Targeting sequence on cargo proteins make specific molecular contacts with coat protein

**Table 14.2 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 soluble proteins</td>
<td>KDEL receptor in cis-Golgi membrane</td>
<td>COP1</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>Sec63/Eg5 (e.g., Sec63/Eg5)</td>
<td>Cargo membrane proteins in ER</td>
<td>COP1, Sec24 subunit</td>
<td>COP1</td>
</tr>
<tr>
<td>Asp–Pro–X–Tyr (NXXD)</td>
<td>LDL receptor in plasma membrane</td>
<td>AP2 complex</td>
<td>Clathrin/AP2</td>
</tr>
<tr>
<td>Tyr–X–X–Tyr (TXXE)</td>
<td>Membrane proteins in trans-Golgi</td>
<td>AP1 (γ1 subunit)</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td>Leu–Leu (LL)</td>
<td>Plasma membrane proteins</td>
<td>AP2 complex</td>
<td>Clathrin/AP2</td>
</tr>
</tbody>
</table>

3-D structure of ternary complex comprising the COPII coat proteins (Sec23, Sec24) & Sar1-GTP.

**CFTR: inherited disease cystic fibrosis**

Mutation of CFTR receptor (chloride channel) phenylalanine 508→ conformational change of di-acidic sorting signal → did not interaction with Sec24→ did not formed COPII → did not transport

**COPII vesicles mediate transport from the ER to the Golgi**

Formation of COPII vesicles: triggered by Sec12 → induced catalyzes the GDP for GTP of Sar1 → binding Sar1 to ER membrane → followed by binding of Sec13/24 → formation of complex → second complex comprising Sec13 and 31 → interact with fibrous proteins Sec 16 → coat polymerization Sec24: interact with integral ER transport to Golgi

3-D structure of ternary complex comprising the COPII coat proteins (Sec23, Sec24) & Sar1-GTP.
COP I vesicles mediate retrograde transport for retrieval of ER resident proteins (recycle protein)

necessary for soluble secretory proteins to move anterograde without loss of ER resident proteins (e.g., PDI, BiP)

ER resident proteins possess ER retrieval signals
- KKXX at C-terminal end for ER membrane proteins interacts w/ COP1 α / β (e.g., PDI)
- KDEL at C-terminal end for ER soluble proteins interacts w/ KDEL receptor (e.g., BiP)

KDEL receptor serves to retrieve KDEL tagged proteins from cis-Golgi and return them to ER
- KDEL receptors localized primarily to membranes of cis-Golgi itself and to small vesicles that shuttle between ER and cis-Golgi

KDEL and KKXX signals are both necessary and sufficient for ER retention

Lys-Lys-X-X in KDEL receptor or membrane receptor (Retrieval of ER-resident membrane proteins from Golgi)
At the very end of C-terminus, which faces the cytosol.
Binds to COP1 α & β subunits and retrograde to ER.
Anterograde transport through the Golgi occurs by **cisternal progression**

Cisternal progression: protein form cis to trans. Trans more large than cis

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Exp demonstrated golgi cisternal maturation in a living cell

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Later stages of the secretory pathway

Three major types of coated vesicles in secretory & endocytic pathways.

- **COPII**: mediate anterograde transport from ER to cis Golgi complex.
- **COPI**: retrograde transport from cis to ER

Secretory proteins: coated protein (usually clathrin), move from cis to trans, is also cisternal progression.

**trans-Golgi network (TGN)**

---

Vesicles coated with clathrin and adapter proteins mediate several transport steps (clathrin-coated vesicle; CCV)

Trans-Golgi → Vesicles, has two layered, outer composed of the fibrous protein **clathrin** and inner layer compose of adapter protein (AP) complex.

- 3 heavy (180k) and 3 light (35-40k) chain (三曲線)

Fibrous cathrin coat around vesicles is constructed of 36 clathrin triskelion AP complex determine which cargo protein specifically to included in or excluded from. AP: has 1, 2, 3 subunit

All vesicles, ARF → initiate coat assembly onto the membrane

Adapter protein (AP and GGA) bind to the cytosolic domain of cargo protein.

