Protein targeting is necessary for proteins that are destined to work outside the cytoplasm.

Protein targeting is more complex in eukaryotes because of the presence of many intracellular compartments.

Prokaryotic protein targeting (secretion)
The chaperone protein **SecB** binds to the nascent polypeptide chain to *prevent premature folding* which would make transport across the plasma membrane impossible. **SecE** and **SecY** are transmembrane components which form a pore in the membrane through which the still unfolded polypeptide is threaded. The translocation process is energy-dependent (ATP) and is driven by **SecA**. Once the protein has passed through the pore, the signal sequence is cleaved off by an extracellular, membrane-bound *protease*.

**N-terminal signal sequences of representative secreted prokaryotic proteins.**

<table>
<thead>
<tr>
<th>Protein</th>
<th>-20</th>
<th>-10</th>
<th>-1</th>
<th>+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Type</td>
<td>Sequence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine-binding protein</td>
<td>MKANAKTI I IAGMIALAI SHTAMA EE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-alkaline phosphatase</td>
<td>MKQSTIALALLPLLFTPVTKA RT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-lipoprotein</td>
<td>MKATKLVLGAVILGSTLLAG CS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hydrophobic residues in red.

**Next:** Eukaryotic Protein Targeting

*Please report typos, errors etc. by [EMAIL](mailto:) (mention the title of this page).*
Eukaryotic Protein Targeting

Targeting in eukaryotes is necessarily more complex due to the multitude of internal compartments:

- nucleus
- mitochondria
- peroxisomes
- chloroplasts
- endoplasmic reticulum (ER)
- Golgi
- lysosomes
- secretory granules

The signals involved are also called sorting signals. They are regions on the targeted protein with certain amino acid sequences.

These signals interact with specific receptors, either on the target organelle or a carrier protein.
There are two basic forms of targeting pathways:

- **post-translational** targeting:
  - nucleus
  - mitochondria
  - chloroplasts
  - peroxisomes

- **co-translational** targeting (secretory pathway):
  - ER
  - Golgi
  - lysosomes
  - plasma membrane
  - secreted proteins
In the absence of targeting signals, a protein will remain in the **cytoplasm:**

- translational machinery
- metabolic enzymes
- cytoskeletal proteins
- many signal transduction proteins

### Nuclear targeting:

- Unusual since 2-way traffic:
  - *in:* proteins, DNA
    - DNA & RNA polymerases
    - transcriptions factors
    - histones etc.
  - *out:* mRNA, tRNA, rRNA
Proteins are not transported through the nuclear membrane but rather through a complex pore called the **nuclear pore**:
- comprised of about 100 different proteins
- proteins smaller than 20 kDa move by diffusion
- proteins larger than 20 kDa move by selective transport (**nuclear localization signal**)
  - cluster of 4-8 positively charged amino acids (example: **PKKKRLV**)
  - signal sequence binds to receptor on the pore called **importin**

**Mitochondrial targeting:**
- not well understood
- usually by post-translational targeting

**Lysosomal targeting:**
- **Lysosomes** are organelles that store enzymes which rapidly degrade other proteins and nucleic acids.
- A famous target sequence is "**KDEL**"
- Initial targeting via **secretory pathway**
- Final targeting occurs in the Golgi

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The Golgi (8.2b)

The secretory pathway

ER targeting (secretory pathway)

- co-translational insertion of protein into or through ER membrane via attached ribosomes (rough ER):
  - **signal sequence** of 16-30 amino acids at N-terminus (hydrophobic)
  - emerging **signal sequence** of nascent protein on free ribosome binds to **signal recognition particle** (SRP) -- translation is **arrested**.
    - SRPs consist of 6 proteins and one RNA molecule (7S RNA).
    - The SRP-signal sequence-mRNA-ribosome complex docks with **receptor** on ER membrane.
      - **signal sequence** crosses ER membrane.
      - translation continues with polypeptide chain being pulled into the ER lumen.
While in the ER, many proteins undergo the first stages of glycosylation. Most proteins then migrate inside vesicles from the ER and enter the cis face of the Golgi where further processing and final sorting occurs:
The Golgi is responsible for further **processing** and final **sorting** of proteins. One example is the formation of **primary** and **secondary lysosomes**:

- Primary lysosomes bud from the *trans* face of the Golgi and subsequently
  - undergo exocytosis (A)
  - fuse with vesicles to digest their contents (B & C)
  - rupture, causing autolysis (D)

---

**Overview of Trafficking**
The Golgi (8.2b)

Next: Targeted Protein Degradation

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Targeted Protein Degradation

Why are proteins degraded?

In order to keep a cell working it needs to remove:

- incorrectly synthesized proteins (with errors in amino acid sequence)
- damaged proteins (i.e. oxidative damage)
- cell-cycle specific proteins
- other signaling proteins which are no longer necessary

One mechanism of protein degradation is via lysosomes. **Lysosomes are acidic vesicles that contain about 50 different enzymes involved in degradation:**

- proteases (cathepsins): cleave peptide bonds
- phosphatases: remove covalently bound phosphates
- nucleases: cleave DNA/RNA
- lipases: cleave lipid molecules
- carbohydrate-cleaving enzymes: remove covalently bound sugars from glycoproteins

- Lysosomes often secrete their contents into the extracellular medium via **exocytosis**.
- Lysosomes can also target damaged organelles in a process called **autophagy**.
- Sometimes, lysosomes are triggered to rupture inside a cell, resulting in **autolysis**,
also called *apoptosis* or *programmed cell death*.

Another major mechanism is via *ubiquitin labeling* of surplus proteins:

- Ubiquitin (a small 76-residue protein) is attached to the protein:
  - First, an *activating enzyme* attaches itself to the carboxy terminus of free ubiquitin in an ATP-dependent process.
  - Then, the activated ubiquitin is transferred onto a second enzyme which at the same time recognizes damaged proteins.
  - The activated ubiquitin is then *covalently* linked to lysine residues on the surface of the damaged protein.

- These *ubiquitin-tagged* proteins are now recognized by specific *proteases* in the cytosol which in turn cleave and degrade the tagged protein.

- These proteases are combined in a very large protein complex called the *proteasome*.

- The proteasome (20S) is comprised of 28 subunits and has a molecular weight of 700 kDa:
Next: Summary

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Summary

- Targeting of newly synthesized proteins is an integral component of protein synthesis.

- In prokaryotes, targeting is usually achieved by an N-terminal signal sequence of about 20 mostly hydrophobic amino acids.

- In eukaryotes, targeting is more complex due to the large number of different cellular compartments:
  - Nuclear targeting via the nuclear pore using a nuclear localization signal.
  - ER targeting (secretory pathway) via N-terminal signal sequences using SRPs with subsequent attachment to the ER,
  - Followed by transport to the Golgi complex

- Protein degradation of damaged or obsolete proteins is carried out
  - by lysosomes, vesicles filled with degradating enzymes
  - in a ubiquitin-dependent process by specific proteases in a large cytosolic complex called the proteasome.

Last Lecture: Expression Systems for Recombinant Proteins

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