Intra- and Intermolecular Domain Interactions of the C-terminal GTPase Effector Domain of the Multimeric Dynamin-like GTPase Drp1*

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Mammalian Drp1 is a dynamin-like GTPase required for mitochondrial fission. Although it exists primarily as a cytosolic homo-tetramer in vivo, it can also self-assemble into higher order structures on the mitochondrial outer membrane, where it is required for proper mitochondrial division. Functional studies and sequence comparisons have revealed four different structural domains in Drp1, comprising N-terminal GTP-binding, middle, insert B, and C-terminal GTPase effector (GED) domains. Here we describe an intramolecular interaction within Drp1 between the GED and the N-terminal GTP-binding and middle domains. A point mutation (K679A) within the C-terminal GED domain inhibits this intramolecular association, without affecting the formation of Drp1 tetramers or the intermolecular associations among isolated C-terminal domains. Mutant Drp1 K679A exhibits impaired GTPase activity, and when overexpressed in mammalian cells it decreases mitochondrial division. Sedimentation experiments indicate that the K679A mutation either increases Drp1 complex formation or, more likely, decreases complex disassembly as compared with wild-type Drp1. Taken together, these data suggest that the C-terminal GED domain is important for stimulation of GTPase activity, formation and stability of higher order complexes, and efficient mitochondrial division.

Drp1 is a member of the dynamin superfamily of multimeric large GTPases. Although the prototype dynamin is involved in severing nascent endocytic vesicles from the plasma membrane, others in this family appear important for vesicle remodeling or fission events in different cellular organelles (1–5). Mammalian Drp1 (also referred to as DLP1, DVLP, dymple, and HdyIV) (6–10) and its Caenorhabditis elegans (DRP-1) and Saccharomyces cerevisiae (Dnm1p) orthologs are involved in mitochondrial division, and mutants of these proteins deficient in GTP binding and GTPase activity have been shown to induce a dominant-negative impairment of mitochondrial fission (9, 11–16).

A common theme throughout the diverse dynamin superfamily is that self-assembly and oligomerization play important roles in the function of these proteins (1–5, 17). In dynamin, dimers/tetramers assemble to form rings and collars at the base of clathrin-coated pits, inducing cooperative increases in its GTPase activity (2–5). Formation of higher order structures and cooperative increases in GTPase activity have also been described for the dynamin-related Mx and guanylate-binding protein families (17–20). In many cases, detailed analyses have been performed to characterize the domains involved in assembly and cooperative GTPase stimulation of these proteins. Most appear to have an N-terminal GTP-binding domain, a middle assembly domain, a short insert (in dynamin, a pleckstrin homology domain), and a C-terminal assembly domain dubbed the “GTPase effector domain” (GED).1 In the case of the dynamin, guanylate-binding protein, and Mx proteins, an intramolecular domain association has been identified, with the GED domain folding back to interact with the GTP-middle domains (18, 21–23). The GED domain is critical for not only assembly of higher order complexes but also for cooperative stimulation of GTPase activity. In fact, the significance of the intramolecular backfolding of the GED domain of dynamin has been emphasized by studies suggesting that it functions as an intramolecular GTPase-activating protein (24, 25). Also, the conservation of amino acid residues within this region has led to the extension of the intramolecular GTPase-activating protein concept to other dynamin-like GTPases such as yeast Dnm1p (26).

A major area of controversy is whether members of the dynamin superfamily act as regulatory GTPases or are involved directly as mechanoenzymes in severing membranes (4, 5, 27). Studies have evaluated effects of mutations in highly conserved Arg and Lys residues within the C-terminal GED domain in dynamin but with conflicting results for both endocytosis and GTPase activity (24, 25, 28). The effects of similar mutations on mitochondrial division have also been evaluated in the yeast Dnm1p protein (26). However, the interactions as well as the functional and structural roles of the GED domain of mammalian Drp1 remain unclear. Here we investigate the inter- and intramolecular associations of dynamin superfamily proteins in vivo.
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**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—Complementary DNAs for full-length *Homo sapiens* Drp1 (GenBank™ accession number AB009865.1) and Drp1 truncation constructs were generated by PCR using *Pfu*Turbo™ DNA polymerase (Stratagene, La Jolla, CA) with EcoRI ends added and cloned into the EcoRI site of the mammalian expression vector pGWI (29, 30). N-terminal c-Myc (EQKLISEEDL, single-letter amino acid code) or HA epitope tags (YPYDVPDYA) were engineered into pGWI after an internal restriction site was introduced (29, 30). For yeast two-hybrid tests, Drp1 deletion constructs were produced by PCR amplification using *Pfu*Turbo™ polymerase, incorporating EcoRI restriction sites for subcloning in-frame into pGAD10 or pBHA (BD Biosciences, Palo Alto, CA). Site-directed mutagenesis was performed using the QuikChange™ method (Stratagene) and confirmed in each construct by DNA sequence analysis of the entire coding sequence.

