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The Synaptic Vesicle Cycle Revisited

Minireview

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Science goes through waves of innovation driven by technical advances. One recent such advance was the discovery of FM dyes as a tool to measure presynaptic activity by optical recordings (reviewed in Cochilla et al., 1999). FM1-43 and its cousins are lipophilic fluorescent probes that spontaneously insert into membranes where they become more flourescent. Importantly, these dyes can be easily washed out but do not cross bilayers. As a result, when a cell surface is exposed to FM dyes and subsequently washed with dye-free solution, only those membranes that have been recaptured and are no longer surface exposed retain the fluorescent dye. Reexocytosis of these membranes then leads to release of the dye. Since membrane-bound dye is much more fluorescent than dye in aequous solution, treatments of cell surfaces with FM dyes followed by washes provide a sensitive way of monitoring exo- and endocytosis. This approach has been particularly useful for studying synaptic vesicle recycling in presynaptic nerve terminals. In the most recent application of this technological innovation, Pyle et al. (2000) describe a new pathway in the synaptic vesicle cycle using two FM dyes that differ in the speed with which they equilibrate between the lipophilic membrane and aqueous solution. Using this ingenious approach, Pyle and colleagues observed that synaptic vesicles can be immediately reused after exocytosis, probably without ever leaving the active zone of the presynaptic plasma membrane. In an independent study, Stevens and Williams (2000) also identify a fast mode of vesicle reuse during exocytosis based on staining and destaining with FM dyes. These observations clarify several unresolved issues and give rise to a new, more economical view of the synaptic vesicle cycle. However, they also raise general questions of how many different vesicle pathways are nested in the synaptic vesicle cycle, how these pathways are interrelated, and how they may be molecularly determined. In the following, I will briefly discuss these issues in light of the new results and attempt to provide an overview of current thinking of the synaptic vesicle cycle in presynaptic nerve terminals.

Synaptic Vesicle Pools and Puddles

The readily releasable pool (RRP) of synaptic vesicles constitutes those vesicles that are immediately available for release. In synapses of cultured hippocampal neurons, arguably the best-characterized synapse, the RRP comprises five to nine synaptic vesicles as measured by FM1-43 staining/destaining (Murthy and Stevens, 1999). In similar cultures, the number of vesicles that are attached to the active zone (i.e., docked) was determined by electron microscopy and also found to be approximately five to ten vesicles per active zone (Schikorski and Stevens, 1997). This finding suggests that the vesicles in the RRP correspond to docked vesicles. The presence of multiple vesicles in the RRP provides a regulatory margin for triggering release and allows the synapse to repetitively release neurotransmitters during bursts of action potentials. During an action potential, Ca^{2+} influx normally triggers neurotransmitter release from a presynaptic terminal only unreliably, with one release event per five to ten Ca^{2+} signals (Dobrunz and Stevens, 1997), resulting in a low overall synaptic release probability. Whenever release is triggered, however, usually only a single synaptic vesicle undergoes exocytosis, although all of the vesicles in the RRP appear to be ready for fusion. The restriction of exocytosis to a single vesicle is probably not an active process, but simply caused by the low release probability of each individual vesicle. This suggests that the overall synaptic release probability is the sum of the individual vesicular release probabilities. Consistent with this idea, the number of vesicles in the RRP appears to be a major determinant of the synaptic release probability (Dobrunz and Stevens, 1997).

The RRP of synaptic vesicles is by definition the most important vesicle pool for neurotransmitter release. However, this pool constitutes only a tiny fraction of the total vesicle pool. The RRP is a component of the recycling pool of vesicles, defined as all vesicles that can be labeled with FM1-43 when synapses are stimulated extensively. After extensive stimulation, the RRP is depleted and additional vesicles are recruited for exocytosis from the so-called "reserve pool" to the active zone. The RRP and the reserve pool together constitute the recycling pool (Figure 1). The total recycling pool has been estimated at 21–25 vesicles/synapse in hippocampal cultures, with ~17–20 vesicles in the reserve pool (Murthy and Stevens, 1999). Presynaptic terminals thus contain a small number of active vesicles (the recycling pool), of which a third is in the RRP. Since morphologically the same synapses were shown to contain ~200 synaptic vesicles (Schikorski and Stevens, 1997), the size of the recycling pool is surprisingly small, with the majority of synaptic vesicles being inactive. We would like to refer to this inactive pool of synaptic vesicles as the resting pool (Figure 1). The overall picture that emerges is that of a hierarchy of pools, of which the smallest pool is at best a puddle but nevertheless the most active in release, while the largest pool appears to participate in release very little under regular conditions.

