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?

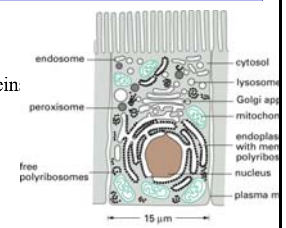


Figure 12-1. Molecular Biology of the Cell, 4th Edition.

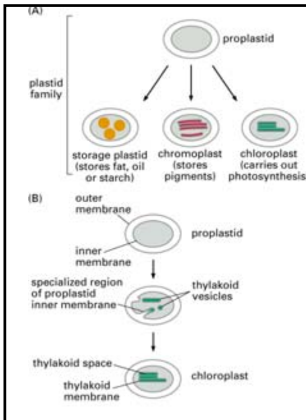


Figure 12-3. Molecular Biology of the Cell, 4th Edition.

A clue from plastid development

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

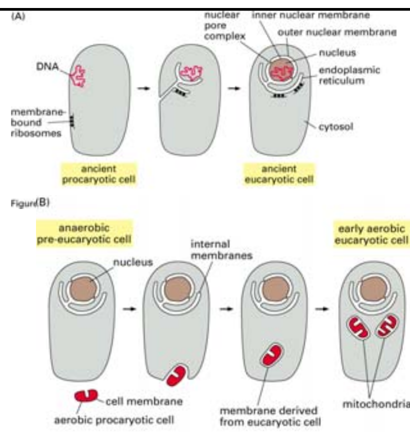


Figure 12-4 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

12-4. Hypothetical model for origin of organelles

12-5. Topological relationships of compartments.

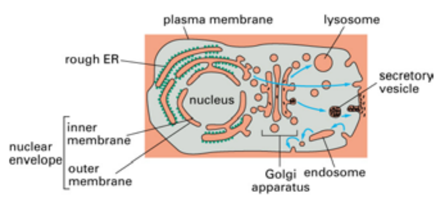


Figure 12-5. Molecular Biology of the Cell, 4th Edition.

Note: lumen = exterior of cell

How do proteins move to their destination?

Membrane can bud and fuse. Vesicular transport

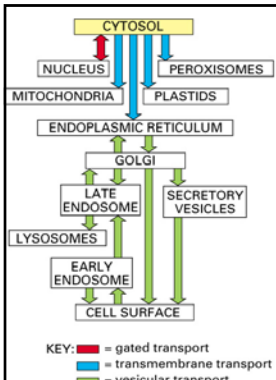


Figure 12-6. Molecular Biology of the Cell, 4th Edition.

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

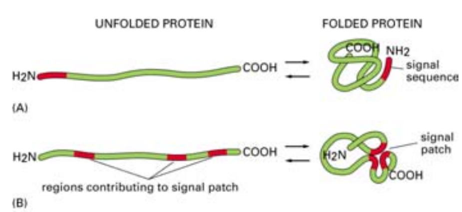


Figure 12-8. Molecular Biology of the Cell, 4th Edition.

Complementary sorting receptors recognize these signals.

TABLE 17-1 Properties of Uptake-Targeting Signal Sequences That Direct Proteins from the Cytosol to Organelles

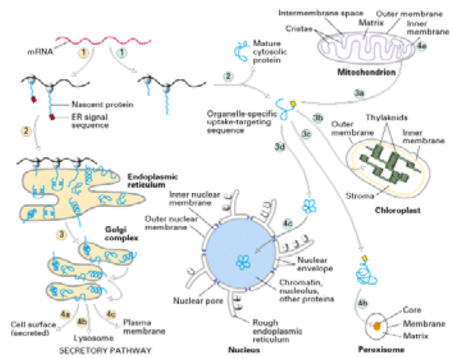
Target Organelle	Usual Signal Location within Protein	Signal Removal*	Nature of Signal
Endoplasmic reticulum	N-terminal	(+)	"Core" of 6–12 mostly hydrophobic amino acids, often preceded by one or more basic amino acids
Mitochondrion†	N-terminal	(+)	3–5 nonconsecutive Arg or Lys residues, often with Ser and Thr; no Glu or Asp residues
Chloroplast†	N-terminal	(+)	No common sequence motifs; generally rich in Ser, Thr, and small hydrophobic amino acid residues and poor in Glu and Asp residues
Peroxisome	C-terminal	(−)	Usually Ser-Lys-Leu at extreme C-terminus
Nucleus	Internal	(−)	One cluster of 5 basic amino acids, or two smaller clusters of basic residues separated by ≈10 amino acids

*Indicates whether signal sequence usually is (+) or is not (−) removed after a protein enters its target organelle.
†These signals direct the protein from the cytosol into the matrix space of the mitochondrion or the corresponding stroma of the chloroplast; other signals discussed in the text redirect proteins into other subcompartments of these organelles.*

