Importance of Protein sorting

Cell organization depend on sorting proteins to their right destination.

Cell functions depend on sorting protein to their right destination.

Examples:
- A. Energy production by mitochondria
- b. Transcriptional regulation: import of proteins, export of RNA
- c. Biogenesis of ER and Golgi, and proper functioning of the secretory system
- d. Signal transduction networks

Q: What is the relationship of intracellular compartments with one another?
What is their evolutionary origin?

A clue from plastid development

12-3. Development of proplastid to differentiated plastid [, e.g. chloroplast] involves membrane invagination.

12-4. Hypothetical model for origin of organelles

12-5. Topological relationships of compartments.

Note: lumen = exterior of cell

How do proteins move to their destination?
Membrane can bud and fuse. Vesicular transport

12-6. Roadmap of protein traffic.
All proteins are made in the cytosol.
Their fate depends on the sorting signals.
3 types of protein transport.

What determines the destination?

12-8. Sorting signals built into a protein

Complementary sorting receptors recognize these signals.
### Synthesis and sorting of nuclear-encoded proteins to organelles

**Major questions**
1. How do proteins recognize their target destination?
2. What is the identity of the molecules that recognize targeting information?
3. How do large molecules pass through membranes? What is the driving force?
4. What controls protein sorting?
5. What approaches are used to study these questions?
6. What lines of evidence support the model?

**Mitochondria: model of transmembrane transport**

- Review of mitochondria structure, function.
- Most proteins coded by nuclear genes, synth in cyt, and imported.
- Method to study import
- Eukaryotic chaperones deliver proteins to mito
- Mito receptors transfer protein to channel
- Import depends on pmf and mito chaperones to keep proteins unfolded
- Eut evidence for the model.

**Import into chloroplast**
17.3. Study protein import into mitochondria in a cell-free system
Biochemical approach – in vitro

A. Label protein with isotope: In vitro synthesis
   mRNA + ^35S-Met

b. Import assay
   Follow isotope-labeled protein over time.
   Check protein is inside by protease resistance.

C. Test requirement for cytosolic factors or energy
   d. Test requirement for mitochondria proteins
      with mutant lacking a mitochondrial protein.

Other approaches to study mechanism of translocation
see panel 12-1

Transfection Approach
Find the sorting signal for mitochondria.
Fuse targeting signal with cytosolic proteins.

Genetic approach
e.g. yeast mutants defective in one protein of the uptake machinery
   cannot uptake mitochondria-destined proteins

Signal peptide is an amphipathic alpha helix
with no sequence homology to other mitochondrial signals.

Surface receptor and translocation pore form a complex

Recognition, insertion, translocation and processing

Energy is needed at 3 different steps:
   ATP and H+ gradient
Repeated Hsp binding and ATP hydrolysis pull in protein

Fig. 17-9. Chloroplast development and structure

Light energy is used to oxidize water. Electrons are transferred to reduce NADPH and proton gradient is used to form ATP.

Targeting proteins to the chloroplast:
- a. matrix Rubisco has single matrix signal sequence
- b. thylakoid protein has 2.
Summary and a problem

**Problem:** Do mito and chloroplast-destined proteins have distinct matrix targeting sequences? Design an experiment to test your hypothesis.

Protein Import into mitochondrial matrix

Evidence:
1. Import depends on cytosolic factors
2. ATP is needed to keep protein unfolded
3. Mitochondrial receptors are needed
4. Import depends on pmf and matrix chaperones

pmf: provides a driving force