Synaptic Vesicle Pathways

Earlier studies have defined two pathways for synaptic vesicles in the vesicle cycle: an extended pathway where the vesicles recycle via an endosomal intermediate after endocytosis (here referred to as endosomal recycling), and a shorter pathway where vesicles recycle directly

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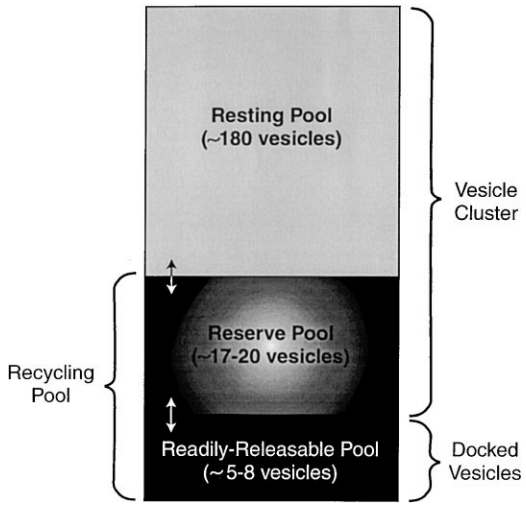

Figure 1. Synaptic Vesicles Pools

The three functionally defined pools of synaptic vesicles are shown schematically, with the estimated pool sizes determined in cultured hippocampal neurons (Murthy and Stevens, 1999) indicated.

without a trafficking intermediate (called here local recycling). These two cycles are sometimes presented as mutually exclusive alternatives, and especially the existence of the endosomal pathway has been questioned. However, strong evidence suggests that both pathways are employed in parallel, although at different rates. Three lines of evidence implicate an endosomal intermediate in the recycling of some synaptic vesicles. First, endosomes are observed morphologically after extensive stimulation of nerve terminals, suggesting that at least a subset of vesicles fuse with endosomes after endocytosis (reviewed in Südhof, 1995). Second, purified synaptic vesicles from brain contain a limited number of Rab proteins that includes Rab5 whose function is restricted to endosome fusion. Organelle immunoprecipitations showed that the majority of synaptic vesicles, and not just a small subfraction, contain Rab5 (Fischer von Mollard et al., 1994), suggesting that these vesicles (possibly the resting pool) pass through endosomes. Third, synaptic vesicles contain high concentrations of the SNARE Vti1a, which functions in membrane fusion involving endosomes but not in membrane fusion at the plasma membrane, again indicating that the majority of vesicles recycles via endosomes at some point in their lifetime (Antonin et al., 2000). However, the presence of Rab5 and Vti1a on the majority of synaptic vesicles does not exclude the possibility that a subpopulation of vesicles lacks Rab5 and Vti1a and correlates very nicely with the assignment of the majority of the vesicles to the resting pool that may be functionally related to endosomes (Figure 1).

Direct local recycling of synaptic vesicles without an endosomal intermediate is also supported by multiple lines of evidence. In classical experiments, Whittaker and colleagues demonstrated that after stimulation of nerve terminals, a small subset of vesicles becomes preferentially filled with newly synthesized neurotransmitters (Barker et al., 1972). Furthermore, this vesicle subset is localized close to the membrane as shown by biochemical techniques and is the first to undergo exocytosis upon stimulation. These observations led to the postulate that synaptic vesicles can be divided into a smaller subpopulation of active vesicles (in current parlance, the "recycling pool"), and a larger population of reserve vesicles (in our terminology, the "resting pool"; Figure 1). The concept of local recycling without endosomal intermediates was resurrected by Murthy and Stevens (1998) who showed that synaptic vesicles labeled with FM1-43 dye during endocytosis are subject to reexocytosis without dilution of the dye. This result is consistent with the notion that the vesicles in the recycling pool do not pass through endosomes as an intermediate.

