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# 1 cellular localization

## 1.1 compartments and organelles overview

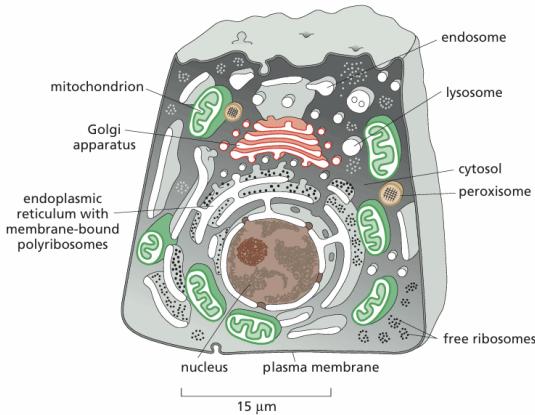


Figure 1: overview of compartments

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**

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#### 1.1.0.1 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  $\mu\text{m}^3$  compared with 1000  $\mu\text{m}^3$  for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000  $\mu\text{m}^2$  and 13,000  $\mu\text{m}^2$ , respectively.

Figure 2: table volumes of membranes

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

Table 1: 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:

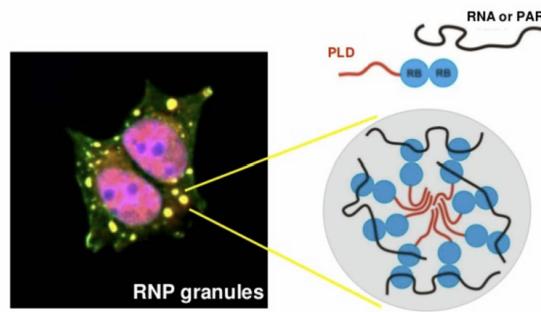


Figure 3: membrane less organelle overview of RNP

#### 1.1.1 Endoplasmatic reticulum

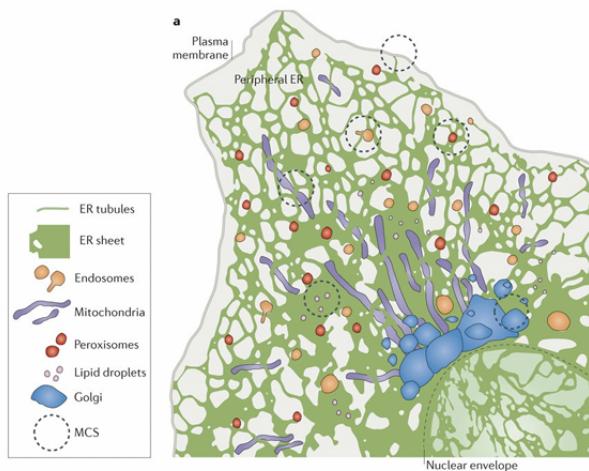


Figure 4: Structure of Endoplasmatic reticulum

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.

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#### 1.1.1.1 Smooth and rough ER separation

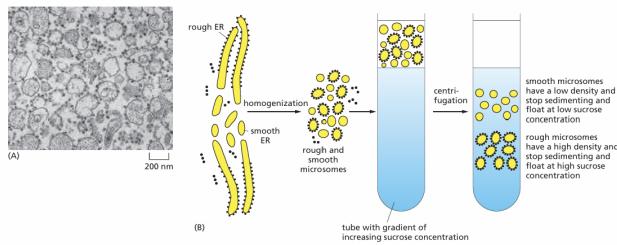


Figure 5: ER separation experiment

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

#### 1.1.2 Golgi system

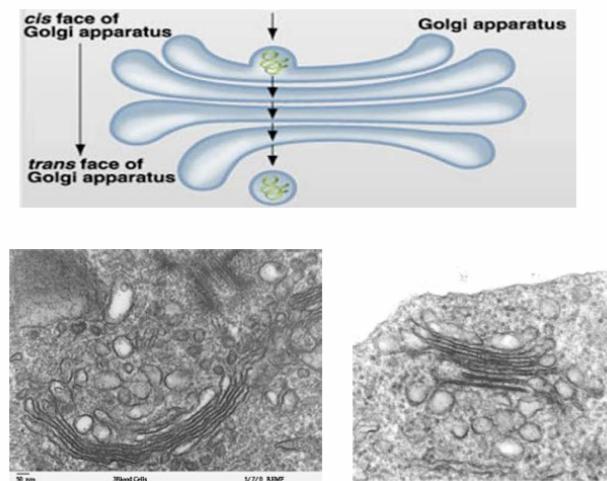


Figure 6: Golgi apparatus overview

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.

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### 1.1.3 lysosomes

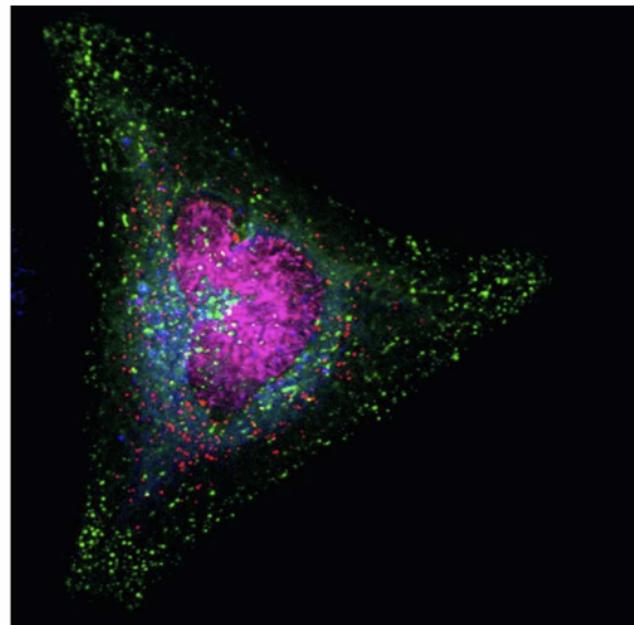


Figure 7: lysosome fluorescent microscopy image

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...)

