Cytoplasmic Membrane Systems (endomembranes; organelles)

- Structure
- Function
- Membrane Trafficking

Intracellular compartmentalization

- eukaryotic cells have a variety of identifiable organelles - intracellular compartments surrounded by membrane
  - nucleus
  - endoplasmic reticulum
  - Golgi complex
  - lysosomes
  - peroxisomes
  - endosomes
  - mitochondria
  - chloroplasts
A plant cell viewed with the electron microscope

An animal cell viewed with the electron microscope

A “generalized” animal cell has a variety of organelles
Why compartmentalize cells?

- 1. allocation of specialized tasks within cells
- 2. membranes themselves can become the site of reactions
- 3. microenvironments to optimize specific reactions
- 4. storage of materials within cells
- 5. cells can become bigger, and more developmentally diverse

Origin of intracellular membranes

- nuclear membrane and the endoplasmic reticulum formed by invagination of the plasma membrane of an ancient prokaryote

Origin of intracellular membranes (cont’d)

- mitochondria and chloroplasts likely originated when one cell engulfed another—endosymbiosis
- explains why these organelles:
  - have two distinctly different inner and outer membranes
  - are semi-autonomous—have their own DNA
  - are not involved in membrane exchange with any other organelles
Origin of mitochondria by endosymbiosis

Organelles exchange membrane and contents

- Within cells, there is a constant “traffic” of membranous vesicles which are shuttling membrane and contents back and forth from one organelle to another.
- The delivery of membrane via vesicles:
  - Is from donor compartment to target compartment and sometimes back again.
  - Maintains membrane integrity.
  - Is highly specific.

Vesicle Transport

Membrane vesicle buds off (fission), donor compartment and migrates to the recipient compartment, where it fuses.
Overview of endomembrane system “traffic”

1. Secretory Pathways (membrane and vesicle contents out of the cell)
   - Constitutive (happens continuously)
   - Regulated (is stimulated to occur)

2. Endocytic Pathways (membrane and vesicle contents into the cell)
   - Lysosome production

Endoplasmic reticulum (ER) and the Golgi complex--two dominant organelles:

- Endoplasmic reticulum (ER):
  - large, flattened cisternae
  - can make up nearly half of a cell's membrane
  - ribosomes attached-- “rough” ER: primary function is synthesis of proteins
  - no ribosomes attached-- “smooth” ER: primary functions are lipid synthesis, detoxification, sequestering ions

Rough ER
Some roles of the rough ER

- Synthesis of secretory proteins—these proteins are “targeted” to RER by signal sequences
- Synthesis of lysosomal proteins
- Synthesis of integral membrane proteins
- Partial glycosylation of proteins (i.e., adding sugars covalently)
- Protein folding, including the formation of disulfide bonds

Both smooth and rough ER occur in many cells

The cisternae of the rough ER and the smooth ER are continuous; during early development of cells, the rough ER forms first, then the smooth ER
Some Roles of Smooth ER

- Synthesis of steroid hormones
- Detoxification—especially in the liver
  - Oxygenase enzymes break down toxins
  - Examples: alcohol; barbiturates; other drugs
- Sequestering of Ca²⁺
  - Example: muscle cells

Separation of smooth and rough ER into microsomes can be done by homogenization and centrifugation

The Golgi complex

- named after Camillo Golgi— a 19th century neuroanatomist
- lies near the nucleus, usually
- 10-12 flattened interconnected cisternae (or "sacs")
- has "cis", "medial", and "trans" regions, which seem to be functional compartments
- connected to RER via "transitional vesicles" on cis face
- concentrates and chemically modifies proteins within its cisternae in an apparent cis------>trans direction
Views of the Golgi Complex

Golgi Traffic

Vesicles from RER

Secretory cells show a strongly polarized structure

The apical surface of the cell is where the secretory granules (mucus in this case) is exocytosed on to the surface of the cell (mature protein is “secreted”)

The medial region of the cell houses the Golgi complex (packages and modifies protein)

The basal surface of cell has the rough endoplasmic reticulum (makes protein)
Experimental demonstration of relationship between RER and Golgi complex

