Molecular Biology of the Cell
Vol. 12, 2756–2766, September 2001

The GTPase Effector Domain Sequence of the Dnm1p GTPase Regulates Self-Assembly and Controls a Rate-limiting Step in Mitochondrial Fission

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Submitted March 6, 2001; Revised May 30, 2001; Accepted June 19, 2001
Monitoring Editor: Lawrence S. Goldstein

Dnm1p belongs to a family of dynamin-related GTPases required to remodel different cellular membranes. In budding yeast, Dnm1p-containing complexes assemble on the cytoplasmic surface of the outer mitochondrial membrane at sites where mitochondrial tubules divide. Our previous genetic studies suggested that Dnm1p's GTPase activity was required for mitochondrial fission and that Dnm1p interacted with itself. In this study, we show that bacterially expressed Dnm1p can bind and hydrolyze GTP in vitro. Coimmunoprecipitation studies and yeast two-hybrid analysis suggest that Dnm1p oligomerizes in vivo. With the use of the yeast two-hybrid system, we show that this Dnm1p oligomerization is mediated, in part, by a C-terminal sequence related to the GTPase effector domain (GED) in dynamin. The Dnm1p interactions characterized here are similar to those reported for dynamin and dynamin-related proteins that form higher order structures in vivo, suggesting that Dnm1p assembles to form rings or collars that surround mitochondrial tubules. Based on previous findings, a K705A mutation in the Dnm1p GED is predicted to interfere with GTP hydrolysis, stabilize active Dnm1p-GTP, and stimulate a rate-limiting step in fission. Here we show that expression of the Dnm1 K705A protein in yeast enhances mitochondrial fission. Our results provide evidence that the GED region of a dynamin-related protein modulates a rate-limiting step in membrane fission.

INTRODUCTION

In the budding yeast *Saccharomyces cerevisiae*, mitochondrial membranes form a branched, tubular network located near the cell surface (reviewed in Hermann and Shaw, 1998). The morphology of the mitochondrial network is maintained by opposing fission and fusion events (Nunnari *et al.*, 1997). Genetic and morphological studies indicate that mitochondrial fission is regulated by a predicted GTPase called Dnm1p, which assembles on the outer mitochondrial membrane at sites where fission occurs (Otsuga *et al.*, 1998; Bleazard *et al.*, 1999; Sesaki and Jensen, 1999). Dnm1p belongs to a large family of dynamin-related GTPases required to remodel different cellular membranes (van der Bliek, 1999a). Recent studies indicate that Dnm1p-catalyzed mitochondrial fission is a multistep process requiring two additional proteins called Fis1p and Mdv1p (Fekkes *et al.*, 2000; Mozdy *et al.*, 2000; Tieu and Nunnari, 2000). Fis1p is an outer mitochondrial membrane protein that plays both an early role in Dnm1p complex assembly and a late role in membrane constriction and/or fission (Mozdy *et al.*, 2000). Mdv1p is thought to bind directly to Dnm1p and is essential for Dnm1p complex function after assembly (Tieu and Nunnari, 2000).

Sequence comparisons have identified four distinct structural domains in Dnm1p including an N-terminal GTPase domain, a Middle domain, Insert B, and a C-terminal domain with alpha-helical character called the alpha helical/GTPase effector domain (AH/GED) (van der Bliek, 1999a). An intact GTPase domain is required for mitochondrial fission because alleles of *DNM1* containing GTPase domain mutations fail to rescue mitochondrial fission defects in a *dnm1* null strain (Otsuga *et al.*, 1998). Moreover, the same GTPase mutant forms of Dnm1p induce dominant mitochondrial fission defects in cells expressing wild-type Dnm1p (Otsuga *et al.*, 1998). These dominant defects could arise because mutant Dnm1p proteins coassemble with, and disrupt the function of, wild-type Dnm1p, increasing the possibility that oligomerization is required for the function of wild-type Dnm1p in vivo.

Self-assembly/oligomerization is thought to play an important role in regulating the activity of dynamin. Dynamin

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tetramers assemble to form rings/collars at the base of clathrin-coated pits that are required for the scission and release of clathrin-coated vesicles (de Camilli *et al.*, 1995; Schmid, 1997; Hinshaw, 2000). Formation of these rings/collars stimulates dynamin's GTPase activity (Warnock *et al.*, 1996). It was recently proposed that this stimulation occurs because a novel domain in dynamin, called the GED, functions as an assembly-dependent GTPase-activating protein (GAP) (Sever *et al.*, 1999). GED mutations that alter either 1) a *cis*-acting catalytic residue required for GTPase activation (dyn R725A), or 2) a residue required in *trans* for both assembly and hydrolysis (dyn K694A), impair dynamin's GED-stimulated GTPase activity (Sever *et al.*, 1999). When overexpressed in mammalian cells, both of these mutant forms of dynamin increase the rate of formation of constricted coated pits, the rate-limiting step in endocytosis. However, membrane fission and coated vesicle release is blocked by dyn R725A but occurs normally in dyn K694A (Sever *et al.*, 1999, 2000). Sever *et al.* (1999, 2000) suggested that these findings argue against a mechanochemical role for dynamin (Kelly, 1999; van der Bliek, 1999b; Yang and Cerione, 1999). Instead, they proposed that dynamin acts as a regulatory GTPase during endocytosis. In this regulatory role, dynamin:GTP would control the formation of constricted coated pits and the recruitment of dowstream partners required for membrane fission. GTP hydrolysis by dynamin would then be required to remove it from the membrane so that the subsequent fission step could occur.

Studies to date have not distinguished between a regulatory and a mechanochemical role for the Dnm1p GTPase in mitochondrial fission. In vivo, Dnm1p localizes to punctate complexes on the cytoplasmic face of the mitochondrial network (Otsuga *et al.*, 1998). We and others have proposed that these complexes are composed of higher ordered Dnm1p structures organized as collars surrounding mitochondrial tubules (Bleazard *et al.*, 1999; Mozdy *et al.*, 2000; Tieu *et al.*, 2000). Immunogold labeling studies reveal that these Dnm1p complexes cluster at sites where mitochondrial tubules are constricted (Bleazard *et al.*, 1999). Based on the dynamin GED analysis published by Sever *et al.* (1999, 2000), we favor a model in which Dnm1p:GTP controls the formation of constricted mitochondrial tubules and the recruitment/activity of downstream partners required for fission. GTP hydrolysis by Dnm1p might then lead to the actual fission event. In this study, we provide experimental evidence that validates several aspects of this model. First, we show that Dnm1p binds and hydrolyzes GTP in vitro. Second, we demonstrate that Dnm1p forms an oligomeric complex with itself in vivo. Third, with the use of a GED mutation predicted by Sever *et al.* (1999) to prolong the GTP-bound state of Dnm1p, we provide evidence suggesting that Dnm1p controls a rate-limiting step in mitochondrial fission. Although these findings do not eliminate a mechanochemical role for Dnm1p in fission, they raise the possibility that Dnm1p functions as a classical GTPase to regulate distinct steps during mitochondrial division.

MATERIALS AND METHODS

Yeast Strains and Methods

All JSY and ADM strains are derivatives of the FY10 strain (Winston *et al.*, 1995). Standard genetic methods were used to grow, transform, and manipulate yeast (Sherman *et al.*, 1986; Guthrie and Fink, 1991) and bacterial (Maniatis *et al.*, 1982) strains. All mutations, disruptions, tag insertions, and replacements were confirmed by polymerase chain reaction, DNA sequencing and, where appropriate, Western blotting. Strains used were as follows: JSY1361, *Mat a ura3-52 leu2Δ1 his3Δ200 dnm1Δ::HIS3 FZO1*; PJ69-4A, *Mat a trp1-901 leu2-3112 ura3-52 his3Δ200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ* (James *et al.*, 1996); JSY3096, *Mat α ura3-52, leu2Δ1, his3Δ200 DNM1 FZO1*; JSY3097, *Mat α ura3-52, leu2Δ1, his3Δ200 dnm1Δ::HIS3 FZO1*; ADM52, *Mat a ura3-52 leu2Δ1 his3Δ200 DNM1 fzo1-1*; ADM755, *Mat a ura3-52 leu2Δ1 his3Δ200 trp1Δ63 dnm1::HIS3 FZO1*; JSY3163 *Mat a ura3-52 leu2Δ1 his3Δ200 dnm1Δ::HIS3 fzo1-1*; and JSY3164, *Mat α ura3-52 leu2Δ1 his3Δ200 dnm1Δ::HIS3 FZO1*.

