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Review

Clathrin-Mediated Endocytosis at the Synaptic Terminal: Bridging the Gap Between Physiology and Molecules

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It has long been known that the maintenance of fast communication between neurons requires that presynaptic terminals recycle the small vesicles from which neurotransmitter is released. But the mechanisms that retrieve vesicles from the cell surface are still not understood. Although we have a wealth of information about the molecular details of endocytosis in non-neuronal cells, it is clear that endocytosis at the synapse is faster and regulated in distinct ways. A satisfying understanding of these processes will require molecular events to be manipulated while observing endocytosis in living synapses. Here, we review recent work that seeks to bridge the gap between physiology and molecules to unravel the endocytic machinery operating at the synaptic terminal.

Key words: adaptors, clathrin, endocytosis, pHluorin, synaptic vesicle

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Since the quantal hypothesis of synaptic transmission was formulated by Bernard Katz, we have known that synaptic vesicles are the basic units of fast signaling between neurons (1,2). Most synaptic terminals in the brain contain no more than 100–200 vesicles, so the maintenance of communication depends on vesicles being recycled from the surface after fusion (3,4). Three basic mechanisms have been proposed to internalize vesicular membrane and proteins: endocytosis of small vesicles coated by clathrin, bulk retrieval of large portions of membrane and fast recapture of vesicles that do not fully collapse into the surface (Figure 1). Of these processes, clathrin-mediated endocytosis (CME) is the best understood. Biochemical experiments have provided a wealth of information about the binding interactions and properties of proteins involved in CME (5,6), allowing detailed network diagrams to be constructed (7). The sequence of molecular events that lead to the recycling of a clathrin-coated vesicle can now be modeled, but the potential interactions are complex and our information is incomplete, so it is essential that such models are tested in the living cell. For this reason, we have argued that 'a major bottleneck in our understanding of endocytosis at the synapse is the gap between physiology and molecules. Bridging this gap will require the coupling of real-time methods for directly assaying endocytosis to preparations that allow manipulation of specific proteins' (3). Here, we revisit this issue and review the significant progress that has been made in understanding the molecular physiology of synaptic vesicle recycling through CME.

The evidence that CME is a key mechanism of synaptic vesicle recycling has accumulated over time and is now overwhelming. Using electron microscopy, Heuser and Reese examined how stimulation altered the ultrastructure of the frog neuromuscular junction (NMJ) after stimulation at 10 Hz (8). They observed decreased numbers of synaptic vesicles and the appearance of membranous cisternae emanating from the plasma membrane, often with coated buds on the surface (Figure 1B). If a rest period was allowed after stimulation, small vesicles reappeared without coats on their surface and the cisternae gradually diminished in size. We now know that these coats are formed by clathrin triskelia (9), and the complex molecular machinery that assembles and disassembles these coats is understood in great detail, at least in the context of non-neuronal cells (5,6,10,11). A wide variety of experiments in a number of species and different functional classes of neuron also confirm that clathrin-dependent mechanisms retrieve synaptic vesicles (4). Nonetheless, a number of important questions remain unanswered. Most fundamentally, is CME the process that *normally* operates at the synapse? How is CME initiated if not by activation of a receptor? Why is CME at the synapse more rapid than in non-neuronal cells? And which adaptors are used to select the appropriate cargo for inclusion into the new vesicle?

Testing the Role of CME at the Synapse

The idea that CME may not be the normal route of synaptic vesicle retrieval stems from the work of Ceccarelli

