Chapter 1: Vesicular traffic
Major Protein-sorting pathways in eukaryotic cells
Secretory and endocytic pathways
Vesicular transport

Unifying principle governs all protein trafficking in secretory and endocytic pathways: transport of membrane and soluble proteins from one membrane-bounded compartment to another is mediated by transport vesicles.

Vesicles collect “cargo” proteins in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment.

The same face of the membrane remains oriented toward the cytosol.

Each step in the secretory and endocytic pathways employs a different type of vesicle, but each of the different vesicular transport steps is simply a variation on a common theme.
Major routes for protein trafficking in the secretory pathway
Anterograde and retrograde transport vesicles

1. Protein synthesis on bound ribosomes; co-translational transport of proteins into or across ER membrane
2. Budding and fusion of ER-to-Golgi vesicles to form cis-Golgi
3. Retrograde Golgi-to-ER transport

cis-Golgi network
ER lumen
Rough ER

Figure 14-1 part 1
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Exocytosis and endocytosis
Studies to establish the order in which proteins move from organelle to organelle in the secretory pathway.

Many components required for the formation and fusion of transport vesicles have been identified in the past decade by a remarkable convergence of genetic and biochemical approaches.

pulse-chase labeling on pancreatic acinar cells
Fluorescence microscopy of VSVG-GFP fusion protein

Gene encoding a temperature-sensitive mutant of the membrane glycoprotein G from vesicular stomatitis virus (VSV), fused to GFP protein has been introduced into cultured mammalian cells by transfection (VSVG-GFP).
Transport from the ER to the Golgi can be assayed based on sensitivity to cleavage by endoglycosidase D.

**Figure 14-3a**
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**Cis-Golgi maturation of VSV-G protein from vesicular stomatitis virus (VSV)**

Tracking movement of VSV-G protein in virus-infected cells pulse-labeled with radioactive amino acids.
Phenotypes of yeast sec mutants identified stages in the secretory pathway

Many of the components required for intracellular protein trafficking have been identified in yeast by analysis of temperature-sensitive sec mutants defective for the secretion of proteins at the nonpermissive temperature. These studies (double mutants) confirmed that as a secreted protein is synthesized and processed it moves sequentially from the cytosol → rough ER → ER-to-Golgi transport vesicles → Golgi cisternae → secretory vesicles and finally is exocytosed.
Basic mechanisms underlying vesicle budding and fusion. Each step in the secretory and endocytic pathways employs a different type of vesicle, studies employing genetic and biochemical techniques have revealed that each of the different vesicular transport steps is simply a variation on a common theme.
In vitro budding reactions of a coated vesicle

Isolated or artificial membranes and purified coat proteins. Polymerization of the coat proteins onto the cytosolic face of the parent membrane is necessary to produce the high curvature of the membrane.
### Types of coated vesicles

**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 <em>cis</em>-Golgi</td>
</tr>
<tr>
<td>COPI</td>
<td>Coatomers containing seven different COP subunits</td>
<td>ARF</td>
<td><em>cis</em>-Golgi to ER Later to earlier Golgi cisternae</td>
</tr>
<tr>
<td>Clathrin and adapter proteins*</td>
<td>Clathrin + AP1 complexes</td>
<td>ARF</td>
<td><em>trans</em>-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>Clathrin + GGA</td>
<td>ARF</td>
<td><em>trans</em>-Golgi to endosome</td>
</tr>
<tr>
<td></td>
<td>Clathrin + AP2 complexes</td>
<td>ARF</td>
<td>Plasma membrane to endosome</td>
</tr>
<tr>
<td></td>
<td>AP3 complexes</td>
<td>ARF</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.*
Major types of coat proteins in vesicular traffic in the secretory and endocytic pathways.

Some vesicles form with the help of coat proteins. Geometrically arranged coat proteins on the surface of the membrane help the vesicle to bud off.

http://learn.genetics.utah.edu/content/cells/vesicles/
The budding of vesicles from their parent membrane

Coated vesicle budding

The budding of vesicles is driven by the polymerization of soluble protein complexes onto the membrane to form a **proteinaceous vesicle coat**. The coat functions:
1) adds curvature to the membrane to form a vesicle
2) also **acts as the filter** to determine which proteins are admitted into the vesicle.

The integral membrane proteins in a budding vesicle include **v-SNAREs**, which are crucial to eventual fusion of the vesicle with the correct target membrane.
A Set of GTPase Switch Proteins Controls Assembly of Vesicle Coats

The coats of all three vesicles contain a **small GTP-binding protein**: acts as a **regulatory subunit** to control coat assembly. **Sar1** is present in the coat of COPII vesicles. **ARF** is the GTPase used by COPI and clathrin vesicles.

