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DYNAMIN AND ITS ROLE IN MEMBRANE FISSION[1]

J. E. Hinshaw
Laboratory of Cell Biochemistry and Biology, National Institutes of Health, Bethesda, Maryland 20892; e-mail:jennyh@helix.nih.gov

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■ **Abstract** Dynamin, a 100-kDa GTPase, is an essential component of vesicle formation in receptor-mediated endocytosis, synaptic vesicle recycling, caveolae internalization, and possibly vesicle trafficking in and out of the Golgi. In addition to the GTPase domain, dynamin also contains a pleckstrin homology domain (PH) implicated in membrane binding, a GTPase effector domain (GED) shown to be essential for self-assembly and stimulated GTPase activity, and a C-terminal proline-rich domain (PRD), which contains several SH3-binding sites. Dynamin partners bind to the PRD and may either stimulate dynamin's GTPase activity or target dynamin to the plasma membrane. Purified dynamin readily self-assembles into rings or spirals. This striking structural property supports the hypothesis that dynamin wraps around the necks of budding vesicles where it plays a key role in membrane fission. The focus of this review is on the relationship between the GTPase and self-assembly properties of dynamin and its cellular function.

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INTRODUCTION

Dynamin was originally identified as a microtubule-binding protein (Paschal et al 1987); however, there is no evidence at present that dynamin interacts with microtubules in vivo (Noda et al 1993, Scaife & Margolis 1990). When dynamin was sequenced it was found to contain a GTP-binding domain (Figure 1*a*, see color insert) homologous to other large GTPases (Mx and Vps1) (Obar et al 1990). This led to the classification of a new group of GTPases referred to as the dynamin family of large GTPases (Obar et al 1990). Dynamin was also identified as a substrate for protein kinase C (PKC) in neuronal tissue and subsequently was called dephosphin in the literature (reviewed in Robinson et al 1994). To date, only the neuronal isoform of dynamin, dynamin 1, has been shown to be phosphorylated by PKC, suggesting a neuronal-specific function for this phosphorylation event.

The role of dynamin in endocytosis was established when the *Drosophila* gene *shibire* was sequenced and found to be a homologue of dynamin (van der Bliek & Meyerowitz 1991, Chen et al 1991). The temperature-sensitive *shibire* fly exhibits a rapid and reversible paralysis at the non-permissive temperature (Grigliatti et al 1973), which has been identified as a block in endocytosis (Kosaka & Ikeda 1983a, Koenig & Ikeda 1989). At the non-permissive temperature the nerve termini of the *shibire* fly lack synaptic vesicles and accumulate clathrin-coated pits at the plasma membrane (Kosaka & Ikeda 1983a, Koenig & Ikeda 1989). This observation led to the speculation that the mammalian homologue, dynamin, also plays a role in clathrin-coated vesicle formation at the plasma membrane. In support of this concept, endocytosis was inhibited in cells overexpressing a dynamin mutant deficient in GTP binding (van der Bliek et al 1993, Herskovits et al 1993a, Damke et al 1994). In recent years, numerous researchers have used these mutants to determine if a particular protein or complex is internalized using a dynamin-dependent pathway (see Table 1).

Structural analysis of recombinant dynamin 1 revealed that dynamin exists as a tetramer (Muhlberg et al 1997) that can assemble into rings and spirals (Hinshaw &

TABLE 1 Compounds internalized in a dynamin-dependent pathway as shown by cells transfected with dynamin mutants

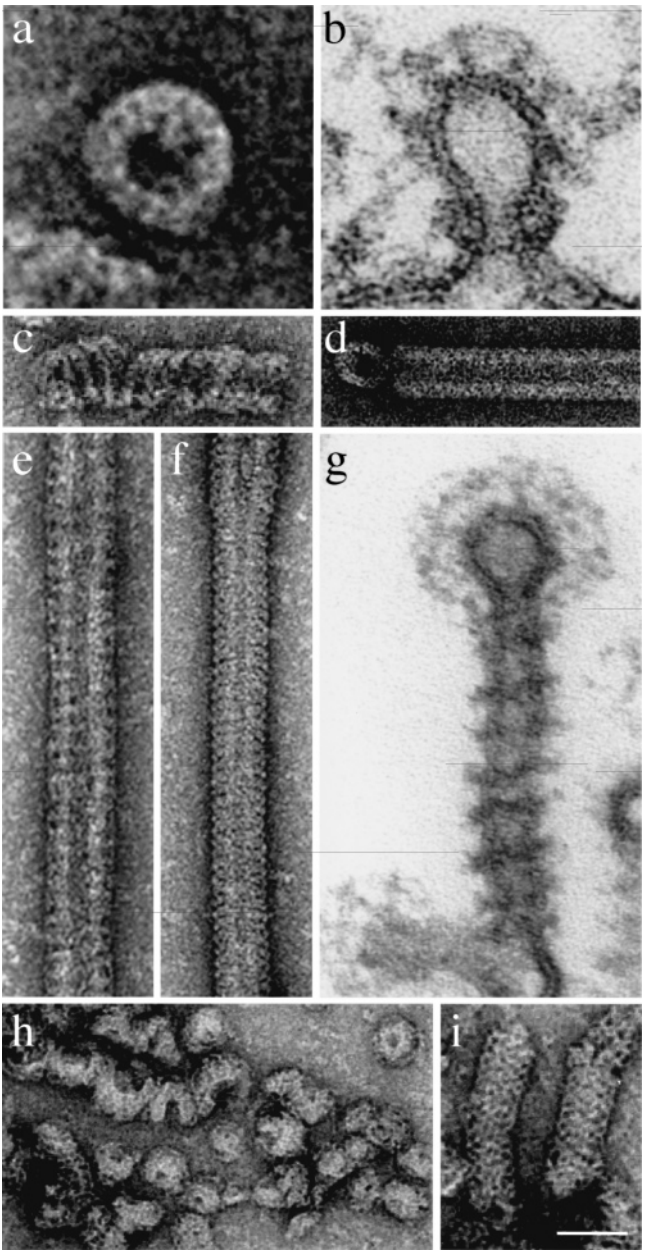
Internalization Blocked by Dynamin Mutants	References
Adenovirus	Wang et al 1998[a]
ARF6-stimulated endocytosis in MDCK cells	Altschuler et al 1999[a]
$\alpha_{2b/2c}$-adrenergic receptor (plus arrestin3)	DeGraff et al 1999[a]
β_2-adrenergic receptor	Zhang et al 1996, Gagnon et al 1998, Jockers et al 1999[a]
Bradykinin B_2 receptors	Pizard et al 1999[a]
Canine parvovirus	Parker & Parrish 2000[a]
CXCR1 (interleukin-8 receptor)	Barlic et al 1999[a]
CXCR2	Yang et al 1999[a]
Diphtheria toxin	Simpson et al 1998, Skretting et al 1999[a]
Dopamine D1 receptors in HEK293 cells	Vickery & von Zastrow 1999[a]
Dopamine D2 receptors in COS-7 cells	Iwata et al 1999[a]
Dopamine transporter	Daniels and Amara 1999[a]
Epidermal growth factor receptor	Damke et al 1994, Vieira et al 1996[a]
Epithelial Na(+)/H(+) exchanger isoform 3	Chow et al 1999[b]
Epithelial sodium channel	Shimkets et al 1997[c]
Glucose transporter, GLUT4	Al-Hasani et al 1998, Kao et al 1998, Omata et al 1997[a]
Influenza virus	Roy et al 2000[a]
Insulin receptor	Ceresa et al 1998[a]
Invariant chain (Ii)	Wang et al 1997[a]
Ionotropic glutamate receptors (AMPARs)	Carroll et al 1999[a]
L1 subfamily of cell adhesion molecules (CAMs)	Kamiguchi et al 1998[a]
Lutropin/choriogonadotropin receptor	Lazari et al 1998[a]
m1, m3, m4 muscarinic cholinergic receptors	Lee et al 1998, Volger et al 1998[a]
δ opioid receptor	Chu et al 1997[a]
κ opoiod receptor	Li et al 1999[a]
μ opioid receptor	Zhang et al 1998[a]
Rat gonadotropin-releasing hormone receptor	Heding et al 2000[a]
Recombinant adeno-associated virus	Duan et al 1999[a]
Semlike Forest, Sidbis and human rhinovirus 14 in HeLa cells	DeTulleo & Kirchhausen 1998[a]
Transferrin	van der Bliek et al 1993, Damke et al 1994[a]
Thromboxane A_2 receptor	Parent et al 1999[a]
Thyrotropin-releasing hormone	Yu & Hinkle 1998[a]

[a]K44A or K44E
[b]S45N
[c]DK44

Schmid 1995, Carr & Hinshaw 1997) and form helical tubes on a lipid bilayer substrate (Sweitzer & Hinshaw 1998, Takei et al 1998) (Figure 2). These structures resemble the collared structures seen at the base of the coated pits in the *shibire* mutant (Kosaka & Ikeda 1983a, Koenig & Ikeda 1989) and the striated tubules seen in synaptosomes treated with GTPγS (Takei et al 1995). These results, along with numerous other findings, including the observation that dynamin is capable of generating force (Sweitzer & Hinshaw 1998), led to the hypothesis that once dynamin wraps around the necks of clathrin-coated pits it is directly involved in pinching off coated vesicles (reviewed in McNiven 1998, Hinshaw 1999) (Figure 3).

