Membranes

Introduction

- 5-7 nm, strong, non-elastic, self-sealing, flexible, deformable, hydrophobic barriers
- Bilayer of amphiphilic lipids (phospholipids)
- Additional lipids (e.g. cholesterols) and proteins (often associated with carbohydrates or covalently bound to lipids)
- Fluid, mostly liquid, crystalline (liquid disorder), semipermeable, electrically insulating
- Asymmetric (cytosolic vs. extracellular)
- Microdomains: Lipid rafts, rab domains and coated areas
- Membranes establish compartmentalization: Boundary of the cell (plasma membrane) and organelles (allows different compositions, ionic milieus and functions)

Growth and Modification

- Membranes are derived from membranes (seed membranes from egg and sperm)
- Modification of size and composition:
    - Synthesis, insertion, degradation or modification of single component molecules one-by-one
    - Fusion/fission of larger membrane entities at once
    - Binding of peripheral components
- Components cycle between membranes and compartments
- Peripheral proteins cycle between membrane-associated and cytosolic states (tight regulation)
- Proteins modify and complement the basic bilayer and make it specific!

Classification of Membrane Proteins

Integral Membrane Proteins

- Can only be extracted with the use of detergents
- Ex.: Single pass transmembrane proteins (type I and II), tail-anchored (SNAREs), loop-anchored (caveolin-1), lipid-anchored and polytopic (multiple passes through membrane)

Peripheral Membrane Proteins

- Can be extracted without the use of detergents (as they are not integrated into the membrane itself, but associated with integrated lipids/proteins)
- Ex.: Lipid-associated, protein-associated and carbohydrate-associated

Transmembrane Proteins

Different plots have different hydrophobicity plots. The secondary structures of the intermembrane part of such proteins can either be an **α-helix** or a **β-barrel**.

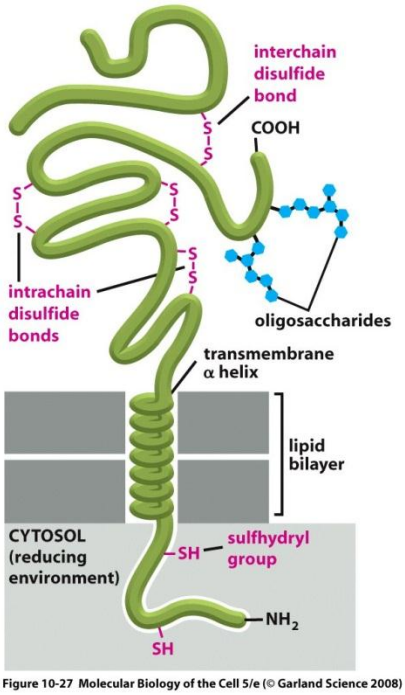


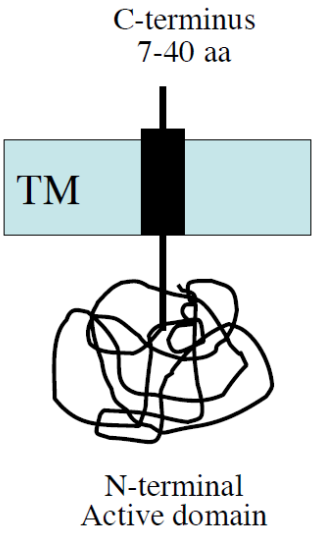
Figure 10-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)

α-Helices

18-28 amino acids (depends on membrane thickness and tilt angle, 10 residues would be enough to span membrane if they were fully extended); mainly apolar residues, positive inside rule: positive residues on the cytosolic site (positive membrane potential, negatively charged lipids concentrated at the inside leaflet); aromatic amino acids often at border of membrane, polar residues may be involved in aqueous channel formation.

Important: Living in three different surroundings, either cytosolic (no disulfide bonds), intermembrane or extracellular. Functionality does not always depend on the presence of all parts (cytosolic parts often work without the other parts).

The majority of membrane proteins are oligomeric.



Tail-Anchored Membrane Proteins

Functional domain is N-terminal and cytosolic, short luminal C-terminal, one transmembrane domain, targeted to the ER (further transported by vesicles), not interacting with SRP (signal recognition particle), membrane insertion post-translational, sometimes inserted into outer mitochondrial membrane. Ex.: SNAREs, cytochrome B_5, BCL2.

Polytopic Membrane Proteins

Helix bundles, single helices associated to each other via side-chain contacts (mainly hydrophobic, space filling, lock and key), center to center distance about 10nm, topology established co-translationally, first formation of helices, then bundling, small transmembrane proteins included in bundles, oligomers formed by larger ones.

Peripheral Membrane Proteins

- Non-covalently attached to lipid head-groups or proteins in the membrane

- Complex mixture on both sides
- Transient and regulated interactions
- Cytosolic side of plasma membrane normally rich in peripheral proteins: extensive, dynamic cortex of actin, adaptor proteins and other proteins (important for stability and local membrane specialization, connections with cytoskeleton, signaling, trafficking of vesicles, cell shape and polarity, endocytosis…)

Lipid Modifications

Some integral membrane proteins do not span the membrane, but contain lipid modifications (mostly on the cytosolic side, only GPI anchor is extra-cytosolic):

	Chemical Composition	**Topology**	**Distribution**	**Examples**
Myristylation	C14 fatty acid via amide bond to amino group of N-terminal glycin.	Added co-translationally to cytosolic non membrane proteins permanently.	Sufficient for membrane binding only when combined with positive charge cluster, fatty acid alkyl group or protein-protein interaction.	Arf1, c-src; exposed or hidden after reversible conformational change.
Prenylation	Farnesyl (C15) or geranylgeranyl (C20) by thioether to cysteine.	Added to cytosolic non membrane proteins. So called CAAX box usually at the C-terminus, permanent modification.	Often combined with nearby fatty acid acyl groups.	Two Ras isoforms, H-ras farnesyl and two fatty acid acyl chains, K-ras farnesyl and cluster of positive charges

Fatty Acylation	Typically C16 palmitic acid by thioester to cysteine.	Reversible modification in cis Golgi, on soluble proteins and endodomains of transmembrane proteins.	Double fatty acid acyl group direct many proteins to lipid rafts.	Caveolin, influenza hemagglutinin.
GPI Anchor (Glycophospha-tidylinositol)	Phosphoinositides, several sugar residues (N-acetyl glucosamine and mannose and others), ethanolamine connected by amide bond to C-terminus of protein.	Extracellular leaflet, GPI anchor provides the only connection to the membrane.	Enriched in apical membranes of epithelial cells, enriched in lipid rafts. Tail can be removed by phospholipase C, releasing the proteins.	Thy-1 antigen, alkaline phosphatase, acetylcholine esterase.

Lipid modifications are important as they allow proteins to come on and off membranes (dynamic processes, signal transduction, molecular sorting, membrane bending, vesicle formation, membrane recognition etc.), they allow a tight and strict regulation (both functions and dynamics) and interaction of proteins with specific membranes and specific microdomains.

The lipid composition differs between membranes in the cell and membranes themselves have more negatively charged lipids on the cytosolic side (and no negatively charged lipids on the extracellular side).

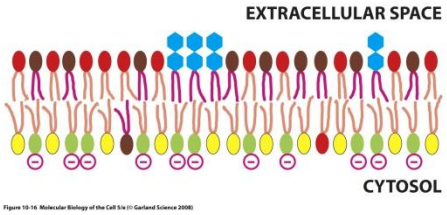


Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	*E. COLI* 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	13	8	23	27	30

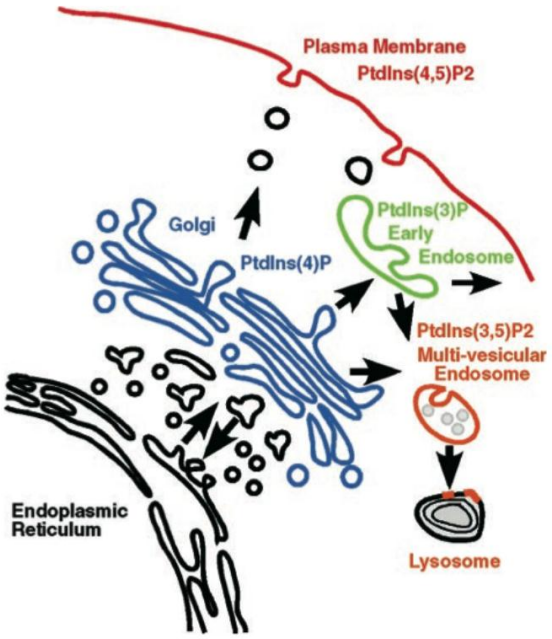
Table 10-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Extracytosolic side: Phospholipids with choline headgroups -> phosphatidylcholine (PC), sphingomyelin(SPH); glycolipids; cholesterol
- Cytosolic leaflet: Phospholipids with terminal amino group -> phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI); cholesterol
  -> Charge difference, as most of the negatively charged phospholipids face the inside (PS and PI)
- Asymmetry generated by flippases and scramblases (enzymes in the ER and the plasma membrane, flip specific P-lipids across)

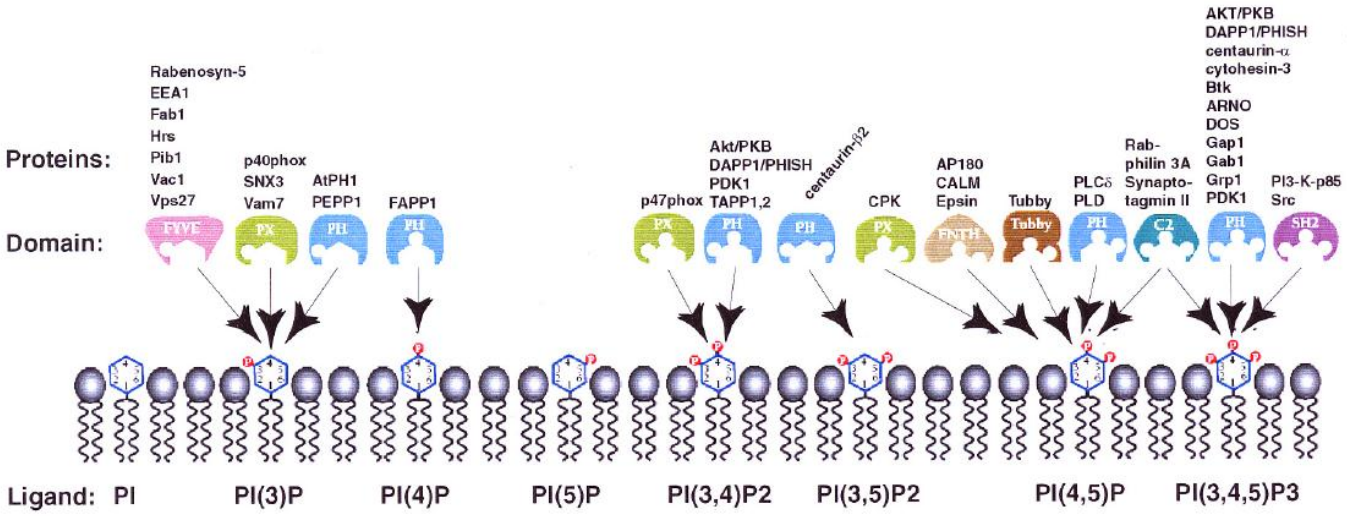
Phosphoinositides (PIs)

- Phosphorylation/dephosphorylation through organelle-specific PI kinases and phosphatases in three positions of the inositol ring: 3', 4' and 5'
- Different lipid products with distinct subcellular distributions control the correct timing and location of many important trafficking events via binding of proteins to the inositol ring
- Examples of proteins: Coat proteins, fusion/fission proteins, membrane bending factors, enzymes, GTPase effectors… Most associate transiently with the membrane

Examples for specific positions (all facing the cytosolic compartment):
PIP(4,5)P2: Plasma membrane
PI(4)P: Golgi complex
PI(3)P: Early endosomes
PI(3,5)P2: Late endosomes



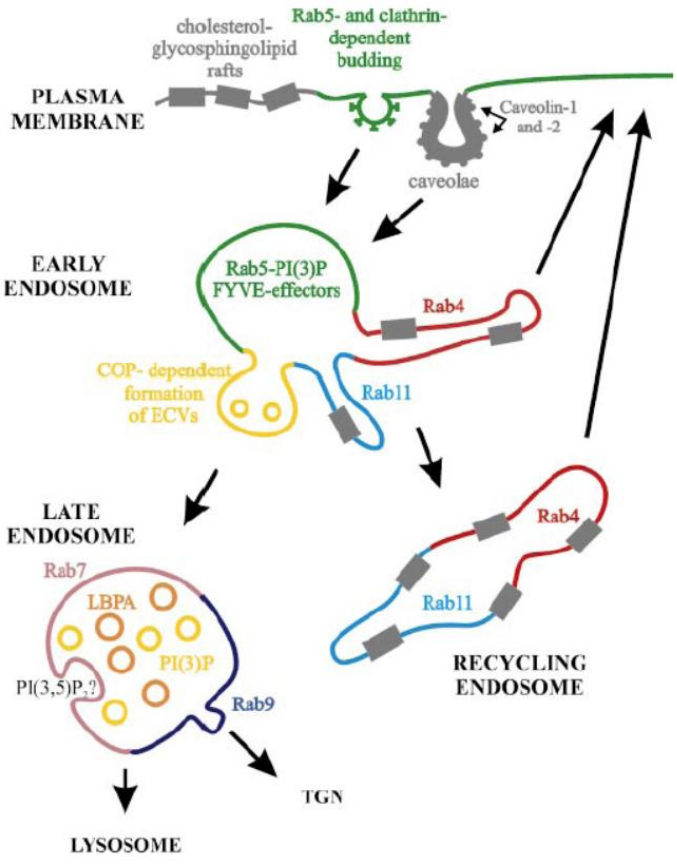
Phosphatidylinositol-binding protein domains: Many different domains, mostly sub-domains of larger proteins, e.g. PH, PX, FYVE, ENTH etc. Such domains regulate signal transduction, cytoskeleton remodeling, cell migration and membrane trafficking. Activation of specific PI kinases and phosphatases is strictly regulated in both time and space.



