13-3. Synthesis-Secretory pathway:

Sort lumenal proteins, Secrete proteins, Sort membrane proteins

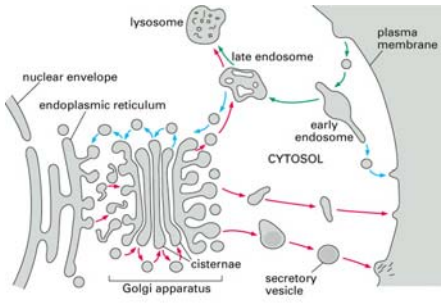


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Molecular sorting: specific budding, vesicular transport, fusion

1. Why is this important?

A. Forming and maintaining membrane identity:
Formation of ER, Golgi, PM,
Nuc envelope assembly

b. Endocytic pathway:
recycle membrane,
transduce signals,
nutrient uptake,
defense against invading microbes.

C. Exocytosis:
synaptic transmission in neuron,
polarized distribution of PM proteins

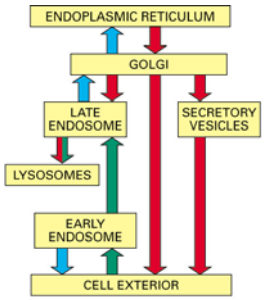
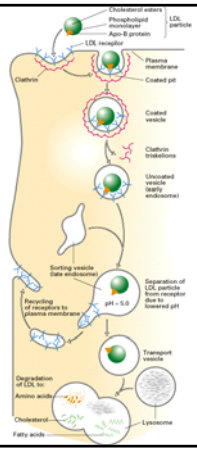


Figure 13–1. Molecular Biology of the Cell, 4th Edition



Cells can take up nutrients by endocytosis and sort the nutrients to the right place.

E.g. 17-46. Receptor-mediated endocytosis.

To bring cholesterol into cells.

I. Vesicular Transport:
1. Budding: specific
2. Transport: targeted vesicle transport
3. Fusion: Recognition, binding and fusion

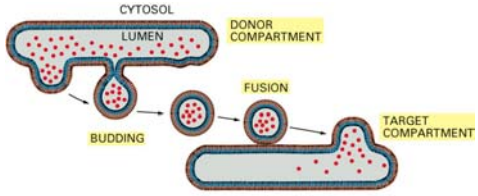


Figure 13–2. Molecular Biology of the Cell, 4th Edition.

Specificity?

A. Markers on the cytosolic side of the membrane

b. Sometimes it is not only 1 specific marker but a combinations of markers serve as the unique address.

II. **Approaches/ Methods**

1. Biochem: In vitro (reconstitution) assays to identify the players. by removing one at a time. (e.g. mutants, inhibitors, or physical removal), or adding one.

2. Genetic: Yeast *sec* mutants deficient in secretion. >25 genes Identify proteins that are essential for each step.

3. Cell biology.-
a. endocytosis of labeled ligand by a living cell, and visualize internalized ligand by Immuno-Gold or flourescence. [To visualize, cell is fixed] Follow path of endocytosis.

b. Transfection with GFP-tagged proteins! [living cell] Follow sorting in real time. Measure time it takes to move from ER-> G. Movie later

Budding with specificity.
17-51. Three types of coated vesicles

Vesicle	Coat and Adapter Proteins	Small GTP-Binding Protein	Transport Step
Clathrin	Clathrin heavy and light chains; AP2	ARF	Plasma membrane → endosome (endocytosis)
	Clathrin heavy and light chains; AP1	ARF	Golgi → endosome
	Clathrin heavy and light chains; AP3	ARF	Golgi → lysosome, vacuole, melanosome, or platelet vesicles
COPI	COP α, β, β′, γ, δ, ε, ζ	ARF	Golgi → ER Retrograde transport between Golgi cisternae
COPII	Sec23/Sec24 complex; Sec13/Sec31 complex; Sec16	Sar1	ER → Golgi

Subclasses within each type:
each specialized for a different transport step

13-5

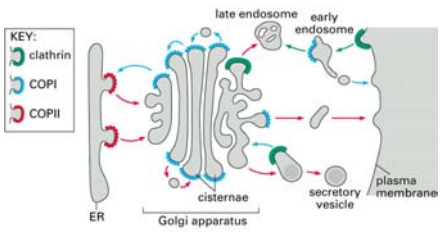


Figure 13-5. Molecular Biology of the Cell, 4th Edition.