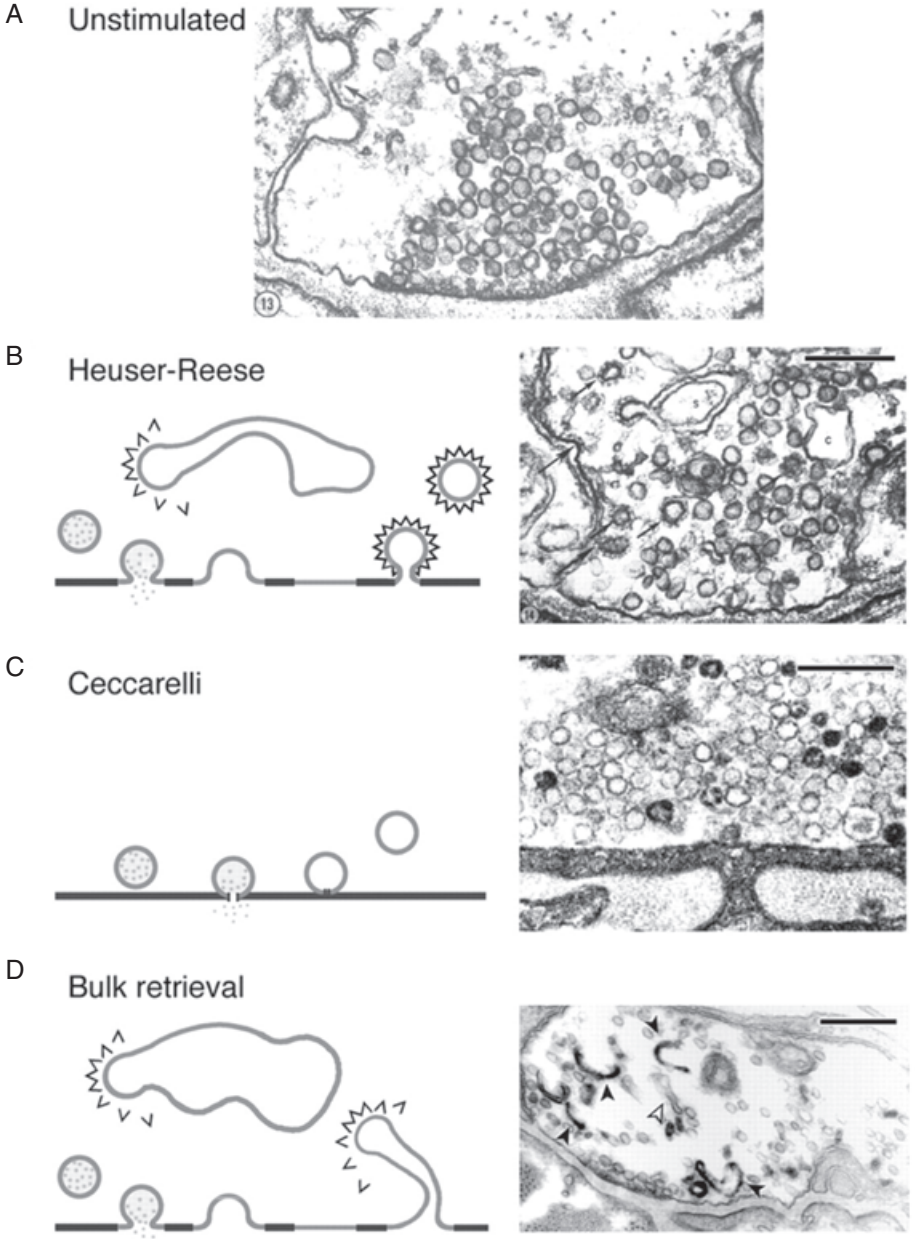

Figure 1: Three modes of synaptic vesicle retrieval at the frog NMJ. A) Electron micrograph of the frog NMJ at rest. B) Heuser-Reese model for retrieval: vesicles collapse fully into the plasma membrane and are retrieved by CME. Coated vesicles may also form from large internal cisternae. Electron micrograph (right) of frog NMJ following stimulation at 10 Hz for 1 min. Coated pits and vesicles (arrows) and cisternae (c) are seen in the terminal. C) Ceccarelli model for retrieval: vesicles do not collapse fully but release neurotransmitter through a transient fusion pore by 'kiss-and-run'. Micrograph (right) of a terminal stimulated at 2 Hz for 2 h showing an absence of clathrin-coated vesicles and cisternae at low stimulation frequency. D) Bulk membrane retrieval: large areas of membrane are internalized following complete vesicle collapse. Subsequently, coated vesicles are 'resynthesized' from large membrane invaginations or internalized cisternae. The micrograph (right) shows cisternae that are empty (open arrowheads) or filled with photoconverted FM1-43 (filled arrowheads). Scale bars, 250 nm in all cases. Figure reproduced from Ref. (3).