Plasmid Construction

For pET23a-*DNM1* (Dnm1–6xHisp), a full-length *DNM1* fragment flanked by *Bam*HI/*Sal*I sites was cloned into pET23a (Novagen, Madison, WI). For pRL-3xHA-*DNM1* (3xHA-Dnm1p) and pRU-3xMyc-*DNM1* (3xMyc-Dnm1p), a *Not*I site was engineered in pRL1-*DNM1* and pRU1-*DNM1* (Otsuga *et al.*, 1998) just after the *DNM1* start codon to create pRL1-*Not*I-*DNM1* and pRU1-*Not*I-*DNM1*, respectively. The 3x HA and 3x Myc fragments generated by *Not*I digest from pMPY-3xMyc and pMPY-3xHA (Schneider *et al.*, 1995) were cloned into pRL1-*Not*I-*DNM1* and pRU1-*Not*I-*DNM1*. To generate two-hybrid expression vectors maintained at low copy in *Escherichia coli*, the *Bam*HI and *Sal*I sites in both YEp24 and YEp213 were destroyed by site-directed mutagenesis. The *GAL4* DNA binding domain from pGBDU-C1 (James *et al.*, 1996) or the *GAL4* DNA activation domain from pGAD-C1 (James *et al.*, 1996) was cloned into the *Sph*I sites of the modified YEp24 and YEp213 vectors to create pRUBD-C1 and pRLAD-C1, respectively. Polymerase chain reaction fragments encoding different *DNM1* domains were cloned into the *Bam*HI and *Sal*I sites of pRUBD-C1 and pRLAD-C1. YEp213-*dnm1*^{K705A} and YEp213-*dnm1*^{R736A} were generated by site-directed mutagenesis of YEp213-*DNM1* (Otsuga *et al.*, 1998).

Expression and Purification of Dnm1p-6XHis

BL21(DE3) *E. coli* cells (Novagen) containing pET23a-*DNM1* (encoding Dnm1–6xHisp) were grown in LB medium plus carbenicillin and induced with 0.4 mM isopropyl β-D-thiogalactoside. The Dnm1–6xHisp fusion protein was purified from cleared cell lysates by sequential chromatography on Ni^{2+} affinity and gel filtration columns.

GTP Binding and Hydrolysis

To perform the UV cross-linking assay, 1 μg of purified Dnm1–6xHisp in buffer containing 20 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 20 mM HEPES 7.0, 2 mM $MgCl_2$, 1 mM dithiothreitol, 10 μCi [α-^{32}P]GTP, and 25% ethylene glycol was incubated on ice and exposed to UV light for 30 min as described previously (Melen *et al.*, 1994; Warnock *et al.*, 1996). Reactions terminated by boiling in Laemmli sample buffer were separated by SDS-PAGE and analyzed by autoradiography. To measure GTP hydrolysis, reactions containing 4 μg of purified Dnm1–6xHisp were initiated in buffer containing 20 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), 20 mM HEPES 7.0, 2 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM GTP, and 1 μCi [α-^{32}P]GTP. Aliquots removed at the indicated times were spotted onto phosphocellulose thin-layer chromatography plates (J. T. Baker, Phillipsburg, NJ). The GDP pool was separated from the GTP pool by chromatography in formic acid:$LiCl_2$ buffer (Melen *et al.*, 1994; Warnock *et al.*, 1996). After drying, radiolabeled nucleotide spots were visualized and quantified with the use of a phosphorimager.

Coimmunoprecipitation Studies and Yeast Two-Hybrid Analysis

Cell lysates prepared by glass bead lysis in Lysis/IP buffer (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 5 mM EDTA, 0.07% TX-100, 1 mM phenylmethylsulfonyl fluoride, leupeptin [1 μg/ml], and aprotinin [1 μg/ml]) were incubated with either anti-hemagglutinin (HA) (Covance Research Products, Richmond, CA) or anti-Myc (Santa Cruz Biochemicals, Santa Cruz, CA) affinity matrix at 4°C for 4 h. Immunoprecipitates collected by centrifugation were washed three times in Lysis/IP buffer, boiled in Laemmli sample buffer, and analyzed by SDS-PAGE and Western blotting with the indicated antibodies. Yeast two-hybrid studies with the use of the plasmids described in Figure 3 were performed essentially as described (James *et al.*, 1996).

Quantification of Mitochondrial Morphology

Mitochondrial morphology was scored by $DiOC_6$ staining in *DNM1* and *dnm1*Δ cells containing YEp213-*dnm1*^{K705A}, YEp213-*dnm1*^{R736A}, or YEp213-*DNM1* as described previously (Hermann *et al.*, 1997, 1998; Otsuga *et al.*, 1998; Bleazard *et al.*, 1999). Digital microscopic images of cells were acquired with the use of a confocal microscope (Carl Zeiss, Thornwood, NY) as described previously (Hermann *et al.*, 1998; Otsuga *et al.*, 1998).

RESULTS

Dnm1p Binds and Hydrolyzes GTP

To test whether the predicted GTPase domain of Dnm1p was functional, we performed GTP cross-linking and hydrolysis assays with the use of a bacterially expressed form of Dnm1p containing a C-terminal 6xHis affinity tag (Dnm1–6xHisp). When analyzed by SDS-PAGE, Dnm1–6xHisp enriched by Ni^{2+} chromatography was the predominant Coomassie blue-stained band, although some lower molecular weight proteins were present (Figure 1A, lane 1; the arrow marks Dnm1–6xHisp). As shown in Figure 1B lane 3 (arrow), only the full-length Dnm1–6xHis protein was labeled when this fraction was incubated with [α-^{32}P]GTP and exposed to UV, indicating that Dnm1–6xHisp bound GTP. No cross-linking was observed in control fractions containing bovine serum albumin (BSA) (Figure 1, A [lane 2] and B [lane 4]). In similar studies, Dnm1–6xHisp was not cross-linked to [α-^{32}P]ATP or [α-^{32}P]CTP (our unpublished results). Moreover, no labeling was detected when cross-linking was performed with Dnm1–6xHisp protein containing K41A or S42N mutations predicted to interfere with GTP binding/hydrolysis (our unpublished results).

As shown in Figure 1C, Dnm1–6xHisp also hydrolyzed GTP. Over a 60-min time-course, [α-^{32}P]GTP was converted to [α-^{32}P]GDP in fractions containing Dnm1–6xHisp but not BSA or buffer alone (Figure 1C; B, buffer alone). The rate of GTP hydrolysis by Dnm1–6xHisp was ~5 moles of GTP hydrolyzed/min/mol of Dnm1–6xHisp, consistent with the rate of GTP hydrolysis determined for dynamin (Warnock *et al.*, 1996). Because we have not yet established conditions for oligomerization of bacterially expressed Dnm1p, this number probably represents the intrinsic or basal rate of GTP hydrolysis.