Both **ARF** and **Sar1** are **monomeric GTPase** of **switch proteins** that exchange GDP/GTP.

Cycling of GTPase switch proteins between the active and inactive forms. Activation is promoted by GEFs (guanine nucleotide–exchange factors).

Binding of GTP to **Sar1** (ARF) promoted by **Sec12** causes a conformational change in **Sar1** that exposes its hydrophobic N-terminus,
Sar1 couples a cycle of GTP binding and hydrolysis to the formation and then dissociation of the COPII coat.

2. The membrane-attached Sar1 GTP drives polymerization of cytosolic complexes of COPII subunits on the membrane, eventually leading to formation of vesicle buds.

3. Once COPII vesicles are released from the donor membrane, the Sar1 GTPase activity hydrolyzes Sar1 GTP in the vesicle membrane to Sar1 GDP.
Disassembly of COPII coat

With mutant versions of Sar1 that cannot hydrolyze GTP, vesicle coats form and vesicle buds pinch off. However, all available coat subunits eventually become permanently assembled into coated vesicles that are unable to fuse with target membranes.

**ARF** protein undergoes a similar cycle of nucleotide exchange and hydrolysis coupled to the assembly of vesicle coats composed either of COPI or of clathrin and other coat proteins.
Different mechanisms of recruitment of cargo to transport vesicles

Vesicle buds must be able to discriminate among potential membrane and soluble cargo proteins.

**Membrane cargo proteins:** the mechanism by which the vesicle coat selects cargo molecules is by directly binding to specific sequences, or **sorting signals**, in the cytosolic portion of membrane cargo proteins.

**Soluble proteins** within the lumen of parent organelles can in turn be selected by binding to the luminal domains of certain membrane cargo proteins, which act as receptor.

Nica Borgese J Cell Sci 2016;129:1537-1545
Targeting Sequences on Cargo Proteins Make Specific Molecular Contacts with Coat Proteins

<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>COPII Sec24 subunit</td>
<td>COPII</td>
</tr>
<tr>
<td>Mannose 6-phosphate (M6P)</td>
<td>Soluble lysosomal enzymes after processing in cis-Golgi Secreted lysosomal enzymes</td>
<td>M6P receptor in trans-Golgi membrane</td>
<td>Clathrin/AP1</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) Plasma membrane proteins (cytosolic domain)</td>
<td>AP1 (μ1 subunit)</td>
<td>Clathrin/AP1</td>
</tr>
<tr>
<td>Leu-Leu (LL)</td>
<td>Plasma membrane proteins (cytosolic domain)</td>
<td>AP2 (μ2 subunit)</td>
<td>Clathrin/AP2</td>
</tr>
</tbody>
</table>

* = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.
Rab proteins are required for the targeting of vesicles to the target membrane

Targeting of vesicles to the appropriate target membrane is mediated by Rab proteins, GTPase superfamily of switch proteins.

Conversion of cytosolic Rab GDP to Rab GTP, enables it to interact with a particular transport vesicle and insert its isoprenoid anchor into the vesicle membrane.

Once Rab GTP is tethered to the vesicle surface, it interacts with one of a number of different large proteins, known as Rab effectors, attached to the target membrane.

After vesicle fusion occurs, the GTP bound to the Rab protein is hydrolyzed to GDP, triggering the release of Rab -GDP, which then can undergo another cycle of GDP-GTP exchange, binding, and hydrolysis.
A different type of Rab and Rab effector appears to function for each vesicle type

Each type of vesicles and organelles has at least one Rab protein on its cytosolic surface. Sec4 mutant (yeast cells) accumulate secretory vesicles that are unable to fuse with the plasma membrane (class E mutants).

Example: Rab5 protein is localized to endocytic vesicles (EE). A long coiled protein known as EEA1 (early endosome antigen 1), which resides on the membrane of the early endosome, functions as the Rab effector for Rab5.

Rab1 is essential for ER-to-Golgi transport reactions, Rab7 associate with late endosome.

Paired Sets of SNARE Proteins Mediate Fusion of Vesicles with Target Membranes

After Rab-mediated docking of a vesicle on its target membrane, the interaction of cognate SNAREs brings the two membranes close enough together that they can fuse. They provide a layer of specificity.

The best-understood examples of SNARE-mediated fusion occurs during **exocytosis** of secreted proteins.

The cognate **SNAREs**:
- **V-SNARE**: = VAMP (vesicle-associated membrane protein)
- **T-SNARE**: Syntaxin and SNAP-25.

