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Rapid Report

The Proline/Arginine-Rich Domain Is a Major Determinant of Dynamin Self-Activation†

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ABSTRACT: Dynamins induce membrane vesiculation during endocytosis and Golgi budding in a process that requires assembly-dependent GTPase activation. Brain-specific dynamin 1 has a weaker propensity to self-assemble and self-activate than ubiquitously expressed dynamin 2. Here we show that dynamin 3, which has important functions in neuronal synapses, shares the self-assembly and GTPase activation characteristics of dynamin 2. Analysis of dynamin hybrids and of dynamin 1−dynamin 2 and dynamin 1−dynamin 3 heteropolymers reveals that concentration-dependent GTPase activation is suppressed by the C-terminal proline/arginine-rich domain of dynamin 1. Dynamin proline/arginine-rich domains also mediate interactions with SH3 domain-containing proteins and thus regulate both self-association and heteroassociation of dynamins.

Dynamins are ∼100 kDa GTPases that promote membrane vesiculation and actin polymerization (*1*, *2*). GTP hydrolysis, which is stimulated upon dynamin polymerization, induces a change in the conformation of the dynamin coil and promotes dynamin polymer disassembly (*3*−*5*). Mammals express three dynamin genes, encoding dynamins (Dyns) 1, 2, and 3 (*6*). Dyn1 is abundant in presynaptic nerve terminals and participates in rapid synaptic vesicle recycling. Ubiquitously expressed Dyn2 has a role in receptor-mediated endocytosis, Golgi budding, and regulation of the actin cytoskeleton. Dyn3, the least studied of the three dynamins, was originally identified in testes but has since been shown to participate in restructuring of dendritic spines (*7*, *8*). In addition, Dyn3 may have a role in synaptic vesicle recycling in presynaptic terminals, as this isoform (but not Dyn 2) can partially rescue this process in Dyn1 knockout mice (*9*).

The three dynamins have the same overall domain structure, including an N-terminal catalytic domain, followed by a middle domain that mediates dynamin−dynamin interactions, a pleckstrin homology (PH) domain involved in phosphoinositide binding, a GTPase effector domain (GED) that interacts with the catalytic domain and promotes concentration-dependent GTPase activation, and a C-terminal proline/arginine-rich domain (PRD) known to mediate interactions between dynamins and other proteins.

The sequences of the three dynamins are reasonably conserved but are most divergent in their PRDs. Dyn1 and Dyn2 have been extensively characterized in vitro. They express basal GTPase activities of ∼1−2 min^{-1} (Dyn1) or ∼10−20 min^{-1} (Dyn2), which can be stimulated to ∼200 min^{-1} in the presence of scaffolds, such as microtubules (*10*−*12*). These scaffolds facilitate dynamin self-assembly, presumably allowing productive interactions between the catalytic and GED domains. Dyn1 and Dyn2 can also self-assemble in the absence of scaffolds at sufficiently low ionic strengths and sufficiently high dynamin concentrations (*3*, *13*, *14*). Dyn2, by itself, polymerizes at lower concentrations than Dyn1 and achieves much higher levels of concentration-dependent self-activated GTPase activity (∼10 min^{-1} for Dyn1 and ∼100 min^{-1} for Dyn2) (*3*).

In contrast to Dyn1 and Dyn2, almost nothing is known about the physical and enzymatic properties of Dyn3. To determine its ability to self-assemble and self-activate, we expressed human Dyn3 in Sf9 cells with a C-terminal His_6 tag and purified the protein by affinity for Ni^{2+} resin. As shown in panels A and B of Figure 1, Dyn3 expressed high GTPase activity which, like that of Dyn2, was concentration-dependent and approximately 10-fold higher (∼100 min^{-1}) than that of Dyn1 (∼10 min^{-1}) (*3*). Using a turbidity assay, we observed that Dyn3 was also similar to Dyn2 with respect to its strong propensity to self-assemble (compare Figure 1C with Figure 3). Moreover, Dyn2 and Dyn3 polymers disassembled at similar rates upon addition of GTP. Sedimentation equilibrium measurements indicated that, like Dyn1 and Dyn2 (*15*, *16*), unassembled Dyn3 exists in a monomer−tetramer equilibrium with a K_a (*1*, *4*) of 8.1×10^{19} M^{-3} (Figure S1 of the Supporting Information).

