The secretory pathway

1. Protein synthesis on bound ribosomes; cotranslational transport of proteins into or across ER membrane

2. Budding and fusion of ER-to-Golgi vesicles to form cis-Golgi network

3. Retrograde Golgi-to-ER transport

4. Cisternal progression

5. Retrograde transport from later to earlier Golgi cisternae

6. Constitutive secretion

7. Regulated secretion

8. Sorting to lysosomes

9. Endocytosis
Addition and initial processing of N-linked oligosaccharine in the rough ER of vertebrate cells

O-linker
Ser/Thr

N-linked
Asn-X-Ser
Asn-X-Thr
Processing of N-linked oligosaccharide chains on glycoproteins in Golgi

- **Trans**: UDP $\rightarrow$ CMP
- **Medial**: UDP $\rightarrow$ GDP
- **Cis**: GDP $\rightarrow$ GDP

- $\text{N} \text{-Acetylglucosamine}$
- Mannose
- Fucose
- Galactose
- $N$-Acetylneuraminic acid

Transport vesicle from ER
Formation and rearrangement of disulfide bonds by protein disulfide isomerase (PDI)

(a) Formation of a disulfide bond

(b) Rearrangement of disulfide bonds
Chaperon proteins facilitate folding and assembly of protein
Unfolded protein response

1. Unfolded proteins are sensed in the ER lumen by BiP.
2. The unfolded proteins stimulate the formation of Ire1 dimers. 
3. The dimerized Ire1 initiates the endonuclease cleavage of the unspliced Hac1 mRNA.
4. The spliced Hac1 mRNA is translated into the Hac1 transcription factor.
Proteasome degradation of misfolded proteins
Major types of proteins involved in vesicle budding and fusion with a target membrane

(a) Coated vesicle budding

- GTP-binding protein
- v-SNARE protein
- Membrane cargo protein
- Membrane cargo-receptor protein
- Coat proteins

(b) Uncoated vesicle fusion

- Target membrane
- t-SNARE proteins
- t-SNARE complex
Budding of ER vesicles

1. Sar1 membrane binding, GTP exchange

2. COPII coat assembly

3. GTP hydrolysis

4. Coat disassembly

Uncoated vesicle
### TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

<table>
<thead>
<tr>
<th>Vesicle Type</th>
<th>Coat Proteins</th>
<th>Associated GTPase</th>
<th>Transport Step Mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPII</td>
<td>Sec23/Sec24 and Sec13/Sec31 complexes, Sec16</td>
<td>Sar1</td>
<td>ER to cis-Golgi</td>
</tr>
<tr>
<td>COPI</td>
<td>Coatomers containing seven different COP subunits</td>
<td>ARF</td>
<td>cis-Golgi to ER</td>
</tr>
<tr>
<td>Clathrin and adapter proteins</td>
<td>Clathrin + AP1 complexes</td>
<td>ARF</td>
<td>Later to earlier Golgi cisternae</td>
</tr>
<tr>
<td></td>
<td>Clathrin + GGA</td>
<td>ARF</td>
<td>trans-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>Clathrin + AP2 complexes</td>
<td>ARF</td>
<td>trans-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>AP3 complexes</td>
<td>ARF</td>
<td>Plasma membrane to endosome</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Golgi to lysosome, melanosome, or platelet vesicles</td>
</tr>
</tbody>
</table>

*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

### TABLE 17-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

<table>
<thead>
<tr>
<th>Signal Sequence</th>
<th>Proteins with Signal</th>
<th>Signal Receptor</th>
<th>Vesicles That Incorporate Signal-bearing Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Asp-Glu-Leu (KDEL)</td>
<td>ER-resident luminal proteins</td>
<td>KDEL receptor in cis-Golgi membrane</td>
<td>COPI</td>
</tr>
<tr>
<td>Lys-Lys-X-X (KKXX)</td>
<td>ER-resident membrane proteins (cytosolic domain)</td>
<td>COPI α and β subunits</td>
<td>COPI</td>
</tr>
<tr>
<td>Di-acidic (e.g., Asp-X-Glu)</td>
<td>Cargo membrane proteins in ER (cytosolic domain)</td>
<td>COPI II / subunit</td>
<td>COPI</td>
</tr>
<tr>
<td>Mannose 6-phosphate (M6P)</td>
<td>Soluble lysosomal enzymes after processing in cis-Golgi</td>
<td>M6P receptor in trans-Golgi membrane</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td></td>
<td>Secreted lysosomal enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn-Pro-X-Tyr (NPXY)</td>
<td>LDL receptor in the plasma membrane (cytosolic domain)</td>
<td>AP2 complex</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Tyr-X-X-Φ (YXXΦ)</td>
<td>Membrane proteins in trans-Golgi (cytosolic domain)</td>
<td>AP1 (μ1 subunit)</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td></td>
<td>Plasma membrane proteins (cytosolic domain)</td>
<td>AP2 (μ2 subunit)</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Leu-Leu (LL)</td>
<td>Plasma membrane proteins (cytosolic domain)</td>
<td>AP2 complexes</td>
<td>Clathrin/AP2</td>
</tr>
</tbody>
</table>

*X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.
Docking and fusion of transport vesicles

1. **Vesicle docking**
   - Transport vesicle
   - VAMP
   - Rab • GTP

2. **Assembly of SNARE complexes**
   - Target membrane
   - Syntaxin
   - SNAP-25
   - Rab effector

3. **Membrane fusion**
   - NSF
   - α-SNAP
   - cis-SNARE complex

The diagram illustrates the process of docking and fusion of transport vesicles, highlighting the role of various molecules and complexes involved in the process.
Vesicle-mediated protein trafficking between the ER and cis-Golgi

Forward (anterograde) transport

Reverse (retrograde) transport
Fluorescence microscopy of cells producing a GFP-tagged membrane protein

VSVG-GFP temperature sensitive mutants
Radioactive amino acids pulse/chase and protein sensitivity to glycosidases

(a) Time at 32 °C (min)  0  5  10  15  20  30  45  60
Resistant  
Sensitive  

(b)

Fraction of total G protein sensitive to endoglucosidase D

32 °C

40 °C

Time (min)
Genetic approach - Yeast sec mutants

**Class A**
- ER
- Accumulation in the cytosol
- Transport into the ER
- Normal secretion

**Class B**
- ER
- Accumulation in rough ER
- Budding of vesicles from the rough ER

**Class C**
- Accumulation in ER-to-Golgi transport vesicles
- Fusion of transport vesicles with Golgi

**Class D**
- Accumulation in Golgi
- Transport from Golgi to secretory vesicles

**Class E**
- Accumulation in secretory vesicles
- Transport from secretory vesicles to cell surface
Cell free system

Cis-Golgi ➔ Medial-Golgi ➔ Trans-Golgi

G protein

VSV-infected wild-type cells

N-Acetylglucosamine transferase I reaction

VSV-infected mutant cells (no N-acetylglucosamine transferase I)

Golgi isolated from uninfected wild-type cells

G protein in Golgi from infected mutant cells

Incubation

Addition of N-acetylglucosamine to G protein

- N-Acetylglucosamine
- Mannose
- Galactose
- N-Acetylneuraminic acid