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#### 1.1.4 Mitochondria

Mitochondria Structural Features

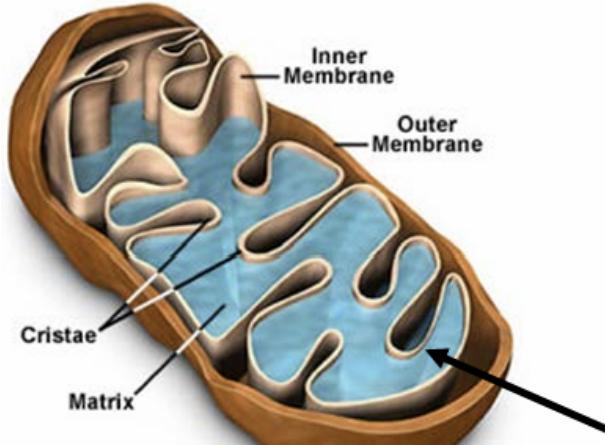


Figure 1

Figure 8: 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.

#### 1.1.5 Endosomes

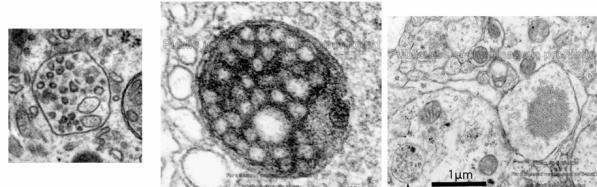


Figure 9: Endosomes

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

### 1.1.6 Peroxisomes

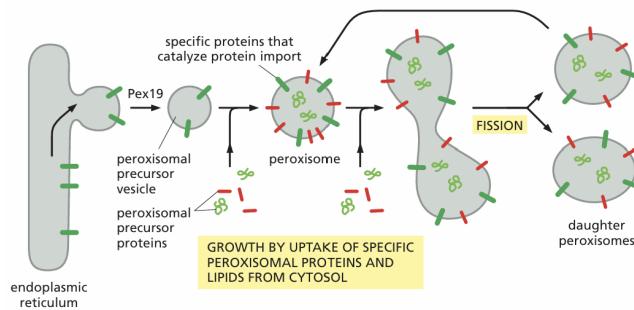


Figure 10: Peroxysomes proliferation

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  $\beta$ -oxidation.

### 1.1.7 lipid droplets

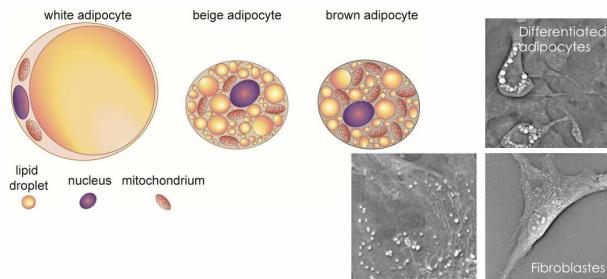


Figure 11: adipocyte overview

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

## 1.2 lipid composition of various organelles

The lipid composition is different across the various organelles.

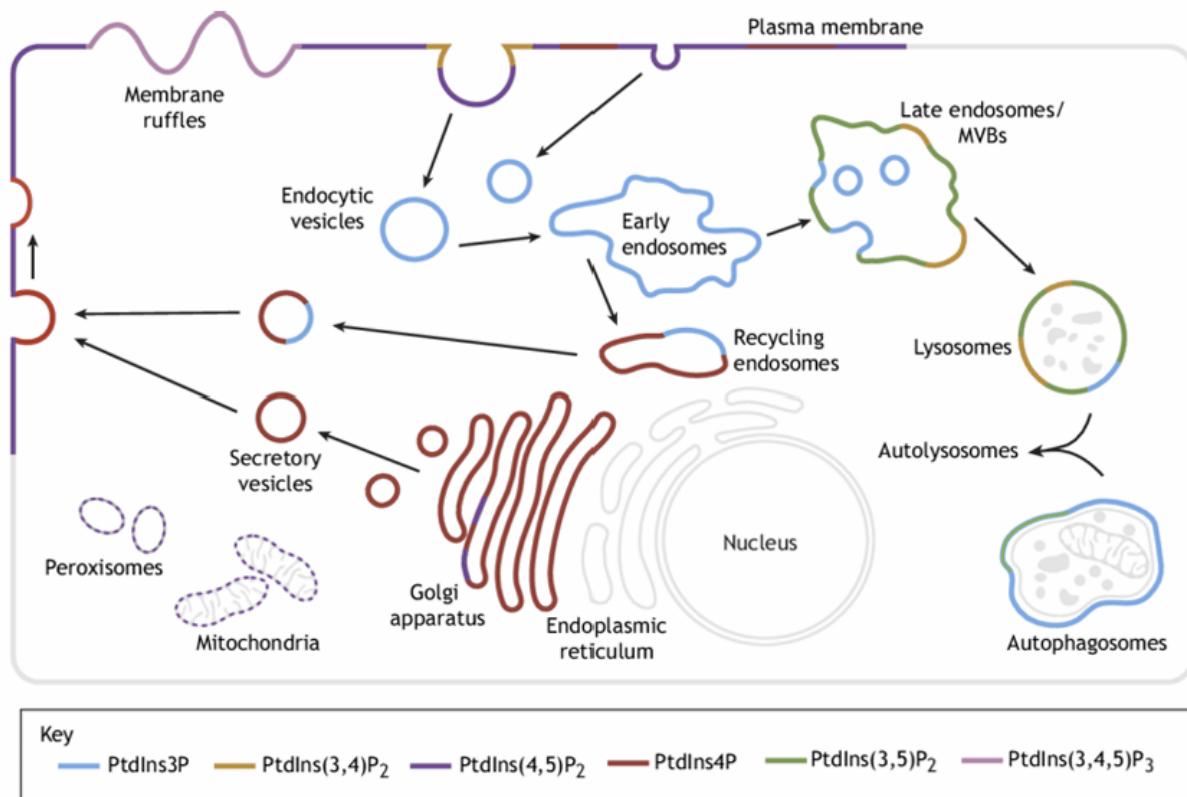


Figure 12: Phosphatidylinositol location and phosphorylation state

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

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

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

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

Figure 13: Membrane composition based on cell type

## 1.3 protein localization

### 1.3.1 types of transport

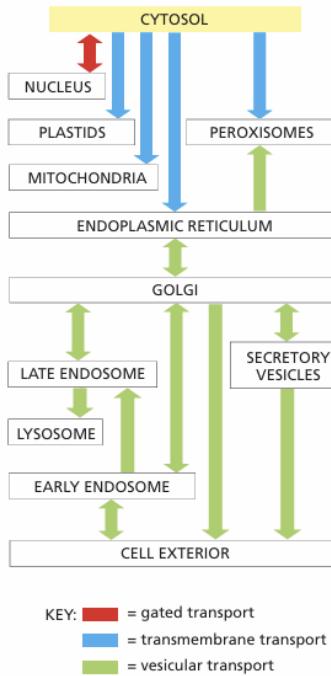


Figure 14: Types of transport overview

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.

