The origins of microbiology

- **Lucretiu** and **Fracastoro** suggested disease was caused by invisible living creatures.
- **Stelluti** seen microoranisms using microscope supplied by Galileo.
- **Hooke** published first drawings of microorganisms
- **Antony van Leeuwenhoek** developed better microscopes- magnification of ~50-300x

Spontaneous Generation: living organisms could develop from non-living matter
Challenged by **Redi** → supported by **John Needham** (life developed in lightly boiled meat broth sealed after heating) → X Chllenged by **Spallanzani** (no life developed in strongly boiled broths, sealed before heating)

Pasteur- swan-necks flasks
Flasks of broth with "swan-necks" were boiled, but not sealed

- If the swan-neck was left intact → no life developed in the broth
- If the swan-neck was broken (letting in both air and dust) → growth occur

Germ theory of disease

People believed in disease spread by miasmas and imbanlance of the 4 humors in the body.

- **Pasteur-** disease was due to microorganisms, not miasmas
- **Koch-** specific microorganisms were the cause of particular diseases
    - Developed agar and petri dishes → produce pure cultures

Koch's (original) postulates

1. The microorganism must be present in every case of the disease but absent from healthy organisms
2. The suspected microorganisms must be isolated and grown in a pure culture
3. The same disease must result when the isolated microorganism is inoculated into a healthy host
4. The same microorganisms must be isolated again from the diseased host

!! Not always Feasible

- Some pathogens are part of the normal microbiota
- Some pathogens cannot be grown/cultured
- Some pathogens grow/cause disease only in humans
- Sometimes more than on pathogen is involved in a disease. E.g. polymicrobial disease (periodontal disease)

Koch's Molecular Postulates

Emphasis is on the **Virulence Genes** rather than the microorganism itself

- VG encode characteristics which allow the microorganism to cause disease

- VG must be found in pathogenic strains
- VG must be expressed during infection/disease
- Mutation (or deletion) of the VG decreases pathogenicity
- Replacement/restoration of the VG mutation/deletion restores pathogenicity

The Host I & II

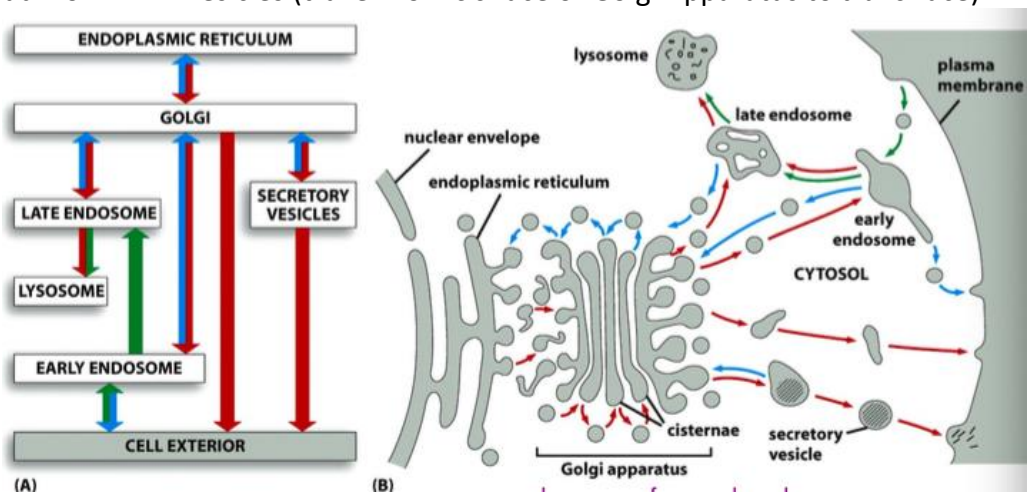
Intracellular transport: move constituents in an out of the cell

- Residency: most proteins made in the ER but may have a function elsewhere
- Secretion: want some molecules to be expelled to the outside of the cell (antibodies)
- Required upon stimulation (Regulatory pathway→hormone release)

The secretory pathway

Protein destined for the PM, endosomes/lysosomes or secretion

- Synthesised by the RER
- Contain specific amino acid sequence (signal sequences) which target them to lumen of RER
- Proteins may be glycosylated while passing through the ER
- Then bud from ER in vesicles (travel from *cis* face of Golgi Apparatus to *trans* face)