The cytosolic region in each of these three SNARE proteins form a **four-helix bundle** that anchor vesicles to the target membrane.
In **liposomes**, formation of SNARE complexes is sufficient to bring about membrane fusion

The cytosolic region in each of these three SNARE proteins contains a repeating **heptad sequence** that allows four helices—one from VAMP, one from syntaxin, and two from SNAP-25 to coil around one another to form a **four-helix bundle** with unusual stability.
Dissociation of SNARE complexes is driven by ATP Hydrolysis

Because of the stability of SNARE complexes, their dissociation depends on additional proteins and the input of energy.

**NSF examer** and **α-SNAP**, are required for regeneration of free SNARE proteins and not for ongoing vesicle fusion.

Sec yeast mutants homologues to NSF and alpha-SNAP belong to the mutants of class C. NSF and α-SNAP proteins are not necessary for actual membrane fusion, but rather are required for regeneration of free SNARE proteins.
NSF is required to recycling of SNARE proteins
Early Stages of the Secretory Pathway

Take a closer look at vesicular traffic through the ER and Golgi stages of the secretory pathway

Vesicular traffic through the ER and Golgi stages of the secretory pathway is mediated by COPII (anterograde transport) and by COPI vesicles (retrograde transport)
**COPII Vesicles Mediate Transport from the ER to the Golgi**

**COPII** vesicles were first recognized when cell-free extracts of yeast rough ER membranes were incubated with cytosol, ATP, and a nonhydrolyzable analog of GTP.

The cytosolic segments of Integral ER membrane proteins are specifically recruited into **COPII** contain a **di-acidic** sorting signal (**Asp-X-Glu**) which binds to the **Sec24** subunit of the COPII coat and is essential for the selective export of certain membrane proteins from the ER.

Few receptors for soluble cargo proteins are known.
The COPI coat assembles upon activation of Arf1

Arf1 in turn recruits the inner coat complex (Sec21/Sec26/Ret2/Ret3) (similar to AP-2 adaptor complex).

The COPI outer coat is formed by 3 proteins which assembles in a triskelion structure via interactions of three domains of Sec27

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COPI Vesicles Mediate Retrograde Transport within the Golgi and from the Golgi to the ER

Yeast cells containing temperature sensitive mutations in COPI proteins have been categorized as class B sec mutants

Functions of retrograde transport from the cis-Golgi to the ER:

☑ Recycling of vesicle membranes
☑ Recycling of v-SNARE

• COPI mutants cannot recycle key membrane proteins back to the rough ER, the ER gradually becomes depleted of ER proteins such as v-SNAREs and eventually vesicle formation from the rough ER is halted.

☑ Retrieval of missorted ER-resident proteins (sorting mistakes).
  • ER contains several soluble resident proteins (chaperone BIP and protein disulfide isomerase) loaded passively into vesicles destined for the cis-Golgi.
Retrograde transport from the cis-Golgi rescues missorted ER-resident proteins (sorting mistakes).

Most soluble ER-resident proteins carry a Lys-Asp-Glu-Leu (KDEL) sorting signal at their C-terminus. KDEL is recognized and bound by the KDEL receptor, found on transport vesicles shuttling between the ER and the cis-Golgi and on the cis-Golgi reticulum.

The KDEL receptor and other membrane proteins that are transported back to the ER from the Golgi contain a Lys-Lys-X-X sequence at the very end of their C-terminal segment, which faces the cytosol. This is necessary and sufficient to incorporate proteins into COPI vesicles for retrograde transport.

Mutant protein disulfide isomerase lacking these four residues is synthesized in fibroblasts is secreted
Anterograde Transport Through the Golgi could occur by Cisternal Progression

Some protein aggregates (e.g. collagen) are too large to be incorporated into small transport vesicles, and aggregates have never found in transport vesicles. It has been suggested that the forward movement of these and perhaps all secretory proteins from one Golgi compartment to another does not occur via small vesicles.
Later Stages of the Secretory Pathway

Properly processed cargo proteins reach the *trans-Golgi network*, the most-distal Golgi compartment where they are sorted into vesicles for delivery to their final destination.
Vesicles Coated with Clathrin and/or Adapter Proteins Mediate Several Transport Steps

The best-characterized vesicles that bud from the trans-Golgi network (TGN) have a two-layered coat:
- an outer layer composed of the fibrous protein clathrin and
- an inner layer composed of adapter protein (AP) complexes.

