NAME OF TEACHER	**Dr. ROLEE SHARMA**
MOBILE NUMBER	**9336576545**
EMAIL ID	**roleesharma@csjmu.ac.in, roleesh@gmail.com**
DESIGNATION	**Professor**
UNIVERSITY NAME	**CSJM University, Kanpur**
COLLEGE NAME	**CSJM University, Kanpur**
STREAM NAME	**BIOLOGY**
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SUBTOPIC NAME	**Protein targeting-2 Vesicular Traffic, Secretion And Endocytosis**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**Vesicular Traffic, Secretion, Endocytosis**

Protein Targeting: 2. Sorting to organelles.

Objectives:

To acquaint the students about:

i) the transport though the Golgi compartment.

ii) the role of vesicles in protein trafficking from ER to Golgi apparatus and to lysosomes/ plasma membrane.

iii) the role of COPI, COPII and clathrin–coated transport vesicles in protein trafficking.

iv) the molecular mechanism of vesicle trafficking.

VESICULAR TRAFFIC, SECRETION AND ENDOCYTOSIS

- Mechanisms that allow soluble and membrane proteins synthesized on RER to move to final destinations via the **secretory pathway.**

- **Transport vesicles (TV) 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.

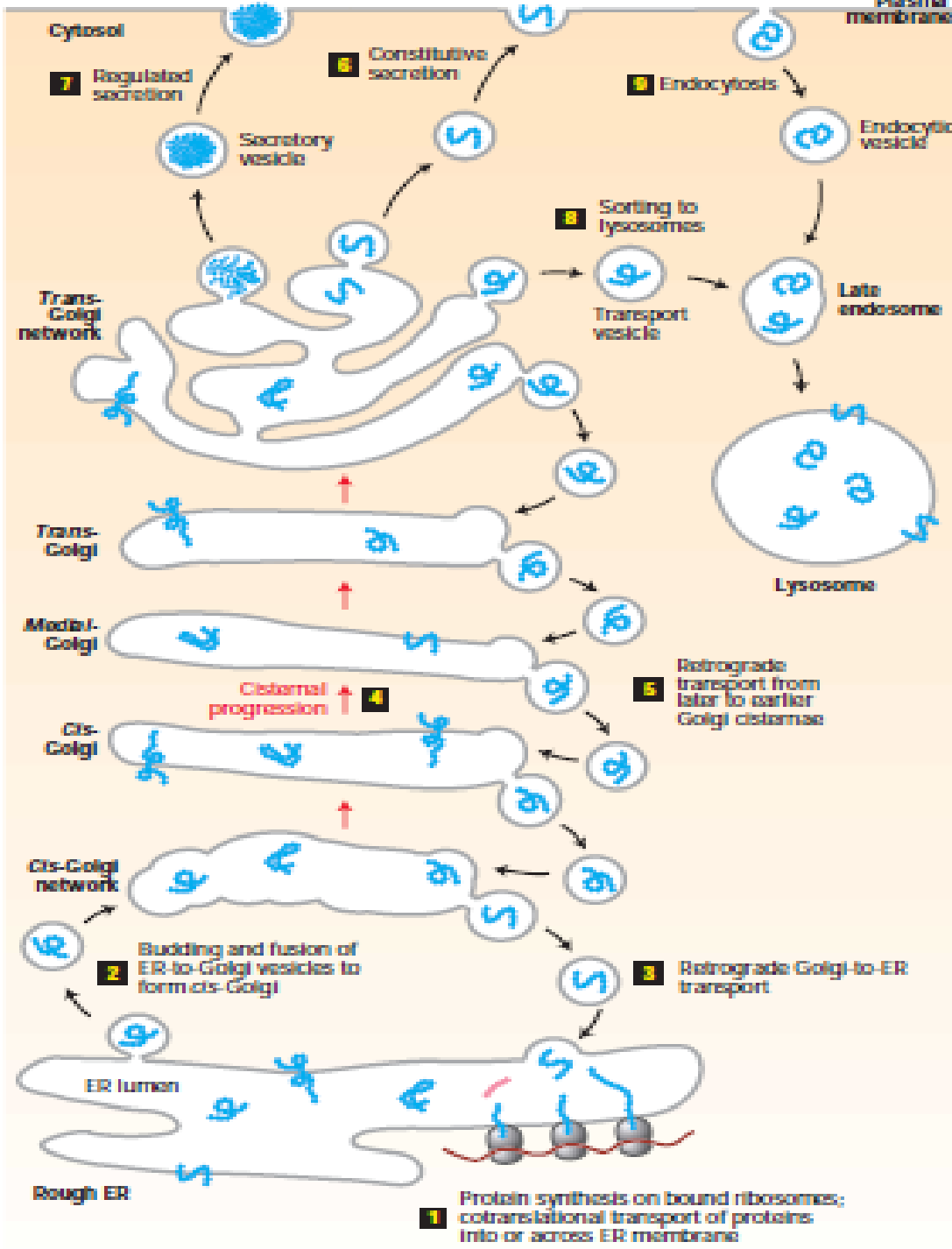
- As TVs bud from one membrane and fuse with the next, the same face of the membrane remains oriented toward the cytosol.

