

A Molecular Motor or a Regulator? Dynamin's in a Class of Its Own[†]

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Dynamin was originally purified from bovine brain extracts as a microtubule-associated protein that could be released in the presence of GTP or ATP (1). Indeed, in the presence of crude preparations of dynamin, microtubules were bundled and individual tubules were seen to slide past each other, suggesting that dynamin might function as a microtubule-dependent motor protein. Cloning and sequencing of the gene for rat brain dynamin revealed consensus GTP binding motifs that are found among regulatory and signaling GTPases (2). The sequence of dynamin's N-terminal GTPase domain is highly identical with the sequences of other high-molecular weight GTPases, including the interferon-inducible Mx proteins and Vps1p, a protein involved in vacuolar sorting in yeast (3). These structurally related enzymes comprise the dynamin subfamily of GTPases, which also includes two mitochondria-associated members, Dnm1p in yeast or Drp1/Dlp1 in higher eukaryotes, and Mgm1p in yeast and higher eukaryotes. Dynamin family members are multidomain proteins characterized by an N-terminal GTPase domain, a middle domain, and a GTPase effector domain (GED)¹ (Figure 1). Dynamin alone contains two additional functional domains: a pleckstrin homology (PH) domain that binds to PI4,5P₂ and a C-terminal proline- and arginine-rich domain (PRD) that binds to numerous SH3 domain-containing proteins. The functionally diverse dynamin family members are also characterized by their low affinity for GTP (10–100 μM) and by their ability to self-assemble into rings and helical stacks of rings. Self-assembly potentially stimulates dynamin's GTPase activity.

Temperature-sensitive mutations in the *shibire* gene in *Drosophila* cause paralysis at elevated temperatures by reversibly blocking endocytosis at the nerve terminal (4–6). This prevents membrane recycling and results in depletion of synaptic vesicles. A role for dynamin in vesicular traffic was proposed on the basis of the finding that the *shibire* gene product is the *Drosophila* homologue of dynamin (7, 8). Mammalian dynamins are also involved in endocytosis (9); however, the exact function of dynamin has not been resolved and remains a subject of much debate (10–13).

Model 1: Dynamin Functions as a Mechanochemical Enzyme

Dynamin spontaneously self-assembles into rings and spirals at low ionic strengths (14) or under physiological

conditions in the presence of either transition state analogues of GTP (i.e., GDP•BeF₃[−] or GDP•AlF₄[−]) or a template (e.g., PI4,5P₂-containing lipid nanotubes or liposomes) (15–17). Self-assembly by dynamin stimulates its GTPase activity ~100-fold. Intriguingly, collar-like structures reminiscent of dynamin rings accumulate at the necks of the endocytic intermediates that are trapped at the synapses of *shibire* flies incubated at the nonpermissive temperature. These observations led to the first models for dynamin function in endocytosis (Figure 1B, model 1), which suggested that dynamin functions as a mechanochemical enzyme, akin to ATPase-driven molecular motors. In these models, GTP hydrolysis by dynamin causes a force-generating conformational change that is physically required to drive membrane fission. Specifically, dynamin is suggested to assemble, in its GTP-bound state, into a collar-like structure at the necks of deeply invaginated coated pits. Subsequent GTP hydrolysis is proposed to cause one of two conformational changes that result in either constriction (model 1, arrow 1) of the assembled structure or expansion of the helical pitch (model 1, arrow 2) such that dynamin functions as either a molecular “garrote” or “spring” to drive vesicle fission.

Model 2: Dynamin Functions as a Regulatory Enzyme

Mechanistic and structural analysis of dynamin's assembly-stimulated GTPase activity led to the discovery that dynamin encodes its own GAP (GTPase activating protein), the GTPase effector domain or GED (18, 19). When added to full-length dynamin, the isolated GED fragment expressed in *Escherichia coli* stimulates dynamin's GTPase activity to almost the same degree as template-driven self-assembly (19). Mutations in the GED have been shown to specifically affect assembly-stimulated GTPase activity without affecting dynamin's basal rate of GTP hydrolysis (19); thus, the GED functions as an assembly-dependent GAP. The mechanism of GED-stimulated GTPase activity is unknown.

Mutations have been identified in the GED that impair dynamin's ability to self-assemble, both in vivo (13, 19) and in vivo (20), and consequently its assembly-stimulated GTPase activity. The observation that endocytosis is stimulated in cells overexpressing these GED mutants led to a second model for dynamin, which suggests that it functions,

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¹ Abbreviations: GED, GTPase effector domain; PH, pleckstrin homology; PRD, proline- and arginine-rich domain; PI4,5P₂, L-α-phosphatidyl-D-myoinositol-4,5-bisphosphate; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein; RGS, regulators of G protein signaling; Abp1, actin filament binding protein 1.

