Importance of Protein sorting

Cell organization depend on sorting proteins to their right destination.

Cell functions depend on sorting proteins to their right destination.

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

To understand sorting mechanisms, we need to know the relationship of intracellular compartments with one another.

What might be their evolutionary

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.
- origin of Nucleus: DNA at PM is invaginated
- origin of ER: PM invaginated
- Mitochondria/plastids: Bacteria origin
- inner membrane = PM of bacteria
- outer membr = PM of host cell

12-5. Topological relationships of compartments.

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.
1. Gated (nuc pore)
2. Transmembrane (ER, mito)
3. Vesicular

Vesicles bud, move and fuse.

What determines the destination?

12-8. Sorting signals built into a protein

Complementary sorting receptors recognize these signals.
12-3. Signal sequences

<table>
<thead>
<tr>
<th>Function of Signal Sequence</th>
<th>Map of Signal Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export into membranes</td>
<td>[Diagram of export]</td>
</tr>
<tr>
<td>Import into mitochondria</td>
<td>[Diagram of import]</td>
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</tbody>
</table>

Proteins of the secretory pathway

- Cytosol
- ER-bound Ribosomes
- Lumenal protein or membrane proteins
- Soluble = lumen
- Extracellular
- Nuclear Env lumen

Mitochondria: model of transmembrane transport

- Most proteins coded by nuclear genes, synth in cyt, and imported.
- Method to study import
- Cyt Chaperones deliver proteins to mito
- Mito receptors transfer protein to channel
- Import depends on pmf and mito chaperones to keep proteins unfolded
- Exp experiment evidence for the model.

Structure

- Two membranes
- Three membranes

17-1. Sorting of nuclear encoded proteins

- Synthesis and sorting of nuclear-encoded proteins to organelles
  - Major questions:
    1. What is the sorting signal?
    2. What serves as the complementary receptor?
    3. How do large molecules pass through membranes? What is the driving force?
    4. What controls protein sorting?
    5. How can we study these questions? Approaches?
    6. What lines of evidence support the model?

- Import into chloroplast

- Mitochondrion function
  - PVA – citric acid cycle
    - $\text{CO}_2 + \text{NADH}$
  - $\text{NADH} + \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{NAD}^+$ + $\text{H}^+$ gradient
  - $\text{H}^+$ gradient – ATP synthase $\rightarrow$ ATP
Most proteins are imported

- **mitochondria genome**
  - Protein-coding sequences:
    - Human: v. small (13)
    - Arabidopsis-ave: 32

- ATP synthase (8 subunits)
- ATP/ADP translocator
- Citric acid cycle enzymes
- Electron transport complexes - cyt c oxidase

**Approaches to study mechanism of translocation**

See panel 12-1

1. **Biochemical approach**: to determine mechanism in vitro synthesis and import assay
2. **Transfection Approach**: define signal sequence
   - Find the putative sorting signal for an organelle (mitochondria).
   - Fuse targeting signal with reporter (cytosolic) protein.
   - Transfect a cell.
3. **Genetic approach**: identify essential players
   - e.g. yeast mutants defective in one protein of the recognition, binding or uptake machinery cannot take up mitochondria-destined proteins.
   - Identify the gene product & its function

**17.3. Study protein import into mitochondria in a cell-free system**

**Biochemical approach**

- Label protein with isotope: In vitro synthesis
  - mRNA + 35S-Met
- Import assay
  - Follow isotope-labeled protein over time.
  - Check protein is inside by protease resistance.
- Test requirement for cytosolic factors or energy
- Test requirement for mitochondria proteins with mutant lacking a mitochondria.

**Transfection approach**

- Test if a sequence is required and sufficient to target protein to a compartment.

**Biochemical approach**

- Label protein with isotope: In vitro synthesis
  - mRNA + 35S-Met
- Import assay
  - Follow isotope-labeled protein over time.
  - Check protein is inside by protease resistance.

**Genetic approach**

- Screen for mutants defective in mito import.
- Identify the mutant x gene product.
- Use the Wt x gene to see if it can restore Wt phenotype.
Signal peptide is an amphipathic alpha helix with no sequence homology to other mito. Signals.

A. Matrix sorting
b. Inner membrane-sorting

Surface receptor and translocation pore form a complex

How does it work?

1-Recognition, 2-insertion, 3-translocation and 4-processing

1. Protein is imported into mito or not?
2. N-terminal target sequence is processed or not?

Is energy required? How would you test for this?
Are other factors required?
If yes, what is energy used for?

Expt finding: in vitro import assay
1. - ATP: no uptake
   + ATP: import
2. - cytosol: no import
   + cytosol: import
3. + CCCP: no import [H+ ionophore]
   - CCCP: import

Interpretation?
Energy is needed at 3 different steps:
ATP and H+ gradient

Why?

Repeated Hsp binding and ATP hydrolysis pull in protein

Paths of non-matrix proteins:
IM protein, multi-TM

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.

CO₂ + RuBP → rubisco→ 2 PGA
PGA → NADPH, ATP → G3P  2G3P → glucose

Targeting proteins to the chloroplast:
a. matrix Rubisco has single matrix signal sequence
b. thylakoid protein has 2.
17-8. Proteins move into the thylakoid by one of four pathways.

A. Sec ATP, ΔpH [PC, OEC33]

b. SRP, GTP, ΔpH [LHCP]

c. ΔpH [OE22, RR-pr]

d. Spontaneous [CFo-II]


Summary of protein import, and a problem

Sorting signal at the N terminus

Signal is recognized by surface receptor

Protein traverses a pore in the protein channel complex.

Energy is needed to keep protein unfolded & generate the pmf.

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

How would you identify the surface receptor complex proteins?

Mitochondria: plasticity

Rapid changes in shape

Wrap around flagellum

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

Growth and division of yeast mito

Arrangement is controlled by rates of division and fusion. Regulated by GTPases. Fly mutants impaired in mitochondria fusion are infertile (male).