Moving Proteins into Membranes and Organelles
DNA → RNA → protein → Protein sorting → different organelles → different functions

The mechanisms or pathway of protein sorting / protein targeting?

In mitochondria:
Matrix, inner membrane, intermembrane and outer membrane has different protein
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 14

**Signal sequences (20-50 aa.),** uptake-targeting sequences, receptors, translocation channel, unidirectional translocation
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 newly made 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?
Overview of major protein-sorting pathways in eukaryote (protein targeting)
<table>
<thead>
<tr>
<th>INTRACELLULAR COMPARTMENT</th>
<th>PERCENTAGE OF TOTAL CELL VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>54</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>22</td>
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<tr>
<td>Rough ER cisternae</td>
<td>9</td>
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<tr>
<td>Smooth ER cisternae plus Golgi cisternae</td>
<td>6</td>
</tr>
<tr>
<td>Nucleus</td>
<td>6</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>1</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>1</td>
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<tr>
<td>Endosomes</td>
<td>1</td>
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</table>
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. Sorting of proteins to mitochondria and chloroplasts

5. Sorting of peroxisomal proteins

6. Sorting of nucleus proteins
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 $^{35}$S)

Incubate with organelle fraction
Density centrifugation
Gel electrophoresis and autoradiography

How do we know protein is inside the organelle?
- protease/detergent treatment
16.1 Translocation of secretory proteins across the ER membrane

Signal sequence: for ER, peroxisome, mitochondria, chloroplast
Translocation of secretory protein across the ER membrane

Secretory proteins are synthesized on ribosomes attached to ER (rough ER).

Free ribosome: for cytosolic protein synthesis

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**Fig13.2A** Electron micrograph of ribosomes attached to the rough ER in a pancreatic acinar cell
Structure of the rough ER

Schematic representation of protein synthesis on the ER

真的是如此嗎??
Labeling experiments demonstrate that secretory proteins are located to the ER lumen shortly after synthesis.

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

In vesicle, protease did not degrade protein
In outside, protease degrade protein

Result: protein did not degraded by protease

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 of secretory protein (from N to C) → 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 is cleaved from the protein while it is still growing on ribosome
not present of signal sequence 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  How to demonstrated it??????
Cell-free experiments demonstrate that translocation of secretory proteins into microsomes is coupled to translation.

(a) Cell-free protein synthesis; no microsomes present

- EDTA - ribosome free microsomes
- Add microsome membranes
- No signal sequence
- No incorporation into microsomes
- Completed proteins with signal sequences
- No incorporation into microsomes; no removal of signal sequence

(b) Cell-free protein synthesis; microsomes present

- Signal sequence
- Incorporation into microsomes
- Microsomes must be added before the 1st 70 aa
- Cotranslational transport of protein into microsome and removal of signal sequence
- Mature protein chain without signal sequence

Cell-free experiments demonstrate that translocation of secretory proteins into microsomes is coupled to translation.
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 $\rightarrow$ RNA $\rightarrow$ cytosol $\rightarrow$ ER + ribosome $\rightarrow$ cotranslation translocation $\rightarrow$ via a specific protein $\rightarrow$ to ER
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; in secretory protein yes.

**Signal-recognition particle (SRP)**

**Bip:** molecular chaperones

Not all signal sequence located at N-terminal; in secretory protein yes.
Two key components involve of contranslational translocation:

1) signal-recognition particle (SRP)
   - is a cytosolic ribonuclear protein particle
   - 300 nt RNA and 6 discrete (分開) 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 ribosome
   - 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 (忠誠的)
Most signal peptides are hydrophobic sequence
Signal-recognition particle (SRP)

- p9 and p14 interact with ribosome
- p68 and p72 are required for protein translocation

Signal peptide about hydrophobic sequence

- Binds ER signal sequence
- Required for protein translocation
- Interact with ribosomes

Hydrophobic binding groove

Bind to signal peptide
Passage of growing polypeptide through the **translocon** is driven by energy released during translation.

At translocon, **Sec1**α is a translocon (bacteria) translocated into ER without energy.

Artificial mRNA has lysine codon (middle) without stop codon + cell free system + chemical modify lysyl-tRNA → translation → light → cross linking reagent attached to lysine side chain.

At translocon, Translocated into ER without energy.
Translation → elongation → push peptides.

