A typical mammalian cell: has about 10,000 different kinds of proteins
- cytosol
- a particular cell membrane, an aqueous compartment, cytosol, or to the cell surface for secretion

Protein targeting or protein sorting:
1) protein targeting to membrane or aqueous interior of intracellular organelle
2) vesicular-based protein sorting (secretory pathway) –chapter 17

Signal sequences (20-50 aa.), uptake-targeting sequences, receptors, translocation channel, unidirectional translocation

DNA → RNA → protein → Protein sorting → different organelles → different functions

The mechanisms or pathway of protein sorting / protein targeting?

Live bovine endothelial cell: Green is ER; Orange is mitochondria

Press Release: The 1999 Nobel Prize in Physiology or Medicine

THE NOBEL ASSEMBLY AT THE KAROLINSKA INSTITUTE

11 October 1999

The Nobel Assembly at Karolinska Institutet has today decided to award the Nobel Prize in Physiology or Medicine for 1999 to

Günter Blobel

for the discovery that "proteins have intrinsic signals that govern their transport and localization in the cell"

Summary
A large number of proteins carrying out essential functions are constantly being made within our cells. These proteins have to be transported either out of the cell, or to the different compartments - the organelles - within the cell. How are these proteins transported across the membrane surrounding the organelles, and how are they directed to their correct location?

These questions have been answered through the work of this year’s Nobel Laureate in Physiology or Medicine, Dr. Günter Blobel, a cell and molecular biologist at the Rockefeller University in New York. Already at the beginning of the 1970s he discovered that newly synthesized proteins have an intrinsic signal that is essential for governing them to and across the membrane of the endoplasmic reticulum, one of the cell’s organelles. During the next twenty years Blobel characterized in detail the molecular mechanisms underlying these processes. He also showed that similar “address tags”, or “zip codes”, direct proteins to other intracellular organelles.
Four fundamental questions:

1. What is the nature of the signal sequence, and what distinguishes it from other types of signal sequences?
2. What is the receptor for the signal sequence?
3. What is the structure of the translocational channel that allows transfer of proteins across the membrane bilayer? In particular, is the channel so narrow that proteins can pass through only in an unfolded state, or will it accommodate folded protein domains?
4. What is the source of energy that drives unidirectional transfer across the membrane?

Moving Proteins into Membranes and Organelles (protein targeting)

1. Translocation of secretory proteins across the ER membrane
2. Insertion of proteins into the ER membrane (glycoprotein to the outside of membrane or release)
3. Protein modifications, folding, and quality control in the ER
4. Export of bacterial proteins
5. Sorting of proteins to mitochondria and chloroplasts
6. Sorting of peroxisomal proteins
7. Sorting of nucleus proteins
16.1 Translocation of secretory proteins across the ER membrane

Secretory proteins are synthesized on ribosomes attached to ER (rough ER). Signal sequence: for ER, peroxisome, mitochondria, chloroplast

**Fig 16.2** Electron micrograph of ribosomes attached to the rough ER in a pancreatic acinar cell

**Translocation of secretory protein across the ER membrane**

**How do we know that signal peptides are “necessary and sufficient”?**

*In vitro* system can be used

- *In vitro* translate mRNA for a mitochondrial protein
  - w/ or w/o signal peptide
  - radiolabeled (e.g., with ^35S)

- Incubate with organelle fraction
- Density centrifugation
- Gel electrophoresis and autoradiography

**How do we know protein is inside the organelle?**
- *protease/detergent treatment*

**How to study of Secretory proteins are localized to the ER lumen shortly after synthesis.**

- Cell + isotope-amino acid → new protein synthesis had isotope → homogenization

**Fig 16.3** Labeling experiments demonstrate that secretory proteins are located to the ER lumen shortly after synthesis

**Translocation of secretory protein across the ER membrane**

**Free ribosome:**
- for cytosolic protein synthesis

**Fig 16.3** Labeling experiments demonstrate that secretory proteins are located to the ER lumen shortly after synthesis
A hydrophobic N-terminal signal sequence targets nascent secretory proteins to the ER

After synthesis secretory protein → signal sequence → ER → modification (glycosylation, ...) → vesicle transport to ...........