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Structure of clathrin coats (36 triskelions)
Targeting sequence on cargo proteins make specific molecular contacts with coat protein

<table>
<thead>
<tr>
<th>SIGNAL SEQUENCE</th>
<th>PROTEINS WITH SIGNAL</th>
<th>SIGNAL RECEPTOR</th>
<th>VESICLES THAT INCORPORATE SIGNAL-RECOGNIZING PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-Luminal</td>
<td>ER resident soluble proteins</td>
<td>KDEL receptor in cis-Golgi membrane</td>
<td>COP1</td>
</tr>
<tr>
<td>Manacine-6-phosphate (KMP)</td>
<td>KMP receptor in trans-Golgi membrane</td>
<td>Clathrin/AP1</td>
<td></td>
</tr>
<tr>
<td>Secreted lysosomal enzymes</td>
<td>KMP receptor in plasma membrane</td>
<td>Clathrin/AP1</td>
<td></td>
</tr>
</tbody>
</table>

**Luminal Sorting Signals**

<table>
<thead>
<tr>
<th>CYTOPLASMIC SORTING SIGNALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys-Lys-Lys-X-Lys (KLKXK)</td>
</tr>
<tr>
<td>DJ-solvak (e.g., Asp-X-Glu)</td>
</tr>
</tbody>
</table>

**Cytosolic Sorting Signals**

<table>
<thead>
<tr>
<th>Cytoplasmic component</th>
<th>COP1 complex</th>
<th>Clathrin/AP1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane proteins (core-Golgi)</td>
<td>AP1 (µ1 subunit)</td>
<td></td>
</tr>
<tr>
<td>Membrane proteins (core-Golgi)</td>
<td>AP2 (µ2 subunit)</td>
<td></td>
</tr>
<tr>
<td>Membrane proteins (core-Golgi)</td>
<td>AP2 complexes</td>
<td></td>
</tr>
</tbody>
</table>

**Mistransport Mechanism; Retrgrade**

Dynamin is required for pinching off (脫離) of clathrin vesicles.

Dynamin is needed for left donor membrane, need GTP hydrolysis.

COPI and II did not need GTP hydrolysis by dynamin is required for pinching off of clathrin-coated vesicles in cell free extract (GTP-γ-S).

No GTP hydrolysis → no pinching off of clathrin-coated vesicles.

GTP hydrolysis by dynamin is required for pinching off of clathrin-coated vesicles in cell free extract (GTP-γ-S).

**Lysosomes and cellular digestion**

和過氧化體不同，蛋白質運送 need vesicle

Containing digestive enzyme

Lipids, carbohydrates, nucleic acids, proteins, extracellular materials (endocytosis), intracellular materials and macromolecules

The endpoint of the endocytosis pathway for many molecules is the lysosome, a highly acidic organelle rich in digestive enzymes.

The V-ATPase maintains the high acidity of the lumen by pumping protons across the lipid bilayer.
Lysosomal enzymes are important for several different digestive processes

Functions of lysosomes
- Nutrition
- Defense
- Recycling of cellular components
- Differentiation
- Phagocytosis, receptor-mediated endocytosis, autophagy, extracellular digestion
- Autophagy: The original recycling system

Lysosomes isolate digestive enzymes from the rest of the cell

To be discovered in 1950s
Enzymes: acid phosphatase, beta-glucuronidase, deoxyribonuclease, ribonuclease, protease
Containing various size and shape (generally ~0.5 μm in diameter)
A single membrane
Have ATP-dependent proton pumps to maintain pH value (4.0-5.0)
→ for denature and degradation of macromolecules → actively or passively transport → to cytosol
Major enzymes are acid hydrolases
- Could digestive entire organelles
- Could not digestive lysosomal membrane by glycosylation of interior membrane

Lysosomes develop from endosomes

Lysosomal enzymes: synthesized in RER → golgi → sorted in TGN → have mannose-6-phosphate → packaged in clathrin-coated vesicles → budded from TGN → to one of the endosomal compartments (early endosome) → late endosome (full complement of acid hydrolases) → proton pump → change pH
Enzymes activation mechanisms
- Moving the enzymes
- More acidic environment
Two ways
- By ATP-dependent proton pump (late endosomal lumen to 4.0-5.0)
- Transfer material to an existing lysosome

Lysosome protein targeting
Mannose 6-phosphate (M6P) residues target soluble proteins to lysosomes. M6P receptors bind M6P specifically and tightly at acidic pH 6.5, at trans-Golgi pH < 6. Bound lysosomal enzymes are released with late endosomes. Phosphatase within late endosomes remove the phosphate from M6P on lysosomal enzymes to prevent rebinding to the M6P. Vesicle budding from late endosomes recycle the M6P receptor back to the trans-Golgi.

Lysosomal enzymes (e.g., acid hydrolases) possess N-linked oligosaccharide as sorting signal. The acid hydrolases in the lysosome are sorted in the TGN based on the chemical marker mannose 6-phosphate. M6P receptors bind M6P specifically and tightly at acidic pH 6.5, at trans-Golgi pH < 6. Bound lysosomal enzymes are released with late endosomes. Phosphatase within late endosomes remove the phosphate from M6P on lysosomal enzymes to prevent rebinding to the M6P. Vesicle budding from late endosomes recycle the M6P receptor back to the trans-Golgi.