Dnm1p Intermolecular Interactions

Intermolecular interactions are required for the higher order assemblies and/or functions of dynamin (Hinshaw, 2000) and

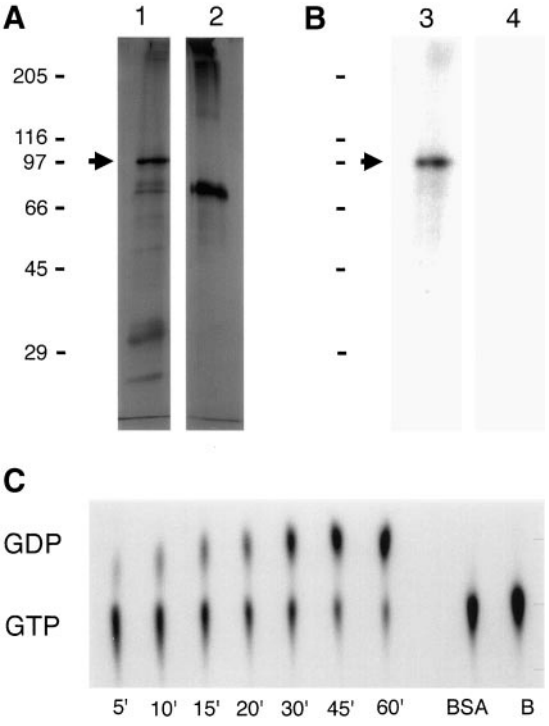

Figure 1. Dnm1–6xHisp Binds and Hydrolyzes GTP. (A) Coomassie blue-stained gel showing partially purified Dnm1–6xHisp (lane 1, see arrow) and BSA (lane 2). (B) Autoradiogram showing [α-^{32}P]GTP UV cross-linking to Dnm1–6xHisp in A (lane 3, see arrow). (C) Autoradiogram of thin-layer chromatography showing the time course of [α-^{32}P]GTP hydrolysis by Dnm1–6xHisp. The calculated rate of GTP hydrolysis is 5 mol of GTP hydrolyzed/min/mol of Dnm1–6xHisp. The 60-min time point is shown for BSA and buffer (B) alone.

dynamin-related proteins, including Dnm1p/Vps1p-like protein (Shin *et al.*, 1999), MxA (Schumacher and Staeheli, 1998; Flohr *et al.*, 1999), and phragmoplastin (Zhang *et al.*, 2000). To determine whether Dnm1p was in a complex with itself in the yeast cytoplasm, we performed coimmunoprecipitation experiments from wild-type yeast cells expressing both N-terminal HA- and Myc-tagged Dnm1p. Both tagged forms of Dnm1p partially rescued the *dnm1*Δ phenotype, indicating that they retain function in vivo (our unpublished results). When HA-Dnm1p was immunoprecipitated from cleared cell lysates containing both tagged proteins with the use of anti-HA affinity matrix (Figure 2, lane 8, anti-HA Western blot), Myc-Dnm1 protein was also detected in the precipitated fraction (Figure 2, lane 8, anti-Myc Western blot). Similarly, when Myc-Dnm1p was immunoprecipitated from lysates expressing both tagged proteins with anti-Myc affinity matrix (Figure 2, lane 9, anti-Myc Western blot), HA-Dnm1 protein was detected in the precipitated fraction (Figure 2, lane 9, anti-HA Western blot). No coimmunoprecipitation was observed when similar experiments were performed with the use of cell lysates expressing only the HA-Dnm1p (Figure 2, lanes 2 and 3) or the Myc-Dnm1p (Figure 2, lanes 5 and 6) proteins. These results indicate that Dnm1p is in a complex with itself in vivo. Presumably, the small amount of HA-Dnm1p coimmunoprecipitated with Myc-Dnm1p (and vice versa) reflects the fact that only a frac-

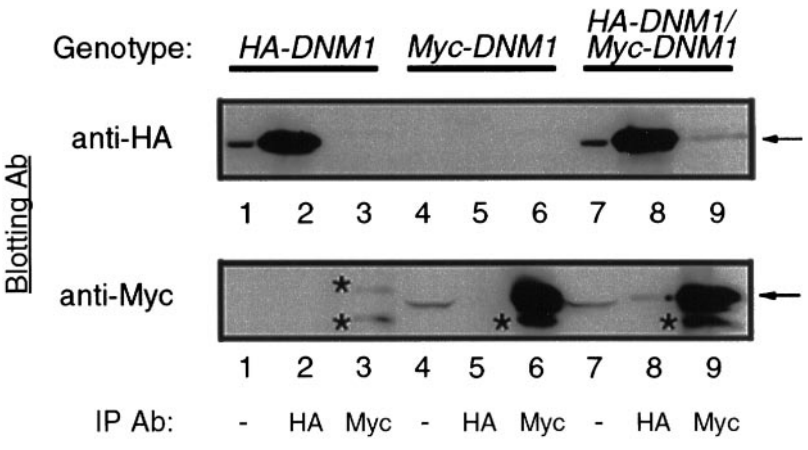

Figure 2. Coimmunoprecipitation of HA- and Myc-tagged Dnm1p from yeast extracts. Cleared cell lysates were prepared from cells expressing HA-tagged Dnm1p (lanes 1–3), Myc-tagged Dnm1p (lanes 4–6), or both HA- and Myc-tagged Dnm1p (lanes 7–9). Lysates (lanes 1, 4, and 7) immunoprecipitated with anti-HA (lanes 2, 5, and 8) or anti-Myc (lanes 3, 6, and 9) affinity matrix were analyzed by SDS-PAGE and Western blotting with monoclonal anti-HA (top) and anti-Myc (bottom) antibodies. The asterisk indicates background bands that precipitate with the anti-Myc antibodies. Arrows mark the position of the full-length tagged Dnm1 proteins.

tion of Dnm1p in the cell is in a complex or oligomerized at steady state (Otsuga *et al.*, 1998). Moreover, we and others have observed that Dnm1p complexes are unstable after cell lysis (Otsuga *et al.*, 1998; Tieu and Nunnari, 2000).

The two-hybrid system described by James *et al.* (1996) was used to determine which Dnm1p domain(s) facilitated Dnm1p–Dnm1p interactions. Figure 3A shows a schematic representation of the Dnm1p constructs cloned into the binding domain (BD) and activating domain (AD) vectors used for these studies. As shown in Figure 3B, yeast host strains containing combinations of empty vector controls, full-length Dnm1p, an N-terminal fragment (GTPase 1–343), and a C-terminal fragment grew well on nonselective medium containing adenine (Figure 3B, +Ade). In additional control experiments, none of the plasmid constructs grew on selective medium when paired with an empty BD or AD vector (Figure 3B, −Ade). In contrast, yeast host strains containing full-length Dnm1p on both BD and AD plasmids grew well on selective medium lacking adenine (Figure 3B, Dnm1:Dnm1, −Ade). Similar results were obtained when interactions were measured with the use of β-galactosidase assays (our unpublished results). This finding is consistent with our coimmunoprecipitation studies (Figure 2), suggesting that Dnm1p oligomerizes in vivo.

Additional constructs were examined to determine the domains required for the intermolecular Dnm1p interactions. The N-terminal GTPase$^{1–343}$ construct did not grow on selective medium in combination with itself (Figure 3B, GTPase:GTPase, −Ade) or with the C-terminal fragment (Figure 3B, GTPase:C-terminal, −Ade). However, strains expressing both the AD and BD domains fused to the C-terminal fragment grew well on selective medium (Figure 3B, C-terminal:C-terminal, −Ade), suggesting that amino acids within the C-terminal fragment were responsible for interactions of full-length Dnm1p with itself in this assay.

The C-terminal fragment contains three subdomains as defined by sequence homology with mammalian dynamin, including the Middle domain, Insert B, and the AH/GED domain (Figure 3A). To define the domain responsible for the C-terminal self-interaction, we cloned these subdomains into the BD and AD vectors and tested them in the two-hybrid assay.

As summarized in Table 1, the Dnm1p AH/GED was sufficient for robust growth on selective −Ade medium in combination with itself or the Insert B-AH/GED construct. This interaction also occurred (although to a lesser extent) when the AH/GED was paired with the Middle domain. Lengthening the AH/GED by the addition of Insert B (Insert B-AH/GED) increased the efficiency of its interaction with the Middle domain. Moreover, strains containing the Insert B-AH/GED fragment grew well in combination with all subdomains of the C-terminal fragment.

Surprisingly, the Middle domain did not interact with the C-terminal fragment, although it did interact with subdomains of the C-terminal fragment. One possible explanation for these results is that binding sites for the Middle domain are somehow masked (i.e., by folding) in the complete C-terminal fragment. Alternatively, intramolecular interactions of the Middle domain may be favored over intermolecular interactions of the Middle domain with the complete C-terminal fragment.

Together, these results suggested that all subdomains contributed to self-interactions of the C-terminal domain, albeit to different extents. In most cases, the strongest interaction occurred between constructs containing the AH/GED. In support of this idea, less growth was observed in strains expressing C-terminal and AH/GED constructs (in both orientations). This reduced growth may be due to self-interactions sequestering AH/GED, thereby preventing AH/GED from interacting with the C-terminal construct. We were unable to assess the role of Insert B alone with the use of the two-hybrid assay because the Insert B construct appeared to interact nonspecifically with all other constructs, including empty vectors. However, we observed that fusion of Insert B to the AH/GED (Insert B-AH/GED) stabilized the interaction of AH/GED with other subdomains of the C-terminal fragment. For this reason, we used the Insert B-AH/GED fusion for the analysis described below.