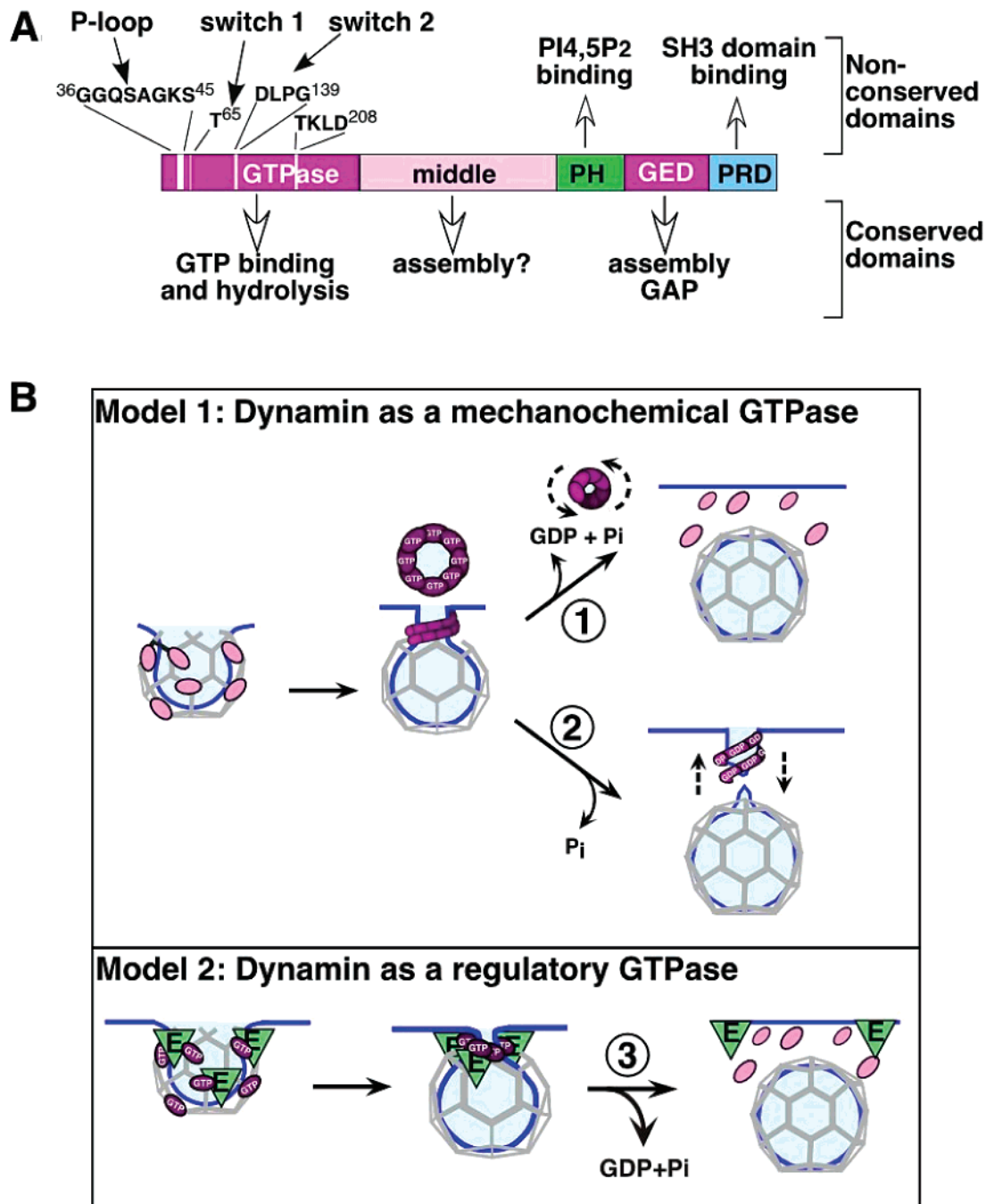


FIGURE 1: Domain structure and function of dynamin. (A) Shown are dynamin's domain structure and their respective functions. The N-terminal GTPase domain has the four consensus binding elements characteristic of all GTPase superfamily members. (B) Two models of dynamin function. As a mechanochemical enzyme, dynamin uses chemical energy released from GTP hydrolysis for fission of the neck of a coated pit, either by constricting dynamin collars (arrow 1) or by expanding (arrow 2) the helical pitch of dynamin rings. As a regulatory enzyme, the dynamin-GTP complex, which is localized to coated pits, recruits and/or activates effector molecules that are required for vesicle formation. GTP hydrolysis triggered by assembly of dynamin at the neck (arrow 3) serves to terminate interactions with downstream effectors.

