Novel Structure of the N Terminus in Yeast Fis1 Correlates with a Specialized Function in Mitochondrial Fission*

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Mitochondrial fission is facilitated by a multiprotein complex assembled at the division site. The required components of the fission machinery in Saccharomyces cerevisiae include Dnm1, Fis1, and Mdv1. In the present study, we determined the protein structure of yeast Fis1 using NMR spectroscopy. Although the six α-helices, as well as their folding, in the yeast Fis1 structure are similar to those of the tetratricopeptide repeat (TPR) domains of the human Fis1 structure, the two structures differ in their N termini. The N-terminal tail of human Fis1 is flexible and unstructured, whereas a major segment of the longer N terminus of yeast Fis1 is fixed to the concave face formed by the six α-helices in the TPR domains. To investigate the role of the fixed N terminus, exogenous Fis1 was expressed in yeast lacking the endogenous protein. Expression of yeast Fis1 protein rescued mitochondrial fission in Δfis1 yeast only when the N-terminal TPR binding segment was left intact. The presence of this segment is also correlated to the recruitment of Mdv1 to mitochondria. The conformation of the N-terminal segment embedded in the TPR pocket indicates an intra-molecular regulation of Fis1 bioactivity. Although the TPR-like helix bundle of Fis1 mediates the interaction with Mdv1 and Mdv1, the N terminus of Fis1 is a prerequisite to recruit Mdv1 to facilitate mitochondrial fission.

Mitochondria are dynamic organelles that change their morphology by fusion and fission. Such processes, apparently counteracting each other, are facilitated by two independent molecular machineries. Mitochondrial outer membrane fusion is regulated by the integral membrane proteins Mfn1 and Mfn2 in the case of mammals (1) or Fzo1 in the case of yeast (2, 3). These proteins span the mitochondrial outer membranes twice, exposing an N-terminal GTPase domain and a C-terminal coiled-coil domain to the cytosol. A recent study of mouse Mfn1 revealed that the C-terminal coiled-coil domain points outward from mitochondrial membranes to form homodimers in an antiparallel fashion, which is implicated in tethering of two mitochondria together at a distance of about 10 nm (4). Subsequent inner membrane fusion is mediated by a protein called OPA1 in the case of mammals (5, 6) or Mgm1 in yeast (7, 8), which localizes to the inner membrane with the GTPase domain facing the intermembrane space. Mitochondrial fission is mediated by a dynamin-related protein, Drp1, identified as Dnm1 in yeast (9–11). Drp1 and Dnm1 localize in the cytosol as well as in foci at the dividing site of the mitochondrial outer membrane surface during mitochondrial fission (9, 12). In contrast to proteins that are involved in fusion, Drp1 and Dnm1 do not span membrane bilayers.

Drp1- and Dnm1-mediated mitochondrial fission is achieved with other accompanying proteins. Genetic studies of Saccharomyces cerevisiae identified Mdv1 and Fis1 as proteins involved in the Dnm1-dependent fission process (13, 14). Mdv1 is a peripheral membrane protein that localizes with Dnm1 at punctate structures along the mitochondrial outer membranes to regulate Dnm1. Fis1 is an integral membrane protein that is uniformly distributed along the mitochondrial outer membranes that is required for immobilization of Dnm1 and Mdv1. A high molecular weight complex of Dnm1, Mdv1, and Fis1 constructs the punctate structures at the dividing site and facilitates constriction and division of mitochondrial membrane (13, 14). Mdv1 orthologs have not been identified in nematode, fruit fly, or vertebrates, and it is not known whether another protein takes its place or whether the molecular machinery of mitochondrial fission in the various species is different. In contrast, human and mouse orthologs of Fis1 have been identified, suggesting that the role of Fis1 in mitochondrial fission is conserved among lower and higher eukaryotes. Although increased levels of human Fis1 in mammalian cells may (15–18) or may not (19) accelerate mitochondrial fission, a reduction in human Fis1 level results in notable extensions in the length of mitochondria (17, 18, 20), indicating that, as in yeast, Fis1 is required for mitochondrial fission in mammals. However, the regulation of Drp1- and Fis1-mediated mitochondrial fission remains unclear in both yeast and mammals.

