The Solution Structure of the FATC Domain of the Protein Kinase Target of Rapamycin Suggests a Role for Redox-dependent Structural and Cellular Stability*

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Regulation of cellular growth is a prerequisite for cells, organs, and organisms to achieve a characteristic size. The target of rapamycin (TOR,1 also known as FRAP, RAFT, RAPT, or mammalian TOR; mTOR) is a highly conserved Ser/Thr kinase that plays a central role in the control of cellular growth. TOR has a characteristic multidomain structure. Only the kinase domain has catalytic function; the other domains are assumed to mediate interactions with TOR substrates and regulators. Except for the rapamycin-binding domain, there are no high-resolution structural data available for TOR. Here, we present a structural, biophysical, and mutagenesis study of the extremely conserved COOH-terminal FATC domain. The importance of this domain for TOR function has been highlighted in several publications. We show that the FATC domain, in its oxidized form, exhibits a novel structural motif consisting of an α-helix and a COOH-terminal disulfide-bonded loop between two completely conserved cysteine residues. Upon reduction, the flexibility of the loop region increases dramatically. The structural data, the redox potential of the disulfide bridge, and the biochemical data of a cysteine to serine mutant indicate that the intracellular redox potential can affect the cellular amount of the TOR protein via the FATC domain. Because the amount of TOR mRNA is not changed, the redox state of the FATC disulfide bond is probably influencing the degradation of TOR.


dependent proteins is mediated by a disulfide-linked cluster; the other domains are assumed to mediate interactions with TOR substrates and regulators. Except for the rapamycin-binding domain, there are no high-resolution structural data available for TOR. Here, we present a structural, biophysical, and mutagenesis study of the extremely conserved COOH-terminal FATC domain. The importance of this domain for TOR function has been highlighted in several publications. We show that the FATC domain, in its oxidized form, exhibits a novel structural motif consisting of an α-helix and a COOH-terminal disulfide-bonded loop between two completely conserved cysteine residues. Upon reduction, the flexibility of the loop region increases dramatically. The structural data, the redox potential of the disulfide bridge, and the biochemical data of a cysteine to serine mutant indicate that the intracellular redox potential can affect the cellular amount of the TOR protein via the FATC domain. Because the amount of TOR mRNA is not changed, the redox state of the FATC disulfide bond is probably influencing the degradation of TOR.

The atomic coordinates and structure factors (code 1WIN) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The known interactions of TOR with other proteins have been reviewed recently (1, 2). The FKBP12-rapamycin binding site is flanked by the FAT domain and the catalytic kinase domain. Binding of the FKBP12-rapamycin complex to the FKBP12-rapamycin-binding domain specifically inhibits TOR (5). The catalytic kinase domain is highly homologous to that of phosphatidylinositol 3- and 4-kinases. Thus, TOR and its relatives have been termed phosphatidylinositol kinase-related kinases. TOR has been shown to fulfill its functions by directly phosphorylating serine and threonine residues of target proteins and by regulating protein phosphatases (1, 2). The carboxyl-terminal ~33 residues of TOR form the FATC domain. In all phosphatidylinositol kinase-related kinases, the FATC domain always occurs in tandem with the FAT domain (6). Previous studies have shown that the FATC domain is indispensable for TOR function in vivo and in vitro (7, 8).

Here, we report the solution structure of y1fatc, the FATC domain of yeast TOR1 (residues 2438–2470), which showed a new structural motif consisting of an α-helix and a disulfide-bonded loop. Reduction of the disulfide bond resulted in strong conformational changes and increased flexibility of the loop region. The structural data, together with the determined intermediate range redox potential (~0.23 V) and the biochemical characterization of a Cys to Ser mutant of the full-length protein, suggest that the cellular stability of TOR toward degradation and possibly its function can be redox-regulated.