et al. (12), who stimulated the NMJ at low frequency (2 Hz), and, in stark contrast to Heuser and Reese (8), observed little change in the ultrastructure of the terminal. A number of studies have since confirmed that when a synapse is weakly active, synaptic vesicles can be recycled fast enough to prevent depletion (13). But Ceccarelli et al. went further, and suggested that the lack of vesicle depletion was indicative of another process in which neurotransmitter was released through a transient connection to the plasma membrane that closed before the vesicle could collapse. This faster mechanism has been termed 'kiss-and-run' (14), and it has also been suggested to operate at small synapses in the brain. A major argument put forward in favor of 'kiss-and-run' has been the idea that CME alone would not be fast enough to prevent vesicle depletion during ongoing activity (15–17). However, a number of recent studies have failed to detect a fast 'kiss-and-run' process at small synaptic terminals (18–22), and it appears that CME may operate fast enough to account for the measured rates of synapse depression and recovery from depression (23).

One of the most obvious ways to test the relative importance of CME at the synapse is to block this process and observe the effects on vesicle retrieval assayed using a real-time method (see Table 1). This experiment has now been carried out by three groups, two studying small synapses formed by cultured hippocampal neurons (18,19) and the other, the NMJ of *Drosophila* (20).

Table 1: Studies that bridge the gap between physiology and molecules: real-time measurements of vesicle retrieval following molecular manipulation

Target	Manipulation	Neuron/species	Effect	References
Clathrin	CHC RNAi	Hippocampal neurons (rat)	Block of retrieval after brief stimulation. Mixture of complete block of retrieval and partial recovery.	(18) (19)
	Photoinactivation	Neuromuscular junction (*Drosophila*)	Run-down of synaptic events with repeated stimulation. Synaptic vesicles do not reform after inactivation. Bulk internalization occurs but no competent vesicles can form.	(20,27)
	Perfusion of CHC N-terminal domain	Retinal bipolar cells (goldfish)	Block of the slow (t ~10 seconds) mode of endocytosis.	(28)
Dynamin	Dynasore	Hippocampal neurons (rat) Calyx of Held (rat)	Complete block of retrieval.	(29,30)
	Peptide interference	Calyx of Held (mouse)	Block of retrieval.	(31)
	GTP analogues	Retinal bipolar cells (goldfish)	Block of both fast and slow modes of endocytosis.	(28) (31)
		Calyx of Held (mouse)	Block of endocytosis.	
	Knockout mouse	Cortical neurons (mouse)	Incomplete block of retrieval.	(32)
Amphiphysin	Peptide interference	Retinal bipolar cells (goldfish)	Block of the slow (t ~10 seconds) mode of endocytosis.	(28)
AP2	μ2 RNAi	Hippocampal neurons (rat)	Minor effects on vesicle retrieval. Possible compensation by AP1.	(33,34)
	Peptide interference	Calyx of Held (rat)	Complete block of retrieval.	(28,29)
		Retinal bipolar cells (goldfish)	Block of slow form of endocytosis.	
AP1	σ1B knockout mouse	Hippocampal neurons (mouse)	Slight delay in retrieval. Role internal SV formation?	(35)
PtdIns(4,5)P2	Knockout of PIPK1γ (type 1 phosphatidylinositol phosphate kinase)	Cortical neurons (mouse)	Slower retrieval of synaptopHluorin	(36)
Synaptojanin 1	Knockout mouse	Cortical neurons (mouse)	Slower retrieval of synaptopHluorin (1.6-fold).	(37)
	Null allele	Neuromuscular junction (*Drosophila*)	Evidence for slower vesicle retrieval.	(38)
Endophilin	Null allele	Neuromuscular junction (*Drosophila*)	Evidence for slower vesicle retrieval.	(38)
Auxilin	Knockout mouse	Hippocampal neurons (mouse)	Slower retrieval of synaptopHluorin.	(39)
Stonin 2	Expression of dominant-negative constructs	Hippocampal neurons (rat)	Effect on synaptotagmin recycling. No effect on retrieval kinetics.	(40)