Although only one dynamin isoform has been identified in *Drosophila* and *Caenorhabditis elegans* (Chen et al 1991, van der Bliek & Meyerowitz 1991, Clark et al 1997), in mammals three closely related isoforms have been identified (for review see Urrutia et al 1997). Dynamin 1, found specifically in neuronal tissue (Scaife & Margolis 1990, Powell & Robinson 1995), has 8 splice variants (see Cao et al 1998) and has been mapped to human chromosome 9q34 (Newman-Smith et al 1997). Dynamin 2 is ubiquitously expressed (Cook et al 1994, Sontag et al 1994, Diatloff-Zito et al 1995) and has 4 splice variants (Cao et al 1998), whereas dynamin 3, expressed predominantly in testes (Nakata et al 1993), but also found in lung and neurons (Cook et al 1996), has 13 splice variants (Cao et al 1998). There is evidence that different isoforms and splice variants function at distinct locations within the cell. In addition, there is a growing number of dynamin-like proteins that have similar GTP-binding and hydrolysis properties but exhibit quite diverse cellular functions. Moreover, most dynamin-like proteins have also been shown to self-assemble, and therefore a general mechanism of action may underlie the function of each specific protein in the cell.

Figure 2 Dynamin rings and spirals and dynamin-lipid tubes resemble the collar structures seen in *shibire* and the striated tubules observed in synaptosomes treated with GTPγS. (*a, c*). Dynamin self-assembles into rings (*a*) and spirals (*c*) in the presence of GDP and γ-phosphate analogues (negative stain). (*b*) A thin-section micrograph of a clathrin-coated pit from *Drosophila shibire* nerve termini with a dense collar material seen at the neck. (Image provided by K Ikeda; Kosaka & Ikeda 1983a.) (*d*) Tight spirals form when ΔPRD dynamin is dialyzed into GMP-PCP. (*e*) Dynamin-lipid helical tube made from incubating ΔPRD dynamin with phosphatidylserine liposomes in the absence of any nucleotide. The diameter of the tubes is $\sim$50 nm. Lipid tubes made with wild type (wt) dynamin (Sweitzer & Hinshaw 1998) are indistinguishable from those shown here. (*f*) The addition of GMP-PCP to the ΔPRD-dynamin lipid tubes caused the tubes to constrict to $\sim$40 nm. Similar constricted tubes, but shorter and irregular, were seen when wild type dynamin tubes were incubated with GTP (*i*) (Sweitzer & Hinshaw 1998). (*g*) Long invaginating tubules decorated with a striation pattern and a terminal clathrin-coated pit are observed in synaptosomes treated with GTPγS. (Image provided by K Takei and P De Camilli; Takei et al 1995.) (*h,i*) Addition of GTP to dynamin lipid tubes results in fragmentation (*h*) and constriction (*i*) of the tubes. Scale bar 22 nm (*a,b*) and 50 nm (*c-i*).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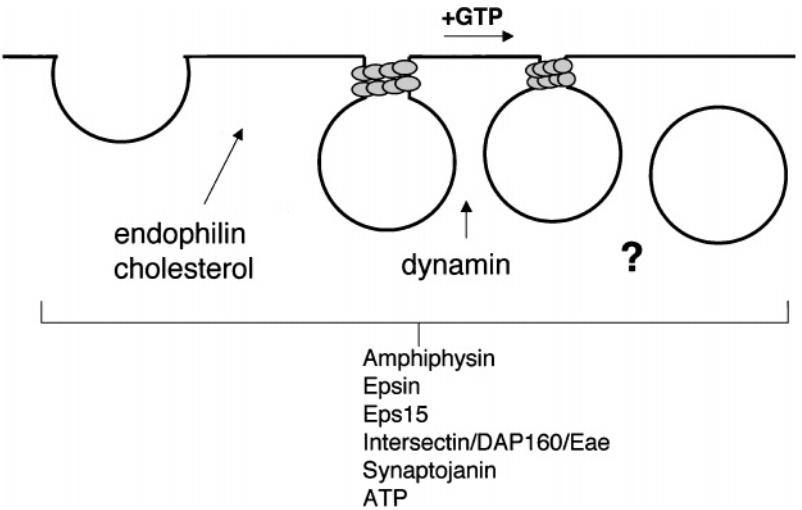

Figure 3 Model of clathrin-coated vesicle formation. Endophilin and cholesterol are necessary to convert the shallow coated pits to deeply invaginated coated pits. Once invaginated, dynamin-GTP wraps around the necks, and upon GTP hydrolysis constrict the necks, which may lead to vesiculation. Alternatively the binding of GTP to dynamin may trigger another molecule to bind to the necks and act in concert with dynamin for membrane fission. Additional molecules that have been localized to the necks or shown to play a role in clathrin-coated vesicle formation are listed.

DYNAMIN DOMAINS

Over the years clues to the mechanism of dynamin function have arisen from studying its five domains: an N-terminal GTP hydrolysis domain, a middle domain; a pleckstrin homology (PH) domain; a GTPase effector domain (GED); and a C-terminal proline-rich domain (PRD) (Figure 1*a*). As discussed below, each one of these domains has been examined and characterized in relationship to its role to endocytosis.

GTP Hydrolysis Domain (Residues 1–299) and GTPase Activity

The GTP hydrolysis domain (GTPase domain) is the most highly conserved region within the dynamin family of proteins (reviewed in Warnock & Schmid 1996, van der Bliek 1999). Although the atomic structure of the GTPase domain of dynamin is unknown, it is likely to have features similar to the GTP-binding domain found in the recently solved structure of human guanylate-binding protein 1 (hGBP1), the first solved structure in the dynamin family of large GTPases (Prakash et al 2000) (Figure 1*b*). This GTPase domain is similar to GTPase domains of other

solved structures such as the small GTP-binding protein, Ras, but contains an eight-stranded β-sheet with nine surrounding helices (instead of the usual six β sheets and five α helices), making this domain slightly larger than the more traditional GTPase structure (Prakash et al 2000).

Compared with the small GTP-binding proteins, dynamin has a high GTPase activity (1–20 min^{-1}) and low affinity for GTP (10–100 μM) (reviewed in Warnock & Schmid 1996). Furthermore, under conditions that promote dynamin assembly into oligomers, the GTPase activity can be stimulated greater than 15-fold over the intrinsic rate (Tuma & Collins 1994; Warnock et al 1995, 1996). In vitro, the binding of dynamin through its proline-rich domain to microtubules (Shpetner & Vallee 1992, Herskovits et al 1993b), to growth factor receptor-bound protein 2 (Grb2; Herskovits et al 1993b), and to antibodies directed against its C terminus (Warnock et al 1995) stimulates the GTPase activity of dynamin and promotes its assembly (reviewed in Warnock & Schmid 1996). In addition, anionic phospholipids (liposomes), which bind to the PH domain of dynamin, also stimulate its GTPase activity and promote assembly (Tuma et al 1993). This stimulation can be further enhanced synergistically by the addition of Grb2, a SH3 (Src homology)-containing protein (Barylko et al 1998). This implies that dynamin bound to anionic lipids in the membrane is accessible for binding to activators such as Grb2. The stimulated and intrinsic GTPase activity increases in a sigmoidal fashion with increasing concentration of dynamin, suggesting positive cooperativity (Tuma & Collins 1994, Warnock et al 1996). Thus a direct correlation exists between the assembly of dynamin into oligomers and its GTPase activity.

Proteolytic analysis of dynamin has revealed that the C-terminal proline-rich domain behaves as a positive regulator of self-assembly and GTP hydrolysis, whereas the PH domain acts as a negative regulator (Muhlberg et al 1997). The GED region, as indicated by its name, is also a positive regulator of the GTPase activity and directly interacts with the GTPase domain of dynamin (Muhlberg et al 1997, Sever et al 1999). The interaction between GED and the GTPase domain is believed to be the underlying mechanism for all stimulated GTPase activity of dynamin (Sever et al 1999).