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### 1.3.2 important signals

**TABLE 12-3 Some Typical Signal Sequences**

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

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

Figure 15: 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)

### 1.3.3 nuclear import

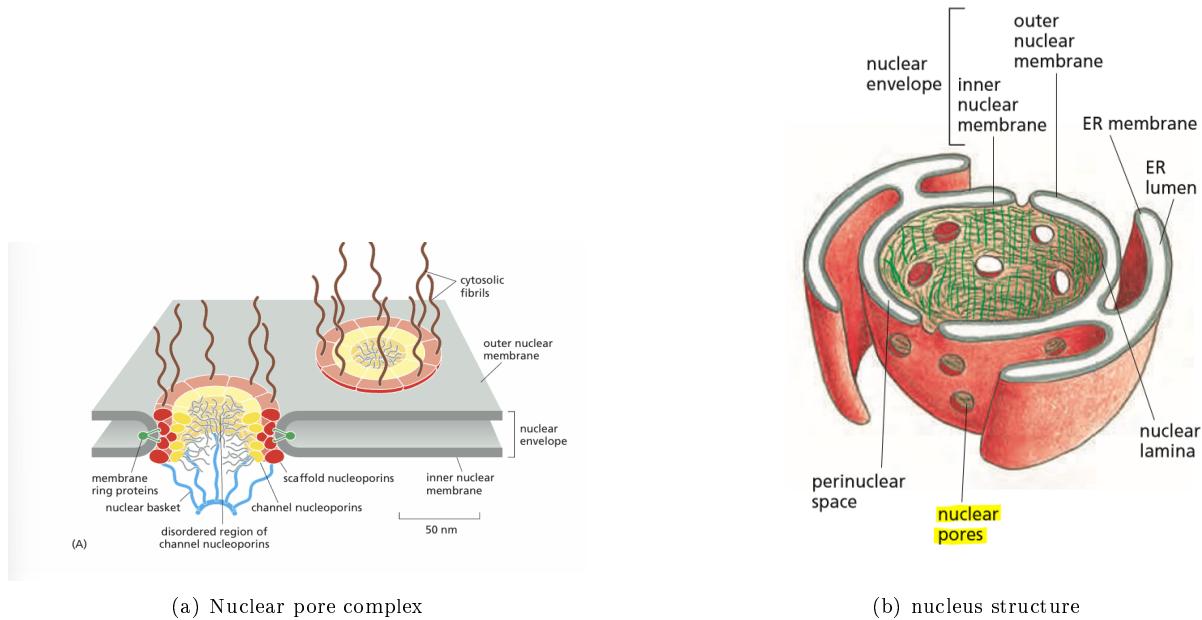


Figure 16: Application of protein design

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**

#### 1.3.3.1 note on ribosome production

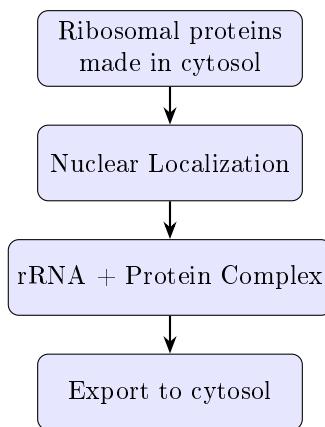


Figure 17: Overview of Ribosome Production and Trafficking

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

### 1.3.3.2 Nuclear import receptors(importins)

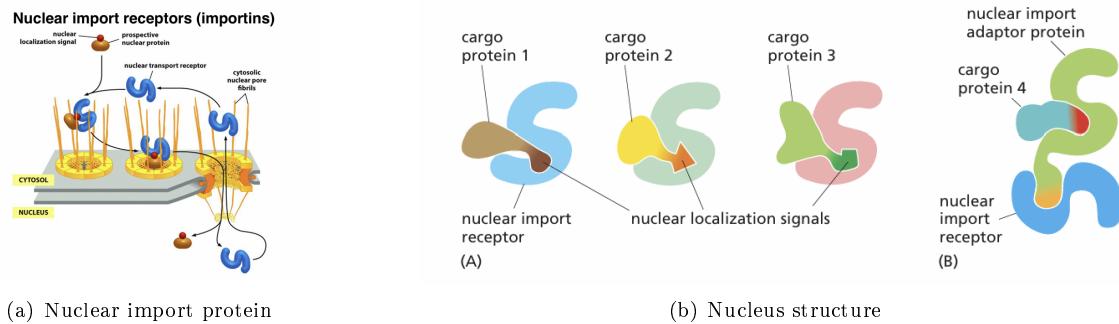


Figure 18: Importins bind to cargo proteins that have NLS

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**.