The new data by Pyle et al. (2000) and Stevens and Williams (2000) now suggest the presence of an additional, third vesicle cycle in the nerve terminal called "reuse." It is proposed that, after exocytosis, the fusion pores of the vesicles in the RRP close quickly, the vesicles refill with neurotransmitters immediately, and the vesicles are then reprimed for another round of exocytosis without ever leaving the active zone. This reuse pathway results in very fast turnaround of vesicles after exocytosis, an extremely attractive idea in view of the economy that it would provide. Pyle et al. (2000) base their proposal of the reuse pathway on two critical findings. First, using FM dyes with different dissociation constants that were already very helpful in defining endocytosis (Klingauf et al., 1998), they demonstrate that vesicles in the RRP have an extremely short time of fusion pore opening, so short that the regular FM1-43 dye is not quantitatively released. Only the faster dissociating dye FM2-10 leaves the membrane. In contrast to this differential destaining reaction, fusion of vesicles recruited from the reserve pool is associated with much longer times of fusion pore opening and equivalent dissociation of FM1-43 and FM2-10 dyes. Second, they show that after depletion of the RRP, this pool refills much faster than can be explained by repopulation from the reserve pool. In other words, vesicles in the RRP recover the ability to reexocytose locally without recycling through the reserve pool. Although these experiments do not unequivocally prove that vesicles in RRP remain attached to the active zone ("docked") during this process, this appears to be the most plausible hypothesis. The data of Pyle et al. (2000) are beautifully complemented by recent results from Richards et al. (2000), who also exploit the different binding constants of various FM dyes to demonstrate that different pools of synaptic vesicles are preferentially stained by FM1-43 and FM2-10 during tetanic stimulation.

Using a different approach, Stevens and Williams (2000) provide independent evidence for a fast reuse pathway of synaptic vesicle recycling. They directly compare glutamate release measured electrophysiologically with the rate of membrane dissociation of FM1-43 during exocytosis. A relative lack of FM1-43 dissociation is noticed under conditions that preferentially stimulate exocytosis from the RRP. This suggests that the fusion pores of vesicles in the RRP close again very quickly after exocytosis. Thus, the studies by Pyle et al. and by Stevens and Williams arrive at similar models that postulate a hierarchy of vesicle cycling pathways, although there is some disagreement between

these studies with regard to the destaining kinetics obtained with FM1-43 and FM2-10. Taken together, these results indicate that synaptic vesicles are trafficked in three nested cycles. The shortest vesicle cycle (the reuse cycle) contains the smallest pool of vesicles (the RRP; five to eight vesicles in hippocampal cultures) but the highest rate of throughput. The intermediate cycle (the local recycling pathway) contains all of the vesicles in the recycling pool (17–20 vesicles); these vesicle actively participate in neurotransmitter release. The longest and most complicated cycle (the endosomal cycle) is comprised of the largest vesicle pool (the resting pool; 200 vesicles) with little participation in neurotransmitter release under normal stimulation conditions (Figure 1).

Dynamics of Pools and Pathways

One important discovery by Pyle et al., in conjunction with previous studies by Barker et al. (1972) and Murthy and Stevens (1998), is that each pool retains its identity in the cycle during activity. During repetitive stimulation, the RRP pool is released first as expected, but unexpectedly the same vesicles are also preferentially reused in the RRP after a short recovery phase (1–10 s depending on whether release was stimulated by Ca^{2+} or hypertonic sucrose). This reuse occurs by immediate closure of the fusion pore, a process which the authors call rapid endocytosis, although their data suggest that this is not true endocytosis since the vesicles may remain attached to the active zone. The immediate closure of the fusion pore after exocytosis is reminiscent of "kiss-and-run", a concept proposed in the 1970s (Ceccarelli and Hurlbut, 1980a), except that it looks more like "kiss-and-stay"—after all, these are more conservative times. When the RRP is depleted, further stimulation leads to a low rate of steady-state release maintained by recruitment of reserve vesicles. The reserve vesicles, however, are subsequently not channeled into the RRP with rapid endocytosis, but remain reserve vesicles. They undergo regular endocytosis and are recycled into the regular reserve pool. Thus, reserve pool and RRP are kept separate even after exocytosis. These results from hippocampal cultures are also consistent with similar data obtained at the neuromuscular junction (Richards et al., 2000).