Lipid Micro-Domains

Have different lipid and protein composition, therefore different fluidity (e.g. liquid ordered state in rafts) and different dynamic properties which allows lateral partitioning of membrane components.

Membranes a mosaic of distinct lipid micro-domains? Many membranes are composed of mosaics of distinct functional and compositional domains. Rabs play a central role in organizing such domains.



Protein Modifications

Introduction

Human genome -> about 19'000 genes which encode for more than 100'000 different proteins. Even higher number if all modified variants are counted as well. Practically, every amino acid side chain can be modified. Some modifications involve selective cleavage of the polypeptide chain. Modifications can be transient (activation/deactivation, association/disassociation, assembly/disassembly) or permanent. Modifications often serve as signals (localization), stabilize proteins (disulfides, glycans), are needed for recognition or signal degradation (ubiquitin, removal of sialic acid).

The Big Four Major Modification Networks

1. Phosphorylation (Tyr and Ser/Thr)
2. Ubiquitination and related modifications (Lys)
3. Glycosylation (extra-cytosolic)
4. Acetylation and methylation (Lys, Arg; histone code -> epigenetics)
   (HATs (histone acetyltransferases) add an acetyl group to lysine sidechains at the N-terminal tails of histones, leading to decompaction of chromatin and activation of transcription. HDACs (histone deacetylases) remove the acetyl groups in the N-terminal tails of histones, leading to compaction of chromatin and repression of transcription. Nowadays, 2'000 proteins are known to undergo acetylation.)

Modifications in the ER

- Signal peptide cleavage
- N-linked glycosylation and trimming
- Disulfide bond formation
- GPI anchor addition
- Proline hydroxylation (collagens)

The ER

- Conditions as in the extracellular space -> pH neutral, high calcium (5 mM, mostly bound to proteins), mildly oxidizing (the cytosol is highly reducing)
- Lipids of the membrane very fluid, little cholesterol, no lipid rafts, no complex cabohydrates or linked sugars

- Lumen filled with chaperones and folding enzymes, mostly associated in complexes, incompletely folded cargo proteins face constraints in lateral diffusion (folded -> no problems)
- Different regions: nuclear envelope, rough (RER) and smooth (SER), ER exit sites, tubular ER and lamellar ER

Folding in the ER

Folding can take from about 10 minutes till half an hour or even longer and often begins co- and finishes post-translocationally. It often involves covalent modifications (signal peptide cleavage, coupling of carbohydrates and formation of intra- and interchain disulfide bonds) and is assisted by molecular chaperones. Quality control systems prevent the exit of misfolded and unassembled proteins

-> ER associated degradation system (ERAD) needed if protein misfolds permanently;

-> unfolded protein response (UPR) regulates the capacity of the ER to process cargo.

Some examples of folding enzymes and molecular chaperones in the ER:

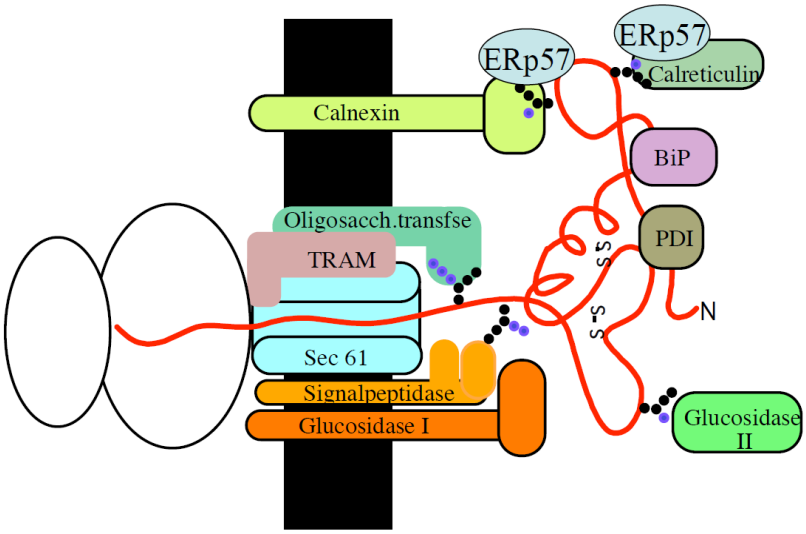
PDI (protein disulfide isomerase) and ERp57 (ER-protein 57): Homologous thiol oxidoreductases responsible for correct oxidation of cysteins to form disulfide bonds and for isomerizing disulfide bonds.

BiP/GRP78 (binding protein/glucose regulated protein 78): Heat shock protein 70 homologue which binds to exposed hydrophobic sequence elements.

Calnexin and calreticulin: Lectins that promote folding and quality control of glycoproteins, bind to monoglucosylated N-linked glycans.

GRP94: HSP90 homologue.

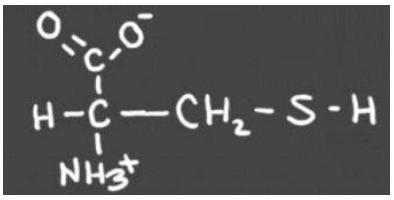
Proline isomerases: Catalyze the cis/trans isomerization on peptide bonds at proline residues.



Folding takes place in three different environments:

- ER lumen: Aqueous, oxidizing, high calcium and glycosylation
- Membrane: Hydrophobic/non-aqueous
- Cytosol: Aqueous, reducing, potassium, low calcium

Disulfide Bridges



Disulfide bonds form between two free cysteines in proteins.

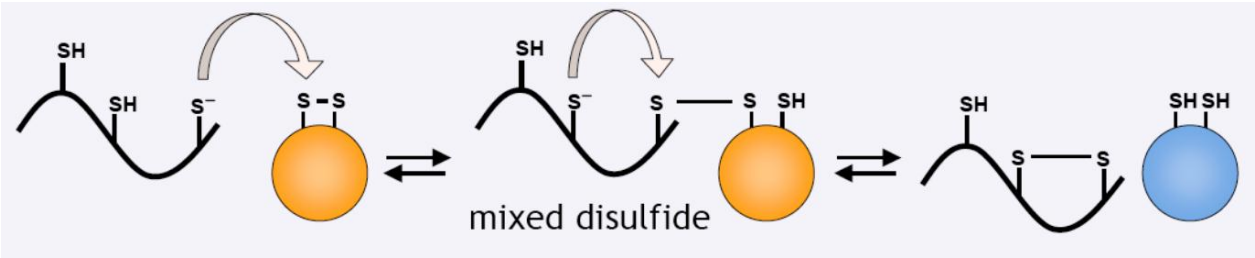
Cysteine is a relatively rare but important amino acid. In cytosolic proteins it is used to bind metal ions. Polypeptide cross-linking became a new function of cysteine as soon as the world became oxidizing (production of oxygen by bioorganisms), but only in the extra cytosolic environment (cytosol and nucleus stay reducing). Great costs involved, however, oxidative phosphorylation provides source of energy.

Disulfide bonds:

- Regulate redox-dependant protein functions
- Provide stability by lowering the conformational entropy of the unfolded state
- Are important during folding of many proteins systhesized
- Free energy of folding provides the driving force for the correct pairing of cysteines
- Formation takes place co-/post-translationally and is catalyzed by an enzymatic reaction *in vivo*
- Spontaneous formation *in vitro* (slow, unspecific)

Thiol-Disulfide Oxidoreductase

Catalyze the oxidation, reduction and isomerization of disulfide bridges. During a thiol-disulfide



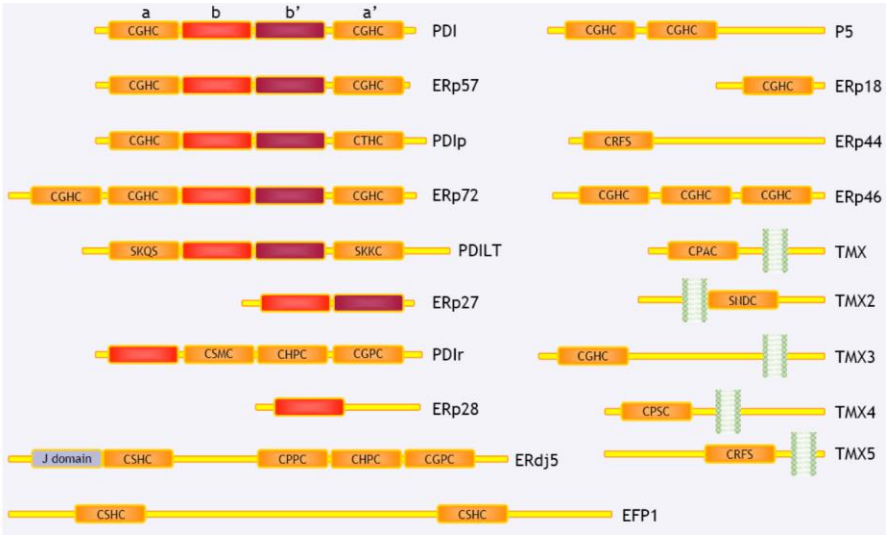
exchange, a reactive species - the thiolate anion S^- - occurs after a first reaction. A second exchange oxidizes the originally reduced protein and reduces the originally oxidized protein. Such reactions can be

either intra- or intermolecular. Enzymes often contain a characteristic CXXC active site motif embedded in a thioredoxin-like domain.

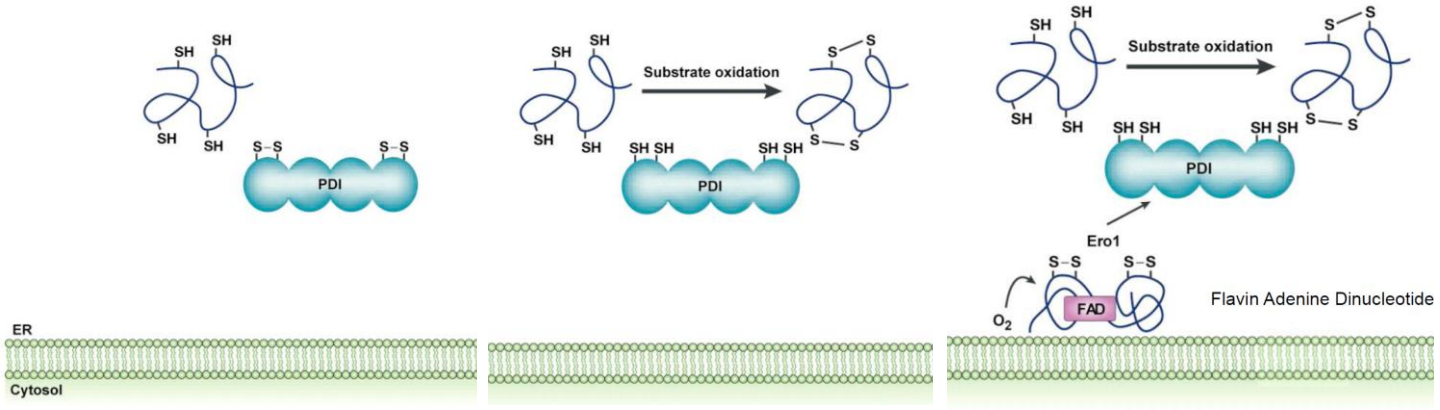
PDI - Protein Disulfide Isomerase: Four thioredoxin-like domains: a, a', b and b'. In a and a', active site cysteines are found in CXXC motifs. PDIs catalyze thiol-disulfide exchange reactions involved in oxidation, reduction and isomerization.

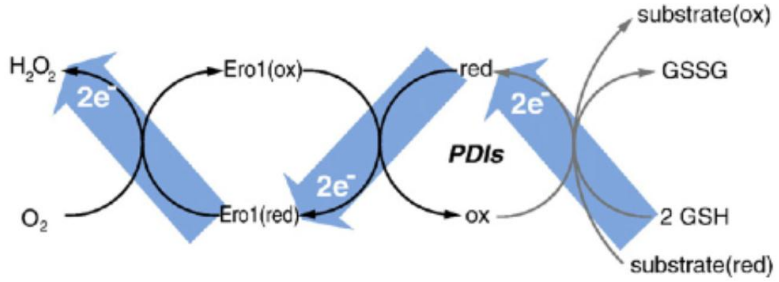
ERp57: Abundant ER thiol-oxidoreductase

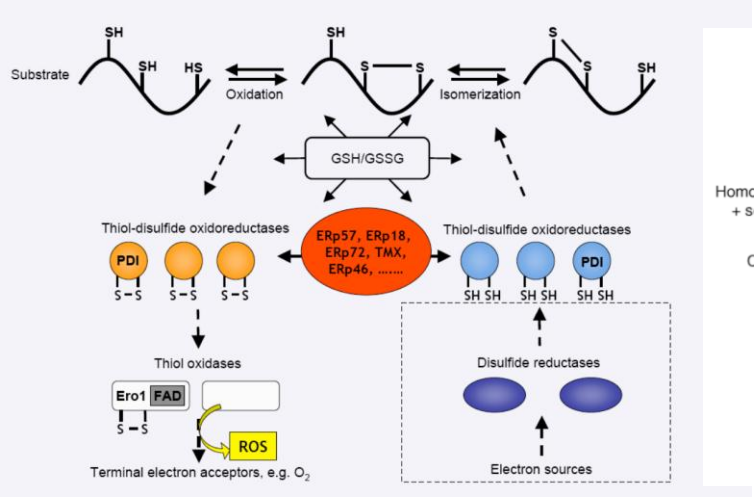
Human PDI family: Why so many? Different substrates, different functions, guarantee highly specialized functions.

