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Dynamin-independent synaptic vesicle retrieval?

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Abstract

A new study proposes that synaptic vesicle endocytosis at a large synaptic terminal is partly independent of dynamin and GTP hydrolysis, suggesting a new mechanism leading to vesicle fission and maintenance of neurotransmission.

Among the most astonishing mutants in flies are the temperature-sensitive alleles of *shibire*. These mutants are paralyzed when their temperature is raised to 30 °C, yet they resume activity rapidly after their return to 25 °C. Their paralysis is mirrored by the trapped endocytic intermediates that decorate vesicle-depleted synapses of *shibire* mutants at the elevated temperature¹. The dynamin GTPase encoded by the *shibire* gene has since been implicated as being important in endocytic events. Dynamin's GTPase activity is required to pinch vesicles off the plasma membrane once a critical curvature is reached during endocytosis². Mutants and biochemicals that inhibit this 'pinchase' activity have become standard tools for assessing the importance of endocytosis at synapses and elsewhere.

In the current issue, Xu *et al.*³ report an elegant set of experiments that led them to propose that at least some components of synaptic vesicle endocytosis operate independently of dynamin and GTP hydrolysis. The authors took advantage of the intracellular accessibility of the calyx of Held, a large nerve terminal in the auditory brainstem, to examine the dependence of synaptic vesicle endocytosis on dynamin. Notably, they found that several manipulations aimed to disrupt dynamin function blocked endocytosis only transiently. Synaptic vesicle endocytosis recovered, despite the continued presence of reagents that potently block dynamin function, and typically endocytic retrieval, in multiple systems.

The authors evoked exocytosis through direct depolarization of the calyx of Held terminal. A critical advantage of using this system is the ability to measure membrane capacitance, which is a direct method for assessing changes in membrane surface area resulting from exocytosis and endocytosis⁴. In such experiments, a swift rise in membrane capacitance as the result of the addition of excess membrane to the terminal surface area during exocytosis is followed by a rapid decrease that is due to endocytosis. In most systems, including the calyx of Held, the endocytic phase has both fast and slow kinetics. In the new experiments, the fast component of endocytosis had a time constant around 1 s, compared with 13 s for the slow component. Furthermore, in agreement with earlier findings, infusion of nonhydrolyzable analogs of GTP blocked fast and slow components of endocytosis, presumably by interfering with dynamin function⁵.

In addition to nonhydrolyzable GTP analogs, Xu *et al.*³ used multiple reagents to interfere specifically with dynamin function³. First, they infused nerve terminals with dynasore⁶, a small membrane-permeable compound that inhibits the GTPase of both dynamins I and II. This

manipulation caused a block or deceleration of rapid and slow endocytosis. Second, the authors took advantage of a proline rich– domain peptide that prevents amphiphysin’s recruitment of dynamin. Third, they used the pleckstrin-homology domain of dynamin to inhibit interactions between dynamin and phospholipids, which are critical for endocytosis. Finally, the authors dialyzed nerve terminals with an antibody against dynamin to block its function. All of the last three agents substantially inhibited slow endocytosis and slowed down the fast component. The manipulations that directly tamper with dynamin function (nonhydrolyzable GTP analogs and dynasore) inhibited both fast and slow endocytosis, whereas the reagents that interfere with dynamin’s interaction with other proteins and lipids selectively impaired slow endocytosis. These results suggest that dynamin interactions may be more important for slow endocytosis, whereas dynamin alone may be sufficient to trigger fast endocytosis. Up until this point, Xu *et al.*’s findings³ generally supported the existing notion that dynamin function is essential for synaptic vesicle endocytosis and were consistent with the selective loss of the fast component of synaptic vesicle recycling in dynamin I mutant mice⁷.

The surprise came when the authors continued to stimulate terminals after a nearly complete block of endocytosis. Repeated application of the stimulation protocol resulted in the recovery of endocytosis with similar fast and slow kinetics in the continued presence of GTP γ S or other reagents. This occurred even after extensive dialysis of the nerve terminals with high concentrations of nonhydrolyzable GTP analogs. Notably, the capacitance measurements of this recovered endocytosis matched other measures of synaptic function, as they were coupled to exocytosis detected by postsynaptic recordings and uptake of the styryl dye FM1-43 that could be observed optically. Overall, recovered endocytosis shared all the basic characteristics of dynamin- and GTP-dependent endocytosis, except that it was insensitive to agents that interfere with GTP hydrolysis and dynamin function.