The studies of CME at hippocampal synapses both used similar experimental strategies: exocytosis and endocytosis were monitored optically using synaptophysin-pHluorin and clathrin heavy chain (CHC) was knocked down using RNAi. Synaptophysin-pHluorin is modeled on synaptopHluorin, a pH-sensitive form of green fluorescent protein (GFP) fused to the intraluminal side of the vesicle protein synaptobrevin (24,25). The pHluorin is quenched at the acidic pH inside a vesicle and fluoresces more brightly when a vesicle fuses. Because vesicles are thought to be reacidified rapidly after retrieval, the rate of endocytosis can be estimated from the rate at which synaptopHluorin is quenched again. Synaptophysin-pHluorin is more selectively localized to synaptic vesicles than synaptopHluorin, and therefore detects vesicle fusion with a better signal-to-noise ratio. The development of this improved reporter made it possible to measure endocytosis after vesicle fusion triggered by single action potentials (18). Use of this minimal stimulus was a key feature of these experiments because it had been suggested that fast 'kiss-and-run' was the retrieval pathway predominating during normal levels of synaptic activity (26). In fact, both studies using hippocampal neurons found that all vesicle retrieval was severely slowed down or blocked by interfering with the expression of CHC (Figure 2) (18,19).

A drawback of using RNAi to interfere with CME is that the knockdown of CHC occurs over about 3 days, making it difficult to assess whether there may be other less specific effects contributing to the inhibition of synaptic vesicle retrieval (see below). To avoid this problem, Heersen et al. designed a method for acutely photoinactivating clathrin

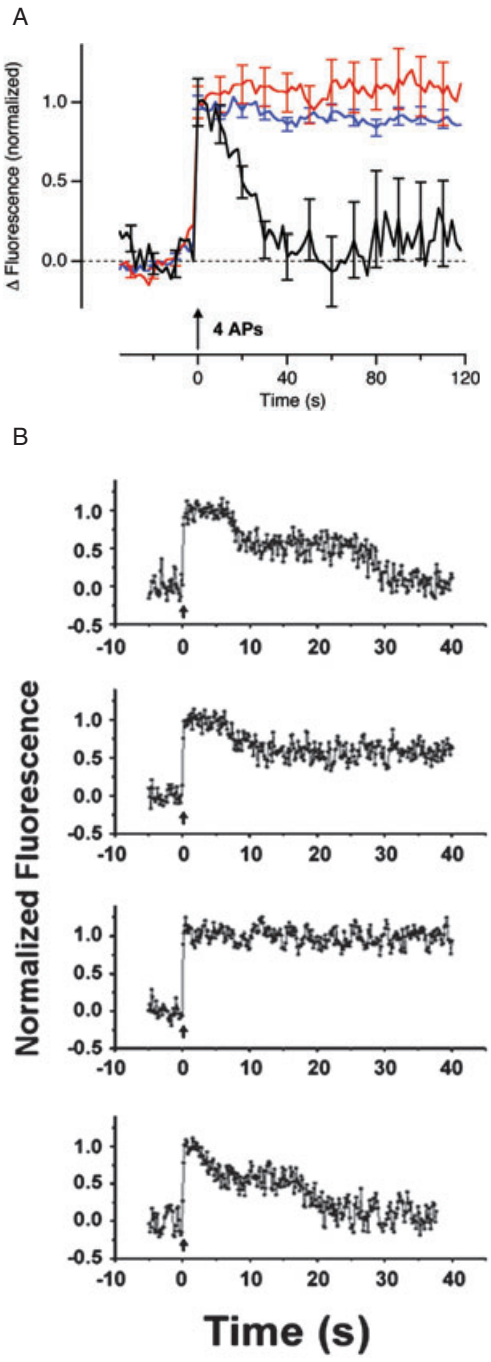

Figure 2: Inhibition of clathrin-mediated endocytosis in hippocampal neurons blocks vesicle retrieval after weak stimuli. A) Synaptophysin-pHluorin traces of hippocampal neurons stimulated with four action potentials at 20 Hz. Normalized fluorescence traces were recorded from cells transfected with synaptophysin-pHluorin and either CHC RNAi (blue) or mRFP-AP180-C (red). Control traces (black) are pooled from cells cotransfected with mRFP or with both mRFP and control siRNA. Figure reproduced from Ref. (18). B) Example traces of synaptophysin-pHluorin single-vesicle responses with clathrin RNAi knockdown (upper three panels), and control (the bottom panel). Figure reproduced from Ref. (19).