EXPERIMENTAL PROCEDURES

Protein Expression—Constructs of the yeast TOR1 FATC (y1fatc) domain were designed based on sequence conservation. Residues 2438–2470 were cloned into GEV2 (9) using the BamHI and XhoI sites and overexpressed in Escherichia coli Rosetta (DE3) (Novagen) in M9 minimal medium. About 40% of the IgG-y1fatc fusion protein was in the soluble fraction obtained after sonication of the cells in 50 mM Tris-HCl, 2 mM EDTA, 2 mM benzamidine, pH 7.5. The remaining 60% of the protein was extracted from inclusion bodies. Inclusion bodies were washed with 20 mM Tris-HCl, 1 M urea, pH 7.5, and extracted using 50 mM Tris-HCl, 6 M guanidinium hydrochloride, pH 7.5, with agitation for 1 h at 4 °C. The extract was dialyzed to TST buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20; equilibration buffer for the
IgG-Sepharose, see below). IgG-y1fatc fusion protein was extracted from the cleared lysate and from the dialyzed inclusion body extract using an IgG-Sepharose 6 fast flow column (Amersham Biosciences) as described in the manufacturer's manual. The purified fusion protein was lyophilized, resuspended in 50 mM Tris, 100 mM NaCl, pH 8, and desalted using a PD-10 column (Amersham Biosciences). The protein was eluted with 50 mM Tris- HCl, 100 mM NaCl, pH 8, and, after the addition of 2 mM CaCl₂, digested with Factor Xa (Amersham Biosciences) overnight. After digestion, the fusion partner was removed by reversed-phase high pressure liquid chromatography in an acetonitrile/ trifluoroacetic acid buffer system, and fractions containing pure y1fatc were lyophilized. Electrospray mass spectrometry confirmed that y1fatc had the correct molecular weight. Analytical ultracentrifugation experiments of air oxidized y1fatc in 50 mM Tris, pH 7, showed that y1fatc was monomeric.

NMR Sample Preparation—Lyophilized ¹⁵N- or ¹⁵N,¹³C-labeled y1fatc was dissolved in 10 mM Tris, pH 8 (in 95% H₂O/5% D₂O or in 100% D₂O), and the pH was adjusted to 6. Reduced samples were obtained by adding 20 mM Tris (2-carboxyethyl) phosphine-HCl. The sample for measuring residual dipolar couplings contained about 25 mg/ml PF1 phages (ASLA Biotech). The protein concentration was always ~0.5 mM.

NMR Spectroscopy—NMR spectra were acquired at 298 K on Bruker DRX600 and 800 spectrometers. The data were processed with NMRPipe (10) and analyzed using NMRView (11). Assignments for ¹³C, ¹⁵N, and ¹H nuclei were based on three-dimensional CBCANH, CBCA(CO)NNH, CBCA(CO)CSNH, H-COSY, TOCSY, and HNCO spectra. Spectral assignments and dihedral angle information were derived from 3D-HACAHB-COSY, 3D-¹⁵N-ROESY, 3D-HNHA, and ¹³C(¹⁵N)-y-SED- and ¹³N-(¹⁵N)-y-SED-¹⁵N-¹H-HSQC spectra. Distance restraints were obtained from ¹³N- and ¹⁵C-edited NOESY spectra. Information about the backbone dynamics was derived from the measurement of ¹⁵N relaxation experiments including T₁ (spin-lattice or longitudinal relaxation rate), T₂ (spin-spin or transverse relaxation rate), and (¹H)-¹⁵N NOE. If not explicitly mentioned elsewhere, references for all experiments are given in Ref. 13 and references therein. ¹⁵N-HResidual dipolar couplings were obtained from the analysis of ¹⁵N-H IPAP-HSQC data (14) acquired on the sample containing PF1 phages. The maximal ΔDᵤₑₐₑ was ~17.4 Hz.