like other GTPase superfamily members, as a regulatory enzyme (Figure 2B). In this model, while in its GTP-bound form, dynamin recruits downstream effectors to the coated pit, which in turn mediate coated vesicle formation. As the neck narrows and vesicle formation nears completion, dynamin self-assembly triggers GTP hydrolysis to terminate its interactions with these downstream effectors (Figure 1B, model 2, arrow 3). By impairing self-assembly, the GED mutants will prolong the period dynamin is in its GTP-bound state and hence accelerate vesicle formation.

Is Dynamin a Mechanochemical or Regulatory Enzyme?

There is yet insufficient evidence to distinguish between these two models. Although evidence suggests that both GTP hydrolysis and conformational changes are essential for dynamin function in endocytosis (20–22), the question of whether the chemical energy from GTP hydrolysis is used to generate a “powerstroke” for mechanochemical work or to “flip a switch” to terminate signaling has not been resolved. Here we compare the enzymatic and structural properties of molecular motors and regulatory GTPases with

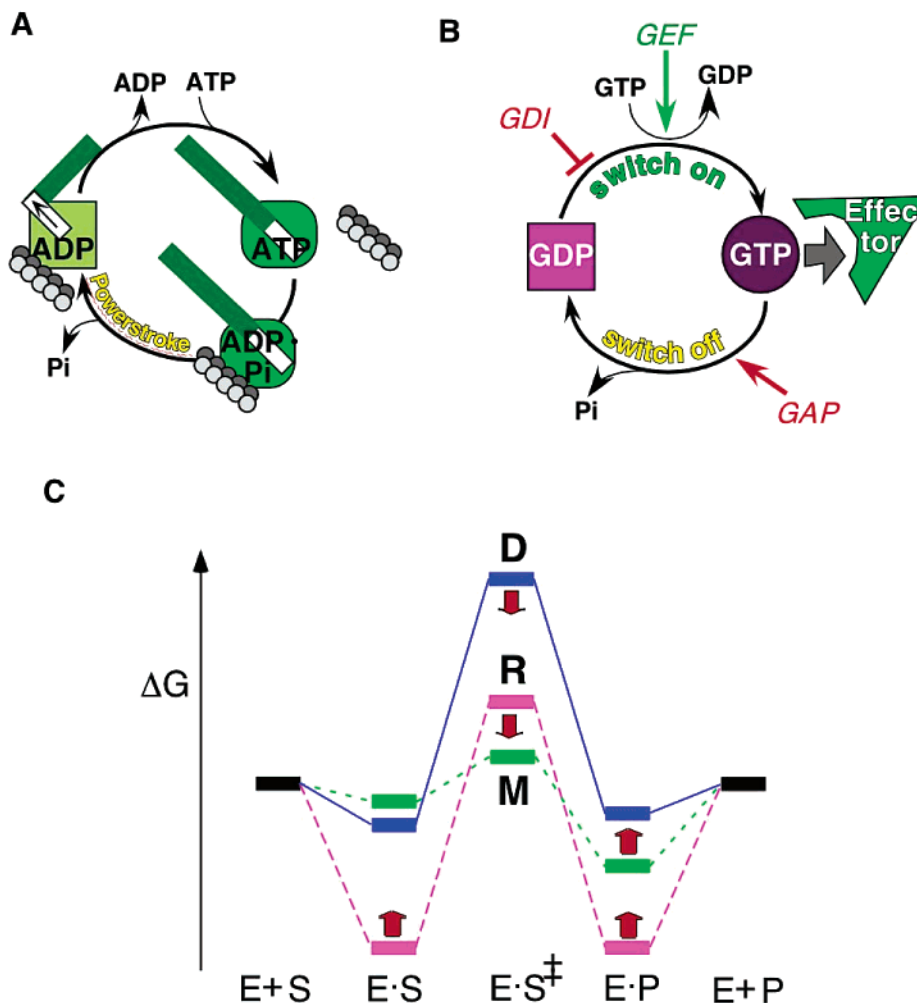


FIGURE 2: Comparison of the NTPase cycles of motors and regulatory enzymes. (A) Typical motor ATPase cycle. The motor head dissociates from the template upon ATP binding. After ATP hydrolysis, the enzyme rebinds to the template with ADP and P_i still bound. The template triggers P_i release and small conformational changes in switch II, which are transmitted through the relay helix (white rectangle) and amplified by the lever arm (dark green), to bring about a powerstroke that moves the motor head relative to the template. The motor is returned to its original conformation by ADP release and ATP binding. (B) Typical regulatory GTPase cycle. Nucleotide binding is tight, and cycling between the structurally distinct GDP-bound (inactive) and GTP-bound (active) conformations is regulated by cellular factors. GEFs (guanine nucleotide exchange factors) release GDP, allowing GTP to bind and activate the GTPase. GAPs (GTPase-activating proteins) accelerate GTP hydrolysis to inactivate the GTPase and terminate signaling. GDIs (guanine nucleotide dissociation inhibitors) bind to and stabilize the inactive E·GDP complex. The active E·GTP complex binds effector molecules, which mediate downstream events. (C) Relative Gibbs free energy profiles are shown for intrinsic NTPase activities of typical motor proteins (M), regulatory GTPases (R), and dynamin (D). E, S, and P represent enzyme, substrate (ATP or GTP), and hydrolysis products (NDP + P_i), respectively. The arrows (red) represent the effects of regulators, templates for motor proteins and GEFs and GAPs for regulatory GTPases. Motors have weak substrate binding and fast ATP hydrolysis, suggesting limited stabilization of the E·S complex and a small activation