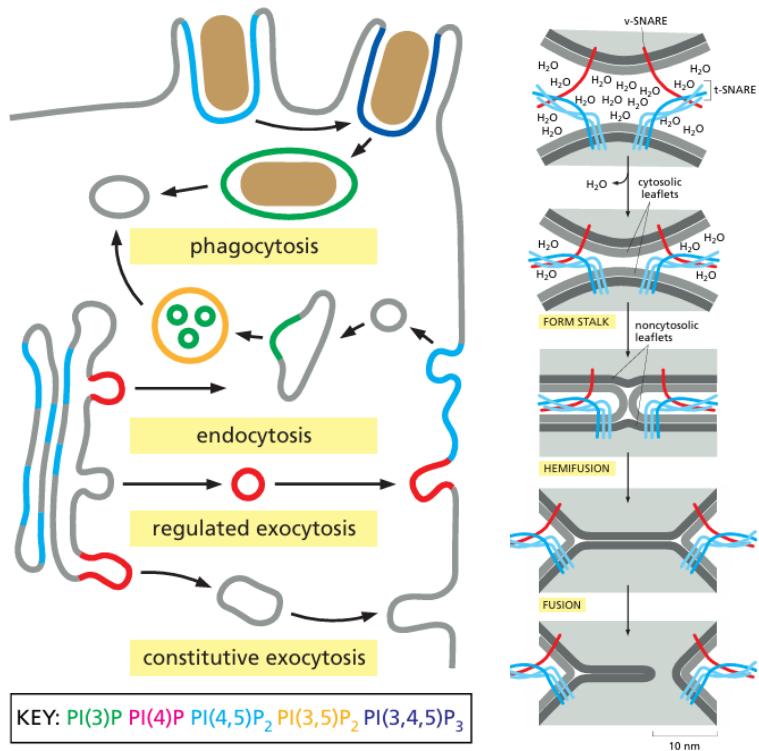
Membrane fusion requires bringing the lipid bilayers of two membranes to within **1.5 nm** of each other so that they can merge. When the membranes are in such close apposition, lipids can flow from one bilayer to the other.

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.



(a) The intracellular location of phosphoinositides. (b) SNARE proteins may catalyze membrane fusion

Figure 92:

Intacting SNAREs need to be pried apart before they can function again. A crucial protein to achieve 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.

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.

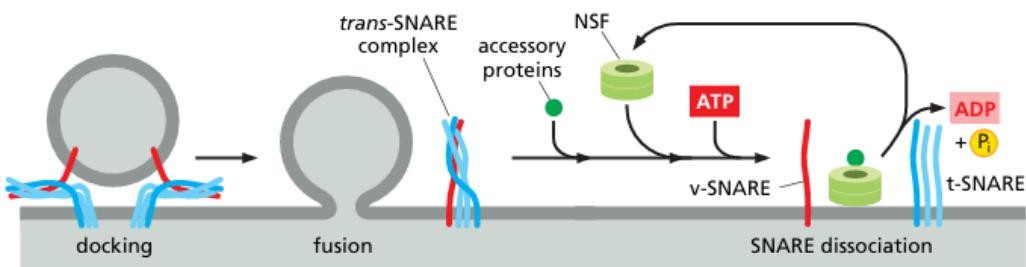


Figure 93: Dissociation of SNARE pairs by NSF after a membrane fusion cycle.

5.1 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 **Glogi 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 ans are destined for the Golgi apparatus or beyond are first packed into **COPII-coated** trasnport 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 signlas on their cytosolic surface that adaptor proteins of the inner COPII coat recognize. *Note some of theses 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 5.7 (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 **retrievel pathway**; They move quickly along **microtubules** (using **motor proteins**) from the ER to the Golgi.

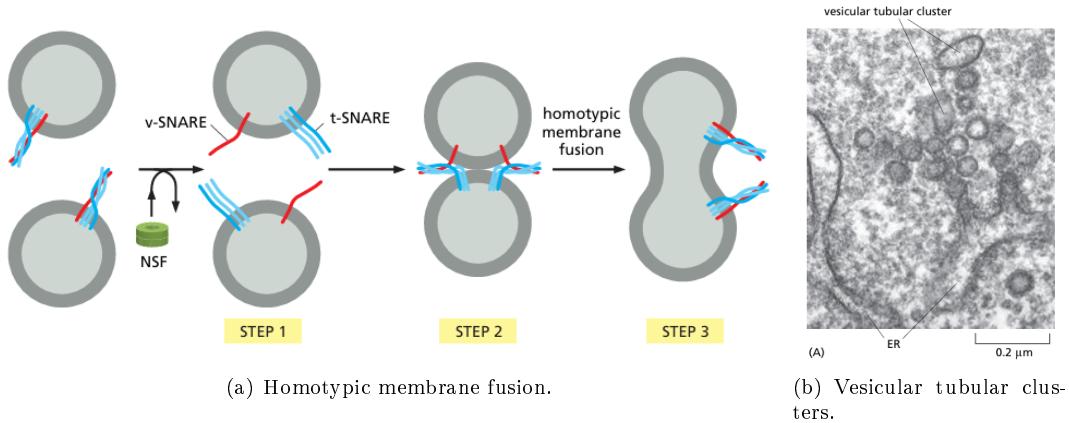


Figure 94: Vesicular Tubular Clusters Mediate Transport from the ER to the Golgi Apparatus

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 **COP1-coated** vesicles. Therefore resident ER membrane proteins contain signals that directly bind to COPI 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**. 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**.

