Chapter 15. Intracellular Compartments

• The importance of compartmentalization.
  - Increased membrane area.
  - Specialized functions

Chapter 15. Intracellular Compartments

• Some important compartments. (Fig. 15-2)
  - Cytosol
  - Nucleus
  - ER
  - Golgi apparatus
  - Lysosomes
  - Endosomes
  - Mitochondria
  - Chloroplasts
  - Peroxisomes
  - See Table 15-1 for functions of major compartments.
Chapter 15. Intracellular Compartments

- Compartments have different evolutionary origins.
  - Membranes of the “endomembrane system” are thought to have evolved from folds of plasma membrane. (Fig. 15-3)

- Mitochondria and chloroplasts evolved from separate organisms. (Fig 15-4)

- Proteins are moved into (or out of) membrane bound organelles in three ways. (Fig. 15-5)
Chapter 15. Intracellular Compartments

- Question: If the cell is divided up into compartments that carry out particular functions, and....
- If nucleic acid synthesis happens in the nucleus and protein synthesis starts in the cytosol......
- How do the right things get to the right compartments?

Chapter 15. Intracellular Compartments

- How do the right things get to the right compartments?
  - A first (wrong) guess -- Suppose that there were “sortases” that recognized every macromolecule in the cell and directed them where to go.

Chapter 15. Intracellular Compartments

- How do the right things get to the right compartments?
  - Correct answer: Proteins have signal sequences within the protein itself. They may be discarded after the protein reached its compartment or they may stay with the protein for its entire lifetime.
Chapter 15. Intracellular Compartments

- Evidence for the statement that proteins have signal sequences within the protein itself:
  - Presence of common sequences in proteins targeted to the same compartment. (Table 15-3)

<table>
<thead>
<tr>
<th>Table 15-3 Some Typical Signal Sequences</th>
</tr>
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<tbody>
<tr>
<td><strong>FUNCTION OF SIGNAL</strong></td>
</tr>
<tr>
<td>Import into ER</td>
</tr>
<tr>
<td>Retention in lumen of ER</td>
</tr>
<tr>
<td>Import into mitochondria</td>
</tr>
<tr>
<td>Import into nucleus</td>
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<tr>
<td>Import into peroxisomes</td>
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</tbody>
</table>

*Positively charged amino acids are shown in red, and negatively charged amino acids in blue. An extended block of hydrophobic amino acids is enclosed in a yellow box. *H2N* indicates the N-terminus of a protein. **OOO** indicates the C-terminus. The ER retention signal is commonly referred to by its single-letter amino acid abbreviation, KOEL.

Chapter 15. Intracellular Compartments

- Better evidence comes from genetic manipulations.
  - Possible to isolate gene for a reporter protein.
  - Cut and paste gene to make a chaemeric gene.
  - Introduce gene into cell by transfection, cell translates gene, and (hopefully) moves it into place.
  - Investigator then examines cell to find location of reporter protein.
Chapter 15. Intracellular Compartments

• An example of this approach. Fig 15-6.

Note well: this figure shows only the final result -- the experiment did not start here!

Chapter 15. Intracellular Compartments

• Let's design a good experiment with controls.
  - Pick a "reporter protein".
  - Pick a cell type.
  - Pick a donor gene for the signal.
  - What would you expect to happen?
Chapter 15. Nuclear transport

- Morphology of the nuclear membrane. (Fig. 15-7)

- Proteins (and nucleic acids) move through the nuclear pores.
- Morphology of the nuclear pores. (Fig. 15-8).

- Small-medium sized molecules can diffuse through the gap in the pore complex.
- Experimental evidence: cell injection experiments.
Chapter 15. Nuclear transport

• Small-medium sized molecules can diffuse through the gap in the pore complex.
• Experimental evidence: cell injection experiments.
• Size limit = 50,000 daltons or 9-11 nm.
  - Therefore all nucleotides, sugars, ions etc freely diffuse.
  - Small proteins can also freely diffuse but large ones cannot.