- Once newly synthesized proteins are incorporated into the ER lumen or membrane, they can be packaged into *anterograde* (forward-moving) transport vesicles which fuse to form a flattened membrane-bounded compartment -*cis-Golgi* ***cisterna.***

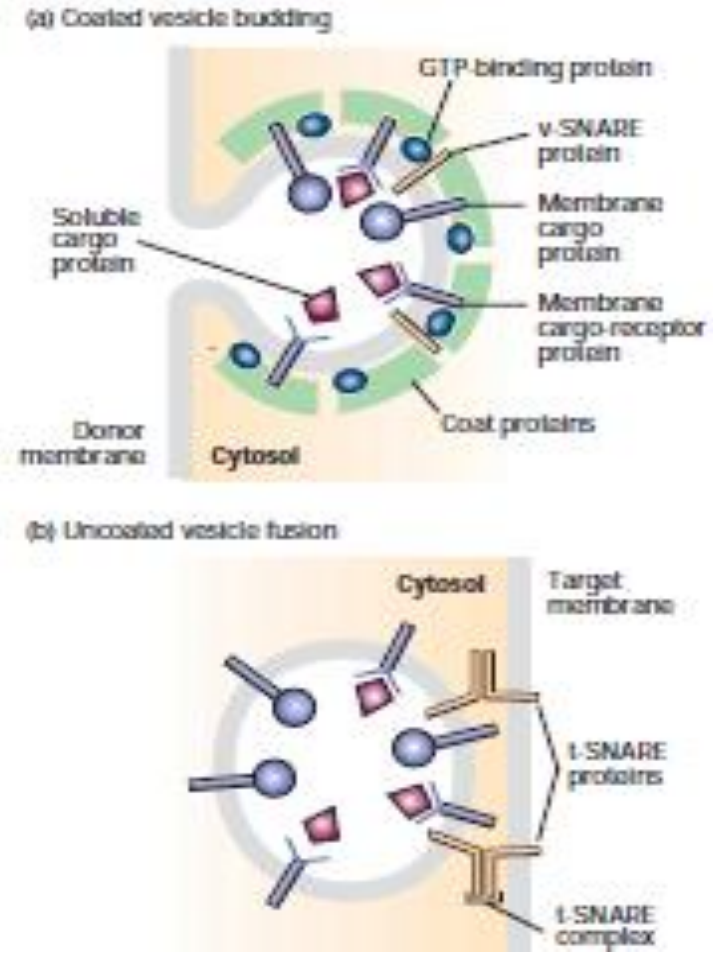
- Certain proteins**,** mainly ER-localized proteins, are retrieved from the *cis-Golgi* to the ER via a different set of *retrograde (backward-moving)* transport vesicles.

Secretory and endocytic pathways of protein sorting

Secretory pathway**:* Newly made polypeptide chains having an ER signal sequence are inserted into ER membrane or enter lumen (**1**). Some proteins remain in ER, some are packaged into transport vesicles (**2**), budding from ER and fuse together to form new *cis-Golgi* cisternae. Missorted ER-resident proteins and vesicle membrane proteins are retrieved to ER by vesicles (**3**) that bud from *cis-Golgi and* fuse with ER. Each *cis-Golgi* cisterna, physically moves from *cis to trans face of Golgi* complex (**4**) by a nonvesicular process called ***cisternal progression. Retrograde transport vesicles (**5**) move Golgi-resident proteins to proper Golgi compartment. In all cells, certain soluble proteins move to cell surface in transport vesicles (**6**) and are secreted continuously (constitutive secretion). In certain cells, some soluble proteins are stored in secretory vesicles (**7)** and are released only after an appropriate signal (regulated secretion). Lysosome-destined membrane and soluble proteins are transported in vesicles budding from *trans-Golgi* (**8**), first move to late endosome and then to lysosome. ***Endocytic pathway**: Membrane and* soluble extracellular proteins taken up in vesicles that bud from PM (**9**) also move to lysosome via endosome.



Molecular mechanisms of vesicular traffic



Assembly of a Protein Coat Drives Vesicle Formation and Selection of Cargo Molecules

Polymerization of the coat proteins on the cytosolic face of the parent membrane is necessary to produce the high curvature of the TV