energy barrier for the transition state. It is possible that the intrinsic binding energy is used in destabilization of the substrate, thereby lowering the activation barrier for catalysis. The rate-limiting step in steady state turnover is product dissociation, indicating a greater barrier for product dissociation than for the ATP hydrolysis step. Regulatory enzymes bind GTP tightly, and the rate of GTP hydrolysis is relatively slow, suggesting a large stabilization of the E·S complex with a large activation energy barrier required for formation of the transition state (E·S[‡]). Since GDP dissociation is slow, the E·P complex is low in free energy and the energy barrier for product dissociation is large. Dynamin has a low affinity for GTP, and GTP hydrolysis is slow, implying limited stabilization of the E·S complex coupled with a larger activation energy barrier for hydrolysis. GDP dissociation is fast and not rate-limiting for steady state GTP hydrolysis.

those of dynamin in an effort to gain some insight into how dynamin might function within the cell.

Interestingly, the catalytic cores of motor proteins, like myosin and kinesin, and of GTPase superfamily members, like Ras and G α subunits, interact with nucleotides in a structurally similar manner, and it has been proposed that they arose from a common ancestor (23, 24). Both classes of NTPases undergo conformational changes depending on the nature of the bound nucleotide. Conformationally sensitive regions in the nucleotide binding sites of both classes of enzyme, termed switch I and switch II, house critical

residues (a conserved Gly in switch I and a Ser or Thr residue in switch II) that interact directly or indirectly with the γ -phosphate through hydrogen bonding to sense the nature of the bound nucleotide. The switch I and switch II regions undergo conformational changes upon hydrolysis of the bound NTP and release of the γ -phosphate that are essential for the function of both mechanochemical ATPases and regulatory GTPases (Figure 2). However, mechanochemical enzymes and regulatory GTPases differ in several respects, including the kinetic mechanism of NTP hydrolysis, the requirement and the effect of a template or an effector, the

Table 1: Kinetic Constants for NTP Hydrolysis

enzyme	regulator	K_d^{NTP} (μM)	$k_{\text{off}}^{\text{NDP}}$ (s^{-1})	k_{hydr} (s^{-1})
dynamain ^a	—	0.5–2.5	60–93	10^{-3} – 10^{-2}
	+	8–15		2–5
Ras ^b	—	7.1×10^{-7}	4.2×10^{-4}	3.4×10^{-4}
	+		0.8	15
G α s ^c	—	8×10^{-4}	5×10^{-3}	3×10^{-2}
	+		10^2	3
myosin ^d	—	10^2 – 10^3	1	50–100
	+	10^3	300–500	30
kinesin ^e	—	50	0.01	9
	+	20	40	100

^a From refs 17, 19, 20, 41, and 67. ^b From refs 35 and 68. ^c From refs 69 and 70. ^d From ref 29. ^e From refs 30 and 31.

nature of the conformational changes during the cycle of NTP hydrolysis, and the step in the NTPase cycle that is most critical for function.

