A Model for Dynamin Self-assembly Based on Binding Between Three Different Protein Domains*

(Received for publication, December 3, 1998, and in revised form, February 9, 1999)

Elena Smirnova‡‡, Dixie-Lee Shurland‡, Erin D. Newman-Smith‡‡, Babak Pishvaee‡, and Alexander M. van der Bliek**‡‡‡

From the ‡Department of Biological Chemistry, the ¶Jonsson Comprehensive Cancer Center, and the **Molecular Biology Institute, University of California, Los Angeles, California 90095-1737

Dynamin is a 100-kDa GTPase that assembles into multimeric spirals at the necks of budding clathrin-coated vesicles. We describe three different intramolecular binding interactions that may account for the process of dynamin self-assembly. The first binding interaction is the dimerization of a 100-amino acid segment in the C-terminal half of dynamin. We call this segment the assembly domain, because it appears to be critical for multimerization. The second binding interaction occurs between the assembly domain and the N-terminal GTPase domain. The strength of this interaction is controlled by the nucleotide-bound state of the GTPase domain, as shown with mutations in GTP binding motifs and in vitro binding experiments. The third binding interaction occurs between the assembly domain and a segment that we call the middle domain. This is the segment between the N-terminal GTPase domain and the pleckstrin homology domain. The three different binding interactions suggest a model in which dynamin molecules first dimerize. The dimers are then linked into a chain by a second binding reaction. The third binding interaction might connect adjacent rungs of the spiral.

The discovery that the Drosophila shibire gene encodes the homologue of dynamin led to the realization that these proteins are important for an early stage in endocytosis (1, 2). Functional assays with transfected mammalian cells demonstrated that dynamin is also required for clathrin-mediated endocytosis in mammals (3). A possible mechanism was suggested by the discovery that dynamin forms a spiral at the neck of budding clathrin-coated vesicles when nerve endings are incubated with GTPγS (a nonhydrolyzable GTP analog) (4). Purified dynamin also assembles into spirals or rings in the absence of membrane (5). These structures are strikingly similar to electron-dense collars that were observed many years earlier in neurons of Drosophila shibire mutants (6). Recent experiments demonstrated the ability of brain cytosol and purified dynamin to form lipid tubules (7, 8). Purified dynamin can sever the lipid tubules by GTP-dependent constriction (8). It is therefore likely that the dynamin spirals at the necks of budding vesicles catalyze the pinching-off of clathrin-coated vesicles.

Dynamin is a member of a family of structurally related but functionally diverse GTP-binding proteins with large C-terminal extensions (9). Three large blocks of sequences are highly conserved within the family of dynamin-related proteins. The N-terminal segment contains a GTPase domain, which is highly conserved between dynamin-related proteins, comprising a distinct subgroup within the GTPase superfamily. The second domain, which we call the middle domain, has no known function. The third domain is a putative coiled-coil, which we call the assembly domain, because it may play an important role in forming dynamin multimers. Most family members have divergent segments inserted between the middle and the assembly domains. The inserted segment in dynamin is a pleckstrin homology (PH) domain, which binds to phosphatidylinositol 4,5-bisphosphate and, therefore, may be important for interactions between dynamin and the plasma membrane (10, 11). The inserted segments of the other family members are completely divergent from each other and from other proteins. This diversity in sequence may reflect different functions performed by dynamin family members.

Dynamin is the only member of the dynamin family with an additional C-terminal extension, which was called the proline-rich domain (PRD) because it has more than 30% proline residues. The PRD interacts with microtubules or SH3 domains in vitro, thereby stimulating the dynamin GTPase (12, 13). However, the intermolecular binding interactions that are necessary for spiral formation are specified by dynamin sequences, because no other proteins are required for in vitro assembly of a dynamin spiral (5). C-terminal fragments help activate the N-terminal GTPases of dynamin (14), but little else is known about the internal organization of the dynamin spiral. There may be as many as three steps in the formation of a dynamin spiral. 1) Cytosolic dynamin forms a tetramer, possibly by joining two dynamin dimers (14). 2) Dynamin oligomers are then linked into longer chains at the plasma membrane. 3) Once the dynamin spiral has made a full circle, the rungs of the spiral might contact each other in a third binding interaction that would help facilitate constriction. Here we describe the discovery of a series of binding interactions between three domains of dynamin that are conserved within the dynamin family: the GTPase domain, the middle domain, and the assembly domain. These binding interactions suggest how dynamin might form a spiral and then constrict.