Structure Calculation—All structure calculations were performed with XPLOR-NIH (15) using torsion angle molecular dynamics. Distance restraints were generated in NMRView and classified according to NOE cross-peak intensities. Upper bounds were 2.8, 3.4 (3.5 for ¹⁵N-bound protons), 4.5, and 5.5 Å. The lower bound was always 1.8 Å. For all NOE restraints, r⁻⁷ sum averaging was used. Backbone dihedral angle restraints for φ were derived based on jHninH and the chemical shift index (16). Stereospecific assignments were obtained for 14 β-methylene protons pairs. Based on 2JHα,hβ coupling constants and NOE/ROE data, side chain χ₁ angles were restrained to one of the staggered conformations (60°, 180°, -60°) ±30°. N-H residual dipolar couplings were included using the SANI statement and an error bound of 0.2 Hz. The distance between the Cα atoms of the disulfide bond-forming cysteines was restrained to be within 4.2–7.0 Å and the dihedral angle around the disulfide bond to be 0° ± 105°, because the dihedral angle χ₃ is always 95° or ~95° ± 10° (17).

Determination of the Redox Potential—The redox potential of y1fatc was determined by following the protein fluorescence at 350 nm for different ratios of reduced and oxidized glutathione (GSH/GSSG) and analyzing the data as described previously (18). Samples contained 1.1 µM y1fatc in 10 mM Tris-HCl, pH 7, 0.1 mM EDTA, 0.1 mM GSSG, and concentrations of GSH ranging from 10 µM to 90 mM. All buffers were degassed and flushed with argon. The concentration of reduced glutathione in the buffer was verified with an Ellman assay (19). All samples were prepared under nitrogen atmosphere. All measurements were performed at 25 °C (298 K) on a Amicon Bowman series 2 luminescence spectrometer using 1 x 1-cm quartz cuvettes (right angle arrangement), an excitation wavelength of 280 nm, and a bandwidth of 4 nm. The bandwidth for detection was 0.5 nm.

Mutagenesis Studies—Plasmid pJM244 (pTOR2C–S) was constructed by PCR amplification of a 1300-bp fragment of the COOH-terminal region of yeast TOR2 using the primers TOR2D (5'-GGT AAA CAG TTG CCA CAA TTA CAA ACT CCT GAA CTA CAT GTG TCG CC) and TOR2Rmut (5'-GAA CTC GAC TAC AAT CAA TGG ATT TTC AAT ACA TTA AAA CTA CAA GAA TGG AGA CC) containing an artificial XhoI restriction site and a single base change (underlined) compared with the TOR2 wild-type sequence resulting in the C2470S mutation. The resulting PCR fragment was digested with SpeI and XhoI and used to substitute the SpeI-XhoI fragment of plasmid p206 (20), a yeast centromeric plasmid containing an NH₂-terminal hemagglutinin epitope-tagged TOR2 expressed under the control of its own promoter. Plasmid pJM232 (pTOR2C) was constructed in a similar way, but with primer TOR2mut as TOR2Rmut and with a C instead of the underlined G, to amplify the wild-type sequence. As a control, the same centromeric plasmid without the TOR2 sequence (pRS314 = pVector) was used. No additional mutations were introduced in plasmids pJM232 and pJM244 as confirmed by sequencing of the SpeI-XhoI fragment. These plasmids were used to transform the Saccharomyces cerevisiae strains SH121 and SH221 (21). Yeast strains were grown in rich medium (yeast extract/peptone/dextrose) or synthetic minimal medium complemented with the appropriate nutrients for plasmid maintenance as described previously (22). Paromomycin and rapamycin were added at the indicated concentration from a concentrated stock to autoclaved yeast extract/peptone/dextrose with 2% agar prior to gelation. Spot tests of yeast growth were performed as described previously (23), but plates were incubated at a restrictive temperature (37 °C) for the thermosensitive allele of TOR2. Immunoprecipitation assays were performed as described previously (24).

RESULTS

The FATC Structure—The FATC domain of the family of TOR kinases is highly conserved. Fig. 1b shows an alignment of the carboxyl-terminal 33 residues of TOR proteins from different species. One-third of the residues are completely conserved, including two cysteines in an i to i + 7 spacing. To elucidate the role of the two cysteines for the structure of the FATC domain, we first acquired ¹⁵N-HSQC spectra of oxidized and reduced...
y1fatc (Fig. 2). Oxidized y1fatc shows a spectrum characteristic for a folded protein with well dispersed amide proton nitrogen cross-peaks for the majority of residues. In the spectrum of the reduced form, resonances for the carboxyl-terminal 15 residues (2456–2470) were broadened beyond detection, indicating increased conformational flexibility because of the reduction of the disulfide bond in this region. To understand the molecular basis of the differences between the oxidized and the reduced forms, we have determined the structure of the oxidized form using standard NMR techniques.

