

# Structural basis for recruitment of mitochondrial fission complexes by Fis1

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**Mitochondrial fission controls mitochondrial shape and physiology, including mitochondrial remodeling in apoptosis. During assembly of the yeast mitochondrial fission complex, the outer membrane protein Fis1 recruits the dynamin-related GTPase Dnm1 to mitochondria. Fis1 contains a tetratricopeptide repeat (TPR) domain and interacts with Dnm1 via the molecular adaptors Mdv1 and Caf4. By using crystallographic analysis of adaptor-Fis1 complexes, we show that these adaptors use two helices to bind to both the concave and convex surfaces of the Fis1 TPR domain. Fis1 therefore contains two interaction interfaces, a binding mode that, to our knowledge, has not been observed previously for TPR domains. Genetic and biochemical studies indicate that both binding interfaces are important for binding of Mdv1 and Caf4 to Fis1 and for mitochondrial fission activity *in vivo*. Our results reveal how Fis1 recruits the mitochondrial fission complex and will facilitate efforts to manipulate mitochondrial fission.**

apoptosis | mitochondrial division | mitochondrial dynamics | tetratricopeptide repeat

Mitochondrial dynamics has emerged as an important process controlling mitochondrial shape, size, distribution, and physiology (1, 2). Mitochondrial fission, balanced by the opposing process of fusion, controls the morphology of mitochondria. Increased fission leads to mitochondrial fragmentation, whereas reduced mitochondrial fission causes elongation and increased connectivity of mitochondria.

Recent results indicate that mitochondrial fission has important physiological functions. In humans, loss of mitochondrial fission results in neonatal lethality with microcephaly, developmental delay, and metabolic aberrations (3). Defects in mitochondrial fission also disrupt mitochondrial distribution in neurons and result in defective synaptic transmission (4). Moreover, mitochondrial fission regulates apoptosis in yeast (5), worms (6), flies (7, 8), and mammals (9). In the early stages of apoptosis, recruitment of fission complexes to mitochondria is increased. The increase in fission leads to mitochondrial fragmentation, which appears to be important for execution of death programs (10). Therefore, a structural and mechanistic understanding of mitochondrial fission may facilitate efforts to regulate apoptosis.

The molecular basis for recruitment of the mitochondrial fission machinery is best understood in budding yeast (2). Mitochondrial fission complexes assemble on the mitochondrial outer membrane protein Fis1 (11–13). Fis1 mediates the assembly of fission complexes consisting of adaptors (Mdv1 or Caf4) and the Dnm1 GTPase, a dynamin-related protein (14–17). Dnm1 is predominantly found in puncta on the mitochondrial surface, and these puncta mark potential sites of future fission. Mdv1p and Caf4p are soluble proteins containing an N-terminal extension, a coiled-coil region, and a COOH-terminal seven-WD40 repeat domain. Acting as molecular adaptors, these proteins bind to Fis1p through the N-terminal extension region and to Dnm1p through the WD40 region (14, 15, 17). Mdv1 and Caf4 are redundant in their ability to work with Fis1 to recruit Dnm1 to mitochondria. *mdv1Δ* and *caf4Δ* mutants show largely normal Dnm1 localization, but *mdv1Δ caf4Δ* mutants, like *fis1Δ*

mutants, show cytosolic localization of Dnm1 (15). Mdv1 is more active than Caf4 in promoting fission.

Because Fis1 initiates assembly of the mitochondrial fission complex, an important mechanistic issue is how Fis1 binds its ligands, Mdv1 and Caf4. The cytosolic domain of Fis1 forms a six-helix bundle, in which the central four helices consist of two tandem tetratricopeptide repeat (TPR)-like motifs. The TPR is a helix-turn-helix motif that is typically organized into a tandem array (18). Such tandem TPR motifs make a right-handed superhelical structure with a concave surface. In all of the solved TPR domains bound to ligand, the ligands are bound in a hydrophobic groove on the concave surface (18–21). The ligands are either helical or in an extended conformation with their axes parallel to the hydrophobic groove.

Given this strong precedent, it has been widely assumed that Fis1 would also bind its ligands through the hydrophobic groove identified on the concave surface of the TPR fold (22–24). In yeast Fis1, surprisingly, the extreme N-terminal residues (termed the N-terminal arm) form a short helix that lies in this predicted ligand-binding site. Therefore, it has been proposed that the N-terminal arm of yeast Fis1 might regulate the binding of Mdv1 and Caf4 to the concave surface (24). Indeed, the N-terminal arm of yeast Fis1 is important for Fis1 function (16, 24). We determined the atomic structure of a Fis1/Mdv1 and a Fis1/Caf4 complex. These structures reveal, in contrast to the previous models, that the TPR domain of Fis1 is used in a unique way to recruit Mdv1 and Caf4. These ligands use a helix-loop-helix motif to pack against both the concave and convex surfaces of the Fis1 TPR domain. Cellular analyses support the physiological significance of these dual molecular interactions. Sequence analysis indicates that mammalian Fis1 probably uses a similar strategy to bind ligands during apoptosis.