### 1.3.3.3 ran-GDP Ran-GTP

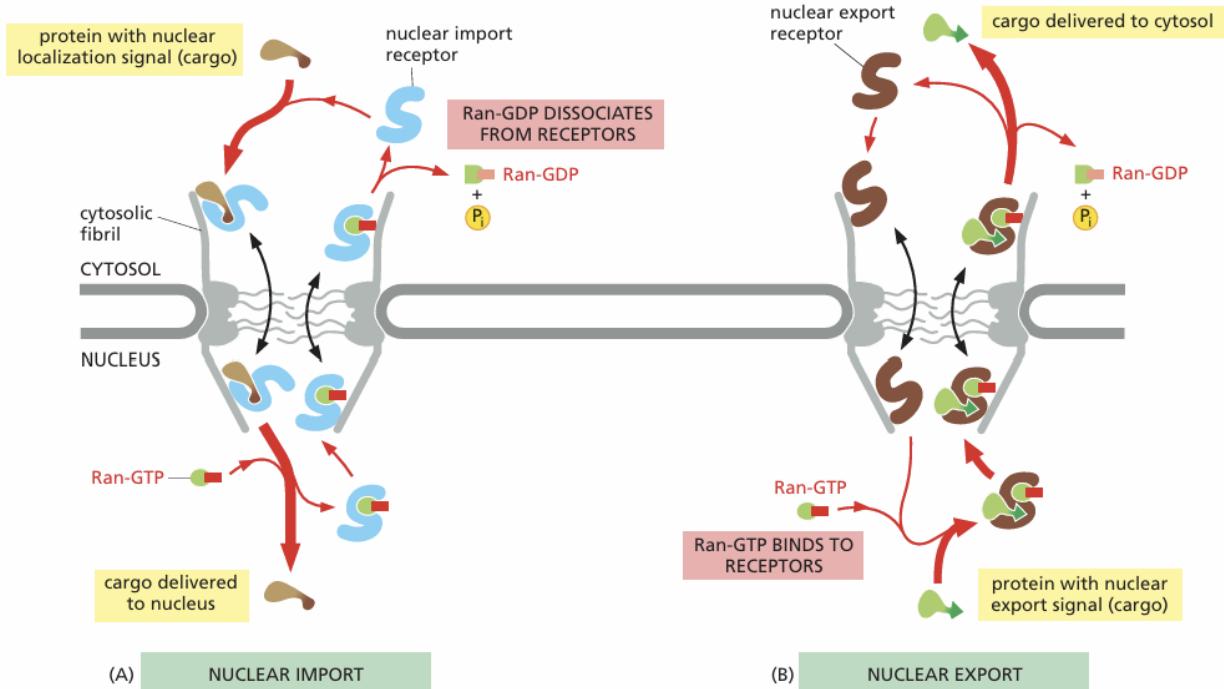


Figure 19: ran-GDP ran-GTP and it's role in shuttling cargo across nuclear pore complexes

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.

#### 1.3.3.4 nuclear import control during T cell activation

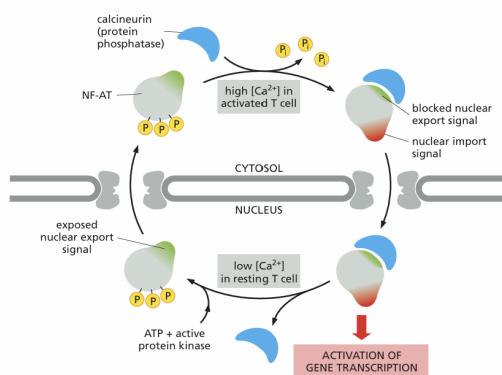


Figure 20: role of nuclear import/ export signals in T cell activation

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

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

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

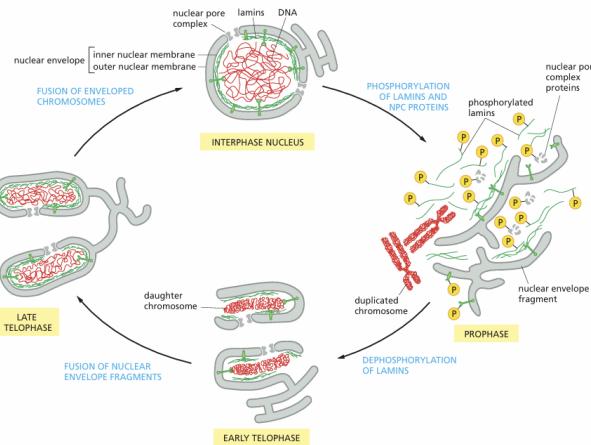


Figure 21: Reformation of the nucleus requires resusable NLS

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**

### 1.3.4 main transport pathways for proteins

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

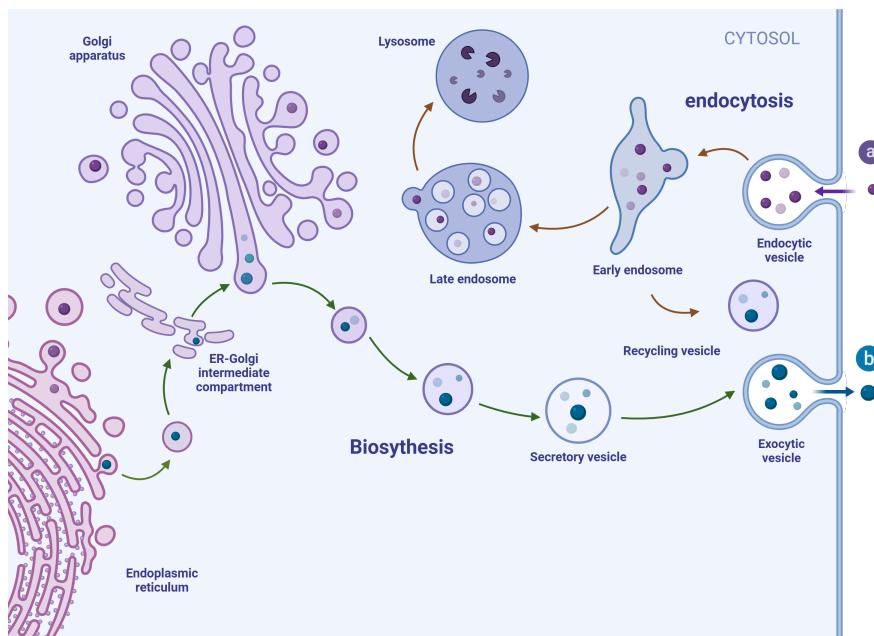


Figure 22: Main Pathways looked at in this chapter

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**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

