Lesson (7)

Inside the neuron III

Organelles and secretion

Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

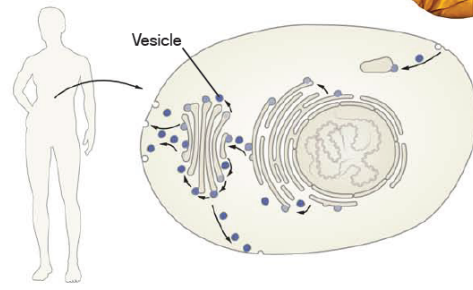
Scientific Background

Machinery Regulating Vesicle Traffic, A Major Transport System in our Cells

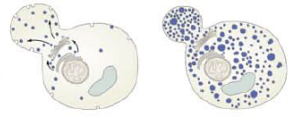
The 2013 Nobel Prize in Physiology or Medicine is awarded to Dr. James E. Rothman, Dr. Randy W. Schekman and Dr. Thomas C. Südhof for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells. This

The Nobel Prize in Physiology or Medicine 2013

Proper functioning of the cells in the body depends on getting the right molecules to the right place at the right time. Some molecules, such as insulin, need to be exported out of the cell, whereas others are needed at specific sites inside the cell. Molecules produced in the cell were known to be packaged into vesicles (pictured in blue), but how these vesicles correctly deliver their cargo was a mystery.



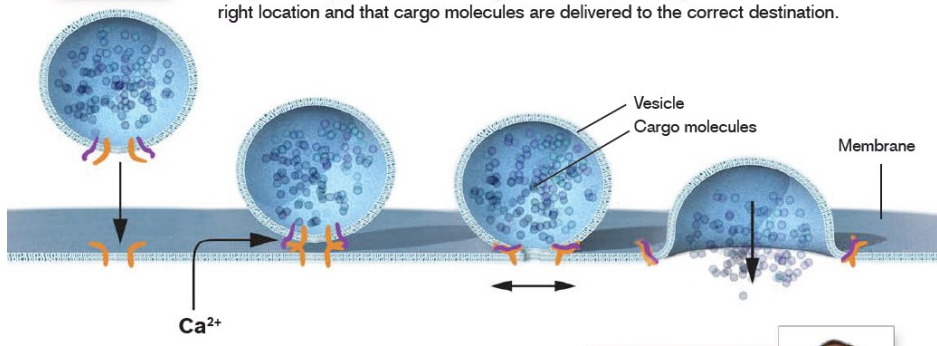
Randy W. Schekman



Randy W. Schekman discovered genes encoding proteins that are key regulators of vesicle traffic. Comparing normal (left) with genetically mutated yeast cells (right) in which vesicle traffic was disturbed, he identified genes that control transport to different compartments and to the cell surface.

James E. Rothman

James E. Rothman discovered that a protein complex (pictured in orange) enables vesicles to fuse with their target membranes. Proteins on the vesicle bind to specific complementary proteins on the target membrane, ensuring that the vesicle fuses at the right location and that cargo molecules are delivered to the correct destination.



Thomas C. Südhof

Thomas C. Südhof studied how signals are transmitted from one nerve cell to another in the brain, and how calcium controls this process. He identified molecular machinery (pictured in purple) that senses calcium ions (Ca^{2+}) and triggers vesicle fusion, thereby explaining how temporal precision is achieved and how signaling substances can be released from the vesicles on command.

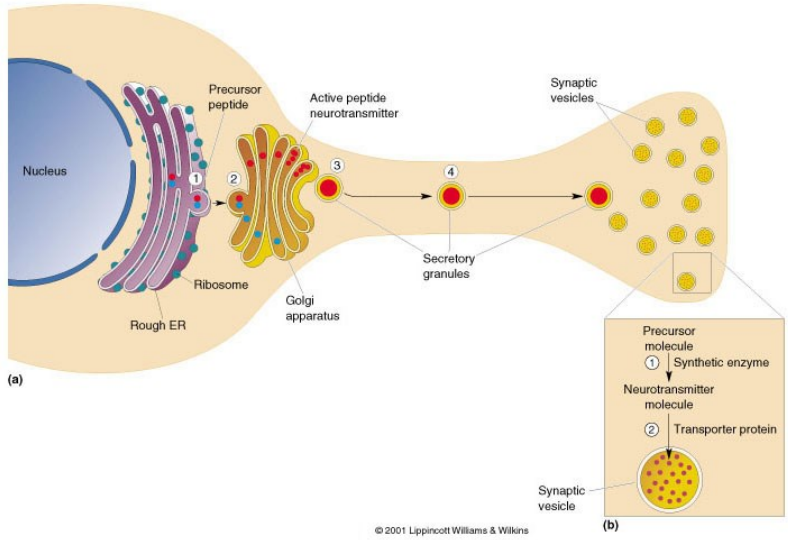
Synaptic transmission, a specialised exocitosis

- **Vesicle targeting (via specific targets guide proteins)**
- **Event-specific and fast release of chemicals**

Molecular biology reveals conserved homologous components involved in synaptic release, from yeast to man.

The classical view of vescicle trafficking in neurons

Figure 5.9
The synthesis and storage of different types of neurotransmitter. (a) Peptides: ① A precursor peptide is synthesized in the rough ER. ② The precursor peptide is cleaved in the Golgi apparatus to yield the active neurotransmitter. ③ Secretory vesicles containing the peptide bud off from the Golgi apparatus. ④ The secretory granules are transported down the axon to the terminal where the peptide is stored. (b) Amine and amino acid neurotransmitters: ① Enzymes convert precursor molecules into neurotransmitter molecules in the cytosol. ② Transporter proteins load the neurotransmitter into synaptic vesicles in the terminal, where they are stored.



