Vesicle formation: **Dynamic dynamin lives up to its name**

Tom Kirchhausen

The GTP-binding protein dynamin was initially thought to be required for just the final stages of clathrin-dependent vesicle formation, but recent results indicate that it can actually catalyse many of the essential steps in the vesiculation pathway.

Address: Department of Cell Biology and Center for Blood Research, Harvard Medical School, Boston, Massachusetts 02115, USA.
E-mail: kirchhausen@crystal.harvard.edu

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Dynamin is unusual among GTPases for two reasons: its affinity for GTP is rather low, and its GTP hydrolysis rate is high and dramatically dependent on polymerization (reviewed in [1–3]). This latter property was one of the first indications that dynamin's *in vivo* function might depend on self-assembly. Pure dynamin spontaneously forms rings and helices in conditions of low ionic strength [4], and decorates microtubules [5,6] and lipid vesicles [7,8] with helices of similar dimensions. Although it was the association between dynamin and microtubules that led to the recognition that dynamin is a GTP-binding protein, it is now believed that most of dynamin's functions are related to intracellular membrane traffic; the relevance of dynamin's microtubule-binding activity remains unclear.

The first clue to dynamin's role in vesicle formation came from the discovery that the defective gene in the *Drosophila* temperature-sensitive mutant *shibire* — in which neurotransmission is blocked by a failure of plasma membrane recycling — encodes a mutant form of dynamin with impaired GTPase activity [9,10]. At the non-permissive temperature, invaginations in the plasma membrane of *shibire* neurons are observed that appear to be arrested just before budding. A dynamin mutation in *Caenorhabditis elegans* has recently been observed to have similar effects [11]. The observations with *Drosophila* were subsequently generalized to mammalian cells, in which overexpression mutant dynamins, defective in either GTP binding or hydrolysis, was found to lead to inhibition of endocytosis through the clathrin pathway, indicating that dynamin has a direct role in the budding of clathrin-coated vesicles from the plasma membrane [1–3].

Recently, however, the variety of roles ascribed to dynamin has dramatically increased. It has been suggested that dynamin itself may directly deform portions of the plasma membrane into tubes, in the absence of clathrin or other coat-forming proteins, and that it might have a very general role as a mechanochemical enzyme that generates the force responsible for membrane fission — called by some a 'pinchase' — in many pathways of vesicular traffic [7,8]. Furthermore, it has now become clear that dynamin interacts directly with a number of proteins, including the adaptor protein complex AP-2, responsible for cargo selection and coat assembly in clathrin-coated vesicles, and amphiphysin, a dimeric protein that, as explained below, has a clear role in endocytosis [3]. Dynamin is also thought to interact directly with lipids; there is a pleckstrin homology (PH) domain in the center of the dynamin sequence, which presumably explains dynamin's tendency to polymerize on the surface of lipid vesicles.

Dynamin may indirectly be linked to much larger networks of proteins via amphiphysin [12]. There are at least two variants of amphiphysin, amphiphysin-1 and amphiphysin-2, which interact with AP-2, clathrin and synaptojanin, as well as with dynamin. Synaptojanin in turn interacts with Eps15, a newly-recognized component of the clathrin coat that localizes to the neck region of budding vesicles [13]. It is plausible that this chain of interactions, Eps15 to synaptojanin to dynamin, is responsible for nucleating the formation of the structure that induces vesicle scission. Amphiphysin interacts with dynamin and synaptojanin via its Src homology 3 (SH3) domain. Overexpression of the isolated SH3 domain of amphiphysin blocks endocytosis, as does microinjection of a peptide based on dynamin's proline-rich SH3-binding site [14,15]. These interactions appear to be controlled by phosphorylation, although the nature of the kinases and phosphatases involved, and the relevant sites for phosphorylation, are currently unknown [12].

Two recent studies [7,8] have provided evidence that dynamin provides the mechanical force for vesicle scission. In the first [7], purified dynamin was shown to decorate large unilamellar lipid vesicles and transform them into elongated tubes. On addition of GTP, many of the tubes rapidly fragmented into smaller vesicles, which remained associated with dynamin. This is something of a surprise, as it is currently assumed that dynamin is always released from the membrane once vesicle scission has occurred, this being clearly the case with clathrin-coated vesicles. It is possible that two modes of dynamin interaction with vesicles exist, one in which it is released immediately after scission, the other in which it is retained until the vesicle reaches its destination. It is also possible that the minimal system in which these studies were performed lacks a factor required for dynamin release.

Sweitzer and Hinshaw [7] observed that some lipid tubes formed when dynamin was added to a lipid vesicle preparation survived the addition of GTP, and these are also informative. The surviving tubes were morphologically distinct from those existing prior to the addition of GTP: they were narrower, and the helices formed by dynamin on their surfaces appeared to be more closely packed. One plausible interpretation is that the addition of GTP causes a conformational change in dynamin that mimics the narrowing of the neck in budding vesicles.

In the second of the two recent studies [8], a cytosol extract, containing clathrin coat proteins, and dynamin were simultaneously added to preparations of protein-free liposomes. The lipid composition of the liposomes was manipulated to optimize the binding of AP-2 and dynamin. Buds and elongated tubes of membrane coated with dynamin were found to be formed, but only occasionally were these tubes capped with a clathrin-coated pit. There is thus no evidence that the deformation of membranes by dynamin is enhanced by the presence of clathrin, at least in the situation where no cargo is available for clathrin-associated proteins to bind to.