light chain (Clc) in *Drosophila* using FlAsH-FALI, which is effective within ~3 min (20). A six amino acid tetracysteine epitope tag was introduced into Clc to bind the membrane-permeable fluorescein derivative FlAsH-EDT_2, allowing blue laser light to selectively inactivate Clc by generation of reactive oxygen species very close by. Using electrophysiology to assay neuromuscular transmission during the course of a train of stimuli, Heersen et al. found that the response ran down much faster after inactivation of Clc, indicating that synaptic vesicle recycling, such as 'kiss-and-run', did not occur (Figure 3) (20). Notably, photoinactivation of CHC also resulted in the internalization of large sections of membrane that could not be recycled or participate in further release (27). The last few years have seen significant advances in our understanding of this process of 'bulk' endocytosis (41,42), and it has recently been reviewed elsewhere (43).

The synapse can adapt to chronic loss of an adaptor, so the timescale of interference is important for experiments designed to interfere with a specific protein. The incomplete block of endocytosis following RNAi of adaptor protein complex 2 (AP2) is reminiscent of studies of organisms with deletions in genes that are important for CME. For example, the deletion of amphiphysin in mice (44) and μ2-adaptin in *Caenorhabditis elegans* (45) produced only subtle changes in synaptic vesicle endocytosis. This could be because of adaptation of the clathrin-mediated endocytic network or a rerouting of retrieval to the bulk retrieval pathway. The generation of a mouse that was null for dynamin-1, the main dynamin isoform in the brain (32), again highlighted the capability for adaptation that synapses have in the face of chronic perturbation of CME. The mouse was viable and only exhibited defects in endocytosis during strong stimulation. This suggests that either dynamin-1 is a catalyst for membrane scission but is not essential for this process, or that other proteins can compensate for the loss of dynamin-1 (32). RNAi of CHC gave a more complete block of endocytosis, perhaps this is because of the lack of a failsafe: another coat protein that can substitute (18,19).

Another approach that has been used to assess the role of CME at the synapse is to introduce peptides or protein domains that interfere with CME through a patch pipette while simultaneously monitoring changes in membrane surface area electrophysiologically using the capacitance technique. Although very direct, the technical difficulties associated with this approach have only allowed it to be applied to two neurons with very large synaptic terminals: the ribbon synapse of retinal bipolar cells isolated from goldfish (46,47) and the giant calyx of Held of neurons in slices of rat auditory brainstem (48). At the bipolar cell terminal, there are two kinetically distinct modes of endocytosis, fast, occurring with time constants of about 1–2 seconds, and slow, in 10–15 seconds (47,49,50). Weak stimuli are followed almost exclusively by fast retrieval, but as more vesicles are released, the slow mechanism recycles