Cells are polar and need vescicle traffic to go asymmetrically

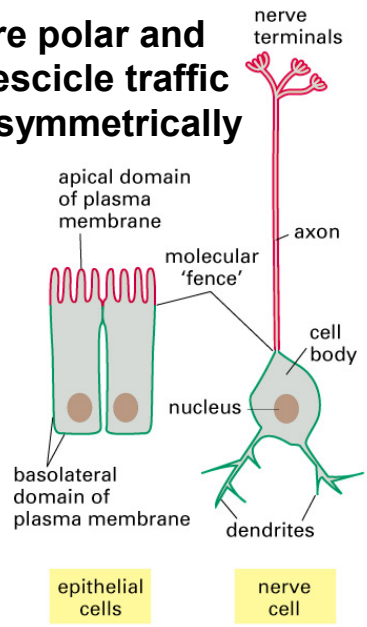
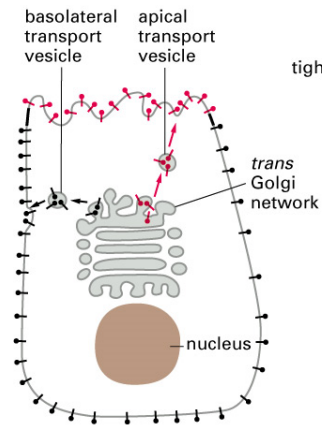


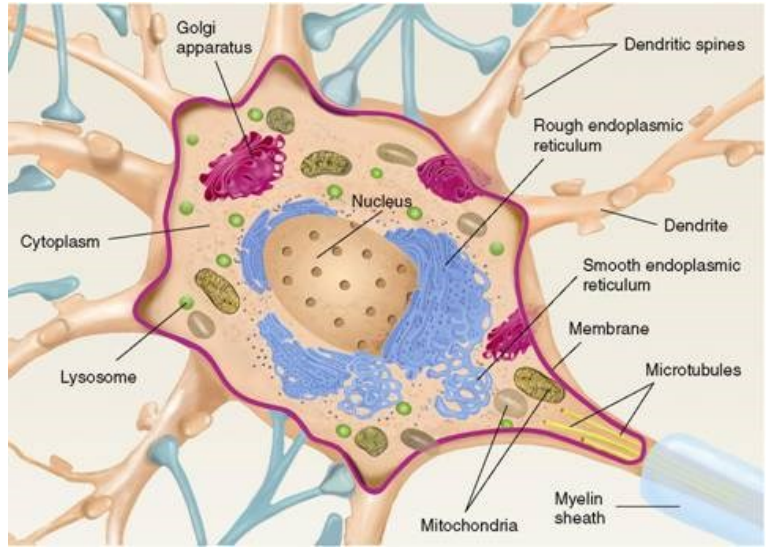
Figure 13–61. Molecular Biology of the Cell, 4th Edition.



(A) DIRECT SORTING OF MEMBRANE PROTEINS IN THE *TRANS* GOLGI NETWORK

Figure 13–62. Molecular Biology of the Ce

Endoplasmic reticulum and Golgi apparatus are key organelles for the biosyntesis of secreted neurotransmitters, peptides and trophic factors (polypeptides)



The Golgi Apparatus has two major functions:

1. Modifies the N-linked oligosaccharides and adds O-linked oligosaccharides ("O-link" is to serine or threonine).
2. Sorts proteins so that when they exit the trans Golgi network, they are delivered to the correct destination.

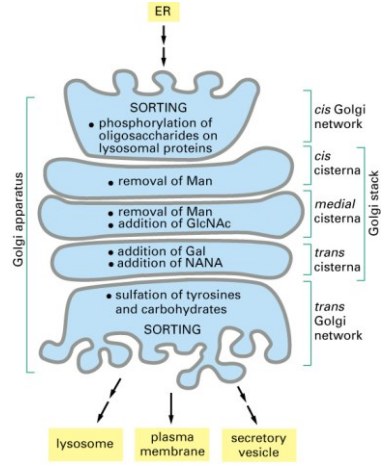


Figure 13-29, Molecular Biology of the Cell, 4th Edition.

Secretory pathway: protein to various organelles by transport vesicles

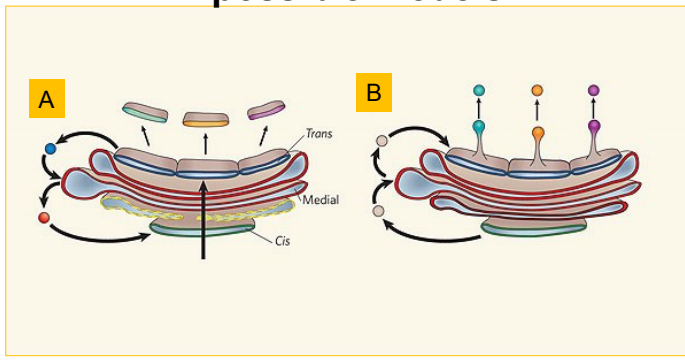
Anterograde: forward moving

Retrograde: backward moving

Trans position: farthest from the ER

Cis position: nearest the ER

Protein trafficking through the Golgi: 2 possible models



Two models of protein trafficking through the Golgi

(A) The cisternal maturation model of protein movement through the Golgi. As a new cis cisterna is formed it traverses the Golgi stack, changing as it matures by accumulating medial, then trans enzymes through vesicles that move from later to earlier cisternae (retrograde traffic).

(B) The vesicular transport model, where each cisterna remains in one place with unchanging enzymes, and the proteins move forward through the stack via vesicles that move from earlier to later cisternae (anterograde traffic).