AtCNX: 1 MRQRQLFSVF LLLLAFVSFQ KLCYCDDQTV LYESFDEPFD

ER Calnexin, type I

17-1. Sorting of nuclear encoded proteins



Protein Synthesis- cytosol

Cytosolic Ribosomes

- **Cytosol**
  - soluble
  - peripheral
- **Organellar:**
  - Nuc: soluble
  - Mitoch: sol + memb
  - Chloroplast: sol + membrane

ER-bound Ribosomes-> secreted protein or membrane proteins

- **Soluble:**
  - ER lumen
  - Golgi lumen
  - Vac lumen
  - Extracellular
  - Nuc Env lumen
- **Membrane:**
  - ER
  - Golgi
  - Nuc
  - PM
  - Vac

MOCB 639, Lodish 2000, ch. 17-1, 17-2; Alberts-ch 12

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?
   What lines of evidence support the model?

Mitochondria: model of transmembrane transport

a. Review of mitochondria structure, function
   Most proteins coded by nuclear genes, synth in cyt, and imported.
b. Method to study import
c. Cyt Chaperones deliver proteins to mito
d. Mito receptors transfer protein to channel
e. Import depends on pmf and mito chaperones to keep proteins unfolded
f. Expt evidence for the model.

Import into chloroplast

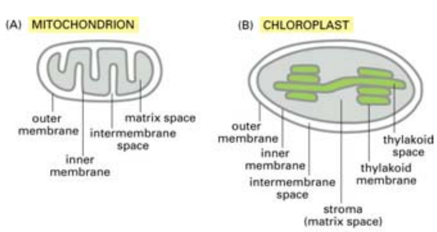
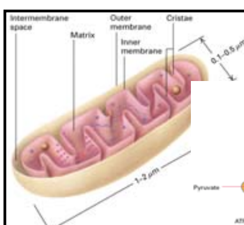
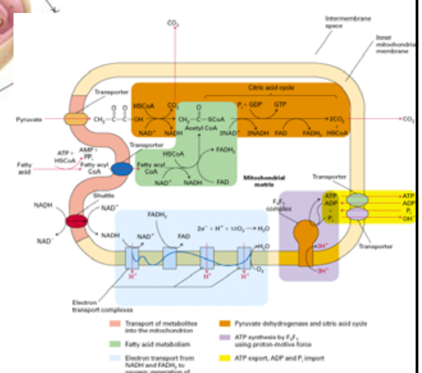


Figure 12-22. Molecular Biology of the Cell, 4th Edition.



16-7, 16-9. Mitochondrion



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

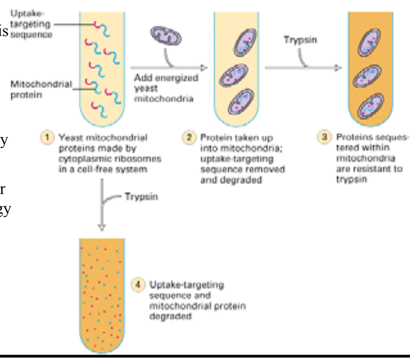
Biochemical approach = in vitro

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 mitoch. 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 mito. Signals.

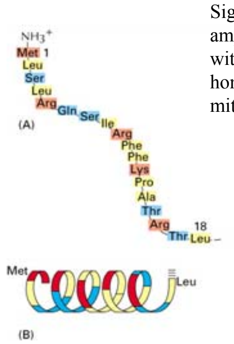


Figure 12–23. Molecular Biology of the Cell, 4th Edition.

Surface receptor and translocation pore form a complex

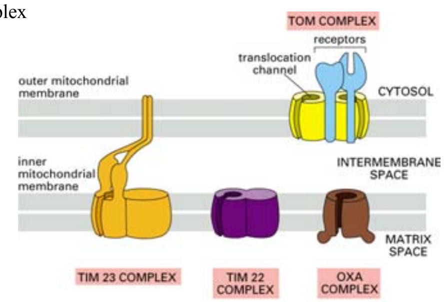


Figure 12–24. Molecular Biology of the Cell, 4th Edition.

Recognition, insertion, translocation and processing

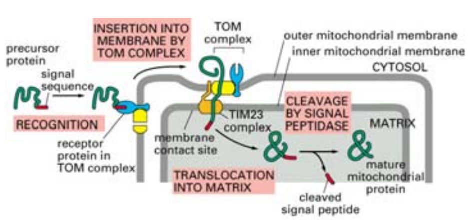


Figure 12–26. Molecular Biology of the Cell, 4th Edition.