## Results

**Fis1 Binds Ligands in a Manner Distinct from Other TPR Domain Proteins.** To understand how Fis1 interacts with the two adaptor proteins, we crystallized complexes of Fis1 (the cytosolic domain, residues 1–129) bound to an N-terminal fragment of Mdv1 (residues 122–171; 2.15 Å) or Caf4 (residues 81–140; 1.9 Å) (Table 1). The binding of Fis1 to adaptors contained several unanticipated features. The Fis1 structures in the two complexes are almost identical to unliganded Fis1 (24). In all cases, helices  $\alpha 1$ – $\alpha 6$  formed the core six-helix bundle, with  $\alpha 2/\alpha 3$  and  $\alpha 4/\alpha 5$  forming tandem TPR-like motifs that lined a hydrophobic,

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Abbreviation: TPR, tetratricopeptide repeat.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, [www.pdb.org](http://www.pdb.org) (PDB ID codes 2PQN and 2PQR).

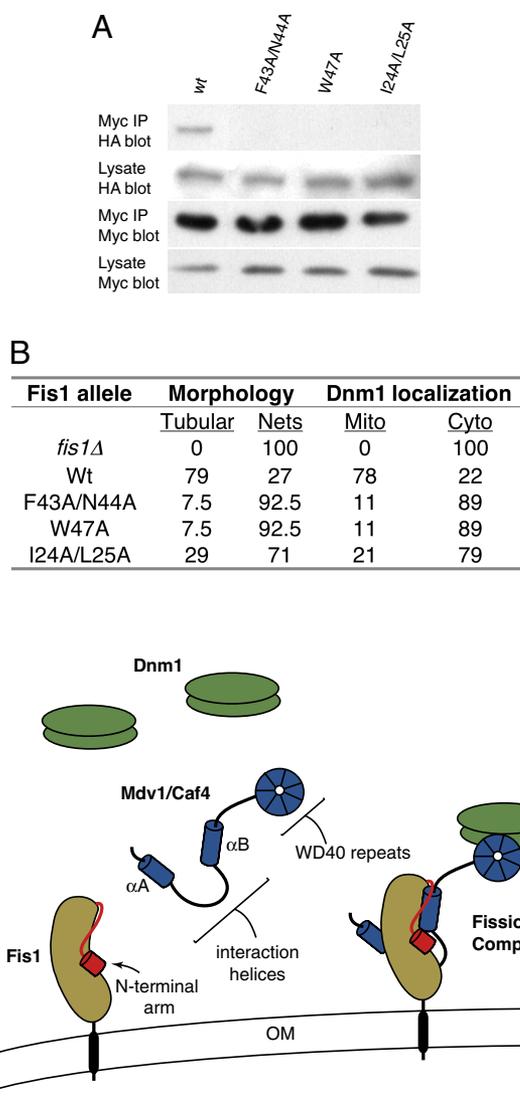
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**Fig. 4.** Surface residues essential for Fis1 function. (A) Immunoprecipitation of HA-tagged Mdv1 by Myc-tagged Fis1 containing mutations in surface residues contacting the  $\alpha$ B helix of adaptors. wt, wild type. (B) Ability of Fis1 mutants to rescue mitochondrial morphology and Dnm1 recruitment in *fis1Δ* yeast. Mitochondrial morphology was scored by using mito-DsRed. Subcellular localization of GFP-Dnm1 puncta was scored by comparison with mito-DsRed. (C) Schematic of fission complex recruitment by Fis1. In the unliganded state (shown at left), Fis1 resides in the mitochondrial outer membrane (OM) with its N-terminal arm (red region) packed against the concave surface of the TPR domain. The adaptors Mdv1 and Caf4 use two N-terminal helices to bind Fis1. The Fis1 N-terminal arm stabilizes adaptor binding by interacting with the  $\alpha$ B helix (complex shown at right). The WD40 region of the adaptors then recruits Dnm1 to the mitochondria. Dnm1 exists as dimers and is activated by assembly into higher-order oligomers (not shown).