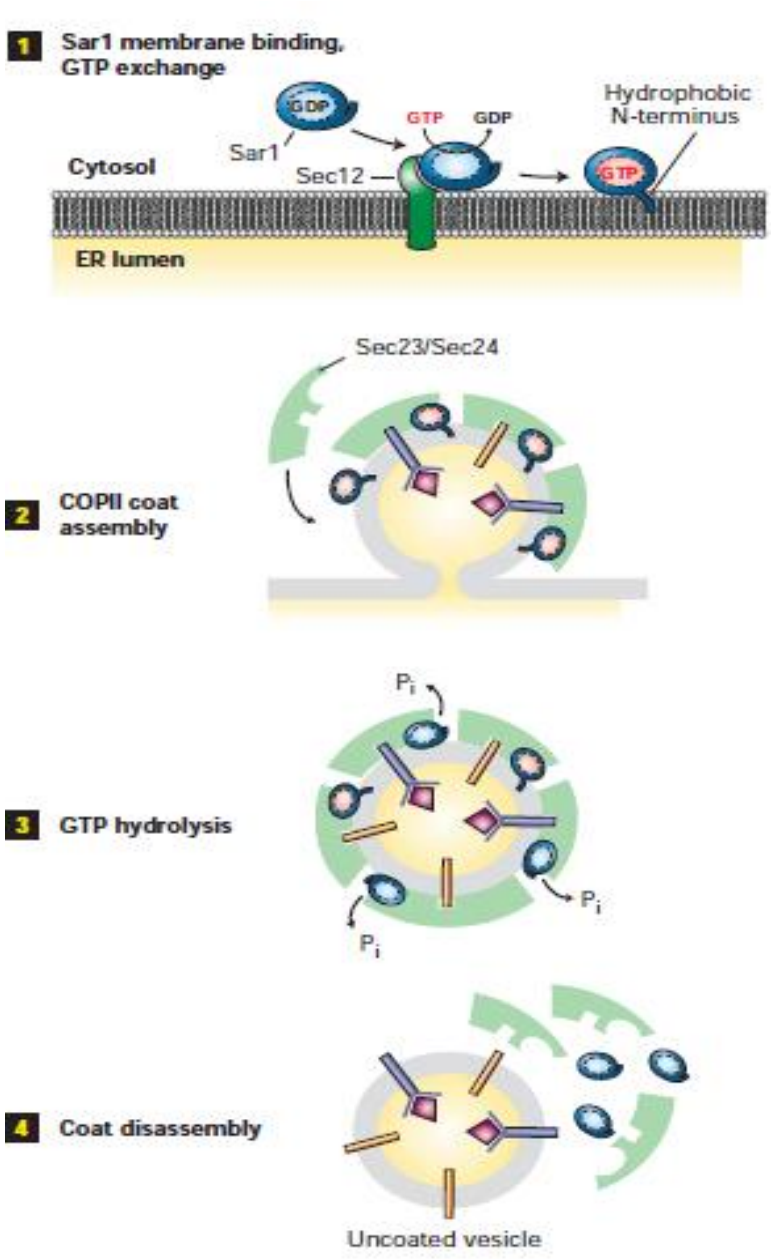
Overview of vesicle budding and fusion with a target membrane. (a) Budding is initiated by recruitment of a small GTP-binding protein to a patch of donor membrane. Complexes of coat proteins in the cytosol then bind to the cytosolic domain of membrane cargo proteins, some of which also act as receptors that bind soluble proteins in the lumen, thereby recruiting luminal cargo proteins into the budding vesicle. (b) After being released and shedding its coat, a vesicle fuses with its target membrane in a process that involves interaction of cognate SNARE proteins.

Coated vesicles involved in protein trafficking

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

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

A Conserved Set of GTPase Switch Proteins Controls Assembly of Different Vesicle Coats



•The coats of all three vesicles contain a small GTP-binding protein that acts as a regulatory subunit to control coat assembly.
-COP-II vesicles - coated with Sar1, and
-COP-I vesicles - coated with ARF
•*Cycle of GTP binding and hydrolysis regulated by ARF and Sar-1 –that controls initiation of coat assembly*.

•Sec-12 is an ER membrane protein.

•Membrane attached Sar1-GTP drives polymerization of cytosolic complexes of COP-II subunit on membrane, leading to formation of vesicle buds

•Sar1 GTP hydrolysis triggers disassembly of the COPII coat.

•*Sar1 couples a cycle of GTP binding and hydrolysis to the formation and then dissociation of the COPII coat.*

Targeting Sequences on Cargo Proteins Make Specific Molecular Contacts with Coat Proteins

- For transport vesicle to move specific proteins from one compartment to the next, vesicle buds must be able to discriminate among potential membrane and soluble cargo proteins, accepting only those cargo proteins that should advance to the next compartment and excluding those that should remain in donor compartment.

- In addition to sculpting the curvature of a donor membrane, the vesicle coat also functions in selecting specific proteins as cargo.

- The *vesicle coat selects cargo molecules by directly binding to specific sequences*, or **sorting signals**, *in the cytosolic portion of membrane cargo proteins*.