### 1.3.5 translocation to ER

#### 1.3.5.1 cotranslational and post translational localization

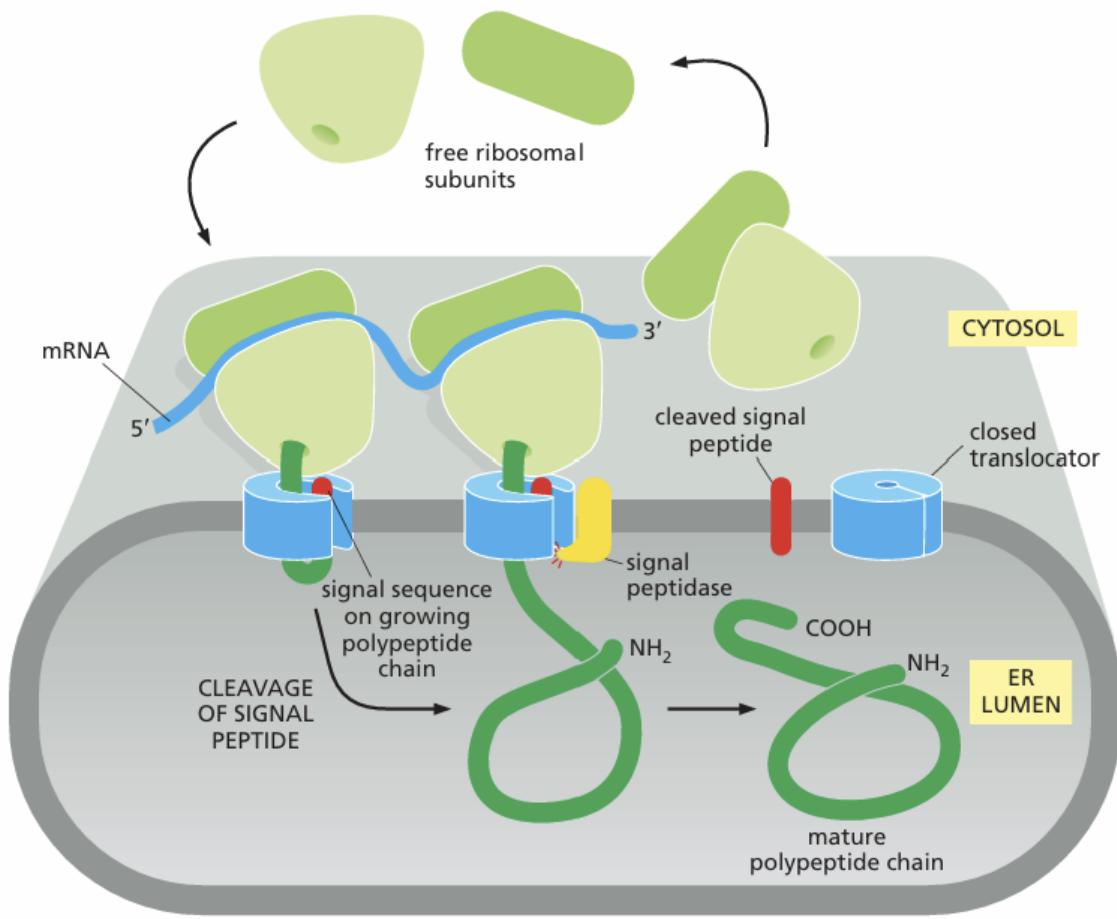


Figure 23: co and post translocation to the ER

**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.

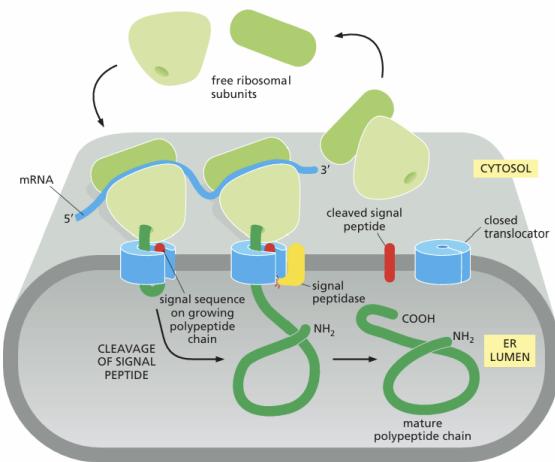


Figure 24: Co translational translocation

### 1.3.5.2 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.

### 1.3.5.3 transmembrane proteins

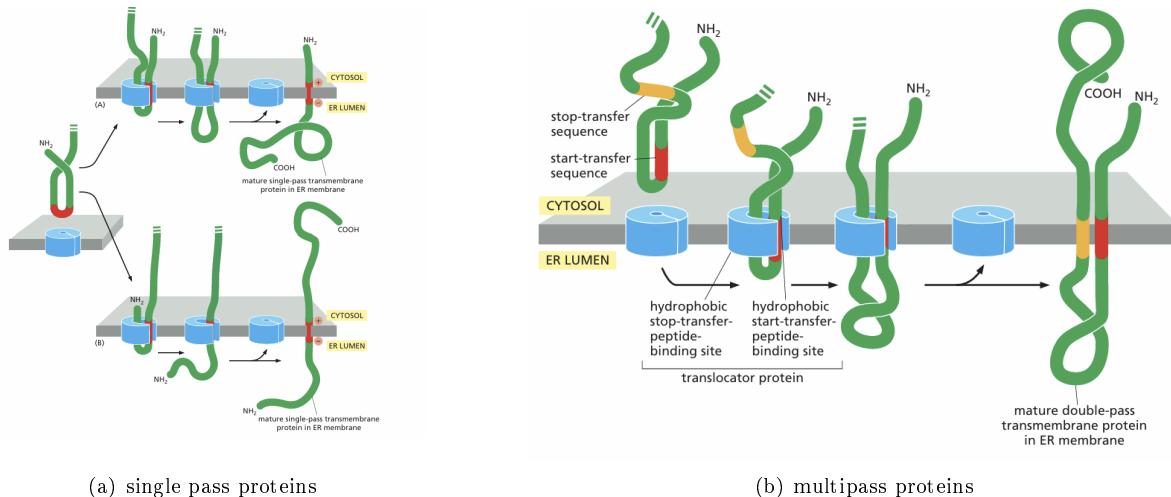


Figure 25: Role of localization in transmembrane protein translation

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.