**Fis1 Residues Lining the  $\alpha$ B-Binding Groove Are Essential for Mitochondrial Fission and Recruitment of Mdv1 and Dnm1.** Our structural studies indicated that helix  $\alpha$ B in Mdv1 and Caf4 bound to the concave face of Fis1 but to a different hydrophobic groove than previously predicted. To experimentally evaluate the functional significance of this Fis1 groove, we mutated key Fis1 residues lining this groove to alanine (I24A/L25A, F43A/N44A, and W47A). In contrast to wild-type Fis1, none of the Fis1 mutants was able to coimmunoprecipitate Mdv1 (Fig. 4A). Moreover, each of the Fis1 mutants showed greatly reduced ability to support mitochondrial fission when expressed in *fis1Δ* cells (Fig.

4B). Consistent with this loss of mitochondrial fission activity, the Fis1 mutants were unable to recruit Dnm1 puncta to mitochondria (Fig. 4B). Because Mdv1 and Caf4 play redundant roles in Dnm1 recruitment to mitochondria (15), these results indicate that Fis1 with mutations at the  $\alpha$ B-binding site are deficient for binding both Mdv1 and Caf4, as predicted by our crystal structures.

## Discussion

Mitochondrial fission is a conserved, fundamental cellular process that regulates mitochondrial shape and function. Fis1 anchors fission complexes onto the mitochondrial outer membrane. In yeast, Mdv1 and Caf4 bridge the interaction between Fis1 and Dnm1, a mechanochemical enzyme thought to constrict mitochondrial tubules during fission. Our current results reveal the structural basis of recruitment of these adaptor molecules by Fis1. Caf4, and by inference Mdv1, uses a helix-loop-helix motif to clasp both the concave and convex surfaces of Fis1 (Fig. 4C). Biochemical and cytological studies demonstrate that key interactions revealed in the structures are critical for maintaining assembly of mitochondrial fission complexes and normal mitochondrial morphology *in vivo*. Our conclusions are supported by previous work showing that Mdv1L148P, analogous to our  $\alpha$ B helix mutant L148A (Fig. 3), is defective for Fis1 binding, mitochondrial localization, and fission activity (25).

These structural insights clarify previous findings. In the unliganded structure of yeast Fis1, it has been perplexing that the N-terminal arm lies in the presumed ligand-binding site (24). This observation has led to suggestions that the N-terminal arm likely relocates to allow ligand binding (16, 24). In contrast, our structures indicate that the presence of the N-terminal arm is fully compatible with Mdv1 or Caf4 binding, because these ligands bind unexpectedly to an adjacent site. Deletion of the N-terminal arm of Fis1 greatly reduces mitochondrial fission (16, 24), but this defect can be complemented by overexpression of Mdv1 (16). Based on the structural data here, helix  $\alpha$ B of Mdv1 binds to a different hydrophobic groove than anticipated, and this interaction is facilitated by packing interactions against the N-terminal arm of Fis1 (Fig. 4C). Therefore, deletion of the N-terminal arm would weaken Mdv1 binding, a defect that can be overcome by increasing the concentration of Mdv1.

In addition to their biological significance, Fis1/Mdv1 and Fis1/Caf4 complexes also display distinctive structural features. TPR motif is widely involved in protein-protein interactions. The interface between known TPR-containing proteins and their partner proteins/peptides are located only in the concave surface formed by TPR motifs (18). In contrast, both TPR surfaces of Fis1 are used to bind ligands. This binding mode increases the interaction interface between the two proteins, resulting in stabilization of the complex. The Fis1/Mdv1 and Fis1/Caf4 structures therefore reveal the versatility of the TPR fold in mediating protein-protein interactions.

A central issue concerning mitochondrial fission is whether mammalian Fis1 functions in the same manner as yeast Fis1 to recruit Drp1 (the mammalian ortholog of Dnm1) to mitochondria. During apoptosis, Drp1 recruitment is enhanced, resulting in activation of mitochondrial fission during the early stages of cell death (9, 10). Sequence alignment indicates that many of the yeast Fis1 residues contacted by the adaptors are conserved in mammalian Fis1 [supporting information (SI) Fig. 5A]. Whereas these Fis1 orthologs have an overall identity of 24%, residues involved in ligand binding are 44% identical. In addition, a comparison of yeast and human Fis1 structures suggests that both binding grooves are conserved (SI Fig. 5B and C). This suggests that mammalian Fis1 has a protein ligand that binds in a similar manner as Mdv1/Caf4 to yeast Fis1. Our results therefore identify residues on the surface of mammalian Fis1 likely to mediate ligand binding and will facilitate efforts to

identify the mechanism of Drp1 recruitment to mitochondria during apoptosis. Moreover, they will likely facilitate efforts to manipulate mitochondrial fission artificially.

