Mitochondria-specific Function of the Dynamin Family Protein DLP1 Is Mediated by Its C-terminal Domains*

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Kelly R. Pitts‡§, Mark A. McNiven‡, and Yisang Yoon¶

From the ‡Center for Basic Research in Digestive Diseases, Mayo Clinic and Foundation, Rochester, Minnesota 55905 and the ¶Departments of Anesthesiology and Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

The mammalian dynamin-like protein DLP1/Drp1 belongs to the dynamin family of large GTPases shown to mediate membrane remodeling during a variety of cellular processes (1). Whereas conventional dynamin is believed to play an important role in the constriction and severing of nascent vesicles at endocytic (2–6) and secretory membrane compartments (7–9), DLP1 and its yeast homologue Dnm1p has been demonstrated to participate in the fission of mitochondria (10, 11) and, more recently, peroxisomes (12, 13). The similarity in biological activity of these two related but distinct proteins is reflected in the similarity of their domain structures. DLP1 and dynamin contain three highly conserved GTP binding elements (3, 4, 14). The middle region (MID)
3 domains are the least characterized of all the dynamin domains, although several studies suggest that it is involved in self-assembly (15, 16). The MID domains are followed by a divergent stretch of ~100 amino acids that, in dynamin, has significant structural homology to pleckstrin. This pleckstrin homology (PH) domain is known to bind phosphatidylinositol 4,5-bisphosphate (17–19) and G-protein βγ subunits (20, 21), facilitating the interaction of dynamin with the plasma membrane. We refer to the correlate region in DLP1 as the unconserved (UC) domain, as it shows little homology (18%) to the dynamin PH domain. Currently, the function of the UC domain is undefined. The PH/UC domains are followed by a region predicted to form a coiled-coil (CC). The CC domain of dynamin is often referred to as the assembly domain, as it is required for dynamin oligomerization and binds both the GTPase and MID domains (15, 16). Because dynamin self-assembly leads to GTPase activation (22, 23), this domain is also referred to as the GED (GTPase effector domain). The CC domain of DLP1 has been shown to bind the DLP1 MID domain (24), indicating that dynamin and DLP1 share common self-assembly mechanisms. Whereas it is unclear whether self-assembly of DLP1 stimulates its GTPase activity, a mutation in the CC domain of Dnm1p was shown to enhance mitochondrial fission by potentially favoring the GTP-bound state of Dnm1p (25). This suggests that the DLP1 CC domain may function in a similar way to that of dynamin, mainly, GTPase regulation. A major distinguishing feature between DLP1 and conventional dynamins is the presence of a C-terminal tail of ~100 proline-rich amino acids in dynamin termed the PRD. This domain has been implicated in targeting dynamin to target membranes via an interaction with a variety of SH3 domain-containing proteins (26–32).

Because of the parallels in structure and organization between DLP and dynamin, it is not surprising that in vitro studies have demonstrated that these proteins possess similar enzymatic and physiological properties. Both are known to self-assemble and constrict membranes in vitro (33–36). Furthermore, these proteins assemble into helical ring structures of a defined dimension (36, 37) that constrict upon GTP hydrolysis (33) resulting in a substantial reduction of the internal dimensions of the helical polymer leading to vesicle scission.

Because DLP1 and the conventional dynamins function at different cellular organelles, it is assumed that these related GTPases contain intrinsically distinct targeting information. As mentioned above, the PH and PRD regions of dynamin are crit-

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‡ Current address: Inflammation Research, Axygen, Inc., 1201 Amgen Court West, Seattle, WA 98119.

§ To whom correspondence should be addressed: 601 Elmwood Ave., Box 604, Rochester, NY 14642. Tel.: 585-275-3856; Fax: 585-244-7271; E-mail: yisang_yoon@urmc.rochester.edu.