ATP-Driven Motors Are Built To Move

An important distinction between switches and motors is the coupling of the conformational changes to force generation along a template. Indeed, in addition to a nucleotide binding site, the catalytic cores of motor proteins contain three other structural features: (1) a distal template binding site, for actin or microtubules, (2) a “relay” helix or “converter”, and (3) a lever arm (Figure 2A). The switch I and II regions are buried in motor enzymes, and are not solvent-accessible. In the presence of bound ATP, they undergo relatively small movements to close over the nucleotide-binding pocket. These are transmitted through the relay helix and amplified by the lever arm (25–27). The relay helix functions like a piston moving up and down in response to the inward and outward shifts of switch II (26).

The nucleotide hydrolysis cycle of motor enzymes has evolved to achieve efficient coupling of ATP hydrolysis to force generation. Indeed, to maximize the rate of ATP hydrolysis, the active sites of motor enzymes have evolved to stabilize the transition state over the ground state of the bound nucleotide (Figure 2C). In this way, most of the available intrinsic binding energies of ATP are used for efficient catalysis, suggesting that the energy barriers between ATP- and ADP-bound conformations are small (Figure 2C and Table 1) (28). Motor proteins exhibit low nucleotide binding affinities ($\sim 100 \mu\text{M}$, Table 1). Indeed, on the basis of structural studies, the two conformations of the switch regions around the nucleotide binding pocket may be in equilibrium. Importantly, force-generating conformational transitions in motor proteins are dependent on both the bound nucleotide and the bound template; interactions with the template stabilize the nucleotide-dependent positioning of the relay helix (25, 26). The rate-limiting step in the ATPase cycle, product dissociation (ADP dissociation in the case of kinesin and P_i dissociation in the case of myosin), is accelerated 300–500-fold by the presence of templates (29–31; Table 1). This ensures that the powerstroke, which accompanies the rate-limiting step of product release, is coupled to force generation and movement along the template. Thus, the functionally critical step in the ATPase cycle of mechanochemical enzymes is the powerstroke that accompanies template-assisted product release (Figure 2A).

GTPase Switches Are Kinetic Timers

Regulatory GTPases use the difference in structural features between GDP- and GTP-bound states for their function; they toggle between GTP- (on) and GDP-bound (off) conformations. In contrast to motor enzymes, “effector” molecules that bind preferentially to the GTP-bound “active” conformation of the enzyme are necessary for implementation of downstream events (Figure 2B). Thus, the functionally critical intermediate in the GTPase cycle of regulatory enzymes is the enzyme•GTP complex.

The switch I and switch II regions of GTPases are surface-exposed and undergo significant conformational changes in response to the bound nucleotide. In the GDP-bound form, the switch I and II loops are flexible and unstructured, whereas in the GTP-bound form, they are structured and provide all or part of the docking site for interaction with effector proteins. In some cases, the conformational changes in the GTPase domain can involve inserts associated with the switch regions in addition to the loops themselves, creating larger binding surfaces (32). P_i release is not rate-limiting in the GTPase cycle (32); therefore, GTP hydrolysis triggers abrupt structural changes in switch I and switch II to control binding specificity.

A key to the function of GTPases as molecular switches is the fact that individual steps of their GTPase cycle, including GTP loading and hydrolysis, are controlled by other cellular factors that are required to turn the switch on or off. For regulatory GTPases, the binding affinities of both GTP and GDP are very high (typically nanomolar to picomolar, Table 1) and both nucleotide-bound forms are stable. This is reflected in the low Gibbs free energy profiles for the ground state E•GTP and E•GDP complexes, especially in comparison to motor enzymes (Figure 2C). GDP dissociation is often the rate-limiting step in the GTPase cycle (Figure 1) and can take from minutes to hours (Table 1). Thus, in the absence of an upstream signal, GTPases exist in a stable GDP-bound, off state. In the cell, turning on a signaling GTPase is tightly regulated and dependent on a guanine nucleotide exchange factor (GEF), which facilitates the release of GDP by 2×10^4 -fold (33). GEFs do not provide nucleotide specificity, but rather, they open up the guanine nucleotide-binding pocket. Selective binding of GTP occurs by mass action, because it exists in an ~ 10 -fold molar excess over GDP in cytosol (34).