A 16- to 30-residue ER signal sequence (in N-terminal):

- One or more positively charged adjacent to the core
- A continuous stretch of 6-12 hydrophobic residues (the core) but otherwise they have little in common
- Cleaved from the protein while it is still growing on ribosome
- Not present in the "mature" protein found in cells
- Signal sequence is removed only if the microsomes are present during protein synthesis
- Microsomes must be added before the first 70 or so amino acids are linked together in order for the completed secretory protein to be localized in the microsomal lumen

Cotranslational translocation

Cotranslational translocation: 必需一同參與
Ribosome and microsome involved; The first 40 aa (include signal sequence) into microsome from ribosome, next 30 aa in ribosome channel.

Cotranslational translocation is initiated by two GTP-hydrolyzing proteins

Secretory proteins are related with ER, but not with other cellular membrane. Has specificity of ER and ribosome interaction

DNA → RNA → cytosol → ER + ribosome → cotranslation translocation → to ER

Fig 16.4 Cell-free experiments demonstrate that translocation of secretory proteins into microsomes is coupled to translation

Cotranslational translocation is initiated by two GTP hydrolyzing proteins

The role of SRP and SRP receptor in secretory protein synthesis

Not all signal sequence located at N-terminal
Two key components involve in contranslational translocation:

1) signal-recognition particle (SRP)
   - is a cytosolic ribonuclear protein particle
   - 300 nt RNA and 6 discrete polypeptides
   - p54 bind to ER signal sequence in a nascent secretory protein
   - homologous to bacterial protein Ffh (hydrophobic residues) p54
   - p9 and p14 interact with ribosomes
   - p68 and p72 are required for protein translocation
   - SRP slows protein elongation when microsomes are absent

2) SRP receptor
   - integral membrane protein (an α subunit & smaller a β subunit)
   - protease – releasing soluble form of the SRP receptor
   - p54 of SRP and α subunit of receptor - GTP – promote interaction
   - GTP hydrolysis – fidelity

Passage of growing polypeptide through the translocon is driven by energy released during translation

Mammalian translocon:
Sec61 complex
Sec61α - integral membrane protein, 10 membrane-spanning α helixes
- interact with translocating peptide (chemical cross-linking exp.)
Sec61β, sec61γ
Signal peptidase

Electron microscopy reconstruction reveals that a translocon associates closely with a ribosome

- 40S subunit
- 60S subunit
- ribosome
- Sec61
- 10 nm
- Translocon
- small ribosomal subunit
- large ribosomal subunit
- protein-conducting channel in ER membrane
- protein-translocating complex
- tRNA
- rRNA
- NAD+
**Energy needs during protein translocation**

1. **Unfolding the protein** in the original location
   - Co-translation translocation: chain elongation during translation post-translational translocation/mitochondrial import:
     - Chaperone (Hsp70) unfolds protein in an ATP-dependent manner

2. **Opening of the “gate”**
   - Mutual stimulation of GTPase activities of an SRP subunit (p54) and the α subunit of SRP-receptor

3. **Pulling through the channel**:
   - Chaperone activity inside the target organelle (Hsp70) that in addition helps fold the protein

**Molecular chaperones**

Up-regulated during heat shock, conserved

2 classes

- **Hsp70**: protect a misfolded or unfolded protein from degradation/folding, Hsp40 and Hsp90 as cofactors
- **Hsp60** (chaperonin), actively helps protein folding

Organelle specific, e.g., Bip in the ER

**ATP hydrolysis powers post-translational translocation of some secretory proteins in yeast**

In most eukaryotes, secretory proteins enter ER by co-translational translocation, using energy from translation to pass through the membrane. But Yeast, post-translational translocation.

**BiP** is HSP 70 family of molecular chaperones, a peptide-binding domain and an ATPase domain. For bind and stabilized unfolded or folded protein.

**16.2 Insertion of proteins into the ER membrane**

How integral proteins can interact with membranes?