¶ The abbreviations used are: MID, middle region; PH, pleckstrin homology; UC, unconserved domain; CC, coiled-coil domain; GFP, green fluorescent protein.
for protein function and targeting, yet DLP1 is missing these domains. Thus, how DLP1 is targeted to, and functions at, mitochondria is undefined. To this end, we exploited the similarities between DLP1 and dynamin domain structures and generated chimeric proteins in which domain(s) of DLP1 were substituted with those of dynamin. We then tested which of these chimeras displayed DLP1-specific mitochondrial function and targeting. Similar chimeric systems have been extensively and successfully used to assess the contributions of individual domains to the structure and function of other motor proteins (38–43). Through transient expression of these chimeras in cultured mammalian cells, combined with fluorescence microscopy and immunoprecipitation, we report that the mitochondrial specificity for DLP1 resides in three consecutive MID, UC, and CC domains. Ablation of any one of these domains resulted in an altered cellular distribution of DLP1 and failed to confer mitochondrial specific function. We also found that the CC domain of DLP1 alone targets exclusively to mitochondria, suggesting a mechanism by which DLP1 is localized to this organelle in vivo. Interestingly, the mitochondrial targeting information within the DLP1 CC domain is suppressed when placed in a dynamin background, although its ability to function as an assembly domain is not compromised. These data suggest that whereas the GTPase domain of DLP1 provides an enzymatic function, the other three domains contain information for organelle targeting and intramolecular interactions.

EXPERIMENTAL PROCEDURES

Generation of Constructs—Chimeric constructs were generated by standard PCR using the Expand High Fidelity PCR System (Roche). A battery of restriction site-containing primers was synthesized with specificity to either DLP1 (DD1 or DD2) or Dyn2ab (DD2), such that any domain or series of domains could be amplified by PCR using the proper DLP1 or Dyn2ab template. PCR products were purified (Qiagen, Hilden, Germany), digested with the proper restriction enzyme, and ligated into the pEGFP-C1 (Clontech, Palo Alto, CA). The validity of each construct was confirmed by DNA sequencing at the Mayo Molecular Biology Core Facility. In some cases, the resulting construct was used as a second or third generation vector for subsequent cloning events leading to a full-length chimera.

Cell Culture and Transfection—Cell lines were maintained at 37 °C, 5% CO2 in Ham's F-12K medium for Clone 9 (ATCC CRL-1439) and in Dulbecco's modified Eagle's medium for BHK-21 (ATCC CCL-10) and COS-7 (ATCC CRL-1651) cells, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT). DNA constructs were purified using NucleoBond columns (Qiagen, Hilden, Germany). Cells were plated 16–24 h before transfection. Transfections were performed by using LipofectAMINE® (Invitrogen) per the manufacturer's instructions. Transfected cells were allowed to recover for 16 to 24 h before processing for immunofluorescence or immunoprecipitation.

Immunofluorescence—Immunofluorescence was performed as described elsewhere (11). Briefly, cells were fixed in 2.5% formaldehyde and permeabilized with phosphate-buffered saline containing 0.1% Triton X-100. After incubation in a blocking buffer containing 5% goat serum, cells were incubated with the appropriate primary antibodies for 1 h at 37 °C. Following a wash with phosphate-buffered saline, cells were incubated with the appropriate secondary antibodies for 1 h at room temperature. Cells were rinsed three times with phosphate-buffered saline and then rinsed in phosphate-buffered saline, fixed, and mounted for fluorescence microscopy. Cells were viewed with a Plan-Appo ×63 lens on an Axiovert epifluorescence microscope (Carl Zeiss Inc.) and transferrin uptake was qualitatively assessed. Representative images selected from three independent experiments for each construct are shown.

RESULTS

Chimera Construction—Both DLP1 and dynamin oligomerize on the membrane surface and, upon GTP hydrolysis, sever membranes of different cellular organelles. Despite the significant similarity in domain structures and physiological properties, DLP1 and dynamin assemble separately to function in distinct cellular processes. The multimeric nature of DLP1 and dynamin has been useful for the study of these molecules as overexpressed GTPase-defective mutants can co-assemble with normal endogenous proteins to confer a dominant-negative effect. The lysine to alanine mutation in the first GTP binding element in both DLP1 (K38A) and dynamin (Dyn2K44A) results in a loss of GTPase activity and greatly reduced binding to GTP (3, 4, 36). Overexpression of this mutant DLP1 protein induces a fission-defective mitochondrial phenotype with cells displaying elongated and entangled mitochondria (Fig. 1) (11). We tested whether this mutant protein, when expressed in cells, co-assembles with normal endogenous DLP1 protein by co-immunoprecipitation. Cells were transfected with a GFP-tagged DLP1-K38A (GFP-DLP1-K38A) construct, and immunoprecipitation was performed using anti-GFP antibodies. Endogenous DLP1 was detected in the anti-GFP immunoprecipitates (Fig. 1B), demonstrating that the dominant-negative effect of GFP-DLP1-K38A was because of the formation of nonfunctional oligomers of DLP1. Taking advantage of this dominant-negative mechanism, we tested whether there were domains in DLP1 that rendered a DLP1-specific function using DLP1/dynamin chimeras bearing the GTPase-defective mutation (K38A for DLP1 and K44A for dynamin; Fig. 1C). The dominant-negative effect of K38A or K44A provides a functional assay for determining DLP1- or dynamin-specific function of a given chimeric construct. For example, if cells overexpressing a chimera exhibit the fission-defective mitochondrial phenotype, this chimera is considered to contain elements for DLP1-specific function. To facilitate testing the ability of mutant chimeric or truncated proteins to co-assemble with endogenous protein, all constructs were tagged with GFP (Fig. 1C), and immunoprecipitations were performed using anti-GFP antibodies.