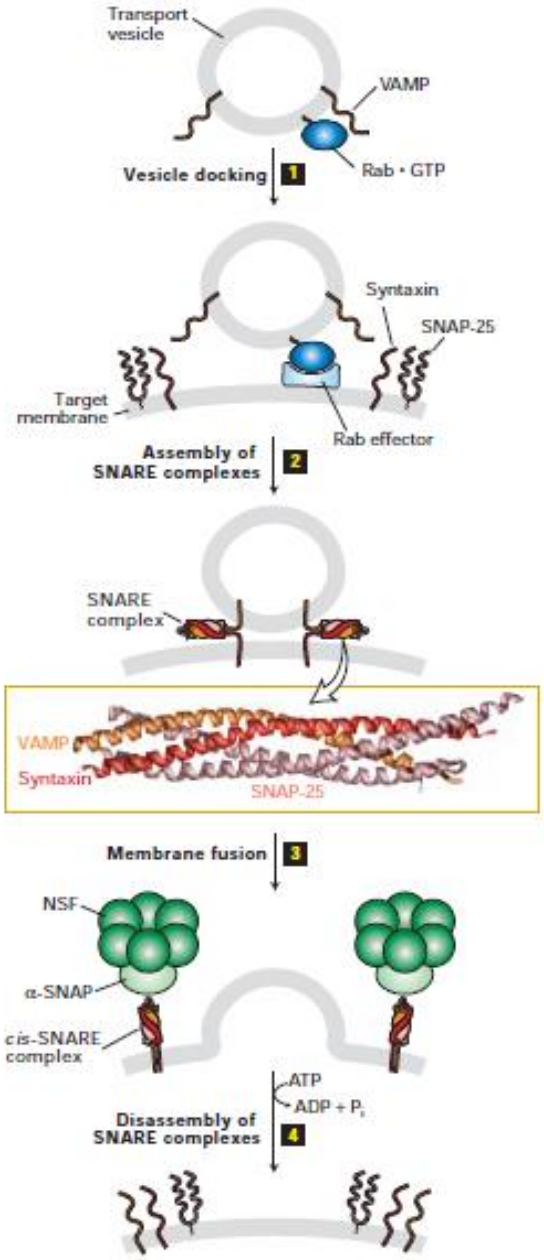
Known Sorting Signals That Direct Proteins to Specific Transport Vesicles (TV)

Signal Sequence*	Proteins with Signal	Signal Receptor	Vesicles That Incorporate Signal-bearing Protein
Lys-Asp-Glu-Leu (KDEL)	ER-resident luminal proteins	KDEL receptor in *cis*-Golgi membrane	COPI
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins (cytosolic domain)	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER (cytosolic domain)	COPII Sec24 subunit	COPII
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2
Asn-Pro-X-Tyr (NPXY)	LDL receptor in the plasma membrane (cytosolic domain)	AP2 complex	Clathrin/AP2
Tyr-X-X-Φ (YXXΦ)	Membrane proteins in *trans*-Golgi (cytosolic domain)	AP1 (μ1 subunit)	Clathrin/AP1
	Plasma membrane proteins (cytosolic domain)	AP2 (μ2 subunit)	Clathrin/AP2
Leu-Leu (LL)	Plasma membrane proteins (cytosolic domain)	AP2 complexes	Clathrin/AP2

*X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.

Polymerized coat acts as an affinity matrix to cluster selected membrane cargo proteins into forming vesicle buds.

Rab GTPases Control Docking of Vesicles on Target Membranes



IInd set of small GTP-binding proteins, ***Rab proteins****, target vesicles to the appropriate target membrane.*

- Rab GTP- conformational change in Rab - interact with a surface protein on a particular TV and insert its isoprenoid anchor into the vesicle membrane.
- After vesicle fusion, GTP is hydrolyzed, triggering release of Rab-GDP.
- **Rab5** –on early endosome, EEA protein on endosome membrane,
- **Rab1** – required for ER to golgi transport, and P115 –tethers COP-II vesicles to target membrane.