For regulatory GTPases, the E•GTP intermediate is the most functionally critical because it activates or recruits downstream effectors. Therefore, nucleotide interactions have evolved such that the E•GTP complex has a lifetime that is sufficiently long for maintenance of a stable signal. This property is reflected in the lower K_d and the lower Gibbs free energy state for the E•GTP complex for regulatory GTPases as compared to those of motor proteins (Figure 2C).

GTP hydrolysis by regulatory GTPases functions to switch the activated GTPase off and to terminate interactions with downstream effectors. Regulatory GTPases typically have a poor intrinsic ability to hydrolyze GTP. The rates of hydrolysis, however, vary considerably between GTPases. Ras, for example, would take ~ 1 h to hydrolyze GTP and turn itself off, whereas G α s does so in ~ 30 s (Table 1). GTPase-activating proteins or GAPs can substantially stimulate this intrinsic rate of GTP hydrolysis. In the presence of

its GAP, the rate of GTP hydrolysis by Ras is accelerated 5×10^4 -fold, yielding a half-life of ~ 50 ms (35) (Table 1). This remarkable degree of stimulation is due to the fact that Ras lacks an essential catalytic arginine in its active site, required for stabilization of the GTP transition state (36). RasGAP functions both to stabilize the switch I and switch II interactions with the bound GTP and to provide, in trans, the necessary catalytic Arg. In contrast, the higher intrinsic GTPase rates of G α subunits reflect the fact that these GTPases encode their own catalytic Arg residue. RGSs (regulators of G protein signaling) are GAPs for G α GTPases and function, like RasGAP, to stabilize switch I and switch II loops, but also to orient the intrinsic residues within the GTPase for efficient catalysis.

Regardless of the mechanisms of hydrolysis, which differ significantly between GTPase superfamily members (32), regulatory GTPases together with their GAPs serve as kinetic timers that control the duration and therefore the degree of amplification of the signal. The timing function of GTPases is also employed in the oversight (termed kinetic proofreading) of complex reactions such as protein translation by elongation factors, or vesicle docking and fusion by Rab proteins (37).

Dynamin Defines a New Class of GTPases

Dynamin has structural features that are reminiscent of both GTPases and molecular motors. Although motor domains and GTPase domains are believed to be evolutionarily related, they have subsequently diverged considerably into two distinct families (23). The recently determined structure of the highly conserved GTPase domain of dynamin A from *Dictyostelium* establishes dynamin as a true member of the GTPase superfamily in that it adopts the core fold of all known regulatory GTPases, including Ras, Rabs, G α subunits, etc. (38). In particular, the switch I and switch II regions are exposed and switch II is immediately upstream of a large, highly charged and highly variable (between dynamin family members) insert that has been suggested to be a docking site for effector proteins (38). However, like motor proteins, in addition to the NTPase "head", dynamin has multiple distal domains through which conformational changes can be transmitted and potentially amplified. The function of the middle domain, in particular, which binds the GED (39) and has α -helical regions (40) is unknown. Possible nucleotide-driven structural changes in the middle and PH domains need to be investigated further.

The enzymological properties of dynamin combine aspects of both mechanochemical enzymes and regulatory GTPases, with aspects that distinguish it from either enzyme class. Dynamin is similar to motor enzymes in terms of its affinity for nucleotides, yet similar to the regulatory GTPases in terms of its rate of GTP hydrolysis (Table 1). Dynamin binds nucleotide with an affinity ($K_d = 0.5$ – 2.5 μ M for GTP) several orders of magnitude lower than those of regulatory GTPases (41), but still higher than those of motor enzymes such as myosin and kinesin (for ATP, $K_d = 100$ – 1000 and 50 μ M, respectively). The basal rate of hydrolysis of GTP by dynamin is 10-fold faster than that of Ras, but comparable to that of G α subunits. It is 10^4 – 10^5 -fold slower than the rate of ATP hydrolysis by myosin or kinesin (Table 1). Dynamin is strikingly distinguished from both motors and

regulatory GTPases by the rapid rate of GDP dissociation [93 s $^{-1}$ (41)] even in the absence of a template or GEF. GDP dissociation from dynamin is 10^5 -fold faster than from Ras, 10^4 -fold faster than for G α , and 10^2 – 10^3 -fold faster than ADP dissociation from myosin or kinesin (Table 1). Because GDP dissociation is much faster than GTP hydrolysis, nucleotide exchange is not rate-limiting for dynamin, distinguishing its NTPase cycle from both motors and regulatory enzymes. The rate of dissociation of GTP from dynamin is also fast (2 s $^{-1}$), although GTP binds ~ 40 -fold more tightly than GDP (41). When the rate of association of GTP with dynamin (7×10^5 M $^{-1}$ s $^{-1}$) and the cellular environment of ~ 100 μ M GTP are considered, dynamin would be expected to predominantly ($>95\%$) exist in the GTP-bound state. Thus, if dynamin functions as a switch, it is an atypical one that is predominantly on.

