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Minireview

Dynamin and receptor-mediated endocytosis

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Abstract The internalization of receptor-bound ligands involves concentration of cell surface receptors in specialized areas of the plasma membrane and subsequent formation of clathrin-coated vesicles. The complex process of invagination, constriction and budding of clathrin-coated vesicles employs the coordinated actions of several proteins. This review is focused on the GTPase dynamin, which plays a key role in the constriction of coated pits.

Key words: Receptor-mediated endocytosis; Clathrin-coated vesicle; Dynamin; GTPase

1. Introduction

Intracellular trafficking involves the continuous formation and fusion of vesicles for the transport of cargo material to its destination and is strictly balanced for maintenance of the cells surface/volume ratio. Within this context the endocytic pathway serves to internalize material from the plasma membrane and transport it to specific intracellular compartments for further processing or sorting. Endocytosis, which constitutively occurs in all eukaryotic cells, requires pinching off plasma membrane to generate new vesicles. Vesicle formation generally involves the assembly of a protein coat from soluble, cytosolic sources [1], although the formation of uncoated endocytic vesicles has been described [2]. Before fusion with the target membrane the vesicle has to be uncoated. In recent years many proteins that direct vesicle formation have been identified [3,4] and we continue to learn of their interaction and regulation by cofactors like ATP, GTP or membrane lipids. The demonstration that the GTPase dynamin is the mammalian homologue to the *shibire* gene product in *Drosophila* implied a function for dynamin in endocytosis. Flies carrying the *shibire*^{ts} allele are rapidly paralyzed due to a block in synaptic vesicle recycling. This review will focus on our current understanding on dynamin's biochemical properties and functional role in the molecular mechanism of receptor-mediated endocytosis via clathrin-coated vesicles (for reviews see [5,6]).

2. Receptor-mediated endocytosis

Many molecules, including viruses and toxins, may enter the cell by the mechanism of receptor-mediated endocytosis. Clathrin-dependent, receptor-mediated endocytosis is essential for the efficient transport of nutrients into the cell and for attenuating the action of signal-transducing receptors by removing ligand-receptor complexes from the cell surface [7]. Multiple steps are required for the efficient uptake of receptor-bound ligands. Internalization of cell surface receptors occurs either constitutively or is ligand-induced. The receptors are concentrated in specialized regions on the plasma membrane, characterized by highly organized lattices and referred to as coated pits. Adaptor proteins (AP proteins) and clathrin are the major constituents of the coated pit and they are recruited from the cytosol to saturable binding sites on the plasma membrane [1,8]. After recruitment of AP complexes in an apparently spontaneous binding reaction that does not require hydrolyzable nucleotides, APs mediate the assembly of clathrin triskelions into an initially planar clathrin lattice. Planar clathrin lattices curve to form deeply invaginated, 'constricted' coated pits. These intermediates of endocytic clathrin-coated vesicle formation have been detected both in vivo and in vitro and are functionally defined by their ability to sequester receptor-bound ligands from exogenously added macromolecules [9]. The contents of a constricted coated pit remain accessible to small, membrane impermeant reagents. In vitro data show that coat protein assembly itself is not sufficient to drive endocytic clathrin-coated vesicle formation and that additional cytosolic proteins as well as ATP and GTP hydrolysis are needed [4,10]. The formation of constricted coated pits in vitro can be supported by GTPγS, but not by ATPγS. The final membrane fusion event at the neck of constricted coated pits that leads to vesicle budding requires both ATP and GTP hydrolysis [10]. Whereas the ATPases involved in these late stages in endocytic coated vesicle budding have not been identified, dynamin has recently been shown to be a required GTPase [11–13].

3. Dynamin is required for coated vesicle formation

Dynamin is a 100 kDa protein originally purified from bovine brain and characterized as a microtubule (MT)-stimulated GTPase with MT-bundling activity [14]. The *Drosophila shibire* gene product is 70% identical to neuronal rat dynamin [15,16], suggesting an in vivo function. Temperature-sensitive *Drosophila shibire* mutants have a pleiotropic defect in endocytosis leading to rapid paralysis. The neuromuscular junctions of paralyzed flies were depleted of synaptic vesicles and accumulated a high number of clathrin-coated and uncoated pits [17].