Topogenic sequence, for basic mechanism used to translocated soluble secretory proteins across the ER membrane

Most important: the hydrophobic sequence for interaction with intra-membrane

**Single-pass**

**Multipass**
Moving Proteins into Membranes and Organelles (protein targeting)

**Type I**: cleavable N-terminal signal sequence (SS), stop-transfer sequence in the C-terminal portion of the protein; most of the protein is on the exoplasmic side **similar to type III, except that there is no signal sequence**

Most cytosolic transmembrane proteins have an N-terminal signal sequence and an internal topogenic sequence.

**Type III also has**

**Type II**: no SS, stop-transfer sequence, start-transfer sequence in the N-terminal portion, often (+) charge N-terminal to the hydrophobic domain

A single internal signal-anchor sequence directs insertion of single-pass Type II transmembrane proteins.

**Fig 16-11** Synthesis and insertion into the ER membrane of type I single-pass proteins

**Fig 16-12** Synthesis and insertion into the ER membrane of type II single-pass proteins

Synthesis of a single pass transmembrane protein with the C-terminal domain in the lumen.
**Type III**: no SS, stop-transfer sequence, flanked by +charged residues on its C-terminal side. 
*same orientation as type I*, but, synthesized without SS, often (+) charge C-terminal to the hydrophobic domain.

High density of positively charged aa at one end of the signal-anchor sequence determine insertion orientation.

**Type IV**: multipass membrane protein (various options)

**Type I**: cleavable N-terminal SS (signal sequence), stop-transfer sequence in the C-terminal portion of the protein, most of the protein is on the exoplasmic side.

**Type III**: *same orientation as type I*, but, synthesized without SS, often (+) charge C-terminal to the hydrophobic domain.

**Type II**: no SS, start-transfer sequence in the N-terminal portion, often (+) charge N-terminal to the hydrophobic domain.

**Type IV**: multipass membrane protein

**GPI** (glycosylphosphatidylinositol): Type I protein is cleaved and the lumenal portion is transferred to a protein. 

*Figure 12–49, Molecular Biology of the Cell, 4th Edition.*
After insertion into the ER membrane, some proteins are transferred to a GPI anchor.

**GPI** (glycosylphosphatidylinositol): Type I protein is cleaved and the lumenal portion is transferred to a preformed lipid anchor.

---

**Arrangement of topogenic sequences in type I, II, III and IV proteins.**

- **Type I**: Usually, enter lumen
- **Type II**: Usually, enter cytosol
- **Type III**: +++ prefer cytosol (mechanism ?)
- **Type IV**: Still move

Even number of a helices: N- & C-termini on the same side; Type IV-B, on the opposite sides.

Whether α helix functions as signal-anchor sequence or stop-transfer anchor sequence is determined by its order.

---

**Protein targeting to ER**

A phospholipid anchor tethers some cell surface proteins to the membrane: GPI-anchored proteins.

GPI (glycosylphosphatidylinositol)-anchored proteins can diffuse in lipid bilayer.

GPI targets proteins to apical membrane in some polarized epithelial cells.

---
The topology of a membrane protein often can be deduced from its sequence: hydropathy profile (親水性係數)

Hydropathic index for each aa.
Total hydrophobicity of 20 contiguous aa

16.3 Protein modifications, folding, and quality control in the ER
m-RNA → ribosome-ER → peptide → modification → mature protein

1. Addition and processing of carbohydrates (glycosylation) in the ER and Golgi
2. Formation of disulfide bonds in the ER
3. Proper folding of polypeptide chains and assembly of multisubunit proteins in the ER
4. Specific proteolytic cleavages in the ER, Golgi, and secretory vesicles

Glycosylation (醣基化)

A preformed N-linked oligosaccharide is added to many proteins in the rough ER

Glycosylation site: ER or golgi complex
All N-linked oligosaccharides on secretor and membrane protein are conserved

N-linked: complex
O-linked: one to four sugar residues

There are two basic types of glycosylation which occur on:
(a)N-linked: asparagine
(b)O-linked: serine and threonine

Fig 16-16 Common 14 residue precursor of N-linked oligosaccharide that is added to nascent proteins in the rough ER
Biosynthesis of dolichol pyrophosphoryl oligosaccharide precursor

**UDP-N-acetylglucosamine**

Strongly hydrophobic lipid (79-95 carbon)

Oligosaccharide side chain may promote folding and stability of glycoproteins

**Consensus:**
Asn-X-Ser/Thr (x did not proline)

The antibiotic tunicamycin acts by **mimicking the structure of UDP-N-acetylglucosamine**, the substrate in the first enzymatic step in the glycosylation pathway.