** EXPERIMENTAL PROCEDURES

* This work was supported by National Institutes of Health Grant GM51866, American Heart Association Grant 965054, and Muscular Dystrophy Association Grant 51866 (to A. M. v. d. B). This article must therefore be hereby marked "in accordance with 18 U.S.C. Section 1734 solely to indicate this fact."

‡ Supported by the Myasthenia Gravis Foundation and the American Heart Association, Western states affiliate.

¶ Supported by the Jonsson Comprehensive Cancer Center, UCLA.

**‡‡ To whom correspondence should be addressed: Dept. of Biological Chemistry, UCLA School of Medicine, P. O. Box 951737, Los Angeles, CA 90095-1737. Tel.: 310-825-9779; Fax: 310-206-5272; E-mail: avan@mednet.ucla.edu.

†† The abbreviations used are: GTPγS, guanosine 5’-O-(thiotriphosphate); PRD, proline-rich domain; PH domain, pleckstrin homology domain; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis.
Three Binding Interactions within Dynamin

Fig. 1. Positions of dynamin fragments that were tested with the yeast 2-hybrid system. The dynamin protein domains are the GTPase domain, the middle domain (M), the PH domain, the assembly domain (Asm), and the PRD. The boundaries of the fragments are indicated by the positions in the dynamin amino acid sequence. The + and − symbols indicate whether these fragments bind to the assembly domain in yeast 2-hybrid experiments.

 restriction sites at their 5′-ends. The GTPase domain was cloned into NdeI and SalI sites of the bait plasmid (pAS1-CYH2) and into BamHI and XhoI sites of the target plasmid (pAct-II) (15). Other fragments were cloned into NcoI and SalI sites of pAS1-CYH2 or into NcoI and XhoI sites of pAct. The positions of the fragments used in yeast 2-hybrid and in vitro binding studies are shown in Fig. 1. Where indicated, mutations were introduced by fusion polymerase chain reaction with complementary mutagenic oligonucleotides as described (3). Polymerase chain reaction constructs were sequenced to rule out replication errors. The two hybrid constructs were transformed into the yeast strain Y190 (15) with polyethylene glycol/lithium acetate, as described (16). A yeast 2-hybrid library made from human brain cDNA (CLONTECH Laboratories, Palo Alto, CA) was screened by growing the transformed yeast on selective plates without leucine, tryptophan, or histidine but with 25 mM 3-amino-1,2,4-triazole (15). Surviving colonies formed yeast on selective plates without leucine, tryptophan, or histidine and were cloned into pBlue-Bac4 (Invitrogen, Carlsbad, CA) with six histidines or GST-coding sequences added by polymerase chain reaction. Recombinant virus was produced in SF9 cells as recommended by Invitrogen. Infected cells were harvested after 48 h, resuspended in lysis buffer (20 mM Hepes, pH 7.2, 160 mM NaCl, 2 mM MgCl₂, 0.5 mM EDTA, 0.5 mM dithiothreitol) with 0.1% Triton and protease inhibitors and lysed with a French pressure cell (American Instrument Co, Inc., Silver Spring, MD) adjusted to 20,000 psi. Recombinant proteins were bound to His-Bind resin (Novagen Inc., Madison, WI) or glutathione-Sepharose (Amersham Pharmacia Biotech) and eluted with 250 mM imidazole in lysis buffer without Triton or with 10 mM glutathione in 50 mM Tris-HCl, pH 8.0. The purified proteins were then dialyzed into lysis buffer and stored at −80 °C. GST fused to the yeast clathrin light chain protein was a gift from Diane Chu.