Mammalian dynamin occurs in at least three different isoforms, all of them with additional splicing alternatives at three different sites The originally isolated dynamin-1 is expressed exclusively in neurons, dynamin-3 in the testes and dynamin-2, which is 79% identical to both dynamin-1 and *shibire*, is ubiquitously expressed. [2,18].

Extensive transfection studies of GTPase defective dynamin mutants in nonneuronal mammalian cells have demonstrated a direct and specific role for dynamin in endocytosis [11–

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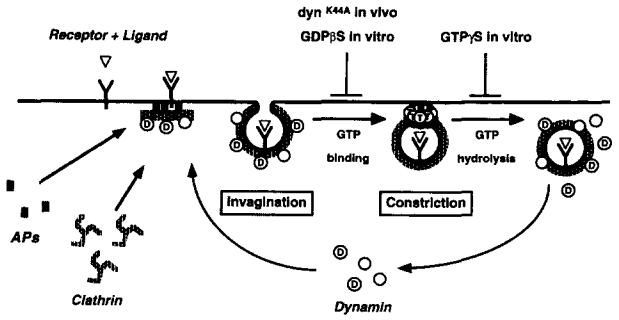

Fig. 1. Model for the role of dynamin in coated vesicle formation. After the assembly of adaptor proteins (APs) and clathrin at the plasma membrane, dynamin is recruited from the cytosol in its unoccupied or GDP-bound form (D) to clathrin-coated areas. On invaginated coated pits dynamin molecules are randomly distributed. GTP binding (T) is required to redistribute dynamin to the neck of coated pits where dynamin assembles into a collar that constricts the coated vesicle. A conformational change correlating with GTP hydrolysis is thought to close the vesicle's neck releasing an isolated vesicle. Dynamin in its GDP form then disassembles and recycles (see text for details).

13,19]. The most extensive phenotypic analysis has been performed in stable transformants of HeLa cells expressing the dynamin^{K44A} mutant defective in GTP binding and hydrolysis under control of an inducible promoter [13], although similar results were obtained from studies of a temperature-sensitive mutant of dynamin homologous to the *shibire*$^{ts-1}$ mutation [19]. Receptor-mediated endocytosis is blocked in cells overexpressing dynamin^{K44A} at a stage following coat assembly and invagination but preceding the sequestration of receptor-bound ligands into constricted coated pits [13]. No other vesicular transport pathway, including protein transport through the biosynthetic pathway, receptor recycling through the endosomal compartment or clathrin-coated vesicle mediated transport of lysosomal enzymes from the TGN to the lysosome were affected by the dynamin^{K44A} mutant [13].

The apparent specificity of dynamin function is consistent with dynamin's exclusive EM immunolocalization to clathrin-coated pits at the plasma membrane. In stably transformed cells, the membrane localization of overexpressed neuronal dynamin-1 is indistinguishable from that of endogenous dynamin-2. Accumulation of overexpressed dynamin in the cytosol suggests that dynamin-1 and dynamin-2 compete for saturable membrane binding sites. The K44A mutant binds at least as effectively to coated pits as wild-type dynamin suggesting that dynamin-1 binds to the membrane in its nucleotide-free or GDP-bound form [13]. A closer examination of endogenous dynamin-2 binding to coated pits in perforated A431 cells revealed two types of distribution [20]. While evenly distributed on flat clathrin lattices and invaginated coated pits, the endogenous dynamin-2 staining of deeply invaginated coated pits indicated a concentration of dynamin at the base of the pit. The K44A mutant remains evenly distributed on the invaginated clathrin pits that accumulate in transformed cells [13] suggesting that GTP binding is required for dynamin's redistribution to the neck of a deeply invaginated coated pit. Since GTP binding, but not its hydrolysis, was required to form a constricted coated pit in vitro [10], these results suggested that the redistribution of dynamin to the necks of coated pits might be required for coated pit constriction.

Insights into dynamin's function in forming constricted coated pits came from the observation that recombinant, purified human dynamin-1 oligomerizes into tetramers which spontaneously self-assemble under low salt conditions into rings and stacks of rings [21]. These dynamin rings resemble electron dense 'collars' observed around the necks of the endocytic vesicles that accumulate at nerve terminals in the Drosophila *shibire*^{ts} mutant [22]. EM immunolocalization of dynamin to stacked rings that accumulated on membrane invaginations in permeabilized synaptosome preparations incubated with the nonhydrolyzable analogue of GTP, GTPγS [23] confirmed that these 'collars' correspond to dynamin rings.