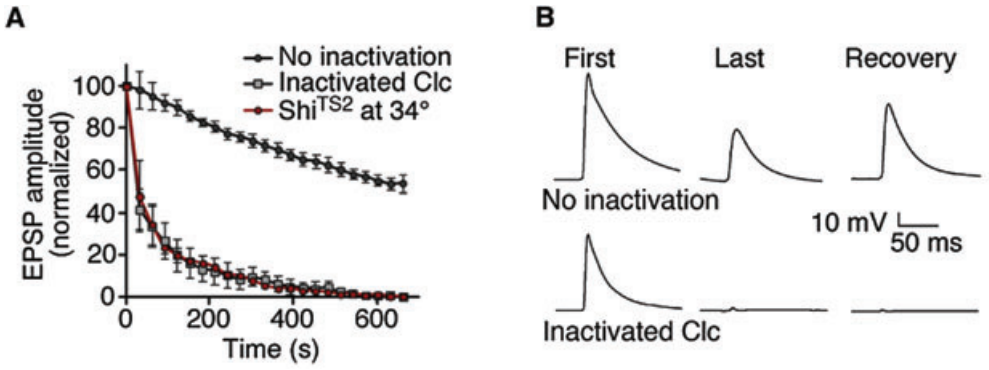

Figure 3: Photoinactivation of clathrin light chain at the *Drosophila* NMJ prevents vesicle recycling. A) Under conditions of repetitive stimulation, excitatory post-synaptic potentials (EPSPs) run-down following photoinactivation of clathrin light chain. This synaptic depression is very similar to that seen with *shibire*^{ts2} mutants at the non-permissive temperature. B) Average EPSPs for control and photoinactivated terminals to illustrate the depression and lack of recovery following photoinactivation. Figure reproduced from Ref. (20).

an increasing proportion of the excess membrane (47). Disrupting the interaction between amphiphysin and the AP2 adaptor complex using a 12mer containing the DNF motif of amphiphysin selectively and completely blocks the slow mode of endocytosis, leaving the fast mode intact (28). Endocytosis at the calyx of Held was also severely inhibited when this peptide and another that targets AP2–synaptotagmin interactions were used (29,51). The complete blockade observed here contrasts with the subtle effects of AP2 RNAi (33,34) or gene deletion (45) and highlights the importance of acute rather than chronic interference methods. An elegant method for rerouting proteins to mitochondria in response to application of rapamycin has now been described (52). The implementation of this method, termed 'knock-sideways', for targeting proteins involved in synaptic vesicle retrieval would be ideal because of the proximity of mitochondria to the active zone and the fast time–course of action (~3 min). This method could be used at synapses where peptide interference is not possible and for proteins where RNAi results in only a mild phenotype.

The absolute requirement for the large GTPase dynamin in synaptic vesicle retrieval has been shown using a variety of methods at different synapses. Classic experiments using the temperature-sensitive *shibire Drosophila* mutant showed that all membrane retrieval at the NMJ is dynamin dependent (53). Perturbation of dynamin function using inhibitory peptides at the calyx of Held (29,31) and the giant reticulospinal synapse of the lamprey (54) or by dynasore, a small molecule inhibitor of dynamin function, at hippocampal synapses (30) and at the calyx (29) resulted in an inhibition of vesicle retrieval. These experiments argue for the importance of dynamin GTPase activity in vesicle retrieval at most synapses.

Auxilin, the neuronal form of the ubiquitously expressed cyclin G-associated kinase (GAK), is a co-chaperone for uncoating of clathrin-coated vesicles (5,55). The auxilin knockout mouse had a number of empty clathrin cages in synapses in the cerebellum, a phenotype that was anticipated following work in non-neuronal cells (56). At hippocampal synapses in culture, synaptopHluorin experiments from a relatively small experimental sample showed defects in synaptic vesicle retrieval following 200 APs at 10 Hz (39). This was in spite of an upregulation of GAK, which is less efficient in clathrin uncoating compared to auxilin (39). The defects in synaptopHluorin retrieval likely reflect the reduced availability of free clathrin, rather than a direct role of uncoating in early stages of endocytosis. In the time since our last review (3), there is now plenty of evidence for a clear involvement of clathrin-mediated retrieval of synaptic vesicles under physiological conditions.

The Speed of CME at the Synapse

It has often been suggested that CME is not fast enough to account for the maintenance of synaptic transmission during ongoing activity (15–17,57). This idea is based on measured rates of receptor internalization by CME in non-neuronal cells, which is on the order of minutes (58). But is CME at the synapse as slow? A number of physiological studies make it clear that the answer is 'no'. The studies of Granseth et al. (18,23) using synaptophysin-pHluorin, and Balaji and Ryan (22) using vGlut1-pHluorin, have produced very similar measurements in cultured hippocampal neurons: CME operates with a time constant of about 14 seconds at room temperature, accelerating to 6–10 seconds at body temperature (22,23). Another study of single vesicle retrieval at hippocampal synapses reported a more complex picture with a fast and slow form of endocytosis. Both forms were clathrin dependent as clathrin siRNA inhibited either form (19). Capacitance measurements in bipolar cell terminals and the calyx of Held also show that CME operates with a time constant of ~10–20 seconds (28,29,31). The rate of

vesicle retrieval is very similar in mossy fiber terminals in the hippocampus (59) and in the ribbon synapse of hair cells (60), although it has not been identified as CME. The dominant mode of endocytosis at most synapses therefore occurs with a time constant of 10–20 seconds.