When the GTPase activity of the different dynamin isoforms is compared, dynamin 2 has a higher intrinsic GTPase activity ($\sim$3- to 10-fold higher than dynamin 1) (Lin et al 1997, Warnock et al 1997), a higher affinity for GTP (12 μM for dynamin 2 compared with 36 μm for dynamin 1), and a higher stimulated GTPase activity under conditions that promote self-assembly (Warnock et al 1997). The difference in GTPase activity between the two isoforms is probably significant for their specific cellular functions, i.e. receptor-mediated endocytosis verses synaptic membrane recycling. The tightly regulated synaptic membrane recycling process in the nerve terminal may require a lower intrinsic GTPase activity, especially considering that the concentration of dynamin is significantly higher in neuronal tissue than in non-neuronal tissue (Warnock et al 1997).

Another mechanism for regulating the GTPase activity of dynamin 1 is possibly through phosphorylation by PKC. The phosphorylation of dynamin 1 by

PKC enhances the GTPase activity up to 12-fold (Robinson et al 1993), whereas dephosphorylation of dynamin by calcineurin inhibits dynamin 1 GTPase activity (Liu et al 1994b). Also, Ca^{2+} has been shown to bind to dynamin 1 and inhibit its GTPase activity (Liu et al 1996). Because dynamin 1 is directed to the membrane when dephosphorylated, it is possible that the dephosphorylation and Ca^{2+} binding allow dynamin time to bind to the necks of coated pits before undergoing stimulated GTP hydrolysis. Once dynamin is in position, another factor may trigger an increase in GTP hydrolysis causing vesiculation and redistribution of dynamin to the cytosol.

Middle Domain (Residues 300–520)

The middle domain of dynamin lacks sequence homology to any known structural motif. Based on proteolysis results, the middle domain can be further divided into N-terminal and C-terminal halves (Figure 1*a*) (Muhlberg et al 1997), with the N-terminal half significantly more conserved than the C-terminal half between dynamin isoforms (92% compared with 72% between dynamin 1 and 2) (Warnock & Schmid 1996). Within the C-terminal half lies alternative splice sites for all three dynamin isoforms. In the N-terminal half there is a predicted coiled-coil region (residues 320–350) that has been implicated in dynamin-dynamin assembly (Smirnova et al 1999, Okamoto et al 1999b) (Figure 1*a*).

PH Domain (Residues 521–622) and Membrane Binding

The PH domain (~100 residues) originates from the protein pleckstrin, a major PKC substrate in platelets. At present the PH domain can be found in hundreds of proteins, including proteins involved in PI3 kinase signaling events, protein-protein interactions, and membrane binding (for reviews see Lemmon et al 1997, Rebecchi & Scarlata 1998, Blomberg et al 1999). PH domains have been shown to bind preferentially to phosphoinositides with each protein conferring different preferences to specific PI head groups. The PH domain of dynamin, which favors binding to $PI(4,5)P_2$, has been shown to be crucial for dynamin membrane localization and receptor-mediated endocytosis (Salim et al 1996). The binding of $PI(4,5)P_2$ to dynamin also gave the highest activation of its GTPase activity (Salim et al 1996). This lipid binding is significantly enhanced when the PH domain of dynamin is oligomerized (Klein et al 1998). Thus dynamin tetramers may confer a stronger lipid-binding property, and the oligomerization of the PH domain may be further mediated at the membrane by the inherent self-assembly property of dynamin.

Phosphoinositide affinities are almost identical for the PH domains of dynamin 1 and dynamin 2 (Klein et al 1998); however, only the addition of recombinant dynamin 1 PH domains blocked rapid endocytosis in chromaffin cells (Artalejo et al 1997). This suggests that there may be another region within the PH domain of dynamin 1 that confers specificity to another molecule that is not a phosphoinositide but is necessary for rapid endocytosis.

The three-dimensional structure of the PH domain of dynamin has been solved by X-ray crystallography (Timm et al 1994, Ferguson et al 1994) and NMR (Downing et al 1994, Fushman et al 1995). The structure was found to be similar to other PH domains with a seven-stranded β sheet sandwich, three variable loops, a C-terminal α-helix, and a strong polarization of charges (Figure 1*a*). The three variable loops are the most divergent regions within the PH domain and are located on the side with a predominant positive charge. This region is believed to be the ligand or membrane-binding site through binding to negatively charged lipids. In support of this concept, a point mutation at K535 in variable loop-1 prevented lipid-stimulated GTPase activity and endocytosis of transferrin (Vallis et al 1999, Achiriloaie et al 1999). Mutational studies have also implicated variable loop 3 (between β6 and β7) in lipid binding and have shown it to be important for receptor-mediated endocytosis (Lee et al 1999).

The PH domain of dynamin has also been shown to bind to G protein $\beta\gamma$ in stimulated neuroendocrine cells (Liu et al 1997), possibly through WD40 repeats in the β subunit (Wang et al 1995a). In contrast to lipid binding, $\beta\gamma$ binding inhibits the GTPase activity of dynamin (Lin & Gilman 1996). The binding of $\beta\gamma$ to dynamin may prevent dynamin self-assembly and domain interactions within the dynamin tetramer that are necessary for GTPase activity. Indeed, $\beta\gamma$ inhibits the intrinsic GTPase activity of dynamin only when dynamin is in the non-assembled state, suggesting that $\beta\gamma$ binding to the PH domain and dynamin assembly are mutually exclusive (Lin & Gilman 1996).

GTPase Effector Domain (GED) (Residues 623–745)

The GED is also referred to as the assembly or coiled-coil domain based on two predicted small coiled-coil segments (residues 650–680 and 710–740) (Lupas et al 1991, Okamoto et al 1999b) (Figure 1*a*). The coiled-coil segments suggest that this domain is involved in protein-protein interactions (Lupas et al 1991), and indeed it has been shown that GED interacts with the GTPase domain of dynamin (Muhlberg et al 1997, Sever et al 1999, Smirnova et al 1999). Addition of isolated GED to unassembled dynamin stimulated the GTPase activity >50-fold over basal rate (Sever et al 1999). This activity was shown to be highly cooperative and suggested that four GED molecules interact with a dynamin tetramer (Sever et al 1999). These results indicate the function of GED is to promote dynamin assembly, leading to stimulated GTPase activity and, therefore, GED acts as a GAP (GTPase-activating protein) for dynamin (Muhlberg et al 1997, Sever et al 1999). The role of the GED in dynamin assembly is discussed further in the Tetramers to Spirals section.

Point mutational analysis of GED indicates that residues K694, R725, and R730 are involved in stimulating GTPase activity (Sever et al 1999). The R725 residue is believed to be involved in the catalytic activity, whereas K694 is believed to play a structural role in dynamin self-assembly (Sever et al 1999). Surprisingly, even though mutating these residues decreased stimulated GTPase activity in vitro,

transfection of cells with the dynamin mutants R725A or K694A caused a stimulated rate of endocytosis (Sever et al 1999) (see Receptor-Mediated Endocytosis and Synaptic Vesicle Recycling section).

Proline-Rich Domain and SH3-Binding Sites (Residues 746–864)

The C-terminal domain of dynamin, containing the last 100 amino acids, is rich in proline and basic residues (pI ~12) and contains several SH3-domain binding sites, as defined by the PXXP motif (where X is any amino acid). SH3-binding domains play an important role in protein-protein interactions in numerous cellular processes, including clathrin-mediated endocytosis (Simpson et al 1999, reviewed in McPherson 1999). For example, Grb2, amphiphysin, and PLCγ all bind to the SH3-binding domains of dynamin (Miki et al 1994, Seedorf et al 1994, Grabs et al 1997, Vidal et al 1998) (Figure 1*a*). Endophilin, another SH3-containing protein, has been proposed to bind to dynamin in a similar manner (Ringstad et al 1997). The role of these SH3-containing proteins in regulating dynamin function is discussed in further detail in the Dynamin Partners section of this review.

REGULATION OF DYNAMIN SELF-ASSEMBLY

Tetramers to Spirals

Evidence suggests dynamin exists as a tetramer (Hinshaw & Schmid 1995, Muhlberg et al 1997) that fluctuates between a monomer-tetramer equilibrium (Binns et al 1999) under physiological salt conditions. The tetramers are capable of further self-assembly into higher-ordered structures that resemble rings and spirals (Hinshaw & Schmid 1995) (Figure 2*a,c,d*). The first hint that dynamin could assemble into helical arrays or spirals came from dynamin-decorated microtubules (Maeda et al 1992). Subsequently, dynamin was shown to assemble into spirals without any underlying support under low-salt conditions (Hinshaw & Schmid 1995) or under physiological salt conditions in the presence of GDP and γ-phosphate analogues (Carr & Hinshaw 1997, Hinshaw 1999). STEM (scanning transmission electron microscopy) analysis on the spirals revealed a ring or rung of the spiral consisting of ~30 dynamin molecules (J Hinshaw, unpublished results). Similar ring structures were also seen in physiological salt conditions when dynamin was incubated with amphiphysin 1, a neuronal-specific protein localized to clathrin-coated pits (Takei et al 1999). In contrast to full-length amphiphysin, isolated SH3 domains of amphiphysin 1 destabilize the dynamin ring formation in low-salt conditions, suggesting there is another region in amphiphysin that stabilizes dynamin oligomerization (Owen et al 1998).