Malhotra, V. & Mayor, S. Cell biology: The Golgi grows up. Nature ***441,*** *939–940 (2006)*

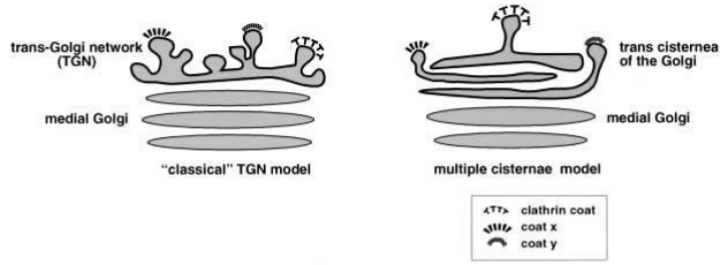
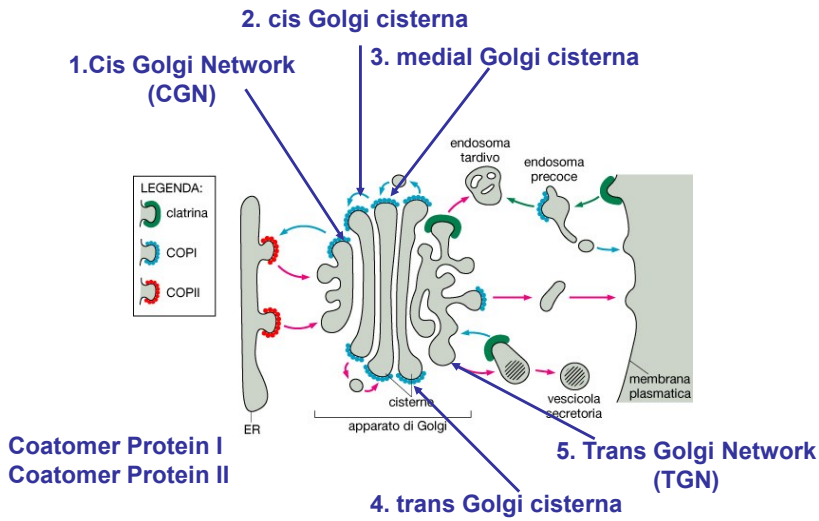
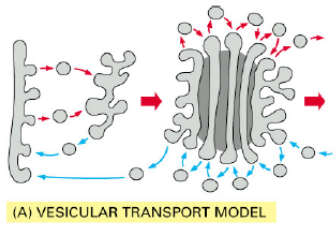


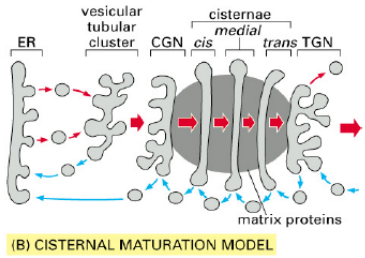
Figure 1.
TGN structure. The left panel represents the classical view of the TGN as a separate tubular network at the trans side of the Golgi compartment, with clathrin-coated vesicles or other unknown coats budding from certain areas of the TGN. This model suggests that all proteins are sorted in parallel, in the same TGN compartment. The right panel represents a different view of the TGN: as the trans-most cisternae of the Golgi; each cisterna projects tubules and buds. This model suggests that protein sorting could occur in a sequential manner and in different cisternae of the TGN.

Transport vescicles through the Golgi are coated by specific proteins





(A) VESICULAR TRANSPORT MODEL



(B) CISTERNAL MATURATION MODEL

Tradional Model - Golgi is a static organelle. Secretory proteins move forward in small vesicles. Golgi resident proteins stay where they are.

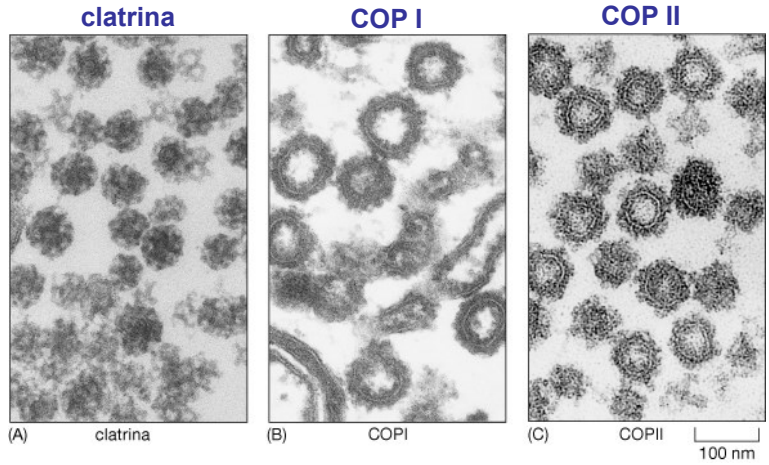
Two Models For Cis to Trans-Golgi Progression

"Radical" Model - Golgi is a dynamic structure. It only exists as a steady-state representation of transport intermediates. Secreted molecules move ahead with a cisterna. Golgi resident proteins move backward to stay in the same relative position.

Vesicular transport model originally developed and advocated by George Palade and Marilyn Farquhar (Farquhar & Palade 1998.) The vesicular transport model posits that the Golgi cisternae are stable compartments that house certain protein modification enzymes that function to add or remove sugars, add sulfate groups, and perform other modifications.

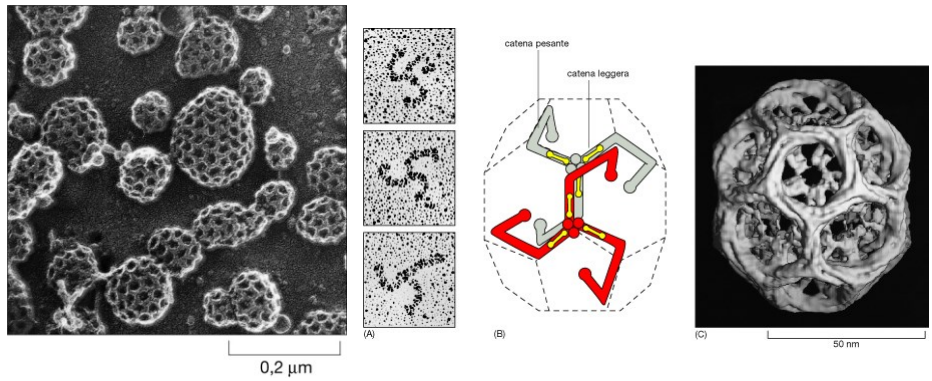
Vesicles arrive at each cisterna carrying cargo proteins, which are then modified by the resident enzymes located within that cisterna. Next, new vesicles carrying the cargo proteins bud from the cisterna and travel to the next stable cisterna, where the next series of enzymes further processes the protein cargo (Rothman & Wieland 1996).