Comparing orthologs is one way to help understand the general scheme of protein function as well as their molecular evolution. In previous studies (19, 21), it was reported that human Fis1 assembles into a novel tetratricopeptide repeat (TPR)1-like helix bundle, and it was suggested, based on similarity to other TPR domain proteins, that its concave surface may provide a means to recruit other proteins such as Drp1.

1 The abbreviations used are: TPR, tetratricopeptide repeat; NOE, nuclear Overhauser effects; NOESY, NOE spectroscopy; PDB, Protein Data Bank.
Here we present the three-dimensional protein structure of yeast Fis1 and show that, in contrast to human Fis1, an extended N-terminal domain binds to the concave surface of the TPR motif. We also show that this self-interacting region of the N terminus that is absent in human Fis1 is required for yeast Fis1 bioactivity.

**MATERIALS AND METHODS**

**Recombinant Protein**—The protein corresponding to residues 1–138 of yeast Fis1 was derived from NMR studies. The residues 139–152, half of the membrane-spanning domain and residues that face the mitochondrial intermembrane space, were excluded to solubilize the recombinant protein. The cDNA of Fis1 was cloned from *S. cerevisiae* genomic DNA library (American Type Culture Collection). NdeI and XhoI sites were introduced next to the initiation and stop codons of the cDNA, respectively, and inserted into pET-17b (Novagen Inc.). Then, the fragment corresponding to nucleotides 1–415 was excised using NdeI and Rsal. pET21b was treated with XhoI followed by Mung bean nuclease and then with NdeI. The excised DNA and the digested pET21b vector were ligated. The resulting plasmid encodes residues Met1–Val138 of Fis1 linked to the C-terminal hexa histidine tag and thus was termed pET21-yFis1-His6. *Escherichia coli* (Novagen Inc.) harboring pET-21a (kindly provided by Dr. Bruceenda) was cultured in Terrific Broth (MTT-250 or MTT-500 Vacuolization, Stable Isotope) to produce uniformly ^15^N-labeled and uniformly ^15^N-,^13^C-labeled protein, respectively. The recombinant protein was isolated from the cytosol by metal chelate affinity chromatography on a Ni^{2+}-resin column (Novagen Inc.) using 20 mm Tris-HCl, pH 7.9, 500 mm NaCl, or 0.1% 100 mm EDTA and then further purified by collecting the flow-through from an ion-exchange chromatography on a mono-Q column (Amersham Biosciences) using 20 mm Tris-HCl, pH 8.9, without NaCl. No detergents were used in any step of the protein purification. All NMR samples contained 0.5–1.0 mm protein in 10 mm Tris acetate, pH 5.5, in 90% H2O/10% D2O or 100% D2O. The recombinant protein of yeast Fis1 for the study of backbone dynamics was prepared as described previously (19).