- structural studies *suggested* a functional relationship as follows (for *secretory or membrane* proteins):
  - RER---*cis* Golgi---*trans* Golgi---cell surface
- there was a need for another approach to more directly show this pathway; i.e., a way of “following” the pathway of protein

The Palade experiment

- George Palade decided to monitor the movement of secretory protein through the *pancreatic acinar cell* (see handout)
- why this cell??
  - chemistry of the proteins made by the cell were well known (digestive enzymes)
  - techniques for maintaining cells in culture were well established
  - ultrastructure of the cell was well characterized

The Palade experiment (cont’d)

- the technique Palade used was *pulse-chase autoradiography*:
  - expose cells in culture to radioactive amino acids for a brief period (“pulse”)
  - these amino acids are then incorporated into protein, which becomes radioactive
  - wash out excess amino acids
  - monitor the movement of radioactive protein over time (“chase”)

- [Handout reference]
An electron micrograph of a pulse-chase experiment in a pancreatic acinar cell

Diagrammatic summary of the pulse-chase experiment in a pancreatic acinar cells

Palade’s observations have been confirmed using green fluorescent protein (GFP) labelling. This cell has been induced to synthesize a viral protein to which the GFP gene is fused—it follows the same ER→Golgi pathway

The VSV used is a mutant form that at the high temperature remains in the ER—it can go to the Golgi only at the lower temperature
Summary of the essential steps suggested by the Palade experiment:

1. Synthesis and segregation of protein into rough endoplasmic reticulum (RER) cisternae
2. Transport via transitional vesicles to the *cis*-Golgi cisternae
3. Transport through the Golgi to *trans* vesicles; protein becomes *glycosylated* along the way
4. Formation of *zymogen* (secretory) granules
5. Exocytosis of granules on to *apical* cell surface

Some features of the “Palade” pathway for secretory protein

- vesicular transport is the centrepiece
- each step is essential—no step can be missed
- found in all eukaryotic cells (with some minor modifications)
- a *highly regulated* pathway; but this pathway also applies to *constitutive* pathways for replacing *membrane* protein
Some questions raised by the Palade experiments

- How does protein get into ER cisternae?
- Is protein selected for transport to the Golgi complex, or is it transported “by default”? Is there “recycling” of vesicles back to ER?
- What is the mechanism of glycosylation of proteins (i.e., the covalent addition of sugars)?
- How do transport vesicles recognize and fuse with their appropriate target organelle?

Proteins are synthesized by the translation of mRNA on cytoplasmic ribosomes

The eukaryotic ribosome is a large RNA/protein complex

Cytoplasmic proteins are synthesized on polyribosomes (i.e., ribosome + mRNA) which occur as a free pool

mRNA encoding a cytosolic protein remains free in cytosol

common pool of ribosomal subunits in cytosol

Figure 12-37 part 1 of 3. Molecular Biology of the Cell, 4th Edition
Synthesis and segregation of protein into the RER occurs by specifically attaching some of the ribosomal pool (*bound* ribosomes) to the ER membrane.

But how does the protein cross the ER membrane?

Two possibilities:
1. **Post-translational insertion**—the protein is synthesized on a *free* polyribosome, and then inserted *after* synthesis is complete.
2. **Co-translational insertion**—the newly forming polypeptide chain is inserted through the membrane *during* the process of translation on a *bound* polyribosome.

**Gunter Blobel and the insertion of proteins into the ER—the signal hypothesis**

- Investigated this problem using isolated RER membrane—called *rough* microsomes.
- Got these to synthesize protein *in vitro*—this is called a *cell-free system*.
- Used the mRNA for *immunoglobulin G* (*IgG*), which had been isolated and purified.
More detail of each of the steps in the co-translational insertion of a soluble protein into the ER lumen

1. Translation begins on a free polyribosome
   - translation begins at 5'-end of mRNA
   - the first portion of the mRNA contains the signal codons
   - signal peptide (signal sequence) is translated first
   - eventually the signal peptide emerges from the large ribosomal subunit
   - the signal peptide then binds to the signal recognition particle (SRP), which is a RNA-protein complex

2. The binding of the SRP to the emergent signal sequence halts further translation until the SRP can bind to an ER-SRP receptor
More detail of each of the steps in the co-translation insertion of a soluble protein into the ER lumen (cont’d)

- 3. The SRP-ribosome complex binds to the ER membrane--SRP released
  Signal peptide orients itself in a “hairpin” formation within a protein translocation channel in the ER membrane.