Different coated Golgi vescicles observed at TEM



Structure of clathrin coating

Triskelion: 3 heavy chains (red) e 3 light chains (yellow)



Formation of coated secretory vescicles in the regulated pathway

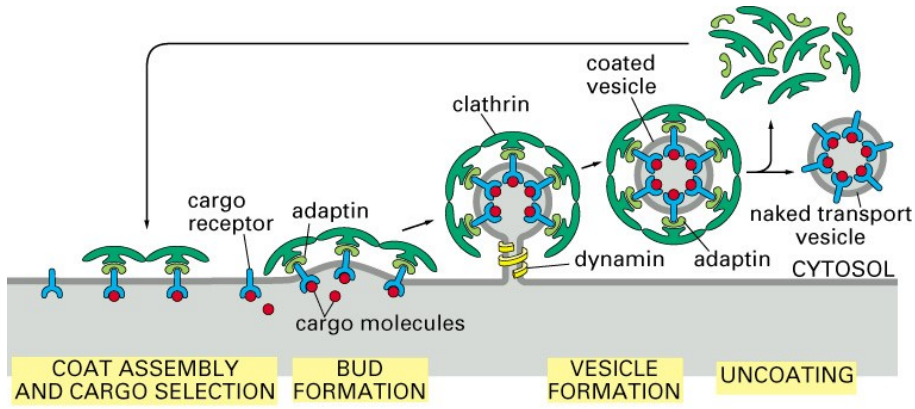


Figure 13–8. Molecular Biology of the Cell, 4th Edition.

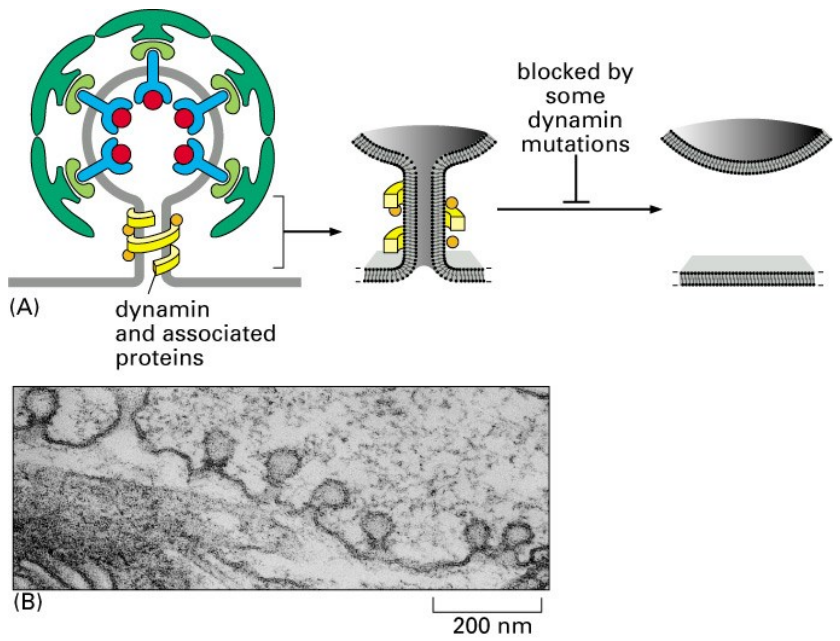
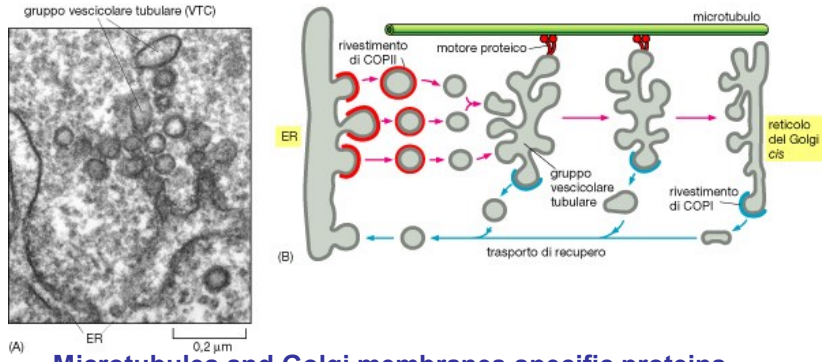


Figure 13–9. Molecular Biology of the Cell, 4th Edition.

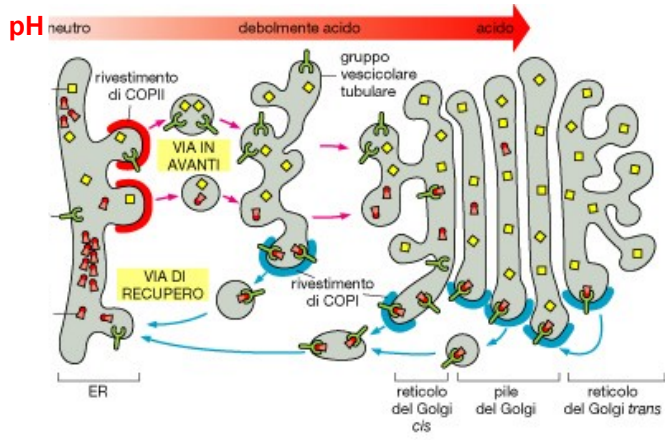
Vescicle trafficking from ER to Golgi is mediated by microtubules