Previous studies of dynamin suggested that the N-terminal GTPase domain interacts with sequences in the C-terminal portion of the protein (Muhlberg *et al.*, 1997; Sever *et al.*, 1999; Smirnova *et al.*, 1999). In addition, extending the C-terminal border of the GTPase domain and introducing a K44A mutation into the GTPase domain reportedly stabilizes a two-hybrid interaction with the GED region (Smirnova *et al.*, 1999). In contrast, we did not observe an interaction between the Dnm1p GTPase$^{1–343}$ domain and the AH/GED region in the two-hybrid assay (Figure 3B). Al-

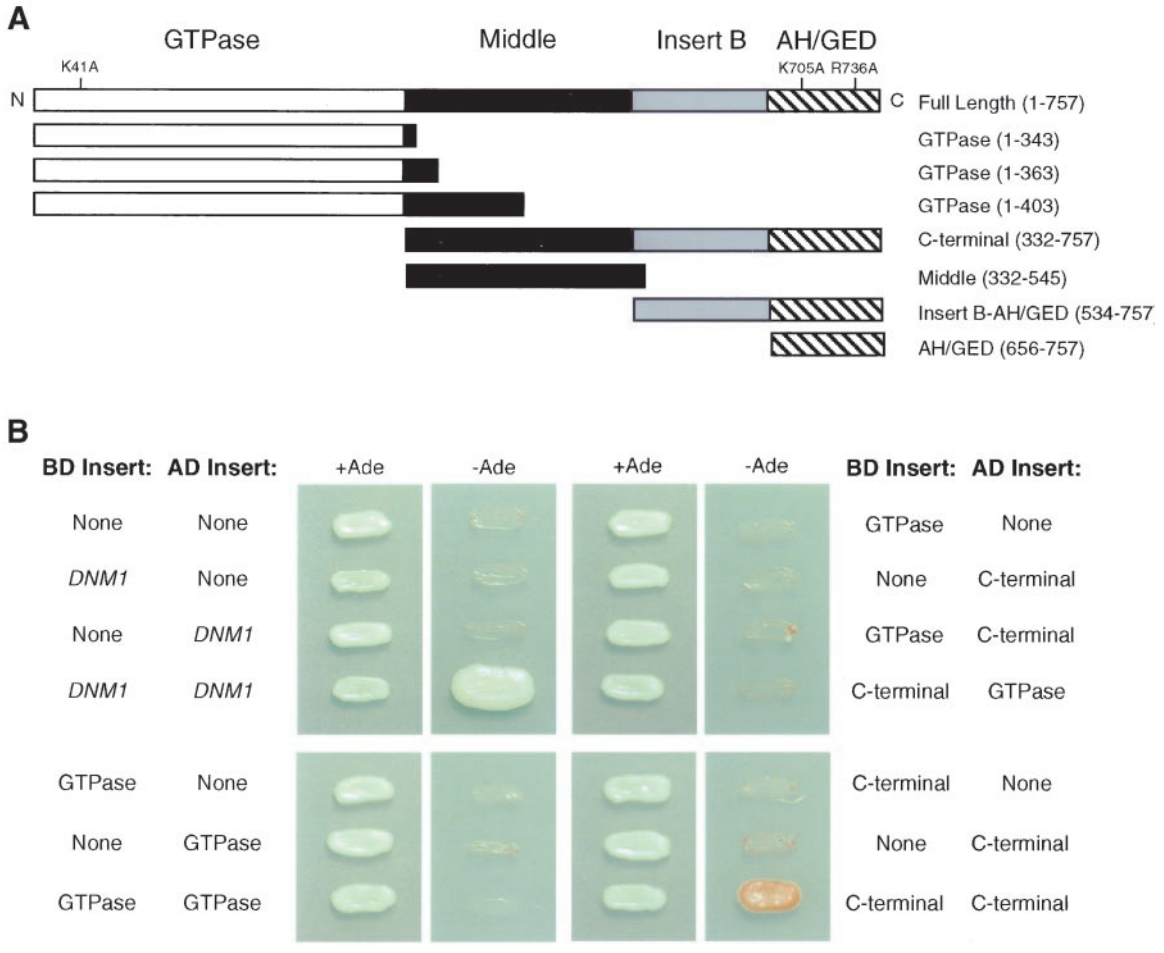

Figure 3. DNM1 domain interactions analyzed by two-hybrid analysis. (A) Schematic representation of the *DNM1* constructs used in the yeast two-hybrid assay. Structural motifs identified in full-length Dnm1p include the GTPase domain, middle domain, insert B, and alpha helical/GED (predicted GED). The amino acids included in the Dnm1p subdomains used for the two-hybrid assay and the point mutations used in this study are indicated. (B) Strains containing the BD and AD plasmids were patched onto SD lacking uracil and leucine (+Ade) and SD lacking uracil, leucine, and adenine (−Ade). Growth on −Ade medium indicates an interaction between the inserts in the respective plasmids.

though lengthening the GTPase domain construct enhanced interactions with the C-terminal fragment (Table 2), this interaction appeared to be due to intermolecular interactions of middle domain sequences on the two-hybrid constructs (Table 2 and Figure 3A; interaction of the middle domain with itself is shown in Table 1). Our inability to detect an interaction between the Dnm1p GTPase domain and another Dnm1p subdomain is puzzling, because the GTPase domain does interact with full-length Dnm1p in the two-hybrid assay (our unpublished results). It is possible that full-length Dnm1p contains a protein interaction domain for the GTPase region that is absent in the shorter subdomains or that the subdomains are not folded properly.

The combined results of our coimmunoprecipitation studies and two-hybrid analyses indicate that the Dnm1p GTPase oligomerizes in vivo and that this oligomerization is due, in part, to intermolecular interactions of the AH/GED region. We currently do not know whether this oligomerization leads to the

Table 1. Two-hybrid interactions within the Dnm1p C-terminal domain

	C-Terminal	Middle	Insert B-AH/GED	AH/GED
C-Terminal	+++	−	+++	++*
Middle	−	++	+++	++
Insert B-AH/GED	+++	+++	+++	+++
AH/GED	−*	++	+++	+++

−, no growth on selective medium; ++, <50 colonies on selective medium; and +++, >50 colonies on selective medium.

* Growth observed only for one of the reciprocal AD and BD plasmid pairs. All constructs paired with empty BD and AD plasmids did not grow on selective medium.

Table 2. Two-hybrid interactions between the Dnm1p GTPase and C-terminal domains

	GTPase$^{1–343}$	GTPase^{K41A}	GTPase$^{1–363}$	GTPase$^{1–403}$
C-Terminal	++*	−	++	+++
Middle	−	−	−	++*
Insert B-AH/GED	++*	−	−	−
AH/GED	−	−	−	−

−, no growth on selective medium; ++, <50 colonies on selective medium; and +++, >50 colonies on selective medium.

* Growth observed only for one of the reciprocal AD and BD plasmid pairs. All constructs paired with empty BD and AD plasmids did not grow on selective medium.

Table 3. Two-hybrid interactions of the full-length Dnm1p mutant GED domains

	Dnm1p	Dnm1p^{K705A}	Dnm1p^{R736A}
Dnm1p	+++	+++	+++
Dnm1p^{K705A}	+++	+++	+++
Dnm1p^{R736A}	+++	+++	+++

+++, >50 colonies on selective medium.

formation of Dnm1p, e.g., dimers/tetramers, higher ordered structures, or both in vivo. To further characterize the role of the AH/GED, we carried out the mutational analysis described below.

Mutational Analysis of Dnm1p GED

The GED has been shown to play two distinct roles in dynamin. First, GED is required for the assembly of dynamin tetramers into rings and collars. Second, the GED acts as a GAP to stimulate GTP hydrolysis after dynamin assembly. Sever *et al.* (1999) showed that introducing a K694A mutation into dynamin's GED reduces its ability to assemble into higher ordered structures (rings/collars). Because GED–GED interactions after ring/collar formation are required for GAP activation, K694A also interferes with GTP hydrolysis. A second dynamin mutation, R725A, does not disrupt binding of the GED to the GTPase domain but does impair assembly stimulated GTP hydrolysis (Sever *et al.*, 1999).