4. Current model

The model emerging from current studies suggests that dynamin is targeted to clathrin-coated pits in its GDP-bound form (see Fig. 1). GTP/GDP exchange might trigger the redistribution of dynamin tetramers from its uniform distribution on flat and curved lattices to the necks of deeply invaginated coated pits. In its GTP-bound form, dynamin would self-assemble around the neck forming collar-like structures as seen in paralyzed *shibire* flies or permeabilized GTPγS-treated synaptosome preparations. These collars have still to be observed in vivo under normal conditions or in nonneuronal cells under any condition, but they are probably very short-lived structures. Stimulation of dynamin's GTPase activity by self-assembly or interaction with other molecular partners (see below) would result in the hydrolysis of GTP driving a conformational change that closes the collar's neck to pinch off isolated vesicles. The GDP-bound form of dynamin would then disassemble and recycle.

While this model is attractive and consistent with in vitro and in vivo observations, a number of questions remain to be answered. Most importantly, how is dynamin initially targeted

Table 1
Molecular interactions with dynamin in vitro

Partner or regulator	Significance
Dynamin [21,37,38]	self-assembly stimulates dynamin's GTPase activity directly
Divalent dynamin antibodies [36]	crosslinking facilitates assembly and stimulates GTPase activity
Microtubules [12,14,31,44,45]	multivalent stimulators of GTPase activity act by facilitating dynamin-dynamin interaction
Acidic phospholipids [24,41]	and bind to PRD of dynamin in C-terminus
SH3 domains containing proteins [34,35]:	
Grb2 [32,46–48]	connection to regulatory events of signal transduction pathways?
Phospholipase Cγ [32,47]	
p85 subunit of PI3 kinase [32]	
Amphiphysin [43]	
α-Adaptin [25,43]	targeting of dynamin to coated pits?
Protein kinase C [39,40]	neuron-specific regulation of dephosphin (= dynamin) by phosphorylation
Calcineurin [49]	Ca^{2+}-dependent dephosphorylation?
Ca^{2+} [41]	Ca^{2+}-sensitive regulation of GTPase?

to coated pits and what is its specific binding site. While dynamin does bind acidic phospholipids [24], it is unlikely that direct interaction with membrane lipids is sufficient to account for the specificity and saturability of the membrane binding site. Dynamin does not appear to bind directly to clathrin (Schmid, S.L., unpublished results), but a direct interaction of dynamin with AP-2 molecules via α-adaptin has been suggested from in vitro observations [25]. Whether this interaction occurs in vivo, given potential accessibility problems, remains to be seen. While factors involved in targeting dynamin to coated pits need to be identified, interactions with dynamin's proline- and arginine-rich domain (PRD) (see below) are likely to be involved [26]. A second unresolved issue involves dynamin's apparent redistribution on coated pits. While initially targeted to and uniformly distributed on the clathrin lattice, after redistribution and assembly into collars, dynamin becomes segregated from clathrin [23] and presumably from APs. What mechanism moves dynamin to its site of action at these new membrane binding sites? Does this redistribution involve dissociation and rebinding to new membrane binding sites or does dynamin migrate along with its putative docking protein to the neck? What controls dynamin's assembly in vivo? Finally, what regulates dynamin's GTP binding and hydrolysis activity in vivo and how is dynamin recycled? Some of these questions can be addressed by studying the biochemical properties of dynamin and its interaction with other proteins in vitro.