**Antibodies**—Affinity-purified antipeptide antibodies against Drp1 (amino acids 511–526; acetyl-NIEEQQRNLAREPS-amide; number 2457) were prepared commercially in rabbits (BioSource, Hopkinton, MA), with the terminal cysteine added to facilitate coupling. Mouse monoclonal anti-Myc (9E10) and rabbit polyclonal anti-HA-probe (Y-11) antibodies were prepared commercially in rabbits (BioSource). CBP-Drp1 fusion proteins were purified from *Escherichia coli* BL21 (DE3) expressing full-length CBP-tagged wild-type Drp1, Drp1 K679A, or Drp1 K38A as described previously (30). Affinity-purified CBP-Drp1 fusion proteins were dialyzed against assay buffer (20 mM HEPES, pH 7.2, 2 mM MgCl₂, and 1 mM dithiothreitol). The reaction mixture for the GTPase assay included 2.3 μM diazylated CBP-Drp1 protein with 0.05% bovine serum albumin and 165 μM [γ-32P]GTP (3000 Ci/mmol; ICN Biomedicals, Irvine, CA) in assay buffer. Samples of the reaction mixtures were spotted onto polyethyleneimine cellulose on polyester TLC plates (Sigma-Aldrich) at various time points. Guanine nucleotides were separated by ascending chromatography in 1.0 M formic acid and 1.2 M LiCl. The [32P]GDP and [32P]GTP spots were identified, and the intensities were quantified using phosphorimaging (Molecular Dynamics, Sunnyvale, CA) and ImageQuant software (Amersham Biosciences). GTPase activity was expressed as the ratio of GDP to total guanine nucleotides (GDP + GTP) at each time point. All of the data points represent the averages of at least three independent experiments ± standard deviation.

**Sedimentation Assay and Chemical Cross-linking**—COS-7 cells were transfected with wild-type or mutant Myc-Drp1 constructs as described above. The cells were washed several times, lysed, and then harvested in 20 mM phosphate buffer (pH 7.4) with 50 mM NaCl. The membrane fraction was pelleted by centrifugation at 120,000 × g; the supernatant was then subjected to ultracentrifugation at 300,000 × g for 1 h. Equal proportions of the resulting pellet and supernatant fractions were subjected to SDS-PAGE and immunoblotted with anti-Myc antibodies (0.2 μg/ml). For chemical cross-linking studies, transfected COS-7 cells were washed twice with PBS and lysed; the post-nuclear supernatant was then incubated with 0.25–1.00 μM bis(sulfosuccinimidyl)suberate (BS²; Pierce) in PBS for 2 h on ice. After the reactions were quenched with 1 M Tris-HCl buffer (pH 7.5), the proteins were resolved on Criterion 12% acrylamide gel electrophoresis (Bio-Rad) and immunoblotted.

**RESULTS**

We generated polyclonal anti-peptide antibodies against the human Drp1 protein (number 2457), and used these to determine the localization of endogenous Drp1 in COS-7 cells (Fig. 1). Punctate staining is found distributed throughout the cytoplasm (Fig. 1, panel A1), often localizing to mitochondrial tubules (Fig. 1, panels A1–A3). In some cases, isolated Drp1-positive puncta are evident at sites of mitochondrial constriction, likely representing sites of subsequent mitochondrial division (Fig. 1B, middle panel). Preadsorption of the antibodies with the immunogenic peptide completely eliminated Drp1 staining, but the same concentration of a control peptide did not (data not shown).