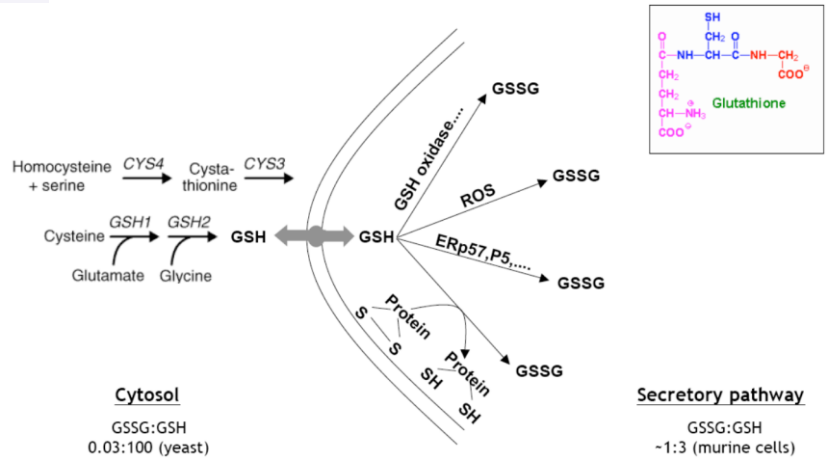


Example: Ero1-PDI oxidative pathway









Additional Information

- Disulfide bonds occur primarily in the extra-cellular and extra-cytosolic proteins and protein domains that live their life in an oxidizing environment
- Since disulfide bonds often define a fold, the location of cysteines in the sequence is highly conserved within homologous protein domains (e.g. immunoglobulin domain)
- Disulfide bonds can be reduced with reducing agents such as 2-mercaptoethanol (2-ME) and dithiotreitol (DTT)
- Since disulfide bonds are usually hidden inside folded domains, denaturation is usually required for efficient reduction of the bonds
- Oxidation of cysteines can be blocked by alkylation

Glycobiology

Introduction

- Glycans are generally conjugated with proteins or lipids
- Glycoconjugates are predominantly found in extracytosolic compartments
- Polysaccharides are often mobile and do not have any secondary structure
- Formation of branched chains
- Diverse and heterogeneous
- Synthesis not directed by genome (but by availability of enzymes and substrates)
- Diversity generators

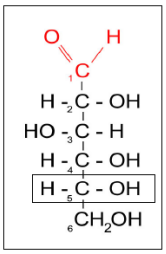
Definitions

Carbohydrates: Polyhydroxyaldehydes or polyhydroxyketones (or larger compounds that can be hydrolyzed to such units).

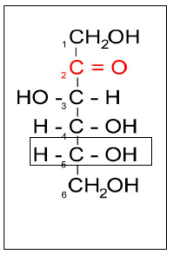
Monosaccharide: Carbohydrate that cannot be hydrolyzed into simpler units.

Oligo-/polysaccharides: Branches or linear chains of monosaccharides connected via glycosidic bonds.

Glycoconjugate: One or more mono-/polysaccharides attached to a non-carbohydrate moiety such as lipids or proteins.



D-Glucose
(aldose)



D-Fructose
(ketose)

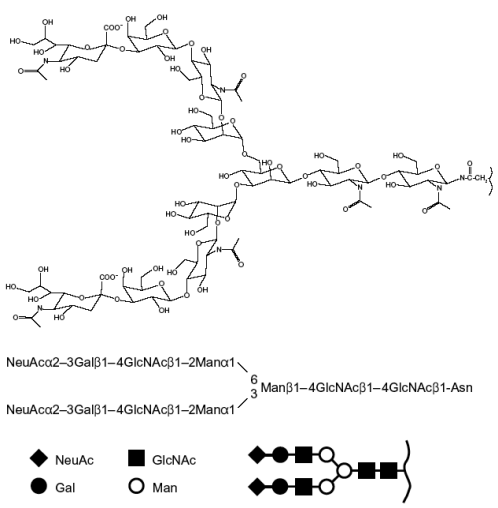
Glycosidic Linkages

In the open form, sugars have one reducing and one non-reducing end (which is no longer valid for the ring form). The reducing end is the aldehyde or ketone group (C1 or C2) and the other end the reducing one. Monosaccharides are linked via glycosidic bonds. The linkage mostly originates from the C1 hydroxyl group (exception sialic acid, C2). Typical bonds: β-1,4; α-1,6 etc.

Each monosaccharide can theoretically generate an α- or a β-linkage to any one of several positions in the other monosaccharide (3 hexoses -> 1'056-27'648 different trisaccharides!).

Glycan code: Large amount of information within a small chemical group.

Example: Mature, N-linked glycan, biantennary, branched, complex; code -> different symbols for different sugars.



Some common sugars in vertebrates:

- Sialic acids: Nine-carbon acidic sugars (e.g. N-acetyl-neuraminic acid in humans or N-glycolyl-neuraminic acid in other animals)
- Hexoses: Six-carbon sugars, including glucose (Glc), galactose (Gal) and mannose (Man)
- Hexosamines: Hexoses with amino group at position 2, either free or N-acetylated (e.g. N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine (GalNAc))
- Deoxyhexoses: Six-carbon neutral sugars without hydroxyl group at position 6 (e.g. Fucose (Fuc))
- Pentoses: Five-carbon sugars (e.g. Xylose (Xyl))
- Uronic acids: Hexoses with negatively charged carboxylate at position 6 (e.g. Glucuronic acid (GlcA) or iduronic acid (IdA), both in GAG chains)

Glycoconjugates

N-linked (Asn) or O-linked (Ser/Thr) sugars.

Glycoproteins: Proteins containing one or more glycans.

Proteoglycans: Have one or more glycosaminoglycan (GAG) chains.

Mucins: large Glycoproteins, carry many O-glycans (often closely spaced in polypeptides).

Glycosphingolipids: Also glycolipids, having a lipid ceramide moiety and sugars attached to the terminal primary hydroxyl group or the ceramide.

Gangliosides: Glycosphingolipids with one or more sialic acid residues.

Glycochalyx: Dense layer of carbohydrates around cells (can be stained with Ruthenium Red).

Glycoproteins: N-linked glycans

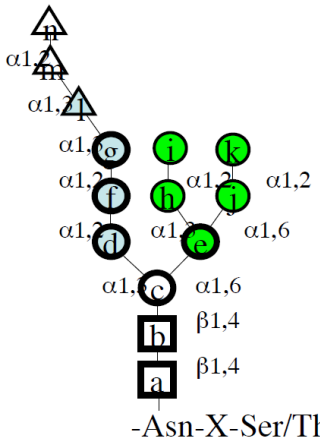
- 50% of human genes are thought to encode for glycoproteins
- 90% of those have one or more N-linked glycans (average of 1.9 per polypeptide, some have more than 10)

- N-linked glycosylation is the most common covalent protein modification (together with phosphorylation)
- Glycans on surface of glycoproteins (usually in regions where secondary structure changes)
- Intermost GlcNAc residue often hydrogen bonded to suface amino acid residues (middle and other sugars are mobile)
- N-linked glycans are semi-independent, can be modified and undergo interactions independently of the protein moiety

Core Oligosaccharide

Form of the N-linked glycan added to growing nascent chains in the ER, 14 saccharides, invariable in eukaryotes. Innermost 7 saccharides inherited from archaeal cell wall glycoproteins. Inner and outer saccharides have distinct biosynthesis and functions.

Biosynthesis of the first seven sugars occurs on the cytosolic side of the ER membrane whereas the last seven are added in the ER lumen. Each sugar means something, the ancient sugars e.g. make the protein more soluble. General functions of the core are help during folding, increased stability, helping the ERAD to work properly.



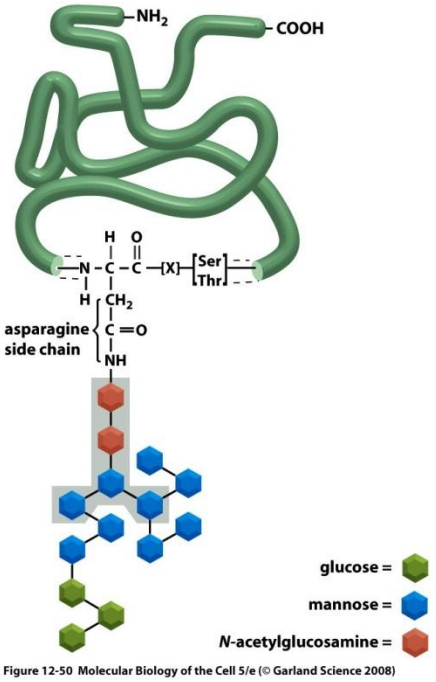
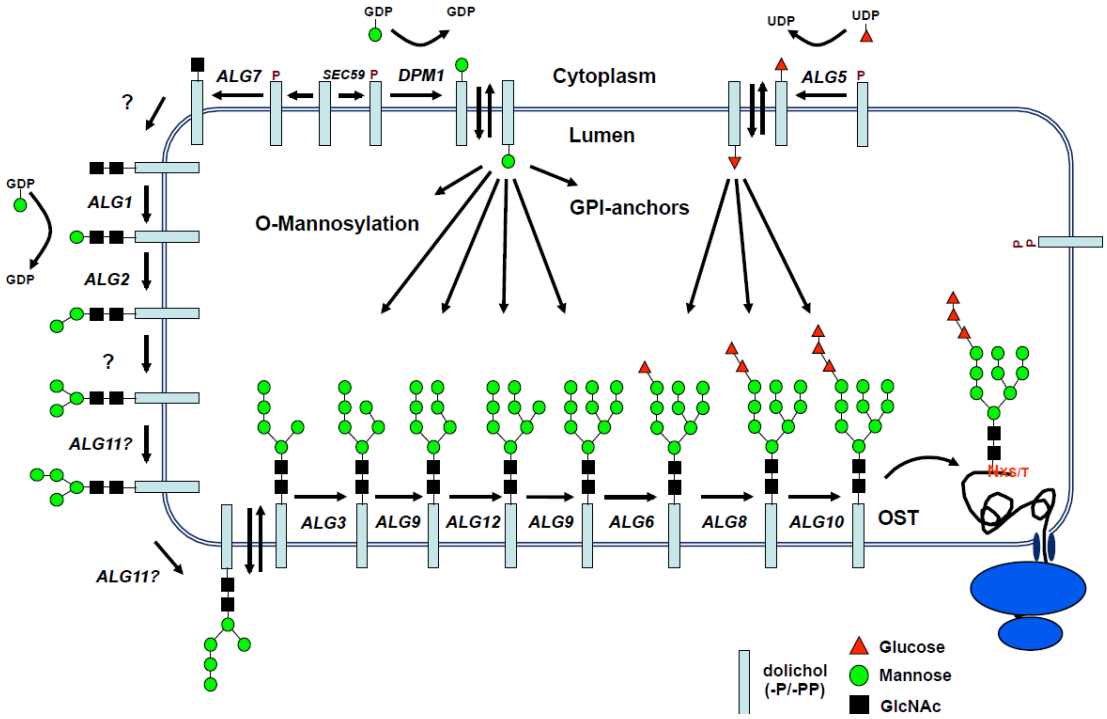


Figure 12-50 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Functions of N-linked Glycans during Glycoprotein Maturation

- Promotion of folding (intermediates more soluble, less aggregation), often essential for proper folding
- Increase stability of proteins
- Binding sites for the lectin chaperones calnexin and calreticulin (indirect function in folding and quality control)
- Binding of Yos9, signal for ERAD of misfolded and unassembled glycoproteins
- With mannose-6-phosphate targeting signal for sorting of lysosomal hydrolases to lysosomes

Trimming: Essential, core sugar added, things removed and added again. Glucosyl-transferase recognizes unfolded proteins and sends them back. Folded proteins go further to the Golgi apparatus and are further modified (by transferases).