Energy is needed at 3 different steps:

ATP and H+ gradient

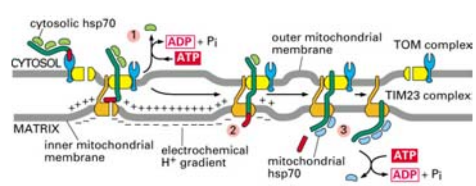


Figure 12–27. Molecular Biology of the Cell, 4th Edition.

Repeated Hsp binding and ATP hydrolysis pull in protein

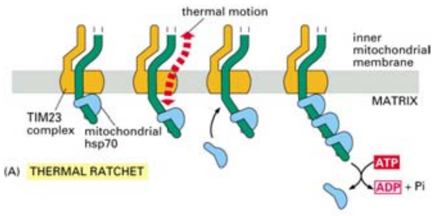


Figure 12–28 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

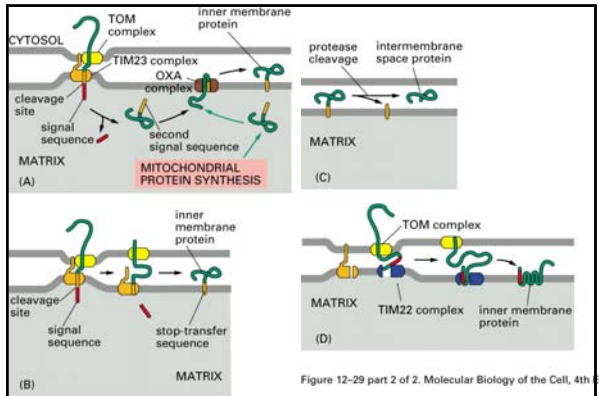
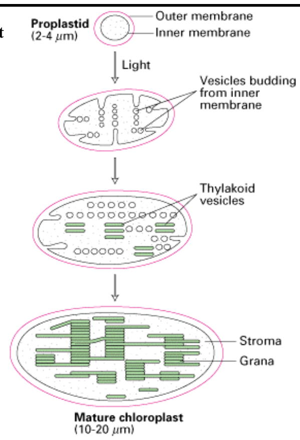


Figure 12–29 part 2 of 2. Molecular Biology of the Cell, 4th E

Figure 12–29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

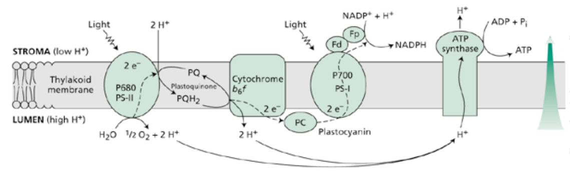
Fig. 17-9. Chloroplast development and structure



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

CO_2 + RuBP -- rubisco--> 2 PGA
PGA --NADPH, ATP --> G3P

2G3P --> glucose



CF1Fo

Taiz + Zeiger (1998) Fig. 7-22

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

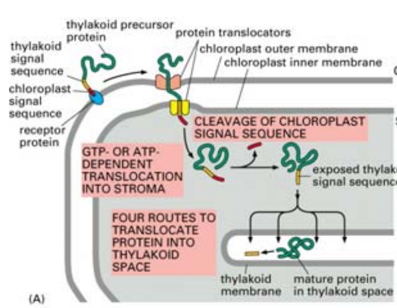


Figure 12–30 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

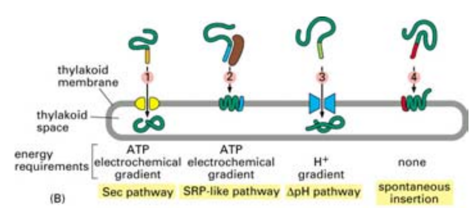


Figure 12–30 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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-p]
d. Spontaneous	[CFo-II]

Keegstra K, Cline K. 1999.
Protein import and routing systems of chloroplasts. Plant Cell. 11(4):557-70.

Summary and a problem

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

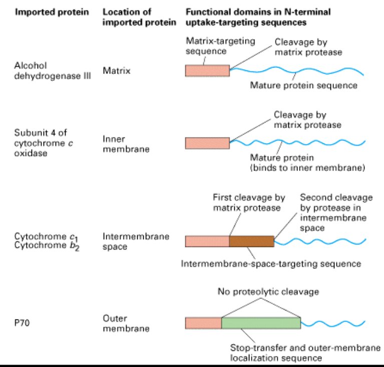
Evidence for matrix targeting signal Fig. 17-2

Yeast ADH3p: 1 **mlrtstlftr rvqpslfsrn ilrlqstaai pktqkgvify**

3-5 non-consecutive Arg (R) or Lys (K),

with Ser (S) and Thr (T),

no Asp (D) or Glu (E)



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

17-4. Protein Import into mitochondrial matrix

Evidence:

1. Need for cytosolic factors
2. cyt ATP is needed to keep protein unfolded
3. Mitochondrial receptors are needed and being identified
4. Import depends on pmf and matrix chaperones