Because the Drp1 foci in Fig. 1 might represent higher order structures of Drp1 and, by analogy with other dynamin super-
family members, it is likely that formation of higher order Drp1 complexes is critical for mitochondrial division, we systematically evaluated the domain determinants of self-association in Drp1 using yeast two-hybrid assays and co-immunoprecipitation experiments (Fig. 2). Two domains that are important for self-association of Drp1 have previously been described, ADH1 and ADH2 (31). Using more recent nomenclature (1, 26), these are within the GTP-binding and neighboring middle domains (GTP-middle) and the C-terminal α-helical GED, respectively (Fig. 2A). Insert B is found between these domains (Fig. 2A) and is the site of variations in Drp1 sequence generated through alternative mRNA splicing (8, 32). As shown in Fig. 2 (A and B), using yeast two-hybrid tests we found that multiple different deletion constructs of Drp1 containing a single association domain (e.g. GTP-middle) interact robustly with deletion constructs containing the other association domain (e.g. GED) but much less or not at all with larger constructs containing both. For instance, the GTP-middle-containing fragment Drp1(1–489) interacts far more strongly with GED-containing fragments Drp1(502–736) and Drp1(637–736) than with full-length Drp1(1–736), which contains both GTP-Middle and GED domains (Fig. 2B). Also, Drp1(502–736) interacts weakly with full-length Drp1, although it interacts strongly with Drp1(1–489). Taken together, these and other results detailed in Fig. 2B indicate that the intramolecular (i.e. cis) interaction between GTP-middle and GED is favored over the intermolecular (i.e. trans) interaction in these assays.

In addition to this robust intramolecular interaction, there is also evidence for intermolecular interactions of Drp1. In agreement with previous studies (26, 31), full-length Drp1(1–736) exhibits a strong intermolecular self-interaction (Fig. 2B). Multiple different N-and C-terminal deletion constructs bind to full-length Drp1, although much more weakly than the full-length protein does to itself, suggesting that multiple domains may be involved in these intermolecular interactions. The C-terminal GED domain interacts with itself, albeit weakly, an interaction reminiscent of those reported for other members of the dynamin superfamily including dynamin, yeast Dnm1p, and MxA (2, 24, 26, 33, 34).

We further assessed the interdomain, intramolecular interactions of Drp1 using cultured COS-7 cells transfected with combinations of full-length Drp1(1–736), Drp1(502–736), and Drp1(1–489) differentially tagged with N-terminal HA or Myc epitopes. We confirmed that full-length HA-Drp1(1–736) associates with full-length Myc-Drp1(1–736) by co-immunoprecipitation studies with anti-HA antibodies followed by immunoblotting with anti-Myc antibodies (Fig. 2C), in agreement with the yeast two-hybrid results. In contrast, Drp1(502–736) could not be co-immunoprecipitated with full-length Drp1(1–736), even though it did bind to Drp1(1–489) (Fig. 2C). These results are consistent with the yeast two-hybrid results and indicate a preference for intramolecular interaction of the two Drp1 association domains, GTP-middle and GED. Thus, although intermolecular associations may be more labile with fragments of the Drp1 protein, the intramolecular interactions are more robust. Drawing upon the results of yeast two-hybrid tests and co-immunoprecipitation studies presented in Fig. 2, the dimeric Drp1 inter- and intramolecular interactions are postulated to be highly reminiscent of those in dynamin dimers (lacking the C-terminal proline-rich domain) reported by Zhang and Hinshaw (23) using cryo-electron microscopy.