Clathrin: three branched shape, called triskelion. Each branch: 1 heavy chain of 180 kDa forming legs from α-helical zigzags and 1 light chain of 35-40 kDa;
Triskelions polymerize to form a polyhedral cage with intrinsic curvature. The clathrin triskelions determine the geometry of the clathrin vesicles.
A clathrin-coated pit on the cytosolic face of the plasma membrane

Triskelions assemble in vitro to form empty lattice cages with open hexagonal and pentagonal faces. They are very similar to those observed in vivo. Assembly does not require ATP and direct binding to membranes.
The adapter complexes (AP)

Clathrin polymerization occurs on a donor membrane in association with **AP complexes** (340,000 MW), which assemble between the clathrin lattice and the membrane. **AP complexes** are heterotetramers containing one copy each of 4 different adapter subunits.

In the presence of **Arf1** binding, AP-1 undergoes a large conformational change to the open state exposing the binding sites for cargo proteins.

clathrin/API: Proteins containing a **Tyr-XX-Φ**, (where Φ is a bulky hydrophobic amino acid), are recruited into clathrin/API vesicles.
Adapter proteins determine which cargo proteins are specifically included in clathrin vesicle

**Vesicles containing different adapter complexes have been found to mediate specific transport steps:**
- **AP1 complex:** cargo selection from the TGN and endosomes.
- **AP2 complex:** cargo selection from plasma membrane
- **AP3 complex:** cargo selection to lysosomes
Dynamin is required for pinching off of clathrin vesicles.

Dynamin is a cytosolic protein that polymerizes around the neck portion and then hydrolyzes GTP. The energy derived from GTP hydrolysis is thought to drive “contraction” of dynamin around the vesicle neck until the vesicle pinches off.

As with COPI and COPII vesicles, clathrin/AP vesicles normally lose their coat soon after their formation. Incubation of cell extracts with a nonhydrolyzable derivative of GTP provides leads to accumulation of clathrin coated vesicle buds with excessively long necks surrounded by polymeric dynamin but do not pinch off.

COPI and COPII vesicles appear to pinch off from donor membranes without the aid of a GTPase.
Mannose 6-phosphate (M6P) residues targets soluble enzymes to lysosomes

The sorting signal that directs soluble lysosomal enzymes from the trans-Golgi network to the late endosome is a carbohydrate residue, **mannose 6-phosphate (M6P)**, which is formed in the cis-Golgi. The N-linked Man8(GlcNAc)2 oligosaccharide present on most lysosomal enzymes undergo a two-step reaction sequence that generates M6P residues.

1) A GlcNAc phosphotransferase transfers a phosphorylated GlcNAc group to C6 of one or more mannose residues.

2) A phosphodiesterase removes the GlcNAc group, leaving a phosphorylated mannose residue on the lysosomal enzyme.

The addition of M6P prevents lysosomal enzymes from undergoing the further processing reactions as other secreted and membrane proteins: clathrin/AP1 vesicles contain the M6P receptor which bounds lysosomal enzymes then bud from the **trans-Golgi network**.
Mannose 6-Phosphate Residues Target Soluble Proteins to Lysosomes

M6P receptor and bound lysosomal enzymes then bud from the trans-Golgi network, lose their coats, and subsequently fuse with the late endosome.

Because M6P receptors can bind M6P at the slightly acidic pH (≈6.5) of the trans-Golgi network but not at a pH less than 6, the bound lysosomal enzymes are released within late endosomes (pH of 5.0–5.5).

A phosphatase within late endosomes usually removes the phosphate from M6P residues on lysosomal enzymes.

Vesicles budding from late endosomes recycle the M6P receptor back to the trans-Golgi network.
Study of Lysosomal Storage Diseases Revealed Key Components of the Lysosomal Sorting Pathway

Lacking the M6P sorting signal, the lysosomal enzymes are secreted rather than being sorted to and sequestered in lysosomes.

Lysosomal storage diseases, are caused by the absence of one or more lysosomal enzymes. As a result, undigested glycolipids and extracellular components that would normally be degraded by lysosomal enzymes accumulate in lysosomes as large inclusions.

I-cell disease: a severe lysosomal storage disease, in which cells from affected individuals lack the N-acetylglucosamine phosphotransferase.


I-cell disease: lymphocytic vacuoles containing round osmiophilic structures
Lysosomal storage diseases are characterized by abnormal lysosomes.

Tay-Sachs GM2 gangliosidosis is an inherited metabolic disorder that results from defects in lysosomal function (Lysosomal storage diseases) due to a Hexosaminidase A deficiency in lysosomes.

Tay-Sachs GM2 gangliosidosis results in cell accumulation of harmful amounts of lipids (gangliosides) in the brain.