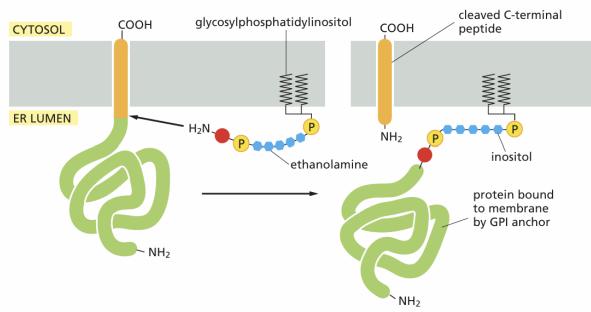


Figure 26: GPI anchor synthesis

**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

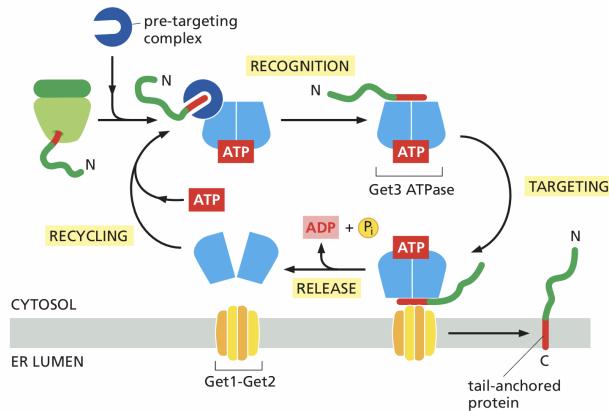


Figure 27: tail anchored proteins

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

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

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

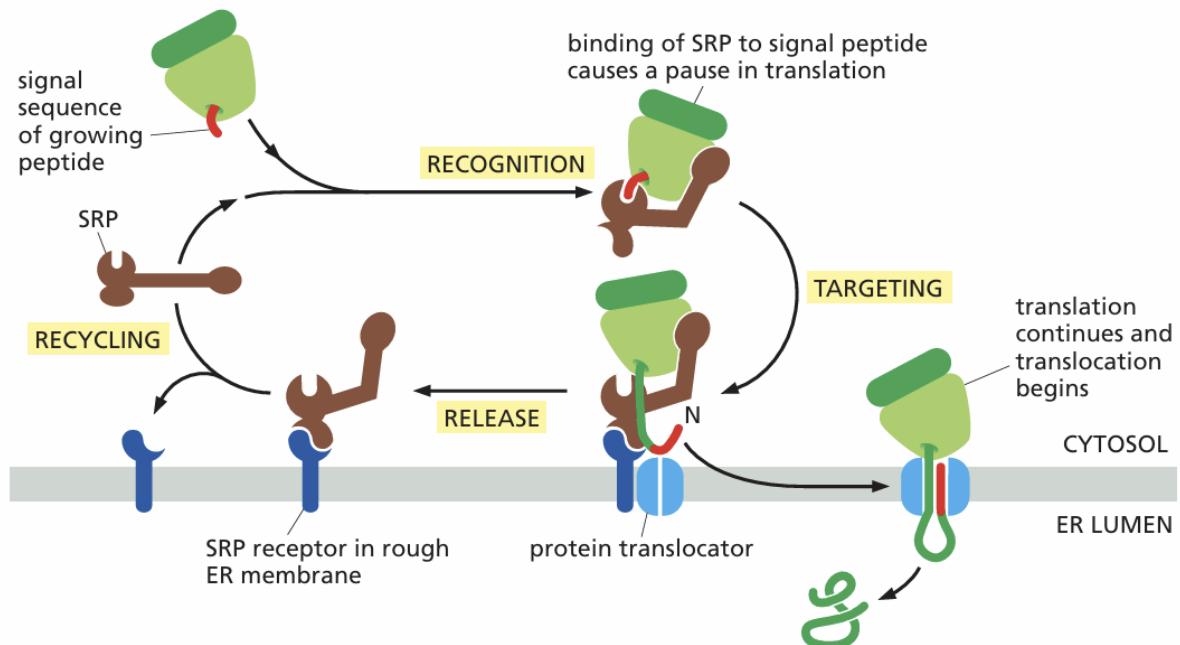


Figure 28: The SRP function in ribosome localization

- 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.

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

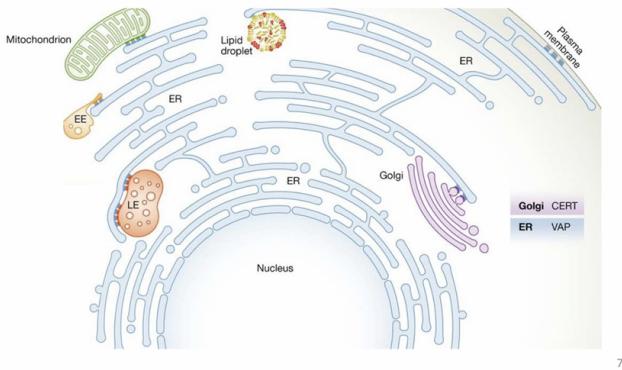


Figure 29: Contact patches role in protein transport

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**

### 1.3.7 topological equivalence principle

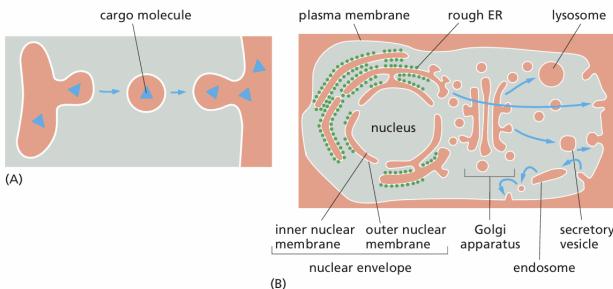


Figure 30: topological equivalence

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**.