C-terminal Domains Are Necessary for Mutant DLP1-induced Mitochondrial Phenotype.—To identify domain(s) that provide DLP1-specific function, we initially made two chimeric proteins in which either the N-terminal half (i.e. the GTPase-MID segment) or C-terminal half (i.e. the UC-CC segment) of DLP1 was replaced with the corresponding domains of dynamin (Fig. 1C, chimeras 1 and 2). Cells expressing either chimera exhibited normal mitochondrial morphology when
compared with mitochondria in adjacent cells not expressing any transgene (Fig. 2, A and B), suggesting that neither the GTPase-MID nor UC-CC segments of DLP1 in a dynamin background were capable of invoking a mitochondrial phenotype. As no dominant-negative effects were observed with these chimeras, we predicted that they were unable to integrate with and disrupt endogenous DLP1 function. We transfected cells with each chimeric construct and performed immunoprecipitations using anti-GFP antibodies. As shown in Fig. 2, the chimeric proteins failed to co-precipitate endogenous DLP1, indicating that these chimeras do not co-assemble with DLP1.

Because chimeras containing either the GTPase-MID or UC-CC segments of DLP1 were unable to confer a mutant phenotype on host cells, we hypothesized that DLP1-specific information might be shared across additional domains. To test these possibilities, a series of GFP-tagged, mutant DLP1 truncation constructs was generated (Fig. 1C, truncations 1–3). Expression of an N-terminal segment containing the DLP1 mutant GTPase and MID domains (truncation 1) had no effect on mitochondrial morphology (Fig. 4A), suggesting that the MID domain alone was not sufficient to serve as a DLP1 determinant. As expected, this truncated protein failed to co-assemble with endogenous DLP1 in immunoprecipitation experiments (Fig. 4A). Also, expression of the DLP1 mutant GTPase, MID, and UC domains (truncation 2) failed to confer a mitochondrial phenotype on cells harboring the transgene, nor did this truncated protein co-assemble with endogenous DLP1 (Fig. 4B). The absence of
the mitochondrial phenotype and co-assembly in cells expressing these truncated DLP1 was not because of the reduced stability of the truncated proteins because similar levels of the truncated forms of DLP1 were detected in cell homogenates and immunoprecipitated materials as shown in Fig. 4. These data suggest that any information intrinsic to the MID and UC domains is insufficient to affect DLP1 function at the mitochondria. Furthermore, these data suggest that the CC domain is required to elicit the mitochondrial phenotype associated with expression of mutant DLP1. To confirm these hypotheses, we deleted the GTPase domain of DLP1 (truncation 3) and tested for co-assembly and a dominant-negative phenotype. As shown in Fig. 4, expression of the MID-UC-CC segment alone was able to disrupt normal mitochondrial morphology. Immunoprecipitation confirmed that this segment co-assembled with endogenous DLP1 within cells. These data indicate that first, the truncated forms of DLP1 were expressed at similar levels and were intact over the course of the experiments, and second, the MID-UC-CC region contains all the necessary information for intermolecular interaction of DLP1, whereas the GTPase domain provides enzymatic function to the molecule. Also, the CC domain of DLP1 appears to have crucial information for DLP1-specific function, as simple removal (Fig. 4, A and B) or exchange (Fig. 3A) of this domain abolished the dominant-negative effect.