It thus blocks protein post-translational modification and hence protein production is inhibited to **kill eukaryotic cells**.

Addition & processing of N-linked oligosaccharides in r-ER of vertebrate cells
The molecule is flipped from the ER membrane to the ER lumen. Additional sugars are added via dolichol phosphate. Finally, the oligosaccharide (14 residues) is transferred to a specific Asn in the lumen.

Before the glycoprotein leaves the ER lumen three glucose units are removed (part of the folding process).

Consensus: Asn-X-Ser/Thr

Red: GlcNAc
Blue: mannose
Green: Glucose

N-glycosylation: Oligosaccharide precursor is attached to the protein co-translationally

Protein glycosylation serves several functions.

Promote proper folding: e.g. influenza virus hemagglutinin cannot fold properly in the presence of tunicamycin or a mutation of Asn to Gln.
Confer stability.
Involved in cell-cell adhesion; Cell adhesion molecules (CAMs).
Protein glycosylation takes place in the ER and Golgi

The endoplasmic reticulum - ER
- A continuous cytoplasmic network studded with ribosomes and functions as a transport system for newly synthesized proteins.

The Golgi complex
- An organelle consisting of stacks of flat membranous vesicles that modify, store, and route products of the ER.

N-linked glycosylation begins in the ER and continues in the Golgi apparatus (via dolichol phosphate).

O-linked glycosylation takes place only in the Golgi apparatus.

In the Golgi:
1. O-linked sugar units are linked to proteins.
2. N-linked glycoproteins continue to be modified.
3. Proteins are sorted and are sent to lysosomes, secretory granules, plasma membrane according to signals encoded by amino acid sequences.

Glycoproteins

Carbohydrates can be covalently linked to proteins to form glycoproteins.
- These proteins have a low percentage of carbohydrate when compared to proteoglycans.

Carbohydrates can be linked through the amide nitrogen of asparagine (N-linkage), or through the oxygen of serine or threonine (O-linkage).

2 classes of glycosylation

O-linked: N-acetylgalactosamine linked to Ser/Thr
Generally short (1-4 sugars)
Sugars added sequentially
N-linked: N-acetylgalactosamine linked to Asn
Complex Preformed oligosaccharide added in ER Modified by addition/removal of sugars in ER and Golgi

Disulfide bonds are formed and rearranged by proteins in the ER lumen via protein disulfide isomerase (PDI) - SH: sulfhydryl group
55 kDa protein - acts as dimer, contains protein binding site

(a) Formation of a disulfide bond

Cystine

Reduction can occur at various stages of post-translational modification

(b) Rearrangement of disulfide bonds

Formation of disulfide bonds in eukaryotes & bacteria

(a) Eukaryotes

ER membrane

Reduction can occur at various stages of post-translational modification

(b) Bacteria

Outer membrane

Periplasmic space

Inner membrane

DsbB

Transfer electrons to a disulfide bond in the luminal protein PDI like DsbA
Functions of The ER

Chaperones: BiP
Glycosylation
GPI-linkages
Disulfide bond formation
Proper Folding - Quality Control
Multisubunit (multimeric) assembly
Specific proteolytic cleavages
Secretory vesicles

ER proteins that facilitate folding & assembly of proteins. Chaperones and other protein facilitates folding and assembly of proteins.

BiP: a chaperone that prevents nascent chain from misfolding or forming aggregates.

PDI: stabilizes proteins with disulfide bonds.