Expression and Purification of Proteins—Protein fragments were expressed with baculovirus, which is known to give high yields of active dynamin (5, 17). To this end, DNA fragments were cloned into pBlueBac4 (Invitrogen, Carlsbad, CA) with six histidines or GST-coding sequences added by polymerase chain reaction. Recombinant virus was produced in SF9 cells as recommended by Invitrogen. Infected cells were harvested after 48 h, resuspended in lysis buffer (20 mM Hepes, pH 7.2, 160 mM NaCl, 2 mM MgCl₂, 0.5 mM EDTA, 0.5 mM dithiothreitol) with 0.1% Triton and protease inhibitors and lysed with a French pressure cell (American Instrument Co, Inc., Silver Spring, MD) adjusted to 20,000 psi. Recombinant proteins were bound to His-Bind resin (Novagen Inc., Madison, WI) or glutathione-Sepharose (Amersham Pharmacia Biotech) and eluted with 250 mM imidazole in lysis buffer without Triton or with 10 mM glutathione in 50 mM Tris-HCl, pH 8.0. The purified proteins were then dialyzed into lysis buffer and stored at −80 °C. GST fused to the yeast clathrin light chain protein was a gift from Diane Chu.

To make radiolabeled protein for in vitro binding studies, cDNA fragments were recloned into PET vectors (Novagen). Proteins were synthesized with the TNT® T7 Coupled Transcription/Translation System (Promega Corp., Madison, WI) using [³⁵S]methionine (Amersham Pharmacia Biotech). Where indicated, unlabeled methionine was added to 100 μM in the transcription/translation reactions, thereby increasing the amount of synthesized protein but decreasing the specific activity approximately 100-fold.

Protein Binding and Cross-linking—In vitro binding assays were conducted with 1 μg of unlabeled GST fusion protein and [³⁵S]methionine-labeled target protein, or both proteins were labeled with [³⁵S]methionine. The proteins were incubated in 250 μl of binding buffer (phosphate-buffered saline with 10 mM EDTA) for 30 min at room temperature and then centrifuged and washed in binding buffer. The bound proteins were eluted with 10 mM glutathione in 50 μl Tris-HCl, pH 8.0, and fractionated by SDS-PAGE. The gels were dried and subjected to autoradiography or quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Cross-linking was essentially as described (19). Briefly, 1 μg of Histagged protein was incubated for 5 min in 20 μl of phosphate-buffered saline with 2 mM nickel acetate on ice. Where indicated, 3 μg of carbonic anhydrase (Sigma) was included as a control. Then magnesium monooxyxyphtallic acid was added to a final concentration of 1 μM, and the mixture was incubated for another 15 min on ice. The reactions were stopped with SDS sample buffer. SDS-PAGE gels were stained with Coomassie Blue and scanned with a Molecular Dynamics densitometer.

RESULTS

Binding between Dynamin Domains Discovered with the Yeast 2-Hybrid System—We conducted a yeast 2-hybrid screen to identify human brain proteins that interact with a 377-amino acid fragment of dynamin containing the N-terminal GTPase domain. In a screen of 600,000 colonies transformed with human brain cDNA, we isolated 300 colonies that could grow without histidine, because they expressed the his-3 reporter gene (15). Upon further analysis, only one of those transformants was found to require both bait and target plasmids to express a second reporter (β-galactosidase), suggesting that this colony contained a target protein that binds to the dynamin GTPase domain. The target plasmid isolated from this colony was sequenced and, much to our surprise, found to encode the C-terminal half of dynamin. The cDNA starts at amino acid position 545, which is in the dynamin PH domain, and then extends into the 3′-untranslated region (Fig. 1). This was a first indication that the dynamin GTPase domain binds to C-terminal sequences.