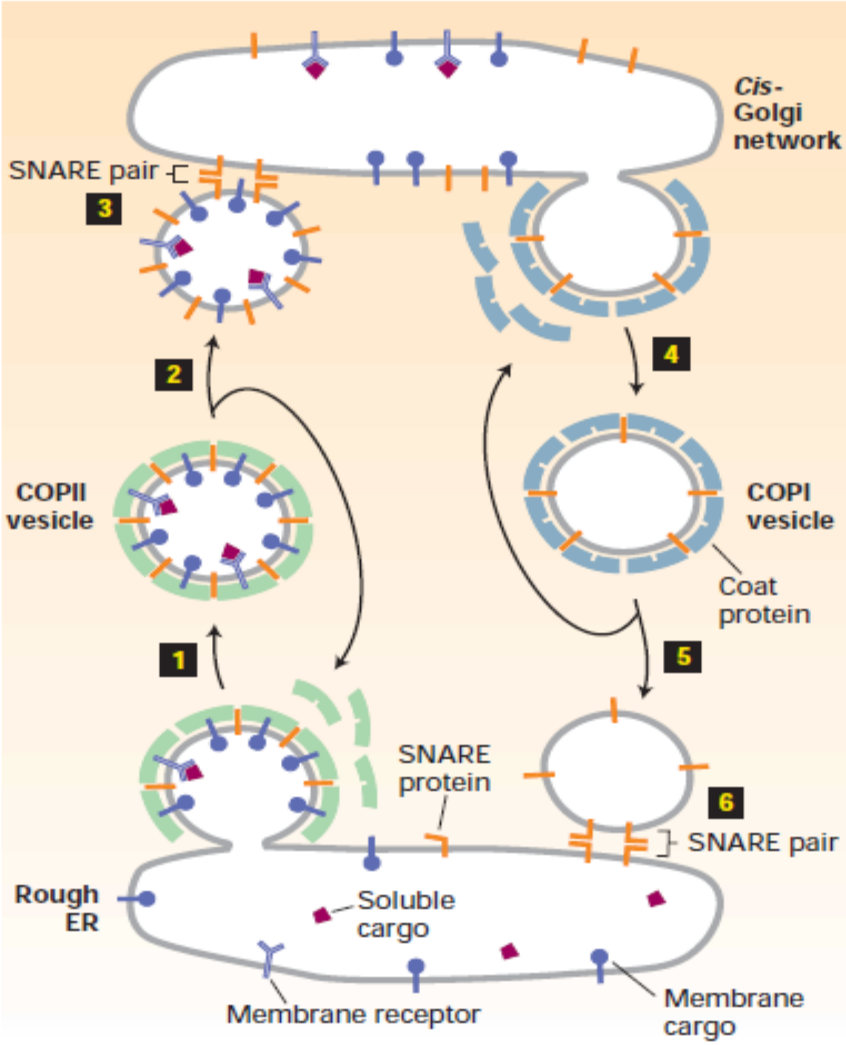
Paired Sets of SNARE Pro Mediate Fusion of Vesicles with Target Membranes

- During exocytosis of secreted proteins, TV bud from TGN-
- v-SNARE, called ***VAMP*** *(vesicle-associated membrane protein)*
- t-SNAREs = ***syntaxin****, are* Integral membrane protein, and
- ***SNAP-25*** *is* attached to PM by a hydrophobic lipid anchor in middle of protein. The cytosolic region in each of these 3 SNARE proteins contains a repeating heptad sequence - forms 4 helix bundle that closes during fusion.

Dissociation of SNARE Complexes After Membrane Fusion Is Driven by ATP Hydrolysis and releases free SNARE proteins.

-SNAP (*soluble NSF attachment protein), NSF- N-ethylmaleimide sensitive factor*

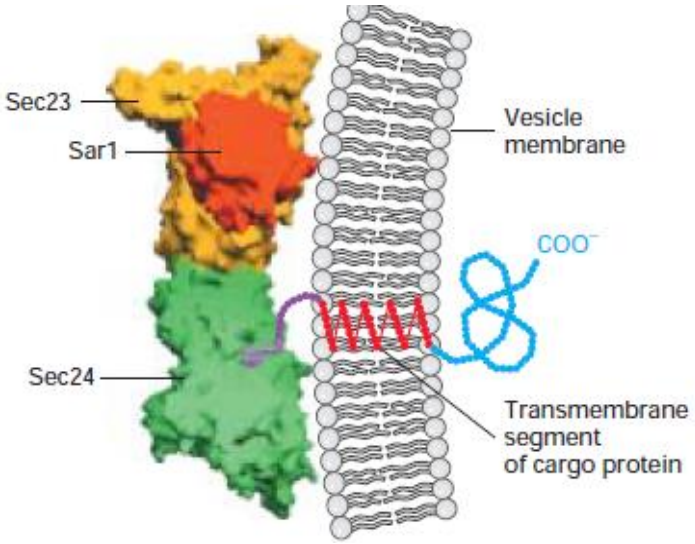
Early Stages of the Secretory Pathway



Vesicle-mediated protein trafficking between the ER and *cis-Golgi.*

<u>COP-I vesicles</u>:mediate transport from golgi to ER
- retrieve v-SNARE proteins and membrane back to ER, and also
- retrieves missorted ER-resident proteins from the *cis-Golgi to correct sorting* mistakes.

