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THE SYNAPTIC VESICLE CYCLE

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■ **Abstract** Neurotransmitter release is mediated by exocytosis of synaptic vesicles at the presynaptic active zone of nerve terminals. To support rapid and repeated rounds of release, synaptic vesicles undergo a trafficking cycle. The focal point of the vesicle cycle is Ca^{2+}-triggered exocytosis that is followed by different routes of endocytosis and recycling. Recycling then leads to the docking and priming of the vesicles for another round of exo- and endocytosis. Recent studies have led to a better definition than previously available of how Ca^{2+} triggers exocytosis and how vesicles recycle. In particular, insight into how Munc18-1 collaborates with SNARE proteins in fusion, how the vesicular Ca^{2+} sensor synaptotagmin 1 triggers fast release, and how the vesicular Rab3 protein regulates release by binding to the active zone proteins RIM1α and RIM2α has advanced our understanding of neurotransmitter release. The present review attempts to relate these molecular data with physiological results in an emerging view of nerve terminals as macromolecular machines.

NEUROTRANSMITTER RELEASE AND THE SYNAPTIC VESICLE CYCLE

Synaptic transmission is initiated when an action potential triggers neurotransmitter release from a presynaptic nerve terminal (Katz 1969). An action potential induces the opening of Ca^{2+} channels, and the resulting Ca^{2+} transient stimulates synaptic vesicle exocytosis (Figure 1). After exocytosis, synaptic vesicles undergo endocytosis, recycle, and refill with neurotransmitters for a new round of exocytosis. Nerve terminals are secretory machines dedicated to repeated rounds of release. Most neurons form >500 presynaptic nerve terminals that are often widely separated from the neuronal cell bodies. Action potentials, initiated in the neuronal cell body, travel to all of the cell body's nerve terminals to be transformed into synaptic secretory signals. Nerve terminals do not convert reliably every action potential into a secretory signal but are "reliably unreliable" (Goda & Südhof 1997). In most terminals, only 10%–20% of action potentials trigger release. The relationship between action potentials and release in a nerve

terminal is regulated by intracellular messengers and extracellular modulators and is dramatically altered by repeated use of a synapse. Thus in addition to secretory machines, nerve terminals are computational units where the relation of input (action potential) to output (neurotransmitter release) continuously changes in response to extra- and intracellular signals.

All presynaptic functions, directly or indirectly, involve synaptic vesicles. Synaptic vesicles undergo a trafficking cycle in the nerve terminal (Figure 1) that can be divided into sequential steps: First, neurotransmitters are actively transported into synaptic vesicles (step 1), and synaptic vesicles cluster in front of the active zone (step 2). Then synaptic vesicles dock at the active zone (step 3), where the vesicles are primed (step 4) to convert them into a state of competence for Ca^{2+}-triggered fusion-pore opening (step 5). After fusion-pore opening, synaptic vesicles endocytose and recycle probably by three alternative pathways: (*a*) Vesicles are reacidified and refilled with neurotransmitters without undocking, thus remaining in the readily releasable pool (step 6, called "kiss-and-stay"); (*b*) vesicles undock and recycle locally (step 7, called "kiss-and-run") to reacidify and refill with neurotransmitters (back to steps 1 and 2); or (*c*) vesicles endocytose via clathrin-coated pits (step 8) and reacidify and refill with neurotransmitters either directly or after passing through an endosomal intermediate (step 9). In the two-dimensional representation depicted here (Figure 1), each step in the vesicle cycle is illustrated by a shift in the position of the vesicle. In reality, however, most successive steps occur without much vesicle movement except for docking (step 3) and recycling (steps 7–9). Investigators sometimes propose that different types of release reactions exist that differ in fusion-pore dynamics (e.g., "kiss-and-run" is used as a description of exocytosis instead of recycling). However, synaptic vesicles are so small (radius 17–22 nm) that even an unstable fusion pore is likely to empty the vesicle rapidly, as reflected in the fast rise times of spontaneous release events (<100 μs). The dynamics of fusion pores are thus unlikely to influence release but are probably of vital importance for endocytosis: Fast recycling may preferentially utilize transient fusion pores, whereas slow recycling likely involves a full collapse of the vesicle into the plasma membrane.