Studies probing the individual domains of dynamin confirmed the hypothesis that GED is essential for self-assembly and interacts strongly with the GTPase domain (Muhlberg et al 1997, Sever et al 1999). Consistent with a role for GED

in dynamin self-assembly, removal of the GED or one or both of the coiled-coil segments within GED eliminated dynamin-dynamin interactions (Okamoto et al 1999b). Additional evidence that GED interacts with the GTPase domain came from a yeast two-hybrid assay using the GTPase domain as bait (Smirnova et al 1999). Only a single potential target protein was identified, the GED region of dynamin (Smirnova et al 1999). GED was also shown to bind to itself, forming homodimers (Smirnova et al 1999) or tetramers (Okamoto et al 1999b), as well as bind to the middle domain (residues 320–349) (Smirnova et al 1999). Similar results were observed with a Mx protein where the middle domain interacted with its C-terminal assembly domain; however, there was no cross-reactivity between the two proteins—dynamin domains did not interact with Mx domains (Smirnova et al 1999).

Although removing the PRD from dynamin does not prevent dynamin assembly into spirals (Carr & Hinshaw 1997), a yeast two-hybrid assay identified a region within the PRD (residues 792–841), as well as in the GTPase domain (residues 49–58) that is involved in dynamin-dynamin assembly (Scaife et al 1998). Furthermore, a dynamin fragment consisting of the C terminus (residues 800–840) bound dynamin (Scaife et al 1998), and addition of a SH3 domain from Grb2 (or PLCγ, P85-SH3, Src) was shown to cause dynamin oligomers to disassemble (Scaife et al 1998). This evidence suggests the C terminus of dynamin is not required for, but participates in, dynamin assembly. Removal of the PH domain, however, significantly increased dynamin-dynamin interactions (Scaife et al 1998) and had little effect on spiral formation (Muhlberg et al 1997).

The binding of guanine nucleotides, GTPγS, GTP, or GDP, destabilized assembled dynamin 1 structures in decreasing degrees of potency (Warnock et al 1996). GTPγS was the most potent with $\sim$50% of assembled dynamin 1 structures destabilized at 10 μM, whereas it took $>$250 μM of GTP and $>$1 mM of GDP to have the same effect (Warnock et al 1996). Dynamin 2, which has a greater tendency to self-assembly than dynamin 1, self-assembles at lower protein concentrations and in the presence of higher GTP concentrations (250 μM) (Warnock et al 1997). Again, the difference between the isoforms probably reflects their individual function in specific cells types, i.e. neuronal versus non-neuronal.

Dynamin Assembly onto Membranes and Lipid Bilayers

The localization of dynamin to membranes (Scaife & Margolis 1990, van der Bliek et al 1993, Herskovits et al 1993a, Damke et al 1994, Liu et al 1994a) and the discovery that anionic phospholipids stimulate dynamin's GTPase activity (Tuma et al 1993) led to the speculation that dynamin directly interacts with membrane lipid. In support of this hypothesis, it was found that mutating residues in the PH domain, predicted to be involved in lipid binding, not only prevented lipid-stimulated GTPase activity of dynamin but also blocked endocytosis (Vallis et al 1999, Achiriloaie et al 1999, Lee et al 1999). The resemblance between the dynamin rings and spirals (Hinshaw & Schmid 1995) to the collar structures

seen in *shibire* at the necks of the clathrin-coated pits (Kosaka & Ikeda 1983a, Koenig & Ikeda 1989) further suggested that dynamin directly associates with the plasma membrane (Figure 2*a-c*). In addition, in synaptosomes treated with GTPγS, the membrane tubules extending from the plasma membrane were decorated with a dense striation pattern (Figure 2*g*) that could be labeled with a dynamin antibody (Takei et al 1995). However, it is now apparent that at least two other proteins have been localized to the striated tubules, amphiphysin (Bauerfeind et al 1997, Takei et al 1999) and endophilin (Ringstad et al 1999). Similar striated tubules, commonly seen with a terminal clathrin-coated bud, were observed when inside-out erythrocytes vesicles or liposomes were incubated with brain cytosol (Takei et al 1998). Among anionic phospholipids, addition of phosphatidic acid (PA) to the liposomes resulted in the most abundant number of striated tubules (PA > PG > PIs > PI > PS > PE; Takei et al 1998).

Purified recombinant dynamin can assemble onto anionic liposomes and form well-ordered helical tubes with a 13 nm ring repeat along the tube axis (Figure 2*e*) (Sweitzer & Hinshaw 1998). As seen by negative stain, these tubes are 50 nm in diameter with a 20-nm central channel (Sweitzer & Hinshaw 1998). Addition of GTP (>10 μM, but not GDP, GTP-PCP or GTPγS) causes these tubes to constrict, from 50 to 40 nm in diameter with a 10-nm central channel, and fragment (Figure 2*h,i*) (Sweitzer & Hinshaw 1998). The distance between the dynamin rings in the constricted tubes also compresses from a 13- to a 10-nm repeat (Sweitzer & Hinshaw 1998). A dynamin mutant lacking the PRD can also assemble onto liposomes, form helical tubes, and constrict and fragment upon addition of GTP (P Zhang & J Hinshaw, personal communication). In contrast to wild-type, the ΔPRD dynamin mutant spirals and tubes also constrict upon GMP-PCP addition (Figure 2*d,f*), which suggests the PRD plays a role in regulating this gross conformational change (P Zhang & J Hinshaw, personal communication). Dynamin lipid tubes made from total brain lipid liposomes also appear to constrict and become disordered upon GTP addition (Takei et al 1998). In each case the helical tubes resemble the striated tubules of synaptosomes but with a tighter packing, or shorter pitch along the tube axis (Sweitzer & Hinshaw 1998, Takei et al 1998). When tubes are formed in the presence of dynamin and amphiphysin, the helical pitch resembles the striated packing seen in synaptosomes (Takei et al 1999). The hybrid dynamin-amphiphysin tubes also fragment to a greater extent upon GTP addition (Takei et al 1999). Overall, the observed tubular constriction and fragmentation support the hypothesis that dynamin is a force-generating molecule responsible for membrane fission and clathrin-coated vesicle formation (Figure 3).

An alternative mechanism of dynamin function came from a study examining dynamin tubes made with galactoceramides, a lipid that spontaneously forms tubes without protein (Stowell et al 1999). Addition of GTP or GDP to these dynamin tubes resulted in an increase in pitch along the helix without constriction or fragmentation (Stowell et al 1999). This observation of helical expansion led to the hypothesis that dynamin stretches the neck upon GTP treatment, causing the vesicle to pop off the membrane (Stowell et al 1999). Further experiments with

additional components of the clathrin-coated vesicle machinery will clarify the precise mechanism of dynamin action.

The observation that dynamin or other proteins bind to lipid and form lipid tubules is not unique to membrane-binding proteins. Certain proteins that have no membrane- or lipid-binding function in vivo can form similar tubular crystals. The helical tubular crystals may then be used to solve the three-dimensional structure of a soluble protein by high-resolution electron microscopy. For example, the structure of RNA polymerase I (Polyakov et al 1995), which has no known membrane-binding function in the cell, and more recently that of IgG and transducin have been solved by such a method (Melia et al 1999). The structure of dynamin is also being solved by a similar method using the tubular crystals seen in Figure 2*f* (P Zhang & J Hinshaw, manuscript in preparation).

DYNAMIN PHOSPHORYLATION AND CALCIUM BINDING

Depolarization of the synapse results in the dephosphorylation of dynamin (Robinson et al 1987) and a relocation of dynamin from the cytosol to a particulate fraction (Liu et al 1994a), possibly the plasma membrane. Preventing dephosphorylation by addition of phosphatase inhibitors results in the disassembly of the endocytic complex consisting of amphiphysin, dynamin, synaptojanin 1, AP2, and clathrin (Slepnev et al 1998). Increased calcium levels also enhance the translocation of dynamin from the cytosol to the particulate fraction (Liu et al 1994a). The calcium effect could be the result of calcium binding directly to dynamin or calcineurin, a calcium-dependent phosphatase known to dephosphorylate dynamin in a Ca^{2+}-sensitive manner (Nichols et al 1994, Bauerfeind et al 1997, Marks & McMahon 1998, Hens et al 1998). In support of calcineurin playing a role in dynamin function, cells transfected with a calcineurin mutant show an inhibition of clathrin-mediated endocytosis (Lai et al 1999). The increase in calcium levels, owing to depolarization of the synapse, could then activate calcineurin, thus leading to the dephosphorylation of dynamin and relocation of dynamin to the plasma membrane where it can directly or indirectly induce membrane fission.