**NMR Spectroscopy**—All NMR spectra were acquired at 32°C on Bruker 600 or 800 MHz NMR spectrometers. The spectra were processed using the NMRPipe (22) and analyzed with PIPPA (23). The following experiments were used for assignments of H, ^13^C, and ^15^N resonances: CBCA(CO)NH (24), HNCACB (25), HBHA(CO)NH (26), CBCAC(CO)N (27), and three-dimensional HCCH-TOCSY (28). For stereospecific assignment of methyls of leucines and valines, a ^13^C/ ^15^N-CT-HSQC experiment (29) was carried out using the protein obtained from a bacterial culture using a minimal medium containing 10% [U-^13^C]glucose-90% [U-^15^N]glucose (30). Proton homonuclear nuclear Overhauser effects (NOEs) were obtained using three-dimensional ^13^C-labeled NOESY (31), four-dimensional ^13^C/^15^N-labeled NOESY (31), and four-dimensional ^13^C/^13^C-labeled NOESY (32) experiments. Residual dipolar couplings for N-H and C-H\(^{\text{a}}\) were calculated from the difference in corresponding NOE cross-peaks measured in the presence and absence of dipolar couplings (31 mg/ml in 150 nmol NaCl) (33). A two-dimensional IPAP ^13^N-H HSQC experiment (34) was used to obtain the one-bond N-H scalar couplings. A CT-(H)CA(CO)NH experiment (35) was used to obtain the one-bond C-H\(^{\text{a}}\) scalar couplings. Relaxation values (^15^N T1 and ^1^H T1) for backbone amides were calculated from the peak intensities measured using conventional pulse programs (36). Steady state heteronuclear NOE for the backbone amides was derived from the ratio of peak intensities of experiments performed with and without ^1^H-preparation using a reported pulse program and corrected for sample errors caused by incomplete ^1^H magnetization recovery (37). The measurements were repeated twice.

**Structure Calculation**—Peak intensities from NOEY experiments were translated into a continuous distribution of proton-proton distance restraints, 124 hydrogen bond distance restraints, 92 φ and ψ angle restraints, and 90 N-H and 41 C-H\(^{\text{a}}\) dipolar couplings. The TALOS program (38) predicted the backbone dihedral angles (φ, ψ) from ^13^C\(^{\text{a}}\), ^13^C, ^15^N, ^1^H, and ^13^C\(^{\text{a}}\) chemical shifts. Statistically significant angles were used as structural restraints with at least 20° margins. Residual dipolar coupling restraints were separated into mobile and rigid regions determined based on the backbone dynamic data. Structures of yeast Fis1 were calculated by a geometry distance and simulated annealing protocol (39) with the incorporation of dipolar coupling restraints (40) using the program XPLOR-NIH (41). Structure calculations employed 2235 inter-residue and 803 intra-residue proton-proton distance restraints, 124 hydrogen bond distance restraints, 92 φ and ψ φ angle restraints, and 90 N-H and 41 C-H\(^{\text{a}}\) dipolar couplings.

**RESULTS**

**Structure Description of Yeast Fis1**—The NMR derived structures of yeast Fis1 are presented in Fig. 1. The core domain of Fis1 consists of six α-helices. The six α-helices are determined based on a combination of NMR data (Fig. 2), including midrange NOE patterns and secondary chemical shifts. We define helix α1 for residues Pro19–Ser21, α2 for Ile39–Lys41, α3 for Val55–Lys70, α4 for Arg76–Lys89, α5 for Tyr93–Glu105, and α6 for Thr112–Ser120.
The first 6 residues of the N-terminal tail do not adopt an ordered conformation. Their $^{13}$C$_{\alpha}$ and $^{13}$C$_{\beta}$ chemical shifts are close to their random coil values, and no $^1$H-$^2$H NOE of midrange or long range can be observed. The region corresponding to residues Leu$^{10}$–Tyr$^{14}$ shows characteristics of an $\alpha$-helix, such as secondary chemical shifts and NOEs between $H^N(i)$ and $H^N(i + 3)$ and between $H^N(i)$ and $H^N(i + 3)$. However, as NOEs between $H^N(i)$ and $H^N(i + 4)$ are absent, this region is not defined to be an $\alpha$-helix. The C-terminal 17 residues of the recombinant protein, which include the hydrophobic region and the artificial His tag, show a disordered conformation in solution. Their secondary chemical shifts for $^{13}$C$_{\alpha}$ and $^{13}$C$_{\beta}$ are close to zero, and no long range $^1$H-$^1$H NOE are observed.

As seen in the ensemble of the 20 lowest energy structures (Fig. 1A), the C-terminal residues Thr$^{9}$–Glu$^{127}$ converged into a well defined conformation. The atomic root mean square deviation about the mean of coordinates for six $\alpha$-helices of the 20 structures was 0.4 ± 0.1 Å for backbone heavy atoms and 1.0 ± 0.1 Å for all heavy atoms.