Sequence alignments revealed that the dynamin K694 and R725 residues are equivalent to K705 and R736 in yeast Dnm1p (Figure 4; van der Bliek, 1999a). To determine the role of these residues in Dnm1p's oligomerization, we generated the equivalent K705A and R736A mutations in our *DNM1* full-length and truncated (Insert B-AH/GED) yeast two-hybrid constructs. Although the dynamin studies suggested that the K705A mutation would disrupt self-interactions of the Dnm1p GED (Sever *et al.*, 1999), we observed no effect of this mutation on Dnm1p AH/GED self-interactions (Tables 3 and 4). Moreover, the K705A and R736A mutations also failed to stabilize/enhance interactions between the Dnm1p Insert B-AH/GED construct and the Dnm1p GTPase constructs (Table 5). Together, these results suggest that 1) the two-hybrid system does not reconstitute some of the Dnm1p GED interactions that normally occur in vivo, or 2) the AH/GED functions differently in dynamin and Dnm1p. To directly test the role of the Dnm1p GED mutations on mitochondrial fission, we performed the two different in vivo assays described below.

Dnm1p GED Regulates Rate-limiting Step in Mitochondrial Division

The tubular mitochondrial network in yeast is maintained by opposing fission and fusion reactions regulated by the Dnm1p and Fzo1p GTPases, respectively (Figure 5). In previous studies, we showed that loss of Dnm1p function blocked mitochondrial fission and converted the wild-type mitochondrial network into interconnected nets of mitochondrial membranes that often collapsed to one side of the cell (Otsuga *et al.*, 1998; Bleazard *et al.*, 1999) (Figure 5). Although *DNM1* alleles that increase the rate or extent of fission have not been described, such alleles are predicted to increase fragmentation of the mitochondrial network.

To determine the effect of GED mutations on Dnm1p function in vivo, full-length Dnm1p K705A and Dnm1p R736A were expressed in both wild-type (WT) and *dnm1*Δ cells, and mitochondrial morphology was analyzed by $DiOC_6$ staining. As shown in Table 6, in a *dnm1*Δ strain containing the YEp213 vector alone, 100% of the cells contained collapsed nets and 0% of the cells exhibited fragmented mitochondrial membranes. The percentage of cells containing partially fragmented mitochondrial membranes increased to 15 or 30% in strains containing one genomic copy or multiple plasmid-borne copies of *DNM1*, respectively. Thus, increasing the dosage of *DNM1* in yeast appears to increase the steady-state level of mitochondrial fission.

*dnm1*Δ cells containing the K705A allele contained partially (47% of cells) or completely (23% of cells) fragmented

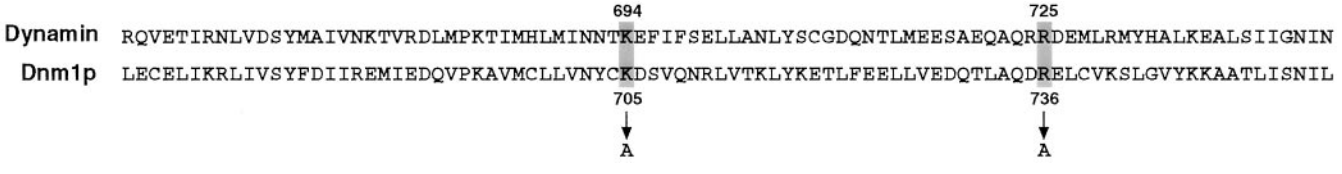

Figure 4. Sequence alignment of GED regions from *Homo sapiens* dynamin^{-1} (aa 657–745) and *S. cerevisiae* Dnm1p (aa 668–757). The residues mutated in dynamin (Sever *et al.*, 1999) and in Dnm1p (this study) are in gray boxes.

Table 4. Two-hybrid interactions of the Dnm1p Insert B-AH/GED mutant GED domains

	Insert B-AH/GED	Insert B-AH/GED^{K705A}	Insert B-AH/GED^{R736A}
Insert B-AH/GED	+++	+++	+++
Insert B-AH/GED^{K705A}	+++	+++	+++
Insert B-AH/GED^{R736A}	+++	+++	+++

+++, >50 colonies on selective medium.

mitochondrial membranes, suggesting that the rate or extent of mitochondrial fission was further increased in this strain. When the K705A allele was expressed in a strain that was also expressing wild-type Dnm1p from the genome, the fragmentation phenotype persisted (Table 6, 55% partially fragmented and 23% completely fragmented). Conversely, an increase in fragmented mitochondrial membranes was not observed in *dnm1*Δ cells containing the R736A allele. Rather, 46% of *dnm1*Δ cells expressing the R736A mutant protein contained wild-type mitochondrial networks. Together, these studies suggest that 1) the K705A mutation accelerates a rate-limiting step in mitochondrial fission, and 2) the R736A mutation partially disrupts Dnm1p function (Figure 6).

dnm1 K705A GED Mutation Enhances Mitochondrial Fragmentation and mtDNA Loss in a Sensitized Background

Whereas the Dnm1p GTPase regulates mitochondrial fission in yeast, mitochondrial fusion is regulated by a GTPase called Fzo1p (Hermann *et al.*, 1998). As depicted in Figure 5, *fzo1* mutations block fusion and result in fragmentation of the mitochondrial network due to unopposed Dnm1p-mediated fission. As a consequence of this fragmentation, *fzo1* mutant cells lose their mitochondrial DNA (mtDNA) and fail to grow on the nonfermentable carbon source glycerol.

With the use of the temperature-sensitive *fzo1-1* allele, we devised a plate growth assay to detect changes in the fission activity of Dnm1p. Previous studies indicated that the fusion activity of the mutant Fzo1-1 protein decreases as the growth temperature increases (Hermann *et al.*, 1998). When *DNM1 fzo1-1* cells are grown at 25°C, the Fzo1-1 protein is functional and the majority of cells contain tubular mitochondrial networks (Hermann *et al.*, 1998). In addition, these *DNM1 fzo1-1* cells retain their mtDNA at the permissive temperature and are able to grow on both fermentable (dextrose, YPD plates) and nonfermentable (glycerol, YPG plates) carbon sources (Figure 6A; 25°C, YEp213, top is YPG, bottom is YPD). In contrast, the Fzo1-1 protein product is unable to function at high (nonpermissive) temperatures (Hermann *et al.*, 1998). As shown in Figure 6A, mitochondrial fusion is blocked at 37°C in *DNM1 fzo1-1* cells, mitochondrial networks fragment and mtDNA is lost, preventing growth on YPG medium (37°C, YEp213). At the semipermissive temperature of 30°C, the fusion activity of the mutant Fzo1-1 protein is reduced but not completely blocked. Thus, *DNM1 fzo1-1* cells can grow on YPG medium (Figure 6A; 30°C, YEp213), even though fusion is compromised.

We used the glycerol growth defect of *fzo1-1* as an assay to monitor the fission activity of the wild-type, K705A, and R736A mutant forms of full-length *DNM1*. The *dnm1*Δ mutation blocks mitochondrial fission and fragmentation in the *fzo1-1* fusion mutant and prevents mtDNA loss, allowing *dnm1*Δ *fzo1-1* cells to grow on glycerol at 37°C (compare 37°C, YEp213; Figure 6, A and B). Conversely, increasing the dosage of the wild-type *DNM1* gene in *fzo1-1* cells increases fission and mtDNA loss and decreases their ability to grow on glycerol at 30°C (Figure 6B; YEp213 *DNM1*). This observation is consistent with our morphological studies (Table 6) showing that extra doses of *DNM1* increase mitochondrial fragmentation in vivo. Similarly, overexpression of the K705A allele in *dnm1*Δ *fzo1-1* decreases the amount of growth on glycerol at 30°C. Together with the observation that mitochondrial membranes are more fragmented in strains containing K705A relative to wild type (Table 6), these results suggest that the K705A allele up-regulates Dnm1p fission activity in vivo. No decrease in glycerol growth was observed in *DNM1 fzo1-1* and *dnm1*Δ *fzo1-1* strains with increased dosage of the R736A allele (Figure 6, A and B; 30°C, YEp213 *dnm1* R736A). In fact, *dnm1*Δ *fzo1-1* strains with increased dosage of the R736A allele were able to grow slowly on glycerol even at 37°C, suggesting that the

Table 5. Two-hybrid interactions between the Dnm1p GTPase and C-terminal mutant GED domains

	GTPase$^{1–343}$	GTPase^{K41A}	GTPase$^{1–363}$	GTPase$^{1–403}$
Insert B-AH/GED	++*	−	−	−
Insert B-AH/GED^{K705A}	−	−	−	−
Insert B-AH/GED^{R736A}	−	−	−	−

−, no growth on selective medium; and ++, <50 colonies on selective medium.