Functions of N-Glycans

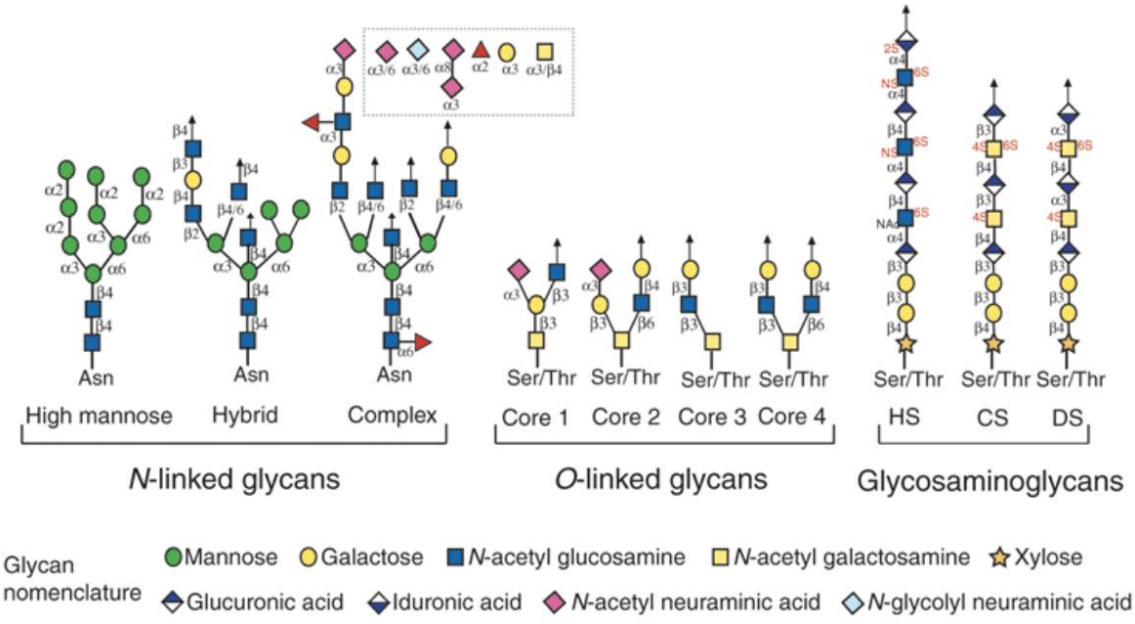
- Recognition markers for binding to lectins
- Protection from proteases
- Inhibition of aggregations, higher solubility
- Stabilization

- Modification of antigenic processes
- Fine-tuning of charge (and other properties)
- Giving cells a “corporate identity”

O-linked Glycosylation

Ser or Thr, via O-glycosidic bond, post-translational, starts in cis-Golgi. No known consensus sequence, often adjacent prolines (position -1 and +3), generate β-turns, Ala, Ser and Thr often next to glycosylated residue. Initially, addition of GalNAc by GalNAc-transferase. O-linked glycans are often bi-antennary structures and clustered in sequence.

A special form are glycosaminoglycans, very long and important, but poorly understood.

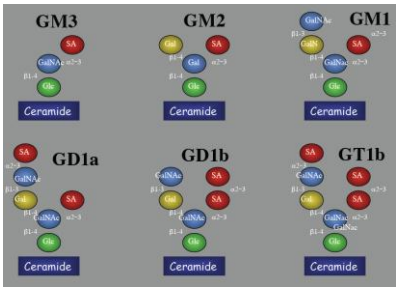


Examples: Chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin… Mostly contain sulfate groups which add additional negative charge. Hyaluronic acid, important in joints…

Functions of O-linked Glycans

- Mucins: Protective barriers in epithelia, lubrication
- ABO blood group antigens
- Components of proteoglycans and extracellular matrix, GAG chains
- Sperm/egg binding
- Lymphocyte homing during inflammation

- Cell recognition
- Pathogen binding



Gangliosides

Ceramide plus oligosaccharide, 4-10% of lipids in the plasma membrane, complex synthesis.

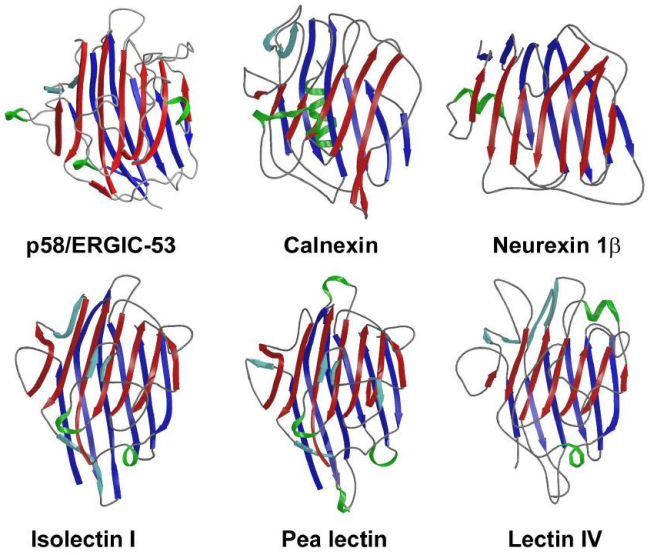
Lectins

Protein binding to carbohydrates. Lectins are receptors, mediate cell-cell interactions and endocytosis of glycoconjugates. Lectins are involved in pathogen/cell interactions and protection against pathogens. They are involved in folding, quality control and sorting in intracellular compartments. Bacterial toxins can be lectins.

Lectins contain one or more carbohydrate-recognition domains (<200 residues, CRDs), highly β-sheet dominated structures.

- C-type (calcium-dependent lectins
- Gal-binding galectins
- P-type Man-6-phosphate receptors
- I-type lectins (immunoglobulin-like domains)
- L-type lectins (related to leguminous plant lectins)

Various CRDs occur in bacterial toxins (Cholera, Shiga…) and viruses (influenza, measles, SV40).



Sugar Recognition

Sugars cannot detect each other, they need proteins to help them. Low affinity binding site for saccharide formed by a shallow indentation on the protein surface. Selectivity is established through hydrogen bonding to the sugar hydroxyls (with or without water molecules) and packing of hydrophobic sugar face against aromatic amino acid side chain. Salt bridges can play an additional role (in charged sugars, such as neuraminic acid). High specificity but low affinity, which can be increased by extending binding over several sugars in oligosaccharides and by clustering of binding sites in oligomers of lectin monomers. Ca^{2+} and Mg^{2+} help fixing the position of amino acids interacting with sugars. Oligomeric

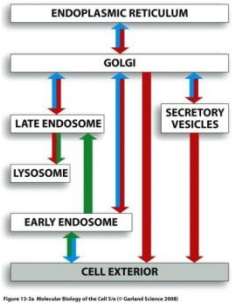
lectins which have more than one binding site have a way higher affinity than only one lectin with one binding site (1 = 1mmol, 2 = $mmol^2$ etc.).

Cell surface lectins can recognize glycans, important role in inflammation, recognized on glycoproteins by selectins on lymphocyte surface.

Example DC-SIGN (dendritic cell-specific intracellular adhesion molecule-grabbing nonintegrin): C-type lectin, binds oligosaccharide ligands found on human tissues as well as on pathogens.

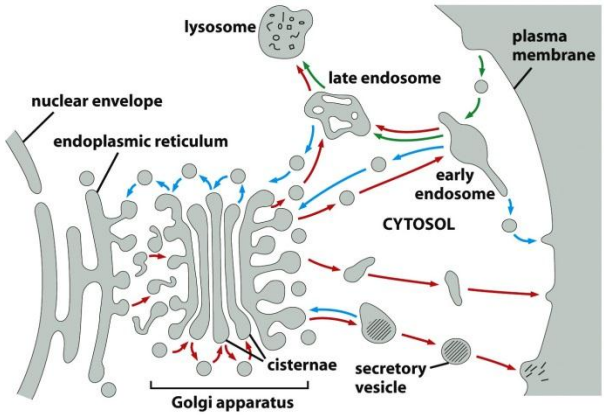
The Secretory Pathway

Introduction



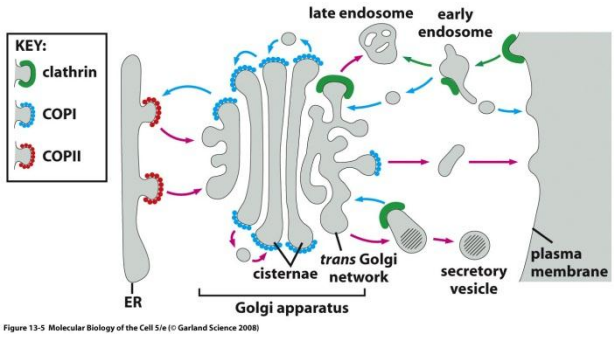
- Cargo: Soluble proteins, membrane proteins, lipids, carbohydrates, lipoproteins, hormones, enzymes, receptors, toxins, extracellular matrix components, growth factors, ion channels, fusion proteins, cell wall components, lysosomal hydrolases
- Synthesis, folding and quality control of cargo in ER
- Processing in Golgi
- Sorting in the TGN
- Vesicular transport to location

How can there be so much membrane movement and still the individual compartments/organelles stay the same? Vesicles! Only take up selected cargo, only to go to the next compartment.



Part 1: Budding, Sorting and Recycling

Traffic mediated by different coated vesicles: Clathrin, COPI and COPII. They drive budding by sculpting a vesicle, membrane curvature, capture cargo proteins (sorting) and transport them (directionality and fidelity). Common features: Coatomers, regulation by small GTPases (sar1p, ARF), binding of sorting signals, packaging of address codes and code readers (rabs, tethers, SNAREs).

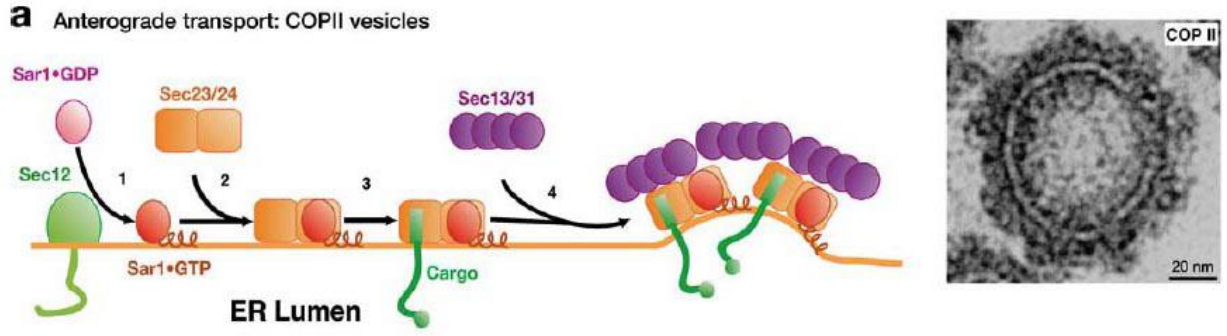


Budding

COPII

Anterograde transport (from ER to Golgi). Coat assembly: Guanine nucleotide exchange factor (GEF)Sec12 promotes the exchange of GDP to GTP in Sar1. Sar1 then exposes the N-terminus and inserts in into the membrane (GDP-bound form soluble, GTP-bound form membrane-associated). The cytosolic Sec23/24 complex binds Sar1-GTP on membrane. Curvature is thought to happen through the insertion of the amphipathic N-terminal helix of Sar1 and the binding of Sec23/24 (curved protein surface, wedges). Sec24 then binds cargo tails and address codes (SNARE's and rabs). Exit signals interact with

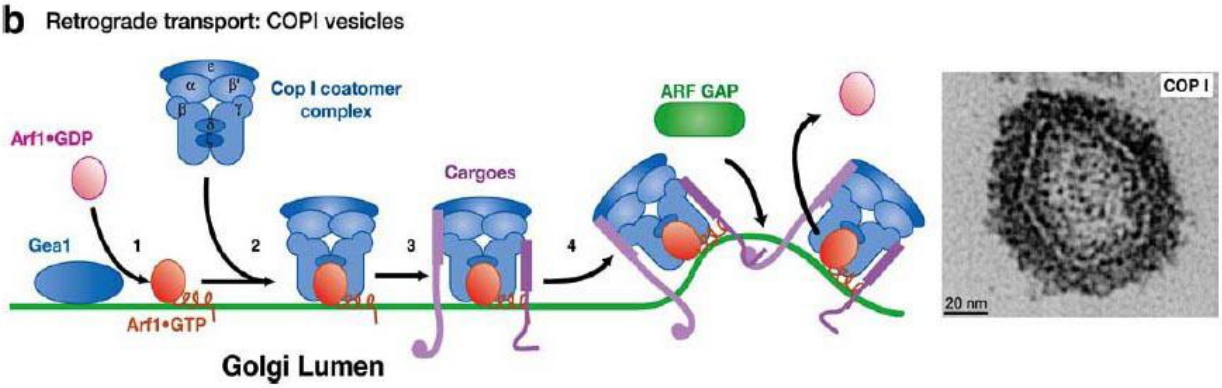
Sec24, e.g. the di-acidic signal binding to the B-site of Sec24. Then, cytosolic Sec13/31 crosslinks complexes and induces budding and fission (cuboctahedron). Sec23 acts as a GTPase activating protein



(GAP), Sar1 hydrolyses GTP and the coat falls off.

