The TOR Kinases Link Nutrient Sensing to Cell Growth*

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Rapamycin is an immunosuppressive natural product that inhibits the proliferation of T-cells in response to nutrients and growth factors. Rapamycin binds to the peptidyl-prolyl isomerase FKBP12 and forms protein-drug complexes that inhibit signal transduction by the TOR kinases. The FKBP12 and TOR proteins are conserved from fungi to humans, and in both organisms the TOR signaling pathway plays a role in nutrient sensing. In response to nitrogen sources or amino acids, TOR regulates both transcription and translation, enabling cells to appropriately respond to growth-promoting signals. Rapamycin is having a profound impact on clinical medicine and was approved as an immunosuppressant for transplant recipients in 1999. Ongoing clinical studies address new clinical applications for rapamycin as an antiproliferative drug for chemotherapy and invasive cardiology.

The TOR kinases are members of the phosphatidylinositol 3-kinase (PI-3K) superfamily that regulate cell growth and differentiation in response to nutrients. In complex with the prolyl isomerase FKBP12, the antifungal and immunosuppressive natural product rapamycin binds and inhibits the TOR kinases. Genetic studies in yeast and biochemical studies in mammalian cells identified the highly conserved proteins, the FKBP12 prolyl isomerase (PI-3K) superfamily that regulate cell growth and differentiation (27) (Fig. 1). The FRB domain was initially identified by mutations that confer dominant rapamycin resistance and was later mapped to a region of −100 amino acids (28–30). The FRB domain binds the FKBP12-rapamycin complex in vitro, and the crystal structure of the tripartite complex has been solved (28, 31). Although rapamycin binding results in only partial inhibition of TOR kinase activity, the importance of the FRB domain in TOR function is beginning to be defined. In human cells, microinjection of the FRB domain blocks cell cycle progression in the G1 phase (32). Similarly, the central TOR toxic domain inhibits cell growth when overexpressed in yeast (33). Tor1 mutants deleted for either the FRB or the toxic domain lack kinase activity and fail to complement tor1 mutants in yeast (33).

The yeast TOR toxic domain shares limited identity over a 240-amino acid region with several PIK family members, including the Atr, Rad3, Mei-41, and Atm proteins (33, 34). The dominant negative effects of the FRB and the toxic effector domains suggest these domains interact with upstream regulators or downstream effectors of the TOR cascade; however, their precise in vivo functions remain to be elucidated.

The TOR proteins exhibit protein kinase activity that is dependent on integrity of the kinase domain and inhibited by FKBP12-rapamycin or the PI-3K inhibitor wortmannin (35, 36–38). Both in vitro and in vivo studies revealed that mTOR phosphorylates and thereby inactivates the translational repressor protein PHAS-I (39, 40). Similarly, phosphorylation and activation of p70 S6 kinase (a regulator of translation) by mTOR has been reported (40). However, the sequences phosphorylated by mTOR in PHAS-I (S/TP) and in p70 S6 kinase (TY) do not conform to a consensus site. Thus, further studies will be needed to reconcile these results. Phosphorylation of both PHAS-I and p70 S6 kinase in response to mitogens that coordinates nutritional and mitogenic signals and controls gene expression, protein biosynthesis, and cell growth.

TOR Kinases Are Targets of the Immunosuppressant Rapamycin

Rapamycin is a microbial natural product with potent antiproiferative activity. Rapamycin blocks cell proliferation in response to either nutrients or mitogens, including interleukin 2, interleukin 3, platelet-derived growth factor, epidermal growth factor, and insulin. In vivo rapamycin binds with high affinity to the prolyl isomerase FKBP12 to form an active drug-protein toxin (1, 2). The targets of the FKBP12-rapamycin complex, first identified in yeast, are the highly homologous Tor1 and Tor2 proteins (targets of rapamycin), which directly interact with FKBP12-rapamycin (1, 3, 14–17). Subsequent biochemical studies identified the mammalian TOR (mTOR) homolog (5, 18–20). Other TOR homologs have been identified in the human fungal pathogen Cryptococcus neoformans (TOR1), in the fission yeast Schizosaccharomyces pombe (TOR1, TOR2), and in Drosophila melanogaster (dTOR) (21–25). Thus TOR is conserved from yeasts to flies to humans.

The TOR proteins are founding members of a family of large proteins that bear resemblance to the PI-3K, named the PIK-related kinases, which regulate cell cycle progression in response to extracellular or intracellular stimuli. This family includes: the mammalian phosphatidylinositol 3-kinase, Atm (ataxia telangiectasia mutated), Atr (ataxia telangiectasia related), DNA-dependent protein kinase, and the yeast Mec1, Rad53, and Tel1 proteins (26). A characteristic of the PIK-related family is a C-terminal kinase domain, which shares homology with both protein and lipid kinases.