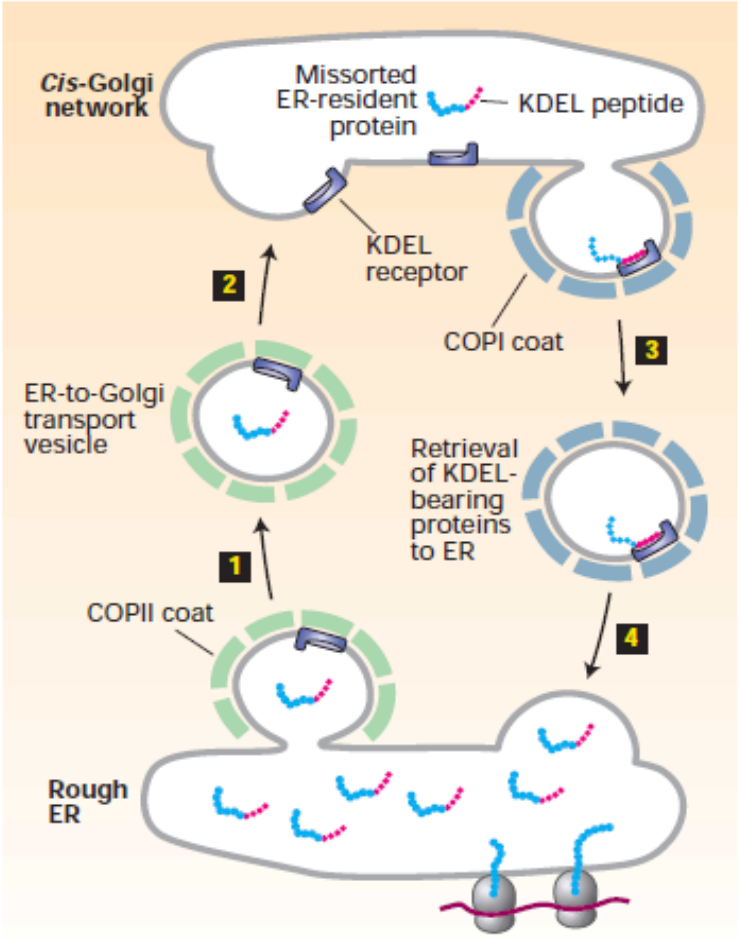
<u>COPII Vesicles</u> Mediate Transport from ER to Golgi



3-D structure of ternary complex comprising COPII coat proteins Sec23 and Sec24 and Sar1 GTP.

Cytosolic segments of these proteins contain a *sorting signal (Asp-X-Glu, or DXE*)- that binds to Sec24 subunit of COPII coat and is essential for selective export of membrane proteins from ER.

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



Role of the KDEL receptor in retrieval of ER-resident luminal proteins from the Golgi.

Coat is formed from large cytosolic complexes, called *coatomers, composed of* 7 polypeptide subunits.

•*Most soluble ER-resident proteins carry Lys-Asp-Glu-Leu (KDEL) sequence at their C-terminus.*

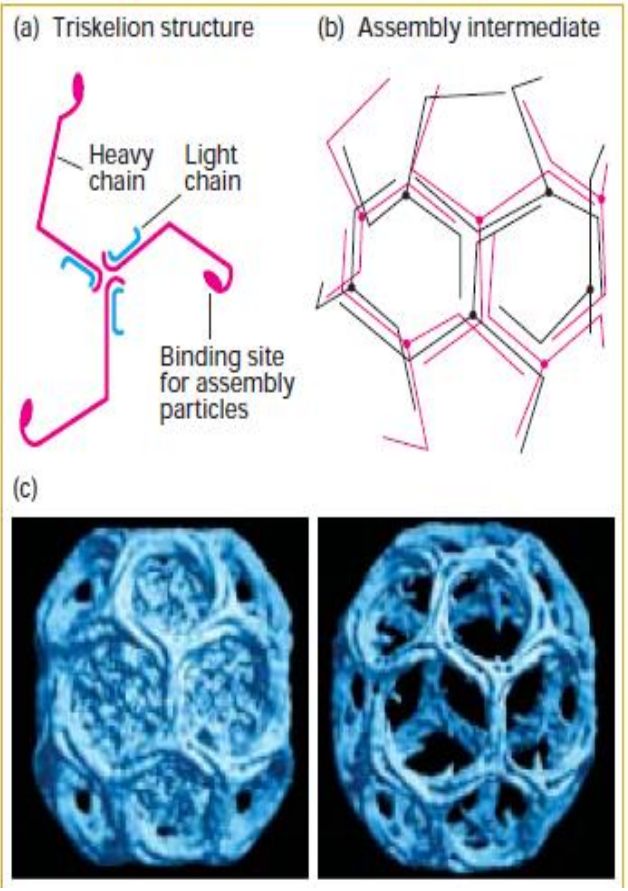
•*KDEL sorting signal is both necessary and sufficient* for retention in the ER.

•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 *KKXX sorting signal which binds to a complex of the COPI and* subunits, is both necessary and sufficient to incorporate membrane proteins into COPI vesicles for retrograde transport to the ER.

Anterograde Transport Through the Golgi Occurs by Cisternal Progression

Later Stages of the Secretory Pathway

Vesicles Coated with Clathrin and/or Adapter Protein Mediate Several Transport Steps