A data base search performed using the DALI program for structural similarities (43) revealed, at the top of the list, 1YG, the structure of mouse Fis1 as a representative structure of Fis1 orthologs. Currently, there are three PDB depositions for Fis1 orthologs: 1PC2 is the solution structure of human Fis1 (19), 1NZN is the crystal structure of human Fis1 (21), and 1IYG is the solution structure of mouse Fis1. The core domain of human, mouse, and yeast Fis1 contains six $\alpha$-helices with the same folds. Due to the high degree of structural similarity between yeast and mouse Fis1, the rest of the list from the DALI search is the same as the list that we obtained previously using the human Fis1 structure, 1PC2, as a query (19). Namely, the overall fold of the six $\alpha$-helices in yeast Fis1 is similar to the fold of helices composed of the TPR motif, although, like other Fis1 sequences, no significant sequence similarity to the typical TPR motif is found within the TPR-like core domain of yeast Fis1. The typical TPR motif contains degenerate 34-amino-acid sequences with 8 loosely conserved consensus residues ($X_3$WLF$X_2$LIM$X_2$GAS$X_2$YLF$X_2$ASE$X_3$$X_3$FVL$X_2$ASL1$X_3$PK$X_7$) and usually presents in a tandem array of multiple copies (44). The TPR motif is found in a number of functionally different proteins. The TPR-containing domains facilitate specific protein-protein interactions at the concave surfaces, although the common features of the interaction partners have not been defined. The structural analogy of yeast Fis1 to the typical TPR-containing proteins, although the protein sequences are discrete, suggests that yeast Fis1 may bind to other proteins at its concave protein surface.

**Three-dimensional structure of yeast Fis1**—To evaluate the structural conservation between yeast and human Fis1, we compared these two structures (Fig. 3). The core domain of both yeast and human Fis1 consists of six $\alpha$-helices. The length of the $\alpha$-helix in yeast Fis1 is shorter than that in human Fis1 (Fig. 3A). For the other five $\alpha$-helices, the lengths of corresponding helices in yeast and human Fis1 are the same. Moreover, the folds of six $\alpha$-helices are similar (Fig. 3, B and C), as indicated by the data base search described in the previous section. The pairwise root mean square difference, calculated using the backbone atoms (N, C$_{\alpha}$, C$_{\beta}$, and O) of the corresponding six helices for yeast and human Fis1, is 1.8 Å. Among the six $\alpha$-helices from the two structures, the $\alpha_1$-helix shows a major difference in its location; it is shifted along its axis and results in the largest deviation in the pairwise comparison. We previously found that human Fis1 contains two TPR-like motifs, where the $\alpha$-loop-$\alpha$ motif forms one TPR-like motif and the $\alpha$-loop-$\alpha$ motif forms the other. The two loops in the yeast Fis1 structure (one between $\alpha_2$ and $\alpha_3$, and the other between $\alpha_4$ and $\alpha_5$) show the same conformation as those in the human Fis1 structure. The pairwise comparison of overall folds between yeast and human Fis1 using DALI results in the Z-score of 12.