A major goal in neurobiology in recent years has been to gain insight into the molecular machinery that mediates neurotransmitter release. More than 1000 proteins function in the presynaptic nerve terminal, and hundreds are thought to participate in exocytosis. In this protein zoo, which proteins are actually important, and which are only bystanders? How do proteins collaborate in shaping the vesicle cycle, and how can we understand the functions of so many proteins? To approach this fundamental problem, I posit that all of the presynaptic functions ultimately converge on the vesicle cycle and that all steps in the vesicle cycle collaborate, directly or indirectly, to make possible rapid, regulated, and repeated rounds of release (Südhof 1995). Because release is mediated by the interaction of synaptic vesicles with the active zone during exocytosis, this interaction is the common final pathway of all nerve terminal functions. In the discussion below, I therefore pursue a "vesicocentric" perspective that focuses on synaptic vesicles as the central

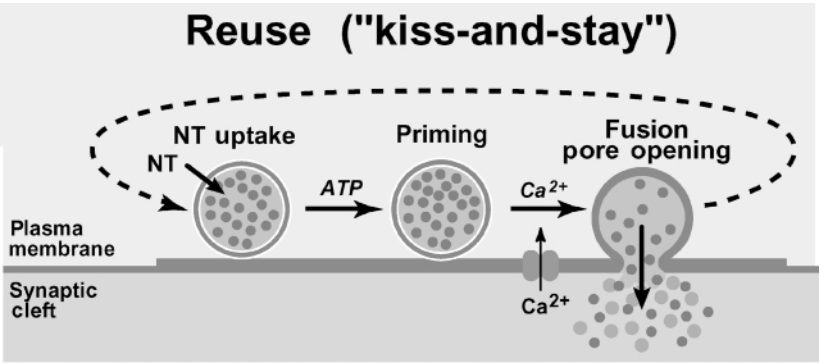
organelle of neurotransmitter release. This perspective is guided by the promise that synaptic vesicles as small, relatively simple organelles are in principle amenable to a complete molecular analysis.

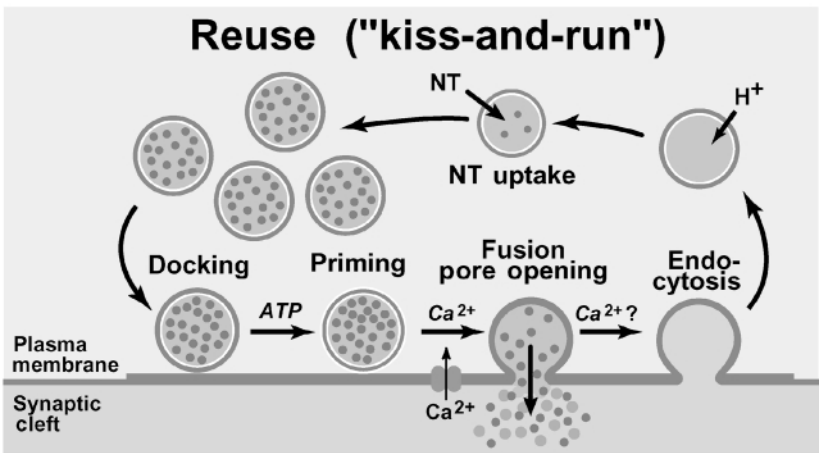
Instead of providing a comprehensive overview of the entire literature (which would be impossible within the constraints of this review), I concentrate on a limited number of proteins for which key functions have been proposed. For many topics, other recent reviews provide a good summary [e.g., see Jahn et al. (2003) for a review of membrane fusion, Slepnev & De Camilli (2000) or Galli & Haucke (2001) for reviews on endocytosis, and Zucker & Regehr (2002) or von Gersdorff & Borst (2002) for reviews on short-term synaptic plasticity]. Furthermore, I primarily focus on work done in mammals because space constraints do not allow a discussion of the extensive and outstanding work in flies and worms (reviewed in Richmond & Broadie 2002). Before discussing the molecular machines that are anchored on synaptic vesicles and drive the vesicle cycle, one must review the salient properties of the two principal limbs of the cycle—exocytosis versus endocytosis and recycling—and the methodology used to elucidate how a given molecule could participate in the cycle.