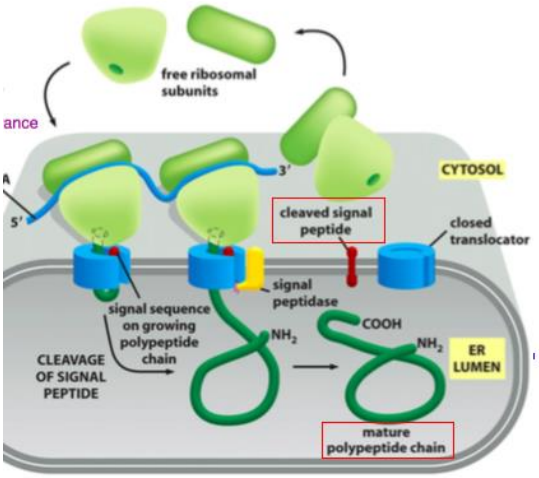
To localize to specific sites within the cell → protein encode signal sequences (Motifs) →recognised by adaptor proteins embedded in the organelle membrane

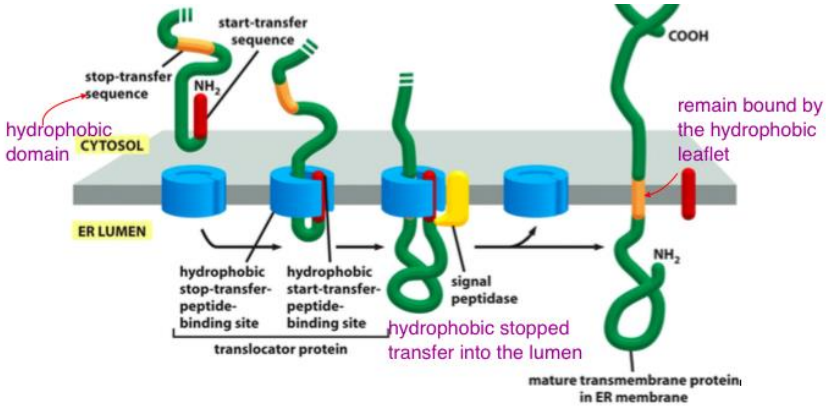
Import into the ER

Motifs: 12-16 hydrophobic amino acid flanked by basic "charged" residues → **signal sequence/peptide**
Once translocation into the ER has occurred → signal peptide is removed and release mature protein into the ER (Co-translational translocation)

IF protein contains additional hydrophobic regions, these will embed the protein within the membrane

- Enable signaling, some part of the protein exposed to the outside, some exposed to the inside
- **Multiple 'membrane spanning'** or 'transmembrane' (hydrophobic) domains may exist within a protein →tightly associated with the membrane →high affinity

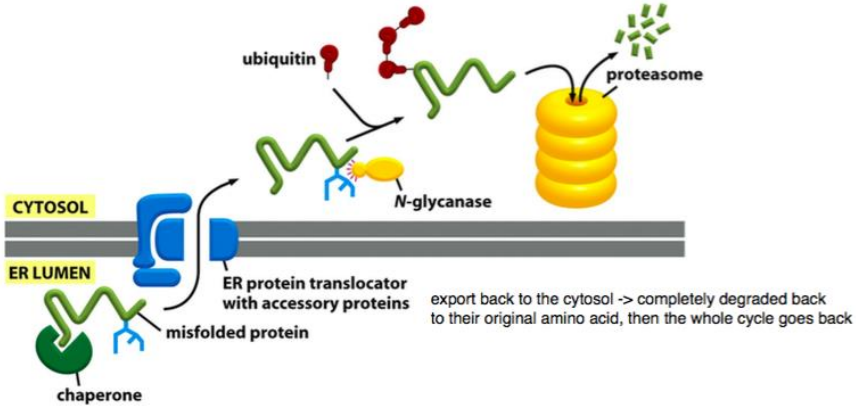




Proteins within the ER

- Require glycan "sugar" modification → Mannose
- Protein maturation: protein **chaperones** → correctly fold the protein (enable full function)
- Protein **calnexin** recognizes the newly attached mannose residue (bind to incompletely folded proteins)

Misfolded/incompletely folded protein → expelled from the ER → Ubiquitined → proteasome for degradation.



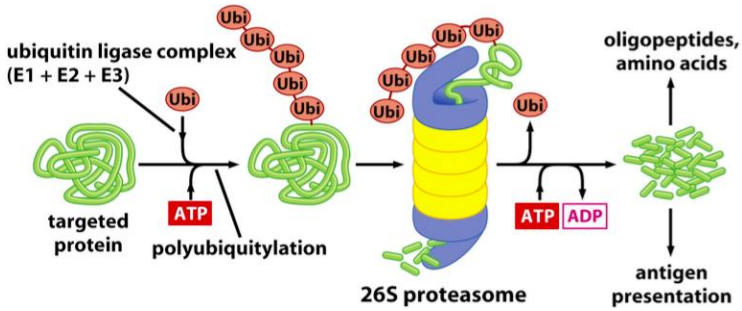
Ubiquitin and Proteasome

Unfolded and mis-folded proteins are

- Secreted into the cytosol
- Targeted for destruction by **ubiquitination**
- Degraded by the **proteasome** → produce small peptides (antigen presentation during immune response) and amino acids (recycling)