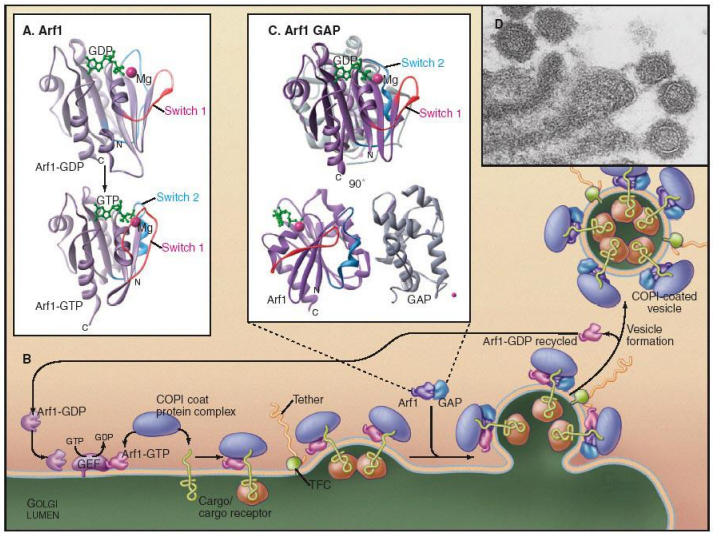
COPI

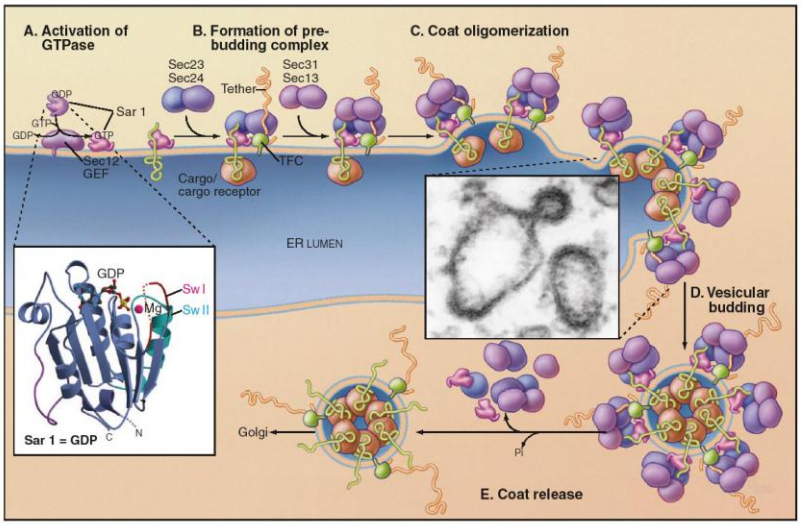
Retrograde transport from Golgi to ER and between Golgi cisternae. Coat assembly induced by ARF GTPases, 6 different ARF isoforms exist (for the different organelles), ARF1 is critical for cis-Golgi and ERGIC (ER-Golgi intermediate compartment). The soluble GEF Ges1 (ARNO in mammals) exchanges GDP for GTP in ARF1 (exchange only happens on membrane). COPI coats consist of several coatomers, α-COP to ζ-COP (7 subunits) which assemble on the membrane by binding of ARF-GTP. Cargo loading requires specific signals (KKxx at C-terminus or xxRR at N-terminus). ARF GAP induces GTP hydrolysis and therefore uncoating. GAP is cytosolic, but only active in the coat, which falls off after hydrolysis. COPI forms so called triskelions which form an icosahedral structure.



The Sar1 and ARF GTPases:

- Contain switch 1 and 2 regions that undergo conformational changes during the GTP-GDP cycle (like other small GTPases)
- The exchange of GDP with GTP triggers the extension of an N-terminal helix for membrane binding (ARF: myristyl group, Sar1: amphipathic helix)
- The GTP-GDP cycle regulates membrane association and effector interaction
- Small GTPases lack the arginine finger in the catalytic domain
- Sec23 is the GAP for Sar1 and provides its arginine finger
- ARF has soluble GAPs, which activate ARF by changing the conformation on the catalytic domain (ARF GAPs are only active within the coat)





Sorting and Recycling

Resident ER chaperones (e.g. calnexin) bind incompletely folded proteins and retain them (quality control). Folded proteins leave by bulk flow, some membrane proteins have signals that bind to COPII components. Soluble cargo can be sorted by cargo membrane receptors (e.g. ERGIC-53). ER resident proteins leak out and migrate to Golgi. The pH gradient assures that cargo receptors and ER resident proteins come back. Cargo detaches from forward receptors at low pH (ER=neutral, Golgi=acidic).

The KDEL receptor constantly migrates out of the ER and binds the KDEL signal of escaped ER proteins at a low pH (in the ERGIC). The COPI coat binds KKxx or xxRR signals of membrane proteins (e.g. KDEL) and transports them back to the ER. Secretory proteins are thereby concentrated about 50fold.

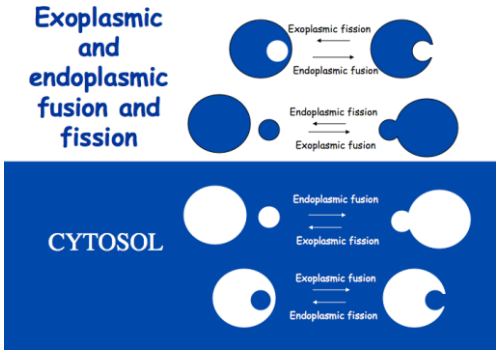
Carriers are transported with the help of motor proteins along microtubules to their right destination.

Summary

- Transport between compartments is mediated by different coated vesicles
- Vesicle formation and cargo-loading depends on assembly of coats from cytosolic protein complexes
- After vesicle fission the coats dissociate
- COPII/Sar1 vesicles mediate the anterograde transport from the ER to the Golgi
- COPI/Arf vesicles mediate the retrograde transport from the Golgi to the ER and the intra-Golgi transport
- Recycling ensures integrity of organelles and concentration of products
- Packaging and sorting are signal dependent

Part 2: Mechanisms and Specificity of Membrane Fusion and the Golgi Apparatus and Beyond

Membrane Fusion



- Thousands of fusions every minute
- Permeability, topology, asymmetry, membrane area and volume are conserved
- Highly regulated, no spontaneous fusions occur
- Specific proteins facilitate fusion which is often triggered by changes in lipids and ionic milieu
- Fusing membranes are carefully matched (SNAREs, tethering factors, rabs) and the process is restricted both to location and time
- Homotypic: Fusion of two identical membranes
- Heterotypic: Fusion of two differend membranes (e.g. ER and vesicle, Golgi and vesicle…)

Spontaneous fusion does not occur because the hydrophobic effect of the lipid bilayer prevents structural changes. Fusion and fission require external energy. Moreover, the hydration force prevents two polar surfaces to get close in water and electrostatic forces and steric effects of membrane proteins keep them away from each other (at least 10-20nm).

Hydration force: Phospholipid heads -> highly hydrated. Hydration shell must be dismantled to get membranes as close as 2nm. Work needed provides enormous energy barrier against fusion -> hydration force, exponential increase the closer membranes get, only collapses at very short distances, where attractive forces take over.

Less hydrated lipids start accumulating at places where membranes get close to each other (e.g. PE). Negatively charged lipids -> increased membrane repulsion, unless calcium is available -> calcium salt between to lipids of different membranes -> fusion. Calcium is a trigger for fusion. Strong curvature locally minimizes the area of strong intermembrane repulsion and promotes fusion. Fusion proteins bring membranes into direct contact, generate curvature and disturb the membrane structure.

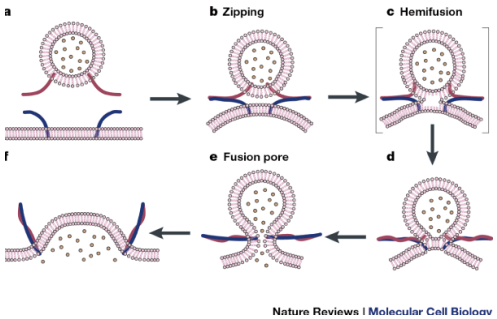
Endoplasmic Fusion

Endoplasmic fusion is mediated by integral membrane proteins (SNAREs) in both membranes and regulated by peripheral membrane proteins recruited to the membrane from the cytoplasm (Rabs, tethering factors, AAATPases NSF…). The components may be used for several times. Fusion is energy dependent (ATP) and mostly triggered by calcium. Membrane pairing, the time and the location are highly regulated.

NSF: N-Ethylmaleimide (NEM) sensitive factor.

SNAP: Soluble NSF attachment protein.

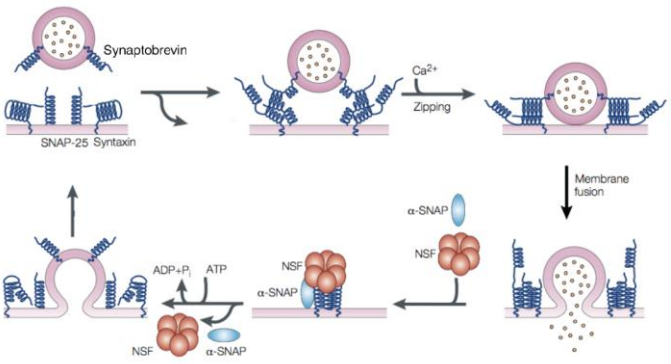
SNARE: Snap receptor.



SNAREs

SNAREs are present in both fusing membranes and are regulated by Rabs, tethering proteins and AAA ATPases-NSF. They act with each other, form coiled coils (with the help of SNAP25) and bring the outer membranes together first (hemifusion), then the inner membrane (total fusion). The oligomeric folding of the SNAREs gives the energy to remove the water so the membranes are forced to approach.

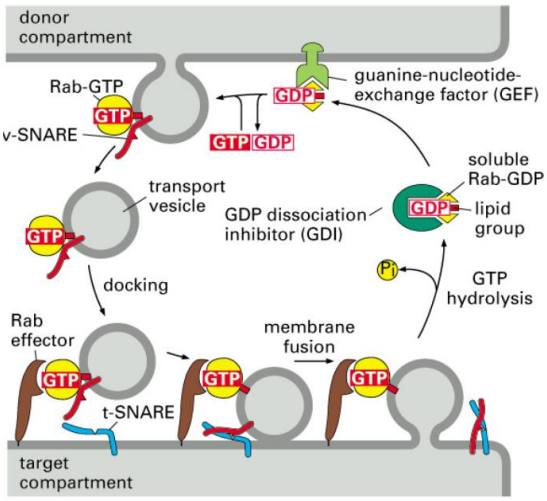
Different t-SNAREs are found on target membranes whereas different v-SNAREs are found on vesicles.



Pairing is crucial for fusion and specificity. SNARE pairs are dissociated by the AAA ATPase NSF that binds via SNAP adaptors and transported back to the ER for recycling.

Tethering factors and Rabs

Ensure specificity. Rabs in their GDP bound form are soluble, isoprenol makes them insoluble, unless they have GDI bound (GDP dissociation inhibitor). GEF exchanges GDP with GTP, Rabs get activated, bound to v-SNAREs and effectors, transport of vesicle takes place, after fusion, GAP hydrolyses GTP -> new cycle. Rab proteins are very specific, more than 60 exist in mammals.



Molecular switches are specific for each fusion event. Rabs ensure fidelity and specificity of transport (recruitment of specific SNAREs and tethering factors to vesicles and interaction with tethering factors on target membranes).

Golgi

Functions

- Glycan chain modification
- Synthesis of O-linked glycans and glucoseaminoglycans
- Synthesis of shingomyelin and glycolipids
- Proteolytic cleavage
- Sorting (TGN)

Subcompartments have defined functions due to a specific set of enzymes. Resident proteins are integral membrane proteins (many type II).

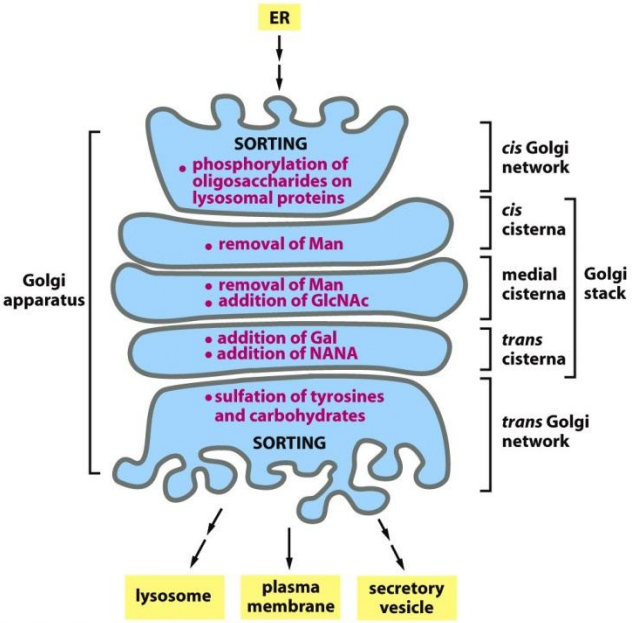


Figure 13-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Problem: How maintain cisternal integrity (by localization of enzymes) and transport of cargo through the stack? Two models have been proposed so far, the vesicular transport model and the cisternal maturation model. The vesicular transport model proposes that Golgi cisternae are static with resident enzymes. Passing from molecules from cis to trans through the Golgi is accomplished by vesicles, that bud from one cisternae and fuse with the next. The cisternal maturation model is favored and claims that each cisternae matures as it migrates outward through the stack. At each stage, the resident proteins are moved backward in COPI vesicles and enzymes are received from the cisternae just ahead.

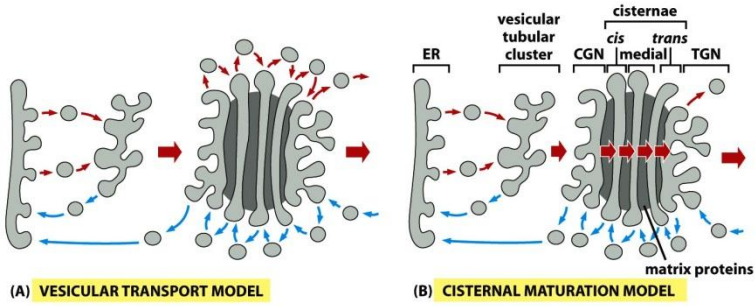
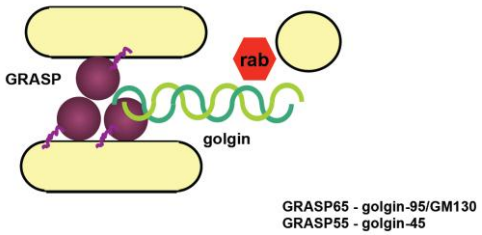


Figure 13-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Stacking and tethering: Golgin (long coiled-coil, rod-shaped proteins, GM130, giantin (on vesicles), golgin-84 (on rims of cisternae)), GRASPs (globular, acylated stacking factors, GRASP65, GRASP55) and GTPases (rabs and Arl1, regulate interaction).