Calnexin & calreticulin: lectins that bind a single glucose attached onto unfolded or misfolded polypeptide chains and prevent their aggregation. (p677)

Peptidyl-prolyl isomerase: facilitates folding by accelerating rotation about peptidyl-prolyl bonds.

In all cases, multimeric constituting in ER

Unfolded protein vs. ER quality control

A glucosyl transferase can recognize an unfolded protein and add one terminal glucose.

Glucose trimming

Carbohydrate binding protein

Quality control by BiP & calnexin: ensuring that misfolded proteins do not leave ER.

In addition to co-translational modifications, the correct folding/assembly may require the presence of a group of proteins called chaperones. Some chaperones (e.g. BiP) have high affinity toward unfolded proteins in general, yet others (e.g. calreticulin or calnexin) recognize more specific features (e.g. glycosylation) during the folding of a protein. (Carbohydrate-binding proteins are called lectins. Calnexin and calreticulin are lectins binding to certain N-linked carbohydrates)
The orientation of a membrane protein is established during synthesis on the ER membrane.

What if unfolded proteins start to accumulate within a cell?

unfolding in the cytosol:
leading to an increase of cytosolic chaperones
(also called heat shock response)

unfolding in the ER:
leading to an increase of ER chaperones
(also called unfolding protein response, UPR)

- Translocated proteins can be exported to the cytosol.
- There they are:
  - ubiquitinated
  - degraded by the proteasome
  - a process known as ER-associated degradation.

How to deal with mis-folded proteins?

Proteasome: an ATP-dependent protease complex composed of three parts: two side caps and one central core, each containing multiple subunits. It is found in the cytosol and the nucleus, and functions to degrade mis-folded proteins.

Mis-folded proteins enter here to be degraded.

How does a proteasome recognize its substrates?
Terminally misfolded proteins in the ER are returned to the cytosol for degradation

Degradation of misfolded or unassembled proteins.

They are transported through the translocon back into cytosol and degraded by ubiquitin-mediated proteolytic pathway.

have very compact structures consisting of two α-helices and two β-sheet structures. The C-terminus of Ubiquitin is extended and unstructured.

Misfolded protein for ubiquitin-dependent proteasome degrade

Unassembled or misfolded proteins are blocked from moving to the Golgi complex

ERAD: ER-associated degradation

misfolded proteins remain bound to ER chaperones (e.g., BiP, calnexin)

Aberrant (不正常) proteins are finally targeted for degradation and extruded back to cytoplasmic compartment through translocon

N-glycanase in cytosol removes N-linked carbohydrate moieties (去除一半)

proteins are ubiquitinated in cytosol and degraded via proteasome complex

– ubiquitin-conjugating enzymes are localized on cytoplasmic face of ER
– Ub-conjugating enzymes interact with integral membrane Ub ligases
– polyubiquitinated proteins are degraded in proteasomes

Emphysema 廣泛性肺泡肺氣腫

Misfolding protein in ER

The α1-antitrypsin mutation (release from hepatocytes, macrophage)

trypsin $\rightarrow$ degrade $\rightarrow$ elastin (ECM) $\rightarrow$ support down

Anti-trypsin inhibited trypsin
Pathway of protein breakdown in mammalian cells

Cytosolic protein
Abnormal protein  →  Ubiquitin proteasome pathway
Short-lived protein
ER-associated protein
Long-lived protein

Endocytosed proteins
Membrane protein  →  Lysosomal pathway
Extracellular protein

Degradation of protein
1. Lysoosome: primarily toward extracellular protein and aged or defective organelles of the cells.
2. Proteasomes: Ubiquitin dependent; for intracellular unfolding, aged protein.
   1. control native cytosolic protein
   2. misfolded in the course of their synthesis in the ER

16.4 Export of bacterial proteins (post-translational translocation)

Cytosolic SecA ATPase pushes bacterial polypeptides through translocons into the periplasmic space.
Bacterial translocon is very similar to eukaryotic Sec61 complex.
Several mechanisms translocate bacterial proteins into the extracellular space.

The secretion mechanisms are important for pathogenic bacteria to secrete extracellular protein to colonize specific tissue or host.