Like motor proteins, dynamin self-assembly and nucleotide-dependent conformational changes can generate force. Dynamin assembly onto PI4,5P $_2$ -containing liposomes causes them to become tubulated (16, 42). Subsequent GTP hydrolysis causes further constriction of the dynamin spirals and vesiculation (16), depending on the composition of the liposome (43). However, while striking, dynamin's ability to tubulate liposomes is not a unique feature of this protein; amphiphysin-1, endophilin, and epsin all have similar abilities (44–46). These proteins are believed to induce the spherical to tubular transition by inserting their N-terminal lipid binding domains into the outer leaflets of the lipid bilayers, thus increasing membrane curvature. Dynamin-dependent tubulation may not reflect its specific mechanochemical properties, because dynamin has also been shown to insert a large portion of itself into lipid membranes (47). Other dynamin family members studied so far also self-assemble onto liposomes and can induce tubulation, including the MxA protein (48). However, mutations in MxA that abrogate its ability to self-assemble have no effect on its cellular function of conferring viral resistance (49). Thus, the in vivo function of these in vitro properties of dynamin needs to be tested.

Template Interactions Constrain Models for Dynamin as a Force-Generating Enzyme

Dynamin, like motor proteins, exhibits template-stimulated NTPase activity. However, unlike motors, the template (liposomes, lipid nanotubules, or microtubules) is not directly responsible for accelerated hydrolysis; rather, it facilitates and orients dynamin–dynamin interactions, in part through the GED. In the context of molecular motors, then, one might suggest that dynamin, by assembling into a helical spiral, provides its own template. In this way, force can be generated by the movement of dynamin along the template, i.e., circumferentially around the spiral. This could occur, as initially proposed (14, 50), in a single powerstroke driven by a concerted conformational change in the assembled collars to cause constriction and membrane fission at the neck.

For dynamin to function as its own template, multiple runs of a helix must assemble to generate force. Thus, a caveat to mechanochemical models lies in the well-established fact that GTP hydrolysis triggers dynamin disassembly (51) and its displacement from templates (2, 52). In vitro studies documenting force generation by dynamin

have depended on preassembling dynamin spirals in the absence of nucleotides, followed by acute addition of GTP, conditions not likely to be encountered *in vivo*. The problems, then, are how collar assembly can be controlled and how the hydrolytic events can be coordinated with collar assembly to generate force. Alternatively, can rapid hydrolysis by a few subunits that overlap to complete the ring be sufficient to cause constriction of the entire collar? These issues must be resolved and the mechanisms incorporated into any mechanochemical model for dynamin function.

Similar dilemmas are encountered for force generation in the “spring” model for dynamin. Quantitative analysis has suggested that at least two complete turns of the spiral, as well as the stable binding to the underlying membrane along the inner surface of the helix, would be necessary to generate sufficient force for neck constriction and vesicle detachment (53). Given that GTP hydrolysis disassembles dynamin, this model must invoke some mechanism for regulating dynamin GTPase activity to complete assembly of the mechanical spring, which then functions as a fission apparatus.

Thus, for either mechanochemical model for dynamin function, a mechanism must be defined that enables assembly of the complete fission apparatus so that the powerstroke driven by GTP hydrolysis can be coupled to force generation at the neck.

A third model for dynamin circumvents the problem of assembling a fission apparatus. On the basis of their observation of nucleotide-dependent dynamin interdomain interactions, van der Blik and colleagues suggested that dynamin’s rapid assembly-stimulated GTP hydrolysis drives sequential stepwise translocations of dynamin along the circumference of the spiral as it assembles (39). Driven by GTP-dependent interdomain interactions, this stepwise translocation would result in ratcheting down a collar of diminishing subunits around the neck of a budding vesicle. This model, with dynamin spirals acting as its own template, is more in keeping with the known stepwise translocation of motor heads along a template.