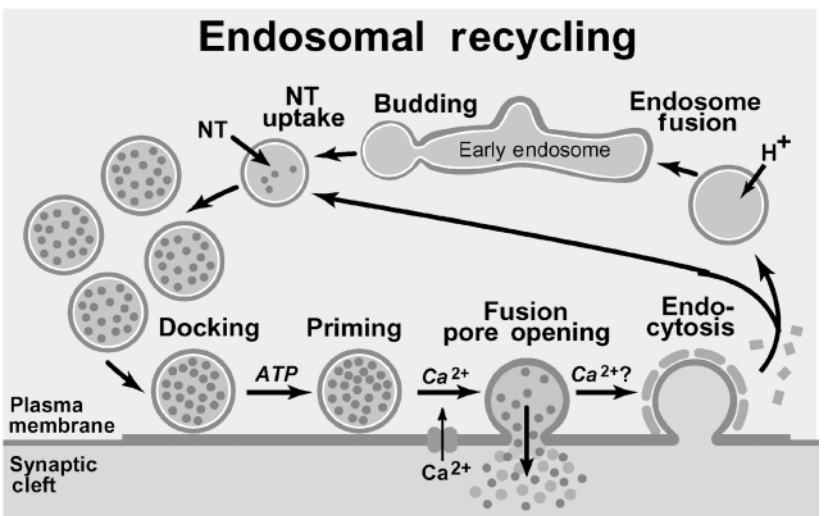
Ca^{2+} TRIGGERING OF NEUROTRANSMITTER RELEASE

In preparation for neurotransmitter release, synaptic vesicles dock at the active zone and are primed to become Ca^{2+} responsive (steps 3 and 4; Figure 1). When an action potential invades a nerve terminal, voltage-gated Ca^{2+} channels open, and the resulting pulse of intracellular Ca^{2+} triggers fusion-pore opening of release-ready vesicles (step 5). In most synapses, release is stimulated by Ca^{2+} influx through P/Q- ($Ca_V2.1$) or N-type Ca^{2+} channels ($Ca_V2.2$), whereas the related R- ($Ca_V2.3$) or the more distant l-type Ca^{2+} channels (Ca_V1 series) are involved only rarely (e.g., see Dietrich et al. 2003). Even at rest, synapses have a finite but low probability of release, causing spontaneous events of exocytosis that are reflected in electrophysiological recordings as miniature postsynaptic currents (Katz 1969). Ca^{2+} influx triggers at least two components of release that are probably mechanistically distinct: A fast, synchronous phasic component is induced rapidly, in as little as 50 μs after a Ca^{2+} transient develops (Sabatini & Regehr 1996), and a slower asynchronous component continues for >1 s as an increase in the rate of spontaneous release after the action potential (Barrett & Stevens 1972, Geppert et al. 1994a, Goda & Stevens 1994, Atluri & Regehr 1998). Both components of release are strictly Ca^{2+} dependent but change differentially upon repetitive stimulation (Hagler & Goda 2001).

The best physiological description of how an action potential induces phasic, synchronous neurotransmitter release was obtained for the synapse formed by the calyx of Held, the only synapse for which models are available that accurately account for all properties of release (reviewed in Meinrenken et al. 2003). The calyx of Held forms a large nerve terminal ($\sim$15 μm diameter) that envelops the soma of the postsynaptic neuron like a cup, hence its name. The calyx terminal makes