The Trans-Golgi Network (TGN)

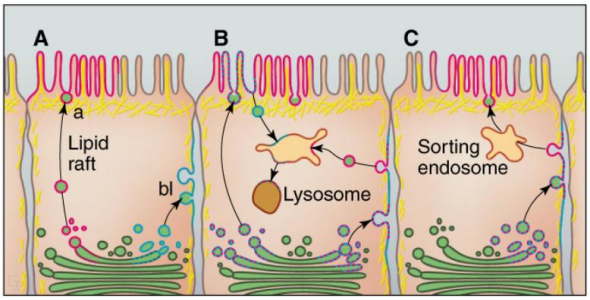
Four routes from the TGN:

1. Constitutive secretion to the plasma membrane via different mechanisms (no triggers needed)
2. Regulated exocytosis via secretory granules (triggers needed)
3. Sorting to other compartments (e.g. lysosome via the mannose-6-phosphate receptor M6P-R)
4. Lipid raft and GPI anchor containing vesicles to the plasma membrane

Secretion is regulated in that cargo can only be transported via secretory granules and exocytosis only takes place after activation (e.g. hormonal triggers).

Example M6P-R: Addition of phosphate on mannose in cis golgi, M6P-R binds mannose-P, cytosolic tail (di-leucine motif) recognized by clathrin adaptors and transport via clathrin coated vesicles.

Example sorting in epithelial cells: Epithelial cells establish and maintain polarity via regulated transport and sorting of specific membrane components to the apical or basolateral membrane. Proteins containing Yxxθ are sorted via special clathrin adaptors μ1B. Apical sorting occurs without a signal, GPI anchored proteins are transported via lipid rafts. Recycling happens via endocytosis and sorting at the sorting endosome ensures maintenance of polarity.



!!!STUFF MISSING!!!

Endocytosis

Curvature

In order to form vesicles, curvature of the membrane must be induced. This can happen either through the cargo or by coats. Proteins assist in curvature establishment, e.g. proteins with the N-BAR domain (BIN, Amphiphysin, RVS) induce or sense curvature, which are recruited from the cytoplasm to trigger formation of plasma membrane extensions, invaginations, tubular organelles and transport intermediates (endocytic vesicles). F-BAR is similar but has a smaller curvature. Proteins can also be inserted into the bilayer (or in only one leaflet) and function as a wedge.

Example Arf1: Arfs are small Ras-like GTPases (function in vesicle formation). They help to recruit coat components and it is supposed that they are also involved in curvature generation or sensing. A curved membrane helps them to keep their active state (GTP bound) where N-terminal myristate and the amphiphilic helix peptide are exposed and can interact with the bilayer.

Receptor-Mediated Endocytosis (RME)

- Internalization of nutrients and their carriers, growth hormones, other regulatory ligands and their receptors, plasma membrane components, damaged proteins, antigens, viruses and toxins…
- Regulation of plasma membrane composition

Cell surface receptors are used as an antenna to collect molecules and particles. The efficiency depends on the concentration of the ligand and receptor, affinity and avidity and the mode of internalization. Ligand and receptor are internalized together, and some receptors are then recycled (others are degraded). This happens not only by clathrin-mediated endocytosis. The low pH in early endocytosis often used to dissociate ligand from receptors allowing recycling.

Bulk fluid uptake can be distinguished from receptor-mediated endocytosis by looking at the saturation. Bulk flow only depends on the concentration of a protein and cannot be saturated whereas receptor-mediated endocytosis can be saturated.

Example low density lipoprotein (LDL): LDL dissociates from its receptor in the acidic environment of the early endosome (at neutral pH, the R domains are extended and free to bind LDL,

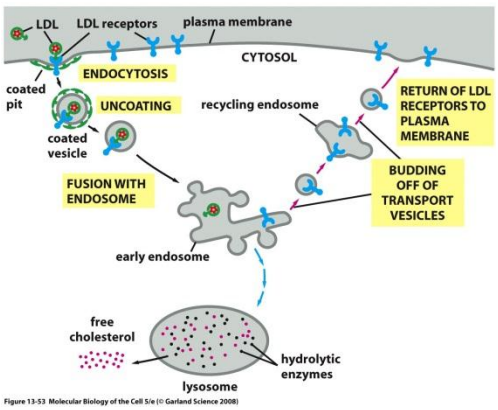
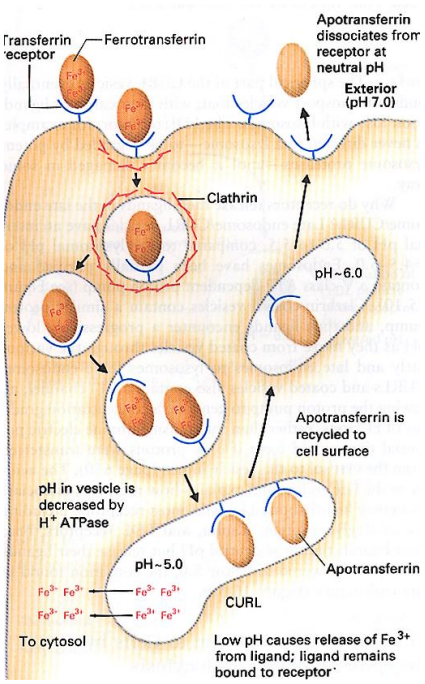


Figure 13-53 Molecular Biology of the Cell 5/e (© Garland Science 2008)

but at endosomal pH, the receptor has another structure, the β-propeller domain now serves as a "competing" ligand for LDL binding to domains R4 and R5). The LDL ends up in lysosomes, where it is degraded to release free cholesterol whereas the receptor is returned to the plasma membrane via clathrin-coated vesicles that bud off from the tubular region of the early endosome. One receptor makes this trip every ten minutes (occupied or not).



Example Transferrin: Transferrin is the carrier for Fe^{3+} and is endocytosed, together with its ligand. At low pH, Fe^{3+} is released from transferrin (which stays bound to the receptor). The receptor and the empty transferrin (apotransferrin) are recycled to the plasma membrane. At neutral pH, the apotransferrin has low affinity for the receptor and is therefore released.

Endosomes and Lysosomes

Early endosomes:

- Pleiomorphic, tubular/vesicular, in the periferal cytoplasm
- Rab5, Rab4, Rab11, Arf1, PI(3)P, EEA1 (early endosome antigen 1), ESCORT
- Sorts receptors and cargo
- pH 6-6.5
- Sorting by geometry: Tubules detach with higher membrane to volume ratio
- Sorting by signals: Mono-ubiquitination of cytoplasmic tail leads to inclusion in internal vesicles and degradation

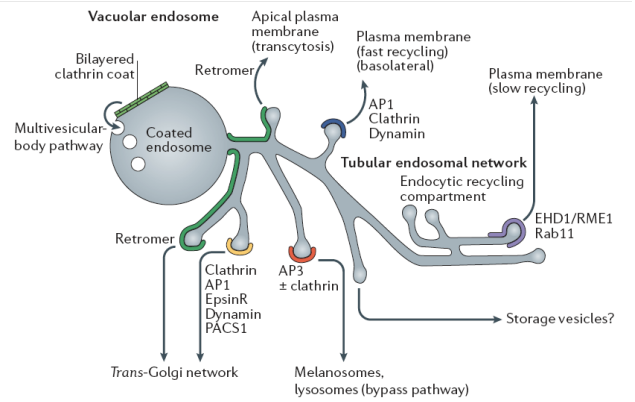
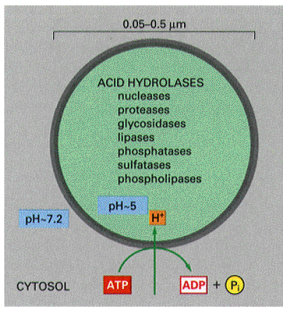


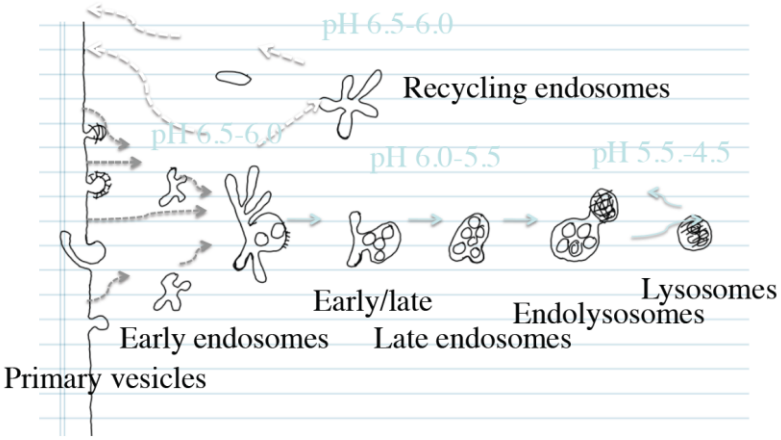
Figure 3 | **A schematic representation of the 'tubular endosomal network'.**

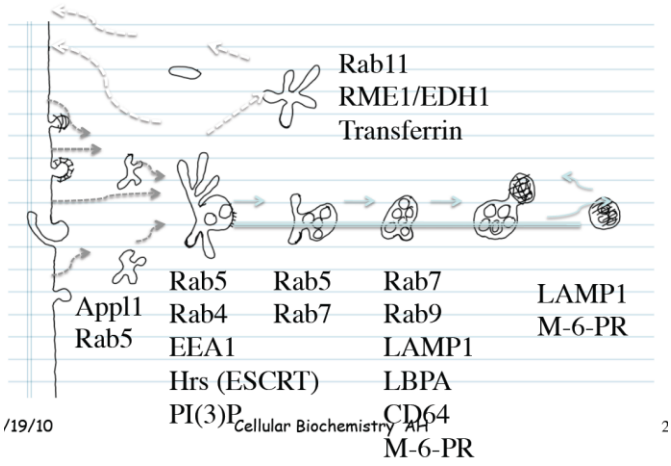


Early endosomes work like an airport, there is a central part where cargo is then sorted and brought to different domains (different pathways) with different composition of coats and proteins.

Late endosomes are closer to the nucleus (perinuclear space, where the MTOC is), also called multivesicular body.

Lysosomes are heterogenous, there are different stages (late endosome -> endolysosome -> lysosome). The pH is acidic (pH 5) and lysosomes have transporters for amino acids, monosaccharides, nucleotides etc. which can bring those back to the cytoplasm for further usage.





Apical and basolateral endocytosis: More complicated, the late endosome is shared by both sides (apical and basal), but the early part of the pathway is separated. A common recycling compartment (CRE) allows transcytosis.

Endosome Maturation

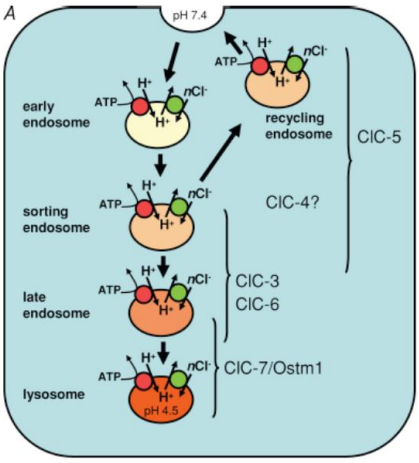
- Vacuolar elements dissociate from most of the tubules in early endosomes
- Recycling receptors leave the organelle
- pH drops to 5.5 and lower
- Rab5 exchanged with Rab7, later addition of Rab9
- Association to microtubules via dynein and dynamitin, transported to perinuclear space
- Loses capacity to fuse with early endosomes, instead fusion with late endosomes and lysosomes
- Formation of intralumenal vesicles (late endosomes are multivesicular bodies)
- Acquisition of mannose-6-phosphate receptors, lysosomal hydrolases and lysosomal membrane proteins from the TGN

All changes are highly interconnected and interdependent. Changes in one process can influence all other processes and inhibition of one step might inhibit transport of cargo to the lysosome.

