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A Pseudoatomic Model of the Dynamin Polymer Identifies a Hydrolysis-Dependent Powerstroke

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SUMMARY

The GTPase dynamin catalyzes membrane fission by forming a collar around the necks of clathrin-coated pits, but the specific structural interactions and conformational changes that drive this process remain a mystery. We present the GMPPCP-bound structures of the truncated human dynamin 1 helical polymer at 12.2 Å and a fusion protein, GG, linking human dynamin 1's catalytic G domain to its GTPase effector domain (GED) at 2.2 Å. The structures reveal the position and connectivity of dynamin fragments in the assembled structure, showing that G domain dimers only form between tetramers in sequential rungs of the dynamin helix. Using chemical crosslinking, we demonstrate that dynamin tetramers are made of two dimers, in which the G domain of one molecule interacts in *trans* with the GED of another. Structural comparison of GG_{GMPPCP} to the GG transition-state complex identifies a hydrolysis-dependent powerstroke that may play a role in membrane-remodeling events necessary for fission.

INTRODUCTION

Clathrin-mediated endocytosis (CME) is a highly regulated pathway wherein nutrients, growth factors, and macromolecules are concentrated in invaginating clathrin-coated pits (CCPs) that pinch off to form vesicles to carry these cargo into the cell (McMahon and Boucrot, 2011). The large, multidomain GTPase dynamin assembles into collars at the necks of deeply invaginated CCPs to catalyze membrane fission in the final stages of CME (Mettlen et al., 2009; Schmid and Frolov, 2011).

Purified dynamin exists as a tetramer (Muhlberg et al., 1997) that can self-assemble into helical structures reminiscent of collars observed in vivo (Hinshaw and Schmid, 1995). Dynamin encodes five domains (Figure S2A available online): a catalytic G domain; a middle domain involved in self-assembly and oligomerization; a membrane-binding pleckstrin homology (PH) domain; a GTPase effector domain (GED); and a C-terminal proline- and arginine-rich domain (PRD) that binds SH3 domains of accessory proteins important for CME (Praefcke and McMahon, 2004; Mettlen et al., 2009) but is not essential for GTPase activities or oligomerization in vitro (Muhlberg et al., 1997). Aside from the PRD, structures of all of dynamin's individual domains or their homologs have been solved by crystallography (Figure S2A). These include the human dynamin 1 PH domain (Ferguson et al., 1994; Timm et al., 1994), the G domains of rat dynamin (Reubold et al., 2005) and dictyostelium dynamin A (Niemann et al., 2001), the middle domain and GED of the related interferon-induced GTPase MxA (Gao et al., 2010), and a fusion linking the C terminus of human dynamin 1's GED (C_{GED}) to its G domain (GG) (Chappie et al., 2010). Crystallographic and biochemical studies have shown that the C_{GED} forms a three-helix bundle with the N and C termini of the G domain (N_{GTPase} and C_{GTPase}, respectively) (Figure S2B) and that this module—the bundle-signaling element (BSE)—transmits the conformational changes associated with dynamin assembly to the G domain (Chappie et al., 2009, 2010). However, as the BSE was structurally characterized in the context of the GG fusion, it is not known whether C_{GED}'s interaction with the G domain occurs in *cis* within the same polypeptide or in *trans* via another polypeptide in the dynamin tetramer.

Dynamin has a low affinity for guanine nucleotides (10–100 μM) and a high basal turnover (~0.4–1 min^{-1}) (Praefcke and McMahon, 2004). Assembly into helical oligomers stimulates dynamin's basal GTPase activity >100-fold (Warnock et al., 1996; Stowell et al., 1999). This enhancement arises from G domain dimerization, which optimally positions dynamin's catalytic machinery and stabilizes conformationally flexible switch regions (Chappie et al., 2010). Mutations that impair GTP binding, assembly, or stimulated GTP hydrolysis also cause defects in endocytic uptake in vivo (reviewed in Schmid and Frolov, 2011), thus establishing the importance of dynamin's GTPase activities in CME.

Despite its essential role in CME, the mechanism of dynamin-catalyzed membrane fission remains poorly understood. Efforts

to recapitulate these activities in vitro using synthetic membranes suggested that dynamin functions as a mechanochemical enzyme that actively severs the membrane via hydrolysis-dependent conformational changes (Sweitzer and Hinshaw, 1998; Stowell et al., 1999; Chen et al., 2004; Mears et al., 2007; Roux et al., 2006) that generate a constricted neck and impose strain on the membrane lipids (Bashkirov et al., 2008; Roux et al., 2010). GTP hydrolysis also promotes partial dissociation of dynamin subunits from membranes (Danino et al., 2004; Ramachandran and Schmid, 2008; Pucadyil and Schmid, 2008; Bashkirov et al., 2008). Loosening of the dynamin scaffold could allow local lipid rearrangements and an energetically favorable hemifission intermediate that promotes nonleaky membrane scission (Bashkirov et al., 2008; Schmid and Frolov, 2011). The hydrolysis-dependent conformational changes that trigger these membrane-remodeling events have yet to be elucidated.