5. Biochemical properties and molecular interactions

Dynamin has a complex domain structure. Its N-terminal, ~300 amino acid GTP-binding domain consists of three consensus elements highly conserved among all GTPase superfamily members. Dynamin's entire GTPase domain is well conserved with other members of a related, but functionally diverse subfamily of GTPases, including the dynamin-like yeast protein Mgm1 (maintenance of mitochondrial genome) [27] and the mammalian Mx protein (viral resistance) [28]. Of these GTPases, the yeast proteins, Vps1p [29] and Dnm1p [30] are the most related to dynamin and play a role in the trafficking of proteins to the yeast vacuole along the exocytic or endocytic pathways, respectively. The remaining two-thirds of the dynamin molecule is more divergent. Dynamin encodes a pleckstrin homology (PH) domain, which is generally thought to be involved in protein-protein or protein-lipid interactions [18]. Based on sequence analysis the regions C-terminal of the PH domain are predicted to be short coiled-coil domains and the final 100 amino acids are characterized as a basic, proline- and arginine-rich domain (PRD) and thought to mediate inter- or intramolecular protein interactions. The other dynamin-like family members lack the pleckstrin homology and proline-rich C-terminal domains characteristic of dynamin isoforms and therefore their functional relationships to the dynamin isoforms remain unclear. Multiple functional domains of dynamin, many implicated in protein-protein interactions, are consistent with the notion that it must interact with a number of 'partners' for its function in endocytosis [6,18].

Dynamin has a high intrinsic rate of GTP hydrolysis of ~100 nmol/min per mg [31]. This GTPase activity can be stimulated in vitro by a group of functionally diverse molecules, including microtubules, src homology 3- (SH3) domain containing proteins like grb2 and acidic phospholipids [31,32,24] (see Table 1). The in vivo significance of these interactions remains unclear. None of the proteins so far identified as interacting with dynamin in vitro have provided much insight into how it functions in the context of clathrin-coated pit endocytosis. Dynamin seems not to interact with microtubules in vivo [33,13] and growth factor induced interaction between SH3 domain containing proteins is inconsistent with dynamin's role in constitutive endocytosis [34]. All of the interacting molecules reported to stimulate dynamin's GTPase in vitro are multivalent proteins that bind to dynamin's PRD [24,35]. Crosslinking experiments using monoclonal antibodies to dynamin's PRD directly established the significance of multivalent interactions for the stimulation of dynamin's GTPase activity [36]. Recent findings that dynamin self-assembly in vitro stimulates its GTPase activity in the absence of any effector molecules clearly suggest that dynamin-dynamin interactions are sufficient for stimulating GTPase activity [37]. These findings, coupled to the observation that microtubule and phospholipid stimulated dynamin GTPase activity was strongly cooperative [38], suggest that these multivalent stimulators of dynamin GTPase act by concentrating dynamin molecules and facilitating its self-assembly. Interestingly, GTP hydrolysis destabilizes assembled dynamin collars [37] suggesting that both dynamin self-assembly and disassembly are directly connected to its GTP cycle. Thus, dynamin represents its most significant binding partner and dynamin-dynamin interactions can account for its function in coated vesicle constriction and regulation of its GTPase activity.

6. Other regulators of dynamin function

Dynamin-1 was shown to be identical to the neuronal protein dephosphin, which is rapidly dephosphorylated upon depolarization of nerve terminals [39]. Dynamin-1 can be phosphorylated by protein kinase C (PKC) in vitro and this phosphorylation stimulates intrinsic dynamin GTPase activity [40]. These studies indicate that interactions with or modifications of the C-terminal portion of dynamin control dynamin's GTPase activity. Dynamin-1 is not phosphorylated by PKC in stably transformed cells in vivo (Damke, unpublished results). Opposite in vivo and in vitro results might reflect the requirement of additional regulating molecules, e.g. Ca^{2+} [41]. Although dynamin-1 and dynamin-2 seem to be identical in function, their regulation might be different as the process of synaptic vesicle recycling requires specific control of neuronal dynamin [5,42]. Amphiphysin, a SH3 domain containing neuronal protein, might be involved in synaptic vesicle endocytosis [43]. It specifically interacts with dynamin in vitro and colocalizes with dynamin. Dynamin-2 association with SH3 domain containing proteins might also be relevant for the regulation in nonneuronal cells. The functional significance of these possible regulatory events, both in neuronal and nonneuronal cells, remains to be established.

7. Concluding remarks

With the general idea of how dynamin functions in receptor-mediated endocytosis in mind, much work needs to be done to fill the gaps in our model. The identification of dynamin's partners in vivo seems to be the biggest challenge. Future studies on dynamin function in vitro, carefully evalu-

ated for their correlation with in vivo studies, will contribute to our understanding of the role of this new class of GTPases in endocytosis.

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