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Mini Review

From membranes to organelles: Emerging roles for dynamin-like proteins in diverse cellular processes

Michelle Williams, Kyoungtae Kim*

Department of Biology, Missouri State University, 901 South National, Springfield, MO 65897, United States

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ABSTRACT

Dynamin is a GTPase mechanoenzyme most noted for its role in vesicle scission during endocytosis, and belongs to the dynamin family proteins. The dynamin family consists of classical dynamins and dynamin-like proteins (DLPs). Due to structural and functional similarities DLPs are thought to carry out membrane tubulation and scission in a similar manner to dynamin. Here, we discuss the newly emerging roles for DLPs, which include vacuole fission and fusion, peroxisome maintenance, endocytosis and intracellular trafficking. Specific focus is given to the role of DLPs in the budding yeast *Saccharomyces cerevisiae* because the diverse function of DLPs has been well characterized in this organism. Recent insights into DLPs may provide a better understanding of mammalian dynamin and its associated diseases.

Dynamin-like protein family

Members of the dynamin family are GTPases found in a broad range of eukaryotic and prokaryotic organisms (Burmann et al., 2012; Ferguson and De Camilli, 2012). Based on structural and functional similarities, the dynamin family proteins are divided into classical dynamins and dynamin-related proteins (DRPs) or dynamin-like proteins (DLPs). Classical dynamins contain five functionally distinct domains (Fig. 1) (Ramachandran, 2011). The N-terminal GTPase domain functions in GTP binding and hydrolysis (Chappie et al., 2010). The middle domain makes up part of the stalk in structure-based models of dynamin. The Pleckstrin homology (PH) domain is responsible for lipid binding and targeting of the protein to membranes. Mutations in the middle and PH domain are linked to human diseases such as centronuclear myopathy and Charcot-Marie-Tooth neuropathy (Gonzalez-Jamett et al., 2013). The GTPase effector domain (GED) contains a bundle signaling element (BSE), which is able to interact with the stalk and GTPase domain of the neighnoring dynamin molocule, and has an inhibitory effect on the GTPase domain (Faelber et al., 2011), and the C-terminal proline-rich domain (PRD) for mediating protein–protein interactions with accessory proteins containing Src-homology 3 (SH3) domains. DLPs contain three of these five domains: the N-terminal GTPase domain, the middle domain, and the C-terminal GED (Smaczynska-de et al., 2012; van der Bliek, 1999; Vater et al., 1992).

Revelation of the structure of a classical dynamin protein has helped decipher the mechanism by which dynamin self-assembly and GTP hydrolysis may induce membrane scission (Chappie et al., 2010; Faelber et al., 2011; Ford et al., 2011). Dynamin oligomerizes into helical collars around the necks of clathrin-coated vesicles during the late stage of endocytosis; this process occurs *via* hydrophobic interactions between the stalks of dynmain proteins. The oligomeric structure of dynamin undergoes a major conformational change in a GTP-hydrolysis dependent manner, which causes constriction of the underlying membrane, and subsequently, scission occurs (for review see Chappie and Dyda, 2013). DLPs are predicted to carry out membrane scission in a similar manner to the model of classical dynamins mentioned above. Three DLPs exist in the budding yeast *Saccharomyces cerevisiae*: mitochondrial genome maintenance (Mgm1), dynamin-related GTPase (Dnm1) and vacuolar protein sorting (Vps1). Mgm1 and Dnm1 function in mitochondrial fusion and fission, respectively (Abutbul-Ionita et al., 2012; Bleazard et al., 1999; Mears et al., 2011; Sesaki et al., 2003; Wong et al., 2000), while Vps1 is implicated in scission of numerous cellular membranes.

In mitochondria, membrane fission and fusion require distinct machinery. Yeast DLP Mgm1 is a mitochondrial inner membrane protein that utilizes GTPase activity for inner membrane fusion, and the formation and maintenance of cristae (Meglei and McQuibban,

* Corresponding author. Tel.: +1 417 836 5440; fax: +1 417 8365126.
E-mail address: kkim@missouristate.edu (K. Kim).

