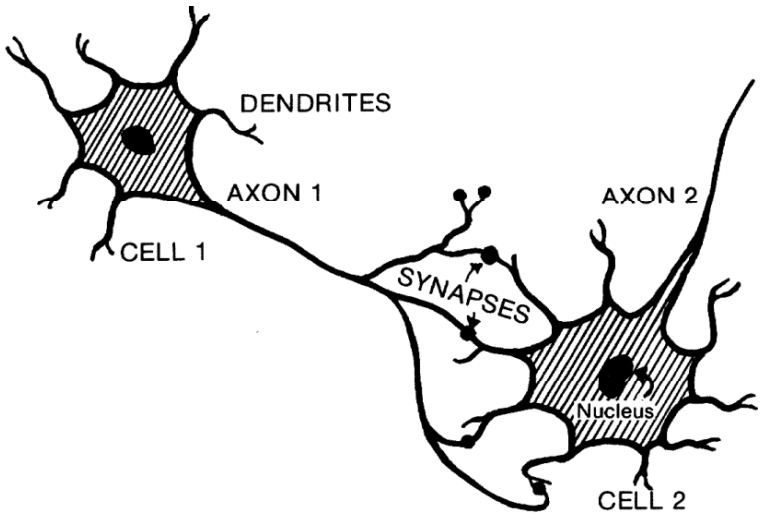
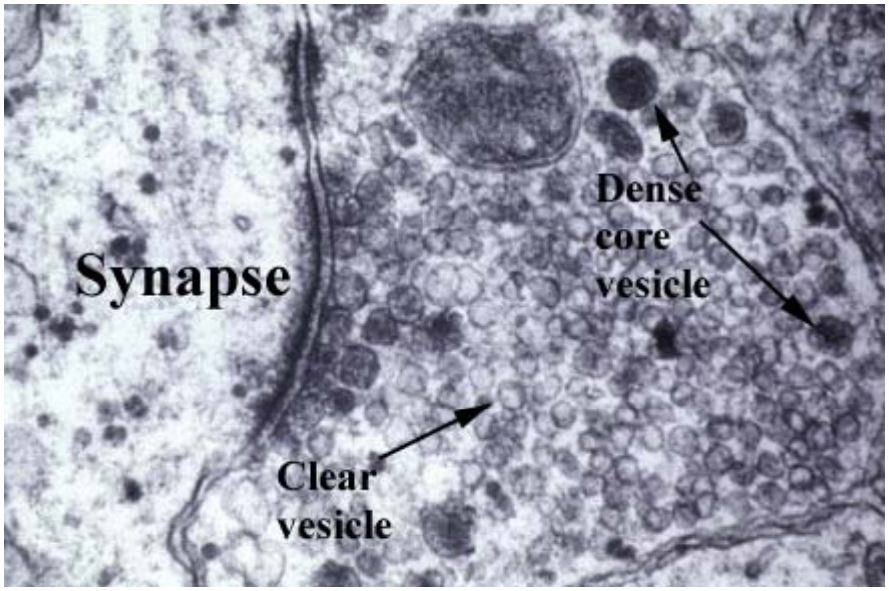
Chap 4. Synaptic transmission: the biochemical basis

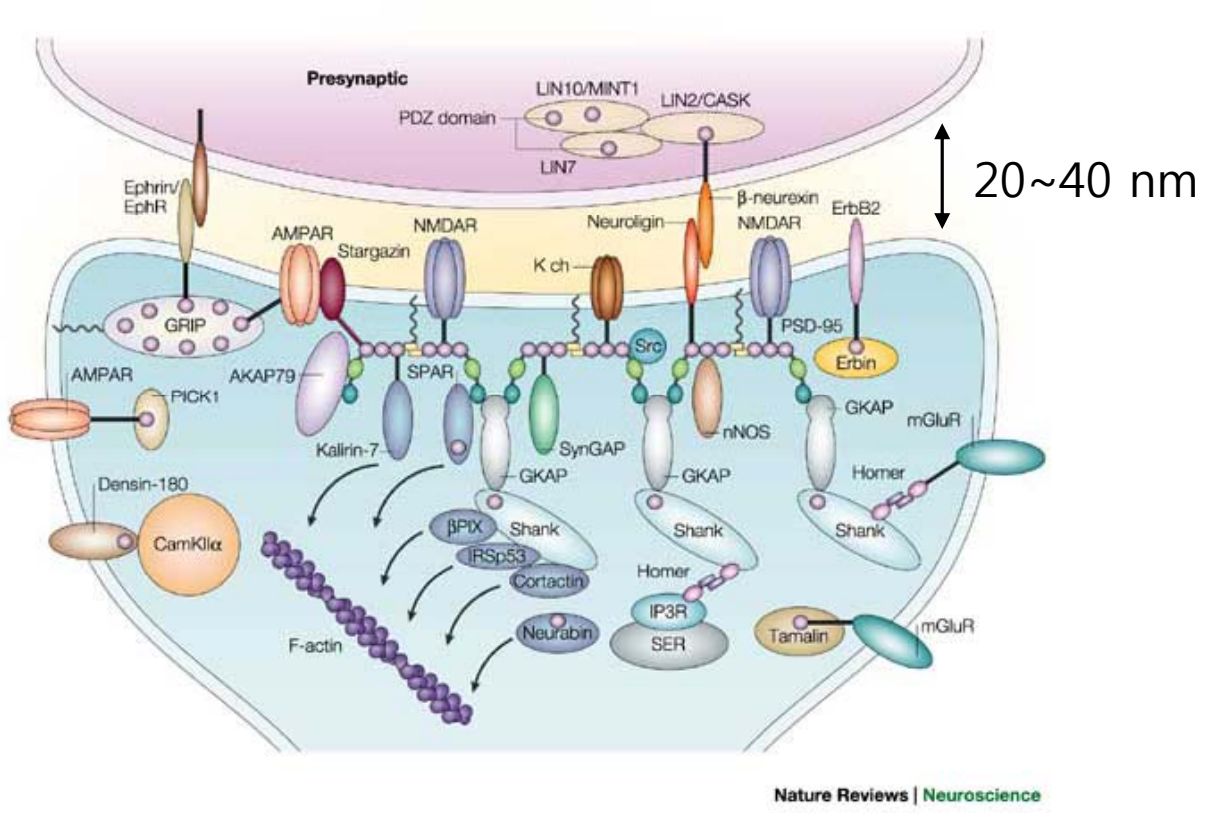
(Chemical) Synaptic transmission is a signal transduction process occurring in 3 steps
(1) Electrical information to a chemical signal (presynaptic terminal)
(2) Transmission of the chemical signal (synapse)
(3) Conversion of the chemical signal into an electrical signal (postsynaptic terminal)





A schematic diagram of the organization of PDZ proteins at a mammalian excitatory synapse

Nature Reviews Neuroscience (2004) 5, 771-781



The PDZ domain is a common structural domain of 80-90 amino-acids found in the signaling proteins of bacteria, yeast, plants, viruses and animals.

PDZ is an acronym combining the first letters of three proteins — **p**ost synaptic density protein (PSD95), Drosophila **d**isc large tumor suppressor (Dlg1), and **z**onula occludens-1 protein (zo-1) — which were first discovered to share the domain. http://en.wikipedia.org/wiki/PDZ_domain

Presynaptic signaling: neurotransmitters

How many kinds of neurotransmitters? >100 types

- Biogenic amines
- Amino acids and peptides
- Nucleosides
- Gases

Does a neuron release only one type of neurotransmitters?

How many vesicles in a presynaptic terminal?

How many neurotransmitters in a vesicle?

- Quantum: the amount of transmitter in each vesicle
- Quantal release: the process of transmitter release

Postsynaptic signaling: receptors

Presynaptic signals: excitatory or inhibitory (depending on receptors)

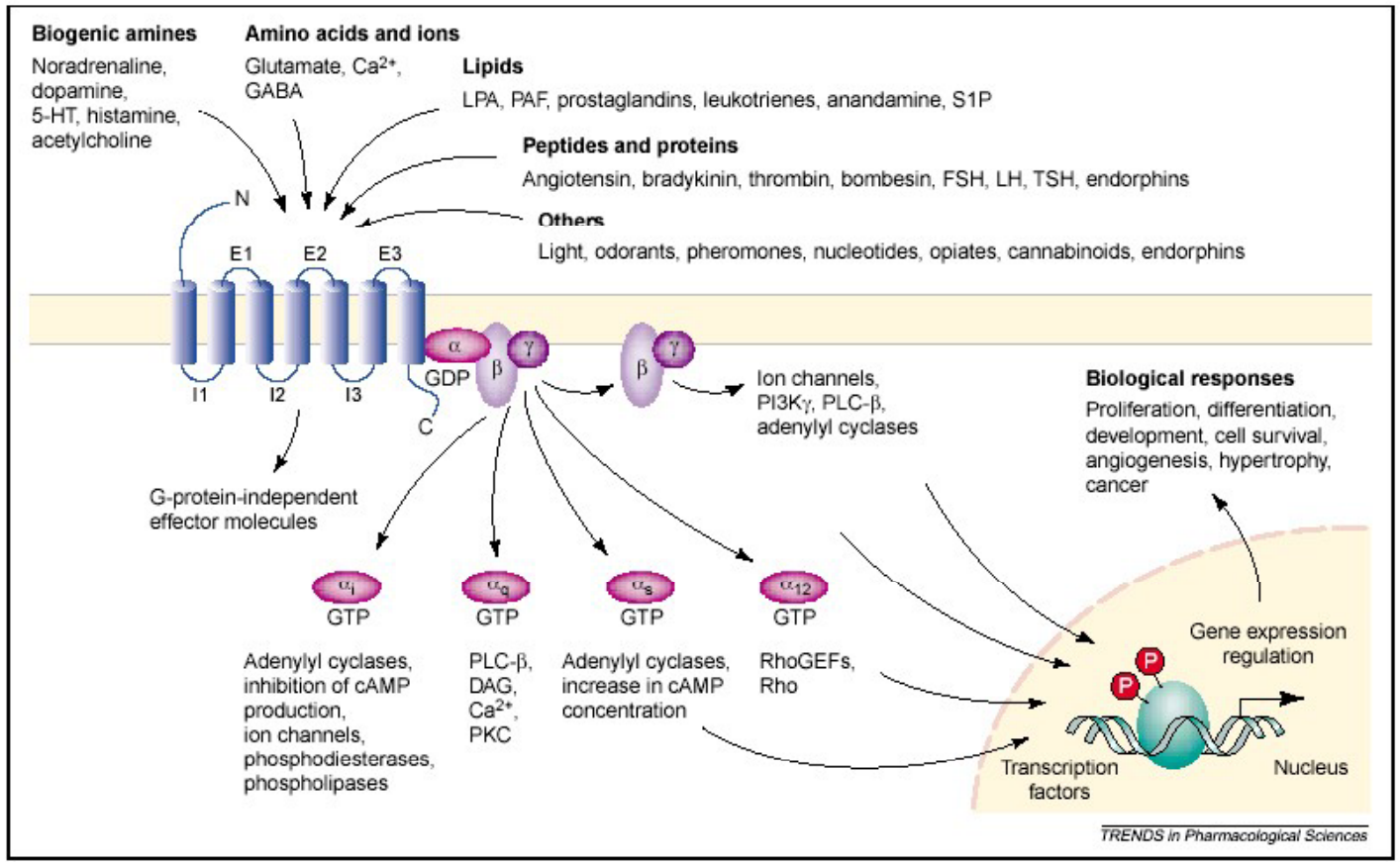
Receptors

- How many kinds of receptors? Multiple subtypes for a transmitter
- One type of receptor for a transmitter?
- One type of receptor in a single neuron?
- Only in postsynaptic neuron?

How fast is the synaptic transmission?

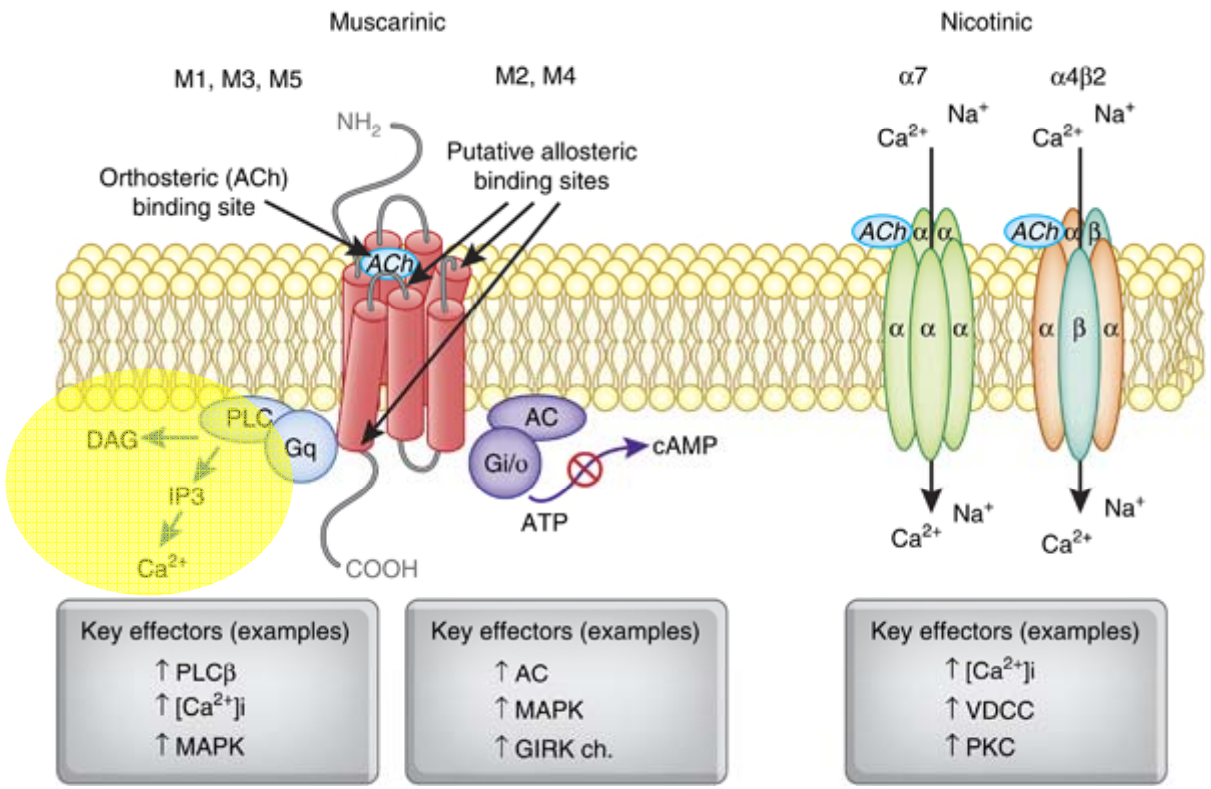
- Fast synaptic transmission: ligand-gated ion channels (ionotropic) receptor ionopores
- Slow synaptic transmission: GPCRs (metabotropic)