* Growth observed only for one of the reciprocal AD and BD plasmid pairs. All constructs paired with empty BD and AD plasmids did not grow on selective medium.

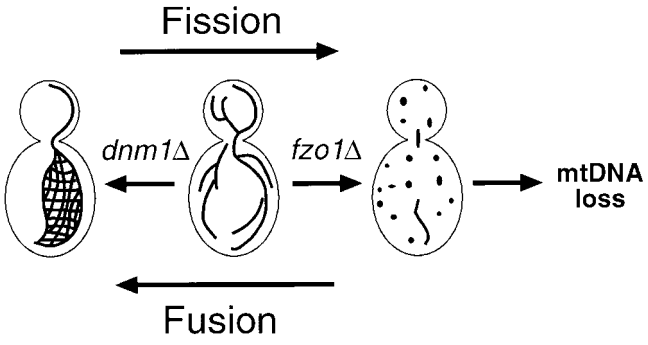

Figure 5. Dnm1p and Fzo1p act in opposing fission and fusion pathways to maintain the yeast mitochondrial network. Opposing fission and fusion events maintain a tubular mitochondrial network in wild-type yeast cells (middle). Loss of the Dnm1p GTPase (*dnm1*Δ) leads to net formation due to unopposed mitochondrial tip fusion (left); loss of the Fzo1p GTPase (*fzo1*Δ) leads to fragmentation due to unopposed mitochondrial fission (right). Fragmentation eventually causes mtDNA loss by an unknown mechanism.

Table 6

Mitochondrial Membrane Morphology in *dnm1*K705A and R736A Mutant Cells

Genotype / Plasmid	Wildtype	*dnm1* null	Partially Fragmented	Fragmented
Wildtype / YEp213	69	13	15	3
*dnm1*Δ / YEp213	0	100	0	0
Wildtype / YEp213DNM1	60	7	30	3
*dnm1*Δ / YEp213DNM1	54	12	31	3
Wildtype / YEp213dnm1K705A	18	4	55	23
*dnm1*Δ / YEp213dnm1K705A	9	21	47	23
Wildtype / YEp213dnm1R736A	52	31	16	1
*dnm1*Δ / YEp213dnm1R736A	46	53	0	1

Numbers are percent of asynchronous culture with a particular mitochondrial morphology (n=300). Mitochondrial morphology was scored in log-phase cells grown in synthetic medium at 25°C.

R736A mutation reduces Dnm1p function in vivo. These data are consistent with the morphological analysis shown in Table 6 and indicate that the protein produced by the R736A allele is partially functional.

DISCUSSION

Dnm1p Is a Functional GTPase

The amino terminal GTPase domain is the most highly conserved domain in the dynamin family of proteins. In this study, we established that Dnm1p can bind and hydrolyze GTP at a rate similar to that of dynamin and other dynamin family members (5 mol of GTP/min/mol of Dnm1p). Conditions that promote the assembly of dynamin tetramers into higher ordered structures (rings/collars) have been shown to stimulate dynamin's intrinsic GTPase activity 10-fold (Warnock *et al.*, 1996). Although other dynamin family members have been reported to form rings/collars in vitro (Schumacher and Staeheli, 1998; Zhang *et al.*, 2000), we have not detected such structures with the use of bacterially expressed Dnm1p (our unpublished results) and have not been able to test the effect of higher ordered structures on the rate of GTP hydrolysis by Dnm1p. Recently, the *Caenorhabditis elegans* (DRP-1) (Labrousse *et al.*, 1999) and mammalian (DRP1/DLP1/DVLP) homologs of yeast Dnm1p were also shown to be required for mitochondrial fission in vivo (Smirnova *et al.*, 2001). Bacterially expressed mammalian DRP1/DLP1 appeared to form stacked rings/collars that tubulated and constricted tubules composed of synthetic lipids (Yoon *et al.*, 2001). Although it is unclear why we have not observed similar Dnm1p rings/collars in vitro, one possibility is that one or more components of the Dnm1p-mediated fission machinery (e.g., Mdv1p and/or Fis1p) are required for the formation of functional, higher order

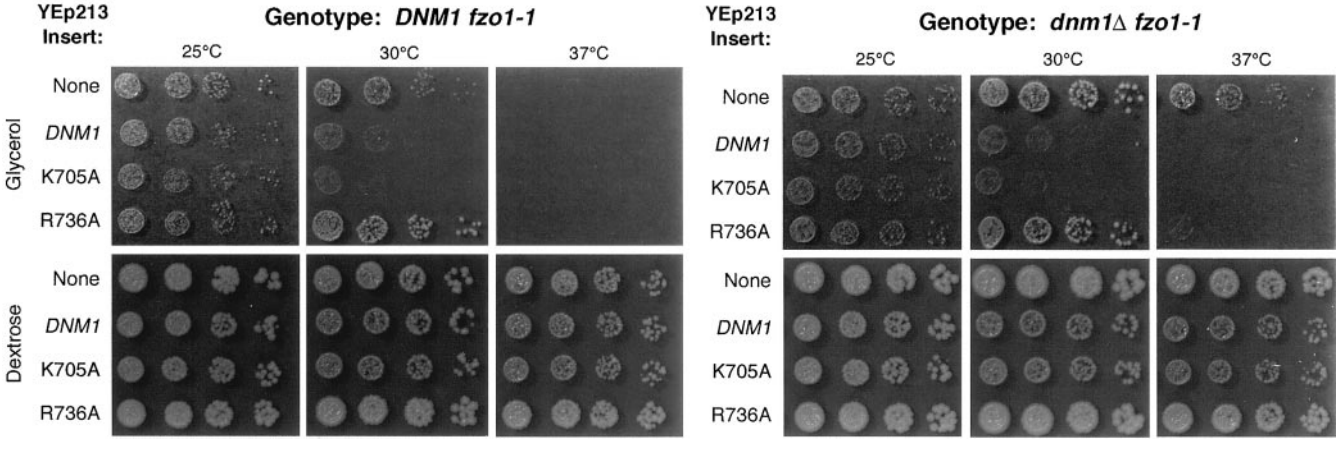

Figure 6. Effect of *DNM1 AH/GED* mutations on the *fzo1-1* conditional glycerol growth defect. Glycerol (top) and dextrose (bottom) growth phenotypes of *DNM1 fzo1-1* (A) or *dnm1*Δ *fzo1-1* (B) strains containing the indicated plasmids at 25, 30, and 37°C.

Dnm1p structures (Mozdy *et al.*, 2000; Tieu and Nunnari, 2000). Based on the observation that the Dnm1p homolog DRP1/DLP1 can form oligomers (Yoon *et al.*, 2001), and our in vivo evidence that Dnm1p is required for mitochondrial fission, we think it is likely that Dnm1p also forms rings or collars around mitochondrial tubules at sites where constriction and fission will occur.

Dnm1p Molecular Interactions

As described above, dynamin exists as a tetramer under physiological salt conditions (Hinshaw and Schmid, 1995; Muhlberg *et al.*, 1997) that can further assemble into higher ordered structures (rings/collars). Our coimmunoprecipitation studies indicate that, like dynamin and other dynamin family members, Dnm1p oligomerizes in vivo. Additional two-hybrid analyses also revealed intermolecular interactions of full-length Dnm1p and identified subdomains of the protein required for these interactions. In most cases, the strongest subdomain interaction observed in these assays occurred between any two constructs containing the AH/GED. Consistent with this observation, a previous study reported that the removal of dynamin's GED reduced or abolished dynamin self-interactions in the yeast two-hybrid assay (Okamoto *et al.*, 1999). We also observed self-interactions of Dnm1p's Middle domain. The Middle domain of mammalian dynamin contains a predicted coiled-coil region required for dynamin self-assembly (Okamoto *et al.*, 1999; Smirnova *et al.*, 1999). The equivalent region in Dnm1p's Middle domain may be responsible for the observed Middle–Middle interactions. We currently do not know whether the Dnm1p interactions reported here contribute to the formation of dimers/tetramers, higher ordered structures, or both.