In animal cells, lysosomal enzymes: TGN → early endosomes → late endosomes → pH 5.5 → lysosomal enzymes to dissociate from the MPRs. Receptors are recycled to TGN.

Some proteins undergo proteolytic processing after leaving the trans-Golgi. Some membrane and secretory proteins initially are synthesized as long-lived, inactive protein, termed proproteins (soluble lysosomal enzyme also called proenzyme) → proteolytic → mature. Proteolytic conversion occurs after the proprotein has been sorted in the trans-Golgi vesicles.
Proteolytic processing of proproteins in constitutive and regulated secretion pathway

(a) Constitutive secreted proteins

Proalbumin

NH₂ Arg Arg COO⁻

Furin endopeptidase

NH₂ Arg Arg COO⁻

Albumin

(b) Regulated secreted proteins

Proinsulin

H₂ B Arg Arg C Lys Arg A COO⁻

PC1 endoprotease

PC2 endoprotease

C Lys Arg

H₂ B Arg Arg A COO⁻

Proinsulin

NH₂ B Arg Arg COO⁻

Cysteine protease

B B Insulin A COO⁻

I-cell disease: human genetic disorder

- Defective phosphotransferase
- Absence of mannose-6-phosphate
- Lysosomal enzymes were released to cell

Protein aggregation in trans-Golgi may function in sorting proteins to regulated secretory vesicles.

Two secretory types: from trans Golgi to cell surface

Constitutive secretion

Regulated secretion: pancreatic β cell regulated by glucose, release insulin

No shared sorting sequence is found.

Protein aggregation is observed in trans Golgi network, buds from trans Golgi, & regulated secretory vesicles.

Regulated secretory vesicles contain 3 proteins, chromogranin A, chromogranin B, & secretogranin II, that form aggregates when incubated at pH 6.5 & 1 mM Ca²⁺.

Aggregates do not formed at the neural pH of ER.

constitutive secretory proteins are sorted into transport vesicles at trans-Golgi network (TGN)
- immediate movement to plasma membrane
- release at PM via exocytosis

regulated secretory proteins are sorted into secretory vesicles at TGN
- proteins are concentrated and stored until stimulus received to elicit exocytosis
  - nerve impulse
  - hormonal stimulus
- ↑ [Ca²⁺] in cytoplasm needed to trigger fusion of vesicles with plasma membrane

sorting to lysosomes via late endosomal compartment
- lysosomal enzymes
- lysosomal membrane proteins

Figure 13-36 part 1 of 2. Molecular Biology of the Cell, 6th Edition.
Several pathways sort membrane proteins to the apical or basolateral region of the polarized cells. Epithelial cells divided into apical and basolateral, have tight junctions. Tight junctions prevent the movement of plasma membrane proteins between different membranes. For different transport distribution, the mechanism is not well known. But they protein trafficking from trans Golgi.

MDCK cell (hepatocytes) are infected with VSV and influenza virus.

Membrane trafficking is critical to Polarity

- Sorting at the Trans-Golgi
- Retention After Secretion
- Sorting After Endocytosis
- Sorting Signals
  - Basolateral: Tyrosine or DiLeucine
  - Apical: N or O-linked Glycosylation Or TM domain

MDCK in specialized container provide a useful experimental system for studying epithelial cells.

Madin-Darby canine kidney

Apical surface

Culture dish

Apical medium

Basal medium

GPI anchored protein only in apical membrane

Lipid raft in apical membrane

Viruses bud only from the apical membrane
Receptor-mediated endocytosis

Also called clathrin-dependent endocytosis
By specific receptors
The primary mechanism for most macromolecules internalization
- Hormones, growth factors, enzymes, serum proteins, Ab, iron, viruses and bacterial toxins
LDL receptor internalization  *Low-density lipoprotein*
Protein molecules essential for new rounds of endocytosis are often recycled.
Recycling of receptor is facilitated by acidification of the early endosome (pH 7.0 $\rightarrow$ 5.9-6.5)
- pH is maintained by an ATP-dependent proton pump in endosome membrane
- Slightly acidic environment $\rightarrow$ decreased receptor-ligand complex

Clathrin-independent endocytosis

Such as fluid-phase endocytosis
Nonspecific endocytosis
No concentrated for material
Materials were transported to early endosomes
Lipid transport in the circulation

Diet → intestine → uptake lipid → package thousands of lipid by protein (apolipoproteins) → lipoprotein → water soluble → circulation

Cholesterol

Proteins (apolipoproteins)

Non polar lipids in core

(TAG and cholesterol esters)

Lipids are insoluble in plasma. In order to be transported they are combined with specific proteins to form lipoproteins

What is cholesterol?