GPCRs



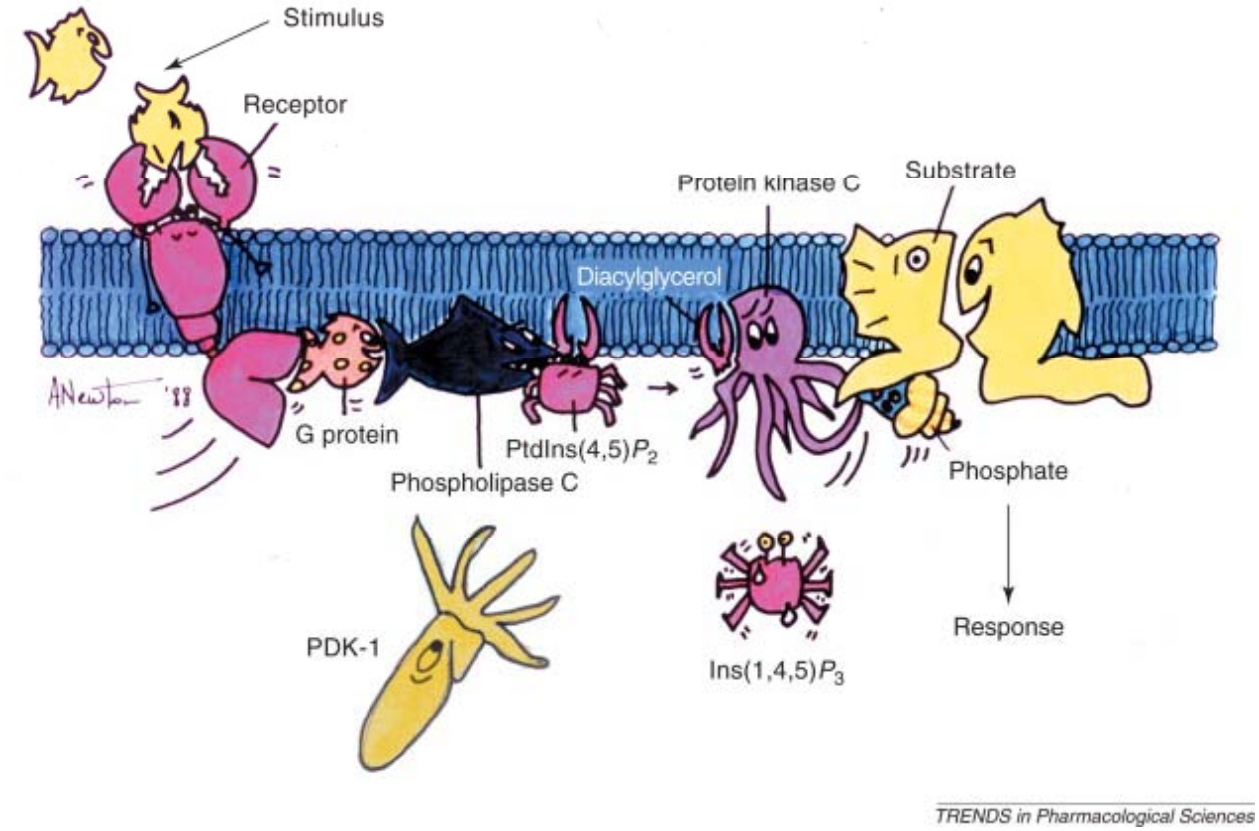
http://www.ibibiobase.com/projects/db-drd4/G_protein.htm

Acetylcholine receptor (AchR)



The structure and signaling pathways of mAChRs and nAChRs. Each mAChR subtype is a seven-transmembrane protein, which belongs to two major functional classes based on G-protein coupling. The M_1, M_3, and M_5 mAChRs selectively couple to the Gq/G11-type G-proteins resulting in the generation of inositol-1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) through activation of the phosphoinositide-specific phospholipase-Cβ leading to increased intracellular calcium levels. The M_2 and M_4 mAChRs preferentially activate Gi/Go-type G-proteins, thereby inhibiting adenylate cyclase, reducing intracellular concentration of cAMP, and prolonging potassium channel opening. All mAChR subtypes show a high sequence homology across species, particularly in the orthosteric ACh-binding sites. Neuronal nAChRs are pentameric ligand-gated ion channels. The most abundant neuronal subunits are α_4, β_2, and α_7, with the heteromeric $\alpha_4\beta_2$ receptor subtype in highest abundance. The heteromeric $\alpha_4\beta_2$ receptor subtype can exist in two different forms: $(\alpha4)_2(\beta2)_3$ receptors show low Ca^{2+} permeability and high affinity to ACh and nicotine, whereas $(\alpha4)_3(\beta2)_2$ receptors have high Ca^{2+} permeability. By contrast, the α_7 nAChR also shows high permeability to Ca^{2+} relative to the heteromeric $\alpha_4\beta_2$ nAChRs. The action of $\alpha_4\beta_2$ nAChRs can enhance intracellular levels of Ca^{2+} by secondary activation of VOCCs, whereas α_7 nAChRs preferentially increase Ca^{2+} release from ryanodine-sensitive intercellular stores through CICR. The capacity of these different nAChR subtypes to couple to VOCC or CICR mechanisms results in distinct patterns of Ca^{2+} signaling that can provide a broader control of synaptic plasticity and neurotransmitter release, as well as gene transcription. *Neuropsychopharmacology Reviews* (2012) **37,** 16–42

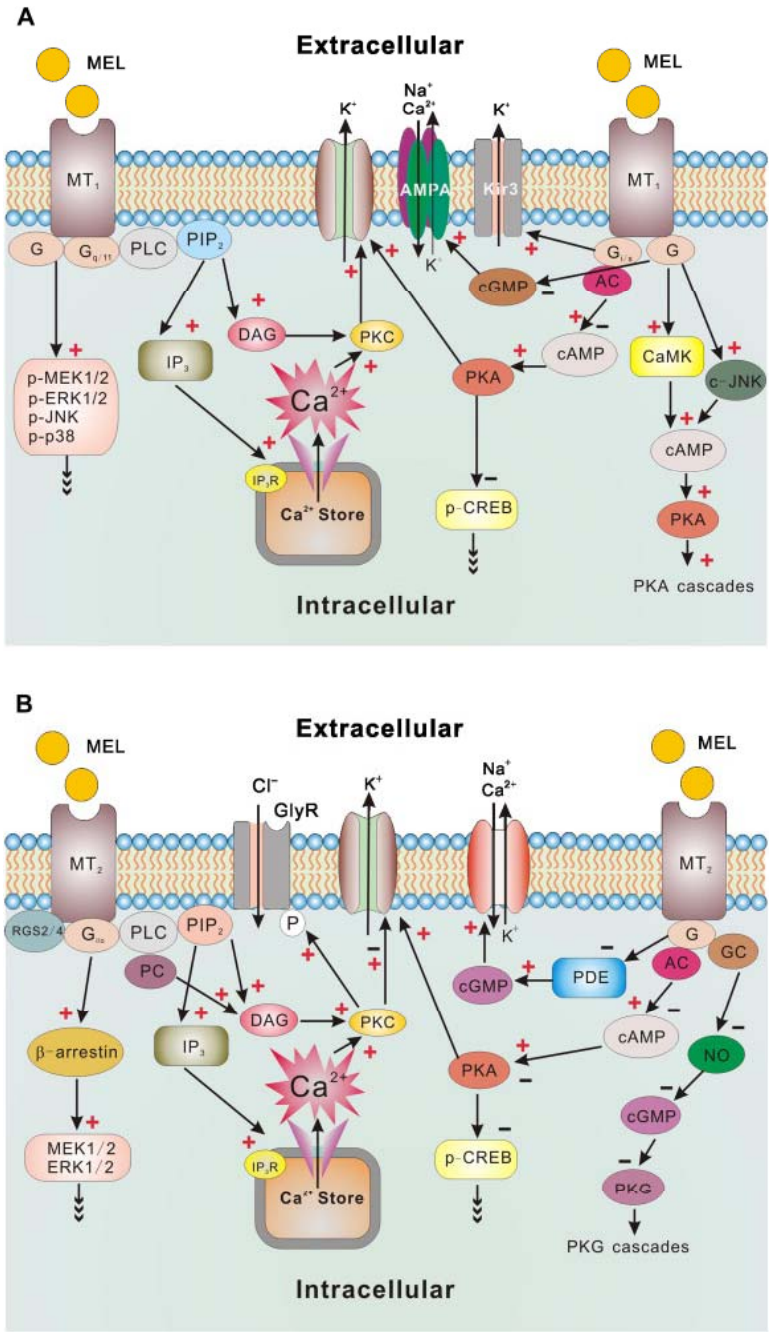
G-protein mediated second messengers (cAMP, cGMP, DAG, Ca^{2+})



Cartoon showing that diacylglycerol, generated by receptor-mediated hydrolysis of phospholipids, tethers protein kinase C (PKC) to the membrane. Hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2 (crab)] generates two second messengers: diacylglycerol, which engages the C1 domain of PKC to membranes, and inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], which releases intracellular Ca^{2+} causing the C2 domain of conventional isozymes of PKC to become tethered to the membrane. Although there are other ways to regulate PKC activity, this scheme represents the classic pathway culminating from the discovery of Nishizuka and co-workers, which is celebrated in this article. Also shown is the upstream phosphoinositide-dependent kinase PDK-1 (see main text), which phosphorylates PKC as part of its maturation process. Note that there are other targets for diacylglycerol, although activation of PKC remains the primary target of this second messenger. TIPS (2004) 25, 175-177.

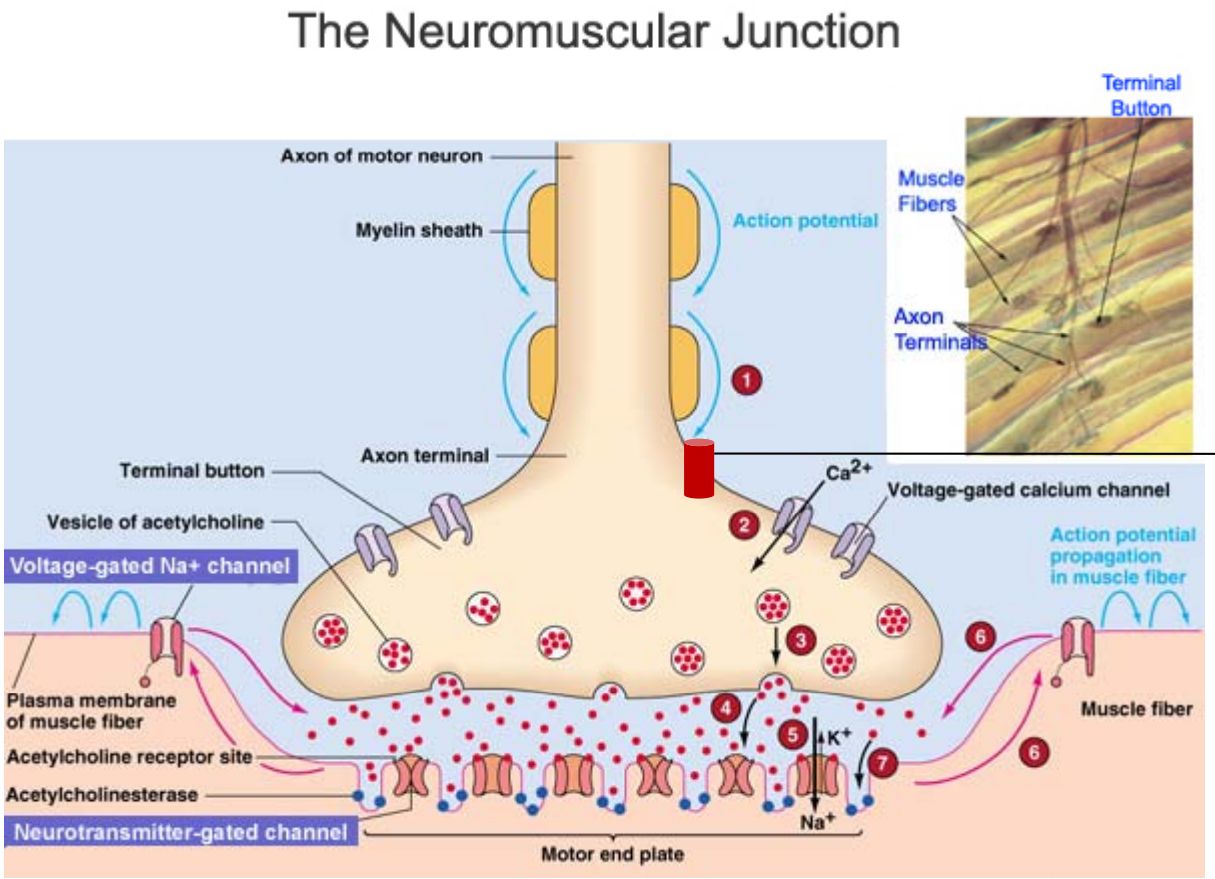
Schematic diagrams showing multiple signaling pathways following activation of MT1 (A) and MT2 (B) melatonin receptors.

Signaling pathway due to the activation of a specific receptor is highly cell type-dependent. AC, adenylyl cyclase; CaMK, calcium-calmodulin dependent kinase; CREB, cAMP responsive element binding protein; DAG, diacylglycerol; ERK1/2, extracellular signal-regulated kinases 1 and 2; GC, guanylyl cyclase; GlyR, glycine receptor; IP3, inositol 1,4,5-trisphosphate; IP3R, IP3 receptor; JNK, Jun N-terminal kinase; MEK1/2, mitogen-activated protein kinase kinases 1 and 2; MEL, melatonin; NO, nitric oxide; PC, phosphatidylcholine; PDE, phosphodiesterase; P, phosphorylation; p-, phosphorylated; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; RGS, regulator of G protein signaling.



II. Neurotransmitter storage, reuptake, and release

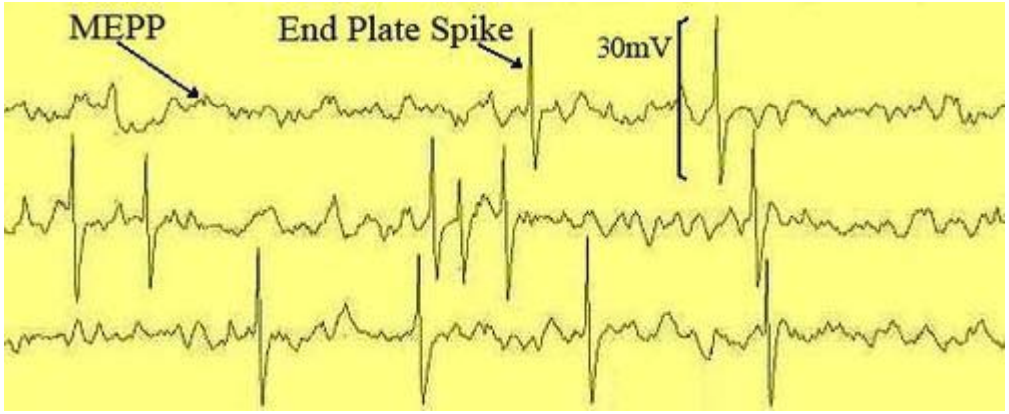
1. The role of calcium ions



→ TTX-sensitive VGSC

http://cmceducation2012.blogspot.kr/2012/09/neuromuscular-junction.html

2. Quantal release



MEPPs and end plate spikes recorded from muscle fiber

Miniature End Plate Potentials (MEPPs): MEPPs are the small (~0.5mV) depolarisations of the postsynaptic terminal caused by the release of a single vesicle into the synaptic cleft. Neurotransmitter vesicles containing acetylcholine collide spontaneously with the nerve terminal and release acetylcholine into the neuromuscular junction even without a signal from the axon. These small depolarizations are not enough to reach threshold and so an action potential in the postsynaptic membrane does not occur. During experimentation with MEPPs, it was noticed that often spontaneous action potentials would occur, called end plate spikes in normal striated muscle without any stimulus. It was believed that these end plate spikes occurred as a result of injury or irritation of the muscles fibers due to the electrodes. Recent experiments have shown that these end plate spikes are actually caused by muscle spindles and have two distinct patterns: small and large. Small end plate spikes have a negative onset without signal propagation and large end plate spikes resemble motor unit potentials (MUPs). Muscle spindles are sensory receptors that measure muscle elongation or stretch and relay the information to the spinal cord or brain for the appropriate response.

Threshold potential ("All or nothing"): When an action potential causes the release of many acetylcholine vesicles, each small depolarization (MEPP) sums together. This summation of MEPPs leads to a greater depolarization of the postsynaptic membrane and become an end plate potential. When the membrane reaches a certain value of depolarization (-65mV), the voltage gated ion channels in the postsynaptic membrane open causes in influx of sodium ions and a sharp spike in depolarization. This spike causes an action potential to occur and propagate down the postsynaptic membrane leading to muscle contraction. In a normal muscular contraction, approximately 35 acetylcholine vesicles are released causing a depolarization that is 35 times greater in magnitude than a MEPP. This causes the membrane potential to depolarize +35mV from -100mV to -65mV where it reaches threshold.

Every action potential leads a quantal release?
A stochastic process, which can vary widely from synapse to synapse and can be modified.