Kinetics Constrain Models for Dynamin as a Regulatory GTPase

Dynamin’s atypical kinetics must necessarily constrain any model for dynamin as a regulatory GTPase because unlike other regulatory GTPases, dynamin will exist predominantly in the “on” conformation. One possibility is to invoke another factor, analogous to Rho or Rab GDI proteins, that binds to dynamin in its GDP-bound state and sequesters the protein in an inactive conformation. Given the relatively high intrinsic rate of GTP hydrolysis and the abundance of dynamin in brain extracts, for example, such a mechanism might also be used to prevent futile cycles of GTP hydrolysis. Intriguingly, dynamin has been shown to interact with $\beta\gamma$ subunits of heterotrimeric G proteins, and this interaction inhibits dynamin’s basal rate of GTP hydrolysis (54). Moreover, the affinity of $\beta\gamma$ subunits for dynamin increases ~10-fold in the presence of PI4,5P₂-containing liposomes. Whether these interactions occur *in vivo* and, if so, whether they function to sequester inactive dynamin•GDP complexes or to stabilize dynamin•GTP complexes at the plasma membrane are unknown.

As an alternative means of regulating dynamin’s signaling, one might speculate that dynamin’s interaction with down-

stream effector molecules occurs with low affinity. As a consequence, dynamin•GTP–effector interactions could be spatially regulated by the effective concentration of these proteins through their mutual targeting to a coated pit. Indeed, there are many examples of scaffolding molecules that bind both dynamin and other endocytic accessory proteins, including candidate effector molecules, which could serve to facilitate low-affinity dynamin–effector interactions (55). Moreover, there is precedence for this type of regulation in that the cellular effects of overexpression of constitutively active mutant forms of Rab or Ras are abrogated by secondary mutations that disrupt their targeting to specific cellular locations.

In sum, its low affinity for, and unstable binding of, both GTP and GDP necessarily constrain models for dynamin as a molecular switch: dynamin is a very loose switch! These constraints suggest that if dynamin functions as a regulatory GTPase, other factors must regulate its GTPase cycle and/or its interaction with effectors.

Other Considerations and Perspectives

Dynamin has been the subject of intensive studies since its isolation. While it is clearly an important component of the endocytic apparatus, its exact role in vesicle formation remains unknown. The two prevalent models, dynamin as a mechanochemical enzyme and dynamin as a regulatory GTPase, can be distinguished by answering the two following critical questions. (1) What is the role of dynamin’s assembly-stimulated GTPase activity, and does it create a powerstroke or terminate signaling? (2) What steps in the dynamin’s GTPase cycle are most critical for its function; is it the process of assembly-stimulated GTP hydrolysis or the accumulation of the dynamin•GTP complex? Or does dynamin combine aspects of both classes of enzymes for its function?

Answers to these questions will require additional mechanistic and structural insight. New cell-free assays that reconstitute dynamin function in the context of vesicle formation from biological membranes will be required to determine how dynamin’s GTPase cycle is linked to vesicle formation. The analysis both *in vitro* and *in vivo* of new classes of dynamin mutants that uncouple self-assembly from assembly-stimulated GTPase activity will be needed to determine the role of these activities. Much has been learned about how motors work by docking high-resolution structures obtained by X-ray crystallography to medium-resolution structures obtained by electron microscopic analysis of motor proteins in different conformational states on their templates [reviewed by Vale and Milligan (26)]. Thus, the recently obtained crystal structure of the dynamin GTPase domain (38) and promising electron microscopic structures of dynamin assemblies on liposomes (56) should provide important structural insight into how dynamin functions. Clearly, the simple models illustrated in Figure 1B will need to be modified as new mechanistic insight is obtained.

Finally, there are additional complexities relevant to dynamin function *in vivo* that must be considered. For example, dynamin binds to numerous SH3 domain-containing proteins through its C-terminal proline- and arginine-rich domain. These proteins can be divided into two groups depending on their targets. Amphiphysin and endophilin are

involved in the formation of coated pits; cortactin, actin filament binding protein 1 (Abp1), profilin, and syndapin are involved in cytoskeleton dynamics, and intersectin is involved in both (57–60). There is no doubt that these protein interactions will add to the complexity of dynamin GTPase activity and function. Recently, it has been shown that dynamin plays a role in regulating actin comet formation and vesicle motility in living cells (61, 62), and in regulating actin assembly in vitro (60). Additional roles in signaling have been suggested on the basis of the induction of p53-dependent apoptosis by overexpressed dynamin-2 (63) and the perturbation of G protein-coupled receptor signaling to MAP kinases by a dominant-negative dynamin mutant that occurs independently of receptor internalization (64–66). These data suggest that whatever dynamin's mechanism of action, the cellular functions of this GTPase are not restricted to a single mechanochemical event.

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