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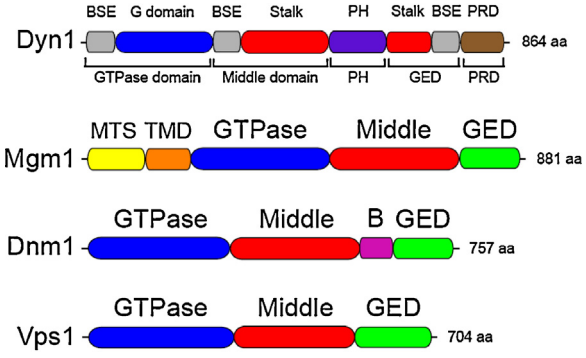

Fig. 1. Domain structure of dynamin and the three yeast DLPs. The lengths of human dynamin and the yeast DLPs are shown on the right as the number of amino acids. The classical five domain assignment of human dynamin 1 (dyn1) is indicated by brackets, including the GTPase domain, middle domain, Pleckstrin homology (PH) domain, GTP effector domain (GED), and proline-rich domain (PRD). Recent structural studies suggest that the bundle signaling element (BSE), which is found in part of the GTPase domain, middle domain and GED domain assemble together and are able to promote self-assembly, and inhibit the GTPase activity of dynamin. Similarly, the stalk domain, which is found partially in the middle and GED domains come together to mediate dynamin dimer formation and oligomerization. The yeast DLPs including Vps1 have a GTPase, middle, and GED domain. In addition, Mgm1 contains a mitochondrial targeting sequence (MTS) that confers mitochondrial localization of Mgm1, and a transmembrane domain (TMD), which anchors Mgm1 in the inner membrane of the mitochondria. Dnm1 contains a B insert (B) domain that binds to the mitochondrial adaptor protein Mdv1.

2009). Mgm1 is homologous to a mammalian DLP Opa1 (Alexander et al., 2000). Mutations in Opa1 lead to dominant optic atrophy, and further studies of yeast Mgm1 may provide better understanding of this disease (Alavi and Fuhrmann, 2013). Another yeast DLP Dnm1 has been shown to regulate mitochondrial fission (Bleazard et al., 1999; Mears et al., 2011). Dnm1 is homologous to another mamalian DLP, Dlp1, which is also implicated in mitochondrial fission. Loss of Dnm1 affects the morphology of mitochondria but not other organelles, and Dnm1 has been localized to the mitochondria using fluorescence microscopy (Otsuga et al., 1998). Dnm1 has mainly been studied in regards to its function in mitochondrial fission, but in this review we discuss additional roles of Dnm1 on other organelles.

An additional yeast DLP, Vps1, shares 45% sequence identity to dynamin (Obar et al., 1990), and is a multifunctional and multi-localized protein that works collaboratively with numerous proteins to carry out membrane remodeling events within the cell. The Vps1 gene encodes an 80 kDa protein (Rothman et al., 1986, 1990) that was originally named Vpl1 (vacuolar protein localization) (Rothman and Stevens, 1986). At one point this protein was also named Vpt26 (vacuolar protein targeting) (Banta et al., 1988), but collaboratively, researchers decided upon the common designation VPS (vacuolar protein sorting) (Rothman et al., 1989). The vacuolar protein sorting function of Vps1 was identified during a screen for yeast mutants that failed to properly deliver Carboxypeptidase Y (CPY) and Proteinase A (PrA) to the vacuole (Rothman and Stevens, 1986). Normally, CPY is synthesized as an inactive zymogen in the endoplasmic reticulum (ER), where it is modified to become p1-CPY (Hasilik and Tanner, 1978). P1-CPY is then transported to the Golgi, where it undergoes further modification to become p2-CPY to be targeted to the vacuole. and is then targeted to the vacuole. In the vacuole, p2-CPY is cleaved to become the active vacuolar hydrolase, mature-CPY (Conibear and Stevens, 1995). In cells lacking Vps1, CPY is secreted out of the cell *via* an intact secretory pathway (Rothman and Stevens, 1986). In addition, cells lacking Vps1 missort Golgi proteins. For example, Kex2, which normally shuttles between endosomes and the Golgi, is redirected to the plasma membrane in *vps1*Δ cells and reaches the vacuole only after being endocytosed (Nothwehr et al., 1995). Since then for almost a decade, the study of Vps1 had focused on the Golgi-to-late endosome traffic. However, in the last ten years our understanding regarding the cellular localization and function of Vps1 has greatly expanded. In the present review, we discuss the most recent findings of the function of Vps1 and other DLPs during vacuolar fission and fusion, peroxisome maintenance, endocytosis and intracellular trafficking.