A major difference between the yeast and human Fis1 structures is the N terminus prior to the $\alpha_1$-helix. The N-terminal tail of yeast Fis1 is located at the concave side of the helix bundle (Fig. 1), whereas that of human Fis1 is not. A difference can also be seen in the backbone dynamics of the region (Fig. 4). In the human Fis1 structure, the entire N-terminal tail is flexible (Fig. 4A). In contrast, the N terminus of the yeast Fis1, which is longer than that of human Fis1 by 8 residues (Fig. 3A), is made of two parts with different characteristics (Fig. 4B). The first portion from the N-terminal end (Met$^1$–Phe$^6$) is flexible, and the second portion (Trp$^7$–Tyr$^{10}$) is rigid. It is not clear...
where the boundary between the flexible and rigid regions is. The backbone dynamics of Trp7 cannot be measured because the cross-peak for the amide $^{15}$N and $^1$H chemical shifts of Trp7 overlaps with those of Val133 and Val134. However, the aromatic side chain of Trp7 appears to be stabilized by hydrophobic interaction as $^1$H-$^1$H NOEs to Tyr81 within $^2$H9251, Leu103 within $^3$H9251, and Val113 within $^4$H9251 are observed. The rigid portion (residues Trp7–Tyr18) is bound to the concave side that is composed of the six helices. Hydrophobic interactions between Trp7, Pro8, Leu10, Ala13 within this loop and hydrophobic residues at the concave surface of the TPR-like domain stabilize the loop (Fig. 5). In addition, two negatively charged residues, Asp12 and Glu15, are proximate of positively charged Lys21 (Fig. 5), indicating that electrostatic interactions assist in the stabilization of the loop. Lack of these interactions in human Fis1 relegates its N-terminal tail out of the concave surface of the TPR-like domain (Fig. 6A).

The rigid region of the N terminus of yeast Fis1 (Fig. 6B) is located in the hydrophobic pocket, similar to that of the N terminus of the mouse Fis1 structure (1IYG) (Fig. 6C). However, in the construction of the recombinant mouse Fis1 protein, a cloning artifact resulted in the extraneous amino acids, Gly110-Ser7-Ser6-Ser5-Gly4-Ser3-Ser2-Gly1, becoming attached to the N-terminal end. The native sequence of mouse Fis1 is identical to that of human Fis1 except for only five residues within $^3$H9251 and $^5$H9251 helices, and therefore, the N-terminal loop of mouse Fis1 that binds into the pocket is not physiological due to the 7-residue extension, and the native mouse Fis1 N terminus would be expected to be flexible as that in the human Fis1 structure (19). Nevertheless, the structure of the N-terminal extended recombinant mouse Fis1 is similar to that of the yeast Fis1 structure, corroborating the clear potential for TPR pocket binding of N-terminal extensions as in yeast Fis1. The first 6 residues (Gly7–Ser2) of recombinant mouse Fis1 do not converge into one conformation, as shown in the 20 ensemble structures, and appears to be flexible, whereas the
The rest of the N terminus prior to the α1 helix appears to be rigid and positions in the hydrophobic pocket made by six α-helices. The residues from 9 to 14 in the recombinant mouse protein (corresponding to Glu2–Asn6 in the native sequence) form a helix, similar to the region corresponding to the residues Leu10–Tyr14 in yeast Fis1. This ironically explains that the N-terminal behavior of yeast Fis1 in solution is physically appropriate. The hydrophobic surface composed by six α-helices is a general binding pocket in the yeast, mouse, and human orthologs. Specific binding can be achieved by electrostatic...
interactions and steric fit, in addition to hydrophobic interactions. To date, the identity of proteins predicted to bind to this region in human and mouse Fis1 is not known, although the current work shows that yeast Fis1 entails at least a self-association with its extended N terminus.