Biochemical studies of dynamin revealed an interaction between the GTPase domain and the GED (Sever *et al.*, 1999; Smirnova *et al.*, 1999). This interaction was shown to stimulate the assembly of dynamin tetramers into rings/collars as well as the GTPase activity of these higher ordered structures (Sever *et al.*, 1999; Smirnova *et al.*, 1999). In contrast, we did not observe stable interactions between Dnm1p's GTPase domain and AH/GED, even when we used longer N- and C-terminal fragments and a mutant form of the GTPase domain predicted to prolong the Dnm1p:GTP-bound state. These data are not surprising because it is unlikely that the two-hybrid assay measures the formation of higher ordered Dnm1p structures and the GTPase domain–GED interaction is predicted to occur in such a higher ordered structure. Our in vivo analysis of Dnm1p AH/GED mutations (see discussion below) may indicate that the AH/GED sequence plays a role in controlling the Dnm1p GTPase cycle (perhaps stimulating GTP hydrolysis). Alternatively, GTPase domain–GED interactions may simply contribute to the formation of Dnm1p dimers/tetramers, higher ordered structures, or both. In either case, it is possible that transient interactions between Dnm1p's GTPase domain and AH/GED region are too weak to be detected by the two-hybrid system.

Two-hybrid analyses of dynamin family members have identified different subdomains that interact (Schumacher and Staeheli, 1998; Shin *et al.*, 1999; Hinshaw, 2000; Zhang *et al.*, 2000). In each case, the interactions detected have been predicted to play a role in oligomerization. These different results may reveal different roles of the various subdomains in each family member. Alternatively, the different results may reflect the limitations of using protein subdomains for interaction studies. For example, in our serial dilution studies and β-galactosidase activity assays, the interaction of full-length Dnm1p with itself was at least 10-fold stronger that the AH/GED self-interaction (10 times more β-galactosidase activity; our unpublished results). One interpretation of these results is that smaller domains of a given protein may fold improperly and fail to interact with other binding partners in the two-hybrid system (or other assays that monitor protein–protein interaction). In support of this interpretation, we previously showed that overexpressing GTPase mutant forms of full-length Dnm1p in a wild-type cell interfered with the function of the endogenous Dnm1 protein and caused dominant defects in mitochondrial fission and morphology (Otsuga *et al.*, 1998). In contrast, overexpressing the subdomains shown in Figure 3A in a wild-type strain had little or no effect on the function of endogenous Dnm1p and did not have striking effects on mitochondrial fission (our unpublished results). This result was surprising and indicated that although all Dnm1p subdomains were able to interact with the full-length Dnm1p construct in the two-hybrid assay (our unpublished results), these subdomains either failed to interact with, or had no effect on the function of, full-length Dnm1p in vivo. Based on these findings, we believe it is most informative to analyze specific domain mutations in the full-length Dnm1 protein expressed in vivo.

Effect of Dnm1p AH/GED Mutations on Mitochondrial Fission In Vivo

According to Sever *et al.* (1999), dynamin's GED is required both for dynamin tetramer assembly into rings/collars and stimulated GTP hydrolysis by these rings/collars during endocytosis. Mutation of arginines and lysines in dynamin's GED (K694A and R725A, equivalent to residues that play a role in rasGAP function) decreased stimulated GTPase activity in vitro. K694 is involved in dynamin tetramer assembly via GED–GED interactions, whereas R725 appears to play a direct role in catalysis. Surprisingly, in vivo analysis indicated that overexpression of both the K694A and R725A GED mutant dynamin proteins increased the rate of formation of constricted coated pits in mammalian cells (Sever *et al.*, 1999, 2000).

Based on these results, Sever *et al.* (1999) suggested that dynamin acts as a regulatory, rather than a mechanochemical, GTPase during endocytosis. A more recent study demonstrated that although dyn (K694A) and dyn (R725A) both increased the rate of formation of constricted, clathrin-coated pits during endocytosis, only dyn (K694A) increased the rate of coated vesicle formation. In contrast, the overall rate of coated vesicle formation decreased in dyn (R725A), suggesting that overexpression of dyn (R725A) may interfere with the GTP-hydrolysis–triggered dynamin disassembly that leads to vesicle fission (Sever *et al.*, 2000).

By analogy with the studies of dyn K694A (Sever *et al.*, 1999, 2000), we expected that the Dnm1p K705A mutant protein would increase the steady-state level of mitochondrial fission. Indeed, the mitochondrial fragmentation we observed when wild-type *DNM1* was overexpressed in yeast increased even further when the mutant K705A pro-

tein was overexpressed in a *dnm1*Δ strain. Thus, like dyn (K694A), which increases the rate of formation of both constricted coated pits and vesicles (Sever *et al.*, 2000), Dnm1p K705A is acting to accelerate a rate-limiting step during mitochondrial fission. Moreover, the Dnm1p K705A mutant protein enhances mitochondrial fission even when it is the only form of Dnm1p expressed in cells. What is the rate-limiting step affected by the K705A mutant protein? Because Dnm1p complexes on mitochondrial tubules are abundant in wild-type yeast cells, it is unlikely that Dnm1p complex assembly is the rate-limiting step in vivo (Otsuga *et al.*, 1998; Mozdy and Shaw, 2000; Tieu and Nunnari, 2000). Instead, we propose that an event after Dnm1p complex assembly, namely, membrane constriction, is accelerated by the mutant Dnm1 K705A protein. If, as predicted by the Sever *et al.* (1999) study, the K705A mutation prolongs the GTP-bound state of Dnm1p, then our results are consistent with the idea that Dnm1p:GTP functions to recruit downstream partners required for membrane remodeling and constriction.

In contrast, we did not observe increased mitochondrial fragmentation in a *dnm1*Δ strain overexpressing Dnm1p R736A. Rather, mitochondrial morphology was shifted toward the *dnm1* null phenotype in these cells. Although this result initially appeared inconsistent with the finding reported by Sever *et al.* (1999), a more recent study (Sever *et al.*, 2000) demonstrated that the early stages of endocytosis (constricted coated pit formation) are stimulated by this mutation, whereas a late step (vesicle formation) is inhibited. Thus, the overall effect of the dyn (R725A) mutation is to decrease the rate of endocytic vesicle formation. Because vesicle formation is analogous to mitochondrial fragmentation in our system, the failure of Dnm1p R736A to increase mitochondrial fragmentation is consistent with the behavior of dyn (R725A) during endocytosis.

While this manuscript was under review, Marks *et al.* (2001) challenged the notions that the dynamin K694A and R725A mutations interfere with assembly-stimulated GTP hydrolysis in vitro and accelerate the rate of endocytosis in vivo. In the Marks *et al.* (2001) study, the GTPase activity of the dynamin K694A and R725A proteins appeared similar to wild-type dynamin. In addition, Marks *et al.* (2001) failed to observe a significant effect on the endocytosis of transferrin when these mutant dynamin GED proteins were overexpressed in COS-7 cells. As reported previously (Herskovits *et al.*, 1993; Damke *et al.*, 1994), overexpression of dynamin mutants deficient in GTP binding/hydrolysis interfered with transferrin internalization in COS-7 cells (Marks *et al.*, 2001). These data suggest that GTP hydrolysis by dynamin is essential during endocytosis and support the idea that dynamin has a mechanochemical function in vesicle scission and release. Clearly, additional studies are required to determine whether dynamin and its related family members act as classical regulatory GTPases, mechanochemical GTPases, or both during membrane remodeling events in cells.

Regardless of the molecular mechanism, our studies support the idea that Dnm1p accelerates a rate-limiting step in mitochondrial fission and that this step is regulated in some manner by the GED sequence. In particular, our in vivo observations with the Dnm1 GED mutant proteins are consistent with those made by Sever *et al.* (1999, 2000) for dynamin proteins containing equivalent GED mutations. The two new components of the Dnm1p fission machinery, Fis1p and Mdv1p, may act together with Dnm1p to accelerate the rate-limiting step in mitochondrial fission. As described above, Mdv1p (Fekkes *et al.*, 2000; Tieu and Nunnari, 2000) appears to assemble with Dnm1p on the outer mitochondrial membrane and is required for the fission activity of Dnm1p complexes. Fis1p is an outer mitochondrial membrane protein required for both the assembly and the fission activity of Dnm1p complexes. The Dnm1p AH/GED mutations described here may reveal subreactions in the mitochondrial fission pathway that require the activities of Mdv1p, Fis1p, or both proteins.