Ubiquitin-proteasome pathway

Protein destined for degradation + **E1, E2, E3 (ubiquitin ligase)** →**polyubiquitylation** → in proteasome **(de-ubiquitylated)** → degrade into **oligopeptides** → amino acids



When cell is under stress (pathogen infection, alcohol, cancer)

- Expand the ER size to accommodate the increase load
- System failed/shut down cellular translation and degrade excessive protein, so the pathogen can't spread on

ER stress or Unfolded Protein Response (UPR)

UPR activation → Increases protein folding, transport and ER-associated protein degradation

PERK stop producing protein, apoptosis**, IRE1** increase the amount of chaperones and proteases**, ATF6** – increase the size of the ER, activate degradation

If protein misfolding is not resolved → cells enter **apoptosis**

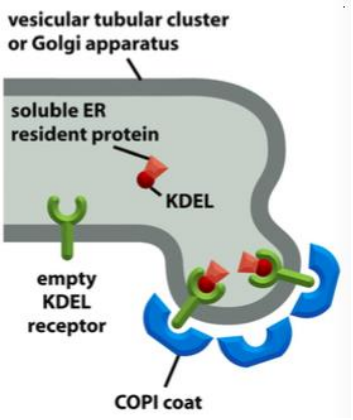
Export from the ER

COP I: Retrograde- returning the Golgi back to the ER, helps to mediate, retrieves protein back

COP II: Anterograde- forward moving from ER o Golgi

Luminal proteins contain the **motif- KDEL** at their c-terminus

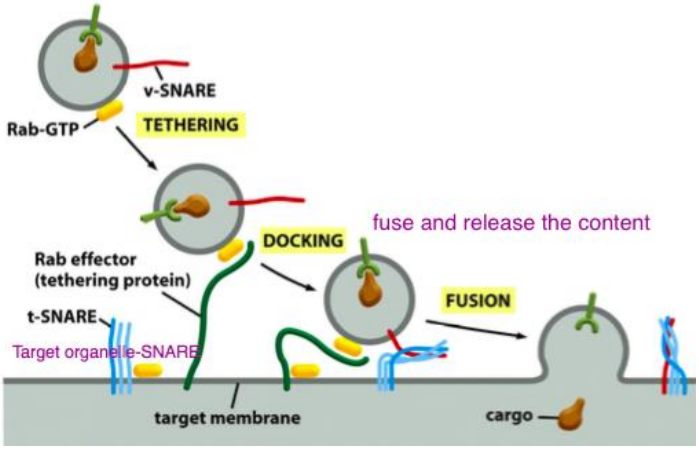
- Recognized by the **KDEL receptor** that interacts with **COP I**



Vesicular Trafficking

Transferring cargo via membrane fusion (similar to virus)

- SNARE proteins make contact and fuse vesicle membrane with target membrane → v-SNARE (vesicle SNARE), t-SNARE (target SNARE)



Glycosylation in Golgi – vital role in protein localization, function and recognition
Different stack of Golgi with different events occurring. (Modification)
Modification of glycan can occur within the ER, Golgi and Endosomes

Constitutive secretory pathway

Operates in all cells. Many soluble proteins are continually secreted from the cell by this pathway, which also supplies the plasma membrane with newly synthesized lipids and proteins.

Regulated secretory pathway

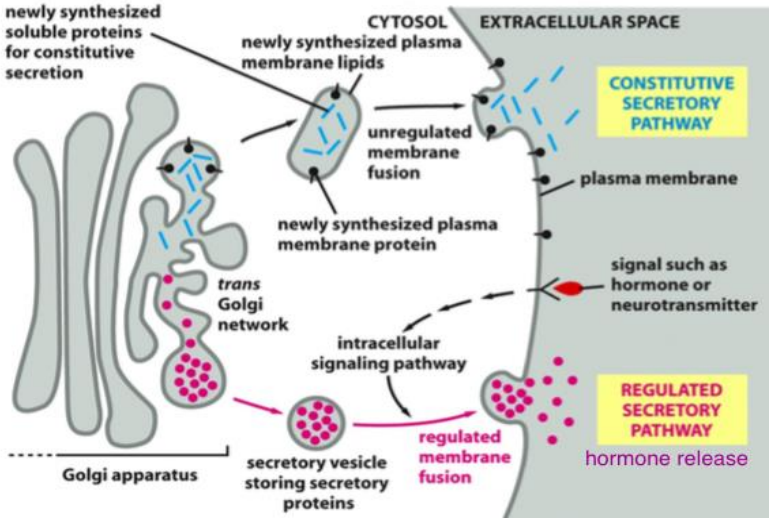
Selected proteins in the *trans* Golgi network are diverted into secretory vesicles, where the proteins are concentrated and store until an extracellular signal stimulates their secretion.