**The biological Role of the N Terminus**—The finding of the N terminus embedded in the binding pocket led us to investigate the importance of the Fis1 N terminus in mitochondrial fission. We constructed plasmids carrying FIS1 (Fig. 7A) and introduced them into a *S. cerevisiae* strain that lacks the fis1 gene. GFP localized to the mitochondrial matrix by import sequence of cytochrome c oxidase subunit IV was used to observe the mitochondrial morphology in the transformed yeast (Fig. 7B). The Δfis1 yeast showed net-like or condensed mitochondria, indicating that the fission machinery is not working properly as initially reported (13, 14). Yeast harboring the empty plasmid, pHH26, showed the same aberrant mitochondrial morphology. Ectopic expression of wild-type Fis1 in the Δfis1 yeast resulted in fragmentation of mitochondria, consistent with the conclusion that it properly rescues the fission machinery. Upon expression of the Fis1 deletion mutant DM1, which lacks the majority of the N-terminal loop (the first 14 residues) in the Δfis1 yeast, the mitochondria appeared mostly fused and condensed, indicating that mitochondrial fission could not be rescued in the absence of the first 14-residue segment. Two other N-terminal deletion mutants were used to explore the role of N-terminal binding to the TPR domain. Expression of the deletion mutant DM2, which lacks the flexible portion of the N-terminal loop (the first 5 residues), reconstituted mitochondrial fission, whereas the deletion mutant DM3, which lacks Phe⁶–Tyr¹⁴ corresponding to most of the rigid region of the N-terminal loop, was inert. To rule out the possibility that the Fis1 deletion mutants DM1 and DM3 are unstable or not expressed, resulting in the failure to reconstitute mitochondrial fission, we confirmed that all of the deletion mutants were present at levels comparable with that of the wild-type protein (Fig. 7C). The loss of function for the deletion mutants DM1 and DM3 is due to the lack of the N-terminal rigid region.

In the multistep process of mitochondrial fission, Fis1 is involved in mitochondrial localization of Mdv1 (13, 14, 45). To investigate whether the N-terminal rigid region of the yeast Fis1 protein is required for binding to Mdv1, we tested the recruitment of GFP-Mdv1 to mitochondria upon expression of the different Fis1 constructs (Fig. 8). GFP-Mdv1 showed a cytoplasmic localization in Δfis1 yeast, and expression of either Fis1 or Fis1-DM2 led to the recruitment of Mdv1 to the mitochondria. However, neither Fis1-DM1 nor Fis1-DM3, both lacking the N-terminal rigid region, was able to relocalize the cytosolic Mdv1 to the mitochondria. Upon co-expression with Fis1 or Fis1-DM2, GFP-Mdv1, through its uniform distribution on the mitochondria, revealed net-like mitochondria, typical of the Δfis1 phenotype (Fig. 8). In contrast to endogenous Mdv1 that produced fragmented mitochondria (Fig. 7B), overexpression of GFP-Mdv1 inhibited mitochondrial fission, as described previously in the literature (46). GFP-Mdv1 is evenly dispersed along the mitochondria, instead of forming punctate structures at discrete foci on the mitochondria, apparently due to the
elevated level of Fis1 on the mitochondrial outer membrane. Nevertheless, it is clear from Fig. 8 that the rigid portion of the N-terminal tail in yeast Fis1 is a required component for the recruitment of Mdv1 to mitochondria.

These data indicate that the N-terminal loop, and specifically the region that binds into the concave face of the TPR domain, is required for yeast Fis1 bioactivity. We propose that the TPR-like helix bundle formulates a module for protein-protein interaction and that the N-terminal loop fitting into the concave surface is a prerequisite for appropriate interactions through the TPR-like helix bundle.

DISCUSSION

Among the many Fis1 orthologs, human and yeast Fis1 are the best characterized. Fis1 exists at the mitochondrial outer membrane and is thought to recruit other proteins that are involved in mitochondrial fission, such as Dnm1 and Mdv1 in the case of yeast and Drp1 in the case of mammals. Fis1 possesses a transmembrane region at its C terminus that anchors it into the mitochondrial outer membrane. The N-terminal core domain is exposed to the cytosol and is proposed to act as a binding module. We previously determined the structure of human Fis1 and characterized the potential TPR-mediated molecular mechanisms by which Fis1 interacts with its binding partners (19). However, it is still not clear how the interaction is regulated. In the present study, we determined the structure of yeast Fis1 and compared it with the human counterpart. It is helpful to consider the differences and similarities between yeast and human Fis1 to investigate the general scheme of their molecular function and their specific functions in the different species.