We evaluated the effects of a GED mutation in Drp1 K679A that is at a position previously shown to be important for dynamin function (24) and that is highly conserved in a number

### Fig. 2. Intra- and intermolecular interactions of Drp1.

**A.** Schematic of Drp1 constructs; boundary amino acids are indicated. The location of alternatively spliced cassettes is indicated by an inverted triangle. **B.** Matrix of yeast two-hybrid assays showing interactions of various Drp1 N- and C-terminal deletion constructs in bait (pBHA) or prey (pGAD10) constructs, as indicated. Strength of interaction was assayed by β-galactosidase and HIS3 induction and semi-quantified as in Blackstone et al. (29). β-Galactosidase activity was assessed by determining the time for colonies to turn blue in X-gal filter lift assays: ++++, <30 min; +++, 30–60 min; ++, 60–90 min; +, 90–180 min; --, no significant activity. HIS3 activity was measured by the percentage of colonies growing on histidine-lacking medium: ++++, >75%; ++, 50–75%; +, 25–50%; +, <25%; --, no growth. In all cases, HIS3 and β-galactosidase induction correlated. **C.** Co-immunoprecipitation (IP) of Myc- and HA-tagged Drp1 constructs. COS-7 cells expressing the indicated pairs of epitope-tagged Drp1 constructs were immunoprecipitated with anti-HA antibodies, and the precipitates were immunoblotted with anti-Myc antibodies. Load represents 20% of the starting material.
of dynamin-like GTPases (Fig. 3A). A striking effect of the K679A mutation is that it strongly inhibits the interaction of the GED-containing Drp1(502–736) and Drp1(637–736) fragments with Drp1(1–489), the latter of which harbors the GTP-middle domain, in yeast two-hybrid tests (Fig. 3B). At the same time, it does not appear to alter the intermolecular associations between full-length Drp1 constructs or the weaker interactions between isolated GED domains (Fig. 3B). Thus, the K679A mutation may result in a more "open" state of Drp1 with altered intramolecular associations (asterisks) between the GTP-middle and AH-GED domains (top). The K679A GED mutation impairs the ability of GTP-middle to interact with AH-GED domain, as shown schematically (bottom). AH, α-helical.

![Fig. 3. Effect of K679A GED mutation on inter- and intramolecular interactions of Drp1.](image)

A. sequence alignment of AH-GED domains from H. sapiens Drp1, S. cerevisiae Dnm1p, H. sapiens dynamin, A. thaliana ADL2b, D. discodeum DymA, S. cerevisiae Vps1p, and H. sapiens dynamin-1. Residues identical in all proteins are shaded in orange, and those identical in three or more are shaded green. The lysine residue mutated in this study is indicated in bold with an asterisk. GenBank™ accession numbers are: Drp1, BAA22193; Dnm1p, NP_013100; ADL2b, BABB56485; DymA, CAA67983; Vps1p, NP_012926; and dynamin-1, Q05193. Boundary amino acid residues are indicated. B, matrix of yeast two-hybrid assays showing interactions of various Drp1 N- and C-terminal deletion bait constructs (as in Fig. 1A) tested against Drp1 prey constructs with or without the K679A mutation as indicated. Strength of interaction was assayed and scored as in Fig. 2. C, model for Drp1 intramolecular interactions (asterisks) between the GTP-middle and AH-GED domains (top). The K679A GED mutation impairs the ability of GTP-middle to interact with AH-GED domain, as shown schematically (bottom). AH, α-helical.

![Fig. 4. Effect of Drp1 mutations on Drp1 oligomerization.](image)

A, standard curve for determination of protein size by FPLC gel exclusion chromatography. The indicated protein standards were applied to a Superdex 200 HR 10/30 column. The peak fractions were used to determine elution volume, and $K_m$ was calculated. These data were used to generate a standard curve, as shown. B, native molecular masses of Myc-Drp1 proteins by gel exclusion FPLC. Aliquots of the indicated fractions (top) were immunoblotted with anti-Myc antibodies, and the peak fractions were identified. C, estimated molecular masses of the indicated Myc-Drp1 proteins, as calculated by linear regression using the average molecular weight calculated from the indicated number (N) of trials ± S.E. WT, wild type.
also seen at –350 and –365 kDa, likely representing higher order structures. At the same time, the native Myc-Drp1(502-736) protein, containing insert B and GED domains, has a single elution peak at –204,000 kDa (Fig. 4, B and C), suggesting that it is able to self-associate. Also, the Myc-Drp1(502-736) fragment cross-links prominently as a dimer but also shows higher order cross-links (Fig. 5B). To investigate the possibility that endogenous Drp1 in the Myc-Drp1(502-736) overexpressing COS-7 cells may be binding Myc-Drp1(502-736) and contributing to these larger structures, we subjected cells co-transfected with Myc-Drp1(502-736) and full-length HA-Drp1(1-736) to chemical cross-linking with BS3. The cross-linked products for full-length HA-Drp1 highly resemble those shown in Fig. 5A for full-length Myc-Drp1 alone. Similarly, the cross-linked products for Myc-Drp1(502-736) in the co-transfected cells are essentially the same as those observed when Myc-Drp1(502-736) is transfected alone (Fig. 5B and data not shown). These results suggest that full-length Drp1 is not involved in the formation of the Drp1(502-736) higher order cross-linked structures. Thus, the GED fragments may be important in not only dimer/tetramer formation but also in higher order structures. This latter result is consistent with previous studies of dynamin structure that identify the C-terminal GED domain as an important assembly domain (23, 33, 34).