The sequences within the C-terminal half that bind to the N-terminal GTPase domain were identified by testing short segments in the yeast 2-hybrid system. We found that those sequences were contained within a 100-amino acid segment, between the PH domain and the PRD (Fig. 2). This segment had previously been suggested to form a coiled coil (20), although the coiled coil-forming probability predicted by programs such as Coils is relatively weak (21). Support for a role in dimerization comes from a comparison with the homologous segment in Mx proteins, which contains leucine zippers. To test whether the dynamin fragment dimerizes, we expressed this fragment both as bait and as target in the yeast 2-hybrid system. Dimerization was readily detectable with a 100-amino acid segment (Fig. 2) but not with shorter fragments (data not shown). We call this segment the assembly domain, because it binds to itself and to the GTPase domain, which suggests that it plays a central role in the assembly process.

To characterize the newly discovered interactions between the GTPase domain and the assembly domain, we introduced mutations that are predicted to alter the GTP binding properties of the GTPase domain and tested their effects on binding between the GTPase domain and the assembly domain. Unfortunately, we were unable to utilize well-characterized Ras-activating mutations, as was done previously with other GTP-binding proteins, because the dynamin GTPase domain is too dissimilar from Ras. We could, however, introduce mutations that are predicted to block guanine nucleotide binding (K44A and S45N). These two mutations cause the GTPase to bind much more strongly to the assembly domain in the yeast 2-hybrid system (Fig. 2). The effect of the K44A and S45N mutations indicates that the strength of the binding interaction is
coupled to the cycling of GTP.

Binding between the Middle Domain and the Assembly Domain—Further experiments with the yeast 2-hybrid system showed that the assembly domain is capable of a third binding interaction besides binding to itself and to the GTPase domain. We found that the assembly domain also binds strongly to the sequences between the GTPase domain and the PH domain, which we call the middle domain (Fig. 3). Our results therefore suggest that the assembly domain has three binding interactions. It can dimerize (1), bind to the GTPase domain (2), and bind to the middle domain (3). These three binding interactions might be needed for dynamin to form a multimeric spiral. However, the number of binding interactions also raises the question of whether these interactions are specific or instead are because of nonspecific “stickiness” of the assembly domain. With the GTPase domain, this issue was addressed by testing point mutations known to affect GTP binding (Fig. 2). With the middle domain, it was not obvious which mutations to test, because nothing else is known about the middle domain.

To investigate the specificity of binding between the middle domain and the assembly domain, we determined whether homologous domains from another dynamin family member could replace them. As a counterpart, we chose Mx1, which is one of the mammalian Mx proteins known to inhibit viral replication. In yeast 2-hybrid experiments, we found that the dynamin middle domain did not bind to the Mx1 assembly domain, nor did the Mx1 middle domain bind to the dynamin assembly domain (Fig. 3). However, the Mx1 middle domain did bind to the Mx1 assembly domain, similar to the dynamin-dynamin interactions described above. This result demonstrates that the middle domains bind specifically to their cognate assembly domains but not to the assembly domains of heterologous proteins. Such specificity could be beneficial by preventing the incorporation of different dynamin family members into heterologous complexes. Curiously, we could not detect dimerization of the Mx1 assembly domain in the yeast 2-hybrid system, although the presence of leucine zippers suggests that this is likely to occur in vivo (3). Dimerization of the Mx assembly domain might not be detectable, because the Gal4 fragment, encoded by the yeast 2-hybrid bait plasmid, also dimerizes, thus favoring a homodimer of bait chimeras over binding between bait and target chimeras. The dynamin assembly domain might be less affected by Gal4 dimerization, because the dynamin coiled coil is much weaker than that of Mx proteins.