Four general types of bacterial secretion systems:

Type I and II: proteins translocated across to inner membrane into periplasmic space → fold and disulfide bond formation → folded protein translocated from periplasmic space to outer membrane by complex of periplasmic proteins; It need energy.

Type III and IV: one step, large protein complex translocated directly from the cytosol to the extracellular space.

**Type III secretion apparatus for injecting bacterial proteins into eukaryotic cells.**

Pathogenic bacteria inject protein into animal.

Type III secretion is similar in size and morphology to the bacterial flagellum.

Yersinia pestis

鼠疫桿菌

Yersinia pestis is an ancient disease transmitted by fleas, causing bubonic plague.

Pathogenic bacteria inject protein → host cell → by type III model.

16.5 Sorting of proteins to mitochondria and chloroplast

All organelles have a lipid bilayer.

Mitochondrial or chloroplast DNA and ribosome → synthesized protein → correct subcompartment.

The mechanisms of Sorting of protein to mitochondria and chloroplast is similar to bacteria.

**TABLE 16.1 Uptake-Targeting Sequences That Direct Proteins from the Cytosol to Organelles**

<table>
<thead>
<tr>
<th>Target Organelle</th>
<th>Location of Sequence Within Protein</th>
<th>Removal of Sequence</th>
<th>Nature of Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoplasmic reticulum</td>
<td>N-terminus</td>
<td>Yes</td>
<td>Core of k-12 hydrophobic amino acids, often preceded by one or more basic amino acids (Arg, Lys)</td>
</tr>
<tr>
<td>Mitochondrion (matrix)</td>
<td>N-terminus</td>
<td>Yes</td>
<td>Amphiphilic k-20 to k-50 residues in length, with Arg and Lys residues on one side and hydrophobic residues on the other</td>
</tr>
<tr>
<td>Chloroplast (stroma)</td>
<td>N-terminus</td>
<td>Yes</td>
<td>No common motifs generally rich in Ser, Thr, and small hydrophobic residues and poor in Gln and Arg</td>
</tr>
<tr>
<td>Peroxisome (matrix)</td>
<td>C-terminus (most proteins), N-terminus (few proteins)</td>
<td>No</td>
<td>PTS1 signal (Asp, Glu) at extreme C-terminus, PTS2 signal at N-terminus</td>
</tr>
</tbody>
</table>

*Different or additional sequences target proteins to organelle membranes and subcompartments.*

See Chapter 11 for targeting sequences required for uptake of proteins into the nucleus.
Amphiphatic N-terminal signal sequence direct proteins to the mitochondrial matrix:

Matrix-targeting sequences:
1. Located N-terminus
2. 20-50 amino acids in length
3. Rich in hydrophobic amino acids, positively charged amino acids (Arg, Lys), and hydroxylated ones (Ser, Thr)
4. Lack negatively charged acidic residues (Asp, Glu)
5. Alpha-helical conformation (one-hydrophobic, opposite side – charged amino acids: amphipathic)
6. Amphipathicity of matrix-targeting sequences is critical to their function

The structure of mitochondria

Mitochondrial protein import requires outer membrane receptor and translocon in both membranes
1. Unfolded protein binding chaperones,
2. Precursor protein bind to an import receptor, which contact with inner membrane
3. Transferred into import pore
4. Translocation protein
5. To adjacent channel in the inner membrane
6. Translocated protein binding to matrix chaperones, remove targeting sequenceby matrix protease, and release chaperones.
7. Folding to mature protein

Fig 16-25 The post-translational uptake of precursor proteins into mitochondria can be assayed in a cell-free system
Tom 20/22 (import receptor) and Tom 40 (general import pore)
Tim 23/17 proteins
Contact sites – close proximity
Tim 44 (translocation channel)/ Hsc70 (a matrix chaperone)
The interaction -ATP hydrolysis by matrix Hsc70
chaperonin- facilitate folding (yeast Hsc60 defect – fail to fold)

**Molecular chaperons:** which bind and stabilize unfolded or partly folded proteins, thereby preventing these proteins from aggregating and being degraded