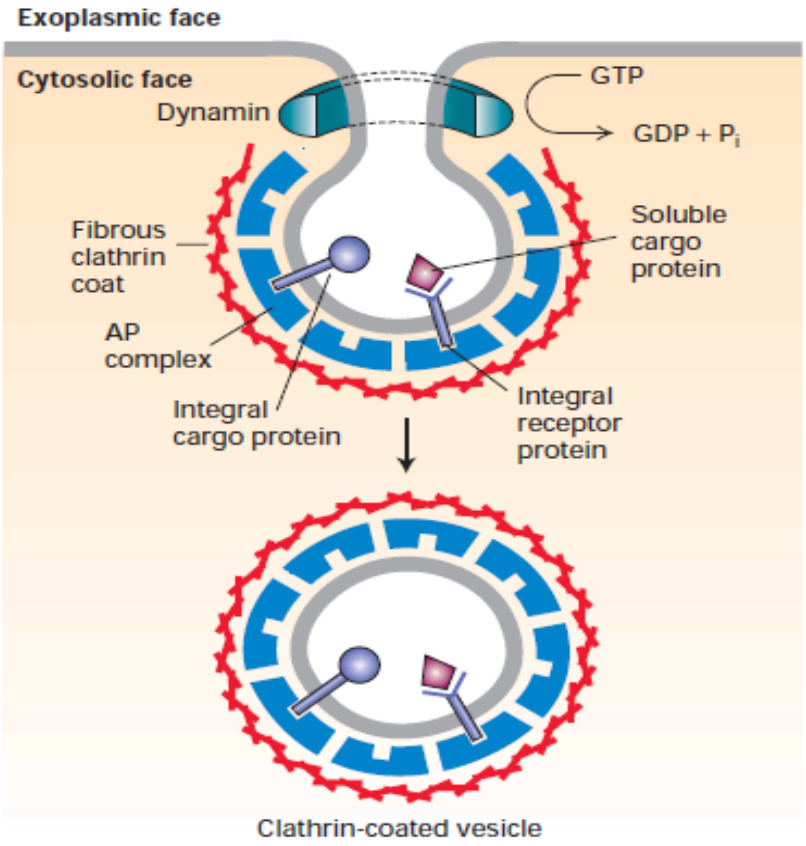


Structure of clathrin coats. A clathrin molecule, called a triskelion, is composed of 3 heavy and three light chains.

- Vesicles that bud from the 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.*
- Purified clathrin molecules 3-limbed shape, are called *Triskelions*. Each limb contains - 1 clathrin heavy chain (180,000 MW) + 1 light chain (40,000 MW).

- Triskelions polymerize to form a polygonal lattice with an intrinsic curvature.
- AP complexes associate with clathrin and assemble between the clathrin lattice and the membrane. Each AP complex (340,000 MW) contains 1 copy each of 4 different adapter subunit proteins.
- These vesicles select membrane proteins containing a Tyr-XX-ϕ sequence, where X is any amino acid and ϕ is a bulky hydrophobic amino acid, are recruited into clathrin/AP1 vesicles budding from *trans-Golgi network. This YXX sorting signal interacts with one of AP1 subunits in* vesicle coat.