Process: Assembly and disassembly of clathrin coat.

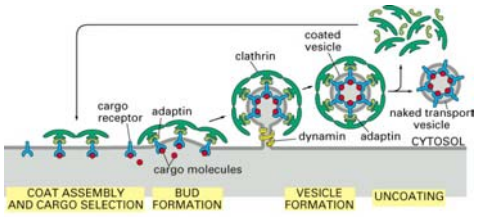


Figure 13-8. Molecular Biology of the Cell, 4th Edition.

1. Adaptin recruited to membrane, & binds coat
2. Adaptin binds to cargo receptor which binds soluble cargo
3. Coat assembly introduce curvature to vesicle
4. Coat is nearly assembled
5. Vesicle pinches off with help of dynamin
6. After budding, coat disassembles

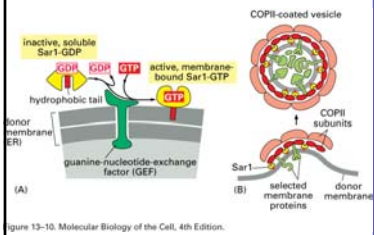


Figure 13-10. Molecular Biology of the Cell, 4th Edition.

13-10. GTP-binding proteins control **Budding** or coat assembly:

1. Initiated by recruitment of GTP-binding protein ARF to membrane (due to Receptor or lipid).

2. GTP-bound protein recruits coat protein and adapter to form complex

3. [Coat protein or assembly factors binds to membrane cargo, and membrane receptor proteins]

4. Coat is assembled

5. Vesicle buds, pinches off

ARF or Sar proteins : Coat recruitment GTPases.

Uncoating: also depends on GTPase
ARF-GTP --> ARF-GDP
Coat dissociates and are released

How does sorting occur during budding??

Tab. 17-6. Sorting signals and receptors

TABLE 17-6 Sorting Signals That Direct Secreted and Membrane Proteins to Specific Transport Vesicles

Signal Sequence*	Type of Protein†	Transport Step	Vesicle Type	Signal Receptor
Lys-Asp-Glu-Leu (KDEL)	Secreted	Golgi to ER	COP I	KDEL receptor (ERD2 protein) in Golgi membrane
Lys-Lys-X-X (KKXX)	Membrane	Golgi to ER	COP I	COP α and β subunits
Di-acidic (e.g., Asp-X-Glu)	Membrane	ER to Golgi	COP II	Not known
Mannose 6-phosphate (M6P)	Secreted	*Trans*-Golgi and plasma membrane to late endosome	Clathrin	M6P receptor in Golgi and plasma membrane; AP1 and AP2 adapter proteins
Tyr-X-X-ϕ (YXXϕ)	Membrane	Plasma membrane to endosome	Clathrin	AP2 adapter proteins
Leu-Leu (LL)	Membrane	Plasma membrane to endosome	Clathrin	AP2 adapter proteins

*X = *any amino acid*; ϕ = *bulky hydrophobic residues*. *Single-letter abbreviations are shown in parentheses.*
†*Signal sequences are located in the cytosolic domains of membrane proteins.*

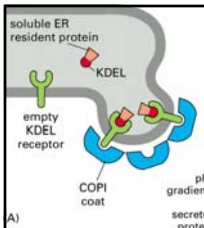


Figure 13-21 part 1 of 2. Molecu

Retrieval of ER resident proteins

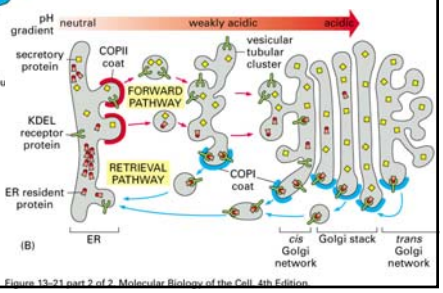


Figure 13-21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

How does a transport vesicle recognize the right target membrane??