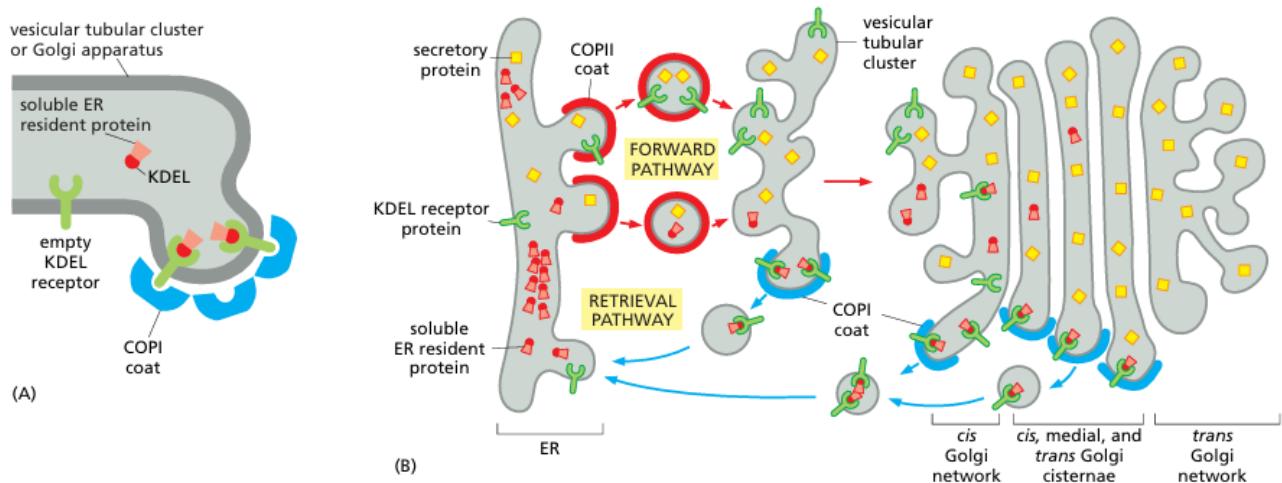


Figure 95: Retrieval of soluble ER resident proteins.

5.2 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.*

During their passage through the Golgi, transported molecules undergo an ordered series of modification. Each Golgi stack has 2 faces: a **cis face** (or entry face) and a **trans face** (or exit face).

5.2.1 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.

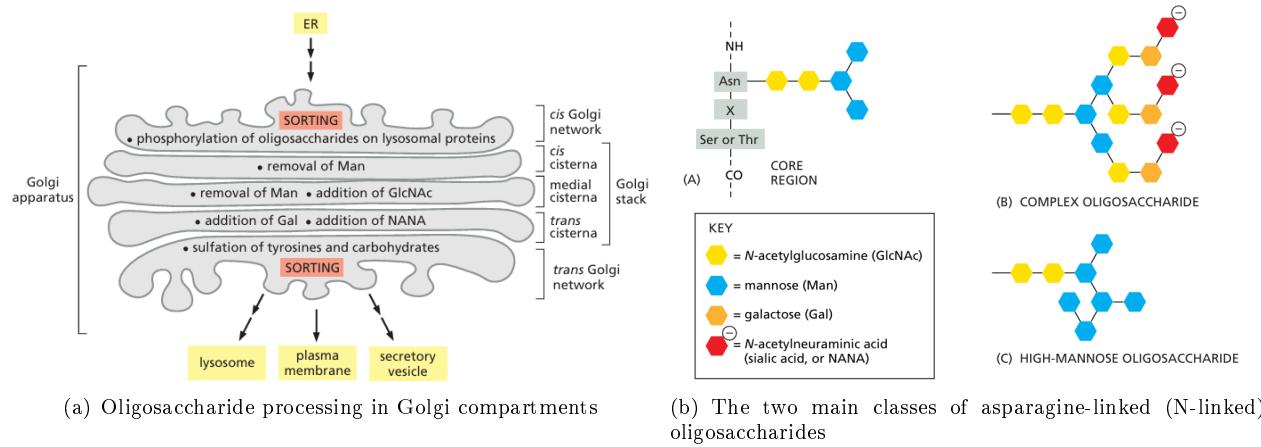


Figure 96: VOligosaccharide Chains Are Processed in the Golgi Apparatus

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

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 5.8 (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.