Chapter 15. Nuclear transport

• How do large molecules get through?
  - Nuclear import signals.
  - Experimental evidence for this.
  - Take a bead of a size that would not be expected to penetrate into the nucleus (cf. above experiments).
  - Coat it with a protein that is normally imported.
  - The combination is obviously too large to enter by simple diffusion, but non the less rapidly accumulates in the nucleus.
Chapter 15. Nuclear transport

- How do large molecules get through?
  - Nuclear import signals.
  - Experimental evidence for this.
  - A proposed mechanism. (Fig. 15-9)
Chapter 15. Nuclear transport

- Large proteins are moved both ways through the pores.

Chapter 15. Nuclear transport

- RNA is transported out in a specific manner
- The RNA is complexed to protein, and the protein/nucleic acid complex might use a mechanism similar to protein transport discussed previously.

Chapter 15. Entry into mitochondria and chloroplasts.

- These organelles make some of their own proteins, but import others from the cytosol.
- The morphology of the process -- remember that the organelles have 2 membranes, not 1.
- Overview -- Fig 15-10.
Chapter 15. Entry into mitochondria and chloroplasts!

• An experiment that showed that the protein passes both membranes at once.

• The two possibilities: (next slide).
Chapter 15. Entry into mitochondria and chloroplasts!

- How could we differentiate between these 2 possibilities?
  - Hint #1. One can incubate isolated mitochondria with proteins and get protein import \textit{in vitro}.
  - Hint #2. At low temperature, the proteins get stuck during import.

- Where is the protein?
  - If 2 steps are required for import, then it could be halted at step #1, #2 or #3.
  - If 1 step is required for import, then it could only be halted at step #1.
Chapter 15. Entry into mitochondria and chloroplasts.

• Where is the protein?
  - An externally applied protease destroyed the C terminal end of the protein.
  - Which possibilities are left?

Possibility A, separate membranes

Possibility B, a contact site

• Where is the protein?
  - The N-terminal import signal is also cleaved.
  - Now which possibilities are left?
Chapter 15. Entry into mitochondria and chloroplasts!

• The importance of chaperones.
  - Cytosolic HSP-70 like chaperones
    • Proteins must be unfolded during transport.
    • Experiment: Proteins cannot be imported into isolated mitochondria unless either unfolded by denaturing agents or incubated with HSP-70 like proteins.
  - HSP-like proteins in the mitochondrial matrix.
    • HSP-70 like proteins.
    • HSP-60 like proteins.

Chapter 15. The ER

• Morphology of the ER:
  - Closed set of tubes
Chapter 15. The ER

• Functions of the ER
  - Detoxification of certain poisons.
  - Sequestration of certain molecules.
    • Especially important in Ca++ sequestration.
  - Synthesis of important molecules:

Chapter 15. The ER

- Synthesis of important molecules
  • Virtually all membrane lipids, even those that end up in other compartments.
  • Virtually all membrane proteins, even those that end up in other compartments.
  • Addition of sugars on many proteins (N-linked glycosylation).
  • Soluble proteins that are moved to another compartment or secreted out of cells.

Chapt. 11/15, Membrane Lipid Synthesis

• Remember that the lipid bilayer is asymmetrical (Chapter 11).
• This asymmetry is generated inside the cell.
  - Membrane lipids are made from components in the cytosol in association with the ER.
  - Thus they get made in the cytosolic leaflet of the ER membrane only.
  - Then specific phospholipids are “flipped”
  - Fig. 11-18 and Fig. 12-53 from Big Alberts
Protein Synthesis & Import into ER

- Virtually all membrane proteins throughout the cell are made in association with the ER.
- In addition, many soluble proteins, including those that end up in the Golgi, endosomes, lysosomes and outside the cell are too.
- Yet protein synthesis starts in the cytosol and many components (mRNA, ribosomes etc) remain there. What gives?