This conclusion is supported by electron microscopic studies of neurons in *shibire* mutant *Drosophila* [16]. At the non-permissive temperature (29°C) exocytosis presumably occurs in *shibire* neurons until all the available vesicles are depleted, inactivation of the dynamin GTPase preventing membrane recycling. When dynamin is reactivated by a temperature reduction, massive recapture of the membrane components that have been delivered to the synaptic face of the nerve terminal is initiated. Interesting differences appear when recovery is performed at the partially permissive temperature of 26°C, were recycling membranes accumulate but not pinched-off vesicles, or at the fully permissive temperature of 19°C.

During recovery at 26°C, flat cisternae, approximately 200 Å wide, develop in the synaptic active zone where exocytosis normally occurs. In other areas of the synapse, branched tubes, approximately 600 Å in diameter, develop that are covered with dynamin at the branching points. Both structures are generally free of clathrin coat components. The reason for these differences in morphology within the active zone is completely unknown. During recovery at 19°C, however, the active zone morphology is quickly restored to normal, with no observable intermediate steps; the branched tubes in the other areas of the synapse develop like the 26°C tubes, but faster. At later time points (10 minutes), the cisternae and tubes fragment into large numbers of vesicles, mostly uncoated. It is only after 20–25 minutes at 19°C that the normal morphology of the synapse is restored, with a full complement of vesicles arrayed below the active zone and throughout the nerve terminal. It is not yet certain whether the flat cisternae that are seen in the active zone during recovery at 26°C are real recovery intermediates, but it is clear that these morphological differences must reflect a fundamental and as yet unidentified difference between the active zone membrane and the bulk of the neuronal membrane.

This model system might help to elucidate the specialized mechanisms for membrane recycling at the synapse, which have been elusive for many years. Several models for rapid membrane uptake in synapses have been proposed. In the standard model [17], membrane uptake occurs via clathrin-coated vesicles at a high rate, but outside the active zone. The 'kiss-and-run' model [18] proposes that vesicles in the active zone fuse with the synaptic membrane only briefly, releasing neurotransmitters through a narrow 'neck' that quickly reseals in a clathrin-independent process to give an endocytic vesicle. A third model proposes that deep invaginations in the synaptic membrane are constantly present and provide a route for membrane uptake by vesicle formation at the bottom of the tube. The observations on *shibire* mutant *Drosophila* would appear to support the last of these models, but the postulated invaginations in unperturbed synapses have been hard to detect, and considerably more work will be required to clarify the processes involved.

A particularly intriguing observation is that *shibire* neurons at the non-permissive temperature contain two types of budding vesicle arrested in the process of vesicle scission. One type has a collar at the neck of the vesicle, which presumably contains dynamin; the other is clathrin-coated and does not have a collar. Few vesicles are observed that are both clathrin-coated and collared. This can be interpreted as supporting the notion that clathrin and dynamin can separately induce the deformation of membranes to form pits; clathrin-coated pits probably require dynamin to complete vesicle formation, but may need only a few molecules, too few to be seen as a 'collar' structure. When dynamin acts alone to form a vesicle directly, a larger array of dynamin molecules may be required, forming the collar structures visible in the electron microscope.

Complex though dynamin's actions may be in *Drosophila*, the situation may be more complex still in mammalian cells. Vertebrates have not one, but at least three dynamin homologs, and in rat more than 25 variants have been shown to be produced by alternative RNA splicing [19]. Dynamin-1 is neuron specific, whereas dynamin-2 is probably expressed everywhere except in neurons; dynamin-3 is found primarily in neurons, with some expression in testis, lung and muscle. All of the mammalian cell experiments described above were performed with dynamin-1, and it is not yet clear how similar the patterns of protein–protein interactions are for dynamin-2 and dynamin-3. Recent work using fusion proteins in which dynamin is linked to green fluorescent

protein (GFP) has indicated that dynamin variants have different subcellular localization patterns [19]. Some of the variants were seen to localize to clathrin-coated vesicles, whereas others were not; the signal at least partially responsible for this differential localization appears to be contained within two very short stretches of amino acid residues in the central region of dynamin.

Dynamin-2 is clearly important for vesiculation from the Golgi complex by both clathrin-dependent and clathrin-independent pathways [20]. Overexpression of dynamin-1 mutants in epithelial cells, which lack endogenous dynamin-1, can interfere with endocytosis from the plasma membrane by the clathrin-dependent pathway and also prevent budding of caveolae [21]. The implication is that several dynamin family members are required for these processes; indeed, microinjected antibodies specific for dynamin-2 inhibit caveolae uptake in epithelial cells [22]. It is thus possible that many vesiculation events require dynamin or a related protein.

Many more questions remain to be answered. If a dynamin is generally responsible for vesicle scission in mammalian cells, what are the specific roles of the many other variants? With such a profusion of dynamin variants, experiments using any individual dynamin must be carried out and interpreted with particular care, and it would seem crucial to use a model cell system appropriate for the particular dynamic variant. And if dynamin is indeed responsible for the mechanical tightening and closure of a vesicle 'neck', how does it work? Are the interactions between dynamin and lipids relevant, perhaps causing microdomains of lipids with special fusogenic properties to form around the neck and facilitate inner-leaflet fusion, or is the process purely protein-driven? And once we understand the direct effects of dynamin, we will still need to understand the roles of its protein partners; obvious possible functions of such partners are in signalling to the actin cytoskeleton, assisting vesicle closure, or providing a way for vesicle closure to occur in response to other signals. Sorting all of this out will require a lot of dynamic people.

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