In addition to the kinase domain, the TOR proteins contain an FKBP12-rapamycin binding domain (FRB), a toxic effector domain, and an N-terminal region that features multiple HEAT repeats (named after the four proteins containing this sequence: huntington, elongation factor 3, the A subunit of type 2A protein phosphatase (PP2A), and Tor) that are thought to mediate protein-protein interactions (27) (Fig. 1). The FRB domain was initially identified by mutations that confer dominant rapamycin resistance and was later mapped to a region of −100 amino acids (28–30). The FRB domain binds the FKBP12-rapamycin complex in vitro, and the crystal structure of the tripartite complex has been solved (28, 31). Although rapamycin binding results in only partial inhibition of TOR kinase activity, the importance of the FRB domain in TOR function is beginning to be defined. In human cells, microinjection of the FRB domain blocks cell cycle progression in the G1 phase (32). Similarly, the central TOR toxic domain inhibits cell growth when overexpressed in yeast (33). Tor1 mutants deleted for either the FRB or the toxic domain lack kinase activity and fail to complement tor1 mutations in yeast (33).

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§§ The abbreviations used are: PI-3K, phosphatidylinositol 3-kinase; mTOR, mammalian TOR; FRB, FKBP12-rapamycin binding domain; eIF, eukaryotic initiation factor; PP2A, type 2A protein phosphatase; RP, ribosomal protein; NCR, nitrogen catabolite repressed.
TOR control of translation in yeast cells involves regulation of PP2A catalytic subunits, including Pph21, Pph22, and Sit4, which are known to associate with Tap42 (57) (Fig. 2). The target of TOR in this process appears to be Tap42. In accord with this view, certain mutations that confer resistance to tap42 mutants, including the association of Tap42 with Pph21/Pph22 and Sit4, is prevented by entry into stationary growth phase or by rapamycin (57). Furthermore, direct phosphorylation of Tap42 by TOR has recently been reported (58). Mammalian cells also contain a homolog of Tap42, the α4 protein, which associates with PP2A phosphatases and modifies the substrate specificity of PP2A (59, 60). However, the rapamycin sensitivity of the α4-PP2A association is at present controversial (59, 60). Furthermore, the regulation of this complex may differ from that in yeast as a recent study has suggested that PP2A is the target of mTOR (61). Although evidence for direct phosphorylation of p70 S6 kinase by mTOR has been presented, other studies failed to find significant kinase activity of recombinant mTOR toward p70 S6 kinase (40, 61). Instead, p70 S6 kinase was found in a complex with a fraction of PP2A, and a model by which TOR phosphorylation of PP2A results in phosphatase inactivation and thereby prevents S6 kinase dephosphorylation has been proposed (61).

Transcriptional Regulation by TOR
Recent studies have uncovered a central role of TOR signaling in the regulation of transcription (Fig. 3). Earlier work established a role for TOR in rRNA and tRNA synthesis (7, 62). Although the targets of this regulation are not known, the TOR pathway may act via PP2A in a manner analogous to translational control. Mutations that affect PP2A function also impair rRNA and tRNA gene expression (63, 64).

Genome array studies reveal that ribosome biosynthetic genes expressed by PolII are also repressed by the addition of rapamycin (65). In a manner that mimics nutritional limitation (8, 10, 65) (Fig. 3). Ribosomal protein (RP) genes are coordinately regulated in response to many environmental changes; however, the molecular details involving the transcription of RP genes are as yet unclear. Most RP gene promoters that contain binding sites for the activator/repressor protein, Rap1, and the transactivator, Abf1 (reviewed in Ref. 66). Studies have linked Rap1-mediated activation of RP genes to the cAMP pathway although the signaling events resulting in cAMP-mediated transcription are at this time unknown (67, 68). The identification of Tor kinases as upstream regulators of RP genes provides a starting point to dissect regulatory events governing these genes and should provide insight to the interplay of the TOR and cAMP nutrient-stimulated signaling pathways.

Recently, the TOR pathway was shown to control the expression of the nitrogen catabolite repressed (NCR) genes, underscoring the central role of TOR in nitrogen sensing (8–10, 65). The NCR genes are repressed by preferred nitrogen sources, such as glutamine or ammonia, and derepressed by limiting or poor nitrogen sources, such as proline or urea. Regulatory factors involved in the repression of these genes to the cAMP pathway although the signaling events resulting in cAMP-mediated transcription are at this time unknown (67, 68). The identification of Tor kinases as upstream regulators of RP genes provides a starting point to dissect regulatory events governing these genes and should provide insight to the interplay of the TOR and cAMP nutrient-stimulated signaling pathways.