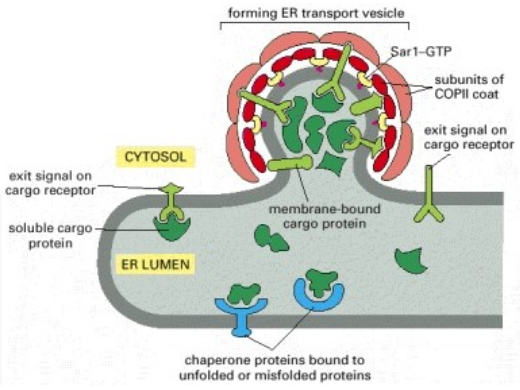
Coalescence of vescicles and formation of tubular vescicular groups



Microtubules and Golgi membranes specific proteins hold Golgi cisternae piled together

Vescicle trafficking through Golgi Follows 2 directions: forward and rescue





The ERGIC53 protein seems to serve as a receptor for packaging some secretory proteins into COPII-coated vesicles. Its role in protein transport was identified because humans who lack it owing to an inherited mutation have lowered serum levels of two secreted blood-clotting factors (Factor V and Factor VIII) and therefore bleed excessively. The ERGIC53 protein is a lectin that binds mannose and is thought to recognize this sugar on Factor V and Factor VIII proteins, thereby packaging the proteins into transport vesicles in the ER.

A conserved set of GTPase switch proteins controls assembly of different vesicle coats.

All three coated vesicles contain a small GTP-binding protein

COP I and clathrin vesicle: ARF (ADP-ribosylation factors)

COP II vesicle: Sar I protein

ARF and Sar I protein can switch GTP (GDP-protein → GTP-protein active; GTPase)

There two sets of small GTP-binding proteins for vesicle secretion. One is ARF and Sar I; another is Rab protein

ARF (ADP Ribosylation Factor) protein exchanges bound GDP for GTP and then binds to its receptor on Golgi membrane

Monomeric GTPase control coat assembly

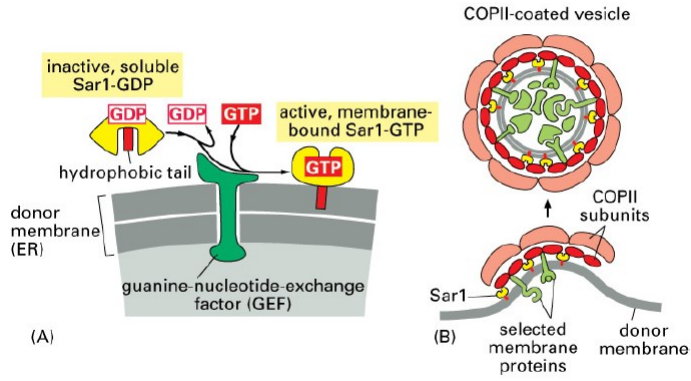


Figure 13–10. Molecular Biology of the Cell, 4th Edition.

Different coated proteins

Clathrin and adapter protein (AP): **vesicles transport proteins** from the plasma membrane and trans-Golgi network to late endosomes

– With AP1: Golgi to endosome

– With AP2: Endocytosis (PM to endosome)

– With AP3: Golgi to lysosome and other vesicles

COPI: Golgi to ER (retrograde transport)

COPII: ER to Golgi (antrograde trnasport)

AP: complex consists of four different subunits

Vescicles that exit from the *trans* Golgi network can undergo constitutive or regulated secretion

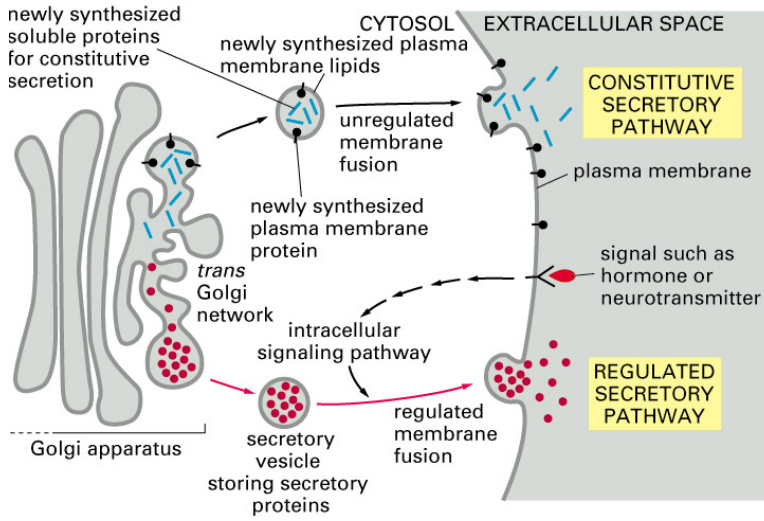
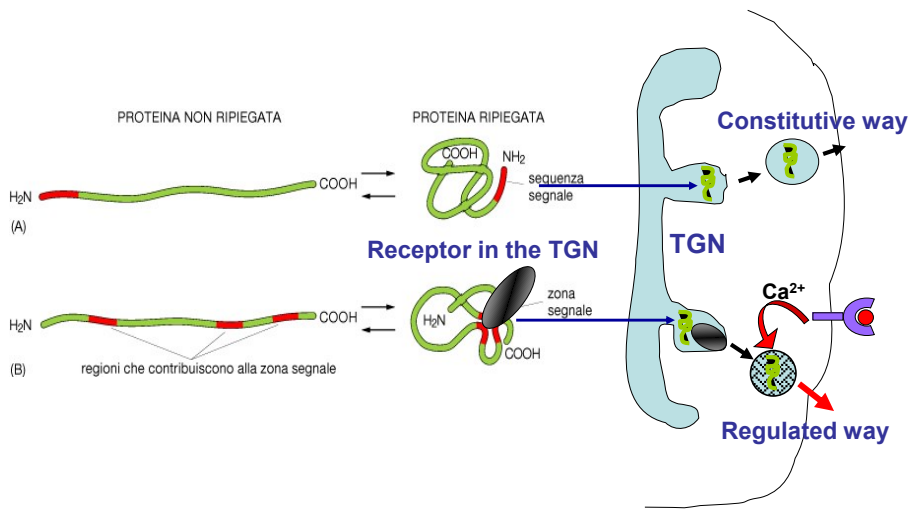


Figure 13–54. Molecular Biology of the Cell, 4th Edition.