Variations in synaptic vesicle content? the quantal size variation

The quantal release of neurotransmitters must be

Localized and rapid
Repeatable at high frequencies
Amenable to up- or down-regulation over time

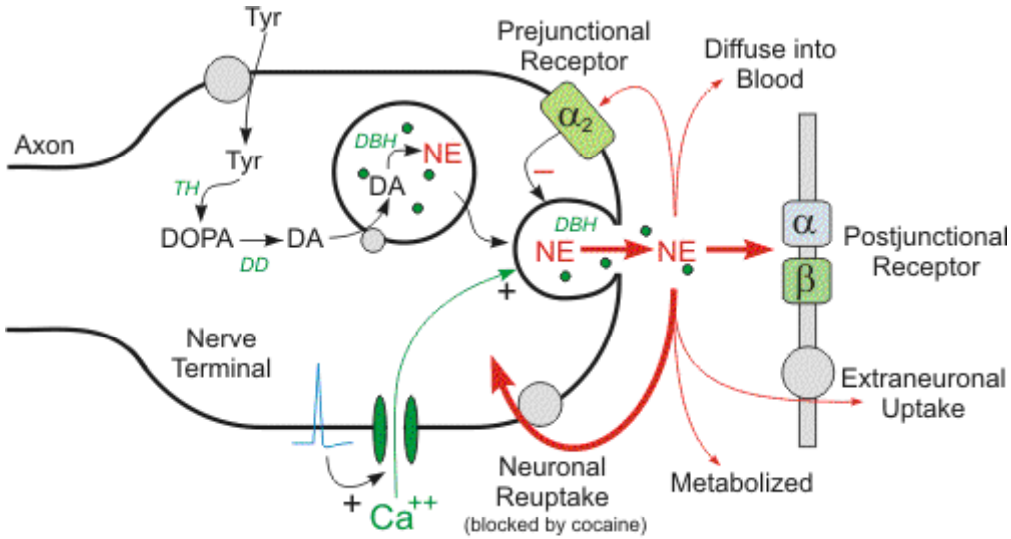
Exocytosis must be precisely coordinated
with the influx of Ca^{2+}
induced by depolarization of the nerve terminal

Terminals must be capable of sustained firing and neurotransmitter release
because neurons often involves repeated trains of stimuli

Finally, exocytosis must be a highly regualted process to accommodate
the neural plasticity that underlies learning and memory

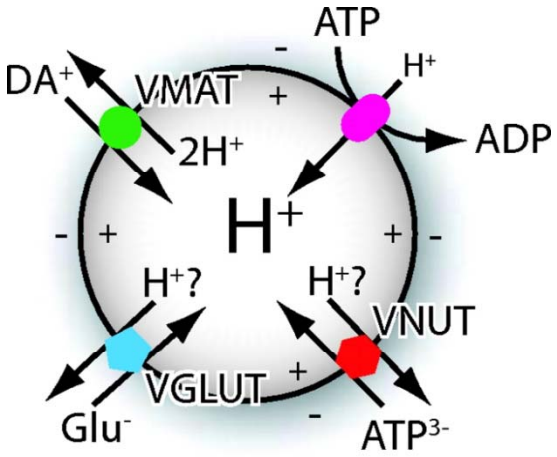
3. Packaging and transport of neurotransmitters

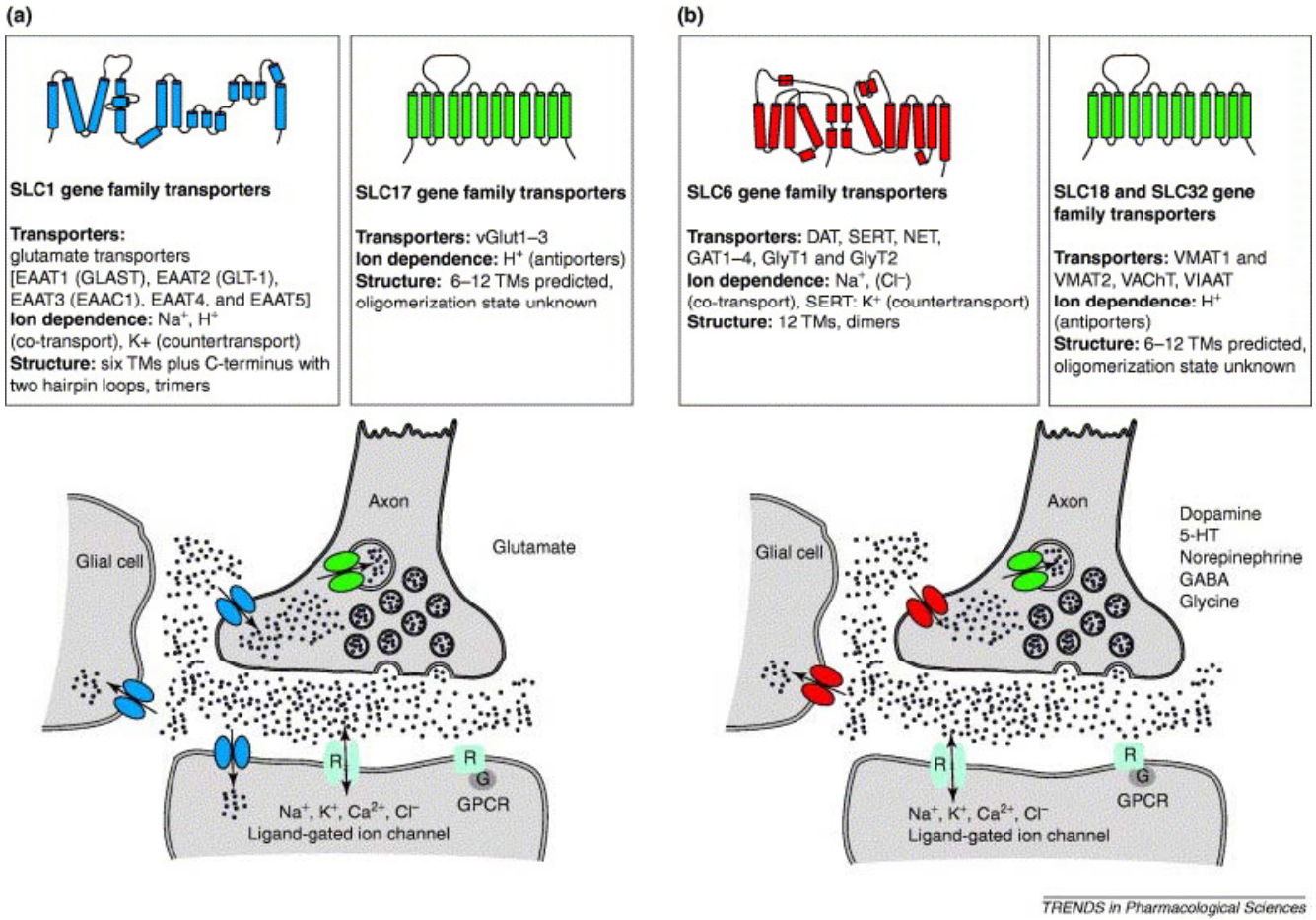
Vesicular ATP transport is a hard (V)NUT to crack.
Schematic depiction of how ATP-dependent proton pumping drives vesicular accumulation of dopamine (DA) through VMAT, glutamate through VGLUT, and ATP through VNUT
PNAS (2008) 105:5949-5950



Tyr = tyrosine; TH = tyrosine hydroxylase; DD = DOPA decarboxylase;
DA = dopamine; DBH = dopamine β-hydroxylase; NE = norepinephrine

Electrochemical gradient for neurotransmitter transport into vesicle





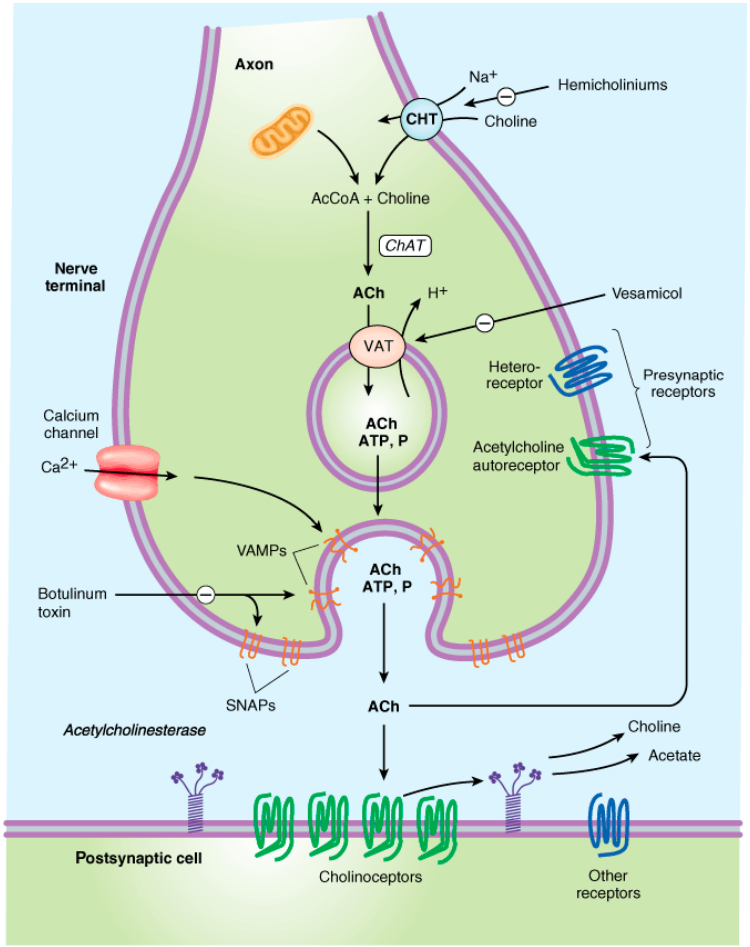
The neurotransmitter transporter families. (a) In the presynaptic nerve terminals of glutamate-containing synapses, vesicular transporters belonging to the SLC17 gene family [vesicular glutamate transporter (vGlut)1–3] (green) sequester glutamate into synaptic vesicles, enabling subsequent vesicular release into the synaptic cleft. Released glutamate exerts its effects via ionotropic glutamate receptors such as NMDA and AMPA receptors (light blue) or via GPCRs (light blue, with associated G protein in gray). The glutamate transporters responsible for removing released glutamate from the extracellular space belong to the SLC1 family (EAAT1–5) (blue) and are present in the plasma membrane of the presynaptic membrane, of adjacent glial cells and of the postsynaptic membrane. (b) In the presynaptic nerve terminals of dopamine-, 5-HT-, norepinephrine-, glycine- and GABA-containing synapses, vesicular transporters belonging to the SLC18 [VMAT1 and VMAT2, and VAChT] and SLC32 [vesicular inhibitory amino acid transporter (VIAAT)] gene families (green) sequester neurotransmitters into synaptic vesicles [3]. Released neurotransmitter exerts its effects via ionotropic receptors such as GABAA receptors, glycine receptors and 5-HT3 receptors (light blue) or via G-protein-coupled receptors (GPCRs) such as dopamine receptors, adrenoceptors, 5-HT receptors and metabotropic GABAB receptors (light blue, with associated G protein in gray). The plasma membrane transporters responsible for removing neurotransmitter from the synaptic cleft belong to the SLC6 gene family (red) and are located in the membrane of the presynaptic neuron (DAT, SERT, NET, GlyT2, GAT-1 and GAT-2) or in the membrane of glial cells (GlyT1, GAT-1, GAT-2 and GAT-3) [3]. TIPS (2006) 27:375-383.

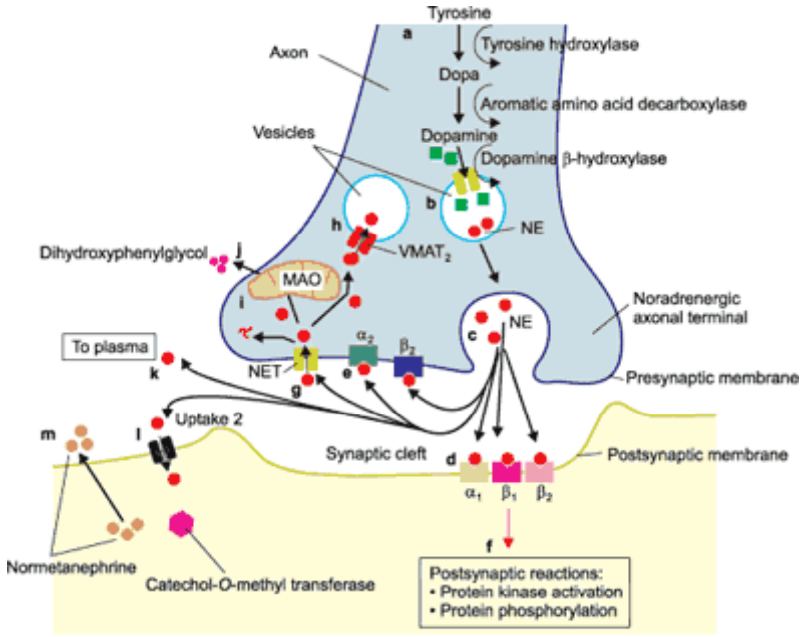
4. Termination of neurotransmitter action: inactivation of neurotransmitters

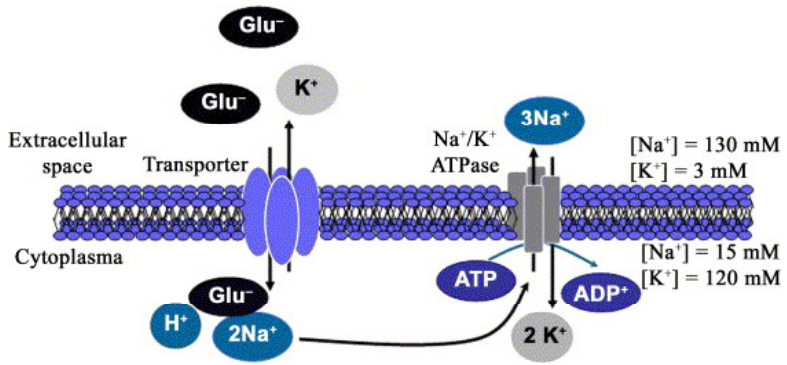
Uptake: plasma membrane transporters (different cellular localization)

Degradation

Diffusion

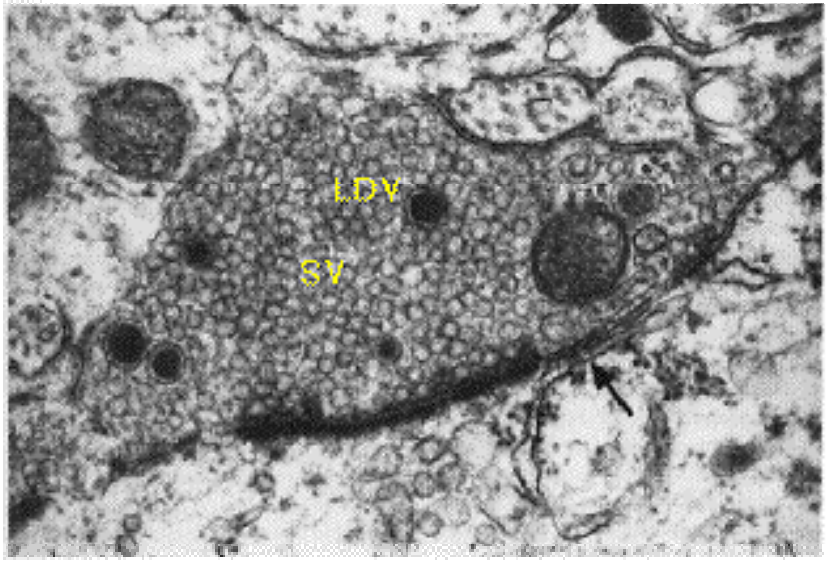


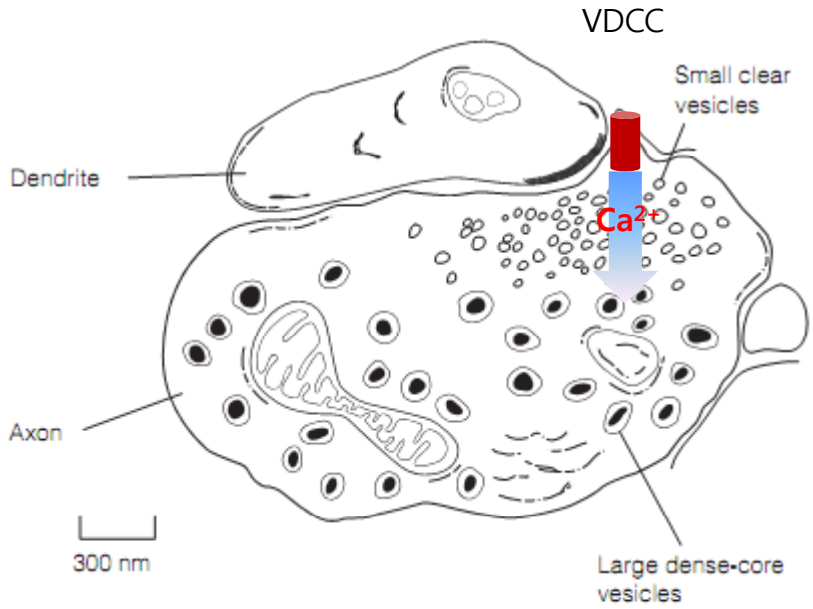




Na⁺ gradient for transport

5. Synaptic vesicles (SV) and large dense core vesicles (LDCV)





Response to different conc of Ca^{2+}

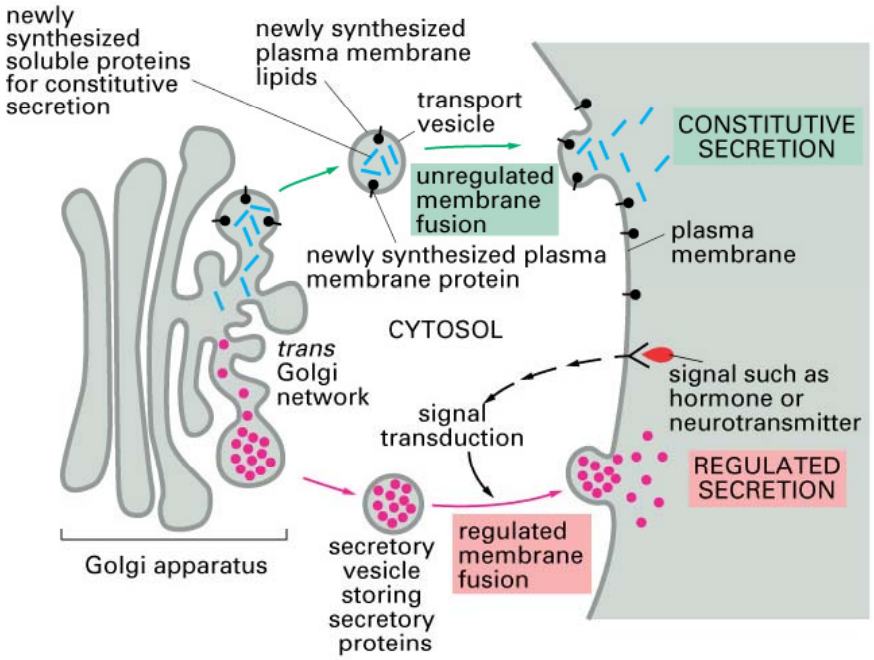
Clear, spherical, small synaptic vesicles (SSVs), 50 nm across, store neurotransmitter, and are scattered all through the terminal in relationship with microtubules which transport them to the presynaptic membrane. Several synapses lack obvious particular contact zones on both pre- and postsynaptic sides and have extremely wide (100–500 nm) synaptic clefts. These frequently secrete a catecholamine and have large dense-core vesicles (LDCVs) 40–120 nm across. The LDCVs are also found in peptide-secreting neurons.