A particular set of genes subject to nitrogen catabolite repression
includes genes required for the accumulation of precursors of α-keto glutarate when yeast are grown on poor nitrogen sources such as urea. Expression of these genes is controlled by the transactivators Rtg1 and Rtg3 and their positive regulator Rtg2. The TOR signaling pathway controls the activity of the Rtg proteins (65). Rapamycin or poor nitrogen sources induce rapid nuclear import of Rtg1 and Rtg3 in an Rtg2-dependent process. In this case, the importin β family member, Msn5, is required for the export of Rtg1 and Rtg3 as msn5 mutations result in constitutive nuclear accumulation of these factors. Interestingly, an msn5 mutation does not cause constitutive activation of the target genes for Rtg1 and Rtg3; instead, addition of rapamycin is still required for Rtg-directed gene expression. Thus, the TOR signaling pathway controls both nuclear localization of the transactivators and downstream signaling events required for gene expression.

The control of nuclear import/export appears to be a general mechanism by which TOR regulates transcription (Fig. 3). Two additional transcription factors, Msn2 and Msn4, are constrained to the cytoplasm through interaction with a negative regulator (the 14-3-3 proteins Bmh1 and Bmh2) (9). The addition of rapamycin induces nuclear import of Msn2 and Msn4 and induction of stress-inducible genes regulated by these factors.

The emerging theme of Tor-regulated nuclear localization of transcription factors may also extend to mammalian cells. The signal transducer and activator of transcription, STAT3, is activated in response to cytokines and translocates into the nucleus where it directs transcription of its target genes. Recent studies indicate both the nuclear localization and the ability to activate transcription may be regulated by both mTor kinase and PP2A (70, 71).

**Regulation of TOR Kinase Activity by Nutrients**

Cells control the rate of translation in response to energy and amino acids. Amino acid levels control amino acid biosynthesis, transport, and expression of the translation machinery in yeast and mammalian cells. Yeast cells use multiple mechanisms to determine the quality and abundance of amino acids and other nitrogen sources. Ammonium availability is sensed by an ammonium-specific permease, Mep2 (72). External amino acids are sensed by the amino acid receptor Ssy1 in a manner analogous to glucose sensing via the Snf3 and Rgt2 glucose sensors (73, 74). Internal amino acid availability is sensed by the general control response that detects uncharged tRNAs through the protein kinase Gcn2, and this pathway is conserved in mammals (70).

In mammalian cells, amino acids such as l-leucine stimulate TOR kinase activity. Both the activity and phosphorylation states
of the mTOR downstream effector p70 S6K are decreased in response to amino acid limitation and stimulated upon their readdition (50, 52). Moreover, a rapamycin-resistant allele of p70 S6K causes cells to be unresponsive to amino acid depletion (52). Amino acid analogs that inhibit amino acid charging of tRNAs were found to suppress p70 S6K activity, and a temperature-sensitive mutation of histidine-tRNA synthetase also impaired p70 S6K activity at the non-permissive temperature (71). These results suggest aminoacylation of tRNAs may regulate TOR in response to amino acids.

**Clinical Perspective**

Rapamycin was originally identified as a potent antifungal agent with an undesired side effect involving bone marrow suppression. The structural resemblance of rapamycin with the immunosuppressant FK506 prompted clinical studies to develop rapamycin as an immunosuppressive drug. During the last decade, the basic mechanisms of rapamycin drug action were elucidated and the targets FKBP12 and TOR identified, and rapamycin was approved by the Food and Drug Administration as an immunosuppressant in renal transplant recipients in August 1999.

Ongoing clinical studies address further uses of rapamycin, alone and in combination with other immunosuppressants and in other transplant settings. Rapamycin is synergetic with cyclosporin A and FK506 and lacks the nephrotoxic effects of cyclosporin A or FK506, providing renal sparing drug combinations. The rapamycin analog everolimus is in phase III clinical trials as an immunosuppressant. Phase II clinical trials of the rapamycin analog CCI-779 as a novel chemotherapy agent for a variety of different solid tumors are ongoing. Finally, rapamycin may also find a novel use in cardiology. Clinical studies in human patients reveal that impregnating cardiac stents with rapamycin inhibits proliferation and restenosis that commonly occur after treatment of coronary artery disease. These clinical advancements illustrate the dramatic impact of rapamycin on modern medicine.

**REFERENCES**