Unraveling the mechanisms governing dynamin-catalyzed membrane fission requires a detailed structural understanding of the architecture of assembled dynamin and the conformational changes induced by stimulated GTP hydrolysis. Dynamin's propensity to form helical arrays in vitro has previously been exploited for cryo-electron microscopy (cryo-EM) structure determination. Three-dimensional reconstructions of truncated dynamin 1 (ΔPRD, Figure S2A) polymers assembled on anionic lipid scaffolds have been obtained both in the absence of nucleotides (Chen et al., 2004) and in the presence of the nonhydrolyzable GTP analog GMPPCP (Zhang and Hinshaw, 2001). In both cases, the asymmetric unit of assembly is a dimer that adopts a T shape when viewed in cross-section ("T view"). The structural differences between these maps suggest that rearrangements in the middle domain and GED mediate a nucleotide-dependent constriction of the ΔPRD assembly (Chen et al., 2004). Constriction alone, however, is not sufficient for membrane fission (Ramachandran and Schmid, 2008; Bashkirov et al., 2008), suggesting that additional conformational changes are required. Although it has been inferred that the middle domain and GED form a coiled-coil "stalk" that connects the PH domain "leg" to the G domain "head" (Zhang and Hinshaw, 2001; Chen et al., 2004), neither the organization nor their connectivity in the polymer is known, owing to the low resolution (>20 Å) of the ΔPRD reconstructions and the lack of a complete, atomic-resolution dynamin structure. These limitations have also hindered our understanding of how assembly promotes G domain dimerization, leading to stimulated GTP hydrolysis and membrane fission. To address these issues, we have used cryo-EM to extend the resolution of the constricted ΔPRD polymer map and employed computational docking and biochemistry to define the underlying subunit interactions. We also present the crystal structure of GG in complex with GMPPCP, which identifies a major hydrolysis-dependent BSE conformational change. Our results provide insights into how dynamin assembly directly facilitates G domain dimerization and stimulated turnover and suggest how the energy of this dimerization and GTP hydrolysis can be converted into large structural movements that may play a role in precipitating membrane fission.

RESULTS

12.2 Å Cryo-EM Reconstruction of ΔPRD in the Constricted State Reveals Additional Structural Features of the Assembled Dynamin Polymer

Our initial attempt to characterize GMPPCP-bound, constricted ΔPRD tubes using cryo-EM and Fourier-Bessel synthesis produced an 18 Å resolution reconstruction (Wilson-Kubalek et al., 2010) that displayed only minor differences compared to previously published structures (Zhang and Hinshaw, 2001; Chen et al., 2004; Wilson-Kubalek et al., 2010). The resolution was limited by variations in the tube diameter, which produced long-range disorder and diminished the overall diffracting power. To circumvent this, we segmented the tubes into individual, overlapping particles that were then aligned, classified, sorted, and averaged with the iterative helical real-space reconstruction (IHRSR) algorithm (Egelman, 2007) (Figure S1A–S1C). This single-particle-based approach produced a 12.2 Å helical map (Figure 1A; Figure S1D) that has an inner lumenal diameter of 7 nm, an outer diameter of 40 nm, 13.2 subunits per turn, and a pitch of 99.3 Å. The improved resolution reveals additional structural features of the ΔPRD polymer. First, the stalk density, which constitutes the base of the characteristic "T view" (Figure 1B; Movie S1), appears to twist in a crisscross fashion (Figures 1B and 1C), intersecting just below the cleft that separates the "head" density regions along the exterior of the polymer. Second, there are two additional strips of density within the cleft that wrap around the tube (Figure 1D, highlighted with dashed boxes). Each strip forms a continuous connection with the alternating head densities of a single helical rung.

Docking of Crystallized Dynamin Fragments Illustrates Ambiguities in Structural Models

To decipher the subunit organization of the dynamin polymer, we docked the crystal structures of the GDP.AlF_4^--stabilized GG dimer ($GG_{GDP.AlF4-}$; PDB 2X2E), the human MxA middle/GED stalk (PDB 3LJB), and the human dynamin 1 PH domain (PDB 1DYN) into our improved ΔPRD reconstruction (Figure 2A). The MxA stalk structure shares a high degree of sequence homology (19.5% identical, 54.9% similar) with dynamin's middle domain and GED (Data S1) and currently represents the best structural model for these domains. Attempts to dock $GG_{GDP.AlF4-}$ as a dimer failed as one monomer always grossly protruded from the density, regardless of its orientation (Figure S3A). The $GG_{GDP.AlF4-}$ dimer from an alternate crystal form (PDB 2X2F) exhibited the same discrepancies (data not shown). We therefore selected only one monomer for docking (monomer A from PDB 2X2E), which allowed more degrees of freedom during the fitting procedures. We similarly positioned the MxA stalks individually, as the crystallized assembly could only be fit into a previously published 23 Å ΔPRD map after a significant rotation between adjacent pairs of monomers (Gao et al., 2010). Fitting was carried out using YUP (Tan et al., 2006, 2008) as described in the Experimental Procedures. In total, 8 GG monomers, 12 MxA monomers, and 8 PH domains were positioned into the cryo-EM density. In agreement with previous biochemical data and structural modeling (Chen et al., 2004; Mears et al., 2007), the PH domain is situated in the "leg" density adjacent to the