Lecture 8:
Chapter 8 Section 8.1, 8.3-8.4

Cytoplasmic membrane systems

Have talked about how membranes are constructed and have been focusing on the membrane that surrounds the cell and what goes on there. Now we will be moving into the cell to see how a eukaryotic cell can manage all its myriad activities.

When looked at with a light microscope the cell has a nucleus and a rather featureless cytoplasm. It wasn’t until the electron microscope was invented that we began to realize the complexity of that region of the cell called the cytoplasm. This is when we realized there were compartments. The compartments can be visualized but to get at the components and look at them in a biochemical fashion the cell had to be physically fractionated. Differential centrifugation was an important method for separating cell compartments and figuring out that each of the compartments was performing separate functions. So now we understand that the reason a eukaryotic cell can perform so many apparently competing functions is because the functions are happening in separate compartments in the cell.

So how is a cell compartmentalized?

This seems like stuff you know but please try to keep the compartments straight. A significant number of students each year miss easy points by forgetting some very fundamental features of these compartments.

<table>
<thead>
<tr>
<th>% = hepatocyte Compartments</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus (6%)</td>
<td>Genome: RNA/DNA SYNTHESIS</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Metabolic functions; Initiation of all PROTEIN SYNTHESIS- but for a small amount in mitochondria</td>
</tr>
<tr>
<td>Cytosol (54%)</td>
<td>ATP synthesis by oxidative phosphorylation</td>
</tr>
<tr>
<td>Mitochondria (22%)</td>
<td>Oxidation of toxic molecules</td>
</tr>
<tr>
<td>Peroxisomes (1%)</td>
<td></td>
</tr>
<tr>
<td>Endomembrane system</td>
<td></td>
</tr>
<tr>
<td>Endoplasmic reticulum (12%)</td>
<td></td>
</tr>
<tr>
<td>Rough</td>
<td>site of synthesis of proteins for secretion, integral proteins and lysosomal proteins</td>
</tr>
<tr>
<td>Smooth</td>
<td>Synthesis of lipids…other lipid associated synthesis</td>
</tr>
<tr>
<td>Golgi (3%)</td>
<td>modification sorting packaging of proteins and lipids</td>
</tr>
<tr>
<td>Cis</td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td></td>
</tr>
<tr>
<td>Trans</td>
<td></td>
</tr>
<tr>
<td>Endosomes (1%)</td>
<td>sorting of endocytosed material</td>
</tr>
<tr>
<td>Lysosomes (1%)</td>
<td>intra cellular degradation of endocytosed material</td>
</tr>
</tbody>
</table>

**FIGURE 1.9**

Focus on the **endoplasmic reticulum** first

Structure:
This is actually an extension of the outer membrane of the nucleus. Nucleus has a double membrane. An inner and outer membrane.

FIGURE 8.2

Smooth and rough endoplasmic reticula have different functions.

I. Smooth--SER
   A. Special cells
      Endocrine cells...Steroid hormone synthesis
      Liver .....Detoxification P450s of hydrophobic compounds
      Conversion of G6P from glycogen to glucose G6Pase is in the membrane
      Glycogen $\rightarrow$ G6P $\rightarrow$ Glucose
      Muscle...sarcoplasmic reticulum
      SER has Ca sequestering protein

   B. All cells – synthesize lipids for membranes
      All cells need their membrane lipids. Lipids are synthesized in the hydrophobic environment of the membrane. They are synthesized by integral membrane proteins that have their active sites on the cytosol side. The new lipids are inserted into the membrane on the cytosolic face of the membrane. Then some are flipped to the other side with the flippase. This is all part of this endomembrane environment so the membrane lipids can be distributed to other regions of the cell by the fluidity of the system and the interrelatedness of them all. As the membranes components move from one compartment to another they get different components and different attachments depending on their travels.

FIGURE 8.15

To get to the mitochondria or chloroplasts there are Phospholipid transfer proteins that take lipids thru the aqueous environment to those membranes.

II. Rough Endoplasmic reticulum--RER
   Where proteins with signal sequences are synthesized.

   Two general groups of proteins identified by the where in the cell they are synthesized

A. Ribosomes of RER
   Secreted proteins
   Integral membrane proteins
   Soluble protein in compartments
      ER
      Golgi complex
      Lysosomes
      Endosomes
      Vesicles

B. Ribosomes free in cytoplasm
   Cytosolic proteins
   Peripheral membrane proteins on cytoplasmic face
   Proteins going to the nucleus
   Proteins going to peroxisomes
   Proteins going to mito, chloroplasts
The next couple of lectures will deal with how this targeting is achieved. Pretty amazing to think about all these different proteins ending up in the compartments they belong to.

So how do proteins get inside this endomembrane system that is the endoplasmic reticulum?

In the early 70s when cell free protein synthesis was being heavily used to study a variety of aspects of protein synthesis. Gunter Blobel made an interesting observation.

**FIGURE 8.5**

In a cell free system...this is the 50,000 x g supernatant from homogenized cells, there were ribosomes, mRNA, tRNA needed to make proteins, radioactive aminoacids could be added and radioactive proteins could be made and looked at on gels.

![Without microsomes](image1.png)

Certain secreted proteins are made in large and detected on gels. The funny thing was that the proteins made this way tended to be slightly smaller than proteins made by the whole cell. Blobel went on to try this same experiment but he added back some of another fraction from centrifuging cell lysates. He added back microsomes and found that then the proteins made were the right size. They were also INSIDE the microsomes!

What he eventually postulated was the **Signal Hypothesis**

Proteins destined for synthesis on the ER contain an amino-acid stretch at their amino terminus that directs the protein and the ribosome to the ER.

So that was quite a concept when it was first proposed but it held up and now we know quite a lot about this.

Who are the players?

**Cytoplasm:**

**Components:**

- ribosome subunits
- mRNA
- SRP (signal recognition particle)
- 6 polypeptides
- 7S RNA

**Steps:**

MRNA + ribosome: in the cytoplasm the start codon of the mRNA is recognized by the ribosomal subunits and the ribosome is assembled on the mRNA.

Protein synthesis begins
The N-terminal amino acids emerge from the large subunit. If these amino acids have a stretch of 5-15 non-polar amino acids with a couple of charged amino acids at the front edge then the SRP will recognize that sequence and bind to it

SRP binding

Protein synthesis basically ceases while the SRP is bound to the ribosome, nascent protein, mRNA complex.

ER membrane

Components:
- SRP receptor
- Translocon:
  - Protein-lined, channel
  - Large central portion that allows passage of proteins

Steps:
- SRP receptor binds SRP
- Moves to translocon
  - Ribosome sits on translocon
  - GTP is bound to both SRP & SRP receptor due to interaction
  - Signal sequence is released to translocon

Protein synthesis resumes

ER lumen--folding and quality control
- Signal peptidase: Signal sequence is cleaved off
- Oligosaccharyltransferase--Carbohydrates are added
- Bip, Calnexin, Calreticulin: Molecular chaperones interact with protein as it emerges from the translocon and helps it fold.

Protein disulfide isomerase—PDI. Makes disulfide bonds.