The NMR structure of oxidized y1fatc is shown in Fig. 3, and structural statistics are given in Table I. The structure consists of a long, NH2-terminal a-helix extending from residue Glu2444 to the first cysteine, Cys2460 (Fig. 3, a and b), and a COOH-terminal disulfide-bonded loop from Cys2460 to Cys2467 that folds the protein chain back onto itself. This chain reversal is facilitated by the completely conserved glycine at position 2465. The NH2-terminal disulfide-bonded loop from Cys2460 to Cys2467 increases the flexibility because the FATC domain is stabilized by only a few tertiary contacts at the COOH-terminal end that are mediated by the disulfide bridge.

The absence of NOEs for the first five NH2-terminal residues preceding Pro2443 indicates a random coil conformation for this part of the sequence. Indeed, the 15N relaxation data (Fig. 4) give evidence of large amplitude backbone motions on the subnanosecond time scale from V2442 onward to the NH2 terminus because of a strong increase in the T2 and T1 relaxation times and a strong decrease of the (1H)-15N NOEs. T2 values for the helical and loop part are more uniform, with an average of 0.57 ± 0.08 s, which is close to values expected for an isotropically tumbling molecule of the size of y1fatc (4 kDa) at 25 °C. In contrast, T1 values (average = 0.10 ± 0.04 s) within this structured part are much smaller (0.4–0.4 s) than expected for a rigid molecule of this size and indicate the presence of considerable line broadening because of chemical exchange on the microsecond time scale. Note also that the asymmetry of the helical molecule contributes to a certain extent to the shortening of the amide 15N T2 values. However, the effect of even very pronounced asymmetry of the diffusion tensor would be too small to account for the observed shortening of T2. A more extended analysis using the program TENSOR (26) and an isotropic model as a first approximation for the rotational diffusion of y1fatc showed that the chemical exchange contributions (R(t)) increase strongly from the beginning to the end of the structured part (Fig. 4, bottom panel). The subnanosecond order parameter S2 also increases from values around 0.3 at the NH2 terminus to values around 0.8 at the COOH terminus (Fig. 4, fourth panel). Thus on the subnanosecond time scale, there are large amplitude motions at the NH2 terminus but only small amplitude motions at the COOH-terminal end of the structure. In contrast, micro- to millisecond time scale motions are stronger in the COOH-terminal half. Therefore, albeit folded, the helix and loop parts of the FATC structure are partly flexible, with the time scale of motions becoming longer toward the COOH terminus. This observation is not surprising because the FATC domain is stabilized by only a few tertiary contacts at the COOH-terminal end that are mediated by the disulfide bridge.

The 13Cα and 1H resonances of Cys2467 are not visible in the NMR spectra of y1fatc, and its side chain and the one following residue Pro2468 show only few and broad NOE contacts. Presumably, this is because of additional conformational exchange from disulfide bond crankshaft motions like the ones that have been characterized in bovine pancreatic trypsin inhibitor (27). Indeed, in the structure calculations, the left-hand conformation (17) of the disulfide bond is only slightly more favored (13 of the 20 best structures) than the right-hand conformation.

A second minor set of resonances was observed for the backbones amides of residues Phe2469 and Trp2470 (Fig. 2, Figs. 3 and 4) and for the total H2O nuclei in the 13C-HSQC spectrum. These resonances indicate the presence of a minor cis-form of Pro2468 in addition to the predominant trans-form. As judged from the spectral intensities, this cis-form is populated to 15–20%. Very similar structures for the rest of FATC were obtained when Pro2468 was constrained to the cis- instead of the trans-conformation (data not shown).