Selection of proteins towards the constitutive secretory way or the regulated secretory way depends upon intrinsic signals receptors within the TGN



Synthesis, storage, and release of neurotrophins

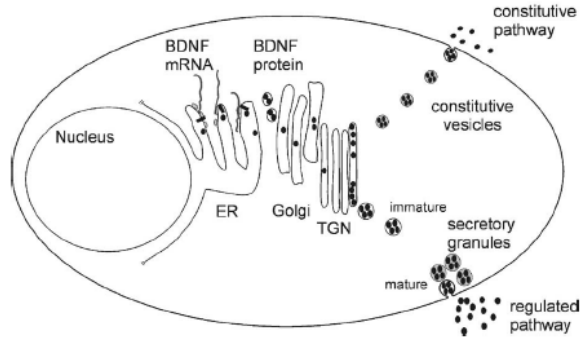
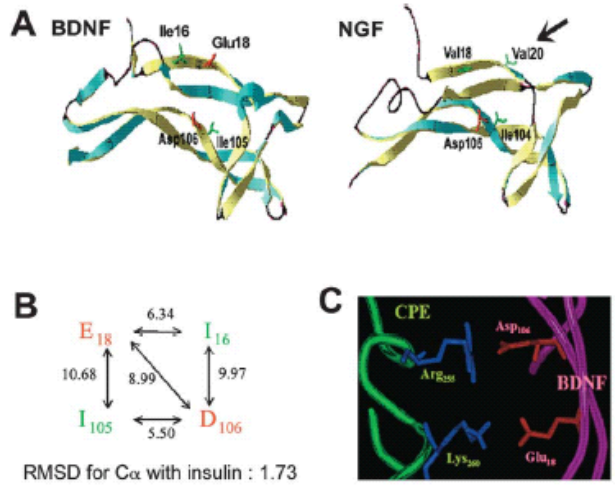


Fig. 2. The route of BDNF from synthesis to secretion. BDNF mRNA is translated from ribosomes (light gray) of the rough ER and the nascent pre-pro-protein is sequestered into the ER (black symbols). BDNF is targeted to the Golgi and subsequently to the *trans*-Golgi network (TGN) by vesicle-mediated transport. TGN resident protein convertases (PCs) can cleave off the pro-sequence and mature BDNF is targeted to constitutively released vesicles. Alternatively, pro-BDNF buds off from the TGN in immature secretory granules (white) containing a distinct set of protein convertases. Mature BDNF is excised in the secretory granules en route to the plasma membrane to yield mature secretory granules (gray). The secretory granules accumulate at the plasma membrane and are eventually released upon triggering signals for regulated secretion (see text).

Neuron, Vol. 45, 245–255, January 20, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2004.12.037

Sorting and Activity-Dependent Secretion of BDNF Require Interaction of a Specific Motif with the Sorting Receptor Carboxypeptidase E

Hong Lou,[1] Soo-Kyung Kim,[1] Eugene Zaitsev,[2] Chris R. Snell,[3] Bai Lu,[3] and Y. Peng Loh[1,*]



A fashionable Golgi Apparatus...

Synaptic transmission is an adaptation of normal vesicle trafficking.

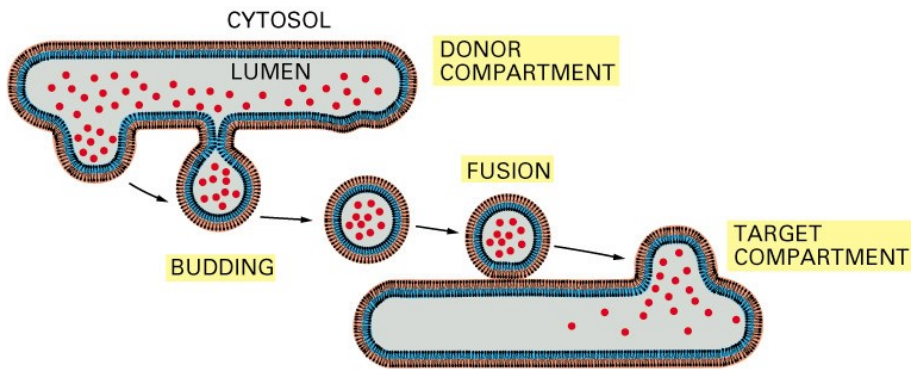
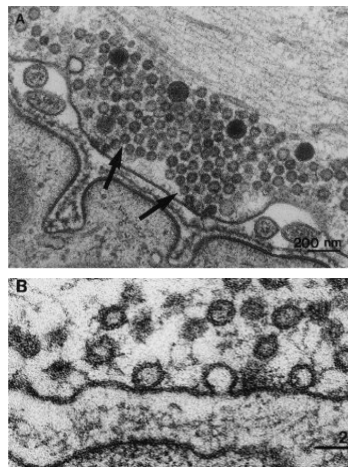


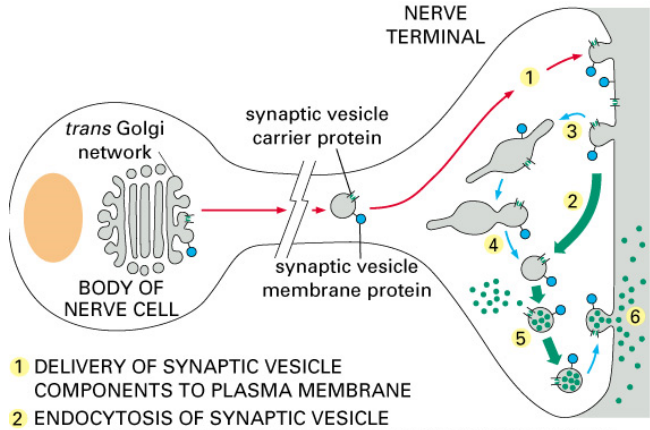
Figure 13–2. Molecular Biology of the Cell, 4th Edition.