Most synapses hold both SSVs and LDCVs as shown in figure. This structural evidence supports physiological studies displaying that many neurons secrete more than one transmitter.

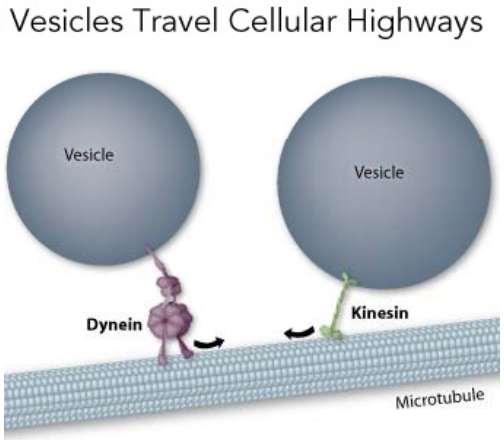
6. Vesicle biosynthesis and transport

Synthesis
SVs: constitutive secretory vesicles
fusion with the plasma membrane
endocytosis

LDCVs: budding directly from the TGN

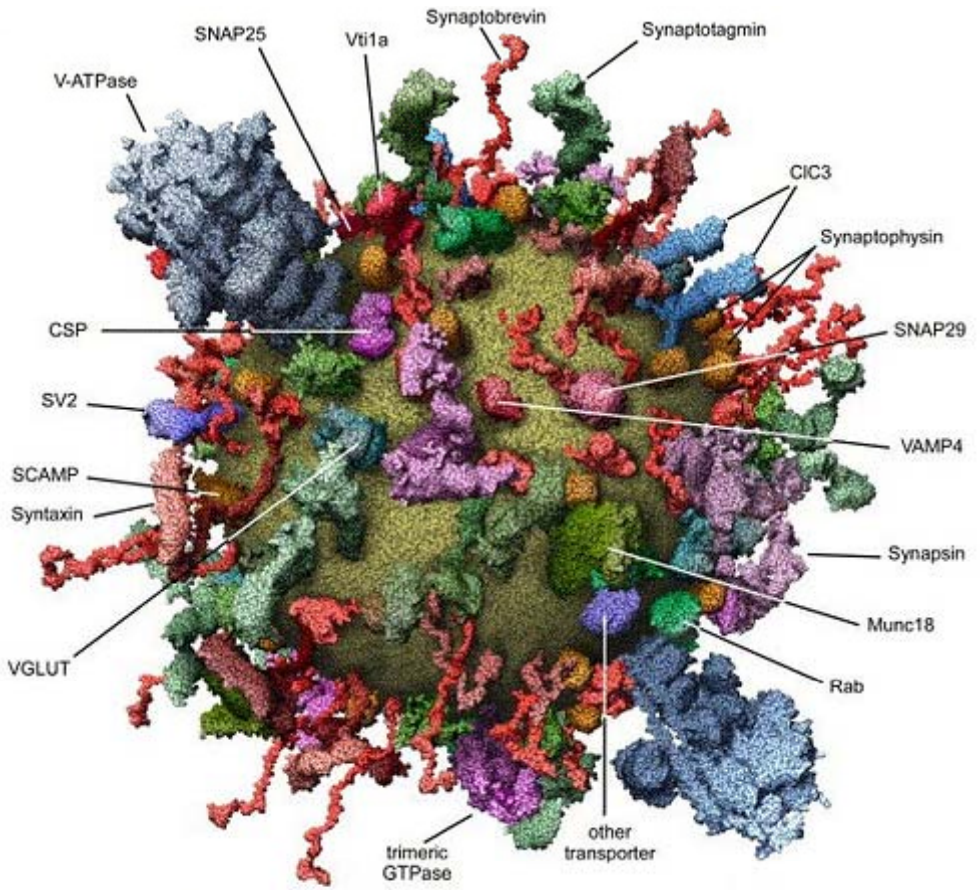


Transport using molecular motors
Anterograde transport: kinesin
Retrograde transport: dynein



Molecular model of a synaptic vesicle

Takamori et al. (2006). Molecular anatomy of a trafficking organelle. Cell, 127, 831-846

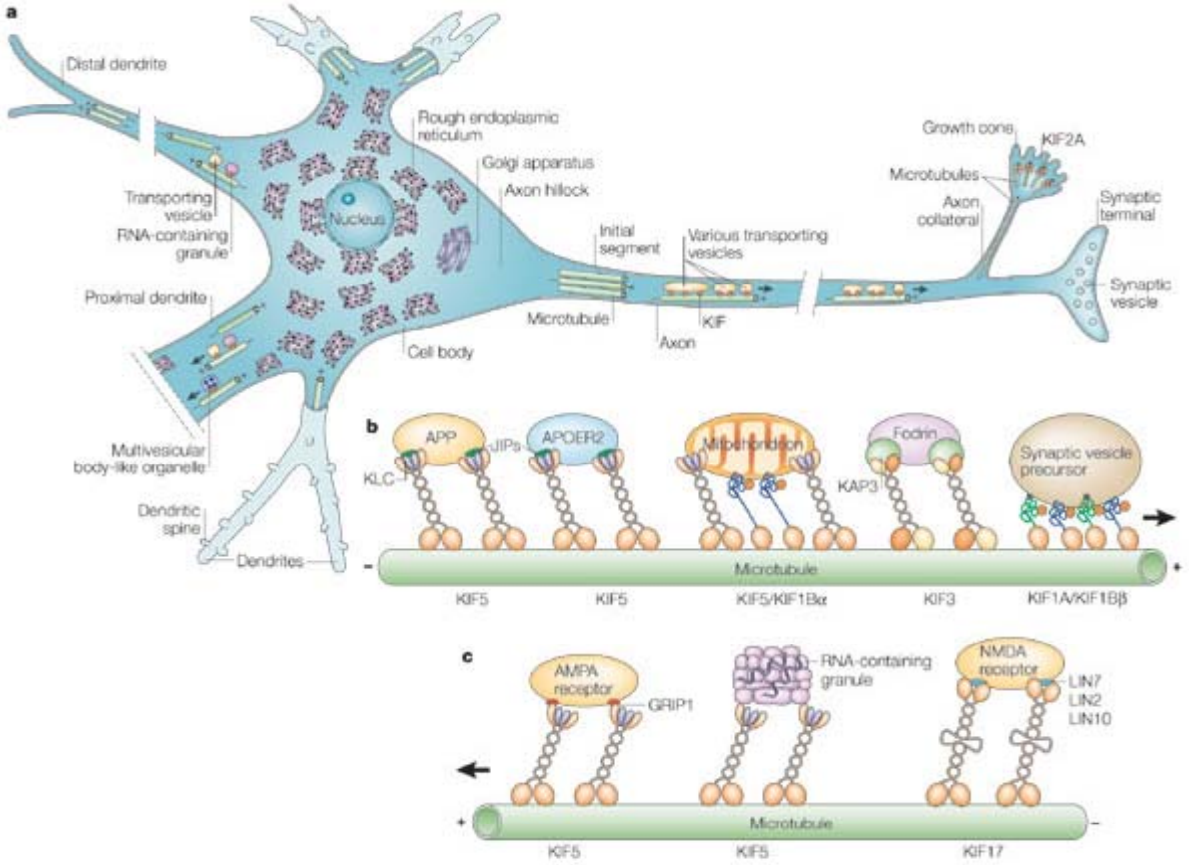


Synaptic vesicles contain two classes of obligatory components: transport proteins involved in neurotransmitter uptake, and trafficking proteins that participate in synaptic vesicle exocytosis, endocytosis, and recycling.

Transport proteins are composed of proton pumps that generate electrochemical gradients, which allow for neurotransmitter uptake, and neurotransmitter transporters that regulate the actual uptake of neurotransmitters. The necessary proton gradient is created by V-ATPase, which breaks down ATP for energy.

Trafficking proteins are more complex. They include intrinsic membrane proteins, peripherally bound proteins, and proteins such as SNAREs. These proteins do not share a characteristic that would make them identifiable as synaptic vesicle proteins, and little is known about how these proteins are specifically deposited into synaptic vesicles. Many but not all of the known synaptic vesicle proteins interact with non-vesicular proteins and are linked to specific functions. http://en.wikipedia.org/wiki/Synaptic_vesicle

Kinesin superfamily proteins (KIFs) and cargoes for axonal and dendritic transport

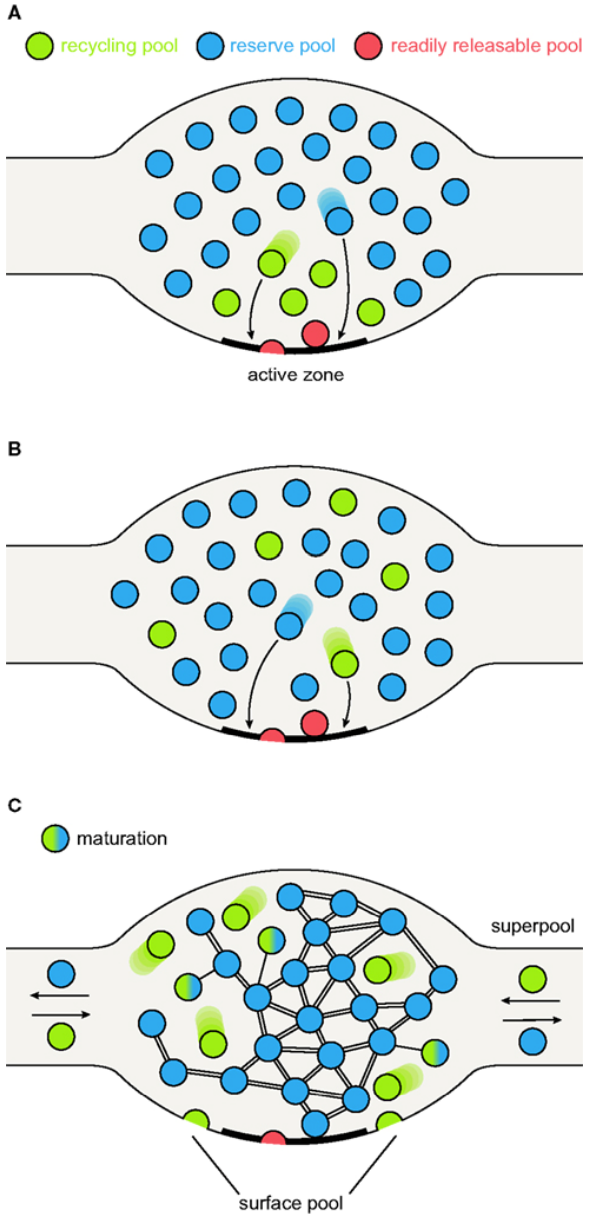


A typical neuron, extending several dendrites (left) and a single thin axon (right) from the cell body. In the axon, microtubules are unipolar, with the plus ends pointing towards the synaptic terminal. Microtubules form special bundles at the initial segment, which might serve as the cue for axonal transport. Tubulovesicular organelles are transported anterogradely along microtubules by KIFs. In the growth cone of an axon collateral, KIF2A controls microtubule dynamics and the extension of collaterals. Rough endoplasmic reticula are abundant in most parts of the cell body, except for the axon hillock. Dendrites contain some rough endoplasmic reticlula. Microtubules have mixed polarity in proximal dendrites, but are unipolar in distal dendrites, with the plus end pointing away from the cell body. Membranous organelles and RNA-containing granules are transported along microtubules by KIFs. b | KIF5 transports vesicles containing APP (amyloid precursor protien) and APOER2 (apolipoprotein E receptor 2) by interacting with KLC (kinesin light chain). Mitochondria are transported by KIF5 and KIF1B27, 45. KIF3 transports vesicles associated with fodrin57. KIF1A and KIF1B both transport synaptic vesicle precursors. JIPs, scaffolding proteins of the c-Jun amino (N)-terminal kinase (JNK) signalling pathway; KAP3, kinesin superfamily-associated protein 3. c | In dendrites, KIF5 transports vesicles containing AMPA (-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors through an interaction between KIF5 and GRIP1 (glutamate receptor-interacting protein 1). RNA-containing granules are also transported by interacting directly with KIF5. KIF17 transports vesicles containing NMDA (N-methyl-D-aspartate) receptors by interacting through the LIN complex, a tripartite protein complex containing mammalian homologues of the Caenhorhabditis elegans presynaptic density zone (PDZ) proteins LIN-2, LIN-7 and LIN-10. Nature Rev Neurosci (2005) 6:201-214.