#### 1.3.7.1 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**

### 1.3.7.2 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.

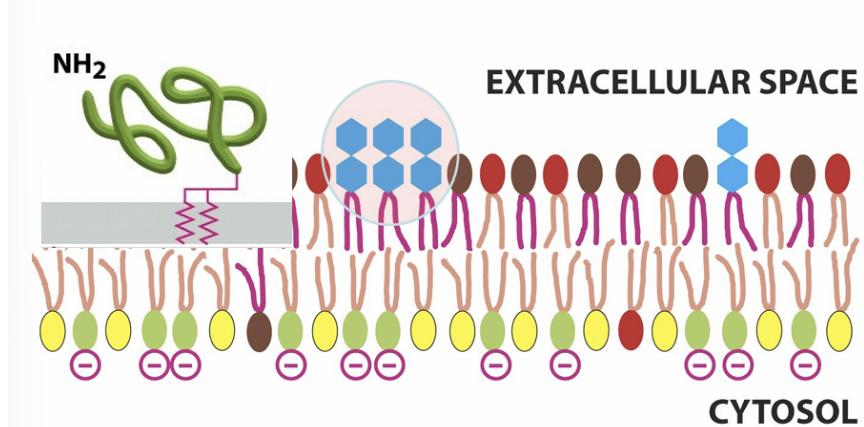


Figure 31: Asymmetry in membrane leaflets

## 1.4 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**

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

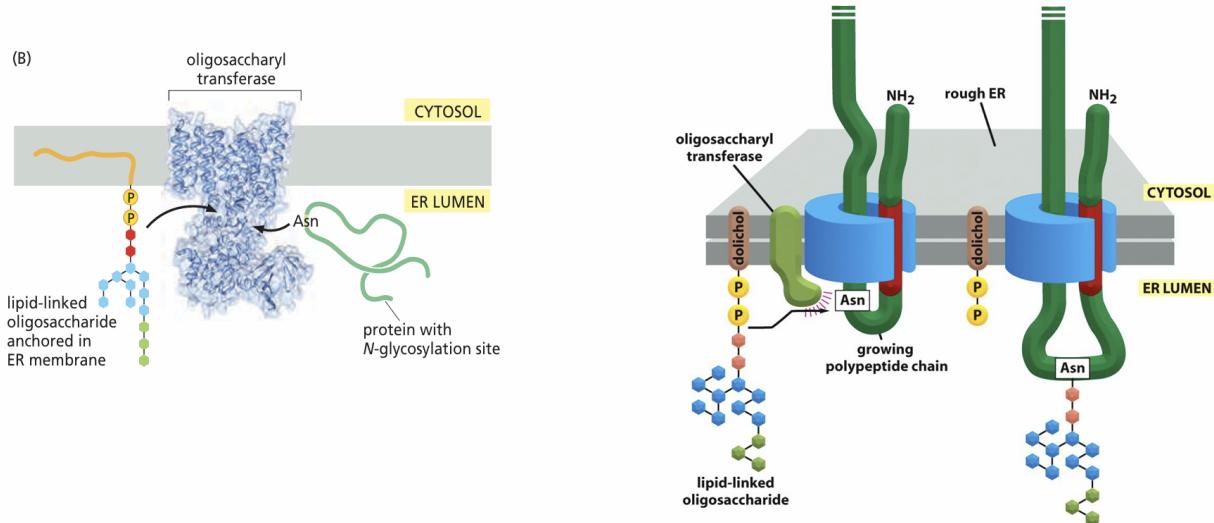


Figure 32: ER linked glycosylation

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.