Why store transmitters in vesicles?



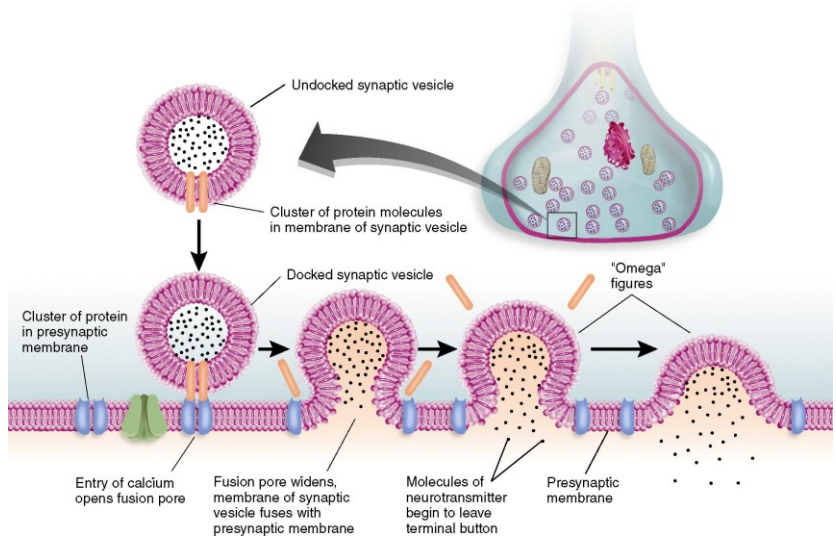
- Protection from degradation - by proteases and esterases
- Allows for regulation
- Provides a storage system
- Can be docked at active zone
- Differ for classical transmitters (small, clear-core) vs. neuropeptides (large, dense-core)

Adaptation at synapses of vesicle cycling found in all cells



1 DELIVERY OF SYNAPTIC VESICLE COMPONENTS TO PLASMA MEMBRANE
2 ENDOCYTOSIS OF SYNAPTIC VESICLE COMPONENTS TO FORM NEW SYNAPTIC VESICLES DIRECTLY
3 ENDOCYTOSIS OF SYNAPTIC VESICLE COMPONENTS AND DELIVERY TO ENDOSOME
4 BUDDING OF SYNAPTIC VESICLE FROM ENDOSOME
5 LOADING OF NEUROTRANSMITTER INTO SYNAPTIC VESICLE
6 SECRETION OF NEUROTRANSMITTER BY EXOCYTOSIS IN RESPONSE TO AN ACTION POTENTIAL

Figure 13–64. Molecular Biology of the Cell, 4th Edition.



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Production and recycling of synaptic vesicles.

• **Synaptic vesicle components** are made in the soma and move through the Golgi, transported to the release sites by fast axonal transport.

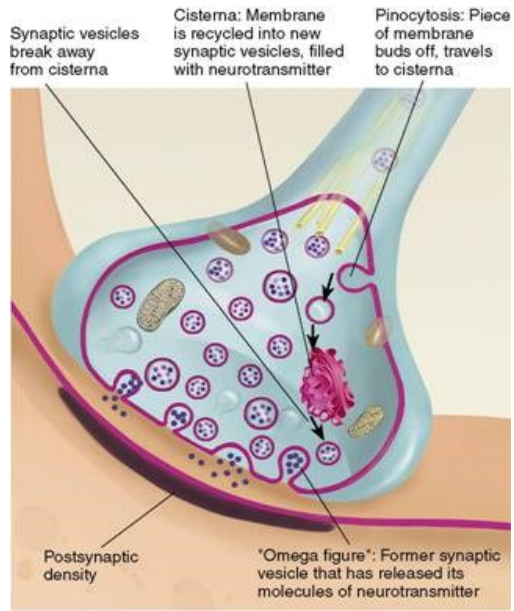
• Since vesicles are **released by exocytosis** need to be recycled or nerve the terminal would grow quite large, also need to conserve synaptic vesicle proteins.

• **Recycling of synaptic vesicles** in extracellular media gets engulfed by endocytosis, visualized with EM and shown to go to: coated vesicles – clathrin endosome sorting to lysosome (destruction) or recyclling (back into vesicle pool).

A problem to solve:

- Vesicles released by exocytosis need to be recycled otherwise nerve the terminal would grow quite large, also need to conserve synaptic vesicle proteins.

Endocytosis and exocytosis processes occur at distinct locations of the presynaptic terminals



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The synapse has adapted forms of vesicle cycling found in all cells.