As stated, dynamin isoforms differ in their abilities to self-assemble into higher-order structures upon dilution into low-ionic strength buffers. To identify the domains responsible for these differences, we analyzed a series of dynamin hybrids with interchanged GED and PRD segments (Figure 2A). Because the GED is critical for both dynamin self-assembly (*17*) and self-activation (*18*) and was shown to interact directly with the catalytic domain (*18*, *19*), we first examined hybrids of Dyn1 and Dyn2 with exchanged GEDs. Surprisingly, the concentration-dependent GTPase activities of Dyn1(GED2) and Dyn2(GED1) were nearly identical to those of Dyn1 and Dyn2, respectively (Figure 2B,C). However, the hybrid protein consisting of Dyn1 with the PRD of Dyn2 [Dyn1(PRD2)] exhibited even stronger self-activation than wild-type Dyn2 (compare panels B and C of Figure 2). Conversely, Dyn2(PRD1) and Dyn3(PRD1) exhibited weaker self-activation than their wild-type parent molecules (for wild-type activities, see Figures 1B and 2C), albeit slightly higher than that of wild-type Dyn1. Confirming the importance of the PRDs in self-activation, we found a construct consisting of Dyn1 with both the GED and PRD of Dyn2 behaved like wild-type

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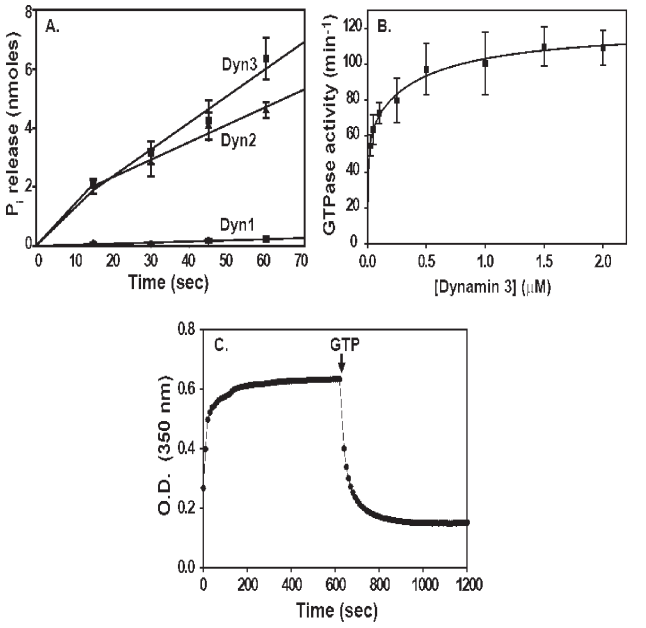

FIGURE 1: Biochemical analysis of Dyn3. (A) GTPase activities of Dyn3, Dyn2, and Dyn 1. (B) Concentration-dependent GTPase activity of Dyn3. GTPase activity was measured at 37 °C in a solution containing 45 mM NaCl, 20 mM Hepes (pH 7.5), 2 mM $MgCl_2$, and 1 mM GTP. Data points represent the averages of triplicate measurements of at least two preparations, and the error bars show the standard error of the mean. (C) Self-assembly of Dyn3 as measured by turbidity of 1 μM dynamin at 350 nm, 45 mM NaCl, and 37 °C.