-SNARE
-Rabs = Targeting GTPases

Snares:
provide specificity and
catalyze fusion

Rabs:
control specificity of docking and tethering, movement & fusion

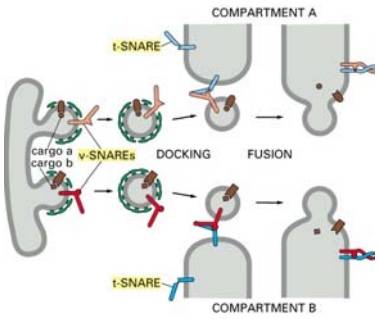


Figure 13-11. Molecular Biology of the Cell, 4th Edition.

V-SNARE pairs with its complementary t-SNARE

The pair must be separated before they can function again.

> 20 SNARES

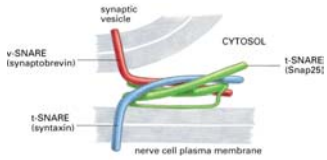


Figure 13–12. Molecular Biology of the Cell, 4th Edition.

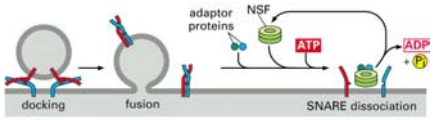


Figure 13–13. Molecular Biology of the Cell, 4th Edition.

Rab GTPases and their effectors determine compartment specificity

Additional layer of regulation to ensure that t-SNARE fuse membranes only at the right time and in the correct place.

Effectors: Protein(s) that bind a Rab-GTPase directly and is required for downstream function determined by that Rab. [e.g. tethering, motor proteins]

Rabs	Organelle	Rab Effectors	Function
Rab1	ER-G	p115	tethering
Rab3a	synaptic ves	rabphilin 3	potentiates fusion
Rab4	early endosome	Rabaptin/NEF	sorting, recycling
Rab6	G ->ER, intra G	Rabkinesin6	vesicle motility

RabGDP = soluble proteins that become attached to membranes when it is converted to Rab-GTP

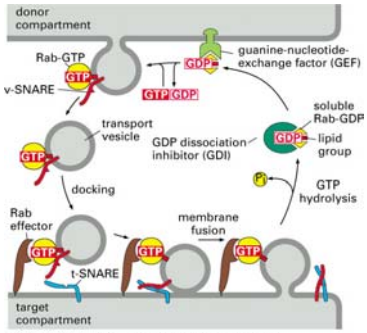


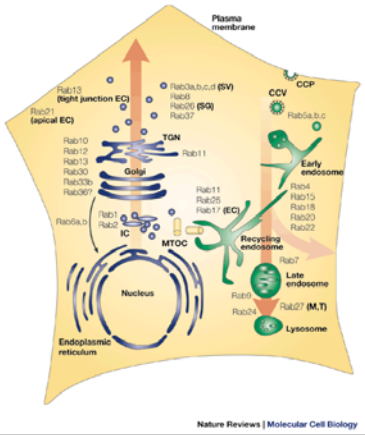
Figure 13–14. Molecular Biology of the Cell, 4th Edition.

E.g. Model

Docking by a specific Rab and its effector

Rab-GTP

Effector- docking protein



Intracellular location of Rab proteins.

Zerial & McBride 2001 Nat Rev MCB

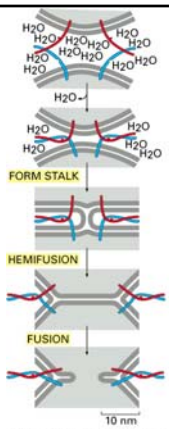


Figure 13–15. Molecular Biology of the Cell, 4th Edition.

13-15 Snares may mediate fusion similar to viral fusion proteins.

Summary

How is vesicular transport specificity determined?

1. **Budding**
a. Recognition of membrane proteins exposed to cytosolic side by ARF or Sar proteins
b. Type of coat protein recruited and assembled

2. **Transport**
motor -cargo recognition by motor receptor on the membrane

3. **Docking and fusion**
a. Rab GTPases (distinct) and their specific effectors
b. SNARE pairs

Arf-, Sar-, Rab-GTPases serve as membrane organizers