Vesicular trafficking- mediated via microtubules and microtubule motor proteins

- **Kinesin** drives transport from ER to PM
- **Dynein** drives transport from PM to ER

Vesicular Trafficking- **cytoskeleton**
Poxvirus utilisation of the cytoskeleton

- Moves from ER to PM via microtubules
- Exits cells via actin



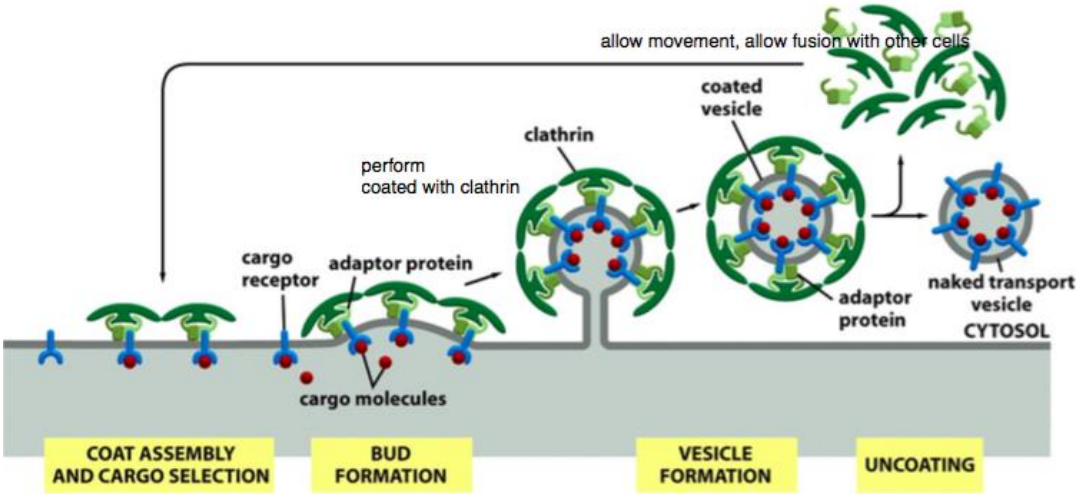
Endocytic Pathways

- in all Eukaryote Cells
- regular occurrence for recycling membrane molecules
- digestion/degradation of molecules/particles/foreign microbes
- **bring extracellular material into the cell**

pinocytosis → intake of solutes

phagocytosis → intake of particles via engulfment by membrane protrusions
receptor-mediated endocytosis → intake of specific molecules which bind to receptor on the cell surface

Clathrin-mediated Endocytosis – engulfment, via vesicles coated in the molecule
A ligand engages with its receptor and promotes association between the cargo molecule, the receptor, adaptor proteins and clathrin to drive the reaction
GTPase Dynamin → pinch the vesicle from the membrane
Clathrin-coated vesicle → transported to the **endosome**

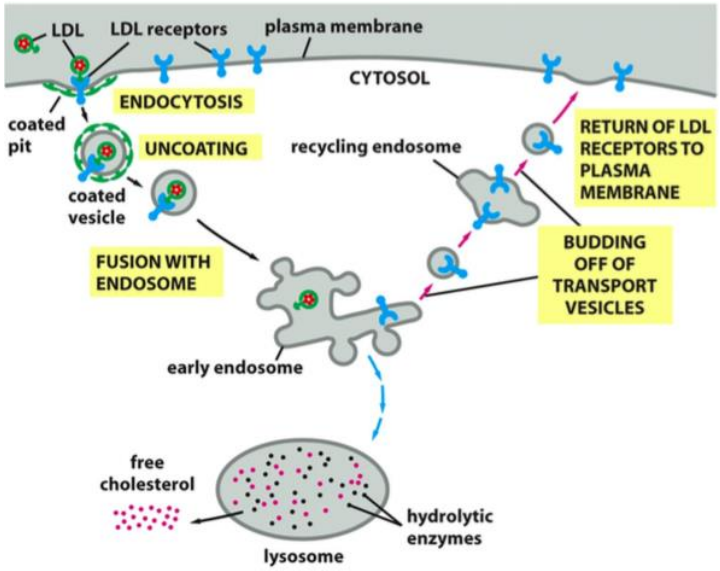


Endosomes

- Aid in the process of endocytosis
- Move material for degradation in lysosomes

Lysosome

- Bud from Golgi
- Maturation pathway: Early endosomes → Late endosomes → Lysosomes
- Function: intracellular digestion/degradation pathways



Autophagy

- Second degradation pathway in lysosomes
- Cell degrades/digests its own constituents and recycles them
- Begins with enclosure of an organelle with a double membrane – **Autophagosome**
- Autophagosome fuses with a lysosome and the internal contents are degraded by **hydrolases** and thus released
- Detection of pathogens and subsequent activation of the immune response