**The Coiled-coil Domain of DLP1 Targets Specifically to Mitochondria**—Because the MID-UC-CC segment of DLP1 is capable of oligomerizing with native DLP1 in the absence of the GTPase domain, we next tested the individual contributions of the MID, UC, and CC domains to DLP1 function by expressing each domain separately and examining the localization and effects on mitochondrial morphology. The MID, UC, and CC domains were tagged individually with a Myc epitope at the C termini (Fig. 1C, MID-Myc, UC-Myc, and CC-Myc) and then ectopically expressed in cultured cells. We found that the MID and UC domains were distributed to the cytosol, showing diffuse localization (Fig. 1C). In addition, mitochondrial morphology was normal in cells overexpressing either the MID or UC domains alone (results not shown). Most notably, however, we found that the CC domain of DLP1 exhibited a punctate, but
organized, distribution seemingly along mitochondria. Expression of the CC domain in cells carrying RFP-labeled mitochondria confirmed that the CC domain localized to these organelles (Fig. 5A). In addition, the distribution of the CC domain alone appeared different from that of endogenous DLP1. As indicated in previous reports, the distribution of DLP1 within cells is heterogeneous, with the majority of DLP1 distributed to the cytosol and subpopulations localized to microtubules, mitochondria, the endoplasmic reticulum, and peroxisomes (10, 11, 13, 44). However, we found that the ectopically expressed DLP1 CC domain was exclusively localized to mitochondria. Furthermore, endogenous DLP1 and expressed CC domain were not co-localized, clearly occupying distinct spots on mitochondria (Fig. 5B). This indicates that the mitochondrial localization of the CC domain is not via binding to the existing DLP1 on mitochondria. Rather, it is likely that the CC domain itself has intrinsic information for mitochondrial binding. In support of a role for the CC domain in mitochondrial targeting, deletion of the CC domain from the wild-type DLP1 (Fig. 1C, trunc 4) resulted in loss of punctate localization typically associated with the wild-type protein (11), showing a diffuse cytosolic distribution without a change in mitochondrial morphology (Fig. 5C). These data further suggest a critical role for the CC domain in mitochondrial targeting of DLP1.

The Coiled-coil Domain of DLP1 Can Function as an Assembly Domain in a Dynamin Background—Although the DLP1 CC domain binds to mitochondria, it alone is not sufficient for mitochondrial targeting, as chimeras 2 and 4 or truncation 3 containing the CC domain did not localize to mitochondria. Whereas we found that the DLP1 CC domain is distinct from the dynamin CC domain in at least one function, that of targeting DLP1 to mitochondria (Fig. 5), it is unknown whether the DLP1 CC domain would function as a mitochondria-targeting domain or simply as an assembly domain in a dynamin background. The CC domain of dynamin has been found to regulate the dynamin GTPase (46) and dynamin self-assembly (15). We tested this hypothesis by using mutant dynamin chimeras containing the DLP1 CC domain (Fig. 1C, chimeras 5 and 6). We have shown in this report that chimera 3 containing the dynamin CC domain diffusely localized with no apparent mitochondrial deformation (Figs. 1C and 3A), suggesting that the dynamin CC domain was unable to substitute for the DLP1 CC domain in a DLP1 background. To our surprise, however, chimeras containing the DLP1 CC domain in a dynamin back-
ground either with or without PRD were unable to endocytose transferrin (Fig. 6, A and B), suggesting that these chimeric proteins interacted with endogenous dynamin and/or its binding partners to inhibit normal dynamin function. As suspected, although these chimeras contained the DLP1 CC domain that has mitochondrial targeting information, they did not localize to mitochondria, nor caused the fission-defective mitochondrial phenotype (data not shown). These data suggest that, although the mitochondrial targeting information in the DLP1 CC domain is suppressed in a dynamin background, the DLP1 CC domain can substitute for the dynamin CC domain in its ability to function as an assembly domain.

DISCUSSION

To provide insight into the function of distinct DLP1 domains, this study utilized in vivo expression of various DLP1-Dyn2 chimeras to assess alterations in localization and mitochondrial morphology. Such chimeric approaches have been utilized in domain studies of other conventional motors such as kinesin and myosin. These studies include: the determination of kinesin directionality using chimeras of conventional kinesin and Ncd (41); the characterization of myosin II ATPase activity governed by head, neck, and tail domain interactions using chimeras of Dictyostelium, Acanthamoeba, and chicken myosin II (42); the discovery that tail chimeras of Dictyostelium myosin II support cytokinesis but not development (43); the elucidation of a role for motor domains defining motor protein using chimeras of myosin 1b and non-muscle myosin IIb (40); the postulation that structures of myosin surface loops could serve as modulators of enzymatic and mechanical properties of myosin heavy chain using loop region chimeras of myosin (38); and the finding that cardiac myosin functionality does not depend on loop 1 or 2 sequences using loop region chimeras of myosin (39). From our chimeric approach, we have made several significant observations that include: first, mitochondrial specificity re-
sides in the three consecutive DLP1 MID, UC, and CC domains, as removal of any one of these domains failed to confer mitochondrial specific DLP1 function; second, the CC domain of DLP1 alone targets exclusively to mitochondria, suggesting a mechanism for localization of DLP1 to this organelle in vivo; and third, the mitochondrial targeting information within the DLP1 CC domain is suppressed when placed in a Dyn2 background. The ability of DLP1 CC to function as an assembly domain is not compromised, suggesting that it may be a key component for regulating the segregation of dynamin family members within the cell.