The dynamin GTPase domain and middle domain fragments used in the experiments described above overlap by 63 amino acids. To rule out the possibility that both binding activities are contained within the overlapping segment, we delineated the boundary between the GTPase and the middle domains with a deletion series (Fig. 1) that we tested with the yeast 2-hybrid system. A boundary at position 320 allowed both the GTPase domain and the middle domain to bind to the assembly domain, thereby demonstrating that these two interactions are independent (Fig. 4). Further deletions at the C terminus of the GTPase domain or at the N terminus of the middle domain completely abolishes binding, suggesting that those domains must be intact to bind to the assembly domain. Interestingly, the C-terminal 20 amino acids of the GTPase domain are predicted to form a coiled-coil with the Coils program (21). However, the interaction between the GTPase and the assembly domain is strongly influenced by mutations affecting GTP binding (Fig. 2), which suggests that an intact GTPase domain is required to achieve optimal binding to the assembly domain. Taken together, our yeast 2-hybrid results indicate that dynamin has three independent binding activities. These interactions may provide the building blocks with which dynamin assembles into a multimeric spiral.

Dimerization of the Assembly Domain Tested with Cross-linking Reagents—We used cross-linking to test whether dimerization of the assembly domain can be replicated in vitro. Initial trials with cross-linkers such as BS3 (3) were unsuccessful, suggesting that the few lysine residues in the assembly domain were not oriented properly for cross-linking. Instead, we used a novel cross-linking strategy, which takes advantage of the histidine tag that was included for protein purification. Nickel, chelated by the histidine tag, can be activated by magnesium monoperoxyphtalate acid to cross-link nearby proteins (19).

The results of in vitro cross-linking of the assembly domain, which migrates as a 15-kDa fragment, are shown in Fig. 5. A reaction product with an apparent molecular mass of 30 kDa is formed upon the addition of the cross-linking reagents nickel acetate and magnesium monoperoxyphtalate acid. No additional products are formed when an unrelated protein (carbonic anhydrase) is added to the reaction. The experiments showed that the assembly domain spontaneously dimerizes in solution, thereby supporting the yeast 2-hybrid results.

In Vitro Binding between Separated Protein Fragments—To further investigate the interactions between the dynamin...
FIG. 4. Delineation of the boundary between the GTPase domain and the middle domain using the yeast 2-hybrid system. The top panel shows GTPase fragments of increasing size in the bait vector tested with the assembly domain (Asm) or with the target vector without insert (pAct). The GTPase fragment used here contained the S45N mutation, which was shown to increase the strength of binding (Fig. 2). The middle panel shows a similar series with middle domain fragments of decreasing size. The lower panel shows controls for the staining reaction. On the left is a streak of yeast transformed with the assembly domain cloned into both bait and target vectors, and on the right is a streak of yeast transformed with vectors alone. The numberings correspond to the fragments depicted in Fig. 1.

FIG. 5. In vitro cross-linking of the dynamin assembly domain. Dimerization of the assembly domain was tested with the cross-linking reagent magnesium monopersphtalic acid (MMPP), which activates the nickel complexed with the C-terminal His tag of the assembly domain fragments. The reaction products were size-fractionated by SDS-PAGE and stained with Coomassie Brilliant Blue. The lanes show assembly domain without cross-linker (1st lane), assembly domain with cross-linker (2nd lane), assembly domain with cross-linker and chicken albumin (Alb.), which was used as a nonspecific competitor (3rd lane), chicken albumin with cross-linker (4th lane), and chicken albumin without cross-linker (5th lane). The arrows indicate the sizes of monomeric and dimeric assembly domain fragments.

GTPase domain and the assembly domain, we performed in vitro binding experiments with dynamin protein fragments. Binding was achieved by incubating radiolabeled dynamin fragments together with a GST fusion protein and glutathione-Sepharose beads. Binding was detected by autoradiography of the glutathione eluates after they were fractionated by SDS-PAGE.