4. Protein translocation begins via a translocation--“translocon”--channel in ER membrane

5. Translocation continues until entire polypeptide is in the lumen

6. Signal peptide is cleaved by signal peptidase (ER membrane enzyme)

The signal hypothesis works for the insertion of transmembrane proteins as well

- think of the signal peptide as essentially a “start-translocation” sequence at the beginning of a protein
- if a “stop” sequence occurs within the polypeptide chain, the translocon open up and releases the protein to remain embedded in ER membrane
In this example, a single internal “start” signal (but no “stop” signals) has a particular amino acid sequence which is oriented:
NH2 end—> positively charged a.a.—> negatively charged a.a.
The protein ends up with its amino terminal end facing the cytoplasm.

In this second example, a single internal “start” signal has a particular amino acid sequence which is oriented:
NH2 end—> negatively charged a.a.—> positively charged a.a.
The protein ends up with its amino terminal end facing the ER lumen, since the start signal is inserted into the translocon differently.

If a “start” signal is followed by a “stop” signal, a double-pass transmembrane protein can be produced.
Post-translational insertion of proteins is used to get membrane proteins into mitochondria

- Proteins are synthesized on free ribosomes, and then bind, via a signal sequence to an outer membrane translocator (TOM), which coordinates with an inner membrane translocator (TIM) to thread the protein across both membranes.

Post-translational insertion of proteins is also used to get membrane proteins into chloroplasts via translocators into the stroma or the thylakoid space

In studying the synthesis of proteins, and translocation of proteins across membranes, it is clear that the folding of proteins into three-dimensional form is a tightly controlled process

- Polypeptides can fold into several possible tertiary conformations spontaneously.
- **But**, only one, unique tertiary conformation is optimal for a particular polypeptide.
- This conformation is obtained with the help of **chaperone proteins**—so called since they "supervise" and promote correct protein folding, and thus prevent "inappropriate" aggregations!!
Chaperone proteins

- discovered by accident!!
- cells in culture exposed to “heat shock” of 42° C synthesized new, unique proteins
- originally called “heat shock proteins” (HSPs)
- produced by the cell for “quality control”-- to prevent aggregation of partially denatured proteins after heat shock, and to cause them to re-fold correctly

Chaperone proteins

- Properties:
  - ATPases
  - recognize hydrophobic regions of polypeptides--prevent mis-folding or aggregation of polypeptides
  - occur in cytoplasm, ER, mitochondrial matrix
  - three types found:
    - Hsp70: variants occur in cytoplasm and mitochondria
    - Hsp60: variants occur in cytoplasm and mitochondria
    - BiP: (similar to Hsp70) in ER cisternae

Hsp70 action in cytoplasmic protein folding—the protein is “wrestled” into the right conformation

Figure 6-83. Molecular Biology of the Cell, 4th Edition.
Action of Hsp60 and Hsp70 machinery in protein folding

- the Hsp60 is like a large barrel—it captures the protein by its rim, then stretches it into the right shape, then it is released. This happens several times, until the protein is folded correctly.

Proteins destined for import into the mitochondria are kept unfolded by a cytoplasmic Hsp70 then folded by mitochondrial Hsp 60 and Hsp70

Modification and Quality Control

In the ER lumen, glucose is used to “tag” proteins so that they can be recognized by the chaperone protein CALNEXIN to obtain their properly folded state.