**Chaperonins:** which directly facilitate the folding of proteins

Studies with chimeric proteins demonstrate important features of mitochondrial import: only unfolded protein can enter

No function sequence

Matrix targeting sequence

DHFR

Unfolded DHFR

in the presence of chaperone

MTX – binds tightly to the active site of DHFR and greatly stabilizes its folded conformation

MTX can not enter

Spacer sequence: >50 amino acids long
Translocation intermediate is formed
<35 residues– intermediate translocated proteins span both membranes in unfolded state

- Chemically cross-linking exp.
- 1000 general import pore (yeast mitochondria)

Fig 16-27 Experiments with chimeric proteins show that a matrix-targeting sequence alone directs proteins to the mitochondrial matrix and that only unfolded proteins are translocated across both membranes

Matrix-targeting sequence alone directs proteins to mitochondrial matrix.
Only unfolded proteins are translocated across both membranes.

Bound to the translocation intermediate at a contact site
Three energy inputs are needed to import proteins into mitochondria

1. Cytosolic Hsc70-ATP hydrolysis - unfolding function
2. Matrix Hsc70-ATP hydrolysis – molecular motor to pull the protein into the matrix (cf. chaperone BiP and Sec63 complex – in post-translational translocation into the ER lumen)
3. H+ electrochemical gradient (proton-motive force) across the inner membrane (inhibitor or uncouple of oxidative phosphorylation such as cyanide or dinitrophenol, dissipates this proton motive force - proteins bind to receptor, but not be imported)

One hypothesis: positive charges in the amphipathic matrix-targeting sequences – electrophoresed or pulled into the matrix by inside-negative membrane electrical potential

Translocation into chloroplast occurs via a similar strategy to the one used by mitochondria

Both occur post-translationally
Both use two translocation complexes, one at each membrane
Both require energy
Both remove the signal sequence after transfer
However chloroplasts have a H+ gradient across the thylakoid membrane and use GTP hydrolysis to drive transfer

Multiple signals and pathways target proteins to submitochondrial compartments

Target:
1. Inner-membrane
2. Intermembrane- space
3. Out-membrane: unknown mechanism
4. Matrix
### Outer-membrane proteins

<table>
<thead>
<tr>
<th>Imported protein</th>
<th>Location of imported protein</th>
<th>Locations of targeting sequences in preprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porin (P70)</td>
<td>Outer membrane</td>
<td>Stop-transfer and outer-membrane localization sequence</td>
</tr>
</tbody>
</table>

- Short matrix-targeting sequence is followed by long stretch of hydrophobic amino acids

### Inner-membrane proteins: three separate pathways (A)

<table>
<thead>
<tr>
<th>Imported protein</th>
<th>Location of imported protein</th>
<th>Locations of targeting sequences in preprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome oxidase subunit CoxVe</td>
<td>Inner membrane (path A)</td>
<td>Cleavage by matrix protease Hydrophobic stop-transfer sequence</td>
</tr>
</tbody>
</table>

Inner membrane has three pathway A,B,C

### Inner-membrane proteins: three separate pathways (B)

<table>
<thead>
<tr>
<th>Imported protein</th>
<th>Location of imported protein</th>
<th>Locations of targeting sequences in preprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP synthase subunit 9</td>
<td>Inner membrane (path B)</td>
<td>Cleavage by matrix protease Internal sequences recognized by Oxa1</td>
</tr>
</tbody>
</table>

### Inner-membrane proteins: three separate pathways (C)

<table>
<thead>
<tr>
<th>Imported protein</th>
<th>Location of imported protein</th>
<th>Locations of targeting sequences in preprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP/ATP antiporter</td>
<td>Inner membrane (path C)</td>
<td>Internal sequences recognized by Tom70 receptor and Tim22 complex</td>
</tr>
</tbody>
</table>

No N-terminal matrix-targeting sequences
Oxa1 also participates in the inner-membrane insertion of certain proteins encoded by mitochondrial DNA synthesized in matrix by mitochondrial ribosomes.