DLP-mediated mitochondrial fusion and fission

Mitochondrial fission is necessary to generate smaller mitochondrial compartments that can be efficiently inherited to daughter cells or degraded during mitophagy (Cerveny et al., 2007; Mao and Klionsky, 2013). In yeast, Dnm1 localizes to mitochondria and plays a major role in mitochondrial fission. Cells lacking Dnm1 display a net-like array of mitochondrial tubules due to a defect in division (Bleazard et al., 1999; Sesaki and Jensen, 1999). Dnm1 is recruited to the mitochondrial membrane by the mitochondrial integral membrane protein Fis1, and the adaptor proteins Mdv1 and Caf4 (for review see Bui and Shaw, 2013). A region between the middle and GED domain of Dnm1 called InsB is thought to facillitate binding of Dnm1 with Mdv1 (Fig. 1) (Bui et al., 2012). Dnm1 is able to tubulate liposiomes *in vitro*, and is thought to cause membrane scission similar to dynamin (Ingerman et al., 2005; Mears et al., 2011). Cryo-electron microscopy analysis of Dnm1-bound lipid tubules provides insight into the three-dimensional structure of GTP-bound Dnm1 oligomers. Dnm1 displays more flexibility than classical dynamin because once recruited to the membrnae, Dnm1 was able to assemble around lipid tubules with a wide range of diameters. Upon addition of GTP, a major conformational change occurs, and the diameter of the tubules decreased by 50 nm, compared to dynmain, which is known to induce a 10 nm constriction. After constriction of the underling membrane, Dnm1 rapidly dissociates from the lipid tubule. Altogether, these data provide insight into the mechanism of DLP scission, which is comparable to dynamin, and may be widely applicable to other DLPs (Mears et al., 2011).

Mitochondrial fusion is important for maintintinence of the mitochondrial network, and promotes cell lifespan. Mgm1 is a DLP found in the intermembrane space of the mitochondria and functions in inner membrnae fusion (Wong et al., 2000). In addition to the three domains characteristic of DLPs, Mgm1 also conatins a an N-terminal mitochondrial tageting secuence, and a hydrophobic transmembrane anchor (Fig. 1). Mgm1 has two isoforms, the larger of which is inserted into the inner membrane, and the smaller isoform is generated by cleavage of the hydrophobic membrane anchor by the serine protease Pcp1, and is freely found in the intermembrane space (Herlan et al., 2003; Zick et al., 2009). An *in vitro* mitochondrial mixing assay provided direct evidence for Mgm1 function in not only mitochondrial fusion but also inner membrane tethering and cristae maintenance (Meeusen et al., 2006). In addition, Mgm1 is able to bind and aggregate liposomes *in vitro* (Rujiviphat et al., 2009). Similar to other DLPs, Mgm1-mediated fusion is dependent on conformational changes induced by binding GTP (Abutbul-Ionita et al., 2012; Meglei and McQuibban, 2009). Mgm1-mediated fusion is aided by two mitochondrial outer membrane proteins Fzo1, a GTPase mitofusion protien, and Ugo1, a multipass outer membrane transmembrane protein that links Fzo1 to Mgm1 (Hermann et al., 1998; Sesaki and Jensen, 2004). Together, Mgm1, Fzo1 and Ugo1 carry out mitochondria outer and inner membrane fusion.

The mitochondrial network is continually sustained by fusion and fission reactions that depend on the conserved proteins Mgm1/Opa1 and Dnm1/Dlp1 in yeast and mammals, respectivly. Further characterization of the structures and functions of DLPs will enhance our understanding of the mechanism underlying