Upon repolarization, dynamin was shown to be phosphorylated slowly by PKC in intact synaptosomes (Robinson 1991, 1992). PKC has been shown to be stimulated by RACK1 (receptor for activated C-kinase) (Ron et al 1994), a homologue to G protein β subunit, and like the β subunit, RACK1 has been shown to bind the PH domain of dynamin (Rodriguez et al 1999). RACK1 may therefore act as a scaffolding protein for the dynamin-PKC interaction (Rodriguez et al 1999). The phosphorylation of dynamin by PKC is inhibited by high concentrations of dynamin and binding to a particulate fraction in the cell (Liu et al 1994a). Dynamin, bound to the membrane and oligomerized, therefore would not be accessible for phosphorylation.

Recently it was demonstrated that dynamin 2 is tyrosine phosphorylated (residues Tyr231 and Try597) upon activation of β_2-adrenergic receptors (Ahn et al 1999) or upon addition of insulin to cells overexpressing insulin receptors (Baron et al 1998). Furthermore, tyrosine phosphorylation of dynamin was required for β_2-adrenergic receptor internalization (Ahn et al 1999). In contrast, in rat mast cells tyrosine-phosphorylated dynamin was dephosphorylated upon aggregation of the IgE receptors, which led to the rapid internalization of the receptors through clathrin-coated pits (Pullar et al 1996). Therefore, just as in the synapse, the dephosphorylation of dynamin 2 in rat mast cells may shift its partitioning to the membrane fraction where it can participate in receptor-mediated endocytosis.

DYNAMIN AND MEMBRANE RECYCLING

It is well established that dynamin is involved in receptor-mediated endocytosis, synaptic vesicle recycling, and caveolae internalization; however, there is conflicting evidence for the role of dynamin in vesicle trafficking from the Golgi (discussed below). Dynamin also has been implicated in rapid endocytosis in a clathrin-independent pathway (Artalejo et al 1995) and shown to play a role in phagocytosis (Gold et al 1999). In addition, different dynamin isoforms can be targeted to different regions of the cell; for example, dynamin 1 was shown to preferentially act at the apical surface of MDCK cells while dynamin 2 played a more predominant role at the basolateral surface (Altschuler et al 1998). Likewise, there is evidence that different splice variants of dynamin are localized to different regions of the cell and participate in specific vesiculation events (discussed below) (Cao et al 1998, McNiven et al 2000).

Receptor-Mediated Endocytosis and Synaptic Vesicle Recycling

The initial link between dynamin and *shibire* revealed the potential role of dynamin in synaptic vesicle recycling through endocytosis. The first direct evidence that dynamin played a role in receptor-mediated endocytosis came from mammalian cells transfected with dynamin mutants (Herskovits et al 1993a; Damke et al 1994, 1995). Cells expressing dynamin mutants deficient in GTP binding (K44A, K44E, or S45N) were unable to internalize transferrin (Herskovits et al 1993a, Damke et al 1994) or EGF receptors (Damke et al 1994), and clathrin-coated pits failed to deeply invaginate (van der Bliek et al 1993, Damke et al 1994). In the transfected (wt or K44A) or non-transfected cells, dynamin was found associated with clathrin-coated pits and coated vesicles (Damke et al 1994). As shown in Table 1, dynamin mutants have been used extensively to identify numerous compounds that are internalized by a dynamin-dependent pathway. The inhibition of receptor-mediated endocytosis by these mutants can be reversed in cells transfected with the K44E mutant that also has deletions in the GED region (Okamoto et al 1999b).

This correlates well with the observation that GED is essential for dynamin self-assembly and, therefore, the GTPase/GED defective protein can no longer interfere with endogenous dynamin function.

Immunogold localization studies suggest that dynamin 2 is located on the clathrin lattice in the GDP state but redistributes to the base of the clathrin pit upon GTP binding (Warnock et al 1997). Nonetheless, the collar structures seen in the thin section at the necks of clathrin-coated pits in the *shibire* nerve termini have not been seen in non-neuronal tissue (Koenig & Ikeda 1989). To address the question as to why dynamin collars are not observed in non-neuronal cells Baba et al (1999) transfected HeLa cells with the human temperature-sensitive dynamin mutant G273D, which corresponds to the *shibire*^{ts} mutation (Baba et al 1999). As expected, this mutant failed to internalize transferrin at the non-permissive temperature, and there was a marked increase in deeply invaginated coated pits with dynamin localized to their necks (Baba et al 1999). In addition, long tubular structures were observed extending into the cytoplasm from the plasma membrane at the non-permissive temperature (Baba et al 1999). However, the collar or striation pattern was not observed even though dynamin was localized to the necks and believed to be present in the GTP state (Baba et al 1999). This observation may be explained by the lack of a neuronal-specific component in the non-neuronal cells that is necessary to visualize the collar by thin-sectioning techniques. In support of this, it has been shown that only when amphiphysin is present with dynamin are striated patterns seen on lipid tubes by thin-sectioning (Takei et al 1999). Whether the ubiquitous isoform of amphiphysin, CALM, is localized to the necks or is capable of forming the striated pattern with dynamin remains to be determined. Therefore, dynamin may be present at the necks of coated pits or on tubule extensions in non-neuronal tissue.

To further explore the GTPase activity of dynamin during receptor-mediated endocytosis, Sever et al (1999) transfected cells with a dynamin mutant defective in GED activity, rendering dynamin incapable of stimulating GTPase activity (Sever et al 1999). Unexpectedly they found the rate of endocytosis in these cells increased $\sim$1.7-fold over wild-type dynamin (Sever et al 1999). This led to the speculation that dynamin acts as a molecular switch, and not a mechanochemical enzyme, and recruits another molecule(s) to the coated pits responsible for membrane fission (Sever et al 1999). The decreased hydrolysis rate of the dynamin GED mutants would shift the equilibrium of dynamin to the active GTP state. In support of the hypothesis that dynamin acts as a molecular switch, endocytosis assays in permeabilized 3T3-L1 cells found dynamin necessary but not sufficient for internalization of transferrin (Simpson et al 1999). However, receptor-mediated endocytosis was inhibited in cells transfected with the *dynamin*^{ts} mutant, G273D, which is believed to be blocked in the GTP state (Damke et al 1995). It is likely additional molecules are necessary for membrane fission, but it is also conceivable that dynamin may be both a molecular switch and a force-generating molecule.

Overexpression of Gα was also found to inhibit endocytosis of transferrin and LDL, and this effect was reversed by coexpression of $\beta\gamma$ subunits, suggesting

a role for the $\beta\gamma$ in endocytosis (Lin et al 1998). The binding of $\beta\gamma$ to the PH domain of dynamin inhibits the GTPase activity of dynamin (Lin & Gilman 1996), and therefore the interaction of $\beta\gamma$ with dynamin in the cell could have a similar effect as the GED mutants, prolonging dynamin in the GTP state and enhancing endocytosis. Conversely, the overexpression of Gα would then pull $\beta\gamma$ off dynamin, increasing GTP hydrolysis and inhibiting endocytosis. However, numerous other interactions between G protein subunits and the endocytic machinery could occur in the cell causing the altered endocytic rates.

The synaptic vesicle recycling pathway uses clathrin-mediated endocytosis to recycle the plasma membrane following stimulated exocytosis (for reviews see De Camilli & Takei 1996, Hannah et al 1999). The same machinery involved in receptor-mediated endocytosis is found in synaptic vesicle recycling but with several neuronal-specific partners and isoforms. Both dynamin 1 and 2 are found in the neuron, although the expression level of the neuronal-specific isoform, dynamin 1 far exceeds the ubiquitous dynamin 2 levels (Warnock et al 1997). The phosphorylation of the neuronal specific dynamin 1 by PKC may play a dual role; preventing membrane binding when the synapse is polarized and inhibiting the redistribution of dynamin to the necks of coated pits by increasing the GTPase rate. The increased GTPase rate would decrease GTP-bound dynamin, the nucleotide state that preferentially binds to the necks (Warnock et al 1997). Further regulation of dynamin 1 through neuronal-specific partners is reviewed in the section Dynamin Partners at the Synapse.

Fluid Phase Pinocytosis

Fluid phase pinocytosis is blocked in *shibire* cells (Kosaka & Ikeda 1983b, Tsuruhara et al 1990) but occurs normally in cells transfected with the dynamin K44 mutations (Herskovits et al 1993a, Damke et al 1994). In cells transfected with the temperature-sensitive dynamin mutant, G273A, fluid phase uptake is partially blocked initially but fully recovers after 30 min of endocytosis (Damke et al 1995). This suggests a dynamin-independent pathway can be upregulated for fluid phase endocytosis (Damke et al 1995).