If there is mis-folding, the protein is translocated OUT of the ER into the cytoplasm, where it is degraded in the cytoplasm by a proteasome—an ATP dependent protease.
Disulfide bonds form in the ER compartment

- disulfide bonds form between cysteine residues in a polypeptide
- in the oxidizing conditions found in the ER, these bonds form
- correct disulfide bond formation is promoted by protein disulfide isomerasers (PDI) in the ER cisternae

As proteins travel from the ER through the Golgi complex, they are chemically modified--this is illustrated by glycosylation of proteins

- many proteins have covalently-linked oligosaccharides added to them
- these sugars are added in stages in the RER and Golgi complex:
  1. In the ER, a "core" oligosaccharide is added, in a bizarre bit of chemistry!!
  2. The oligosaccharide is then "trimmed" by the removal of sugars
  3. Further trimming of oligosaccharides and addition of terminal sugars (terminal glucosylation) occurs in the Golgi complex

Synthesis of the core oligosaccharide takes place on a membrane lipid on the RER membrane

- 1. a specialized lipid (dolichol) in the ER membrane is phosphorylated and glycosylated, one sugar at a time, on the cytoplasmic face of the RER membrane
- 2. when 2 GlcNAc and 5 mannose sugars have been added to Dol-P, the entire Dol-P/sugar intermediate is flipped across the membrane to the luminal side!!
- 3. on the luminal side of the ER, a further 4 mannose and 3 glucose sugars are added, completing the core oligosaccharide
The formation of the core oligosaccharide on the ER membrane

Note that the addition of sugars to dolichol starts on the CYTOPLASMIC side of the ER membrane, then it “flips” to orient the sugars to the LUMINAL side of the ER.

The core oligosaccharide is transferred “en bloc” to the NH$_2$ side chain of an ASPARAGINE amino acid of a protein in a single step by an oligosaccharyl transferase -- these are called “N-linked sugars”.

Summary diagram of the glycosylation of an N-linked glycoprotein
Oligosaccharide side chains are trimmed and modified as they move through the ER and Golgi complex

1. In the ER, 3 glucose sugars and 1 mannose sugar are removed by specific enzymes (glucosidases or mannosidases respectively)
2. In the Golgi stack, both trimming and addition of sugars (terminal glycosylation) takes place
3. Terminal glycosylation is highly variable (depending on the protein), giving a wide variety of sugars by the time the protein reaches the trans Golgi compartment

Enzymes for steps in oligosaccharide processing are highly compartmentalized in the Golgi complex so as proteins move through the cisternae, they encounter them in series.
The Golgi complex - a packaging, sorting and chemical modification centre in cells

Transport from the ER through the Golgi complex
- current models suggest that the Golgi complex is a series of compartments interconnected by vesicular traffic
- James Rothman likens the Golgi to a “distillation tower”:
  - crude export from the RER enters on the cis side
  - forward and reverse (retrograde) transport occurs between the stack of cisternae in the Golgi complex--ER proteins are retrieved
  - “refined” export goes forward to trans compartment
  - fate of the refined export is variable

Vesicular traffic swarms around the Golgi complex!
There are several “pathways” through the Golgi complex

1. Regulated secretory pathway to cell surface:
   - vesicles take secretory protein to be exocytosed from the cell at the plasma membrane

2. Constitutive secretory pathway to cell surface:
   - vesicles take membrane protein, soluble protein and lipid to the plasma membrane--“wear and tear” replacement

3. Regulated pathway to lysosomes:
   - vesicles take acid hydrolases to lysosomes

There are three main pathways of protein through the Golgi complex: two are secretory, and the other is to lysosomes

Regulated and constitutive secretory pathways are distinct—the proteins are “sorted” into different pathways, and then into different types of vesicles
Vesicular transport occurs from the ER to Golgi and back again!!

- transport vesicles move in both directions:
  - ER--------> cis Golgi is forward transport
  - cis Golgi----> ER is reverse transport
- properly folded proteins “escape” from the ER in vesicles in forward transport to the cis—Golgi cisternae
- any protein with the C-terminal amino acid sequence --KDEL (ie lysine-aspartic acid-glutamic acid-leucine) is "retrieved" by reverse transport from the cis—Golgi cisternae---these proteins are thus considered “resident ER proteins” (even though they have gone on a trip to the Golgi and back!!)