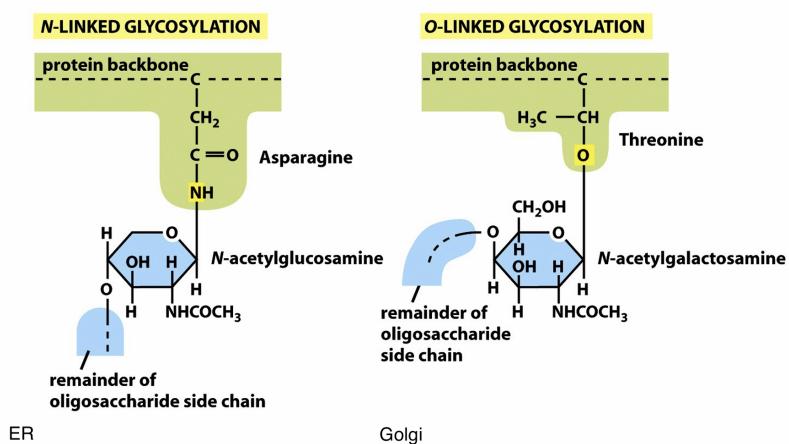


Figure 33: N linked vs O linked glycosylation

**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)

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

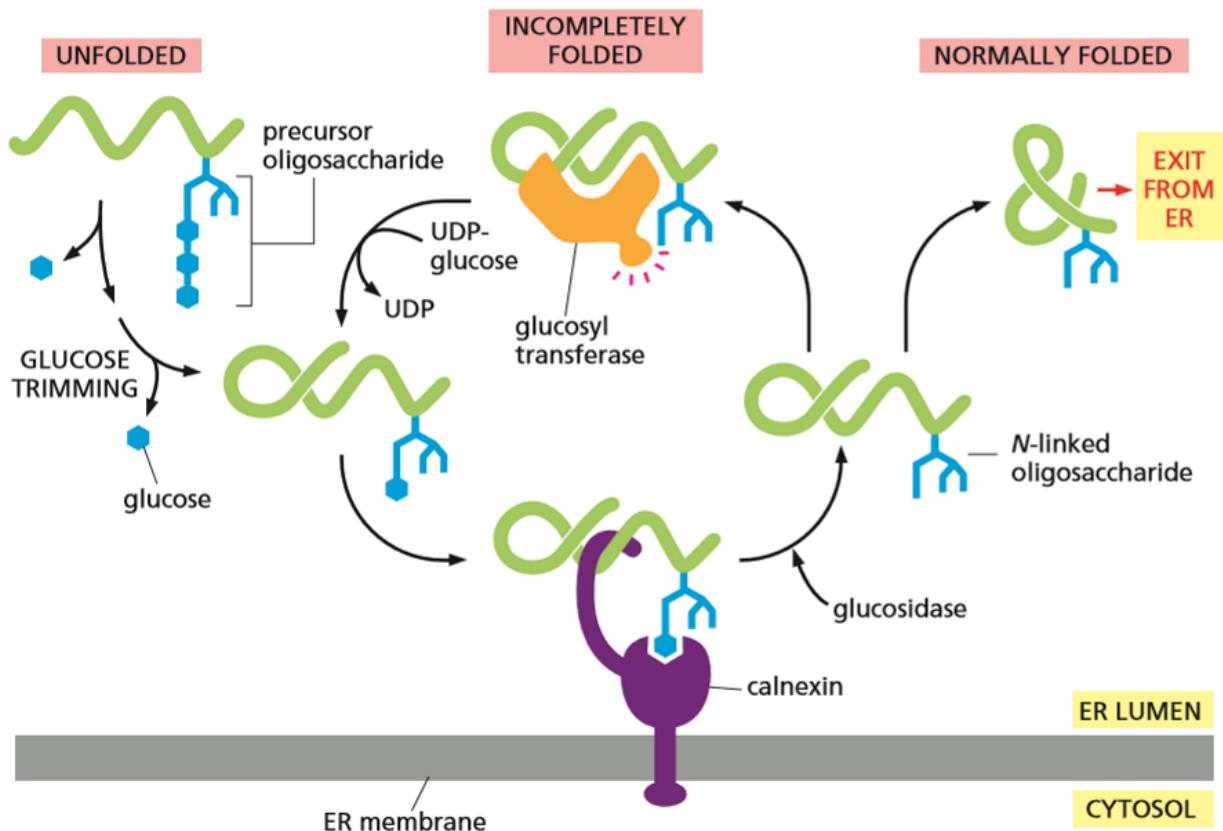


Figure 34: Calnexin/Calreticulin cycle cycle in ER

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.

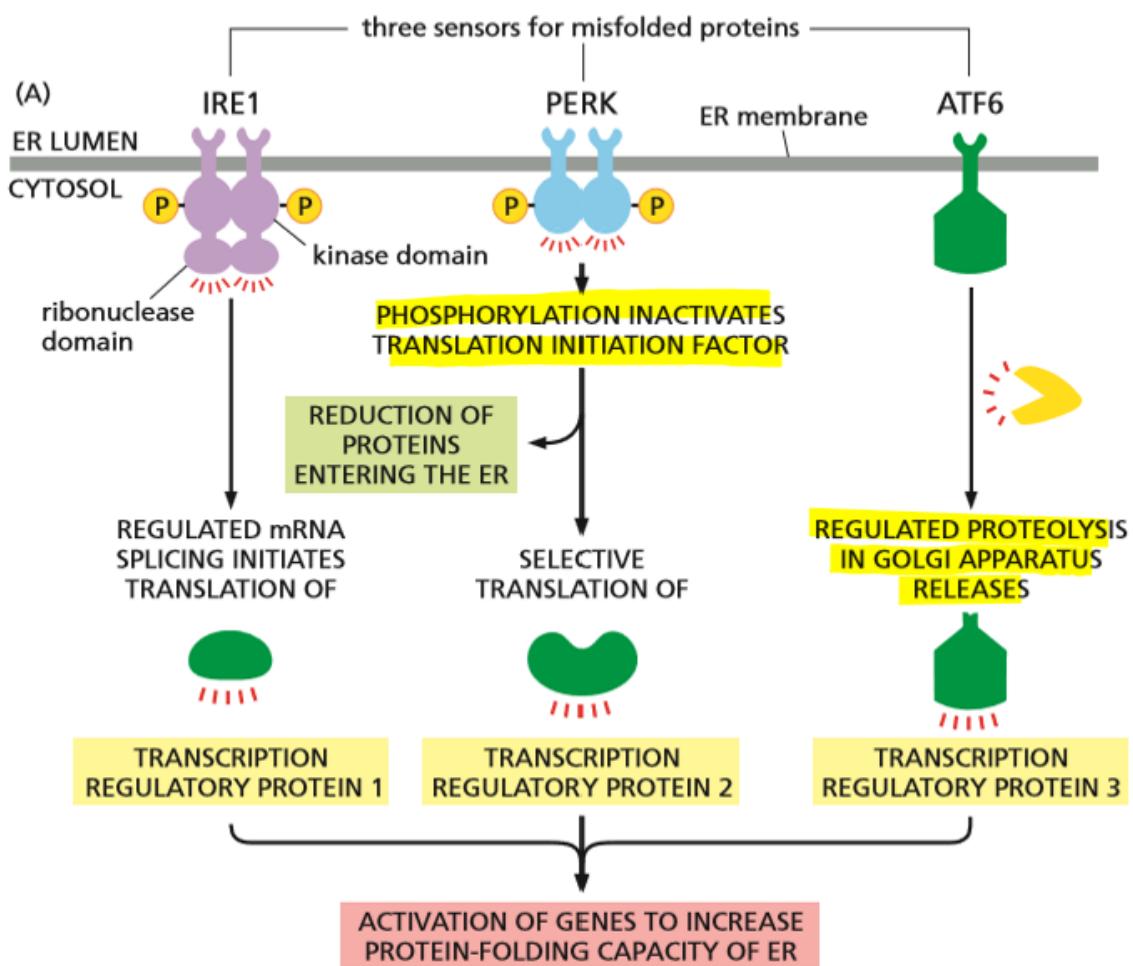


Figure 35: the unfolded protein response

### 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.

## 1.5 phosphatydilcholine sythesis in Er

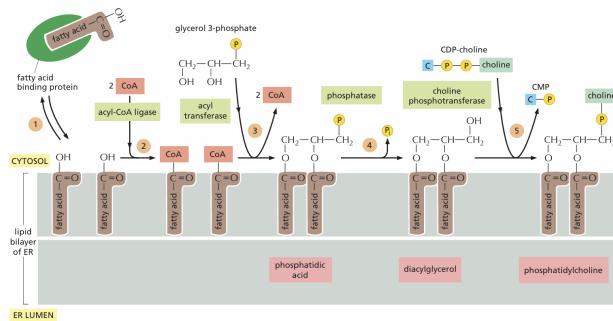


Figure 36: Phosphatidyl Choline Synthesis

Phosphatidyl 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.