Is endocytosis on the timescale of 10 seconds really too slow to account for the maintenance of exocytosis at the synapse? In many areas of the nervous system, synapses become depressed after periods of activity, reflecting a reduction in the pool of readily releasable vesicles docked and primed at the active zone (the RRP). This pool can be replenished by the much larger number of vesicles held in reserve behind the active zone, but is this process of refilling sufficiently fast to account for the speed with which synapses recover from depression? It has been suggested that synaptic vesicles must be retrieved intact within ~1 second of fusion by the process of 'kiss-and-run', after which they immediately dock again rather than mixing in with the reserve pool distributed further within the terminal (15,16,61–63). Surprisingly, there has not so far been any quantitative justification of this idea, and recent experimental tests suggest that it is not correct. Using cultured hippocampal neurons, Granseth and Lagnado found that vesicle retrieval was accelerated by increasing the temperature from 25°C to 35°C, while the recruitment of reserve vesicles to the RRP was not significantly affected (23). Quantitative modeling of the vesicle cycle indicated that the rate-limiting step for supplying vesicles to the RRP was endocytosis operating with a time constant of 10–15 seconds at room temperature and ~6–10 seconds at 35°C. Crucially, it was not necessary to invoke a faster mode of endocytosis to account for changes in the size of the RRP during and after activity (23).

What is the Adaptor for Clathrin-Mediated Synaptic Vesicle Retrieval?

During CME, it is well established that cargo in the membrane is recognized by adaptor proteins, which in turn bind clathrin (9). It is not possible for clathrin to link to the membrane without adaptors (64,65). Adaptors, by definition, are proteins that allow clathrin to engage with the membrane. They can bind to clathrin, phosphatidylinositol (4,5) bisphosphate in the plasma membrane and to cargo proteins (9,66). In non-neuronal cells, CME of the majority of cargo in the plasma membrane is mediated by AP2 (67). However, there are a number of alternative adaptors that serve to link specific cargo molecules to the clathrin coat (6).

In neurons, the situation is much less clear: the adaptors for clathrin-mediated synaptic vesicle retrieval are not defined. Cargo selection by adaptors is especially important at the synapse: all the necessary proteins of the synaptic vesicle must be sorted correctly to prevent non-functional vesicles being formed, e.g. vesicles missing v-SNAREs or neurotransmitter pumps (4). The current picture is that AP2 is one adaptor for synaptic vesicle endocytosis, but that other adaptors are required for retrieval of specific vesicle components and the endocytic machinery has numerous fail-safe mechanisms and a tremendous capacity for redundancy.

There is some evidence that cargo plays an important role in triggering endocytosis, presumably by recruiting adaptor proteins. In the case of one synaptic vesicle protein, synaptotagmin, either deletion (68), photoinactivation (69) or mutation (70) results in impaired synaptic vesicle endocytosis. The presence in the presynaptic plasma membrane of proteins normally residing in the synaptic vesicle must be efficiently detected and internalized. There is some debate whether the vesicle proteins remain clustered on the surface following exocytosis (71,72) or whether they diffuse and are internalized at distal sites (18,73–75).