Retrieving ER proteins: proteins with the -KDEL motif do not bind to receptors in the ER but do in the cis-Golgi cisternae as the pH is more acidic

Two models to explain the organization of the Golgi complex

- in the vesicular transport model, vesicular transport is bidirectional within the Golgi stack
- in the cisternal maturation model, vesicular transport is ONLY retrograde, and the cisternae gradually “mature” carrying membrane and contents forward
- not conclusive as to which model is correct
Vesicular formation is at “coated” regions

- vesicular formation is induced by the interaction of “coat proteins” with the membrane:
  - coat protein assembles on membrane
  - coated pit, then coated vesicle forms
  - coat disassembles from membrane

- different coat proteins exist:
  - clathrin (polymers of polypeptides as triskelions)
  - coatomer (polymers of 7 COP subunit polypeptides)—occurs as COPI and COPII variants

Types of coated vesicles

![Clathrin](image1.jpg) ![COPI](image2.jpg) ![COPII](image3.jpg)

Different kinds of coated vesicles are found in different places in the ER and Golgi complex

- **coatomer (COP) coated vesicles** are found:
  - travelling from ER to *cis* Golgi in forward transport (COP II variant)
  - travelling back from *cis* Golgi to ER in the retrieval pathway (COPI variant)
  - travelling in both directions in the Golgi stack (COPI variant)
  - travelling in the constitutive pathway from the trans Golgi to the cell surface (COPI I variant)

- **clathrin** coated vesicles are found in the *trans* Golgi region
  - in the secretory pathway—from trans Golgi to cell surface
  - lysosomal pathway—from trans Golgi to lysosomes, and in endocytosis from cell surface
Summary of COP-coated and clathrin-coated vesicular movement in cells

The formation of clathrin coats

Clathrin assemblies include:

1. **clathrin triskelions**
2. **adaptins**--which link clathrin to membrane receptor proteins, which in turn can pick up “cargo” for the vesicle
3. **dynamins**--GTP-binding proteins which pinch off membranes in the formation of vesicles

Clathrin coat assembly
**Formation of a clathrin-coated vesicle**
- clathrin binds to adaptins
- adaptins bind to cargo receptors; dynamin pinches off membrane (hydrolyses GTP in doing so); coat from vesicle

**If a variant of GTP is introduced that cannot be hydrolysed, vesicles cannot pinch off, and remain coated with clathrin**

**Assembly of a coatamer coat**
- (A) small GDP/GTPase protein (SAR1) is activated; becomes stuck in the membrane
- (B) coatamer subunits attach to them
How do vesicles get to the right place (target) in the cell?

Steps from budding to fusion
1. **Movement** toward specific target compartment
2. **Tethering** vesicles to the target compartment
3. **Docking** vesicles to the target compartment
4. **Fusion** between vesicle and target membranes

Vesicular recognition is accomplished by “SNAREs”

- vesicular “docking” is very specific
- accomplished by transmembrane proteins called **SNAREs**
  - vesicular SNAREs are called **v-SNAREs**
  - target organelle SNAREs are called **t-SNAREs**
- there are many different specific pairs of SNAREs in cells

Docking of vesicles: note the cargo and SNARE present in each vesicle
Tethering and docking transport vesicles

The v- and t-SNARE complex, along with SNAP, causes membrane fusion to occur

An important example of a precise Golgi pathway is the formation of lysosomes
- lysosomes are membrane-enclosed vesicles which contain acid hydrolases
- lysosomes are formed by clathrin-dependent vesicular budding from the trans Golgi cisternae—these vesicles are termed primary (1st) lysosomes
- when 1st lysosomes fuse with other vesicles, their contents are digested—ie 2nd lysosomes
- the internal content of lysosomes is kept acidic (pH = 5) by a membrane H⁺ pump
- acid hydrolases, in this acidic compartment, are capable of digesting virtually all biological molecules
There are three intracellular pathways that “deliver” materials to the lysosome for digestion: (1) phagocytosis, (2) endocytosis, and (3) autophagy.

A macrophage feasting on a two red blood cells—an example of phagocytosis.
A neutrophil (a white blood cell) engulfing a bacterial cell—another example of phagocytosis

Phagocytosis by protozoans is a way of feeding—they form food vacuoles, use lysosomes to digest the food, then absorb the nutrients

Summary of the phagocytic pathway
Autophagy: “doomed” organelles are engulfed by an isolation membrane (from ER) and then digested by lysosomal enzymes.