Examination of the sequence alignment (Fig. 3A) between human and yeast Fis1 reveals that the region corresponding to residues Met1–Gln40 of yeast Fis1 is the least similar to that of the human ortholog. Nevertheless, yeast Fis1 forms an α-helix (residues Pro19–Ser31) that is unexpectedly similar to the α-helix of human Fis1. The rest of the human and yeast Fis1 is very similar in both sequence as well as structure. Based on the analysis using DALI against the data base of protein structures, overall folds of the two proteins are statistically identical. Both human and yeast Fis1 contain a TPR-like helix bundle that forms a hydrophobic pocket on the concave side. A helix
bundle from a typical TPR motif constructs a hydrophobic pocket that mediates protein-protein interactions (47, 48), and due to the structural similarity between Fis1 and other TPR-containing proteins, we propose that Fis1 mediates protein-protein interactions at this concave surface. It has been reported that a proline substitution within the TPR-like domain of yeast Fis1 results in an inhibition of mitochondrial fission, due to the lack of binding between Fis1 and Mdv1 (45). Proline is known to be unfavorable for the formation of α-helices, thus inhibiting the functional TPR-like structure. Interestingly, many of the hydrophobic residues in yeast and human Fis1 are conserved, suggesting that they may be critical for the stabilization and/or the assembly of the helices into a TPR-like helix bundle.

In contrast to the highly conserved structure of the TPR-like helix bundle, the N-terminal tail shows major structural differences between human and yeast Fis1. The sequence alignment (Fig. 3A) and the details on interactions between the N-terminal tail and the concave surface formed by the TPR-like domain in yeast Fis1 (Fig. 5) provide reasonable explanations for why the N-terminal tail of human Fis1 does not adopt a fixed conformation. The residues involved in hydrophobic interactions, Trp, Pro, and Leu in yeast Fis1, are missing in human Fis1. The residue involved in electrostatic interaction, Asp in yeast Fis1, is also missing in human Fis1. The length of N-terminal tail of human Fis1 is so short that the hydrophobic residues Val and Leu cannot reach deep enough into the hydrophobic pocket to establish stable hydrophobic interactions. Given that the N terminus of yeast Fis1 regulates protein function (Fig. 7), the distinct N termini of these proteins suggest a different regulation of human and yeast Fis1.

Comparison of the human and yeast Fis1 sequences with those from Arabidopsis, Drosophila, Caenorhabditis elegans, and pufferfish (Fig. 9) shows that the length and sequence of the N terminus differs markedly and more than any other part of the molecule. The truncated N terminus of Drosophila Fis1 would lack both helices α1 and α2 in the yeast structure, and Arabidopsis has an 11-residue N-terminal extension beyond that of even the long S. cerevisiae N terminus. The residues in the N terminus of yeast Fis1 that have contact with the TPR bundle are not well conserved in other sequences. The fact that the artificial extension of mouse Fis1 forms a similar conformation and interacts with residues in the homologous pocket in mouse Fis1 indicates that the binding specificity of the concave pocket in the TPR domain for N-terminal extensions is not highly specific.

Mdv1, a protein that binds to Fis1 and is required for mitochondrial fission in yeast, is not found in mammals. Interestingly, we find that the N-terminal region of yeast Fis1 protein that docks to the TPR-like domains and is absent in mammalian Fis1 is required for appropriate interactions between Mdv1 and Fis1 in yeast (Fig. 8). The Fis1 function in binding to Mdv1 is directly related to its function in mitochondrial fission. Deletion of the rigid Phe-Tyr region of yeast Fis1, essential for recruiting Mdv1, abolished its bioactivity. This explains why human Fis1 cannot reconstitute the defect of mitochondrial fission in fisl1 yeast (17), consistent with our finding that the unique N-terminal region of yeast Fis1 is required for mitochondrial fission. The required portion is embedded in the pocket formed by the TPR-like domain, suggesting that this intra-molecular association is a regulatory mechanism for the function of Fis1 in yeast. Since this pocket in other TPR domain proteins is involved in protein-protein interactions, the occupation of the yeast Fis1 hydrophobic pocket by its N-terminal residues may provide a way to compete for and thus regulate interaction of Fis1 between different proteins.

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