Because the predominant cytosolic form of Drp1 K679A expressed in cells migrates at the same size as wild-type Drp1 on gel exclusion FPLC and because chemical cross-linking results are very similar, we sought to determine whether the K679A mutation might alter higher order oligomerization (Fig. 5C). Previous studies have shown a significant salt dependence of this sedimentation for dynamin-like proteins, with lower salt concentrations favoring higher order complex formation (20, 31, 35). We used a low salt concentration isolation buffer (20 mM phosphate with 50 mM NaCl) to assess the effects of these mutations on Myc-Drp1 overexpressed in COS-7 cells. We found that sedimentable complexes are most prominent with Myc-Drp1 K38A and least prominent with wild-type Myc-Drp1, whereas Myc-Drp1 K679A is intermediate (Fig. 5C).

We assessed the functional effect of the Drp1 K679A mutations using in vitro GTPase assays. The CBP fusion protein of wild-type Drp1(1–736) demonstrates efficient GTP hydrolysis (Fig. 6). As expected, the K38A mutation abolishes Drp1...
Mitochondrial tubules are commonly seen in these cells (1). Does not form cytoplasmic aggregates (panel C1, long, extended mitochondrial tubules are commonly seen in these cells (arrows in panel C2).)

Finally, we assessed the effect of the Drp1 K679A mutation on mitochondrial morphology. In contrast to the study by Fukushima et al. (26) did not for Drp1/Dnm1p interaction in cis of the GED and GTP-Middle domains (31). Drp1 structure that we propose, based on yeast two-hybrid and co-immunoprecipitation studies (Fig. 3C), is highly reminiscent of the dynamin structure lacking the proline-rich domain, as determined by cryo-electron microscopy (23). Similar to dynamin (36, 37), Drp1 seems to exist predominantly as a tetramer in the cytoplasm based on our cross-linking and gel exclusion FPLC results as well as the previously reported cross-linking results of Shin et al. (31).

Like dynamin, Drp1 forms higher order structures such as rings and spirals (12, 38). Several studies have indicated that the C-terminal GED of dynamin plays a key role in this formation (2, 3, 5). A domain within the dynamin C-terminal GED (termed D3) forms structures probably larger than tetramers and may in fact be competent to form helical bundles (33). Also, Klockow et al. (39) found that a model of GED assembly is similar to findings for yeast Dnm1p (26) and in agreement with studies of dynamin (23, 24, 33, 34). The Drp1 structure we propose, based on yeast two-hybrid and co-immunoprecipitation studies (Fig. 3C), is highly reminiscent of the dynamin structure lacking the proline-rich domain, as determined by cryo-electron microscopy (23). Similar to dynamin (36, 37), Drp1 seems to exist predominantly as a tetramer in the cytoplasm based on our cross-linking and gel exclusion FPLC results as well as the previously reported cross-linking results of Shin et al. (31).

**DISCUSSION**

In this study, we have investigated the inter- and intramolecular interactions of the human dynamin-like GTPase Drp1, focusing on the structural and functional roles of the C-terminal GED. We have demonstrated that the Drp1 molecule has an intramolecular back-folding of the GED domain onto the GTP-middle domain. A mutation within the GED, K679A, inhibits this intramolecular back-folding and is associated with markedly decreased Drp1 GTPase activity as well as an increased proportion of higher order, sedimentable Drp1 complexes.