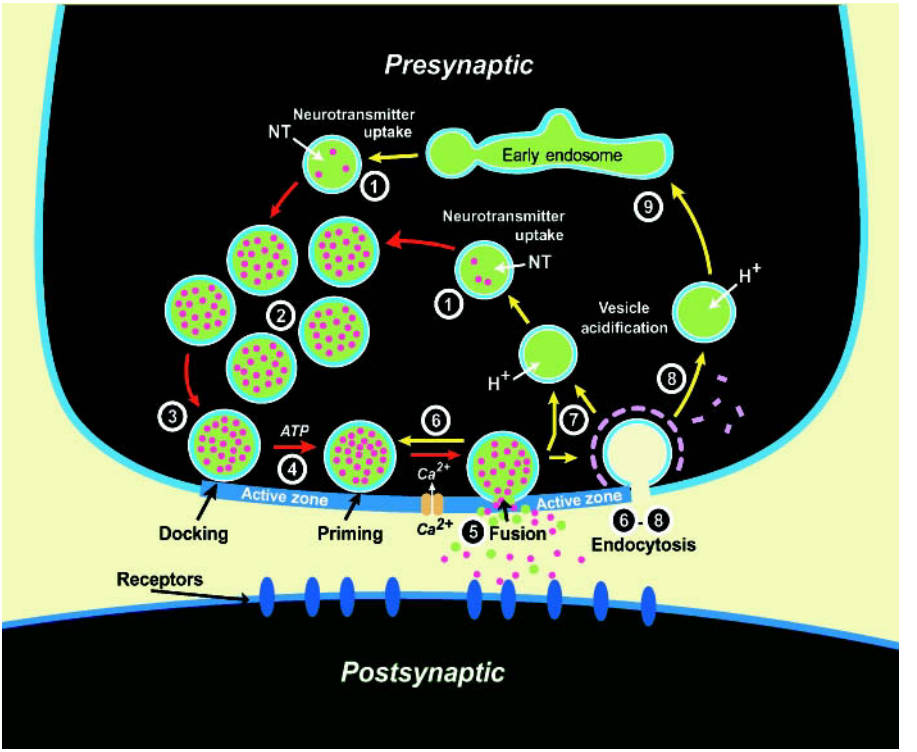


Figure 1 The synaptic vesicle cycle. Synaptic vesicles are filled with neurotransmitters by active transport (step 1) and form the vesicle cluster that may represent the reserve pool (step 2). Filled vesicles dock at the active zone (step 3), where they undergo a priming reaction (step 4) that makes them competent for Ca^{2+} triggered fusion-pore opening (step 5). After fusion-pore opening, synaptic vesicles undergo endocytosis and recycle via several routes: local reuse (step 6), fast recycling without an endosomal intermediate (step 7), or clathrin-mediated endocytosis (step 8) with recycling via endosomes (step 9). Steps in exocytosis are indicated by red arrows and steps in endocytosis and recycling by yellow arrows.