Although the various pools retain their identities during activity, vesicles move between pools. The RRP and reserve pool appear to be in equilibrium, with continuous exchange between the two pools occurring with an estimated time constant of $\sim$100 s in the absence of Ca^{2+} (Murthy and Stevens, 1999; Pyle et al., 2000). Vesicles in the resting pool are not in a "vesicle graveyard" but can be recruited into the recycling pool. Resting pool vesicles undergo exocytosis upon extensive stimulation, as most vividly demonstrated upon stimulation with α-latrotoxin, which depletes a nerve terminal of all synaptic vesicles, including the resting pool (Ceccarelli and Hurlbut, 1980b). It seems likely that the resting pool serves as a true reserve for recycling vesicles, maybe also as a detour for vesicles to be checked and rejuvenated in the endosome. Furthermore, this pool could provide a vesicle reservoir for growing synapses, for example, during synaptic plasticity.

Finally, the individual cycles, and the transitions of vesicles between cycles, are probably subject to extensive regulation. Most of this regulation may be mediated by Ca^{2+}. This is vividly illustrated by Pyle et al. in the 10-fold acceleration by Ca^{2+} of the recovery of the RRP after depletion and of the repopulation of the RRP from the reserve pool under steady-state conditions. Another example is the regulation of the size of the RRP by PKC (Stevens and Sullivan, 1998; Lonart and Südhof, 2000), which most likely means the transfer of vesicles from the resting and reserve pools to reserve and readily-releasable pools, respectively. The regulatory capacity of the pathway provides another possible rationale for the large size of the resting pool because this represents the ultimate reservoir for all expansions of the recycling pool.

Molecular Correlates: Proteins Chasing Vesicles in the Cycle

What are the molecular mechanisms that guide the synaptic vesicle pathway? The components of synaptic vesicles have been largely characterized, several molecules of active zones are known, and a large number of proteins with a function in endocytosis has been identified. Nevertheless, except for membrane fusion, little is known about the molecular correlates of the various stages of the different vesicle cycles. Descriptions of general functions, of vesicles moving here or there, have been much easier to come by than an understanding of the mechanisms involved. For the most part, we only have reasonable hypotheses coupled with negative data. The actin or microtubule cytoskeleton is unlikely to be centrally involved since it is primarily, if not exclusively, localized outside of the vesicle cluster (Dunaevsky and Connor, 2000). Intrinsic proteins of the synaptic vesicles must be the final endpoints of all molecules acting on the vesicles during their different pathways; a systematic analysis of these proteins along the lines that have already started may provide a definitive test of this hypothesis (reviewed in Fernandez-Chacon and Südhof, 1999). However, up to now genetic deletions of various synaptic vesicle proteins mostly cause changes in release rates and synaptic plasticity. In particular, no synaptic vesicle protein that is selectively essential for the biogenesis or recycling of synaptic vesicles has been identified. There is a general lack of insight into what keeps a vesicle together and how it is routed in the presynaptic nerve terminals. Although endocytosis has been exceedingly well studied in nonneuronal cells and the components of the endocytic machinery are highly enriched in nerve terminals (e.g., see Maycox et al., 1992), it is unclear, for example, if clathrin coats are only involved in slow endocytosis or also in the fast "kiss-and-stay" reuse pathway. The molecular understanding of the nerve terminal is far behind the exquisite description of vesicles moving in and out of the active zone. Synaptic vesicle cycling is functionally relatively simple compared to the mechanistic complexity of molecular interactions. Even with three nested pathways, the vesicle cycle is still less complex than the interaction cycle of a protein such as Rab3A which interacts with at least five other proteins during the vesicle cycle in an ordered sequence (Rabphilin, RIM, Rab3-GAP, Rab3-GDP/GTP exchange protein, GDI; Südhof, 1995). The need for such a complexity is given by the plasticity of the terminals, both structurally and functionally, that allows synapses to change release dramatically over short time periods.