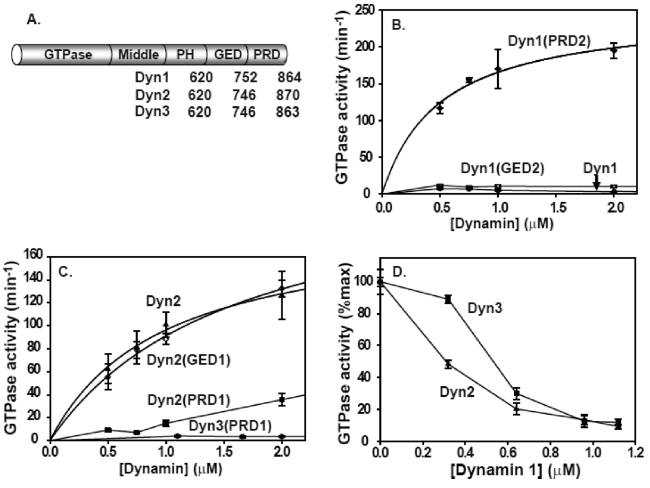

FIGURE 2: GTPase activities of dynamin constructs assayed as a function of dynamin concentration. (A) Schematic presentation of domain structure of dynamins indicating the residues of interchanged domains (see the Supporting Information). (B) Activity profiles of wild-type Dyn1, Dyn1 with the GED of Dyn2 [Dyn1(GED2)], and Dyn1 with the PRD of Dyn2 [Dyn1(PRD2)]. (C) Activity profile of wild-type Dyn2, Dyn2 with the GED of Dyn1 [Dyn2(GED2)], Dyn2 with the PRD of Dyn1 [Dyn2(PRD1)], and Dyn3 with the PRD of Dyn1 [Dyn3(PRD1)]. (D) Effect of Dyn1 on the GTPase activities of Dyn2 and Dyn3. Concentrations of Dyn2 and Dyn3 were 0.5 μM. GTPase activity was measured at 37 °C in a solution containing 45 mM NaCl, 20 mM Hepes (pH 7.5), 2 mM $MgCl_2$, and 1 mM GTP. Data points represent the averages of triplicate measurements of at least two preparations, and the error bars show the standard error of the mean.

Dyn2 while a construct consisting of Dyn2 with the GED and PRD of Dyn1 behaved like Dyn1 (data not shown). Thus, the PRD is a major determinant of the abilities of the three dynamins to self-activate. To verify that the hybrid dynamins were properly folded, we compared the PI(4,5)P_2-stimulated GTPase activities of the PRD-swapped mutants with those of wild-type dynamins. These assays were conducted at low dynamin concentrations (0.1 μM) to minimize self-activation of Dyn2 and Dyn3. All six dynamins expressed PI(4,5)P_2-activated GTPase activities of approximately 80−100 min^{-1} (Figure S2 of the Supporting Information), suggesting that the mutations did not alter protein conformation. The activities of constructs analyzed in this study are compiled in Table S1 of the Supporting Information.

We next asked whether Dyn1 interferes with the strong self-activating abilities of Dyn2 and Dyn3. Assays were performed on mixtures consisting of fixed (0.5 μM) concentrations of Dyn2 or Dyn3 and various concentrations of Dyn1. Figure 2D shows that the activities of both Dyn2 and Dyn3 were dramatically reduced as a function of Dyn1 concentration, indicating that the dynamin isoforms copolymerize and that the presence of Dyn1 in these copolymers has a negative effect on GTPase self-activation. These results suggest that the PRD of one dynamin in the polymer interacts with one or more domains of an adjacent dynamin, as Muhlberg et al. speculated previously (*18*).

Because concentration-dependent GTPase activation is tightly coupled to dynamin self-assembly, we asked whether the differential abilities of the dynamin isoforms to polymerize are also controlled by their PRDs. Using turbidity assays to monitor dynamin assembly, we found that Dyn2(PRD1) polymerized to approximately the same extent as wild-type Dyn2 (Figure 3A). However, Dyn2(PRD1) polymers consistently disassembled more rapidly and extensively than wild-type Dyn2 polymers upon addition of GTP (Figure 3A and Figure S3 of the Supporting Information) and were more susceptible to disassembly by increased ionic strengths (Figure S4 of the Supporting Information). The turbidity of Dyn1(PRD2) increased more markedly than that of wild-type Dyn1, and these polymers were even more resistant than Dyn2 polymers to GTP-induced disassembly. The latter observation may explain the high level of concentration-dependent GTPase activation of Dyn1(PRD2) relative to that of Dyn2 (Figure 2B,C). The Dyn3(PRD1) construct was essentially unable to self-assemble under our conditions (Figure 3A).

This study demonstrates the importance of the PRD in determining the weaker propensity of Dyn1 to self-assemble and to express concentration-dependent GTPase activation compared to that of Dyn2 and Dyn3. At present, there is insufficient information to provide a molecular basis for this finding, as no X-ray structures containing the PRD are available, and cryo-electron microscopy images have been obtained using truncated dynamins lacking the PRD (*20*).

