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. 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

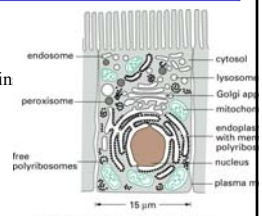


Figure 12–1. Molecular Biology of the Cell, 4th Edition.

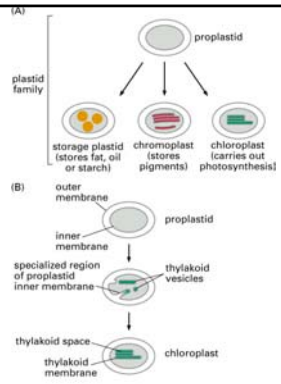


Figure 12–3. Molecular Biology of the Cell, 4th Edition.

A clue from plastid development

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

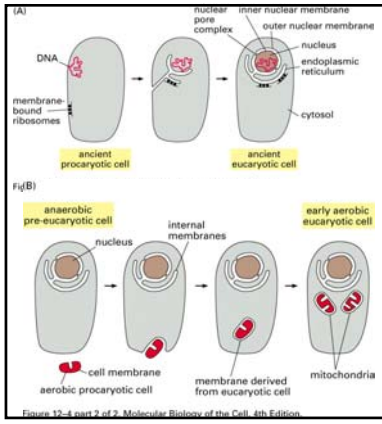


Figure 12–4 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

12-4. **Hypothetical model for origin of organelles.**

-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.

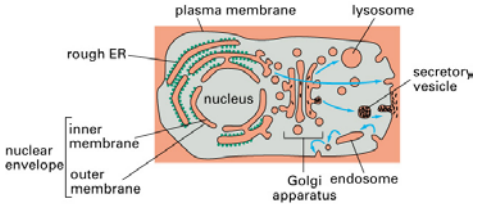


Figure 12–5. Molecular Biology of the Cell, 4th Edition.

Note: lumen = exterior of cell

How do newly synthesized proteins move to their destination?

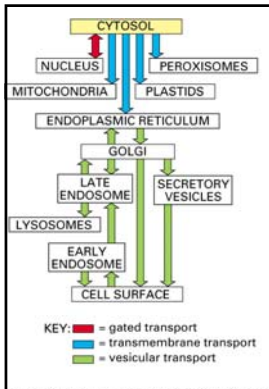


Figure 12–6. Molecular Biology of the Cell, 4th Edition.

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.
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

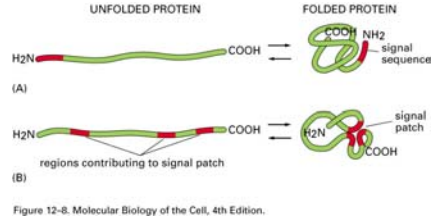


Figure 12–8. Molecular Biology of the Cell, 4th Edition.

Complementary **sorting receptors** recognize these signals.

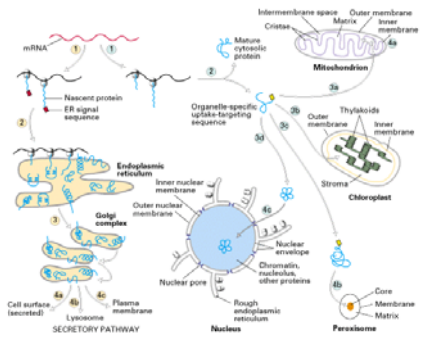
12-3. Signal sequences

TABLE 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3$N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3$N-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^-
Import into ER	$^{+}H_3$N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^-

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in green. Similarly, important hydrophobic amino acids are shown in *yellow* and hydroxylated amino acids are shown in *blue*. $^{+}H_3$N indicates the N-terminus of a protein; COO^- indicates the C-terminus.

17-1. Sorting of nuclear encoded proteins



Proteins of the secretory pathway

Protein Synthesis- cytosol

Cytosolic Ribosomes

ER-bound Ribosomes-> lumenal protein or membrane proteins

Cytosol
soluble
peripheral

Organellar:
Nuc: soluble
Mitoch: sol + memb
Chloroplast: sol + membrane

Soluble = lumen = extracellular:
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. 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?
   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

Structure

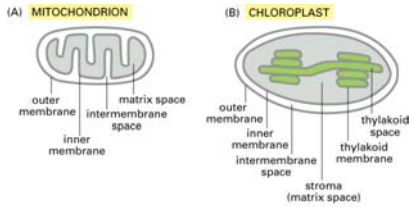


Figure 12–22. Molecular Biology of the Cell, 4th Edition.