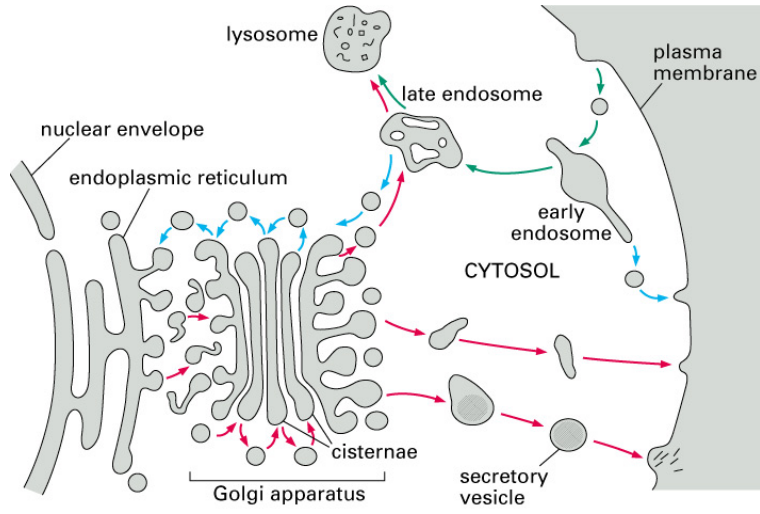
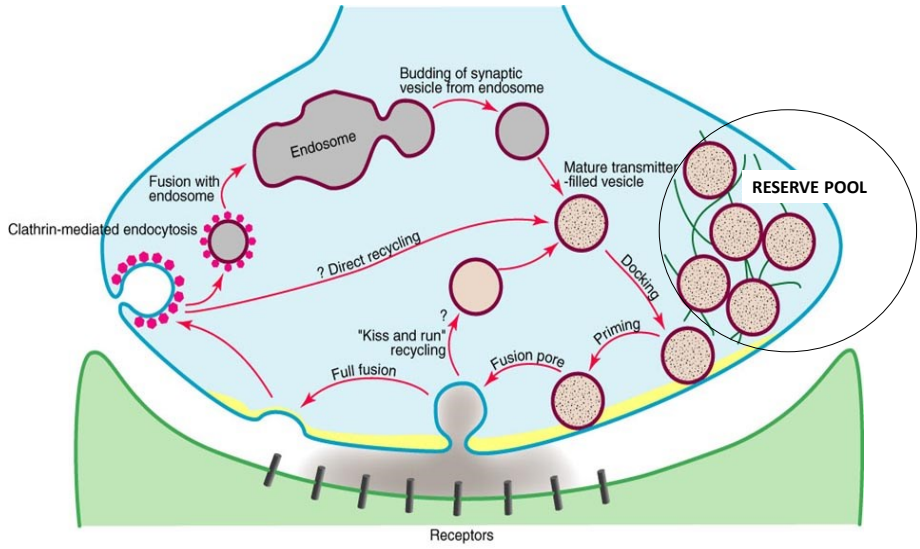


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Modes of release and recycling.

- In neurons Heuser and Miller originally propose two pathways, coated pits and cisternae (endosome) for most synaptic transmission and non-coated pit pathways for times of high activity.
- However the situation in neurons may be more complicated and can include transient forms of fusion such as “kiss and run”.

A complication: the kiss and run recycling model



Three types of membrane retrieval mechanisms coexist

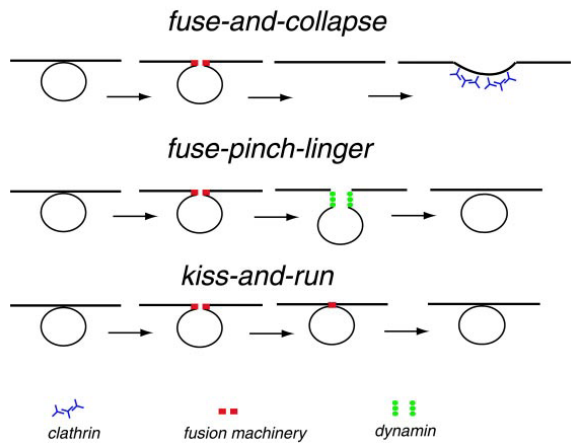


Fig. 1. Three types of membrane retrieval mechanisms may coexist for neurosecretory vesicles. (*Top*) A conventional intermixing of the membrane follows the fusion event eventually leading to a clathrin-mediated retrieval of the vesicle components (fuse-and-collapse). (*Middle*) A new mechanism whereby dynamin serves to cause fission of the intact fused vesicle before collapse and intermixing with the plasma membrane (fuse-pinch-linger). (*Bottom*) The fusion event is terminated by a direct closure of the fusion pore (kiss-and-run).

T. Ryan PNAS 2002

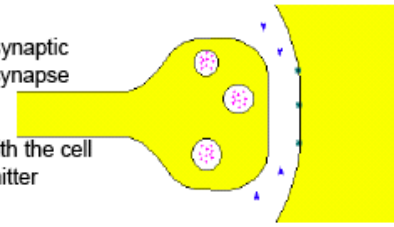
How the impulse is transmitted across the synaptic cleft

1. action potential reaches the presynaptic terminal

2. voltage-gated calcium channels at the end of pre-synaptic neurone open when an action potential reaches the synapse

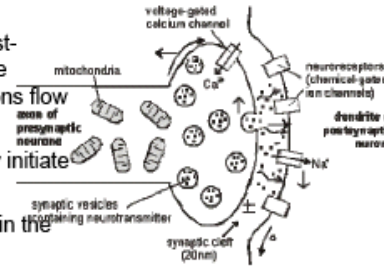
3. calcium ions flow into the cell.

4. calcium ions cause the synaptic vesicles to fuse with the cell membrane, releasing their contents (the neurotransmitter chemicals) by exocytosis.



5. neurotransmitters diffuse across the synaptic cleft.

6. neurotransmitter binds to the neuroreceptors in the post-synaptic membrane, causing the channels to open. In the example shown these are sodium channels, so sodium ions flow in.



7.depolarisation of the post-synaptic cell membrane (may initiate an action potential, if the threshold is reached)

8. neurotransmitter is broken down by a specific enzyme in the synaptic cleft (**acetylcholinesterase**)

9. breakdown products absorbed by the pre-synaptic neurone by endocytosis and used to re-synthesise neurotransmitter, using energy from mitochindria