Determination of the Redox Potential—Because both tryptophans of y1fatc are in the vicinity of the disulfide bond-forming cysteines (Fig. 3d), the redox state-dependent fluorescence at 350 nm was used to determine the redox potential (18) of the Cys2460–Cys2467 disulfide bond of y1fatc. For y1fatc equilibrated in buffers containing different ratios of oxidized and reduced glutathione, we detected a two-state transition curve (Fig. 5a) that corresponds to an equilibrium constant (Keq) of 0.45 M. Using the Nernst equation E0 y1fatc = E0 o GSH/GSSG – (RT/2F) In Keq with E0 o GSH/GSSG = −0.24 V, this results in a redox potential of −0.23 V for y1fatc. Apparently, this value is

FIG. 2. Two-dimensional 1H-15N HSQC spectrum of oxidized (black peaks) and reduced (red peaks) y1fatc. The amino acid sequence of y1fatc is shown at the bottom. Reduction of the intramolecular disulfide bond between Cys2460 and Cys2467 increases the flexibility of the COOH-terminal half (depicted in black letters in the sequence). This increased flexibility broadens the resonances of the respective residues in the reduced form beyond detection.
very close to the one for glutathione. Other cellular components (Fig. 5b) are more reducing or more oxidizing than y1fatc, such as thioredoxin (−0.27 V) or cytochrome c (0.22 mV), respectively.

Mutagenesis Studies—In yeast, two highly homologous TOR proteins have been identified. TOR1 is only found as part of TOR-C1 (complex 1), a multiprotein complex that regulates cell growth in a rapamycin-sensitive manner. In contrast, TOR2 can be found as part of TOR-C1 but additionally can participate in a second protein complex called TOR-C2. The known function of the complex TOR-C2 is to regulate the polarization of the actin cytoskeleton in a rapamycin-insensitive manner (24). To study the influence of the FATC disulfide bonds on both complexes, mutagenesis studies were carried out on the TOR2 protein.

In detail, the second of the disulfide bond-forming cysteines in TOR2 (Cys2470 = Cys2467 in TOR1) was changed to serine (C2470S). To investigate the effect of this TOR2 mutation on the function of the rapamycin-insensitive complex TOR-C2, a plasmid containing the C2470S mutation (pTOR2C2S) was introduced into the yeast strain SH121 (21) that carries a thermosensitive allele of TOR2 (Cys2470 = Cys2467). The pTOR2C2S plasmid was able to complement the thermosensitive phenotype under restrictive temperature conditions (37 °C), where expression of wild-type TOR2 protein is suppressed (data not shown). Experiments with an isogenic wild-type plasmid (pTOR2) yielded

![Figure 3](image-url) **Fig. 3. NMR structures of y1fatc (with Pro2468 in the major trans-conformation).** a, view of the 20 best structures of y1fatc after a least square alignment of the backbone atoms of residues 2443–2470. The backbone of the α-helix is red. The disulfide bond between cysteines 2460 and 2467 is yellow, and the conserved glycine (Gly2465) is orange. Hydrophobic and aromatic side chains are light green in the picture foreground and dark green in the picture background, and polar or neutral amino acids side chains are light blue. b, ribbon representation of the 20 superposed structures from a. c, surface representation of the lowest energy structure from a. The surface is colored according to the charge distribution (red, negative; blue, positive). d, detailed view of the disulfide-bonded loop. All structure pictures were made with the programs MolMol (52) and POV-Ray.