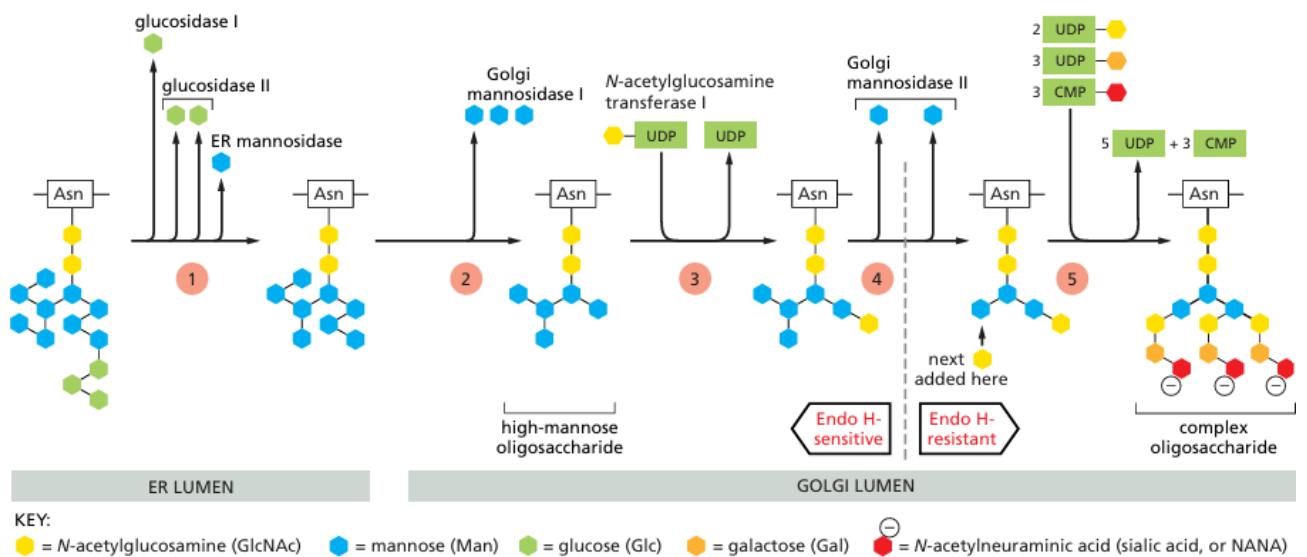


Figure 97: Oligosaccharide processing in the ER and the Golgi apparatus.

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**.

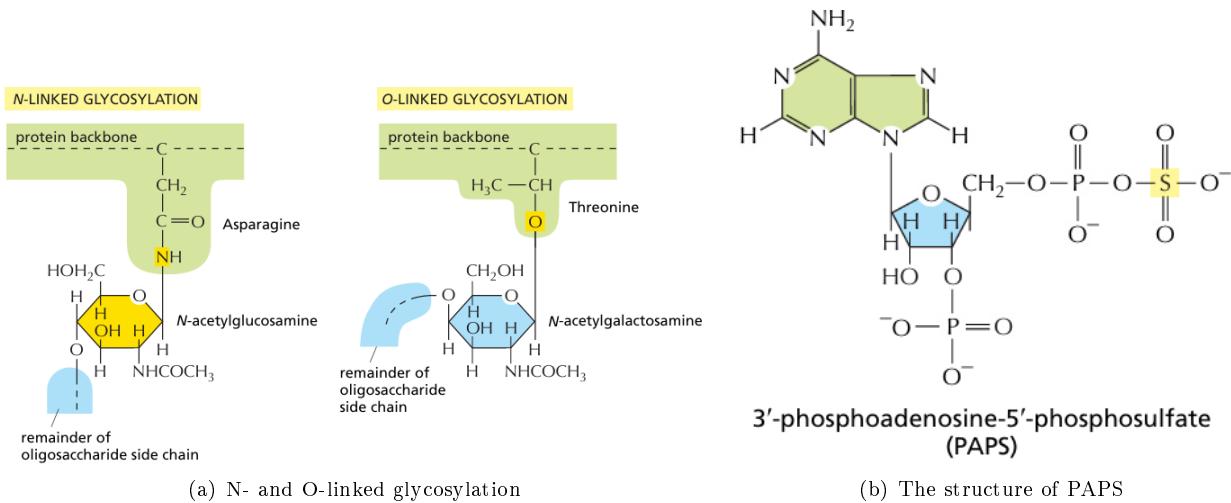


Figure 98: Proteoglycans Are Assembled in the Golgi Apparatus

5.3 Golgi to Lysosomes

5.4 Endocytosis

5.5 Exocytosis

Glossary

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

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.. 7

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

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

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

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