The vacuolar ATPase:

- Electrogenic proton pump
- Responsible for acidification of endosomes, lysosomes, TGN, chromaffin granules…
- Similar to the F_0F_1 ATPase in mitochondria but runs in the opposite direction

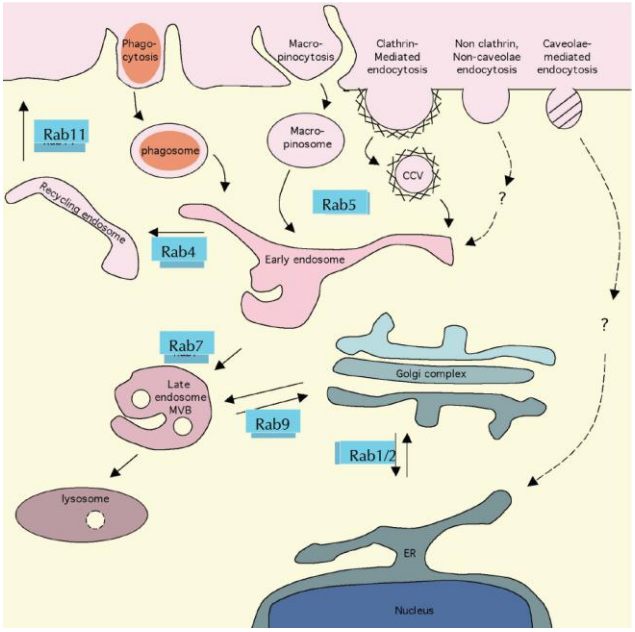
- Protons are pumped from the cytosol into the lumen of organelles
- Membrane potential builds up (positive on the inside, negative on the outside)
- At some point, protons can no longer get in, unless there are chloride channels and transporters (ClCs)
- ClCs regulate the pH, they are voltage-regulated chloride channels that mediate the influx of chloride ions which balances the membrane potential and allows the pH to drop further
- Different organelles, different ClCs to adjust pH
- vATPase can be inhibited by Bafylomycin A



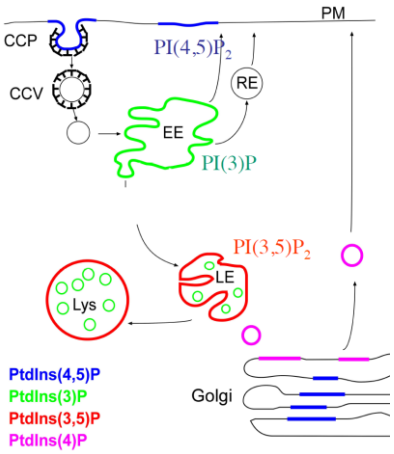
The endosomal pH can experimentally be elevated by using lysosomotropic weak bases (ammonium chloride, chloroquine, membrane permeable molecules that bind protons), carboxylic ionophores (monesin, nigericin, allow transport of monovalent cations) or Bafylomycin A (inhibition of vATPase).

Rabs

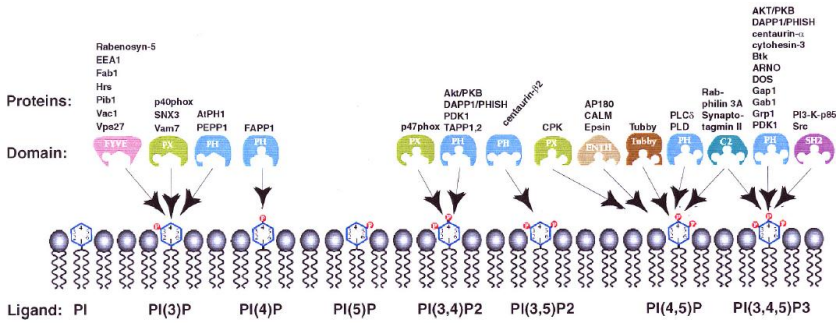
Involved in all steps, soluble when GDP bound, binding to GDI which hides the prenyl group of Rab, GEF exchanges GDP with GTP, gets hydrophobically attached and activated, binding to SNAREs and other factors, dissociation after fusion.



Example Rab5: Rab5GDP activated by its GEF Rabex-5 in the early endosome membrane, then dissociates from the GDI. The geranylgeranyl group exposed in Rab5GTP binds it to the membrane and to Rabaptin-5, a Rabex-5 associated protein. Rab5GTP then recruits a variety of effector proteins, such as VPS34p (a PI(3)kinase that generates PI(3)P, EEA1 (which binds to PI(3)P through its FYVE domain, it is a tethering factor for incoming vesicles and for fusion between early endosomes. So a platform of associated effector proteins and lipids is formed. This platform recruits motor proteins for transport, SNAREs etc.



Functionally and structurally distinct domains are organized by different Rabs, moreover, phosphoinositides are differently distributed. They play a central role in endocytosis as many proteins involved have phosphatidylinositide recognition domains.



Intralumenal Vesicles (ILV)

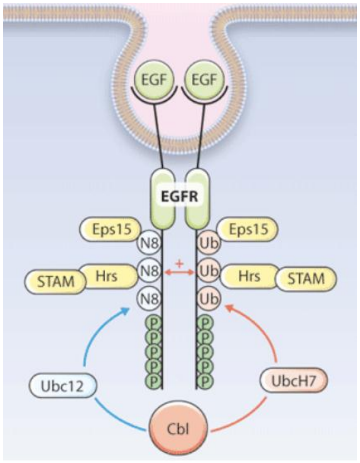
They accumulate in the late endosome and endolysosome and contain proteins and lipids targeted for lysosomal degradation. They form by invagination of the limiting membrane of the early endosome and require the ESCRT complex for cargo selection (monoubiquitin) and for invagination.

Vesicles bud into the endosome which generates a multivesicular body, proteases and lipases eventually digest all the membranes which leads to digestion of the contents. The signal for uptake into intralumenal vesicles are monoubiquitin tags added to cytosolic tails of receptors to be degraded in the lysosome.

Mono-Ubiquitination

- Regulates clathrin-mediated endocytosis of certain receptors
- Endocytosis of some cell surface receptors (such as EGFR) is accelerated by mono-ubiquitination of their cytosolic tail
- Process involves specific receptors with more than one ubiquitin binding domain (UBD) that recognize those tails
- Many ubiquitin receptors are mono-ubiquitinated themselves
- Intra-molecular interaction between mono-ubiquitin and UBD serves to inhibit the receptor (negative control)

Example EGFR: The ligand-dependent endocytosis and down-regulation of the epidermal growth factor receptor (EGFR) are promoted by mono-ubiquitylation mediated by the E3 ligase Cbl. Cbl is recruited to the EGFR upon stimulation with EGF, and mediates the transfer of multiple Ub molecules to the EGFR

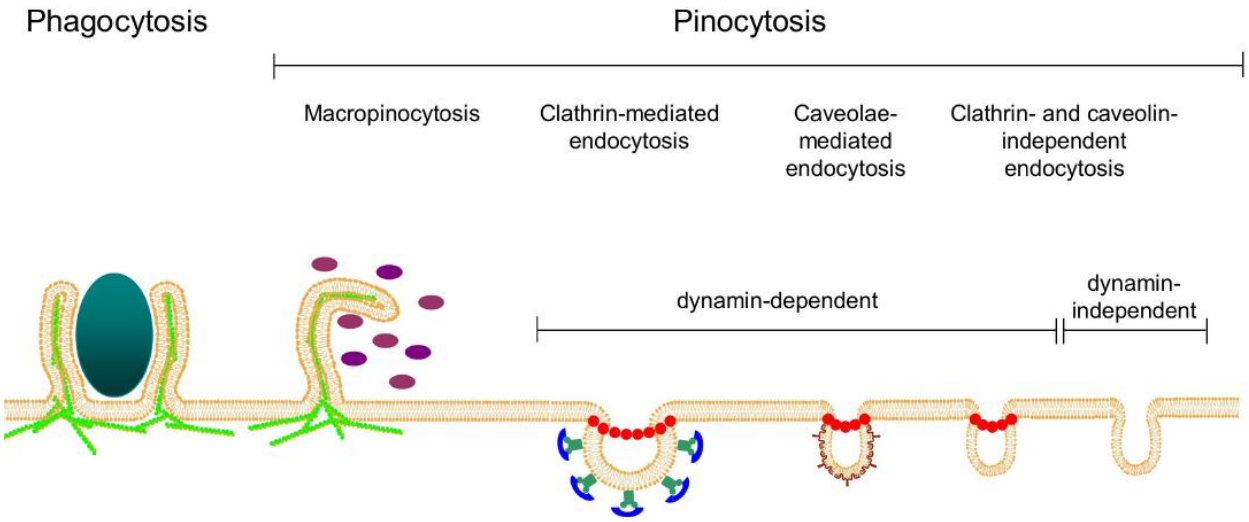


cytosolic domain (also Neddylation occurs). This initiates the internalization and down-regulation of the activated receptor complex, because the attached Ub molecules are recognized by Ub receptors (eps15) in the endocytic sorting machinery. This leads to accelerated endocytosis, and uptake of the receptor into internal vesicles of multivesicular bodies (Hrs). This terminates the EGF-induced signal. The receptor and the EGF are destroyed by degradation.

Formation of internal vesicles involves a machinery of four ESCRT (endosomal sorting complex for transport) complexes. Membrane of the forming vesicle is enriched in monoubiquitin tagged cargo membrane proteins destined for degradation in the lysosome. The ESCRT complex is unusual as a vesicle forming machinery because it generates a vesicle from the cytosolic side to an extracellular compartment and is not part of the formed vesicle. ESCRT components are also involved in other events that involve fission of vesicles away from the cytosolic compartment (HIV-1 virus budding).

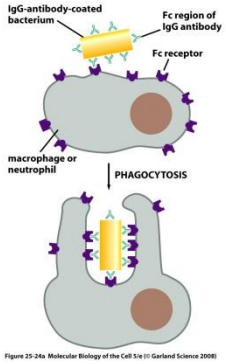
Phagocytosis, Macropinocytosis and Caveolae

Repetition Endocytosis

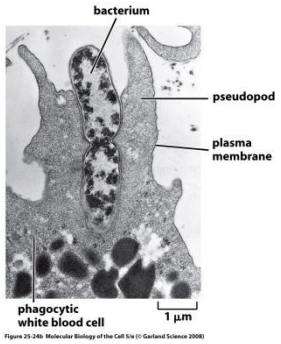


Many different types of endocytosis!

Phagocytosis

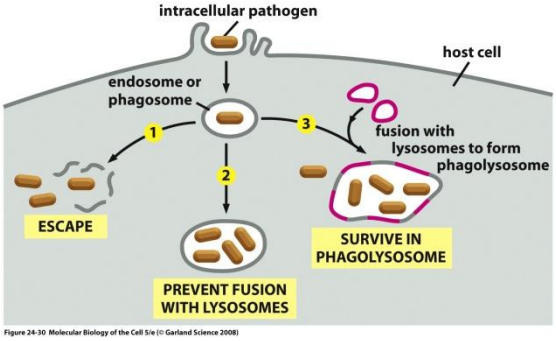


- Only takes place in specialized cells, such as macrophages, polymorphonuclear lymphocytes, amoebae
- Particle-triggered/receptor-mediated: Fc-receptors (ligands are immunoglobulins), complement receptors, phosphatidylserine receptors (uptake of cell debris after apoptosis), scavenger receptors, lectins, integrins, toll-like receptors
- Actin-dependent (inhibition of actin inhibits phagocytosis)
- Leads to degradation in lysosomes (phagolysosome)
- Some pathogens are phagocytozed and escape their fate



Phagocytosis is a cargo particle induced process. It involves a zipper-like interaction between cell and particle. Phagocytosis is a very tight process, the cell grows around the particle, no liquid is internalized, only the particle.

Some pathogens are able to enter cells via phagocytosis but then subvert it and replicate inside the cell.



Why is phagocytosis interesting?

- Massive, transient reorganization of the plasma membrane and cytoplasm (functional differentiation in a local region of the PM)
- Membrane around particle displays highly dynamic properties and exerts forces
- Trafficking of membranes is transiently altered
- Microbial killing mechanisms (hydrolases, oxygen, radicals) activated
- Triggers synthesis of pro- and anti-microbial cytokines, chemokines and interferons
- Activates the antigen presentation machinery
- Part of the native immunity
- All changes are transient

Step by Step Fcγ-Receptor Mediated Phagocytosis

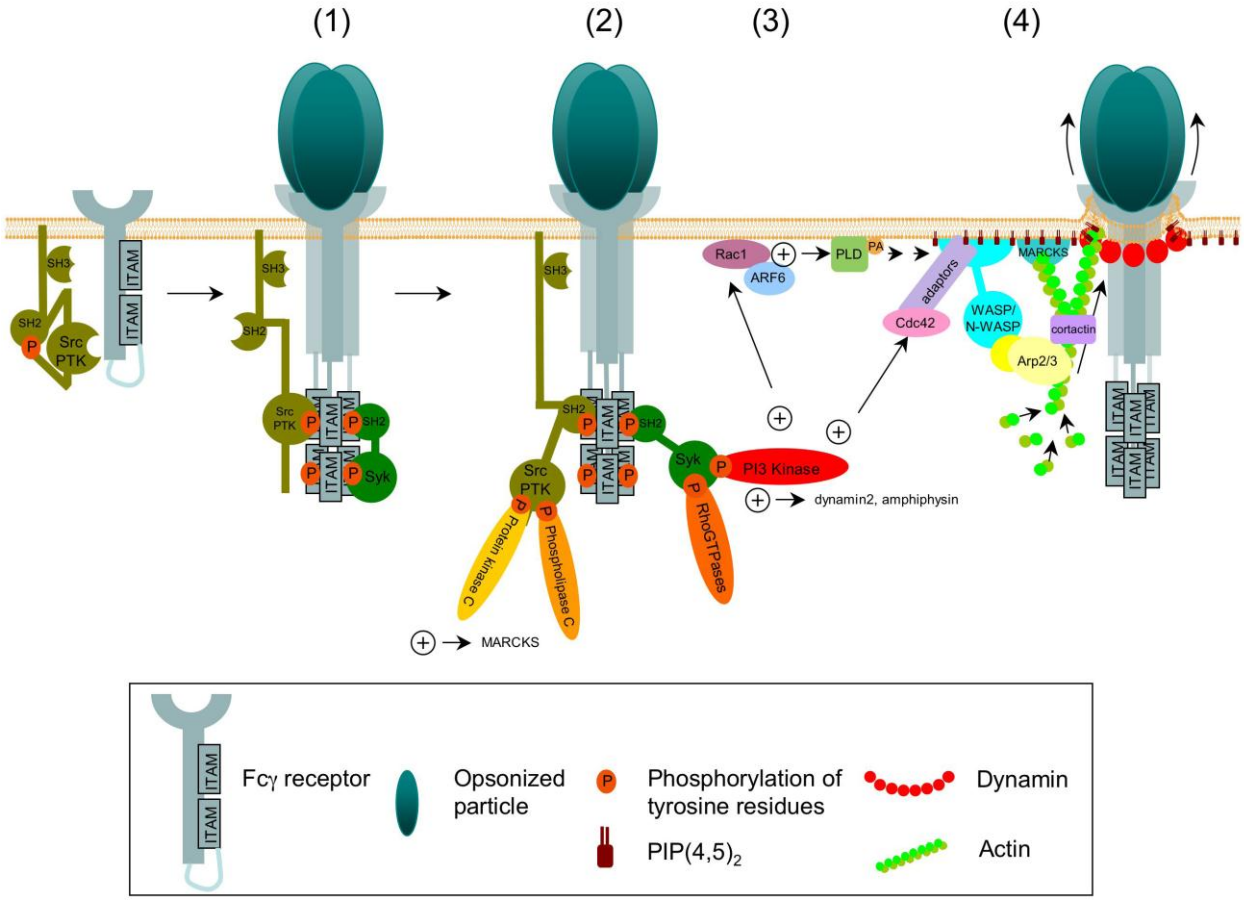
Particle binding and transmembrane signaling -> actin recruitment -> membrane expansion and extension, formation of phagocytic "cup" -> membrane fission to form phagosome -> phagosome internalization -> dissociation of actin -> fusion phagosome with lysosome.