![Figure 4](image-url) **Fig. 4. Backbone dynamics of y1fatc.** 15N-T1 (top panel), T2 (second panel), and NOE (third panel) values and uncertainties were determined for the oxidized form of this 33-residue protein. Secondary structure elements and the location of the disulfide bond (SS) are indicated at the top. A model-free analysis (53, 54) of the 15N relaxation data was performed using the program TENSOR2 (26). The analysis was based on an isotropic tumbling model with a rotational correlation time of 1.9 ns. The Lipari-Szabo model free order parameter S2 and the contribution of chemical exchange Rm to the observed transverse relaxation rate 1/T2 are displayed in the bottom two panels.
Thus, the C2470S mutation reduces the amount of TOR2 was expressing either wild-type or mutated TOR2. The amount of TOR2 that leads to smaller cellular concentrations. To investigate this effect further, we performed an immunoprecipitation of TOR2 extracted from yeast cells that were grown identically at the restrictive temperature (37 °C) and that were watered in selective synthetic minimal media. We used synthetic minimal media. Water dilutions of each strain (1/10, 1/100, and 1/1000) were spotted on yeast extract/peptone/dextrose medium containing the indicated concentration of rapamycin or paromomycin, and growth was recorded after 4 days. Identical results were obtained from at least six independent colonies of each strain in two separate experiments. b, TOR2<sup>C2470S</sup> protein is less abundant than TOR2 protein. Cells expressing hemagglutinin-TOR2 (wild type (<i>wt</i>)) and hemagglutinin-TOR2<sup>C2470S</sup> (<i>C</i>→<i>S</i>) were grown (at 37 °C) and harvested, and the protein was extracted in the same way. The protein concentration was determined by a Bradford assay. Both cell lysates were subjected to immunoprecipitation with anti-hemagglutinin, and the resulting precipitates were resolved by SDS-PAGE and Western blotted to detect TOR2. As a control, actin was immunoprecipitated in parallel (bottom panel).

**FIG. 6.** The cysteine to serine mutation in yeast TOR2 (C2470S) induces rapamycin and paromomycin hypersensitivity. These phenotypes correlate with less TOR2 protein. a, at a restrictive temperature (37 °C), pTOR2<sup>C2470S</sup> is unable to grow in the presence of rapamycin or paromomycin. Precultures of the indicated strains were grown on selective synthetic minimal media. Water dilutions of each strain (1/10, 1/100, and 1/1000) were spotted on yeast extract/peptone/dextrose medium containing the indicated concentration of rapamycin or paromomycin, and growth was recorded after 4 days. Identical results were obtained from at least six independent colonies of each strain in two separate experiments. b, TOR2<sup>C2470S</sup> protein is less abundant than TOR2 protein. Cells expressing hemagglutinin-TOR2 (wild type (<i>wt</i>)) and hemagglutinin-TOR2<sup>C2470S</sup> (<i>C</i>→<i>S</i>) were grown (at 37 °C) and harvested, and the protein was extracted in the same way. The protein concentration was determined by a Bradford assay. Both cell lysates were subjected to immunoprecipitation with anti-hemagglutinin, and the resulting precipitates were resolved by SDS-PAGE and Western blotted to detect TOR2. As a control, actin was immunoprecipitated in parallel (bottom panel).

**FIG. 5.** Determination of the redox potential of yl FATC domain. The shown transition curve (a) was obtained by monitoring the fluorescence at 350 nm of samples containing 1.1 μM yl FATC, 0.1 mM oxidized glutathione, and varying concentrations of reduced glutathione (10 μM–90 mM). The experimental data were analyzed as described under “Experimental Procedures.” b, scale of the redox potentials of some cellular components. Redox pairs to the left of yl FATC are more reducing, and those to the right of yl FATC are more oxidizing.

**FIG. 3.** a, localization signal of yl FATC domain. As a control, actin was immunoprecipitated in parallel (bottom panel).

**FIG. 2.** Structure of the TOR FATC Domain.

**FIG. 1.** a, localization signal of yl FATC domain. As a control, actin was immunoprecipitated in parallel (bottom panel).
environment. Very few values of the redox potential in living cells have been reported. The determination of appropriate values is hampered because the intracellular redox potential may depend on the location, metabolic state, differentiation, and type of the cell. The redox potential for the overall intracellular environment of a mammalian cell line was determined to range from −0.221 to −0.236 V (28). Using a green fluorescent protein with an engineered disulfide bond (rxyFP), the redox potential of the cytosolic glutathione pool of wild-type yeast cells under normal growth conditions was determined to be −0.289 V but changed to −0.227 V upon mutation of genes involved in the regulation of the cytosolic glutathione pool (29). During the life cycle of mammalian cells, the cytosolic redox potential was found to range between −0.260 and −0.160 V (30). In proliferating mammalian cells, the redox potential is −0.24 V (28, 31, 32), whereas more oxidizing conditions are found in differentiating cells (−0.2 V) (30) and at the onset of apoptosis (−0.17 to −0.19 V) (31, 32). TOR has also been found in the nucleus (33). However, to our knowledge, no information is available on the redox potential within the nucleus.