The GTPase domain fused to GST pulls down a small fraction of radiolabeled assembly domain (Fig. 6), which indicates that in vitro binding is weak. However, the amount of assembly domain that was pulled down by GST-GTPase was increased by EDTA, which inhibits GTP binding by chelating the excess Mg$^{2+}$ in the in vitro transcription/translation mixture. The strength of the binding interactions was determined by competition with unlabeled assembly domain. The amount of radiolabeled assembly domain, pulled down in binding reactions with EDTA, was reduced by half when approximately 1 $\mu$M unlabeled assembly domain was added to these reactions (data not shown). Introducing the K44A mutation into the GST-GTPase construct caused increased binding between the assembly domain and the GTPase domain (Fig. 6), similar to results obtained with the 2-hybrid system (Fig. 2). We conclude that binding interactions between the GTPase domain and the assembly domain are regulated by the nucleotide-bound state of the GTPase domain. In vitro binding between the middle domain and the assembly domain was tested in pull-down experiments. The middle domain was pulled down with GST-assembly domain but not with GST-clathrin light chain, which was used as a negative control (Fig. 7). We conclude that the three protein domains that are present in all dynamin family members are capable of binding to each other both in vitro and in the 2-hybrid system.

DISCUSSION

A wealth of literature describes the interactions of the dynamin PH domain and PRD with other molecules. The PH domain and PRD may cooperate to localize dynamin to clathrin-coated pits (22, 23). However, the ability of dynamin to self-assemble in vitro without the addition of other proteins indicates that all the binding interactions necessary for spiral formation are contained within dynamin. Here, we describe the discovery of three binding interactions between different do-
mains of dynamin, which may account for the assembly process.

Two of the binding interactions were anticipated. Binding of the assembly domain to itself is consistent with the weak coiled coil predicted by the Coils program (21), and the homologous domain in Mx proteins contains leucine zippers (24). Our yeast 2-hybrid experiments and cross-linking demonstrate that the dynamin assembly domain dimerizes. The second binding interaction, between the GTPase domain and the assembly domain, is consistent with previous results obtained with proteolytic fragments of dynamin (14) and C-terminal deletions of Mx proteins (25). Our results add to those previous reports by delineating the binding sequences and investigating the nucleotide requirements.

The C-terminal 20 amino acids of the GTPase domain might form a coiled coil (21), but this cannot be the sole determinant for binding between the GTPase domain and the assembly domain, which is regulated by nucleotides. GTP weakens binding of the GTPase domain to the assembly domain, whereas the GDP-bound and nucleotide-free states strengthen binding between the GTPase domain and the assembly domain. We could not determine whether the assembly domain induces nucleotide release or, alternatively, promotes GTP hydrolysis, because hydrolysis and release occur at very high rates even without assembly domain (data not shown). However, binding between the GTPase and the assembly domain might resemble binding between other GTP-binding proteins and guanine nucleotide release proteins, which is also stronger in the nucleotide-free state or with mutations like K44A and S45N (26, 27).

Tight binding between the K44A mutant GTPase domain and the assembly domain might lock up the dynamin spiral. This is reflected by the potent dominant negative properties of dynamin K44A mutants in transfection experiments (3). Intact dynamin with the K44A mutation can assemble into a multimolecular form, whereas a second group has mutations in the middle domain (28). Animals that are heteroallelic for these two complementation groups are viable, suggesting that the GTPase domain and the middle domain have independent functions. Those independent functions are most likely the different binding activities that we discovered.

Binding of the assembly domain to itself suggests that the three domains are linked by a chain in which the middle domains of one dimer bind to the assembly domains of the next dimer. The GTPase domains of one rung could then bind to and release assembly domains of an adjacent rung, thereby ratcheting the rungs of the spirals along each other. The GTPase cycle would thereby provide the force needed for constriction using a mechanism similar to the movement of myosin along actin filaments. For simplicity, the PH domains and the PRDs were omitted from the drawings.
basic unit within a dynamin complex is a dimer. Other domains might also contribute to dimerization. For example, the C-terminal segment of the GTPase domain might form a short coiled coil (21), and the PH domain might dimerize, too (29). Linking dimeric dynamin into a chain requires a different binding interaction. We found two possibilities. The assembly domains could bind either to the GTPase domains or to the middle domains of an adjacent dimer. It seems likely that dynamin dimers are linked in a head to tail configuration, similar to the filaments formed by septins, which are GTP-binding proteins with a C-terminal coiled coil analogous to dynamin (30). Head to tail interactions might also contribute to the formation of tetramers, which is the predominant state of cytosolic dynamin (14).