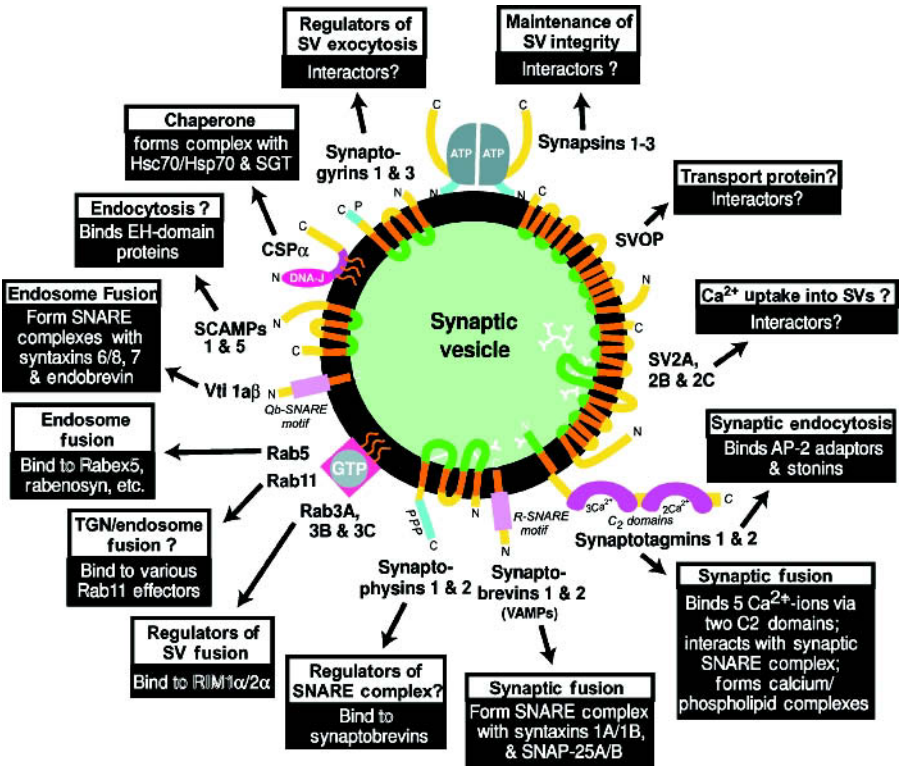


Figure 4 Structures, proposed interactions, and putative functions of synaptic vesicle trafficking proteins. Proteins are shown schematically (*green*, intravesicular sequences; *orange*, transmembrane regions; *blue*, phosphorylation domains; *pink*, SNARE motifs; *red* and *gray*, folded domains; *yellow*, other sequences). The white connecting lines in the intravesicular space identify disulfide bonds, and the branched white lines indicate sugar residues. In the boxes corresponding to the individual proteins, proposed functions are shown on a white background and purported interactions on a black background.