ACKNOWLEDGMENTS

We thank J. Nunnari (University of California, Davis) and members of the Shaw lab for careful review of the manuscript. This work was supported by grants from the American Cancer Society (CB-97) and the National Institutes of Health (GM-53466) awarded to J.M.S., grants from the University of Utah Huntsman Cancer Institute and the University of Utah Research Committee (07119) awarded to N.F. and J.M.S, and a grant from the University of Utah Primary Children's Medical Center Foundation awarded to E.B.

REFERENCES

Bleazard, W., McCaffery, J.M., King, E.J., Bale, S., Mozdy, A., Tieu, Q., Nunnari, J., and Shaw, J.M. (1999). The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. Nat. Cell Biol. *1*, 298–304.

Damke, H., Baba, T., Warnock, D.E., Schmid, S.L. (1994). Induction of mutant dynamin specifically blocks endocytic coated vesicle formation. J. Cell Biol. *127*, 915–934.

de Camilli, P., Takei, K., and McPherson, P.S. (1995). The function of dynamin in endocytosis. Curr. Opin. Neurosci. *5*, 559–565.

Fekkes, P., Shepard, K.A., and Yaffe, M.P. (2000). Gag3p, an outer membrane protein required for fission of mitochondrial tubules [In Process Citation]. J. Cell Biol. *151*, 333–340.

Flohr, F., Schneider-Schaulies, S., Haller, O., and Kochs, G. (1999). The central interactive region of human MxA GTPase is involved in GTPase activation and interaction with viral target structures. FEBS Lett. *463*, 24–28.

Guthrie, C., and Fink, G.R. (1991). Guide to yeast genetics and molecular biology. In: Methods in Enzymology, ed. J.N. Abelson and M.I. Simon, San Diego, CA: Academic Press.

Hermann, G.J., King, E.J., and Shaw, J.M. (1997). The yeast gene, *MDM20*, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. J. Cell Biol. *137*, 141–153.

Hermann, G.J., and Shaw, J.M. (1998). Mitochondrial dynamics in yeast. Annu. Rev. Cell Dev. Biol. *14*, 265–303.

Hermann, G.J., Thatcher, J.W., Mills, J.P., Hales, K.G., Fuller, M.T., Nunnari, J., and Shaw, J.M. (1998). Mitochondrial fusion in yeast requires the transmembrane GTPase Fzo1p. J. Cell Biol. *143*, 359–373.

Herskovits, J.S., Burgess, C.C., Obar R.A., and Vallee, R.B. (1993). Effects of mutant rat dynamin on endocytosis. J. Cell Biol. *122*, 565–578.

Hinshaw, J.E. (2000). Dynamin and its role in membrane fission. Annu. Rev. Cell Dev. Biol. *16*, 483–519.

Hinshaw, J.E., and Schmid, S.L. (1995). Dynamin self-assembles into rings suggesting a mechanism for coated vesicle budding. Nature *374*, 190–192.

James, P., Halladay, J., and Craig, E.A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics *144*, 1425–1436.

Kelly, R.B. (1999). New twists for dynamin. Nat. Cell Biol. *1*, E8–E9.

Labrousse, A.M., Zappaterra, M.D., Rube, D.A., and van der Bliek, A.M. (1999). *C. elegans* dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. Mol. Cell *4*, 815–826.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Marks, B., Stowell, M.H.B., Vallis, Y., Mills, I.G., Gibson, A., Hopkins, C.R., and McMahon, H.T. (2001). GTPase activity of dynamin and resulting conformation change are essential for endocytosis. Nature *410*, 231–235.

Melen, K., Ronni, T., Lotta, T., and Julkunen, I. (1994). Enzymatic characterization of interferon-induced antiviral GTPases murine Mx1 and human MxA proteins. J. Biol. Chem. *269*, 2009–2015.

Mozdy, A.D., McCaffery, J.M., and Shaw, J.M. (2000). Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. J. Cell Biol. *151*, 367–80.

Muhlberg, A.B., Warnock, D.E., and Schmid, S.L. (1997). Domain structure and intramolecular regulation of dynamin GTPase. EMBO J. *16*, 6676–6683.

Nunnari, J., Marshall, W.F., Straight, A., Murray, A., Sedat, J.W., and Walter, P. (1997). Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. Mol. Biol. Cell *8*, 1233–1242.

Okamoto, P.M., Tripet, B., Litowski, J., Hodges, R.S., and Vallee, R.B. (1999). Multiple distinct coiled-coils are involved in dynamin self-assembly. J. Biol. Chem. *274*, 10277–10286.

Otsuga, D., Keegan, B.R., Brisch, E., Thatcher, J.W., Hermann, G.J., Bleazard, W., and Shaw, J.M. (1998). The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. J. Cell Biol. *143*, 333–349.

Schmid, S.L. (1997). Clathrin-coated vesicle formation and protein sorting: an integrated process. Annu. Rev. Biochem. *66*, 511–548.

Schneider, B.L., Seufert, W., Steiner, B., Yang, Q.H., and Futcher, A.B. (1995). Use of polymerase chain reaction epitope tagging for protein tagging in *Saccharomyces cerevisiae*. Yeast *11*, 1265–1274.

Schumacher, B., and Staeheli, P. (1998). Domains mediating intramolecular folding and oligomerization of MxA GTPase. J. Biol. Chem. *273*, 28365–28370.

Sesaki, H., and Jensen, R.E. (1999). Division versus fusion: Dnm1p and Fzo1p antagonistically regulate mitochondrial shape. J. Cell Biol. *147*, 699–706.

Sever, S., Damke, H., and Schmid, S.L. (2000). Dynamin:GTP controls the formation of constricted coated pits, the rate limiting step in clathrin-mediated endocytosis. J. Cell Biol. *150*, 1137–1148.

Sever, S., Muhlberg, A.B., and Schmid, S.L. (1999). Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis. Nature *398*, 481–486.

Sherman, F., Fink, G.R., and Hicks, J.B. (1986). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Press.

Shin, H.W., Takatsu, H., Mukai, H., Munekata, E., Murakami, K., and Nakayama, K. (1999). Intermolecular and interdomain interactions of a dynamin-related GTP-binding protein, Dnm1p/Vps1p-like protein. J. Biol. Chem. *274*, 2780–2785.

Smirnova, E., Griparic, L., Shurland, D.-L., and van der Bliek, A.M. (2001). The Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol. Biol. Cell *12*, 2245–2256.

Smirnova, E., Shurland, D.L., Newman-Smith, E.D., Pishvaee, B., and van der Bliek, A.M. (1999). A model for dynamin self-assembly based on binding between three different protein domains. J. Biol. Chem. *274*, 14942–7.

Tieu, Q., and Nunnari, J. (2000). Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, dnm1p, to trigger mitochondrial division. J. Cell Biol. *151*, 353–366.

van der Bliek, A.M. (1999a). Functional diversity in the dynamin family. Trends Cell Biol. *9*, 96–102.

van der Bliek, A.M. (1999b). Is dynamin a regular motor or a master regulator? Trends Cell Biol. *9*, 2–3.

Warnock, D.E., Hinshaw, J.E., and Schmid, S.L. (1996). Dynamin self-assembly stimulates its GTPase activity. J. Biol. Chem. *271*, 22310–22314.

Winston, F., Dollard, C., and Ricupero-Hovasse, S.L. (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S228C. Yeast *11*, 53–55.

Yang, W., and Cerione, R.A. (1999). Endocytosis: is dynamin a 'blue collar' or 'white collar' worker? Curr. Biol. *9*, R511–R514.

Yoon, Y., Pitts, K.R., and McNiven, M.A. (2001). The mammalian dynamin-like protein DLP1 tubulates membranes. Mol. Biol. Cell *12*, 2894–2905.

Zhang, Z., Hong, Z., and Verma, D.P. (2000). Phragmoplastin polymerizes into spiral coiled structures via intermolecular interaction of two self-assembly domains. J. Biol. Chem. *275*, 8779–8784.