7. Segregation of vesicles

Readily releasable pools (RRP): to be released at a regular rate in response to electrical stimulation

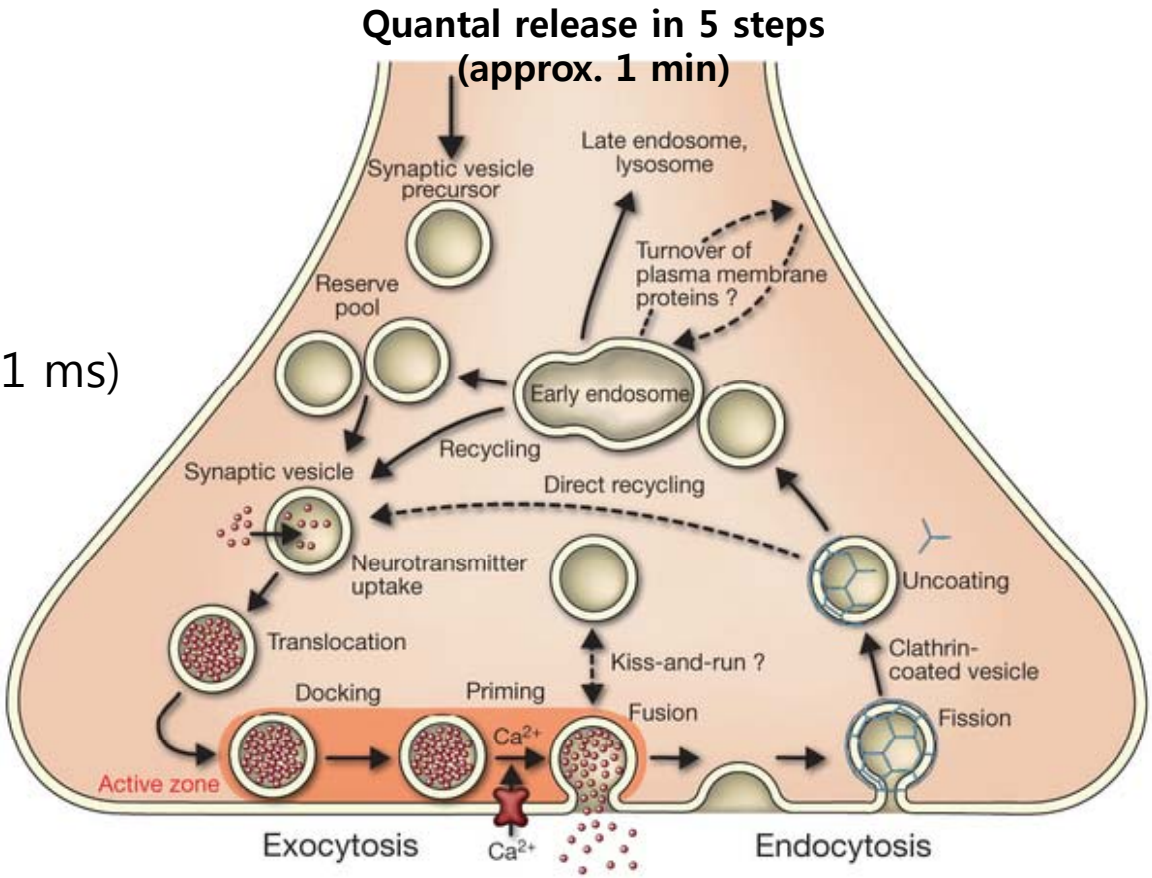
Storage pools



Synaptic vesicle pool models. (A) The classical model of three distinctly localized synaptic vesicle pools. The readily releasable pool (RRP) consists of the vesicles docked at the active zone and primed for release. After depletion of the RRP, the recycling pool vesicles come into play: these vesicles were thought to be located directly behind the RRP. Under moderate stimulation conditions, they are recruited to the active zone (left arrow) and released. Very high stimulation causes the depletion of the recycling pool and recruits the reserve pool vesicles from areas even further away from the active zone (right arrow).
(B) A pool model taking into account the spatial intermixing of vesicles (Rizzoli and Betz, 2005). In contrast to the previous model, in which localization within the nerve terminal determines release kinetics and consequently pool affiliation, here the recycling and reserve pool vesicles are thought to be spatially (but not functionally) intermixed. Upon arrival of an action potential, RRP vesicles (which are in this model only the "lucky" recycling pool vesicles finding themselves docked and primed at the active zone) are released first, followed by release of recycling pool vesicles (right arrow). As above, continuous stimulation at high-frequency results in recycling pool depletion and recruitment of reserve pool vesicles (left arrow).
(C) A new pool model implementing recent findings. As above, recycling and reserve pool vesicles are thought to be spatially intermixed, but display different mobilities: recycling pool vesicles are highly mobile; the movement of reserve pool vesicles is restricted by binding to some scaffolding molecule. With time, recycling pool vesicles can "mature" into reserve pool vesicles, by binding the scaffolding molecule(s) and integrating into the vesicle cluster, as indicated by the green-blue intermediate forms. These are not connected to the cluster as tightly as the reserve pool vesicles (indicated by single or double bonds). Recycling pool vesicles reach the active zone, due to their permanent mobility; stimulation does not "move" them toward the active zone, it just allows them to fuse. Furthermore, the surface pool of fused vesicles is indicated; they would be endocytosed to form part of the recycling pool (see main text). The frequent exchange of both recycling and reserve vesicles between synapses forms what has been termed a super-pool (see main text). **Synaptic vesicle pools: an update (2010)** Front. Synaptic Neurosci.

III. Biochemistry of neurotransmitter release: the exocytotic cycle

Docking
Priming
Fusion/exocytosis (<1 ms)
Endocytosis (<5 s)
Recycling



Synaptic vesicles are filled with neurotransmitter and stored in the cytoplasm. Active vesicles are translocated to release sites in the active zone where they dock. Priming involves all steps required to acquire release readiness of the exocytotic complex. Although usually assumed to occur after docking, priming and even triggering may precede docking during sustained activity, resulting in immediate fusion of an arriving vesicle. After exocytosis, the vesicle proteins probably remain clustered and are then retrieved by endocytosis. Despite some lingering controversies, consensus is emerging that retrieval is generally mediated by clathrin-mediated endocytosis. After clathrin uncoating, synaptic vesicles are regenerated within the nerve terminal, probably involving passage through an endosomal intermediate. Actively recycling vesicles are in slow exchange with the reserve pool. Nature (2012) 490:201-207.

1. Proteins involved in the exocytotic cycle
   (the precisely defined molecular events)

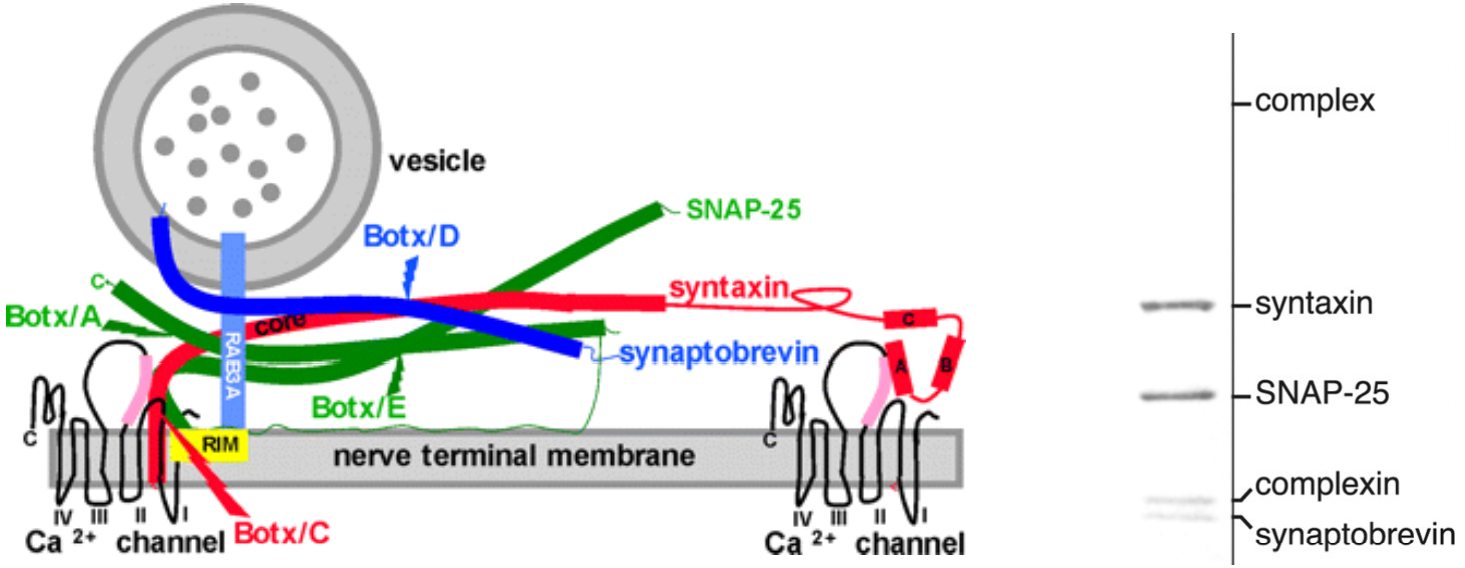
A. The function of SNAREs: targeting or fusion?
B. Disassembly of the SNAREs by NSF
C. Synaptotagmin: A Ca^{2+} sensor?
D. Regulating SNAREs: SECs and Muncs
E. Regulation of storage pools: synapsin isoforms
F. Regulation of vesicle exocytosis by Rab3a

The SNARE complex

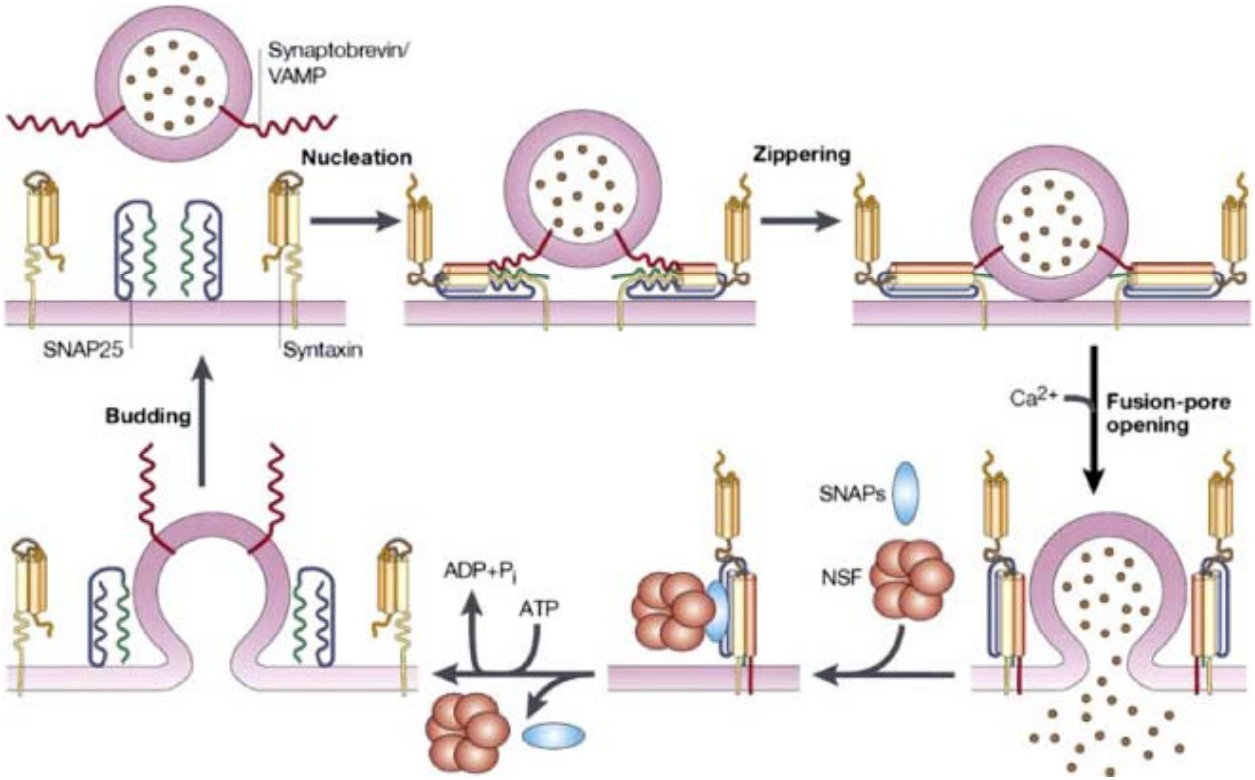
SNARE: SNAP (Soluble NSF Attachment Protein) **RE**ceptor"
Members of a large protein superfamily with more than 60 members

v-SNARE: Synaptobrevin (18 kDa) (VAMP, vesicle-associated membrane protein)
t-SNARE: Syntaxin (35 kDa), SNAP-25 (synaptosomal associated protein of 25 kDa)

NSF (N-ethylmaleimide-sensitive factor): a homohexameric AAA ATPase
involved in membrane disassembly
AAA ATPases: ATPases associated with diverse cellular Activities

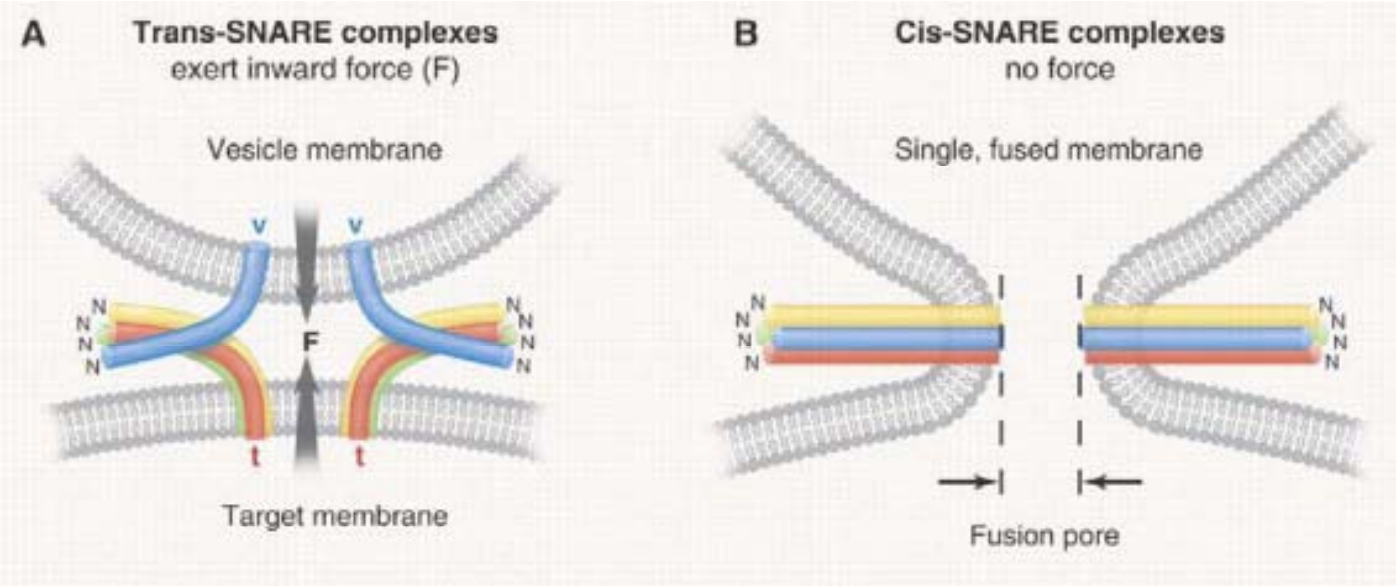


NSF and SNAPs for disassembly of the SNAREs



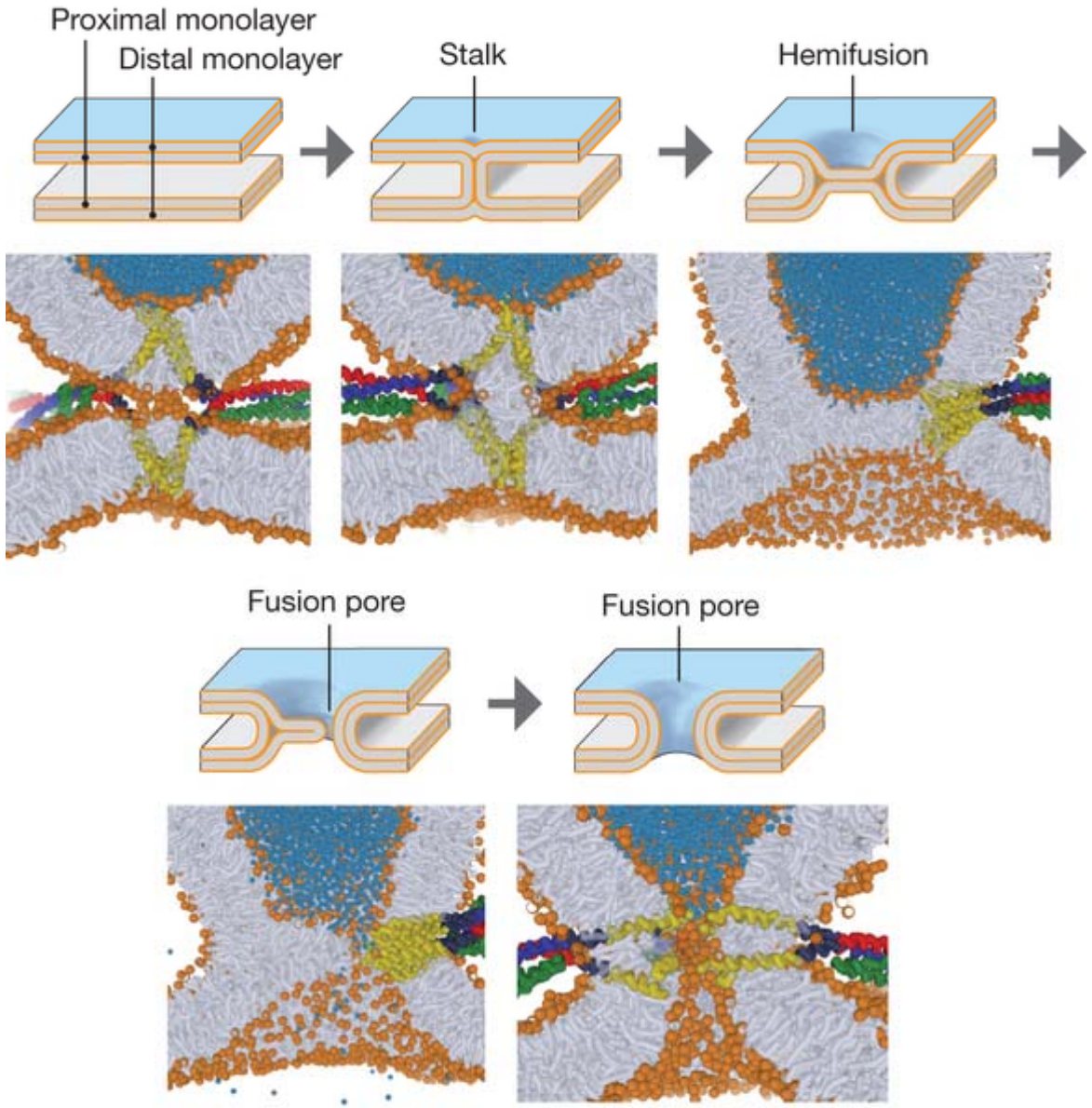
Syntaxin exists in a closed conformation that needs to open to initiate core-complex assembly (nucleation). "Zi ppering" of the four-helix bundle towards the carboxyl terminus brings the synaptic vesicle and plasma memb ranes towards each other, which might lead to membrane fusion. After fusion, *N*-ethylmaleimide-sensitive fusi on protein (NSF) and soluble NSF-attachment proteins (SNAPs) disassemble the cis-core complexes that remai n on the same membrane to recycle them for another round of fusion.

