addition, the UIM-domain protein Hrs may, through direct interactions with ubiquitin and clathrin, sort endosomal ubiquitin conjugates into clathrin-containing membrane microdomains, thus taking proteins that would otherwise be recycled to the plasma membrane and shunting them to the lysosome to be degraded in a proteasome-independent manner[24]. In principle, both soluble and membrane-bound conjugates may be further distinguished on the basis of known variations in the topology of their multi-ubiquitin chains. Whether these various features of the conjugates will provide a sufficient basis for different ubiquitin receptors to distinguish specific conjugates among the hundreds present in eukaryotic cells is anyone's guess. Now pursued into every corner of the cell, ubiquitin remains unpredictable. □

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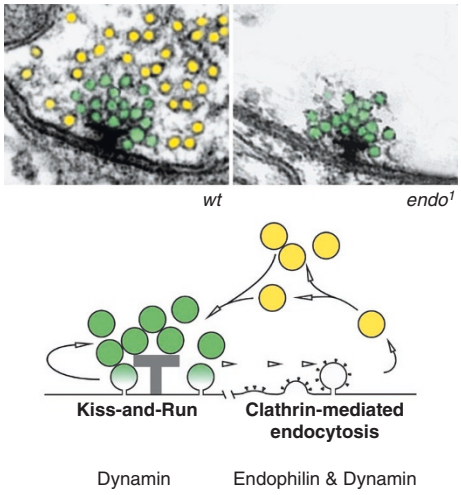
# An encore for kiss and run?

After synaptic vesicles fuse with the plasma membrane of nerve terminals to release neurotransmitters, they must be recycled so that they can be refilled and undergo the next round of release. If vesicle recycling by endocytosis is blocked and nerve terminals run out of vesicles, synaptic transmission in response to repetitive stimuli is severely impaired. New research in *Drosophila melanogaster* by Verstreken *et al.* (*Cell*, **109,** 101–112, 2002) and Guichet *et al.* (*EMBO J.*, **21,** 1661–1672, 2002) now sheds further light on this process.

Flies have two homologues (*endo A* and *endo B*) of endophilin— a protein thought to influence membrane curvature by modifying membrane lipids. Although previous studies have implicated endophilin at multiple steps during clathrin-mediated endocytosis, these papers, which analyse various mutant alleles of *endo A*, are the first to study the loss of endophilin function *in vivo*. *ENDO A* is expressed in the nervous system and localizes to the presynaptic membrane of nerve terminals in neuromuscular junctions (NMJs). *endo A* mutants have defects in motility and die as larvae or early pupae. In *endo A* null mutants, exocytosis of synaptic vesicles at low stimulation frequencies is not impaired. However, the retrieval of vesicles from the presynaptic membrane is blocked, synaptic boutons are larger than in wild-type animals, and ultrastructural studies of NMJs identify a severe depletion of presynaptic vesicles (yellow vesicles in the figure) and an increased number of early endocytosis intermediates. Taken together, these results clearly demonstrate an essential role of ENDO A in endocytosis at NMJs *in vivo*.

Intriguingly, however, Verstreken *et al.* noted that NMJs of a null mutant retain a small pool of vesicles that undergo multiple rounds of exocytosis and maintain about 15–20% of normal neurotrasmitter release during repeated stimulation. This residual activity may well explain why *endo A* mutants survive as long as they do. So what is going on? One possibility is that ENDO B replaces some of the function of ENDO A in endocytosis. But based on a careful analysis of the detection limits for vesicle recycling, Verstreken *et al.* conclude that endocytosis in *endo A* mutants is almost completely blocked. Thus, the authors reason

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that some synaptic vesicles at the active zone (green vesicles) carry out 'kiss-and-run' (a transient fusion of vesicles with the plasma membrane, such that neurotransmitters are released through the transient fusion pore). Why then does the lack of dynamin — another protein that is essential for endocytosis — in *shibire* mutants not have a similar residual activity? An exciting possibility is that dynamin functions not only in clathrin-mediated endocytosis, but also in the rapid fission of vesicles during kiss-and-run. It is safe to assume that kiss and run will remain a controversial subject, and further studies of *endo A* and *shibire* mutants will undoubtedly be important in the resolution of these questions.

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