Caveolae Internalization

As with receptor-mediated endocytosis, cells transfected with the dynamin mutant K44A were also defective in caveolae internalization (Oh et al 1998). Cells transfected with K44A dynamin, or injected with antibodies against dynamin, were incapable of internalizing cholera toxin B chain, a toxin previously shown to be internalized by caveolae (Schnitzer et al 1996, Oh et al 1998, Henley et al 1998). Further support for dynamin playing a role in caveolae internalization came from an in vitro caveolae internalization assay (Oh et al 1998). Addition of dynamin antibodies to the assay prevented the release of caveolin by potentially inhibiting caveolae fission (Oh et al 1998). Moreover, the release of caveolin was enhanced upon addition of overexpressed wild-type but not K44A dynamin and required GTP

hydrolysis by dynamin (Oh et al 1998). Dynamin was also shown to be abundant in a caveolae preparation and colocalized with caveolae by immunofluorescence in endothelial cells (Oh et al 1998, Henley et al 1998). Finally, immunolocalization on cryo-thin sections further showed dynamin localized to necks of caveolae (Oh et al 1998).

Vesicle Transport to and from the Golgi

Evidence suggests dynamin is involved in vesicle transport from endosomes to the Golgi (Llorente et al 1998, Nicoziani et al 2000); however, dynamin-dependent transport from the Golgi remains controversial. Immunolabeling studies using different domain-specific antibodies gave contradictory results. Certain dynamin 2 splice variants (labeled aa and bb) were localized by immunofluorescence to the Golgi (Cao et al 1998, Henley et al 1999); however, other studies found no evidence for Golgi localization of dynamin 1 (aa) or dynamin 2 (aa and ba) (Kasai et al 1999; see Table 2). An antibody that recognizes the C-terminal domain of both dynamin 1 and 2 (Hudy-1) localized endogenous dynamin to the plasma membrane but not to the Golgi (Damke et al 1994, Altschuler et al 1998). In contrast, a dynamin 2 C-terminal-specific antibody associated with isolated Golgi membranes and localized endogenous dynamin to the *trans*-Golgi network (TGN) by immunoelectron microscopy (Maier et al 1996).

The first transfection studies using a dynamin 1 mutant, K44A, showed that transport from the ER to the Golgi (van der Bliek et al 1993) and from the Golgi to the cell surface or to lysosomes was dynamin independent (Damke et al 1994). Also, the expression of K44E only affected the location of α-adaptin (a plasma membrane–specific AP2 subunit) and clathrin at the plasma membrane but had no effect on γ-adaptin (a Golgi-specific AP1 subunit) (Herskovits et al 1993a). Vesicular stomatitis virus glycoprotein transport through the Golgi and cathepsin D export from the Golgi to lysosomes were also unaffected by transfected cells with dynamin 1 or 2 (aa) K44A mutants (Kasai et al 1999). Cells that overexpressed

TABLE 2 Location and function of dynamin 2 slice variants at the plasma membrane (PM) or *trans*-Golgi network (TGN)

	PM	**Non-PM**	**TGN**	**Non-TGN**
dyn2(aa)	Kasai et al 1999 Cao et al 1998		Cao et al 1998	Kasai et al 1999
dyn2(ab)	Cao et al 1998			Cao et al 1998
dyn2(ba)	Altschuler et al 1998			Altschuler et al 1998
dyn2(bb)		Henley et al 1999	Henley et al 1999 Kreitzer et al 2000	

a dynamin 2 (ba) mutant, defective in GTP binding, also displayed no defect in either endosomal recycling to the plasma membrane or trafficking from the TGN to the lysosome (Altschuler et al 1998). In contrast, a recent report found MDCK cells transfected with the dynamin 2 (bb) mutant, D2K44E, inhibited p75 (an apical membrane protein) exit from the Golgi (Kreitzer et al 2000). Also, a cell-free assay demonstrated that dynamin is necessary for clathrin-coated vesicle budding from the TGN (Jones et al 1998). These conflicting results have led to an ongoing debate over the role dynamin plays in vesicle transport from the Golgi and the answer may depend on the marker (i.e. apical versus basolateral) and/or the splice variant selected.

DYNAMIN PARTNERS

Signaling Proteins

Dynamin has been shown to play a direct role in endocytosis, but it may also have a more general role in coupling receptor activation to endocytosis. Dynamin contains SH3-binding domains in its C terminus, which, when interacting with SH3-containing proteins, may trigger a signaling cascade. Protein-protein interactions through Src homology (SH) domains can play a key role in tyrosine kinase receptor signal transduction. Dynamin was shown to bind to a subset of SH3 domain–containing proteins with the strongest binding and highest GTPase stimulation coming from Grb2 (Gout et al 1993, Herskovits et al 1993b). Grb2 has been shown to associate with dynamin in a variety of cell lines and under conditions that stimulate signal transduction. For example, the association of Grb2 with dynamin was shown to increase after (*a*) insulin stimulation of hepatocytes (Wada et al 1998), smooth muscle cells (Karoor et al 1998), or CHO-IR cells (Ando et al 1994); (*b*) ras-transformation of NIH3T3 cells but not non-transformed cells (Yoon et al 1997); (*c*) treatment of monocytes with macrophage colony–stimulating factor (M-CSF) (Kharbanda et al 1995); and (*d*) EGF stimulation of canine epithelial cells (Wang & Moran 1996). There is also evidence that Grb2-bound dynamin is tyrosine phosphorylated in response to lysophosphatidic acid (LPA), and this may be an important step in the Gi-mediated activation of the Ras-MAP kinase cascade (Kranenburg et al 1999). Dynamin tyrosine phosphorylation was also increased fivefold in insulin-stimulated over unstimulated cells (Baron et al 1998).

Grb2 and its two isoforms, Ash-m and Ash-s, contain two SH3 domains; however, only the N-terminal SH3 domain is necessary for high-affinity binding of dynamin (Watanabe et al 1995, Vidal et al 1999). The interaction between the N-terminal SH3 domain of Grb2 and dynamin in ER 22 cells[2] could be blocked by addition of the dynamin peptide GPPQVPSRPNR from its proline-rich C-terminus

[2]ER 22 cells are hamster fibroblast cell line overexpressing human epidermal growth factor receptor.

(residues 827–837) (Vidal et al 1998) (Figure 1*a*). A similar peptide inhibited Grb2 and dynamin interactions in PC12 cells (Miki et al 1994). Two other proteins, Grap, a protein that shares 58% homology to Grb2, and Nck, may also associate with dynamin via its SH3 domain upon cellular stimulation (Trub et al 1997, Wunderlich et al 1999).

Immunoprecipitation and coprecipitation studies have shown that in addition to Grb2, dynamin also associates with PLCγ upon cellular stimulation (Seedorf et al 1994). For example, PDGF stimulation of mammalian cells results in PLCγ binding to dynamin in a growth factor–dependent manner (Scaife et al 1994). The predicted binding site for PLCγ is upstream of Grb2, corresponding to residues 812–820, APPVPSRPG in the PRD of dynamin (Seedorf et al 1994) (Figure 1*a*). Interactions between dynamin and SH3 domain of Src in synaptosomes have been shown by overlay and coprecipitation assays (Foster-Barber & Bishop 1998). Finding no evidence that dynamin was phosphorylated by Src, the authors suggest that the interaction between dynamin and Src is to guide Src to membrane trafficking areas (Foster-Barber & Bishop 1998). However, whether any of these signaling proteins such as Grb2 or Src trigger the cell to internalize receptors by binding to dynamin is not known.

Dynamin Partners at the Synapse

Among the proteins identified as potential dynamin partners, the majority have been characterized from neuronal tissue. Furthermore, all the proteins discussed below are predicted to bind directly or indirectly to dynamin through SH3 domains, a common theme for endocytic protein interactions.

Amphiphysin and Synaptojanin Of all the potential dynamin partners, amphiphysin, a 125-kDa SH3-domain containing protein, has been the best characterized. The neuronal-specific isoform, amphiphysin 1, has been shown to colocalize with dynamin and synaptojanin in nerve terminals (McPherson et al 1994, 1996; David et al 1996; de Heuvel et al 1997) and colocalize with dynamin to the striated tubules extending from the plasma membrane of synaptosomes treated with GTPγS (Bauerfeind et al 1997). Dynamin was also shown to colocalize with synaptojanin, a 145-kDa neuronal-specific protein with inositol-5-phosphatase activity, in nerve termini (McPherson et al 1994, 1996). Both dynamin and synaptojanin bind to SH3 domains of amphiphysin (McPherson et al 1996, Grabs et al 1997). Mutational and peptide library studies suggest amphiphysin binds to a unique SH3-binding domain on dynamin, PSRPNR (residues 833–838) (Figure 1*a*) (Grabs et al 1997).