Two membranes Three membranes

16-7, 16-9. Mitochondrion function

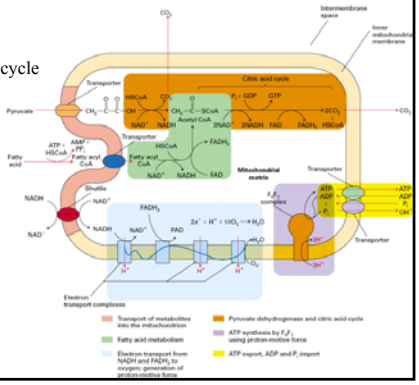
14-10 Albert

PVA – citric acid cycle → CO_2 + NADH

NADH + O_2 → H_2O + NAD^+ + H^+ gradient

H^+ gradient – ATP synthase→

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 to 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

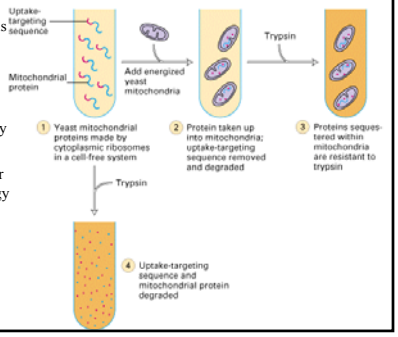
17.3. Study protein import into mitochondria in a cell-free system
Biochemical approach = in vitro

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

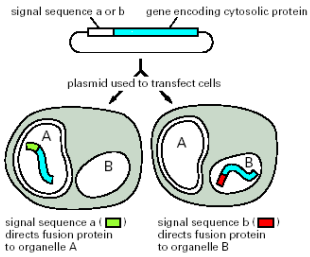
b. import assay Follow isotope-labeled protein over time. Check protein is inside by protease resistance.

C. **Test requirement** for cytosolic factors or energy

d. Test requirement for mitochondria proteins with mutant lacking a mitoch. protein.



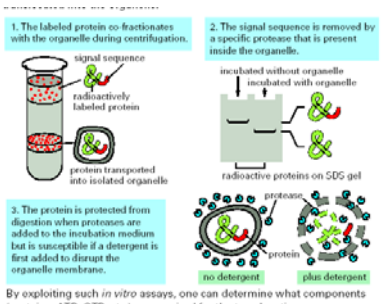
Transfection approach



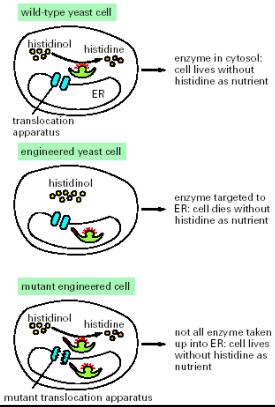
Test if a sequence is required and sufficient to target protein to a compartment.

By altering the signal sequence using site-directed mutagenesis, one can determine which structural features are important for its function.

Biochemical approach



By exploiting such *in vitro* assays, one can determine what components (proteins, ATP, GTP, etc.) are required for the translocation process.



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.

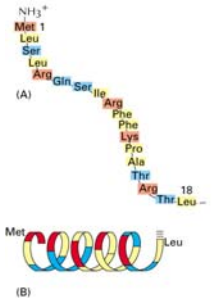


Figure 12–23. Molecular Biology of the Cell, 4th Edition.

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?

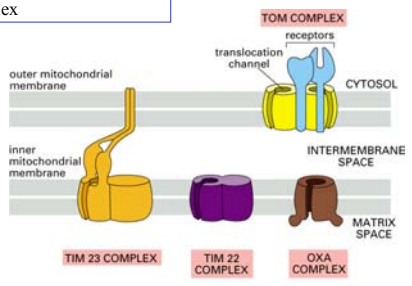


Figure 12–24. Molecular Biology of the Cell, 4th Edition.

Matrix multi-TM inner memb

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

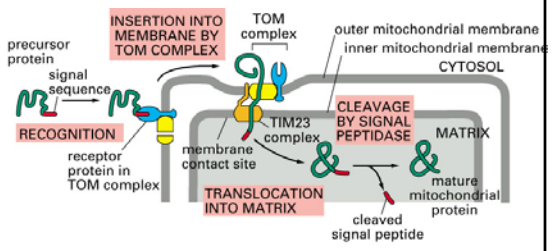


Figure 12–26. Molecular Biology of the Cell, 4th Edition.