The interdomain interactions of Drp1 could be predicted based upon results of the guanylate-binding protein 1 crystal structure and structural studies of the related dynamin and Mx proteins (18, 21–23). However, to date results for Drp1/Dnm1p interactions have been inconclusive. Although Shin et al. (31) reported a robust interaction with GED and GTP-mid domain (9, 11, 13–15), Myc-Drp1 K38A overexpression (panel B2) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B1) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B1) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B1).

**TABLE I**

| Drp1 construct | Predominant mitochondrial morphology | Wild-type, fragmented | Elongated, tubules | Clumped, aggregated |
|---------------|------------------------------------|-----------------------|-------------------|------------------|
| None (mock)   | %                                  | 88 12 0               |                   |                  |
| Wild-type Drp1| %                                  | 87 13 0               |                   |                  |
| Drp1 K38A     | %                                  | 5 37 58               |                   |                  |
| Drp1 K679A    | %                                  | 20 67 14              |                   |                  |

**Fig. 7.** Disruption of mitochondrial fission by Drp1 K679A. COS-7 cells were transfected with wild-type Myc-Drp1, Myc-Drp1 K38A, or Myc-Drp1 K679A as indicated and co-stained with anti-Myc antibodies (for Drp1 detection) and MitoTracker (for mitochondria). A, wild-type Drp1(1–736) overexpressed in COS-7 cells is homogeneously distributed throughout the cytoplasm (panel A1). There are no significant changes in mitochondrial morphology, size, or distribution (panel A2). B, overexpression of Drp1 K38A results in prominent Drp1-positive cytoplasmic aggregates (panel B1) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B2). C, although overexpressed mutant Drp1 K679A does not form cytoplasmic aggregates (panel C1), long, extended mitochondrial tubules are commonly seen in these cells (arrows in panel C2).

**DISCUSSION**

In this study, we have investigated the inter- and intramolecular interactions of the human dynamin-like GTPase Drp1, focusing on the structural and functional roles of the C-terminal GED. We have demonstrated that the Drp1 molecule has an intramolecular back-folding of the GED domain onto the GTP-middle domain. A mutation within the GED, K679A, inhibits this intramolecular back-folding and is associated with markedly decreased Drp1 GTPase activity as well as an increased proportion of higher order, sedimentable Drp1 complexes. Finally, overexpression of the mammalian Drp1 K679A mutant in cells is associated with decreased mitochondrial division.

The interdomain interactions of Drp1 could be predicted based upon results of the guanylate-binding protein 1 crystal structure and structural studies of the related dynamin and Mx proteins (18, 21–23). However, to date results for Drp1/Dnm1p interactions have been inconclusive. Although Shin et al. (31) reported a robust interaction with GED and GTP-mid domain (9, 11, 13–15), Myc-Drp1 K38A overexpression (panel B2) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B1) and markedly decreased mitochondrial division, with prominent perinuclear aggregation of mitochondria (arrows in panel B1).
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strin homology domain of dynamin can self-associate (40).

Although the role of GED in dynamin assembly is widely
accepted, the effects of mutations in this region on properties
such as cooperative increases in GTPase activity and endocy-
tosis have been controversial. Sever et al. (24, 25) found that
a mutation in the GED (K694A) inhibited trans interactions of
neighboring GED domains, prolonging the GTP-bound state
and stimulating endocytosis. However, in another study Marks
et al. (28) reported that the same mutation in the dynamin
GED affected neither GTPase activity nor endocytosis. A mu-
tation equivalent to dynamin K694A in yeast Dnm1p (K705A)
accelerated mitochondrial fission, possibly by prolonging an
active Dnm1p-GTP-bound state, although the effects of this
mutation on GTPase activity were not reported (26).