The discovery of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor complex and the molecular regulation of synaptic vesicle transmitter release: the 2010 Kavli Prize in neuroscience. Neurosci. (20 11) 190,12–20



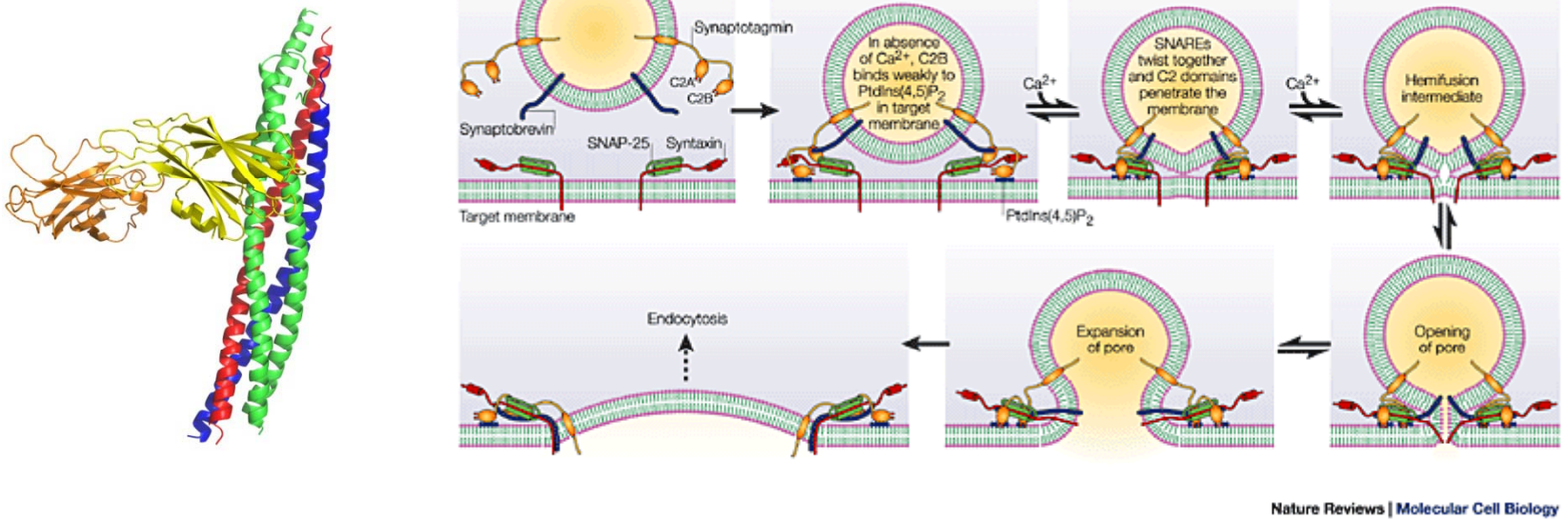
The zippering model for SNARE-catalyzed membrane fusion. Three helices anchored in one membrane (th e t-SNARE) assemble with the fourth helix anchored in the other membrane (v-SNARE) to form trans-SNARE complexes, or SNAREpins. Assembly proceeds progressively from the membrane-distal N termini toward the membrane-proximal C termini of the SNAREs. This generates an inward force vector (F) that pulls the bilayers together, forcing them to fuse. Complete zippering is sterically prevented until fusion occurs, so that fusion a nd the completion of zippering are thermodynamically coupled. (B) Therefore, when fusion has occurred, the force vanishes and the SNAREs are in the low-energy cis-SNARE complex.

Transition states during membrane fusion



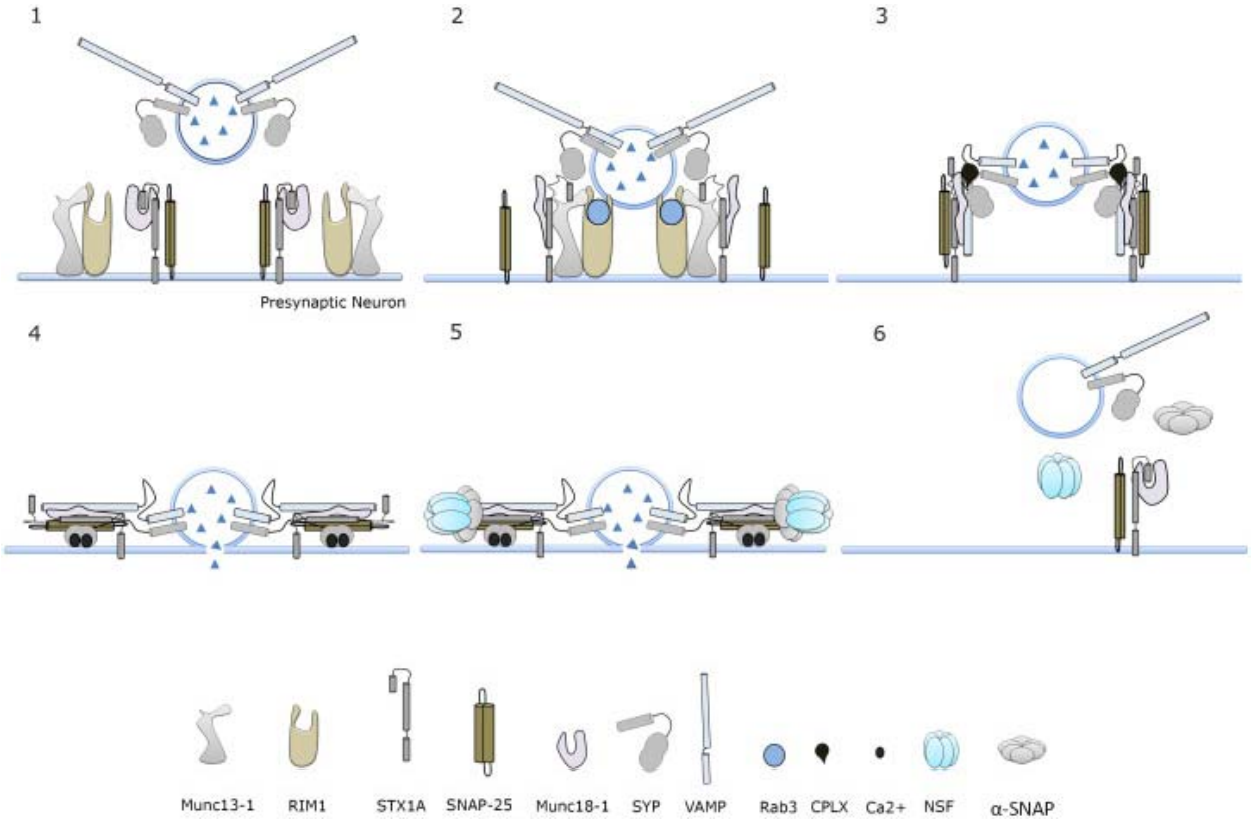
Intermediates of the fusion pathway. The top drawings represent intermediate states of the membrane along the pathway as predicted by the elastic theory. Below, snapshots of intermediate states of a simulation of SNARE-mediated fusion are shown, which, although roughly corresponding to the elastic model, differ in detail and in their energy predictions

How exocytotic machinery senses Ca^{2+}: synaptotagmin



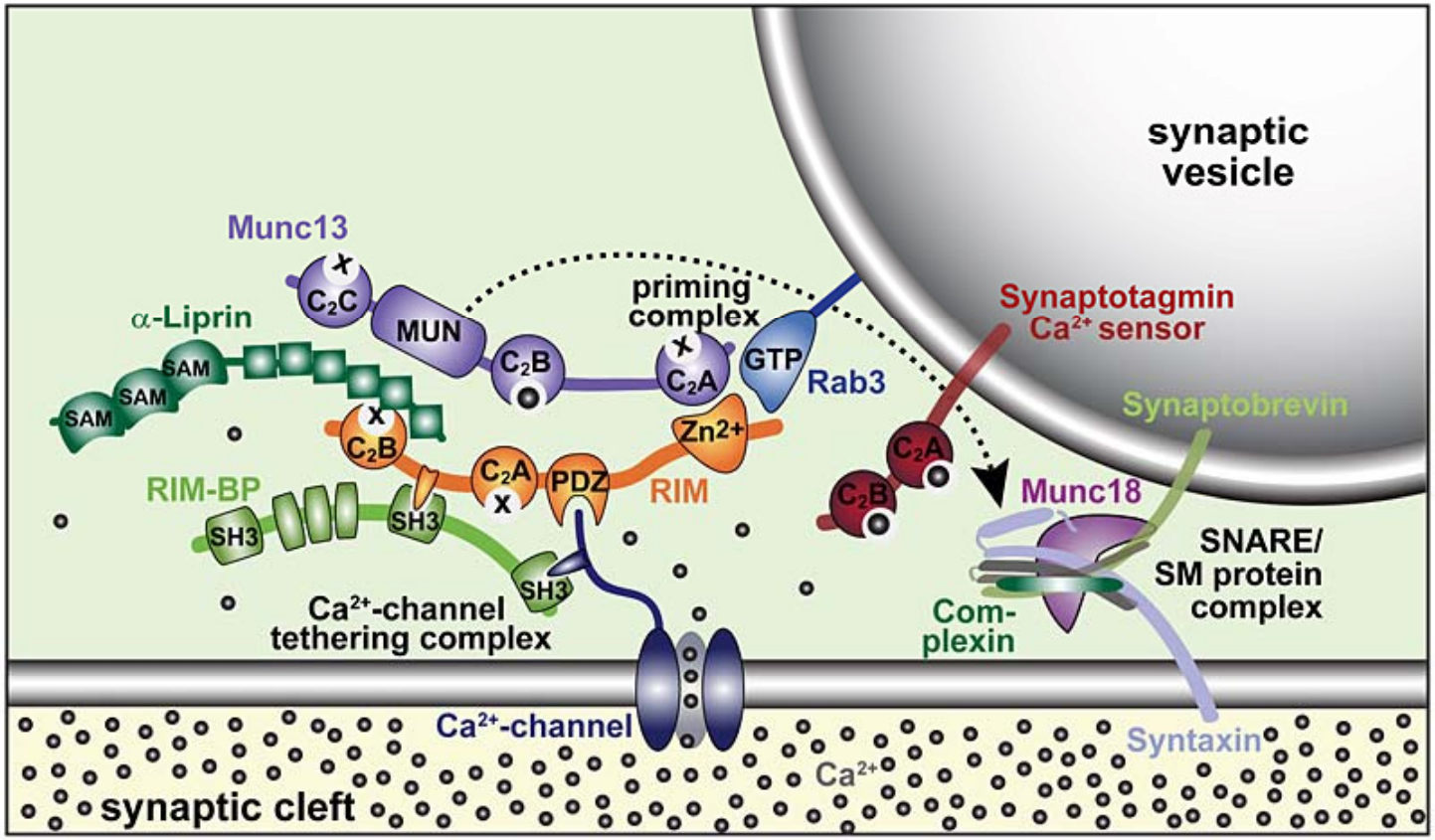
A cutaway view of the putative fusion 'machine', which is composed of synaptotagmin, SNAREs (synaptobrevin, syntaxin and SNAP-25) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), is shown. Other factors clearly have roles in exocytosis but are not considered here[1]. The fusion pore might be composed of phospholipids, th e membrane anchors of the SNARE proteins, or a combination of both. Nature Reviews Molecular Cell Biology 3, 498-508 (July 2002)

More regulators for SNARE function: Muncs



Schematic representation of regulatory components of the SNARE complex in the control of the neurotransmitter release through synaptic vesicle docking. (1) STX1A is in a close conformation due to its binding to Munc 18-1, anchored to the presynaptic membrane. (2) Rab3 catalyzes Munc 18-1/STX1A complex segregation that favors a STX1A open conformation and as a consequence the Rab3/RIM1/Munc13-1 complex is formed to contribute to synaptic vesicle fusion. (3) STX1A and SNAP-25 form the t-SNARE complex that interacts with the v-SNARE complex (SYP, VAMP and synaptic vesicle). Complexin acts on the regulation of synaptic vesicle release. (4) Membrane-vesicle fusion and the neurotransmitter release to the synaptic cleft is triggered by the increase of local Ca2+ concentration. (5 and 6) NSF and α-SNAP contribute to the disintegration of the SNARE complex with ATP hydrolysis. Evaluation of common variants in 16 genes involved in the regulation of neurotransmitter release in ADHD. European Neuropsychopharm. (2012)

Molecular protein complexes that organize the secretory machinery at the presynaptic active zone

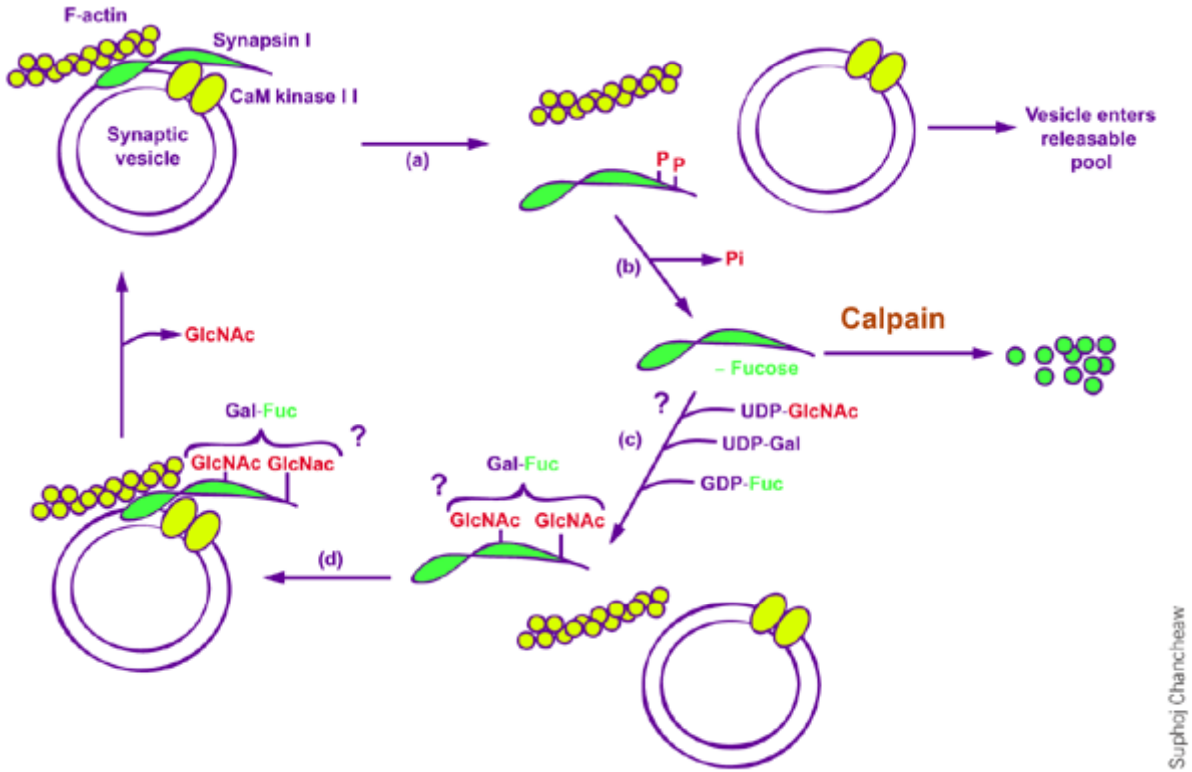


The image depicts the role of RIM proteins in placing the priming factor Munc13 and Ca^{2+}-channels into close proximity to synaptic vesicles and SNARE/SM protein dependent fusion machinery. In addition to Ca^{2+}-channels, RIM proteins directly bind to the vesicle protein Rab3, to the adaptor protein RIM-BP, to the priming factor Munc13, and to the active zone scaffolding protein αlipring (not shown). Munc13 in turn directly activates the SNARE/SM protein assembly. Both RIM and Munc13 proteins are tightly regulated in a manner that determines presynaptic plasticity. Adapted from Kaeser, P.S., et al. 2011. *Cell* 144:282-295.