A dynamin mutant lacking its PRD was prevented from colocalizing with clathrin-coated pits, suggesting a SH3-containing protein was necessary for dynamin recruitment to coated pits (Shpetner et al 1996). Amphiphysin has been proposed to be the SH3-containing molecule that directs dynamin to the coated pits by binding to both dynamin and the α-adaptin of AP2 (David et al 1996). In

support of this hypothesis, microinjecting amphiphysin's SH3 domain or a peptide of dynamin containing the SH3-binding site blocked endocytosis and increased coated pits at the plasma membrane in synapses (Shupliakov et al 1997). Cells overexpressing the SH3 domain of amphiphysin, but not Grb2 or spectrin, prevented transferrin uptake (Wigge et al 1997), and microinjecting the SH3 domain of amphiphysin or the dynamin SH3-binding domain in adipocytes prevented internalization of the glucose transporter, GLUT4 (Volchuk et al 1998). In agreement with a recent report, this evidence further demonstrates the importance of SH3 domain interactions in endocytosis (Simpson et al 1999).

Phosphorylation and dephosphorylation of synaptic vesicle recycling components appear to play a key regulatory role in complex assembly and disassembly necessary for endocytosis. Similar to dynamin 1, amphiphysin 1 and synaptojanin are also phosphorylated and undergo a stimulated dephosphorylation, again possibly by calcineurin, triggered by the influx of Ca^{2+} (McPherson et al 1994, Bauerfeind et al 1997). Consistent with the model that Ca^{2+} influx triggers a priming event for synaptic vesicle recycling (McPherson et al 1994), dephosphorylation of brain extract increased the interactions of dynamin with amphiphysin in a complex with clathrin and AP2 (Slepnev et al 1998). Furthermore, phosphorylation of dynamin 1 and synaptojanin 1 inhibited their binding to amphiphysin, and phosphorylation of amphiphysin prevented its binding to clathrin and AP2 (Slepnev et al 1998). Both amphiphysin and dynamin have been shown to interact with α-adaptin but at different regions of the molecule (Wang et al 1995b, David et al 1996, Wigge et al 1997).

Endophilin Another important SH3-containing protein found to bind to dynamin, amphiphysin, and synaptojanin and shown to play a role in endocytosis is endophilin (originally called SH3p4) (Micheva et al 1997; Ringstad et al 1997, 1999). Endophilin, along with dynamin and amphiphysin, was also localized to the striated tubules found in synaptosomes treated with GTPγS (Ringstad et al 1999). Endophilin, a 40-kDa protein, was further shown to have lysophosphatidic acid acyl transferase activity, which converts lysophosphatidic acid (LPA) to phosphatidic acid (PA) (Schmidt et al 1999). One possible consequence of this lipid modification is to convert an inverted cone-shaped lipid to a cone-shaped lipid, thereby mediating lipid curvature that may be necessary for vesicle formation (Schmidt et al 1999). In support of this hypothesis, a lamprey synapse microinjected with antibodies against endophilin resulted in an accumulation of clathrin-coated pits at the plasma membrane that failed to deeply invaginate (Ringstad et al 1999). Removing the SH3 domain from endophilin also prevented synaptic-like microvesicle formation from the plasma membrane, although it retained lysophosphatidic acid acyl transferase activity (Schmidt et al 1999). The endophilin mutant, lacking the SH3 domain, may no longer bind endocytic proteins such as dynamin or synaptojanin and thus may be mistargeted in the cell, no longer directed to clathrin-coated pits (Schmidt et al 1999).

Intersectin and Syndapin I Dynamin has also been shown to coimmunoprecipitate and colocalize with two additional proteins: intersectin (Hussain et al 1999), a protein containing 2 Eps15 homology domains and SH3 domains (Yamabhai et al 1998) and syndapin I (a synaptic, dynamin-associated protein), a 52-kDa protein shown to bind to the proline-rich domain of dynamin, probably through its SH3 domain (Qualmann et al 1999). In addition, a yeast two-hybrid assay identified intersectin, also called Ese in mammalian cells, as a binding partner to dynamin (Sengar et al 1999). A direct interaction of dynamin with intersectin was shown in vitro to be possible through the SH3 domains of intersectin (Okamoto et al 1999a). Syndapin also interacts with the neuronal Wiskott-Aldrich syndrome protein (N-WASP), a protein involved in regulating the actin cytoskeleton (Qualmann et al 1999). Another link between clathrin-coated vesicle formation and the cytoskeleton is the observed interaction of profilin I and II to dynamin and clathrin and the observation that in vivo profilin colocalizes with dynamin and synapsin in axonal and dendritic processes (Witke et al 1998).

Phospholipids

Other significant potential binding partners of dynamin are the phosphatidylinositides. Dynamin may bind directly to the membrane via its PH domain to anionic phospholipids, which in turn would stimulate its GTPase activity. In support of this hypothesis, it has been shown that phosphatidyinositol-4,5-bisphosphate is required for clathrin-mediated endocytosis (Jost et al 1998).

DYNAMIN-LIKE PROTEINS

The dynamin family of proteins is continually growing in numbers and has been shown to be involved in a wide variety of function, with most involved in some aspect of membrane morphology (Table 3). The dynamin-like proteins have been identified based on their sequence homology primarily with the GTPase and middle domains of dynamin (see van der Bliek 1999). In addition, the dynamin-like proteins are large in size (70–100 kDa), have a relative low affinity for GTP and high GTPase activity. The dynamin-like proteins, however, lack both the PH and proline-rich domains but appear to contain the GED-like or assembly domain. Thus this family of proteins may share a common phenomena of self-assembly into oligomers and assembly-dependent stimulated GTPase activity.

Yeast Vps1 and Mgm1

Although yeast does not contain a dynamin homologue, the only dynamin-like protein identified so far that also plays a role in vesicle trafficking is the yeast protein Vps1. Vps1 was identified in a yeast mutant as essential for vacuolar protein sorting (Rothman et al 1990, Vater et al 1992) and later argued to be required for vesicle

TABLE 3 Dynamin family of proteins—proposed function

Protein	Proposed Function
Dynamin	Synaptic vesicle recycling, receptor-mediated endocytosis, caveolae internalization and trafficking in and out of the Golgi
Drp1, Dlp1, DVLP, Dymple	Mitochondria and ER morphology
VPS1	Vesicle formation at the Golgi
Dmn1	Mitochondrial morphology
Mgm1	Mitochondrial morphology
Mx1	Inhibition of viral RNA transcription
MxA	Inhibition of viral nucleocapsid transport to nucleus
Phragmoplastin	Plant cell wall formation
ADL1(2)	Thylakoid biogenesis
Human dynamin-like protein (hdynIV)	Cell signaling (Hong et al 1998)

formation at the Golgi (Nothwehr et al 1995). More recent evidence suggests that Vps1 is involved in clathrin-mediated vesicle formation at the TGN (Bensen et al 2000). Another dynamin-like protein in yeast, Mgm1 (Jones & Fangman 1992), has been shown to be involved in maintaining mitochondrial morphology (Guan et al 1993) and is localized to the outer mitochondrial membrane (Shepard & Yaffe 1999). In addition, a mutation in the GTPase domain of Mgm1 had a dominant-negative effect just as dynamin did for endocytosis, although in this case it resulted in aggregated mitochondria (Shepard & Yaffe 1999). Finally, Mgm1 also appears to exist in an oligomeric state based on gel filtration results (Shepard & Yaffe 1999).

Drp1 (Dynamin-Related Protein 1)

In addition to Mgm1, there is good evidence that the dynamin-like protein, Dmn1 in yeast (Gammie et al 1995) and Drp1[3] in mammals or *C. elegans*, is also involved in mitochondrial morphology. Both Dmn1 and Drp1 have been localized to mitochondria constriction or fission sites in cells, and cells transfected with a Drp1 or Dmn1 mutant reveal a defect in mitochondria fragmentation (Otsuga et al 1998, Smirnova et al 1998, Bleazard et al 1999, Labrousse et al 1999, Sesaki & Jensen 1999). Furthermore, overexpressing wild-type Drp1 in *C. elegans* caused excessive mitochondria fragmentation (Labrousse et al 1999). Purified Drp1 also

[3]Drp1 (dynamin-related protein) has also been referred to in the literature as Dlp1 for dynamin-like protein (Yoon et al 1998), dymple (Kamimoto et al 1998) and DVLP for Dnm1p-Vsp1p-like protein (Shin et al 1997).

has been shown to exist as an oligomer, possibly a tetramer, that can assemble into large aggregates under low-salt conditions (Shin et al 1999). The N-terminal half of Drp1, containing the GTPase domain, interacts with the C-terminal half corresponding to the GED of dynamin (Shin et al 1999). Together these observations suggest Drp1 is similar to dynamin by assembling into oligomers and directly playing a role in membrane fission.