Two different binding interactions are needed to account for dimerization and head to tail linkage. A third binding interaction might provide structural support, for example by joining the rungs of the dynamin spiral (Fig. 8). It was previously proposed that dynamin spirals are constricted by concerted conformational changes in which the subunits are shortened when they bind or hydrolyze GTP (Fig. 8B) (8). Our results suggest an alternative model in which dynamin spirals are constricted by ratcheting one rung of the spiral along the next rung of the spiral (Fig. 8C). The lengths of the individual subunits need not change if the contacts between the rungs are moved up one subunit at a time. Movement might occur when the GTPase domains of one rung bind and subsequently release the assembly domains of the next rung. Such stepwise movement would be similar to the movement of ATP-driven motor molecules such as kinesins or myosins. Indeed, it had been previously noted that there is significant structural similarity between GTP-binding proteins and the ATP binding domains of kinesin and myosin (31). The lengths of the steps along the dynamin spiral are similar to the steps taken by kinesin (32). In this ratchet hypothesis, dynamin combines the enzymatic properties of GTP-binding proteins with the motor properties of kinesin and myosin.

The ratchet hypothesis is consistent with previous electron microscopy data. Some of the spirals made with purified dynamin appear to be partly constricted, as if caught in the act of sliding shut (5). The earliest report of dynamin isolation also describes the ability of dynamin to decorate microtubules, cause microtubule bundling, and then cause the microtubules to slide along each other (33). Microtubule bundling might occur when the dynamin GTPases on one microtubule binds to the dynamin assembly domains on an adjacent microtubule. Runaway GTP hydrolysis by the dynamin molecules may then cause the decorated microtubules to slide along each other. The ratchet hypothesis departs from the classic perception of GTP-binding proteins as regulatory molecules. If this hypothesis is born out, then ATP-driven motor molecules, such as myosin and kinesin, might provide more meaningful paradigms (31). However, the debate on whether dynamin truly is a severing enzyme or, alternatively, acts as a regulatory GTPase needs to be settled first (34). Future experiments will decide between the different hypotheses that describe dynamin function.

The three domains that bind to each other are conserved throughout the dynamin family and are therefore likely to reflect common structural features (9). While this work was in progress, it was shown that the middle and assembly domains of Mx proteins bind to each other, similar to the interactions described in this paper for dynamin (35). Furthermore, purified Mx protein can form spirals as shown by electron microscopy (36). Sequence similarity, the similar binding interactions, and the ability to form spirals raise the possibility that dynamin and Mx proteins perform similar functions but with different targets. Other dynamin family members were not yet tested in vitro nor have their cellular functions been characterized in detail. Nevertheless, the high degree of sequence similarity suggests that those other proteins might also form spirals in vitro. It is therefore possible that all these proteins use assembly and constriction mechanisms similar to dynamin.

Acknowledgments—We thank G. Payne, A. Labrousse, D. Rube, and I. Davydov for their many helpful suggestions.