In view of this complexity, what then are the primary

questions that we can hope to address molecularly in the near future? The fusion process, and its relation to the RRP, probably holds the greatest promise for understanding. It seems likely that vesicles in the RRP are prefused when they are stimulated for exocytosis by Ca^{2+} in order to allow the extraordinary speed of Ca^{2+} action. Recent results suggest that this prefused state may correspond to assembled SNARE complexes (Lonart and Südhof, 2000). Although it is still unclear if the SNAREs are executive or merely catalytic in fusion, elucidation of how and where they act in synaptic vesicle fusion in conjunction with the essential fusion protein munc18-1 will provide decisive insight into the mechanism of release. A second promising approach is the focus on proteins that reversibly associate with synaptic vesicles during the pathway. For example, Rab3A dissociates from synaptic vesicles after extensive stimulation while Rab5 does not (Fischer von Mollard et al., 1994). It is possible that this dissociation occurs only in the direct recycling and the endosomal recycling pathways because docked vesicles (which presumably correspond to the RRP) contain Rab3A (reviewed in Südhof, 1995). Is there a cycle of GTP hydrolysis/GDP to GTP exchange on docked vesicles, or does GTP hydrolysis only occur in preparation to true endocytosis? As mentioned above, synaptic vesicles quantitatively contain Rab5, a marker for endosomes. However, since most of the vesicles belong to the resting pool of vesicles that are not actively participating, it is impossible to tell if locally recycling vesicles (i.e., the recycling pool) contain or lack Rab5. Biochemically, the lack of Rab5 on 10% of the vesicles would easily have been missed. One idea thus is that the transition of vesicles from one pool to the next could be guided by the acquisition or loss of Rab5 from these vesicles and that different complements of Rab proteins identify different vesicle pools. The elegant physiological description of presynaptic terminals that has now been achieved by the work of Pyle et al. (2000), Stevens and Williams (2000), and others will provide the opportunity to elucidate the molecular mechanisms that underlie these pathways.

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Neurotransmission is sustained by endocytosis and refilling of synaptic vesicles (SVs) locally within the presynapse. Until recently, a consensus formed that after exocytosis, SVs are recovered by either fusion pore closure (kiss-and-run) or clathrin-mediated endocytosis directly from the plasma membrane. However, recent data have revealed that SV formation is more complex than previously envisaged. For example, two additional recycling pathways have been discovered, ultrafast endocytosis and activity-dependent bulk endocytosis, in which SVs are regenerated from the internalized membrane and synaptic endosomes. Furthermore, these diverse modes of endocytosis appear to influence both the molecular composition and subsequent physiological role of individual SVs. Neurotransmission is sustained by endocytosis and refilling of synaptic vesicles (SVs) locally within the presynapse. Until recently, a consensus formed that after exocytosis, SVs are recovered by either fusion pore closure (kiss-and-run) or clathrin-mediated endocytosis directly from the plasma membrane. However, recent data have revealed that SV formation is more complex than previously envisaged. For example, two additional recycling pathways have been discovered, ultrafast endocytosis and activity-dependent bulk endocytosis, in which SVs are regenerated from the internalized membrane and synaptic endosomes. Furthermore, these diverse modes of endocytosis appear to influence both the molecular composition and subsequent physiological role of individual SVs. In a neuron, synaptic vesicles (or neurotransmitter vesicles) store various neurotransmitters that are released at the synapse. The release is regulated by a voltage-dependent calcium channel. Vesicles are essential for propagating nerve impulses between neurons and are constantly recreated by the cell. The area in the axon that holds groups of vesicles is an axon terminal or "terminal bouton". Up to 130 vesicles can be released per bouton over a ten-minute period of stimulation at 0.2 Hz. In the... The Synaptic VesicleCycle Revisited. December 2000. Neuron 28(2):317-20. DOI: 10.1016/S0896-6273(00)00109-4. Source. PubMed. AuthorsÂ Study on the Dynamic Changes in Synaptic Vesicle-Associated Protein and Axonal Transport Protein Combined with LPS Neuroinflammation Model. Article. Full-text available. Sep 2013. Rui Zhang. Ming Zhao. Hai-Jie Ji. At the chemical synapse, electrical signals trigger controlled secretion of neurotransmitter via exocytosis of synaptic vesicles (SV) at the presynaptic site. The fusion of SVs releases neurotransmitters that diffuse across the synaptic cleft and activate postsynaptic receptor channels. That generates a new wave of electric signal in the postsynaptic cell (Katz and Miledi, 1965, 1967). Given the limited number of SVs in a presynaptic terminal, complete fusion of many SVs with the plasma membrane would result in fast depletion of fusion-competent SVs and thereby the loss of the ability to trans...