Protein Synthesis & Import into ER

- Proteins are imported into the ER during synthesis.
  - Terminology:
    - Translation - protein synthesis.
    - Translocation - Literally "across location". This is the physical act of moving a protein across the ER membrane.
  - Key Point: Most ER proteins are translocated as they are translated. This is also called cotranslational import.
Chapter 12/15, Protein Synthesis & Import into ER

- We will first consider the case where a protein is completely imported into the ER during translation.

Protein Synthesis & Import into ER

- Initial events
  - Translation starts just as for protein that will remain in the cytosol.
  - The first part synthesized (the NH₂ end), is an N-terminal cleavable signal.
Protein Synthesis & Import into ER

• The SRP (Signal Recognition Particle)
  - Soluble
  - Composed of both protein and nucleic acid.
  - Binds to both signal and ribosome and therefore stops translation. (Translational pause)

Protein Synthesis & Import into ER

• The SPR receptor:
  - Membrane associated.
  - Binds to SRP

Protein Synthesis & Import into ER

• The translocation pore:
  - Made of transmembrane proteins
  - Assembles to translocate proteins and disassembles afterward.
  - Binds to the original signal after transfer from the SRP.
Protein Synthesis & Import into ER

- Synthesizing an ER luminal protein.

Compare with Figs 15.13, 15.14

Protein Synthesis & Import into ER

- A Simple mechanism to synthesize a transmembrane protein.

Fig 15-15, 15.16

Protein Synthesis & Import into ER

- A modification of this general pattern is important in the synthesis of some single pass membrane proteins and some multipass transmembrane proteins.

- The internal stop/start sequences.
A problem: We have considered one way to make a single pass transmembrane protein with the NH$_2$ end luminal, and a general way to make multipass membrane proteins.

Yet there are single pass membrane proteins with the NH$_2$ end cytosolic.

There are multipass membrane proteins with all possible combinations of orientations.

How is this possible?

The solution: The first internal start sequences can be placed in 2 alternate orientations. Generally the more positive side of the signal get placed in the pore near the cytosolic side.
Protein Synthesis & Import into ER

- The orientation of the first sequence determines the orientation of any subsequent sequences.

Protein Glycoylation in ER

- Only N-linked glycosylation happens in ER.
  - N-linked vs. O-linked glycosylation.
  - N-linked glycosylation happens during translocation.
  - The process of N-linked glycosylation. (Fig 15-22)
Protein Folding in the ER

- Reason why it is a problem.
  - Non-reducing environment
  - Filled with partially folded proteins
  - As a protein is co-translationally imported, only a portion of the protein is present in the lumen until synthesis is completed. Therefore the proper folding may be impossible until the protein is fully imported.

The Solutions:
- BiP (Binding Protein), a chaperone.
- PDI (Protein Disulfide Isomerase).
  - Breaks high energy (improper) disulfide bridges and allows them to reform.
  - Hopefully, the correct ones will eventually form. These low energy bonds are not easily broken by PDI and hence are retained.

Quality control in the ER

- As we will see, material from the ER is normally swept into the Golgi by bulk transport.
- However there is a quality control of this process such that missfolded proteins are retained.
  - Complex process that is not completely understood.
  - One component is due to the role of chaperones. (Fig 15-23).
Protein Folding (or degradation) in the ER

- Proteins that do not fold correctly are eventually ubiquinated and destroyed.
- In complex or large proteins, this may be significant.
  - 90% of t-cell receptors, acetylcholine gated cation channels are tagged and destroyed.
- Cystic fibrosis - a consequence of E.R. quality control.

Protein Folding (or degradation) in the ER

- How does the quality control system recognize state of protein folding?
  - Probably involves N-linked glycosylation.
  - Certain oligosaccharide tags mean that the proper enzymes have not been able to interact (and change) the oligosaccharide. Seems to label proteins as incorrectly folded.