但整個 protein sorting 還是需要的

**Figure 13-7**
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Sec1α is a translocon (bacteria)
Structure of a bacterial sec61 complex

Mammalian translocon:
  Sec61 complex
  Sec61α
  - integral membrane protein,
  - 10 membrane-spanning α helixes
  - interact with translocating peptide (chemical cross-linking exp.)
  Signal peptidase
Electron microscopy reconstruction reveals that a translocon associates closely with a ribosome.
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 \( \alpha \) subunit of SRP-receptor

3. Pulling through the channel:
   chaperone activity inside the target organelle (Hsp70) that in addition helps fold the protein
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 form translation to pass through the membrane. However, also has 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. Specific located in ER
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
**Insertion of proteins into the ER membrane (also called membrane protein)**

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 **hydorphobic** sequence for interaction with intra-membrane

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**Single-pass**

**Multipass**

Not all signal peptide located at N-terminal
Moving Proteins into Membranes and Organelles (protein targeting)
Insertion into the ER membrane of type I proteins

**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 external signal sequence*

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

Type III also has

Synthesis and insertion into the ER membrane of type 1 single-pass proteins
**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

Positive charge amino acids face to cytosol ??

**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**: similar to type II, positive charge residues on the c-terminal side of the anchor sequence.
**Insertion into the ER membrane of type III proteins**

**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)

Synthesis of multiple pass transmembrane protein

Protein with N-terminus in cytosol or
Protein with N-terminus in the exoplasmic

Figure 12-49. Molecular Biology of the Cell, 4th Edition.
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 luminal portion is transferred to a preformed lipid anchor.

**Lipid anchored proteins**: palmitoylation, myristoylation,
    prenylation
Arrangement of topogenic sequences in type I, II, III and IV proteins.

**Figure 13-13**
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Even number of α 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.

Some cell surface proteins are anchored to the phospholipid bilayer by GPT
Covalently atached
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.
Covalently attached hydrocarbon chains anchor some protein to membrane

Acylation attached: attached to the N-terminal glycine residue
Prenylation: to C-terminal cysteine residue
GPI (glycosylphosphatidylinositol): such as proteoglycans.

Acylation involves the generation of the acyl group $R-C=O$

All transmembrane proteins and glycolipids are asymmetrically oriented in the bilayer
The topology of a membrane protein often can be deduced (推論) from its sequence: hydropathy profile (親水性行爲)

- **(a) Human growth hormone receptor (type I)**
  - Signal sequence
  - Stop-transfer sequence

- **(b) Asialoglycoprotein receptor (type II)**
  - Signal-anchor sequence

- **(c) GLUT1 (type IV)**
  - Transmembrane sequences

**Figure 13-15**
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Hydropathic index for each aa.
Total hydrophobicity of 20 contiguous aa
Usually for cytosol
13.3 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
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

Common 14 residue precursor of N-linked oligosaccharide that is added to nascent proteins in the rough ER.
A performed N-linked oligosaccharide is added to many proteins in the RER

Biosynthesis of dolichol pyrophosphoryl oligosaccharide precursor

Strongly hydrophobic lipid (79-95 carbon)

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

Oligosaccharide side chain may promote folding and stability of glycoproteins
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).
N-glycosylation: Oligosaccharide precursor is attached to the protein co-translationally

Consensus: Asn-X-Ser/Thr

Red: GlcNAc
Blue: mannose
Green: Glucose
Function of the ER: Glycosylation

- Core mannose oligosaccharide linked to dolichol by a high-energy phosphate bond
- Transfer across the ER membrane
- Addition of more mannose units
- Transfer of core oligosaccharide to an asparagine side chain
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).

Oligosaccharide side chains may promote folding and stability of glycoprotein.
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-acetylglicosamine 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: sulphydryl group

55 kDa protein - acts as dimer, contains protein binding site

**Formation of a disulfide bond**

- **Oxidized PDI**
- **Reduced PDI**
- **Oxidized Ero1**

**Rearrangement of disulfide bonds**

- **Reduced PDI**
- **Protein with incorrect disulfide bonds**
- **Protein with correct disulfide bonds**

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Cystine
Formation of disulfide bonds in eukaryotes & bacteria

Transfers electrons to a disulfide bond in the luminal protein
PDI like DsbA