Regulation of storage pools by synapsin I

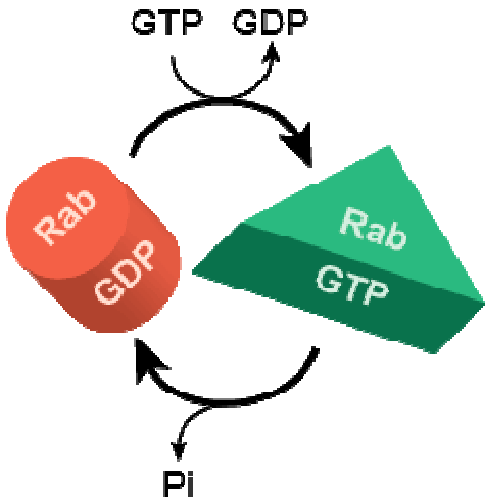
How is the number of vesicles docked at the active zone determined?

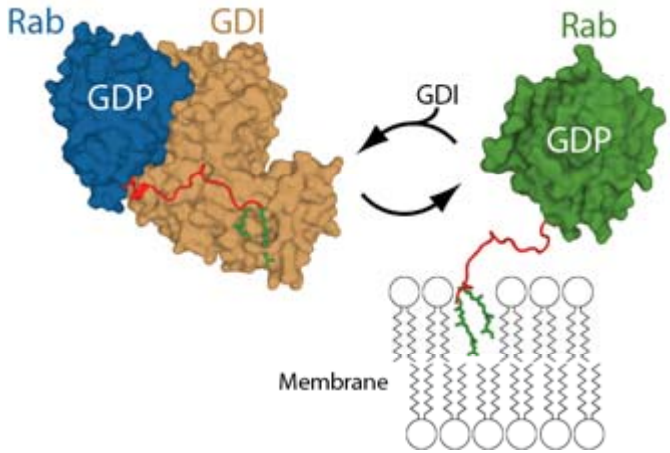
Functions of synapsin I in synaptic vesicle trafficking



Synapsins help tether the vesicles to the actin cytoskeleton. (a) Phosphorylation of synapsins releases the vesicles from the cytoskeleton to the 'releasable pool', where they can rapidly fuse with the plasma membrane, releasing their contents at the synapse upon depolarization of the neuron. (b) Dephosphorylation and prevention of fucosylation allows synapsins to be rapidly degraded by the protease calpain. (c) Fucosylation of synapsins prevents their rapid degradation. Synapsins are both O-GlcNAc10 and Fuc(1-2)Gal modified4 within their 'D' domains, but as indicated by the '?', the order of addition of each modification is unknown. The brackets indicate that the structural relationship between O-GlcNAc and Fuc(1-2)Gal is also undefined. (d) Glycosylated synapsin 1 appears to be involved in tethering vesicles to the cytoskeleton. (e) At some point before phosphorylation, O-GlcNAc (and perhaps Fuc(1-2)Gal residues) are removed. P, phosphate; Gal, galactose; Fuc, fucose; Pi, inorganic phosphate; UDP-GlcNAc, uridine diphospho-N-acetylglucosamine; UDP-Gal, uridine diphospho-galactose; GDP-Fucose, guanidine diphosphofucose; GlcNAc, N-acetylglucosamine. Nature Chemical Biology 2, 67 - 68 (2006)

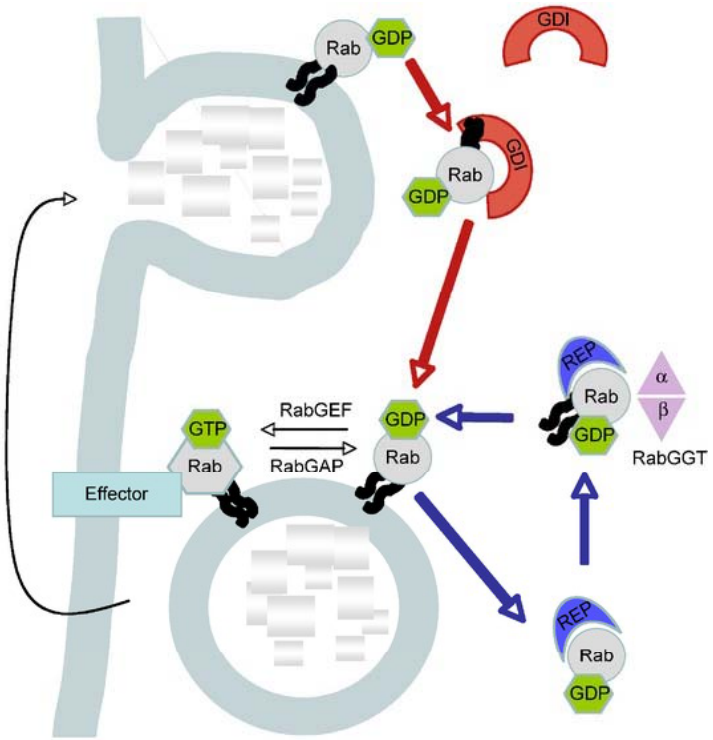
Regulation of vesicle exocytosis by Rab3a



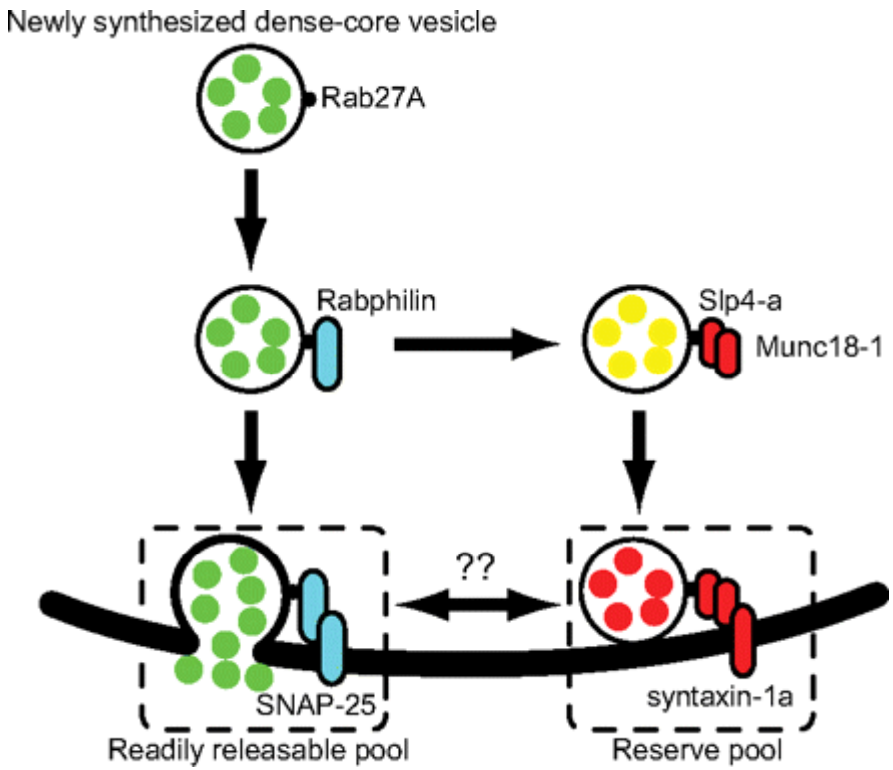


One of the major classes of proteins that control membrane traffic are Rab proteins. These proteins are bound to a nucleotide and change conformation (or shape) according to whether this nucleotide is GDP or GTP. A fascinating feature of Rab proteins is that they are covalently modified at their COOH-terminus with two geranylgeranyl lipid modifications.

This modification enables the protein to associate with lipid bilayers. The GDP-bound Rab can partition into the cytosol with the aid of a chaperone protein called GDI (GDP dissociation inhibitor).
So, in addition to alternating between GDP and GTP-bound forms, Rab proteins also can cycle on and off membranes. http://blogs.cornell.edu/collinslab/research-overview/



The *Rab* protein–GTPase cycles. Rab proteins are intrinsically soluble and require a post-translational modification for membrane association. They first associate with a Rab escort protein (*REP*) and form a stable complex that is the substrate for the subsequent dual prenylation of C-terminal cysteine motifs via Rab geranylgeranyl transferase (*RabGGT*). RabGGT consists of two different functional subunits (RabGGTα and RabGGTß). After lipid tranfer, REP delivers the prenylated Rab to the donor membrane (the REP cycle is shown with *blue arrows*). In the absence of REP or RabGGT, Rab proteins remain in the cytosol in an inactive state. The transfer of Rab proteins between acceptor and donor membranes is facilitated by the GDP dissociation inhibitor (*GDI*) (the GDI cycle is shown with *red arrows*). Both REP and GDI bind the GDP-bound inactive form of Rab. After REP or GDI dissociate from Rabs at the donor membrane, Rabs can cycle between the inactive (GDP-bound) and active (GTP-bound) states. Rab proteins are activated by guanine nucleotide exchange factors (*RabGEFs*) and deactivated by GTPase activating proteins (*RabGAPs*), which accelerate the slow intrinsic rates of nucleotide exchange and GTP hydrolysis. In the active state, Rabs interact with structurally and functionally diverse effectors, including cargo sorting complexes on donor membranes, motor proteins involved in vesicular transport and tethering complexes that regulate vesicle fusion with acceptor membranes

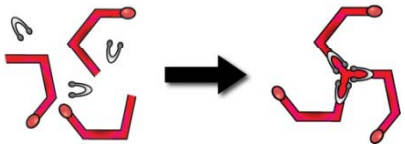


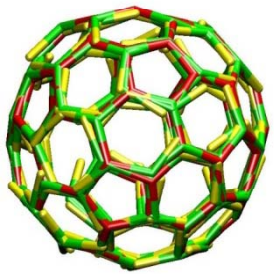
Proposed model for Slp4-a/rabphilin–dependent preferential dense-core vesicle exocytosis in neuroendocrine cells. Rabphilin and Slp4-a promote docking of dense-core vesicles to the plasma membrane through interaction with SNAP-25 and Munc18-1/syntaxin-1a complex, respectively. Dense-core vesicles docked to the plasma membrane by the rabphilin/SNAP-25 complex (blue bars) undergo preferential exocytosis (presumably corresponds to the readily releasable pool), whereas dense-core vesicles docked to the plasma membrane by the Slp4-a/Munc18-1/sytnaxin-1a complex (red bars) do not undergo exocytosis (corresponds to the reserve pool). The molecular switch between readily releasable pool and reserve pool is currently unknown. Green-, yellow-, and red-colored vesicles correspond to newly synthesized vesicles, middle-aged vesicles, and old vesicles, respectively. MCB (2010) 21:87-94.

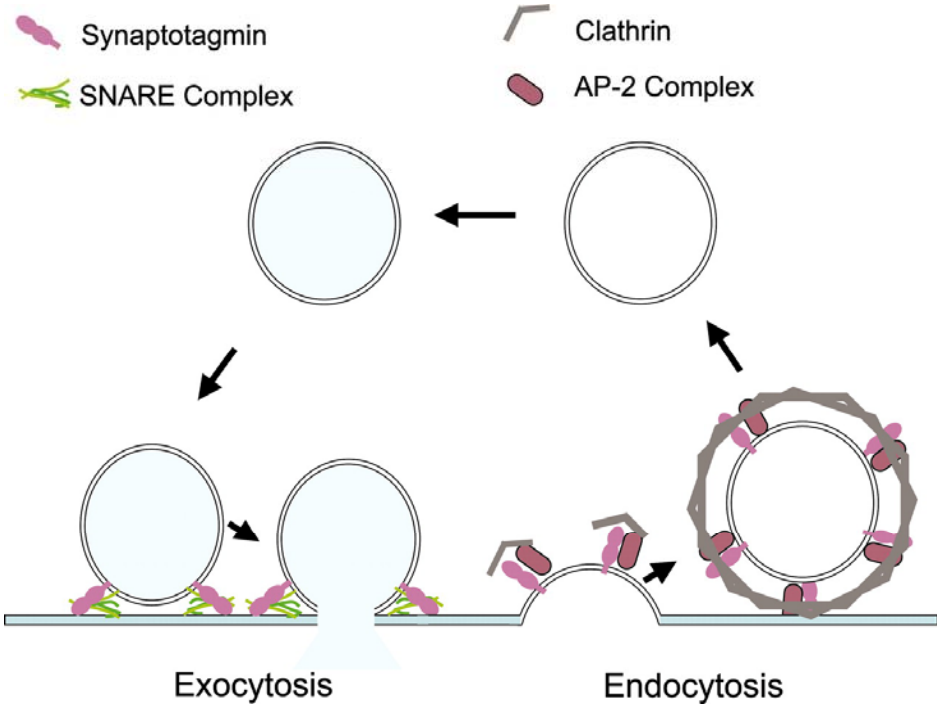
2. Endocytosis and recycling

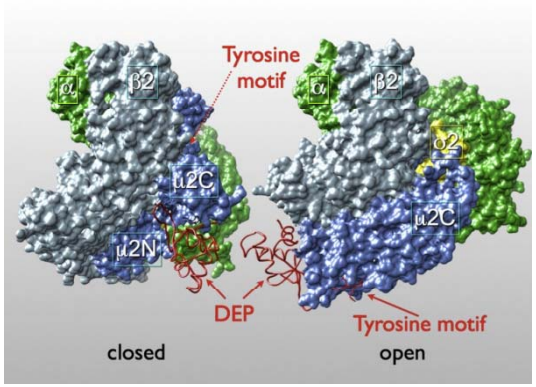
A. Clathrin-mediated endocytosis
B. Endocytotic machinery
C. Regulation of endocytosis
D. Vesicle recycling