Knockdown of CHC in hippocampal neurons was successful in that it showed an involvement of clathrin in synaptic vesicle endocytosis (18). Knockdown of AP2, on the other hand, produced results that were less clear-cut. Chronic (6–12 days) depletion of μ2 by RNAi resulted in an inhibition of endocytosis but not a complete blockade. The AP2-independent endocytosis that persisted was sensitive to brefeldin-A treatment and further experiments indicated that AP1 might be able to compensate for AP2 under the RNAi conditions used in these experiments (34). Later work from the same group replaced the endogenous μ2 with mutated forms that are predicted to interfere with endocytosis (33). The experiments indicated that no single mutation was sufficient to block endocytosis and that the addition of all mutations was required to cause the most efficient block of retrieval (33). In these experiments, vGlut1-pHluorin was used to monitor vesicle endocytosis. This cargo protein uses a dileucine sorting signal to associate with α and σ2 hemicomplex of AP2 (6,33,34,76,77). What other adaptors are employed for synaptic vesicle retrieval? Stonin 2 is proposed to be an adaptor that mediates the sorting of synaptotagmin in an AP2-dependent manner (78). Stonin 2 has a μ2 homology domain that interacts with C2 domains in synaptotagmin and a WVXF motif that can bind to sites on AP2 (78). However, inhibition of AP2 or stonin 2 does not result in a total blockade of endocytosis, at least on the timescale of these experiments. These observations suggest that CME of synaptic vesicles is driven by an ensemble of adaptors that select the various components of the synaptic vesicle (4). If synaptic vesicles are retrieved as intact clathrin-coated entities (79), rather than clathrin-coated vesicles containing only one type of cargo, then the partial inhibition of endocytosis seen in these experiments using vGlut1-pHluorin (33,34) or synaptotagmin-pHluorin (40) is an indication that these cargoes can be internalized in a vesicle along with others whose adaptors are still functional.

Synaptic Vesicle 'Resynthesis': Roles for Other Adaptor Proteins

In the original Heuser-Reese model of synaptic vesicle recycling, CCVs budded from the plasma membrane and fused to form an internal cisterna. These cisternae – equivalent to early sorting endosomes – were broken down into new synaptic vesicles (8). Later work indicated that a sorting endosome was not necessarily involved in the synaptic vesicle cycle (79,80). Clathrin coats can clearly be seen on these synaptic endosomes (8) and the question arose over how they were formed. An analysis of vGlut1 recycling at hippocampal synapses concluded that this transporter could be internalized by two routes: a direct AP2-mediated retrieval from the plasma membrane and a slower AP3-mediated budding of vesicles from cisterna (33,34,77). There is some evidence that vGlut1 binds to endophilin, a protein that can in turn bind dynamin, and this interaction promotes the AP2-mediated retrieval of the transporter (77). The selection of the different routes may be stimulation dependent: with mild stimulation promoting AP2-mediated retrieval and stronger stimulation evoking bulk endocytosis and AP3-mediated budding. The functional importance of synaptic vesicle 'resynthesis' from internal membranes was highlighted in a recent study of mice null for σ1B subunit of AP1 (35). Knockouts of γ1- or μ1A-adaptins were not viable, but σ1B is not required for the ubiquitous functions of AP1, allowing the study of AP1 perturbation. Hippocampal synapses in these animals had fewer vesicles than controls at rest and following stimulation. SynaptopHluorin imaging experiments indicated that this phenotype was because of a defect in vesicle recycling rather than the initial biogenesis of synaptic vesicles (35) Behavioral experiments with σ1B null mice suggest these defects in vesicle recycling cause problems in motor co-ordination and spatial memory. The forming picture is that synaptic vesicles can be retrieved from the plasma membrane or pinched off from internal membranes. The source of the internal membranes may be the large cisternae that are internalized via bulk endocytosis.

Conclusions

In the last few years, the gap between physiology and molecules has narrowed considerably, and we have a much clearer understanding of the role of CME compared to other potential mechanisms of synaptic vesicle retrieval. Key to this improvement in understanding is the use of real-time methods, such as imaging and the capacitance technique, that allow endocytosis to be assayed in living neurons. A number of these measurements now indicate that CME is the normal mechanism of vesicle retrieval. Although it has been suggested that 'kiss-and-run' also operates under some circumstances (15,62), the observations that lead to this conclusion have been difficult to reproduce or the interpretations questioned (21,81). Certainly, we are far from understanding the molecular basis of kiss-and-run, should it actually occur. A major challenge now is to understand how CME is accelerated at synapses compared to non-neuronal cells. The answer almost certainly lies in understanding how the endocytic machinery is assembled after a synaptic vesicle has fused. We therefore need to detail the role of adaptors and accessory proteins during CME at the synapse.

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