– Cholesterol is a fatty substance found in all parts of the body

– Used by the body to make:
  • Cell membranes
  • Steroid hormones
  • Vitamin D

– Sources of cholesterol
  • Liver and diet

PROBLEM: cholesterol is hydrophobic!

Cholesterol is hydrophobic (water-hating)

In order to be transported in the blood, cholesterol must be assembled into “packages”

• **LDL (low density lipoprotein)** = deliver cholesterol to cell via blood vessels
  • apoB protein
  • 2000 cholesterol molecules shielded from blood by monolayer of phospholipids
• **HDL (high density lipoprotein)** = remove cholesterol from cell/blood vessel back to liver
  • apoA protein

An epitope on apoB interacts with the LDL receptor on the cell surface. Each LDL contains 1500 molecules of cholesteryl esters.
Uptake of low-density lipoproteins (LDL) is one of the best understood examples of receptor-mediated endocytosis. LDL is a protein-lipid complex that transports cholesterol-fatty acid esters in the blood stream. LDL normally supplies cholesterol to cells. Defects in the endocytic process result in high blood levels of LDL. High LDL predisposes individuals for atherosclerosis.

**Pulse-chase experiment demonstrates precursor-product relations in cellular uptake of LDL**

Binding → internalization → degradation

![Graph showing the uptake and degradation of LDL over time at 37 °C.](image)

**Endocytic pathway for internalizing LDL. Pinocytosis**

Normally, LDL binds the receptor and the receptors collect in coated pits through association with AP2 (adapts) and clathrin. LDL particles, water soluble carriers, transport cholesterol. LDL receptors bind LDL particle via Apo-B, undergo endocytosis and are transported to late endosome compartment. LDL receptors are recycled back to cell surface. LDL particles are sorted into transport vesicle & targeted to lysosomes in lysosomal compartment, lysosomal hydrolases convert:
- apo-B → amino acids
- cholesterol esters → cholesterol + fatty acids

**Cell membranes, lipid droplets, steroid hormones, bile acids**

- phospholipids, triglycerides
Model for pH-dependent binding of LDL by LDL receptor.

- **7 repeat (R1-R7) in the ligand-binding domain.**
  - R4 and R5 most critical for LDL binding.
- Histidine rich in propeller domain → acid condition → positively charged propeller → high affinity to ligand binding arm (negative) → release LDL particle
- LDL + LDL-receptor → endocytosis → to lysosome → more positive → conformational change → LDL release

Some hypercholesterolemia receptor mutants have been useful in discovering various sorting signal motifs
- one LDL receptor mutant, but can bound LDL normally; however, ligand-receptor complexes failed to internalize and to cluster in clathrin coated pits Tyr → Cys
  - mutation in cytosolic domain, which is within Tyr-X-X-φ motif and unable to bind μ 2 of AP2 heterotetramer complex
- general sorting signal motifs should be used as a CLUE, not a fact

LDL-receptor did interact with clathrin/AP2 formed complex

Studies of familial hypercholesterolemia

Lead to discovery of LDL receptor & mechanism of receptor-mediated endocytosis.
- Mutant LDL receptors -> identify NPXY sorting signal that binds to a subunit of AP2 complex, which is also mutated in some patients.
  - LDL can bind to LDL-receptor, but did not formed clathrin + AP2 complex → did not endocytosis.
- Some patients, AP2 mutant

Other genetic defects that result in elevated blood levels of LDL:
  - absence of LDL receptor.
  - defective LDL-binding site in the LDL receptor.
The endocytic pathway delivers iron to cells **without** dissociation of receptor-transferrin complex in endosomes.

Apotransferrin (iron free system) → bind two Fe3+ → ferrtransferrin → → → → →

pH <6, unknown mechanism, two Fe3+ ferrtransferrin → Fe2+

Free Fe++ level high – promotes radical formation

Acidic pH of late endosomes causes most receptor-ligand complexes to dissociate.

Directing membrane proteins and cytosolic materials to the lysosome

Delivery of plasma-membrane proteins to the lysosomal interior for degradation

Lytic virus

Has envelope ssRNA

Vacuolar H+ pump and many internal vesicles

Viral RNA polymerase replication RNA

Induced viral fusion glycoprotein conformational change

Fusion of viral envelope with endosomal lipid bilayer membrane and release of the nucleocapsid into cytosol
Retrovirus bud from the plasma membrane by a process similar to formation of multivesicular endosomes

Mechanism for budding of HIV form the plasma membrane

The autophagic pathway delivers cytosolic proteins or entire organelles to lysosomes
What are the functions of and pathways to the lysosome?

Vesicular transport from the cell membrane
-- endocytosis, phagocytosis vs pinocytosis