In addition to the changes in mitochondrial morphology it has also been observed that cells transfected with a Drp1 mutant have a dramatic redistribution and decrease in number of ER cisternae (Pitts et al 1999). Drp1 has also been localized to a vesicle population that appears to associate with microtubules (Yoon et al 1998, Pitts et al 1999). Another study suggests that Drp1 plays a role in vesicle trafficking from the Golgi to the plasma membrane (Imoto et al 1998); however, other groups found no effect on the endocytic or secretory pathway (Smirnova et al 1998, Pitts et al 1999).

Plant Phragmoplastin and ADL Proteins

The dynamin-like proteins identified in plants include phragmoplastin and ADL1, 2, and 3 (*Arabidopsis* dynamin-like). Phragmoplastin has been shown to associate with cell plate formation (Gu & Verma 1996), form oligomers, and possibly play a role in membrane fusion at the cell plate (Gu & Verma 1997, Zhang et al 2000). In addition, phragmoplastin may interact with microtubules since treatment with taxol, a microtubule-stabilizing drug, prevented phragmoplastin redistribution during cell plate formation (Gu & Verma 1997). During cell plate formation, numerous vesicles derived from the Golgi fuse to form a tubular network that eventually becomes the new cell wall. It has been postulated that phragmoplastin may coat membranes to create a network of narrow tubes (25–30 nm in diameter) (Gu & Verma 1997).

In *Arabidopsis*, ADL1 (Park et al 1997) has been localized to thylakoid membranes and may play a role in thylakoid membrane biogenesis (Park et al 1998). Similarly, ADL2 has been localized to chloroplasts (Kang et al 1998). However, the location of ADL3 within the plant cell remains unknown (Mikami et al 2000). Surprisingly, unlike all the dynamin-like proteins identified to date, ADL3 contains a PH domain (Mikami et al 2000) and hence may resemble the function of dynamin more closely. Although the precise role of the ADL proteins has yet to be determined, they appear to be similar to other members of the dynamin family in participating in membrane morphology.

Mx Family of Proteins

Unlike the dynamin-like proteins described above, the Mx family of proteins does not appear to associate with membranes. These proteins are interferon-induced and provide resistance to viral infection (Pavlovic et al 1993). As with the other dynamin-like proteins, the Mx family contains a N-terminal GTPase domain and a variable C terminus, which modifies the function and location of the different

Mx proteins (Pavlovic et al 1993). For example, mouse Mx1 is located in the nucleus and is believed to interfere with RNA transcription (Krug et al 1985), whereas human MxA is found in the cytoplasm and is believed to be involved in blocking viral transport into the nucleus (Kochs & Haller 1999b). Mx proteins have also been shown to assemble into homooligomers (Melen et al 1992, Kochs et al 1998), with several domains involved in oligomerization. The first region identified was a segment between the first and second conserved consensus GTP-binding sequences—deleting this region prevented oligomerization of the protein (Nakayama et al 1993). The oligomers, described as horseshoe-shaped, transformed into tightly stacked helices upon GTP treatment (Nakayama et al 1993). Similar to the GED domain of dynamin, MxA also contains a region in its C-terminal half that is involved in activating its GTPase activity (Schwemmle et al 1995, Flohr et al 1999) and promoting oligomerization (Ponten et al 1997). This region is also involved in interaction with the virus nucleocapsids (Flohr et al 1999, Kochs & Haller 1999a). MxA also contains two leucine zippers at its C terminus that appear to be involved in MxA oligomerization (Di Paolo et al 1999).

Human Guanylate-Binding Protein 1 (hGBP1)

The first atomic structure of a dynamin-like protein has recently been solved by X-ray crystallography (Figure 1*b*) (Prakash et al 2000). This protein, hGBP1, is similar to the Mx family of proteins because it is induced by interferon and possibly promotes an anti-viral response. hGBP1, like dynamin, has a low GTP-binding affinity and a relatively fast GTP hydrolysis rate but is unique among the dynamin family of proteins in its ability to convert GTP to GMP (Praefcke et al 1999). The structure of hGBP1 can be divided into two main domains: a globular GTP-binding domain and an extended α-helical domain (Prakash et al 2000). The helical domain can be further divided into two three-helix bundles followed by a long helical stretch that ends at the globular GTPase domain. In addition, the C terminus contains a *CAAX* motif (C = cysteine, A = an aliphatic amino acid, and X = one of several amino acids) that is commonly modified by isoprenylation, proteolytic removal of the *AAX* tripeptide, and carboxylmethylation (Zhang & Casey 1996). For some small GTP-binding proteins, this C-terminal modification is necessary for proper cellular membrane location. Dynamin may resemble this structure but with variations in the less conserved C terminus. For example, dynamin contains two prolines in the region that would correspond to the long extended α-helix.

SUMMARY

The function of dynamin has been studied extensively; however, several questions still remain. Do the different isoforms and splice variants bestow distinct function and location within the cell? A recent report found that dynamin 1 and 2 coimmunoprecipitate, which suggests that the isoforms coassemble in the cell

and do not confer specific function (Okamoto et al 1999b). What are the relevant dynamin partners in the cell, and is there a direct connection between dynamin and cell signaling through signaling proteins such as Grb2 or Src? At present there is good evidence that amphiphysin may direct dynamin to clathrin-coated pits (Shupliakov et al 1997), and once at the plasma membrane dynamin interacts directly with the membrane lipid (Vallis et al 1999, Achiriloaie et al 1999) and possibly endophilin (Ringstad et al 1999). Finally, is dynamin a molecular switch that recruits other components to the membrane for vesicle formation, or is dynamin a mechanochemical enzyme that generates the force necessary for membrane fission? Just as dynamin contains its own GAP, dynamin may also function as a regulator of its own force-generating mechanism.

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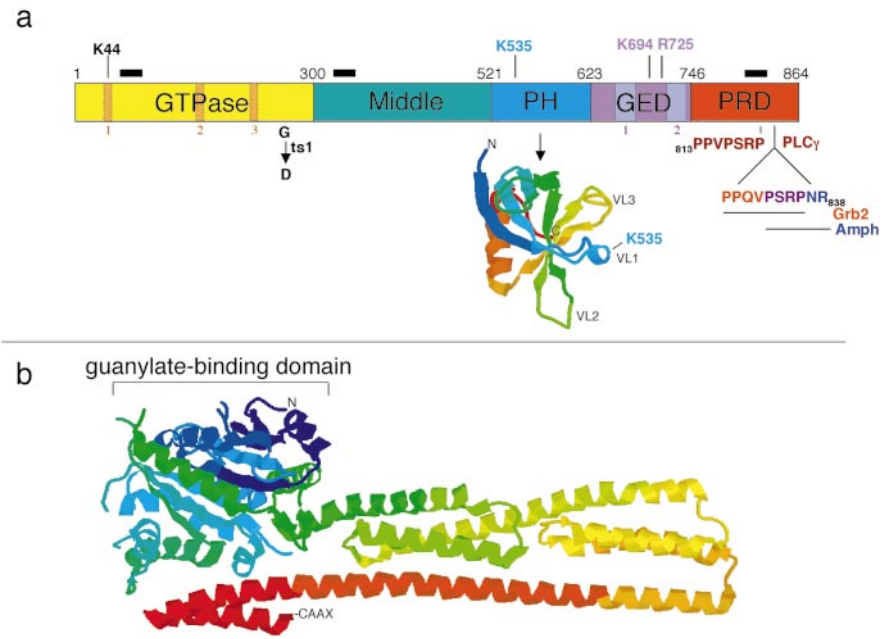

Figure 1 Schematic diagram of the domains within dynamin and the X-ray structure of a dynamin-like protein, human guanylate-binding protein 1 (hGBP1). (*a*) Dynamin consists of five distinct domains: the GTP hydrolysis domain (GTPase), a middle domain, a pleckstrin homology domain (PH), a GTPase effector domain (GED), and a highly basic C-terminal proline-rich domain (PRD). The three GTP-binding consensus sequences and the two presumptive coiled-coil regions in the GED are highlighted and labeled below in the GTPase domain and GED, respectively. The residues within dynamin that were mutated for functional and structural studies are marked K44, K535, K694, R725. Regions within dynamin involved in self-assembly are marked above with a black bar (residues 49–58, 320–349, 792–841). The crystal structure of the PH domain of dynamin (Ferguson et al 1994) and the binding sites for the SH3-containing proteins, PLCγ, Grb2, and amphiphysin (Amph) are marked below. (*b*) Crystal structure of human guanylate-binding protein 1 (hGBP1) showing the globular guanylate-binding domain and an extended α-helical domain ending with a CAAX motif (Prakash et al 2000).