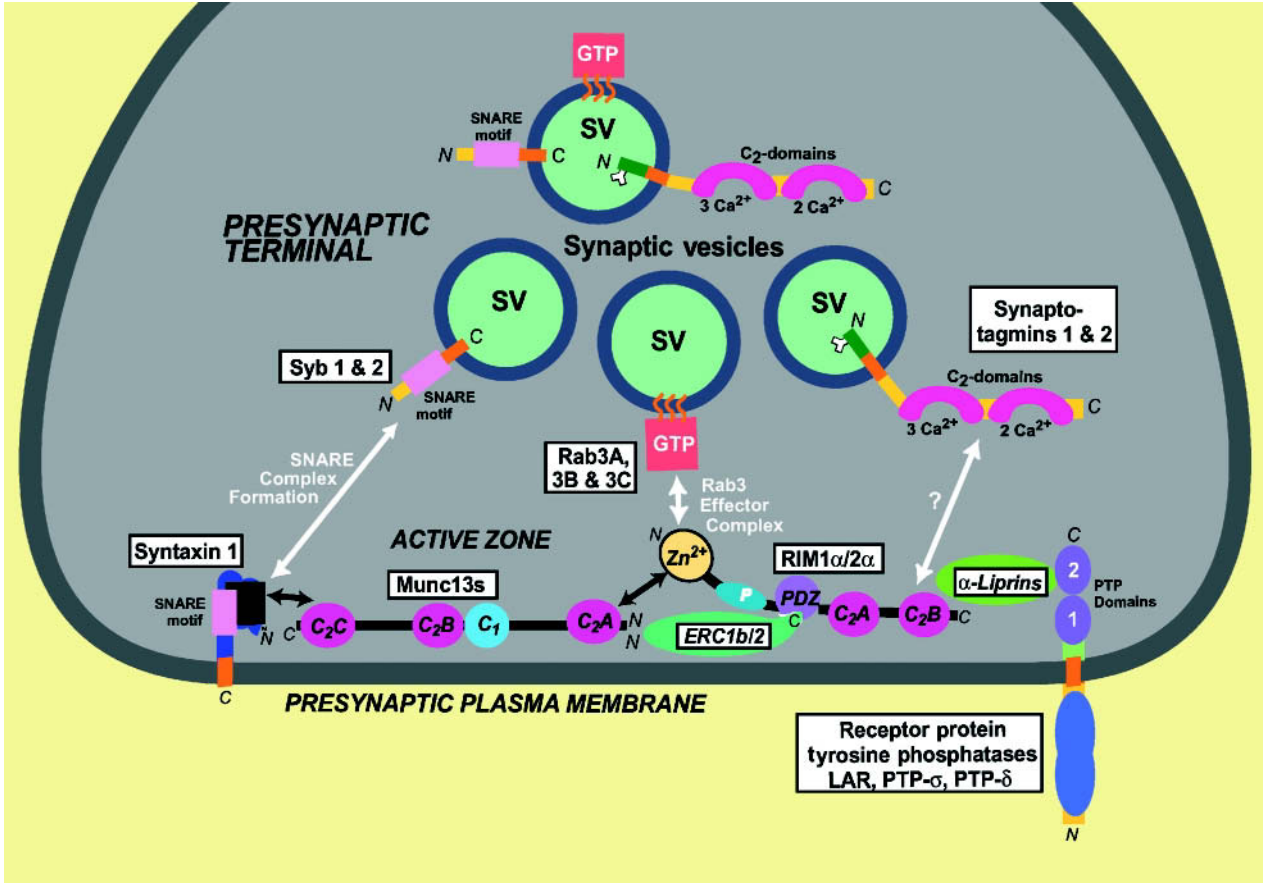


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Figure 5 Protein-protein interactions at the active zone: putative mechanisms of synaptic vesicle attachment. The active zone includes, among others, an interaction of the SNARE protein syntaxin 1 with the active zone component Munc13-1 (*left*), a direct binding of the N-terminal domain of Munc13-1 to the N-terminal zinc-finger of RIM1α/2α (*center*), and additional interactions of RIM1α/2α with the coiled-coil proteins ERC1a and ERC2 (*center*) and with α-liprins that in turn bind to receptor-tyrosine phosphatases (*right*). Two direct connections of synaptic vesicles with the active zone are established: binding of the vesicle SNARE synaptobrevin to syntaxin and SNAP-25 (*not shown*), and binding of the vesicle Rab proteins Rab3A, 3B, 3C, and 3D to the same N-terminal domain of RIM1α and RIM2α that also binds to Munc13-1. In addition, an interaction of the C_2 domains of synaptotagmins with the C_2B domain of RIMs has been observed but is not yet validated (*question mark*).

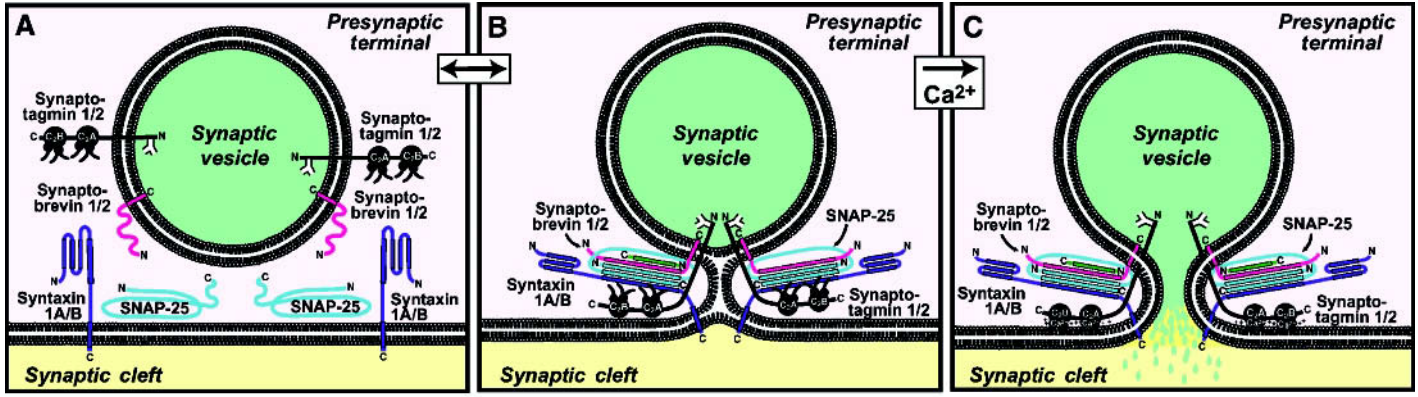

Figure 6 Model for the functions of SNARE proteins, complexins, and synaptotagmins 1 and 2 in synaptic vesicle exocytosis. In docked vesicles (*panel A*), SNAREs and synaptotagmins are not engaged in direct interactions. During priming (*panel B*), SNARE complexes form, complexins (*green*) are bound to fully assembled complexes, and synaptotagmins constitutively associate with the assembled SNARE complexes. The synaptic vesicle membrane and plasma membranes are forced into close proximity by SNARE complex assembly, which results in an unstable intermediate that is shown as a speculative fusion stalk. Ca^{2+} influx (*panel C*) further destabilizes the fusion intermediate by triggering the C_2 domains of synaptotagmin to partially insert into the phospholipids. This action is proposed to cause a mechanical perturbation that opens the fusion pore. Note that the nature and stability of the putative fusion intermediate is unclear and that SNARE complex assembly in panel B is suggested to be reversible, whereas Ca^{2+} triggering is not.