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

How do you test if

1. *Protein is imported into mito or not?*

2. *N-terminal target sequence is processed or not?*

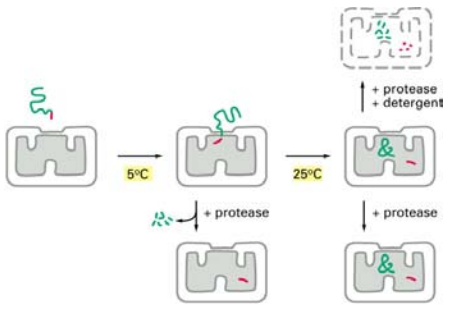


Figure 12–25. Molecular Biology of the Cell, 4th Edition.

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

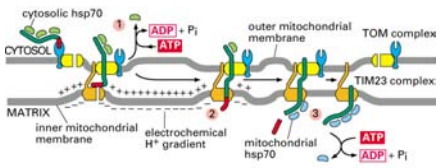


Figure 12-27. Molecular Biology of the Cell, 4th Edition.

Why?

Repeated Hsp binding and ATP hydrolysis pull in protein

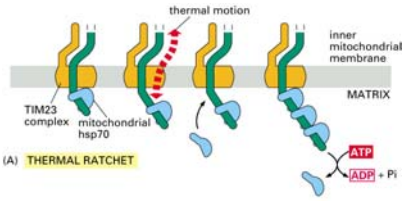


Figure 12-28 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

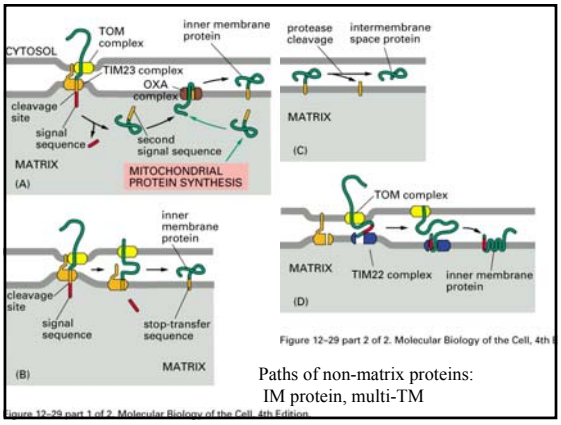
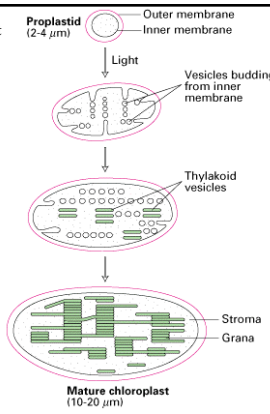


Figure 12-29 part 2 of 2. Molecular Biology of the Cell, 4th E

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

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

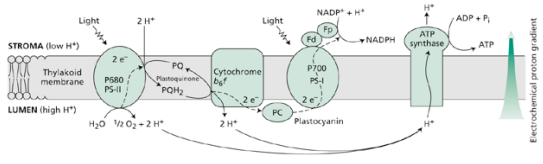
Fig. 17-9. Chloroplast development and structure



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

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.

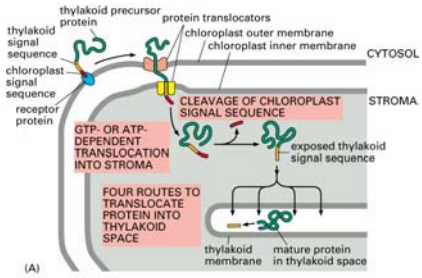


Figure 12-30 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

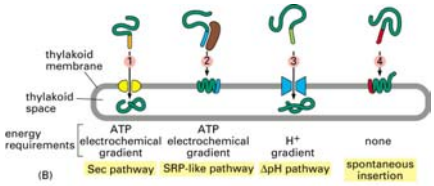


Figure 12-30 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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.

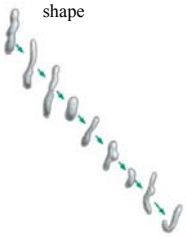
Summary of protein import, and a problem

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



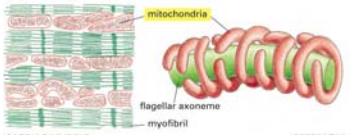


Figure 14–6. Molecular Biology of the Cell, 4th Edition.

Wrap around flagellum

20 minutes

Figure 14–4. Molecular Biology of the Cell, 4th Edition.

Growth and division of yeast mito

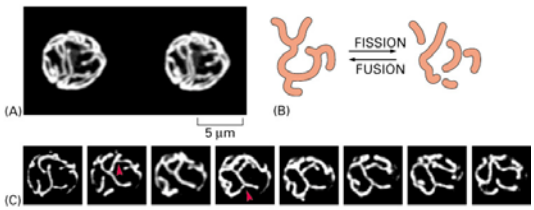


Figure 14–53. Molecular Biology of the Cell, 4th Edition.

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

Do not use

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