Clathrin-mediated endocytosis

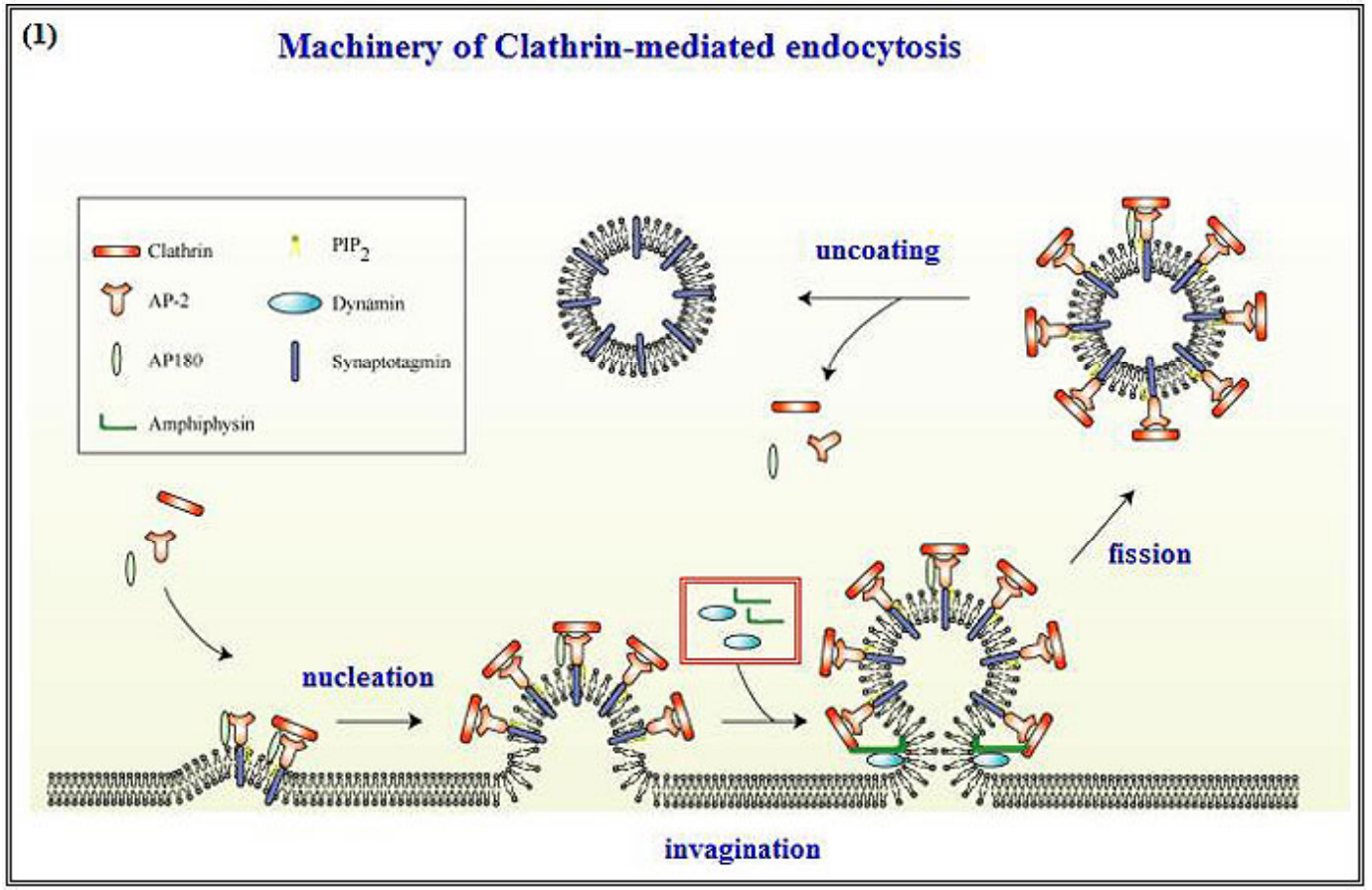






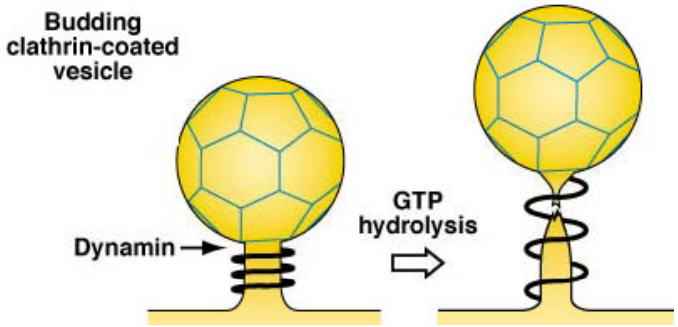


Synaptotagmin coming and going. Vesicles in the nerve terminal are recycled for repeated use. In promoting exocytosis, synaptotagmin interacts with membranes and the SNARE complex, the complex of syntaxin, SNAP-25, and synaptobrevin that likely mediate vesicle fusion. In promoting endocytosis, synaptotagmin interacts with the clathrin adaptor complex AP-2. PNAS (2004) 101:16401-16402

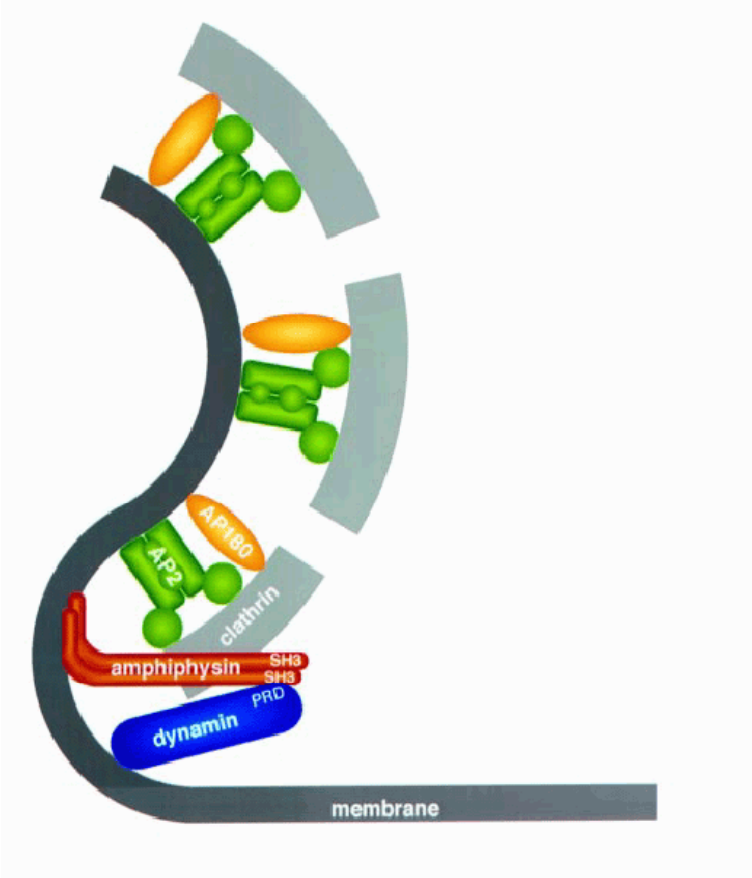


Clathrin-mediated synaptic vesicle endocytosis is divided to four steps: nucleation, invagination, fission and uncoating. Amphiphysin I and dynamin I cooperating on the collar of invaginated vesicle.

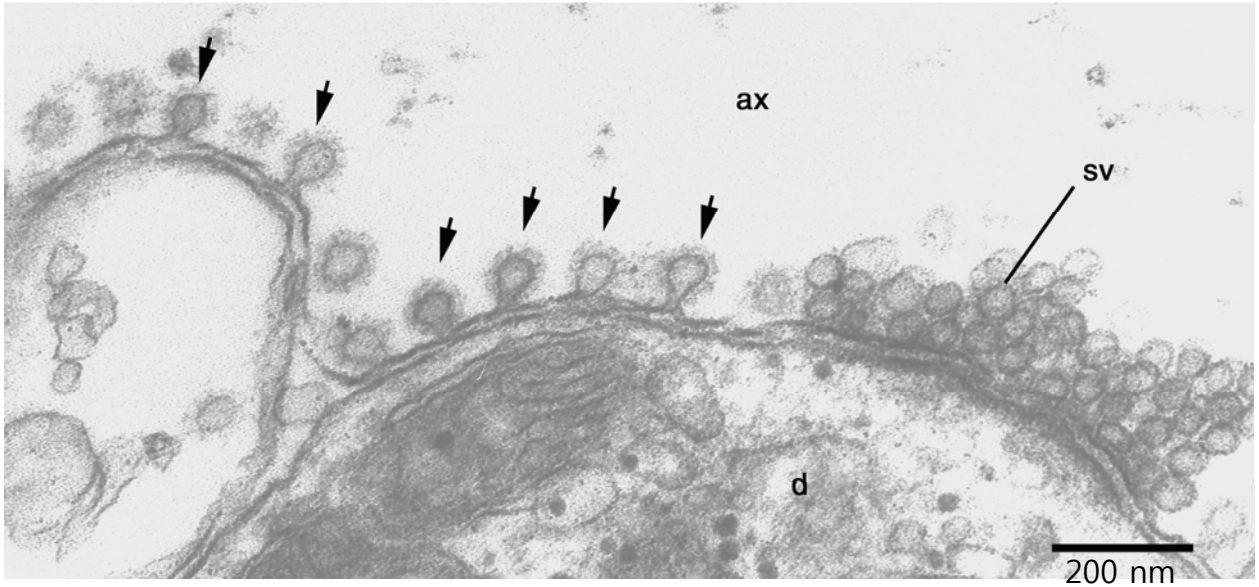
Endocytotic machinery: Dynamin



http://www.endocytosis.org/Endocytosis/CCVAssembly.html



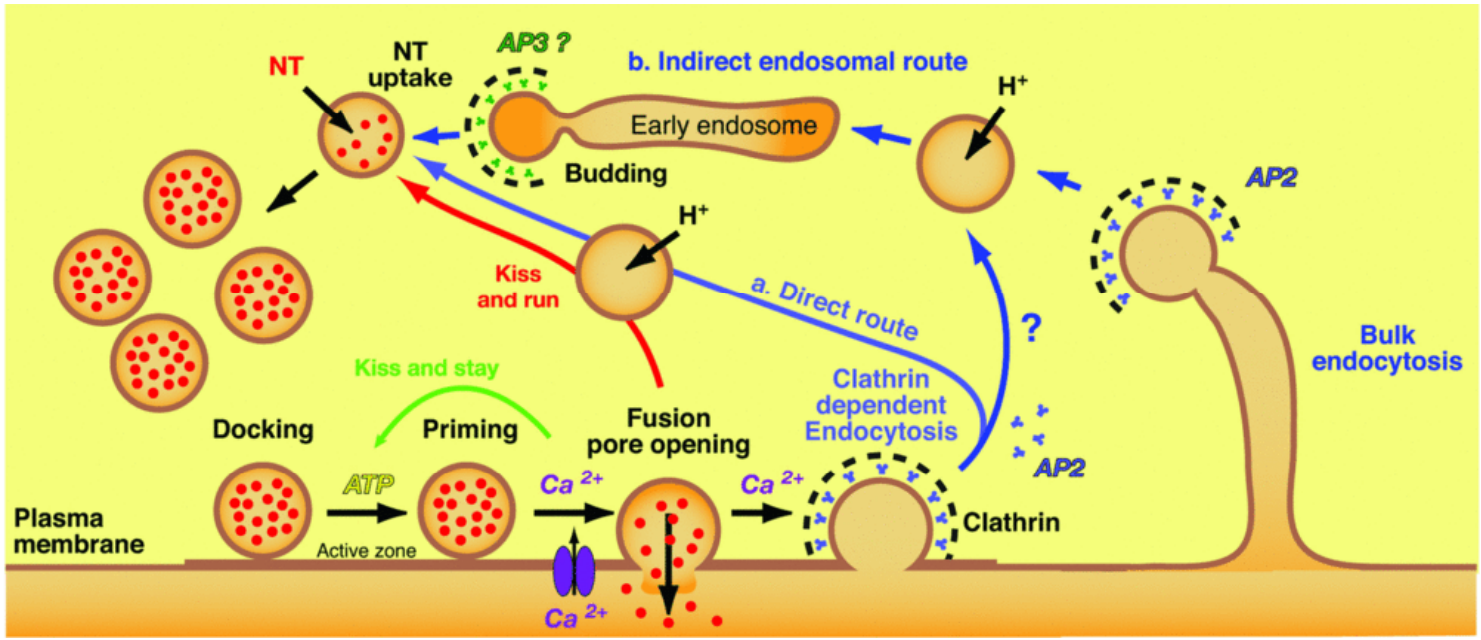
Interactions of the amphiphysin dimer at a clathrin-coated endocytotic pit. Nature Cell Biology 1, 33 - 39 (1999)



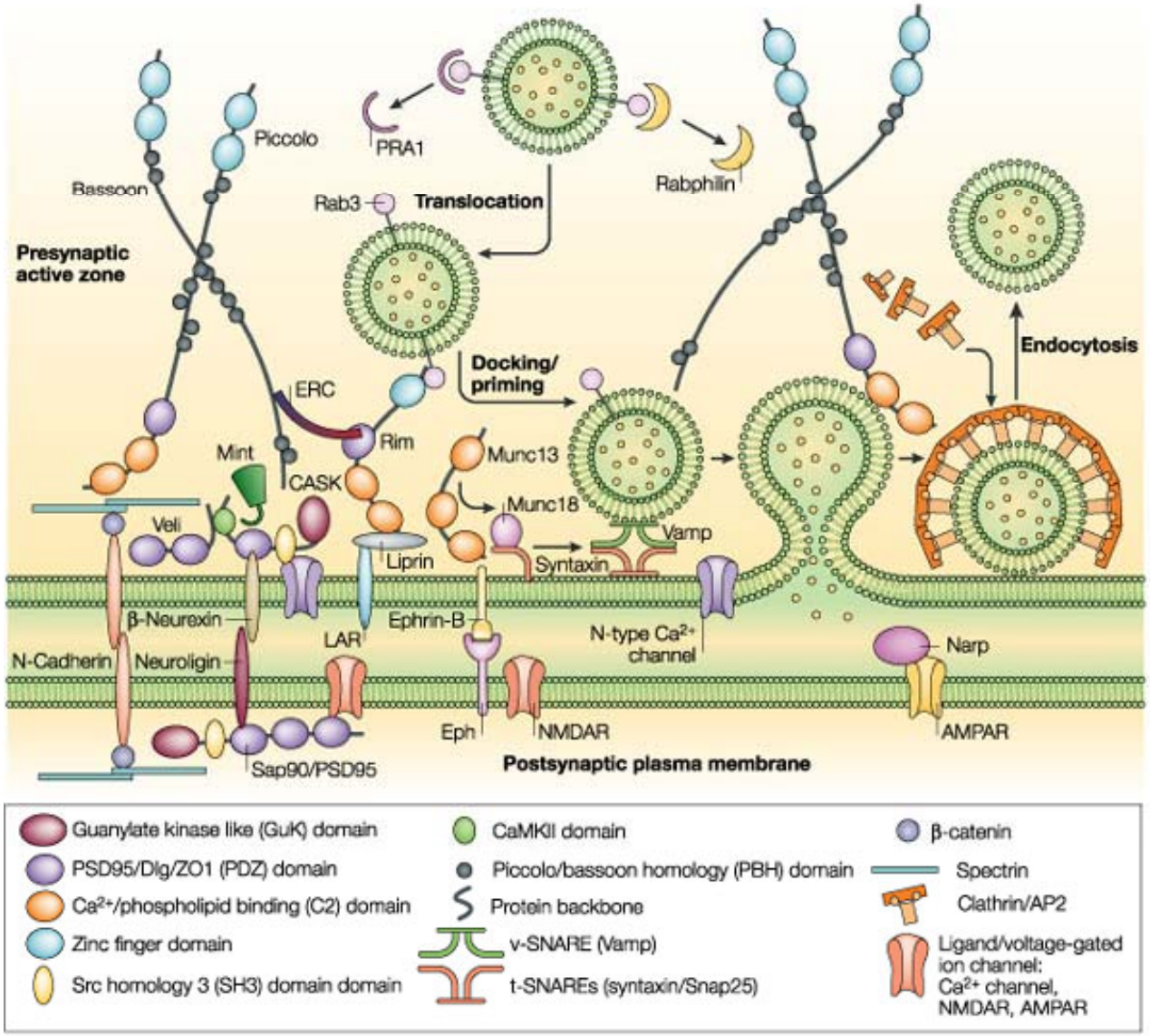
Microinjection of the SH3 domain of amphiphysin traps coated pits with narrow necks

SV cycle at the nerve terminal

Endosomal budding employs AP-3 and does not require clathrin



SVs dock and are primed at the active zone. Upon stimulation, the SV fuses with the membrane allowing for neurotransmitters (NT) to be released in the synaptic cleft. If the SV does not fuse entirely with the membrane, but forms a transient fusion pore, it can be retrieved by the short routes of recycling, i.e. kiss-and-run (in red) and kiss-and-stay (in green) mechanisms. In the kiss-and-run mechanism, the SV is retrieved by endocytosis at the active zone and rapidly refilled with NT. In the kiss-and-stay mechanism the SV is rapidly refilled without leaving the active zone. If the SV fuses completely with the plasma membrane, then it is retrieved by clathrin-dependent endocytosis. Following strong stimulation, bulk endocytosis has been shown to occur at the nerve terminal and to form large membrane invaginations. Budding from the plasma membrane by clathrin-mediated endocytosis and budding on large invaginations were shown to be positive for AP-2 and clathrin. SVs are then uncoated and follow the direct route ('a'; light blue) to be refilled with NT, or the indirect route ('b'; dark blue) which passes through the endosome. AP-3 mediates budding of SVs from endosomes in PC12 cells. Biology of the Cell (2007) 99, 349-361.


Piccolo
PRA1
Bassoon
Rab3
Translocation
Rabphilin
Presynaptic active zone
Endocytosis
Docking/ priming
ERC
Rim
Mint
CASK
Munc13
Munc18
Veli
Vamp
Liprin
Syntaxin
β-Neurexin
Ephrin-B
LAR
N-type Ca2+ channel
Narp
N-Cadherin
Neuroligin
Eph
NMDAR
AMPAR
Sap90/PSD95
Postsynaptic plasma membrane
Guanylate kinase like (GuK) domain
PSD95/Dlg/ZO1 (PDZ) domain
Ca2+/phospholipid binding (C2) domain
Zinc finger domain
Src homology 3 (SH3) domain domain
CaMKII domain
Piccolo/bassoon homology (PBH) domain
Protein backbone
v-SNARE (Vamp)
t-SNAREs (syntaxin/Snap25)
β-catenin
Spectrin
Clathrin/AP2
Ligand/voltage-gated ion channel: Ca2+ channel, NMDAR, AMPAR