Dynamin Is Required for Pinching Off of Clathrin Vesicles

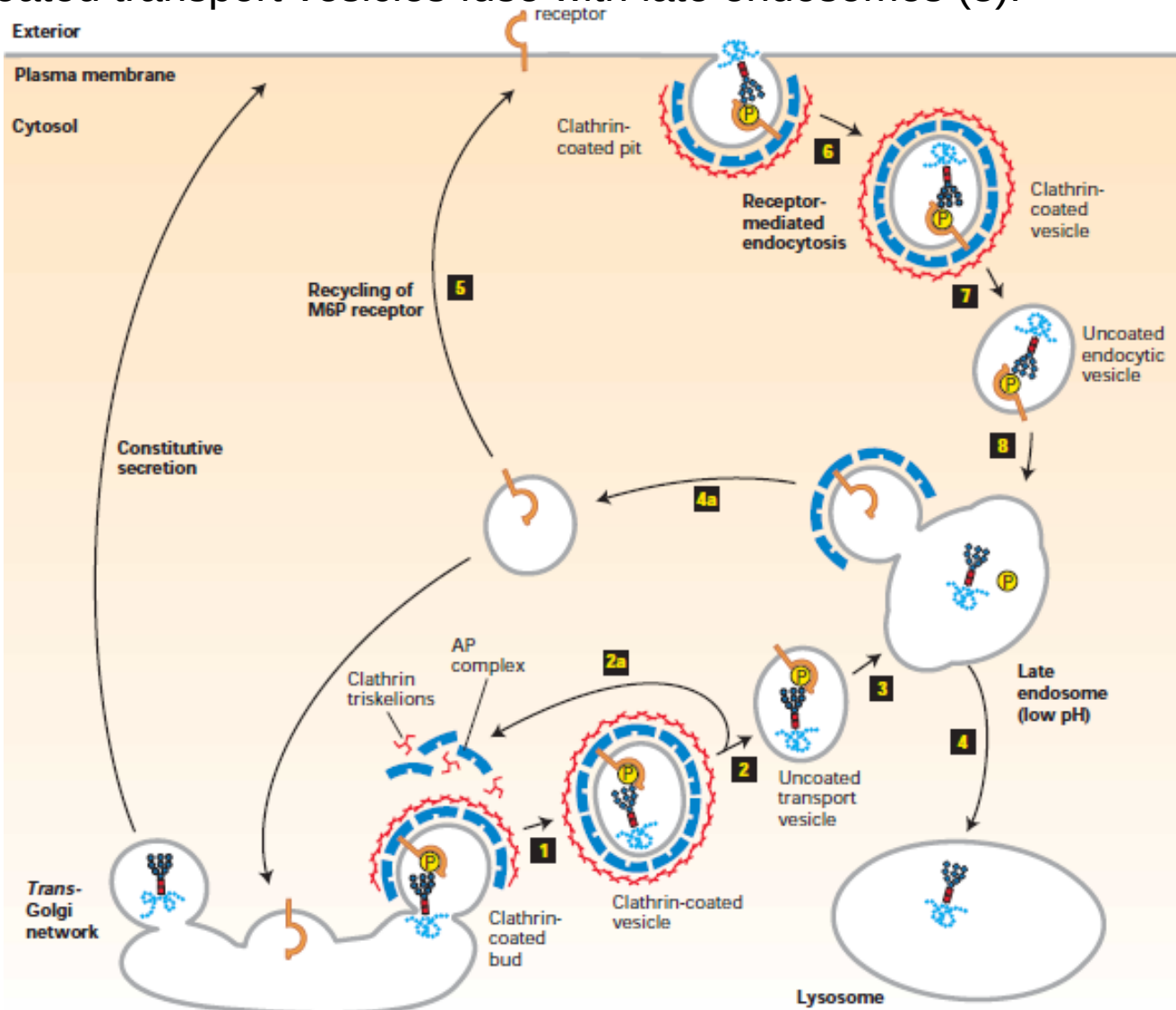


Model for dynamin-mediated pinching off of clathrin/AP-coated vesicles. After a vesicle bud forms, dynamin polymerizes over neck. Dynamin-catalyzed hydrolysis of GTP leads to release of vesicle from donor membrane. The membrane proteins in donor membrane are incorporated into vesicles by interacting with AP complexes in coat.

Mannose 6-Phosphate Residues Target Soluble Proteins to Lysosomes

Lysosomal enzymes synthesized in ER, acquire mannose 6-phosphate (M6P) residues in *cis-Golgi*. In *trans-Golgi network,* proteins with M6P signal interact with M6P receptors in membrane and are directed into clathrin/AP1 vesicles (1). Vesicle coat is depolymerized (2), and uncoated transport vesicles fuse with late endosomes (3).

Phosphorylated enzymes dissociate from M6P receptors and are dephosphorylated, late endosomes subsequently fuse with lysosome (4). Coat proteins and receptors are recycled (2a,4a), some receptors are delivered to cell surface (5). Phosphorylated lysosomal enzymes are sorted from *trans-Golgi to cell* surface and secreted. These secreted enzymes can be retrieved by receptor-mediated endocytosis (6-8), a process that closely parallels trafficking of lysosomal enzymes from the *trans*-Golgi network to lysosomes.



•Because Mannose-6-Phosphate receptors can bind Mannose-6-Phosphate (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, which have an internal pH of 5.0–5.5*.

•Furthermore, a *phosphatase within late endosomes usually removes the phosphate from M6P* residues on lysosomal enzymes, preventing any rebinding to the M6P receptor that might occur in spite of the low pH in endosomes.

•Vesicles budding from late endosomes recycle the M6P receptor back to the *trans*-Golgi network or, on occasion, to the cell surface.

•Eventually, mature late endosomes fuse with lysosomes, delivering the lysosomal enzymes to their final destination.

Protein Aggregation in the *Trans-Golgi May* Function in Sorting Proteins to Regulated Secretory Vesicles

All eukaryotic cells continuously secrete some proteins, a process called *constitutive secretion. Specialized secretory cells store* other proteins in vesicles and secrete only when triggered by stimulus, e.g. *pancreatic cells store* insulin in special secretory vesicles and secrete it in response to elevation in blood glucose. Thus, 2 types of vesicles formed by secretory cells to move proteins from *trans*-Golgi network to cell surface: regulated transport vesicles (secretory vesicles), and unregulated transport vesicles (constitutive secretory vesicles).

Sorting into the regulated pathway is controlled by selective protein aggregation.
Immature vesicles —those that have just budded from the *trans-Golgi network—contain diffuse* aggregates of secreted protein that are visible in the electron microscope.
Proteins destined for regulated secretory vesicles selectively aggregate together before their incorporation into the vesicles.

Regulated secretory vesicles from mammalian secretory cells contain three proteins, *chromogranin A, chromogranin B, and secretogranin II, that together* form aggregates when incubated at the ionic conditions (pH ≈6.5 and 1 mM Ca2) thought to occur in the *trans-Golgi* network; such aggregates do not form at the neutral pH of ER. The selective aggregation of regulated secreted proteins together with chromogranin A, chromogranin B, or secretogranin II could be the basis for sorting of these proteins into regulated secretory vesicles. Secreted proteins that do not associate with these proteins, and thus do not form aggregates, would be sorted into unregulated transport vesicles by default.

DISCLAIMER:
All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

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