REFERENCES
1. Chen, M. S., Obar, R. A., Schroeder, C. C., Austin, T. W., Poodry, C. A., Wadsworth, S. C., and Vallee, R. B. (1991) Nature 351, 583–586
2. van der Bliek, A. M., and Meyerowitz, E. M. (1991) Nature 351, 411–414
3. van der Bliek, A. M., Redelmeier, T. E., Danke, H., Tisdale, E. J., Meyerowitz, E. M., and Schmid, S. (1993) J. Cell Biol. 122, 553–563
4. Takei, K., McPherson, P. S., Schmid, S. L., and De Camilli, P. (1995) Nature 374, 186–190
5. Hins lays, J. E., and Schmid, S. L. (1995) Nature 374, 190–192
6. Kosaka, T., and Ikeda, K. (1983) J. Neurobiol. 14, 207–225
7. Takei, K., Hauke, V., Stumpf, V., Farsad, K., Salazar, M., Chen, H., and De Camilli, P. (1998) Cell 94, 131–143
8. Sweitzer, S. M., and Hins lays, J. E. (1998) Cell 93, 1021–1029
9. van der Bliek, A. M. (1999) Trends Cell Biol. 9, 96–102
10. Salim, K., Bottomly, M. J., Querfurth, E., Zvelebil, M. J., Geut, I., Scalf e, R., Margolis, R. L., Gigg, R., Smith, C. J., Drzecki, P. C., Waterfield, M. D., and Panayotou, G. (1996) EMBO J. 15, 6241–6250
11. Aartoje, C. R., Lemmon, M. A., Schlessinger, J., and Palfrey, H. C. (1997) EMBO J. 16, 1565–1574
12. Shpetner, H. S., and Vallee, R. B. (1992) Nature 355, 733–735
13. Gout, I., Dhand, R., Hilds, I. D., Fry, M. J., Panayotou, G., Das, P., Truong, O., Totty, N. F., Hsuin, J., Booker, G. W., Campbell, I. D., and Waterfield, M. D. (1995) Cell 78, 25–36
14. Muhlb erg, A. B., Warnock, D. E., and Schmid, S. L. (1997) EMBO J. 16, 6676–6683
15. Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Elledge, S. J. (1993) Genes Dev. 7, 555–569
16. Giez, D., St. Jean, A., Woods, R. A., and Schmid, S. L. (1993) Cold Spring Harbor Symp. Quant. Biol. 58, 22310–22314
17. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (eds.) (1989) Current Protocols in Molecular Biology, pp. 20.2.1–20.2.7 Wiley Interscience, New York
18. Schwei, M., Richter, M. F., Herrmann, C., Nassar, N., and Staeheli, P. (1995) J. Biol. Chem. 270, 13518–13523
19. Lai, C. C., Boguski, M., Borek, D., and Powers, S. (1993) Molec. Cell. Biol. 13, 1345–1352
20. Okamoto, P. M., Herskovits, J. S., and Vallee, R. B. (1993) J. Biol. Chem. 272, 11629–11635
21. Melon, K., Romi, T., Broni, B., Krug, R. M., von Bonsdorff, C. H., and Julkunen, I. (1992) J. Biol. Chem. 267, 25988–25997
22. Schwemmle, M., Richter, M. F., Herrmann, C., N asar, N., and Staeheli, P. (1995) J. Biol. Chem. 270, 13518–13523
23. Grant, D., Unalakt, S., Kazene, A., Krishnan, K. S., and Ramaswami, M. (1998) Genetics 149, 1019–1030
24. Klein, D. E., Lee, A., Frank, D. W., Marks, M. S., and Lemmon, M. A. (1998) J. Biol. Chem. 273, 27275–27273
25. Field, C. M., Al-Awar, O., Rosenblatt, J., Wong, M. L., Alberts, B., and Mitchison, T. J. (1996) J. Cell Biol. 133, 605–616
26. Vale, R. D. (1996) J. Cell Biol. 133, 291–302
27. Schnitzer, M. J., and Block, S. M. (1997) Nature 388, 386–390
28. Shpetner, H. S., and Vallee, R. B. (1989) Cell 59, 421–432
29. Roos, J., and Kelly, R. B. (1990) Trends Cell Biol. 7, 257–259
30. Schumacher, B., and Staeheli, P. (1991) J. Biol. Chem. 273, 28365–28370
31. Nakayama, M., Yazaki, K., Kusano, A., Hanai, N., and I shihama, A. (1993) J. Biol. Chem. 268, 15033–15038

Three Binding Interactions within Dynamin