Based on the reported cellular redox potentials, it is possible that TOR exists in both reduced and oxidized forms in the cellular environment. The ratio of oxidized to reduced TOR during the cellular life cycle could be altered through the action of typical cellular redox regulators (glutathione, thioredoxin, glutathione S-transferase, cytochrome c, reactive oxygen species, etc.), in which concentrations depend on metabolic activities in response to the availability of nutrients, the action of growth hormones, and the influence of stress (oxidative, UV, etc.). Furthermore, the local redox potential for the FATC disulfide bond may be modulated within the various TOR complexes.

Several examples exist of cytosolic and nuclear proteins that are regulated through the reversible oxidation of a cysteine side chain. The roles of redox-sensitive proteins involved in stress responses (34), regulation of cell death (35), and cancer proliferation (36) have been reviewed recently. Within the TOR signaling cascade, various redox-dependent proteins or processes have been reported. For example, the lipid phosphatase PTEN, a tumor suppressor, is redox-regulated. Mutations or loss of PTEN are associated with an increased activation of mTOR (37, 38). PTEN is inactivated through the reversible formation of a disulfide bond in its active site (39). Furthermore, it has been suggested that mTOR is involved in the regulation of hypoxia-inducible factor 1α (40–43) and that hypoxia can trigger hypophosphorylation of mTOR and some of its effectors in a hypoxia-inducible factor-independent manner (44). Obviously low oxygen pressure (hypoxia) should influence the redox potential within the cell. Additionally, it has been suggested that the UV response pathway induces the phosphorylation of p70 S6 kinase in an mTOR-dependent manner via the generation of reactive oxygen species (44). Finally, TOR can associate with the mitochondrial outer membrane (45), which can make it directly susceptible to (redox) signals from this organelle.

The effect of mutations within the mTOR FATC domain has been investigated in two studies (7, 8). Both show that deletion of the carboxyl-terminal tryptophan as well as small deletions (three or more residues) of the COOH terminus abolish mTOR autophosphorylation activity as well as mTOR-dependent phosphorylation of eukaryotic initiation factor 4E-binding protein and p70 S6 kinase. Thus, the COOH-terminal deletions abolished mTOR function. These findings indicate that the FATC domain interacts with other parts of TOR or other components of TOR complexes (24, 46–49) to activate kinase function. Notably, a C2546A mutation in the FATC domain of mTOR did not prevent mTOR from autophosphorylating or activating p70 S6 kinase (7). Nevertheless, from the autoradiogram presented, it can be concluded that the degree of p70 S6 kinase activation was lower for mutated than for wild-type mTOR. In agreement with these data, our C2470S yeast TOR2 mutant is also still active, but its cellular concentration is lowered.

In conclusion, we have presented the structure of the FATC domain of yeast TOR1. FATC forms a novel structural motif consisting of an α-helix and a COOH-terminal disulfide-bonded loop. Upon reduction of this disulfide bond, the flexibility of FATC increases dramatically. Considering that several redox-dependent processes can be connected to the TOR signaling cascade, it seems attractive to propose that the function of TOR can be redox-regulated via the highly conserved FATC disulfide bond. Our data indicate that the redox potential of the disulfide bond is in an intermediate range and that the cellular amount of TOR is affected by the C2470S mutation. An analogous redox-regulatory mechanism for the cellular stability of a protein has been suggested for the phosphatase Cdc25C, which is involved in the regulation of cell cycle progression (50). Future biochemical and structural experiments will be required to shed light on the influence of changes in the cytosolic redox potential on the FATC domain and its interactions with other TOR components or domains of TOR complexes.

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