Summary of the autophagic pathway

Endocytosis of yolk proteins by an egg cell—in this case, the yolk proteins are binding to specific membrane receptors, which are clustered together by interactions with clathrin coats.
Summary of the endocytic pathway

Summary features of the three pathways to the lysosome

1. Phagocytosis:
   - non-specific uptake of insoluble material--even whole cells can be taken up!!
   - only found in a few specialized cell types; eg macrophages, white blood cells, protozoans
   - pathway is:
     - phagocytosis -> phagosome -> lysosome -> digestion

2. Autophagy:
   - isolation and destruction of “worn-out” organelles within the cell
   - common during development as cells change function
   - pathway is:
     - autophagic vacuole -> autophagosome -> lysosome ---> digestion

Features of the three pathways to the lysosome (cont’d)

3. Endocytosis:
   - can be specific or non-specific uptake of soluble material from the cell surface
   - in protozoans--called pinocytosis (“cell drinking”) and it is a non-specific uptake of fluid
   - in multicellular organisms, endocytosis is usually specific, receptor-mediated uptake of proteins from the cell surface--receptor-mediated endocytosis
   - Pathway is:
     - endocytosis -> early endosome --> late endosome -> lysosome -> digestion
How are lysosomal enzymes segregated into lysosomes in the Golgi complex?

- Acid hydrolases are synthesized in the rough endoplasmic reticulum.
- They are glycosylated, and transported to the Golgi complex by vesicular transport.
- A unique sugar—mannose-6-phosphate (M6P)—is then added to the hydrolase oligosaccharides in the cis Golgi cisternae.
- In the trans Golgi cisternae, there are transmembrane mannose-6-phosphate receptors present, which bind to M6P.
- Clathrin coats pinch off these receptors into lysosomes at the trans Golgi cisternae.
- These lysosomes are delivered to late endosomes, where the M6P is unloaded, and the receptor recycled to trans Golgi membrane.

The transport of acid hydrolases to lysosomes via the Golgi complex

A good example of the role of lysosomes is the receptor-mediated uptake of cholesterol by animal cells.

- A large proportion of cholesterol is carried around in the bloodstream in large particles called low-density lipoprotein (LDL)—these need to be taken up by cells to provide cholesterol for membrane synthesis.
Excess LDL in the circulatory system can lead to the formation of plaque—this is called atherosclerosis.

- Injury to the endothelial cell lining attracts leukocytes (white blood cells)
- Leukocytes ingest LDL which results in formation of plaque bulging into blood vessel

LDL binds in clusters on the cell surface, since the LDL receptors are clustered there by association with clathrin on the cytoplasmic side.

LDL is taken up at the surface of animal cells by specific LDL receptors which are clustered at clathrin coated membrane regions.
LDL is delivered to lysosomes so that the cholesterol can be released from the LDL particles

1. LDL is taken up into clathrin-coated vesicles
2. The vesicles lose their coat, and fuse to form an early endosome
3. The endosome is acidified by H+ pumps in the membrane, and the LDL dissociates from its receptor
4. The receptor is segregated into a transport vesicle for return to the plasma membrane
5. The remaining endosome acquires lysosomal enzymes, which digests the LDL, releasing the cholesterol into the cell cytoplasm

NB: this pathway operates even if LDL is not present!—ie it is constitutive—more like an escalator than an elevator

Mutations in the LDL receptor can cause high cholesterol since LDL is not taken up from the blood

Two types of receptor mutations are possible:

1. Mutations in the extracellular portion of the receptor:
   - if the part of the receptor which faces the outside of the cell is altered, then LDL binds at a lower affinity
2. Mutations in the cytoplasmic portion of the receptor:
   - if the cytoplasmic side of the receptor is mutated, then it does not recognize clathrin-coated membrane regions, thus the receptor is not taken up into the cell

Mutated LDL receptors are not taken up in clathrin-coated membrane vesicles

Figure 13-44, Molecular Biology of the Cell, 4th Edition.