Previous studies have indicated that the PRD is a positive regulator of Dyn1 assembly and self-activation. For example, Dyn1 polymers fail to form in vitro at low ionic strengths if the PRD is removed by limited proteolysis (*18*) or by introduction of a stop codon after the GED (*3*). Also, deletion of residues 792−841 from the PRD reduces the strength of Dyn1 self-interactions in a yeast two-hybrid assay (*21*). The PRD is also important for self-assembly and self-activation of Dyn2 (*3*) (see also Figure S5A,B of the Supporting Information). To reconcile these earlier observations with our current results, we suggest that whereas the PRD is necessary to initiate self-assembly of all three forms of dynamin, the Dyn1 PRD is less effective than the PRDs of Dyn2 or Dyn3 in stabilizing dynamin polymers once they are formed. Recent evidence demonstrates that membrane fission is likely to occur concomitantly with dynamin disassembly (*4*, *5*).

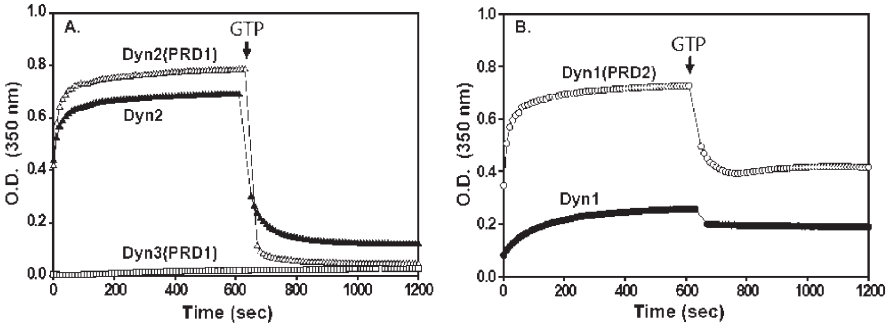

FIGURE 3: Polymerization of dynamin constructs. (A) Absorbance at 350 nm of solutions containing wild-type Dyn2, Dyn2 with the PRD of Dyn1 [Dyn2(PRD1)], and Dyn3 with the PRD of Dyn1 [Dyn3(PRD1)]. (B) Absorbance at 350 nm of solutions containing wild-type Dyn1 and Dyn1 with the PRD of Dyn2 [Dyn1(PRD2)]. Turbidity was measured at 1 μM dynamin at 37 °C upon reduction of the NaCl concentration from 300 to 45 mM. After the turbidity had reached a plateau, 50 mM GTP and 100 mM $MgCl_2$ were added (arrows) to produce final concentrations of 1 mM GTP and 2 mM $MgCl_2$.

Therefore, PRD-dependent destabilization of Dyn1 polymers may contribute to the rapidity of synaptic vesicle retrieval in neurons, which is not an essential feature of Dyn2-catalyzed receptor-mediated endocytosis (*22*). Interestingly, point mutations in the Dyn2 middle and PH domains of centronuclear myopathy patients were found to enhance Dyn2 polymerization and GTPase activity (*23*, *24*), further highlighting the importance of properly controlled dynamin disassembly.

Replacement of the Dyn2 PRD with that of Dyn1 profoundly reduced the GTPase activity of Dyn2 without affecting its ability to self-assemble, demonstrating that dynamin polymerization alone is insufficient to promote catalytic activation. In support of this conclusion, Dyn1 polymers formed at high dynamin concentrations and very low ionic strengths were also shown to express low GTPase activity ($\sim$10 min^{-1}) (*13*). In contrast, assembly of Dyn1 on microtubules or anionic liposomes elicited GTPase activities of $>$200 min^{-1} (*12*). We suggest that these microtubule and liposome scaffolds stabilize dynamin polymers, overcoming the destabilization caused by the presence of the Dyn1 PRD and allowing multiple cycles of the GTPase reaction to occur prior to polymer disassembly.

SUPPORTING INFORMATION AVAILABLE

Supplementary methods, Figures S1−S5, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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