Machinery:

- Receptor
- Tyrosine kinases (c-Src and Syk)
- Serine/Thr kinases (PKC)
- PI kinases
- Phosphatases
- Phospholipases
- Rac and Arf (small GTPases)
- Adaptors and activators
- Actin cytoskeleton components and regulators
- Focal adhesion site components
- Calcium and calmodulin

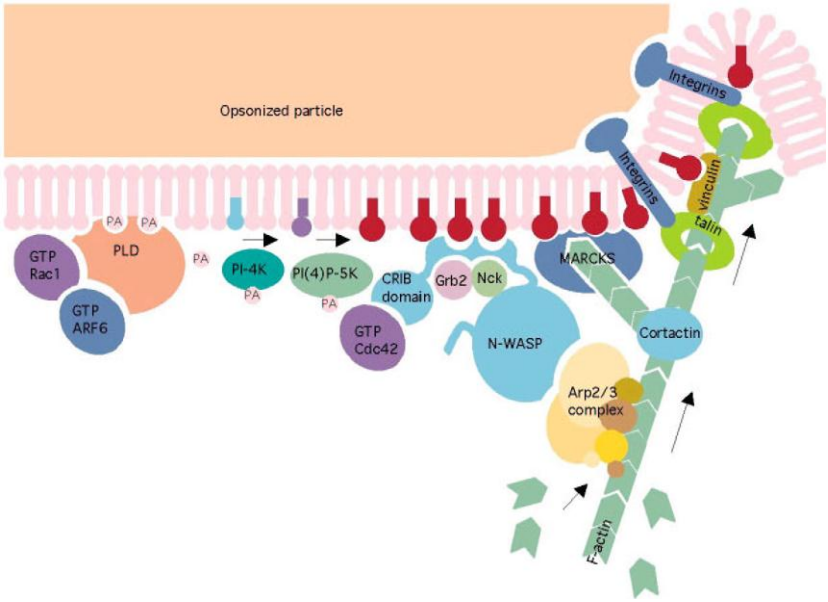
First, Fc-receptors bind Fc-domains of IgG on opsonized particles (coated with IgG). The receptors undergo a conformational change and activate a tyrosine phosphatase (e.g. CD45). CD45 then activates the Src-family tyrosine kinases by dephosphorylating a C-terminal tyrosine. Active Src-kinases phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAMs) in the Fc receptor cytosolic domain. The ITAM then exposes its Src-homology 2 domain (SH2), which binds the Src kinase and a

domain in the Syk kinase. The Syk kinase is phosphorylated by Src kinase and therefore activated. The formed complex serves as a launching pad that sets off the assembly of the endocytic machinery.



Once activated, both Syk and Src kinase complexes phosphorlylate a wide range of substrates such as PI(3) kinase, phospholipase C, protein kinase C, Rho GTPases, adaptor proteins, etc. These induce a cascade of protein-protein and lipid-protein interactions that induce the assembly of the phagocytic machinery and generation of the cup through multiple feed-forward and feed-back mechanisms. Molecular classes involved are phosphoinositides (PIs) and the corresponding kinases, small GTPases of the Rho and Arf families, phospholipases that produce phasphatidic acid (PA) which activates PI kinases and molecules that regulate actin assembly.

Role of PI(4,5)P_2 and PI(3,4,5)P_3: PI(4,5)P_2 serves as a signal in the plasma membrane for vesicular traffic, membrane movement and actin assembly. It recruits proteins with plekstrin homology domains (PH), epsin N-terminal domain homology domains (ENTH) and Lys/Arg-rich effector domains to local sites at the plasma membrane. PI(3,4,5)P_3 is important locally in pseudopod extension and closure of the phagosome. Together, PI(4,5)P_2 and PI(3,4,5)P_3 recruit a number of proteins: WASP and N-WASP family members (interact with PI(4,5)P_2), Arp2 and Arp3; dynamin2, epsin, amphiphysin IIm (PI(4,5)P_2 binding



domains); Arf6 (ADP-ribosylation factor GTPases); PI specific phospholipase C (PI-PLC) which generate diacylglyceride and IP_3 -> calcium release from the ER -> activation of PKC -> activation of MARCKS; myristylated alanine-rich C-kinase substrate proteins (MARCKS) need PKC for dissociation from actin and membranes.

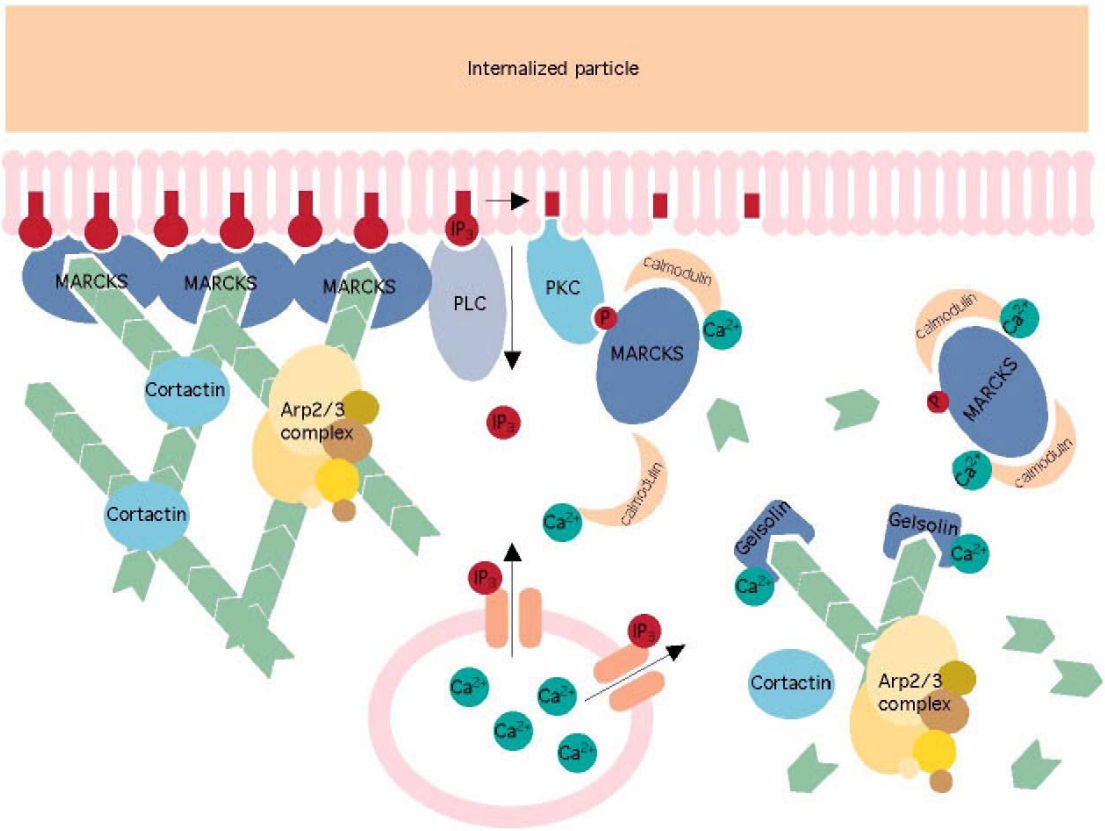
Role of small Rho family GTPases: Activated Arf6 activates plasma membrane phospholipase D (PLD) that produces phosphatidic acid (PA) which in turn recruits PI(4)P- and possibly P(4)-kinases establishing local $PI(4,5)P_2$ generation. GEFs for Rho-family GTPases bind to $PI(4,5)P_2$ enriched phagocytic cups by their plekstrin homology domain (PH). In Fcγ-receptor mediated phagocytosis, activated Rho GTPases, Rac1 and Cdc42, bind to Cdc42/Rac interactive (CRIB) domains present on WASP family proteins. RhoA, Rac1 and/or Cdc42 also recruit focal adhesion kinases (FAKs).

Role of actin dynamics: WASPs are activated by Cdc42 and $PI(4,5)P_2$ and thus expose Arp2/3 binding sites in the C-terminus. The Arp2/3 complex is the main actin nucleator in the phagocytic cup (and recruited by activated WASP family members). Arp2/3 binds monomeric actin and stimulates actin polymerization. Actin cross-linking and bundling is induced by interaction with cortactin and α-actinin. Pacillin, talin and vinculin (focal adhesion components) help to link the actin with the particle surface. At the end of phagocytosis, actin severing proteins (gelsolin and cofilin) play a role in breaking up the actin network.

Sealing the Phagosome: Poorly understood membrane fusion event, PI(3)-kinases, dynamin2 (GTPase capable of pinching off internalized vesicles), amphiphysin IIm and actin depolymerization seem to be required for this process. As soon as the phagosome is released into the cytoplasm, actin filaments are rapidly depolymerized.

Scaffolds involved in Phagocytosis: Multiprotein scaffold complexes, such as phosphotyrosine/Src phosphotyrosine kinase based scaffolds, PI-based scaffolds, actin based scaffolds, adaptor protein based scaffolds (Cbl, Nck, Grb2; contain multiple SH2 (bind pYXXI) and SH3 domains (bind proline rich

sequences). The adaptor proteinscaffolds may serve as integrators, linking other scaffolds functionally to each other.



Macropinocytosis

- Signaling-induced, particle-independent endocytic uptake of fluids, membrane and sometimes particles into larger vacuoles called macropinosomes
- Property of most cell types
- Triggered by growth factors or hormones and activation of complex signaling pathways involving protein and lipid kinases and Rho GTPases
- Transient (15-30 min) rise in fluid uptake
- Dynamics of actin and the formation of cell surface lamellopodia or filopodia which are thought to fold back and fuse with the plasma membrane (engulfing fluid)
- Dependent on actin and a proton/sodium exchanger activity in the plasma membrane (inhibition through Amiloride)
- Macropinocytosis does not trigger immuneresponse

Caveolae

- Allow protein transcytosis across endothelial cells
- Flask-shaped, 50-100nm, smooth invaginations of the plasma membrane, can occur in grape-like clusters
- In many cell types (endothelial, epithelial (on basal lamina of ECM, waste products and proteins and nutrients are transported in and out of the blood stream) and smooth muscle cells, fibroblasts, adipocytes, pneumocytes…), not present in neurons and lymphocytes
- Stable, stationary structures, fixed to actin network
- Internalization can be activated upon signaling (appropriate ligand and tyrosine kinase), endocytosis takes place, then fusion with the early endosome
- Caveolae contain lipid rafts (cholesterol and sphingolipids) and are TX100 resistant
- Three layers:
    - Lipid bilayer rich in lipid rafts
    - Scaffold of integral membrane proteins caveolin-1 and 2 (or 3 in muscle cells)
    - Shell of peripheral proteins called cavins
- Stable and long-lived, no cycle of assembly and disassembly as part of the function

Caveolin-1

- Cav-1
- Essential for formation of caveolae
- Integral membrane protein, 22kDa
- Hairpin-membrane topology with both termini facing the cytoplasm
- Interacts with cholesterol
- Multiple phosphorylation sites
- Palmitoylation of cysteins (not required for plasma membrane targeting)
- Present in higher order complexes, 7-10mers further associated in a scaffold that defines the caveolar domain

Functions

- Transcytosis in vascular endothelia
- Endocytosis and vesicular transport
- Cholesterol homeostasis

- Modulation of signal transduction
- Tumor suppression
- Formation of T-tubules in striated muscle
- Internalization of viruses and toxins

Summary

- Phagocytosis: Complex process, portion of plasma membrane transiently transformed to specialized function of internalizing a large particle
- Macropinocytosis: Similar transient, actin-mediated change in plasma membrane, mainly for internalization of fluid
- Caveolae: Stable specialized microdomains in plasma membrane, can be activated to become endocytic vesicles