## Methods

**Cloning and Protein Expression.** Fis1 (1–129) was amplified by PCR (primers, 5'-AAAAACATATGACCAAAGTAGATTTT-TGG-3' and 5'-TTTTTCTCGAGGAGTGTTCCTTCTG-GAT-3'), digested with NdeI and EcoRI, and cloned into the corresponding sites in the pBB75 vector. Mdv1 (122–171) was amplified by PCR (primers, 5'-GCACATATGGATGCAGAT-GGCAAGCTTCTA-3' and 5'-GCCGGATCCCTAATAGTT-TAATCTTTCAGTGTTTTC-3'), digested with NdeI and BamHI, and cloned into the NdeI/BglII sites of the pET15 vector, which encodes an N-terminal His<sub>6</sub> tag. In a similar strategy, Caf4 (81–140) was amplified (primers, 5'-GGGAT-CATATGCAGAAAGGACAAGTAGGG-3' and 5'-GCCA-GATCTCTATCTCTGTTGAATGATGGAAACGG-3') and cloned into the pET15 vector.

Fis1/Mdv1 and Fis/Caf4 complexes coexpressed in Rosetta (DE3) cells were purified by Ni<sup>2+</sup>-nitrilotriacetic acid chromatography, followed by cleavage of the His tag with thrombin. The protein complexes were further purified to >95% purity with ion exchange and gel filtration chromatography. The purified protein complexes were dialyzed into 20 mM Tris (pH 8).

**Crystallization and Structure Determination.** All of the crystallization trials were carried out at 22°C by using the hanging-drop vapor diffusion method. One microliter of the protein complex was mixed with an equal volume of reservoir solution and equilibrated against 400- $\mu$ l reservoir solution. The reservoir solution for the Fis1/Mdv1 crystals contained 20% PEG3350 and 0.1 mM Tris (pH 8.0). The reservoir solution for the Fis1/Caf4 crystals contained 16% PEG3350 and 0.2 mM NaH<sub>2</sub>PO<sub>4</sub>. Phases were determined by molecular replacement by using the NMR structure of yeast Fis1 (24) as the starting model. Details of the crystallographic analysis and statistics are presented in Table 1.

**Yeast Two-Hybrid Assay.** Fis1 (1–127) was cloned into the EcoRI and BglII sites of pGAD-C1. Mdv1 (1–300), Mdv1 (88–108), Mdv1 (142–164), Mdv1 (88–164), Caf4 (1–251), Caf4 (90–109), Caf4 (122–142), and Caf4 (90–142) were cloned into EcoRI and BglII sites of pGBDU-C1. Mdv1 (301–714) and Caf4 (251–659)

were cloned into the ClaI and SalI sites of pGBDU-C1. Two-hybrid analysis was performed as described previously (15). pGAD-C1 vectors containing cytosolic portion of Fis1 were transformed into PJ69–4 $\alpha$  cells. pGBDU-C1 vectors containing Mdv1 and Caf4 fragments were transformed into PJ69–4 $\alpha$  cells. Transformants for each vector were mated on YPD plates. Diploids were selected by replica plating to SD plates lacking leucine, uracil, and lysine. Positive interactions were detected by growing on replica SD plates lacking adenine, leucine, uracil, and lysine.

**Coimmunoprecipitation.** Wild-type and mutants of Myc-Fis1, HA-Mdv1, and HA-Caf4 were expressed from endogenous promoters in integrating vectors. The transformed yeast cells were grown in yeast extract/peptone/dextrose media and harvested at an OD<sub>600</sub> of  $\approx$ 1.0. Coimmunoprecipitations were performed as described previously (15). Briefly, the cells were lysed by using glass beads. The cleared cell lysate was applied to 9E10-conjugated protein A-Sepharose beads and incubated at 4°C for 90 min. The cell lysates and eluted protein were subjected to Western blotting by using 9E10 hybridoma supernatant (anti-Myc) or 12CA5 ascites fluid (anti-HA).

**Mitochondrial Morphology Analysis.** Yeast strains for morphology analysis contained mitochondrially targeted GFP or DsRed. Cells were grown to midlog phase and fixed by 3.7% formaldehyde at 30°C for 10 min. The cells were washed four times with PBS and scored for mitochondrial morphology. To determine the localization of Mdv1 mutants, mutations were introduced into the plasmid pRS416MET25 + GFP-MDV1 (a gift from J. M. Shaw, University of Utah, Salt Lake City, UT) and analyzed in yeast expressing mito-DsRed. In Fis1 mutant cells, GFP-Dnm1 localization was scored by comparison with mito-DsRed. When >50% of GFP-Dnm1 puncta were on mitochondria, it was scored as mitochondrially localized.

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