In our study, we report several new findings. First, we dem-

onstrate directly that the corresponding GED mutation in hu-
mans Drp1 (K679A) decreases GTPase activity. Interestingly, it
also diminishes an intramolecular interaction between the
GED and GTP-middle domains, indicating there may be a
formational change in the GED that interferes with inter-
domain interactions. Interestingly, although Fukushima et al.
(26) were able to demonstrate an interaction between the GTP
binding and GED domains by yeast two-hybrid assays in only
one reciprocal bait/prey pair, the K705A mutation abolished
the interaction (see Table V in Ref. 26), in agreement with our
results for Drp1. Both the human Drp1 K679A (this study) and
yeast Dnm1p K705A (26) mutants retain the ability to oligomer-
ize by yeast two-hybrid tests. We find that the wild-type Drp1,
GTPase-deficient mutant Drp1 K38A, and the GED mutant
Drp1 K679A retain the ability to form oligomers, most likely
tetramers. However, these mutations may still affect the for-
mation or stability of higher order structures, either through
formational changes or effects on GTPase activity. The
K38A and K679A mutant forms of Drp1 possess less efficient
GTPase activity, and the higher sedimentation of K679A and
K38A suggests that this may result in either increased
assembly or, more likely based on results of previous
dynamin studies, impaired disassembly. The decreased
GTPase activity of Drp1 K679A may be due to a loss of proper
GED-GED interactions, as suggested for dynamin (24), or
intramolecular GED-GTP-middle domain interactions important
for higher order structures, either of which could result from
changes in the conformation of the GED domain. At the same
time, a change in Drp1 conformation may alter interactions
with other proteins required for mitochondrial division.

In contrast to the results for the yeast Dnm1p K705A mutant
(26), where acceleration of mitochondrial fission was reported,
we found that overexpression of mutant Drp1 K679A results in
a marked decrease in mitochondrial fission, reminiscent but
less severe than that seen with Drp1 K38A. How can we ac-
count for this difference? In the Dnm1p study, the K705A
mutant was expressed in a dnm1Δ yeast strain, whereas in our
study the Myc-Drp1 K679A mutant protein was overexpressed
in cells already expressing Drp1 endogenously; thus, heteroge-
eous oligomers containing both wild-type and mutant Drp1
proteins may have properties different from those composed of
mutants alone. Furthermore, our results show that K679A alters
the intramolecular GTP-middle and GED interaction; it is
less clear whether this is also the case in yeast Dnm1p (26).

Lastly, there is sequence divergence in the regions surround-
ing the conserved Lys residue (Fig. 3A), and thus corresponding
mutations in yeast Dnm1p and mammalian Drp1 might still
have different effects on interactions with downstream part-
ners. In this regard it is interesting that no mammalian or-
thologs of Mdv1p, a yeast protein that interacts with Dnm1p to
trigger mitochondrial division (41), have been identified. Iden-
tification of downstream partners for mammalian Drp1 in mi-
tochondrial division pathways may clarify this issue, because
multiple proteins required for mitochondrial division have al-
ready been identified in yeast (16, 41–47).

We conclude that the K679A mutation alters the conforma-
tion of the C-terminal GED of Drp1, affecting tertiary and
possibly quaternary structure and leading to impairment in
GTPase activity. Because Drp1 K679A is still able to form
tetramers, it may not represent an optimal mutation to inves-
tigate the functional importance of Drp1 assembly. The iden-
tification of other assembly domain mutations may help clarify
the role of assembly on GTPase activity and mitochondrial
division. Even so, our results demonstrate the importance of
the GED domain in the inter- and intramolecular interactions of
the Drp1 GTPase and reinforce the structural similarities to
dynamin, including the assembly and disassembly of higher
order structures. Our results do not clearly discriminate
whether Drp1 is a mechanoenzyme or regulatory GTPase, and
in fact it may have features of both. Most of our results for the
K38A and K679A mutants can be explained by relative defi-
ciencies in GTPase activity, leading proportionately to im-
paired mitochondrial division and probably impaired disassem-
bly of higher order complexes. By analogy with dynamin, then,
cytosolic Drp1 is likely recruited into a large spiral that wraps
around a mitochondrion, leading to constriction and/or recruit-
ment of other protein effectors that promote constriction and
fission, followed by rapid disassembly dependent on GTP hy-
drolysis and finally return to the cytosolic pool (2, 5). The
identification of downstream effectors for mammalian Drp1
will help clarify the role of the GED assembly domain in down-
stream interactions.

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