**Key Domains within the Dynamin Superfamily**—One of the hallmarks of the dynamin superfamily of proteins is the high degree of homology shared at the N termini (1, 47). The GTPase domains of dynamin and DLP1 are 62% homologous, suggesting that they are sufficiently homologous to be functionally interchangeable. Mitochondrial deformation by chimera 4 (a mutant Dyn2 GTPase domain in a DLP background) supports this notion (Fig. 3B). However, when a wild-type, functional dynamin GTPase domain was inserted into a DLP1 background, cells expressing this chimera still exhibited collapsed and tubulated mitochondria (results not shown). These data suggest that although this chimera contains a wild-type GTPase domain, it is non-functional, resulting in a mutant phenotype. Indeed, a GTPase assay using this purified recombinant chimeric protein showed a 65% reduction in GTPase activity compared with wild-type DLP1 (results not shown), confirming a functional impact on enzymatic activity. These data are in good agreement with previously published in vitro observations showing that the dynamin CC domain (referred to as the “assembly domain”) can bind to the dynamin GTPase and MID domains, but not the MID domain of the distant dynamin family member, Mx1 (15). It is likely that a mismatch between key regions in the GTPase and downstream domains (e.g. the CC domain) leads to improper regulation of GTPase activity, causing a mutant phenotype. A similar mechanism has been demonstrated for MxA, a distant relative to DLP1 within the dynamin family of GTPases (48).

**Zhang et al.** (49) identified two distinct self-assembly domains (SA1 and SA2) in phragmoplastin, the putative dynamin homologue in plants, that appear to “key” certain domains to...
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Each other. SA1 is located in the GTPase domain between GTP binding elements 1 and 2, whereas SA2 is located in the MID domain. Interestingly, the authors report that SA2 can interact with either SA1 or SA1, but SA1 can only interact with SA2. Moreover, SA1 consists of two ~25-amino acid regions, the first of which is highly conserved among dynamin family members, yet the second region (as well as SA2) is highly divergent, suggesting that GTPase and MID domains may be key to one another. In a similar vein, if the putative SA1 and SA2 regions in DLP1 are not properly matched (chimera 4; Fig. 3B) or are missing (truncation 3; Fig. 4C), the resulting chimeras may still be able to interact with endogenous dynamin and DLP1 (and/or their binding partners) by virtue of the remaining intact domains, yet self-assembly, and subsequent GTPase activation, cannot proceed. Notably, the DLP1 GTPase domain has a stretch of amino acids between the first and second GTP binding elements that is absent in dynamin. It is interesting to speculate that this region may be specifically recognized by downstream domains of DLP1 to bring about DLP1-specific function and/or GTPase regulation.

Matching of GTPase and MID domains is necessary but not sufficient, as chimeras 1 and 2 (where SA1 and SA2 are matched) do not exhibit phenotypes, nor do they bind endogenous DLP1 (Fig. 2). Recent studies with dynamin have suggested that the GTPase and MID domains interact with the CC domain (15, 16). It is possible that specific GTPase and MID domains are, in turn, key to a respective CC domain, thus providing tight regulation to prohibit classes of dynamin family members from interacting in vivo. Regardless of the inability of the wild-type dynamin GTPase to substitute for the wild-type DLP1 GTPase, our data indicate that the MID-UC-CC segment of DLP1 contains necessary information for DLP1 oligomerization, whereas the GTPase domain confers enzymatic activity on DLP1. Future studies elucidating the keying regions in dynamin and DLP1 will further our understanding of how the dynamin superfamly is segregated within the cell.

