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Complexity of the TOR signaling network

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The target of rapamycin (TOR) is a serine/threonine kinase of the phosphatidylinositol kinase-related kinase family and is highly conserved from yeast to mammals. TOR functions as a central regulator of cell growth and is itself regulated by a wide range of signals, including growth factors, nutrients and stress conditions. Recent studies in eukaryotic cells have identified two distinct TOR complexes, TORC1 and TORC2, which phosphorylate different substrates and have distinct physiological functions. Here, we discuss new findings that have extended the complexity of TOR signaling and the different roles of the TORC complexes in yeast, flies and mammals.

Rapamycin is an antifungal agent isolated from *Streptomyces hygroscopicus* [1]. Rapamycin analogs are clinically used as immunosuppressants in organ transplantation and as inhibitors of restenosis of arteries after angioplasty [2,3]. Current clinical trials indicate the potential of rapamycin as an anti-cancer drug because it inhibits cell growth [4]. The target of rapamycin (TOR) was initially isolated by genetic screens in yeast [5] and is a member of the phosphatidylinositol kinase-related kinase (PIKK) family [6], which consists of large molecular weight protein kinases, including TOR, ATM (ataxia-telangiectasia mutated), ATR (ATM and Rad3-related), DNA-PK (DNA-dependent protein kinase) and hSMG1 (suppressor with morphological effect on genitalia) [7]. Although the sequence of the catalytic domain of PIKK family members is closer to that of phosphatidylinositol kinases, PIKK enzymes have only protein kinase activity [7]. Despite the name 'target of rapamycin', rapamycin binds to the immunophilin FKBP12 [8]. Together, the rapamycin–FKBP12 complex inhibits TOR function by binding TOR specifically [5,9]. Because of its high potency and specificity to TOR, rapamycin has been used to probe the biological functions of TOR.

TOR is structurally and functionally conserved from yeast to mammals. Yeast have two TOR genes, *TOR1* and *TOR2* [10], whereas higher eukaryotes have a single TOR gene [11]. TOR exists in multiprotein complexes and has an essential role in regulation of cell growth and size by modulating transcription, translation, ribosomal biogenesis and cell morphology [11]. Rapamycin treatment causes a significant size reduction of mammalian cells in culture [12]. Genetic inactivation of the *Drosophila TOR* gene (*dTOR*) also causes a dramatic reduction in cell size [13,14].

The TOR pathway is regulated by many intracellular and extracellular signals. For example, mammalian TOR (mTOR) is activated by growth factors and nutrients, such as amino acids [15]. Conversely, it is inhibited by numerous stress conditions, such as cellular energy depletion, hypoxia and osmotic stress [16–18]. Therefore, the TOR pathway must integrate both positive and negative signals to regulate cell growth in a coordinated manner. Several recent reviews have covered the regulation and function of TOR in cell growth [11,19,20]. Here, we focus mainly on new developments in the TOR field, specifically those regarding the TOR complexes (TORC) and their functions.

TOR complexes in yeast

Early studies in *Saccharomyces cerevisiae* indicated that TOR has at least two separable cellular activities because not all of its functions are sensitive to inhibition by rapamycin [21]. Mutations in either *TOR1* or *TOR2* confer resistance to growth inhibition by rapamycin [5], indicating that these genes overlap in a function required for cell growth at G1. However, despite this convergence in function, *TOR1* and *TOR2* are not completely redundant with respect to each other. Moreover, rapamycin treatment causes a G1-specific arrest in yeast, whereas deletion of *TOR2* is lethal but does not cause the same arrest [21,22], suggesting that some functions of *TOR2* are not inhibited by rapamycin. Significantly, *TOR2*, but not *TOR1*, is involved in cellular polarization and cytoskeletal reorganization [23]. These observations indicate that the yeast TOR genes have two distinct functions, one of which is sensitive to rapamycin.

The molecular mechanism for the two distinct TOR functions in yeast was not appreciated until the purification of two separate TOR complexes (TORC1 and TORC2) by Hall and colleagues [24]. TORC1 contains Kog1 (Kontroller of growth 1), Lst8 (Lethal with *SEC* thirteen) and either Tor1 or Tor2 (Table 1). The purified TORC2 contains Avo1 (Adheres voraciously to Tor2), Avo2, Avo3, Lst8 and Tor2 (Table 1) [24]. *In vitro* biochemical characterization shows that the rapamycin–FKBP12 complex directly binds to TORC1 but not to TORC2; therefore, only TORC1 function is inhibited by rapamycin. More recently, Tco89 and Bit61 were found to be novel

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Table 1. Components of TOR complexes

	Saccharomyces cerevisiae	*Caenorhabditis elegans*	*Drosophila melanogaster*	*Homo sapiens*
TORC1	Tor1, Tor2	ceTOR	dTOR	mTOR
	Lst8	AAB42347.1	dGβL	mLST8/GβL
	Kog1	ceRaptor	dRaptor	Raptor
	Tco89			
TORC2	Tor2	ceTOR	dTOR	mTOR
	Lst8	AAB42347.1	dGβL	mLST8/GβL
	Avo1			
	Avo2			
	Avo3	CAB042201.1 CAB54288.1	dRictor	Rictor/mAVO3
	Bit61			

components of TORC1 and TORC2, respectively [25]. Consistent with the protein interaction data, deletion of *TCO89* increases sensitivity to rapamycin, a phenotype similar to *TOR1* deletion. Two additional proteins, Slm1 and Slm2, interact with components of TORC2 [26,27]. However, further studies are needed to conclude whether these proteins are integral components of TORC2. Taken together, these studies demonstrate that TOR functions in two distinct multi-molecular complexes and this explains early observations that rapamycin only inhibits part of yeast *TOR2* function.

