The focus of this chapter of our hypertext will be on the endomembranes of the eukaryotic cytosol: Endoplasmic Reticulum; Golgi; Lysosomes; Peroxisomes; and their dynamic interconnections.

Flow of material through the endomembrane system is both anterograde (outward directed, toward the cell surface) and retrograde (inward directed, from the cell surface); each compartment has its resident proteins as well. Delivery from the ER supplies these compartments, as well as the cell surface, with lipids and protein components.

Internal compartmentation by semi-permeable membranes permits accumulation/exclusion/microenvironments/non-compatible biochemistries.

http://www.olympusmicro.com/primer/techniques/fluorescence/gallery/cells/apm/apmcellslarge.html cached 060122

A pseudocolored image of African water mongoose cells showing labeling of the nuclear pore complexes (green), the microfilament cytoskeleton (blue) and Golgi (magenta; the specific antigen is "giantin", a structural protein of the Golgi).

### Cellular Membranes

<table>
<thead>
<tr>
<th>Endomembrane Structure</th>
<th>% total membrane in Hepatocyte</th>
<th>% total in Pancreatic Exocrine Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoplasmic Reticulum (smooth)</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Endoplasmic Reticulum (rough)</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td>Golgi</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>0.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>0.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>Endosomes</td>
<td>0.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td>Nuclear envelope (inner only)</td>
<td>0.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The total amount of each membrane class is plastic and responsive to changes in the metabolic activities of the cell in which they are located.
Flows of material through the endomembrane system deliver materials to different places in the cell.

Each step involves specific sorting, packaging, transport and delivery, as well as associations between membranes and cell structure that maintain functions in specific distributions. For example, Golgi Bodies must stay organized with respect to structure and biochemistry despite the bulk flow of materials (membrane and protein) through the organelle.

There are even systems that return “mistakes” to their appropriate locations, such as the recycling of ER residents (proteins that normally function within the ER, such as molecular chaperones) that escape to the Golgi (via the C-terminal KDEL sequence receptor).

Pancreatic acinar cells were pulsed with radioactive amino acids and subsequently fixed at various times after wash-out and processed for EM autoradiography. The rapid movement of label through the secretory system was revealed by the subsequent localization of label over the:

- ER lumen, 1-5 minutes
- Golgi, 7-10 minutes
- Forming secretory vesicles, 30-40 minutes
- Mature secretory vesicles, 60+ minutes

Release from secretory vesicles to the extracellular space can be triggered by hormonal application

Primary literature images from Palade’s work (for which he shared the Nobel Prize in Physiology or Medicine in 1974).
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The ER is a branching network of membranes that extends throughout the cytoplasm in a reticulate topology.

3,3'-dihexyloxacarbocyanine iodide (also known as DiOC6(3)) is a lipophilic dye that vitally stains the ER network in living cells (such as this 3T3 cell).


HeLa cell expressing ER-targeted GFP and mitochondrially-targeted DsRed2. 5 min time course of an unusually dynamic cell showing branching, fusion and movement of the endoplasmic reticulum. Note too the movement of mitochondria in this time-lapse video. Scale bar is 5 μm.

Both the ER and mitochondria can be transported along (or anchored to) microtubules via motors and microtubule-associated proteins (MAPs).

Movie source [http://www.uhnresearch.ca/facilities/wcif/gallery3.html](http://www.uhnresearch.ca/facilities/wcif/gallery3.html) cached 060120; As of 141104 the original sequence is unavailable but a similar video may be found at [http://www.youtube.com/watch?v=WLYnSSzqGpE](http://www.youtube.com/watch?v=WLYnSSzqGpE).

The SER and RER are contiguous. Segregation of proteins may be accomplished by fence-like corals of IMPs that permit certain lipids to pass but retaining resident proteins in their appropriate domains.

The rER is easily recognized by the presence of membrane-associated ribosomes transcribing integral membrane proteins / secretory proteins. Nascent peptides and their translating ribosomes are recruited to insertion channels in the rER membrane. The amino-terminal targeting signal is hydrophobic in nature and is recognized by a signal recognition particle (SRP) that threads the nascent chain into docking/transfer channels on the rER. The signal sequence is cleaved by a signal sequence peptidase following transit into the ER lumen. IMPs are released laterally into the plane of the membrane, while secretory proteins are released into the rER lumen where they fold, assisted by resident chaperones such as Hsp70 and disulfide isomerase. Glycosylation begins in the rER (later completed downstream in the Golgi apparatus).

TEM image http://www.medicine.unige.ch/morpho/orci/images/30me.jpg cached 060122
Freeze fracture image http://www.medicine.unige.ch/morpho/orci/images/31f.jpg cached 060122

Lipid and protein moves from the rER to the Golgi by a cycle of vesicle formation, transport and fusion. This process is facilitated by a smooth coat formed of coatamer proteins that form the vesicle.

