Major Protein-sorting pathways in eukaryotic cells
Key elements for the Protein targeting events

1. **Signal sequence**: the amino acid sequence of a protein that provides the information to target the protein to a particular organelle.

2. **Receptors** for the signal sequences.

3. **Translocation channels** that allow transfer of proteins across the membrane bilayer.

4. **Energy** that drives unidirectional transfer across the membrane.
Where are the endoplasmic reticulum, Golgi, and mitochondria?
Synthesis of secretory proteins and their cotranslational translocation across the ER membrane

Wenxia:
1. ER signal sequence emerges
2. The binding by a signal-recognition particles (SRP)
3. SRP delivers the ribosome/nascent polypeptide complex to the SRP receptor in the ER membrane, and GTP binding
Signal-recognition particles

Wenxia:
1. Six subunits
2. All proteins binding to 300-nucleotide RNA except p54
3. P54 bind to ER signal sequences and have hydrophobic binding groove
Wenxia:

1. First found in yeast by mutations
2. Sec61 complex, consisting of 3 proteins
3. Sec61a contacts translocating polypeptide chain
4. A cylinder 5-6 nm high, 8.5 nm in diameter, and pore 2 nm diameter
5. Open and close of translocon is regulated
Major topological classes of integral membrane proteins synthesized on the rough ER

Type I
- Glycophorin
- LDL receptor
- Influenza HA protein
- Insulin receptor
- Growth hormone receptor

Type II
- Asialoglycoprotein receptor
- Transferrin receptor
- Sucrase–isomaltase precursor
- Golgi galactosyltransferase
- Golgi sialyltransferase
- Influenza HN protein

Type III
- Cytochrome P450

Type IV
- G protein–coupled receptors (e.g., β-adrenergic receptor)
- Glucose transporters (e.g., GLUT1)
- Voltage-gated Ca_{2+} channels
- ABC small molecule pumps
- CFTR (Cl−) channel
- Sec61
- Connexin
Synthesis and insertion into the ER membrane of type I single pass proteins

Wenxia: Hydrophobic transmembrane segment is the stop-transfer anchor sequence
Type II single-pass proteins

Experiments:
Mutation of the positive charged amino acids changes the orientation of the protein.
Arrangement of topogenic sequences of different membrane proteins

\[ \text{STA} = \text{Internal stop-transfer anchor sequence} \]
\[ \text{SA-II} = \text{Internal signal-anchor sequence} \]
\[ \text{SA-III} = \text{Internal signal-anchor sequence} \]
GPI anchored proteins

Cytosol

COO⁻

GPI transamidase

Preformed GPI anchor

Precursor protein

NH₃⁺

NH₃⁺

Mature GPI-linked protein

ER lumen
<table>
<thead>
<tr>
<th>Nature of Sequence</th>
<th>Removal of Sequence</th>
<th>Location of Sequence Within Protein</th>
<th>Target Organelle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coro of 6–12 hydrophobic amino acids (or longer)</td>
<td>Yes</td>
<td>N-terminus</td>
<td>Endoplasmic reticulum (lumen)</td>
</tr>
<tr>
<td>Amphipathic helix, 20–50 residues</td>
<td>Yes</td>
<td>N-terminus</td>
<td>Mitochondrion (matrix)</td>
</tr>
<tr>
<td>No common motifs generally rich in set Trp and small hydrophobic residues and poor in Glu and Arg</td>
<td>Yes</td>
<td>N-terminus</td>
<td>Chloroplast (stroma)</td>
</tr>
<tr>
<td>PTSS signal (Ser-Lys-Arg) at extreme C-terminus; PTSS signal at N-terminus</td>
<td>No</td>
<td>C-terminus</td>
<td>Peroxisome (matrix)</td>
</tr>
</tbody>
</table>

* Differences or additive sequences target proteins to organelle membranes and subcompartments. See Chapter 12 for targeting sequences required for uptake of proteins into the nucleus.
Protein import into the mitochondrial matrix

Tom-translocon of outer membrane
Tim-translocon of the inner membrane

Wenxia:
Targeting signals
Signal receptor
Translocation channel
Energy
Transporting protein from the cytosol to the inner mitochondrial membrane

Path A:
- Preprotein
- Stop-transfer sequence
- Matrix-targeting sequence
- COO⁻
- NH₃⁺
- Tom20
- Tom22
- Tom40
- Outer membrane
- Intermembrane space
- Tim23/17
- Tim44
- Inner membrane
- Mitochondrial matrix
- Hsc70
- Cleaved matrix-targeting sequences

Path B:
- Preprotein
- Oxa1-targeting sequence
- Matrix-targeting sequence
- COO⁻
- NH₃⁺
- Tom40
- Tim23/17
- Oxa1
- Hsc70
- Assembled protein

Path C:
- Preprotein
- Internal targeting sequences
- COO⁻
- NH₃⁺
- Tom40
- Tim70
- Tim9/10
- Tim22
- Tim54
- Assembled protein
Transporting proteins from the cytosol to the mitochondrial intermembrane space
How to analyze protein movement into membranes and organelles

The in vitro system

1. Uptake-targeting sequence
2. Protein taken up into mitochondria; uptake targeting sequence removed and degraded
3. Proteins sequestered within mitochondria are resistant to trypsin

1. Yeast mitochondrial proteins made by cytoplasmic ribosomes in a cell-free system
2. Protein taken up into mitochondria; uptake targeting sequence removed and degraded
3. Proteins sequestered within mitochondria are resistant to trypsin

Trypsin

Uptake-targeting sequence and mitochondrial protein degraded
How to identify signal sequences

Genetic manipulations
1. Sequence homology
2. Targeted mutagenesis
3. Fusion proteins

Hydropathy profiles
How to identify a translocon component that contacts nascent secretory proteins

Chemical cross-link

Artificial mRNA

5'

tRNA

40S

60S

Ribosome

Cytosol

Microsomal membrane

Microsomal lumen

Sec61α

Cross-linking agent

Nascent protein

NH₃⁺