DLP1 Is Targeted to Mitochondria via Its Coiled-coil Domain—Much is known regarding the targeting of dynamin to specific cytoplasmic locations, which occurs via its PH and PRD domains. It has been shown that the CC domains of dynamin self-associate and interact with the GTPase and MID domains for self-assembly as well as regulating GTPase activity (15, 23). According to domain mapping studies of dynamin on synthetic lipid membranes (50), the dynamin CC domain loops back to interact with the GTPase and MID domains, whereas the PH domain binds to membranes. Whereas dynamin localizes to specific membrane regions via its PH and PRD domains, DLP1 lacks a PRD, and the function of its UC domain is currently unknown. Because the DLP1 UC domain (the correlate of the dynamin PH domain) is the most divergent region of the molecule, one hypothesis is that the UC domain might harbor the mitochondrial targeting information of DLP1. We found, however, that expression of the DLP1 UC domain alone gave a diffuse localization and failed to invoke a mitochondrial defect (data not shown). In addition, truncations 2 and 4, each lacking the DLP1 CC domain, produced diffuse localizations and did not induce mitochondrial collapse (Figs. 4B and 5C), suggesting that the CC domain, not the UC domain, may provide DLP1 mitochondrial targeting information. The finding that the Myc-tagged DLP1 CC domain localizes exclusively to mitochondria (Fig. 5) strongly supports the hypothesis that the CC domain confers mitochondrial targeting information to DLP1 in vivo. This targeting is specific, as a Myc-tagged dynamin CC domain did not localize to mitochondria, but was diffuse and cytosolic (data not shown).

Co-localization studies showed that the DLP1 CC domain associates with mitochondria but its distribution is distinct from endogenous DLP1 on mitochondria (Fig. 5B). This suggests that the CC domain may play a dual role in the overall function of DLP1 as both an assembly domain and a targeting motif. How the CC domain facilitates both of these functions is currently unknown. Our in vivo findings, however, are in good agreement with a recent report describing intra- and intermolecular interactions of DLP1 (51). Using the yeast two-hybrid system, the authors showed that CC domain-containing fragments (e.g. the MID-UC-CC and UC-CC segments) interact far more strongly with N-terminal DLP1 fragments (e.g. the GTPase-MID segment) than with full-length DLP1. These results were confirmed by co-immunoprecipitation of ectopically expressed DLP1 segments. The reduced affinity of the CC domain for full-length DLP1 may explain why we did not observe co-localization of the CC domain with endogenous DLP1. The localization data presented here suggests that the CC domain itself harbors some mitochondrial targeting affinity and binds either directly to the mitochondrial membrane, or via an interaction with other binding partners at the mitochondrial surface. Given that DLP1 puncta are found at sites of subsequent mitochondrial fission (52), the CC domain may facilitate initial mitochondrial binding of DLP1, whereas localization to actual fission sites may be determined by factors such as hFs1, a putative DLP1-binding protein (53). Studies are underway to explore this possibility.

Are Coiled-coil Domains Interchangeable?—Although chimeras containing the DLP1 CC domain in a dynamin background were unable to target dynamin to mitochondria, they did block transferrin uptake (Fig. 6), suggesting that the DLP1 CC domain is competent to bind dynamin effector proteins and/or endogenous dynamin proper. Furthermore, although the mitochondrial targeting information in the DLP1 CC domain is suppressed in a dynamin background, it can still function as an assembly domain capable of interacting with other dynamin domains in a dynamin context. Smirnova and colleagues (15) reported that the CC domain of Mx1, a distant dynamin family member, could not interact with the dynamin GTPase domain in vitro, suggesting a mechanism by which the cell prevents the incorporation of different dynamin family members into heterologous complexes. The dynamin and Mx1 CC domains, however, are only 16% homologous, compared with 35% for dynamin and DLP1 CC domains. Whereas a divergent CC domain may not be competent to substitute, it appears that a more homologous CC domain is capable of interacting with the dynamin GTPase domain. Interestingly, the dynamin CC domain does not appear to be able to function as an assembly domain in a DLP1 background, as this chimera exhibits a diffuse intracellular localization pattern with no phenotype (data not shown). It is possible that the other domains of DLP1 may contain specific regions “keyed” to the DLP1 CC domain by virtue of its mitochondrial targeting information, thereby providing extremely tight regulation over which dynamin family members facilitate changes in mitochondrial morphology. Future studies directed at determining the regions responsible for keying DLP1 domains, and how these differ from dynamin, will prove enlightening regarding the functional regulation of the dynamin superfamly.

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