Quality control mechanisms in the ER mediated by calnexin/calreticulin retains misfolded protein; ultimately, if not folded properly, misfolded chains are exported back to cytoplasm, ubiquitinated and destroyed by proteosome.

http://bio.winona.msus.edu/berg/IMAGES/rer-pan.jpg cached 040123
Vesicular Traffic

<table>
<thead>
<tr>
<th>donor membrane</th>
<th>recipient membrane</th>
<th>vesicle type</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell surface</td>
<td>endosomes</td>
<td>clathrin-coated</td>
</tr>
<tr>
<td>rER</td>
<td>Golgi</td>
<td>coatomerII-coated</td>
</tr>
<tr>
<td>Golgi</td>
<td>Golgi</td>
<td>coatomer-coated</td>
</tr>
<tr>
<td>Golgi</td>
<td>lysosomes</td>
<td>clathrin-coated</td>
</tr>
<tr>
<td>Golgi</td>
<td>cell surface</td>
<td>coatomer-coated</td>
</tr>
</tbody>
</table>

There are at least several (probably many more) distinct classes of vesicle coats/sorting mechanisms that function at different places in the cell.

Vesicle formation from a target membrane is an energetically-unfavorable event, requiring special mechanisms to pinch off a bud that contains the appropriate cargo. The primary motive force for these events is the formation of a protein polymer coat on the cytoplasmic face of the donor membrane that both distends the membrane as well as engaging receptors/adaptors that trap cargo into the forming vesicle (better yet, occupied receptors economically appear to trigger coat recruitment, so every package is always full!). Major coat proteins are clathrin and coatomer; these operate at unique places to sort and direct material along the secretory pathway.

VSVG<sub>ts</sub>::GFP Transport

Vesicular stomatitis virus glycoprotein, temp-sensitive folding mutant

COS cells transfected with a construct encoding GFP fusion to a temperature-sensitive folding mutant of bovine vesicular stomatitis virus coat protein (VSVG) were incubated overnight at non-permissive temperature (40°C) to accumulate marker within the rER (retained by quality control mechanisms). Upon shifting to permissive temperature (32°C) the accumulated material quickly translocates to the Golgi and then out to the cell surface.

http://dir2.nichd.nih.gov/nichd/cbmb/sob/movies.html cached 060122; as of 141103 this video was relocated at http://www.youtube.com/watch?v=UcQE_YOeTtA
Membrane and protein travel from the rER by vesicles that bud and deliver material to the cis, or forming, side of the Golgi (often convex). As materials transit from this early compartment through the stack either by cisternal maturation (“ageing” of the whole cisterna) or budding/refusion at the cisternal edges they are subject to a sequence of modifications (glycosylations begun in the rER and other post-translational modifications) before reaching the trans, or leaving, side of the Golgi (often concave). Lipid tethers are added to some luminal proteins that keep them associated with membrane surfaces, ultimately retaining some of them on the cell surface following exocytosis.

Some of the PTMs mark proteins for sorting from the default, exocytosis pathway. The best known example is the recognition of proteins bearing a mannose-6-phosphate, which are specifically packaged into a subset of vesicles bound for the lysosome (a degradative organelle). A major issue is how "resident" proteins, such as enzymes that catalyze the PTMs in specific compartments (and subcompartments) of the endomembrane system are retained and/or returned to the appropriate place while the bulk flow of materials moves forward. This “swimming up stream” is accomplished by attachment to specific IMPs that are structurally anchored in place, or by trapping them with receptors for resorting backward (as is the case for ER residents which bear a -KDEL carboxyterminal sequence (lysine-aspartic acid-glutamic acid-leucine) that interacts with a protein in the cis Golgi that recycles them back to the rER. Lipids are also sorted, and the vesicles leaving the Golgi for the cell surface are rich in cholesterol and thicker than those of the Golgi and thus may exclude Golgi IMPs passively.

http://cytochemistry.net/cell-biology/golgi.htm cached 141103
http://micro.magnet.fsu.edu/cells/golgi/golgiapparatus.html cached 070130

Lysosomes

The lysosomes are the cellular recycling center, full of proteases, DNases, RNases. Cellular components, even dysfunctional mitochondria, are broken down in this compartment, as are endocytosed/phagocytosed materials. The lysosomes also participate in cell death by releasing their enzymes into the cytosol in the act of self-destruction.

Lysosomal enzymes are marked early in the secretory pathway by the addition of mannose-6-phosphate. This is recognized in the trans-Golgi by a mannose-6-phosphate receptor that sorts these proteins into clathrin-coated vesicles that detach, uncoat and then fuse/mature into lysosomes. Significantly, as the lysosome begins to function, membrane-bound ATPases are turned on that drop the internal pH of the compartment to activate these destructive enzymes, some of which also require a proteolytic step before becoming functional. These multi-layered mechanisms ensure that the lysosomal enzymes do not become active in the wrong place or at the wrong time.

There are several inherited diseases that affect lysosomal function. **I-cell disease** is caused by a loss of function of enzyme that phosphorylates the mannose residues on lysosomally-targeted enzymes. As a consequence, all lysosomal enzymes are secreted (in an inactive form) and the lysosomes will with material that is not degraded, eventually clogging the system. In **Tay-Sachs**, an enzyme required for membrane breakdown does not bear the appropriate signals for mannose-6-phosphorylation so it is secreted and the lysosomes fill with layer within layer of membrane material. In humans, this results in a poisoning of cells of the nervous system and death at an early age.