What kind of a mechanism could underlie the recovery of endocytosis? One possibility is macropinocytosis, which operates independently of dynamin, but requires the actin cytoskeleton. However, in the authors’ hands, recovered endocytosis was insensitive to disruption of actin filaments by cytochalasin D³. Moreover, the kinetics of recovered endocytosis did not match the properties of bulk endocytic retrieval that were previously characterized by the same group in the calyx of Held⁸.

Taken together, these experiments and their unexpected results leave us with a major puzzle. Two questions come to mind. What is the mechanism that activates this dynamin and GTP-independent endocytosis? How does membrane scission operate without the perennial pinchase dynamin? The authors’ experiments provide some clues for a future answer to the first question. They found that recovered endocytosis was recruited by repetitive stimulation, but was not a response to prolonged dialysis of nonhydrolyzable GTP analogs. In addition, recovered endocytosis was typically triggered after a reproducible increase in the membrane surface area. From these results, the authors propose that the number of vesicles that can be retrieved via a dynamin- and GTP-dependent mechanism is fixed, and therefore only vesicles released in excess of this pool are recovered via an alternative mechanism. They favor a scenario in which the vesicles are predestined for one form of retrieval or the other, and therefore they form separate pools (Fig. 1). The GTP- and dynamin-dependent pool has a higher priority for release and retrieval, but limited capacity, saturation of which leads to release, and thus retrieval, of GTP- and dynamin-independent vesicles. Alternatively, dynamin- and GTP-dependent mechanisms can be exhausted by the onslaught of a large number of vesicles (~92 vesicles per active zone as estimated by Xu *et al.*³) during intense stimulation, and thus require activation of the dynamin-independent mechanisms to preserve structural and functional homeostasis of nerve terminals.

Both possibilities, however, would require a molecular tag to recruit the dynamin-independent endocytosis. In the authors' preferred scenario, this tag would be selectively located on vesicles with low fusion propensity, but would activate retrieval immediately after fusion. In the latter scenario, this tag may be present on all vesicles and would steadily accumulate on the surface membrane, but would lead to dynamin-independent endocytosis only after reaching a critical density. Some possible candidates for such tags are the synaptic vesicle proteins synaptotagmin-1 and synaptobrevin-2 (also called VAMP-2), both of which are implicated in facilitating synaptic vesicle retrieval^{9,10}. However, further work is clearly needed to identify synaptic vesicle proteins that actively participate in synaptic vesicle retrieval and ensure the proper, functionally effective protein composition of synaptic vesicles for consecutive rounds of vesicle reuse.

Although it may seem heretical to envision a dynamin-independent form of endocytosis from the current perspective of synaptic vesicle trafficking, there are multiple types of membrane fission that occur without dynamin in other cell biological systems. One example is the budding of COPII vesicles. Their fission from the endoplasmic reticulum requires the Sar1 GTPase, which (like dynamin) hydrolyzes GTP to trigger membrane fission¹¹. However, because GTP γ S dialysis does not block recovered endocytosis, it is not likely to be driven by any such GTPase.

Another intriguing possibility is that the cargo delivered by exocytosis may actively promote the budding of vesicles during recovered endocytosis, as has long been the prevailing idea about the budding for viruses from the cell surface. The matrix and membrane proteins of some viruses may suffice to form virus particles¹², whereas other viruses, like HIV, must recruit ESCRT proteins from the host to facilitate budding¹³. The three multi-protein ESCRT complexes were originally described in the context of the formation of the small internal vesicles of multivesicular bodies late in the endocytic pathway in yeast¹⁴. The interaction between ESCRT proteins and cargo sequestered into internal vesicles seems to be critical, but the mechanism that drives vesicle fission from the limiting membrane is still poorly understood¹⁵.

The answers to the questions raised by these recent papers^{3,7} probing the requirement of dynamin in synapses will not only further our understanding of the mechanisms of synaptic vesicle recycling, but also provide critical insight to several cell biological processes that are hard to access with high-precision functional measurements similar to those available at synapses.

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Figure 1. Xu *et al.*³ propose that the number of vesicles that can be retrieved via a dynamin and GTP-dependent mechanism is fixed, and therefore only vesicles released in excess of this pool are recovered via an alternative mechanism. Vesicles are suggested to be predestined for one form of retrieval, and thus form separate pools (orange versus red vesicles).