In yeast, TORC1 controls cell growth by regulating multiple processes including transcription, translation, ribosomal biogenesis and autophagy [24,28,29]. Recent studies have shown a molecular mechanism by which TORC1 stimulates expression of genes encoding ribosomal proteins [30–32]. The yeast forkhead transcription factor Fhl1 is important for ribosomal gene expression, as it binds directly to ribosomal gene promoters [33]. To stimulate ribosomal gene expression effectively, Fhl1 must associate with Ifh1, a transcription co-factor; however, the interaction between Fhl1 and Ifh1 can be competed for by Crf1, a transcriptional repressor. Crf1 is regulated by Yak1, which is indirectly inhibited by TORC1 [32]. Under nutrient-deficient conditions, the Yak1 protein kinase is active and phosphorylates Crf1, which then relocates into the nucleus and displaces Ifh1 from Fhl1 at the ribosomal gene promoters; therefore, transcription of ribosomal genes is inhibited. Under nutrient-sufficient conditions, active TORC1 inhibits Yak1 through Pka. Inhibition of Yak1 leads to dephosphorylation and the relief of inhibition of Fhl1 by Crf1; therefore, ribosomal gene transcription is increased. Taken together, these studies reveal a biochemical mechanism connecting ribosomal gene regulation and TOR pathway activity [32].

TORC1 in cell-growth regulation

Rapamycin treatment inhibits mammalian cell growth [12]. Expression of a rapamycin-resistant mTOR mutant confers resistance to growth inhibition by rapamycin [34,35]. Therefore, the growth-inhibitory effect of rapamycin on mammalian cells is mediated solely through mTOR inhibition. The best-known targets of mTOR are ribosomal S6 kinase 1 (S6K) and the eukaryotic initiation factor 4E binding protein 1 (4EBP1) [11]; rapamycin potently and rapidly induces dephosphorylation of these proteins [11]. mTOR directly phosphorylates Thr389 of S6K, which is essential for S6K kinase activity and S6K, in turn, phosphorylates ribosomal S6 protein [36]. A knock-in of S6 protein with the S6K phosphorylation site eliminated ($rpS6^{p-/-}$) does not affect 5′-*TOP* (track of polypyrimidine) mRNA translation but instead enhances total protein synthesis [37]. Interestingly, $rpS6^{p-/-}$ mouse embryo fibroblasts (MEFs) are significantly smaller than wild-type MEFs. Furthermore, the size of $rpS6^{p-/-}$ cells, unlike wild-type MEFs, is not decreased by rapamycin. These observations indicate that S6 phosphorylation has a crucial role in mediating the effect of mTOR on mammalian cell-size regulation. Phosphorylation of 4EBP1 by mTOR relieves the inhibitory activity of 4EBP1 towards eukaryotic initiation factor 4E (eIF4E) [38]. eIF4E recognizes the cap structure at the 5′ end of most eukaryotic mRNA; this provides a potential mechanism by which mTOR can stimulate translation initiation [39,40]. Together, regulation of cell growth and protein translation are the best-characterized functions of mTOR in mammalian cells.

Using chemical crosslinking and immunoaffinity purification, Raptor was isolated as an mTOR-associated protein [41,42]. Interestingly, Raptor is homologous to the yeast Kog1 protein in TORC1 that was identified by Loewith *et al.* [24]. These authors have also isolated Lst8 as a component of TORC1 and TORC2 in yeast. They identified the mammalian counterpart of yeast Lst8 (mLST8) and demonstrated its interaction with mTOR. mLST8/GβL has an essential role in the phosphorylation of S6K and 4EBP1 by TORC1 and in the regulation of cell size [43]. These studies demonstrate the structural and functional conservation of TORC1 in eukaryotes (Table 1).

Both S6K and 4EBP1 contain a short sequence termed a TOR signaling (TOS) motif that is important for phosphorylation by mTOR [44] and, interestingly, is important for TORC1 substrates to bind to Raptor [45–47]. This demonstrates that mTOR is the catalytic subunit in TORC1 and that Raptor is involved in substrate recognition. However, the function of Raptor is more complex than being a simple substrate-recruiting component in TORC1. Knockdown of Raptor also significantly reduces mTOR protein levels, suggesting that Raptor stabilizes mTOR [41]. Furthermore, the binding between Raptor and mTOR is sensitive to stimulations that affect mTOR kinase activity, such as by amino acids [41].

One group has proposed that Raptor and mTOR form a nutrient-sensitive complex [41]. Based on this model, nutrient deprivation, such as leucine starvation, results in