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Nutritional Control via Tor signaling in *Saccharomyces cerevisiae*

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Abstract

The yeast *Saccharomyces cerevisiae* senses and responds to nutrients by adapting its growth rate and undergoing morphogenic transitions to ensure survival. The Tor pathway is a major integrator of nutrient-derived signals that in coordination with other signaling pathways orchestrates cell growth. Recent advances have identified novel Tor kinase substrates and established the protein trafficking membranous network and the nucleus as platforms for Tor signaling. These and other recent findings delineate distinct signaling branches emanating from membrane associated Tor complexes to control cell growth.

Introduction

All living organisms sense and respond to nutrient-derived signals to adapt their physiology and adopt appropriate developmental decisions to promote survival. In eukaryotic organisms ranging from yeasts to humans, the Tor signaling pathway is a global regulator that controls cell growth. The central components of this signaling cascade are the Tor protein kinases, which were first identified in yeast as targets of the antifungal and immunosuppressive agent rapamycin. Treatment of cells with rapamycin results in dramatic physiological changes including: G1 cell cycle arrest, protein synthesis inhibition, glycogen accumulation and autophagy, which closely resemble those observed in cells deprived of nutrients. These and other findings support the view that Tor is activated by amino acid-derived signals to positively govern a myriad of anabolic processes, including translation, transcription, ribosome biogenesis and actin deposition to sites of active cell growth, while negatively regulating catabolic processes, such as protein degradation, mRNA destabilization, and autophagy [1*].

The versatility of the Tor kinases in impacting this wide range of cellular responses stems from their ability to physically associate with other proteins and thereby functionally coordinate diverse signaling pathways. The Tor proteins form two distinct, evolutionary conserved multimeric protein complexes known as TORC1 and TORC2 [2-6*]. Recent studies reveal

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these Tor complexes associate with internal membranes of the protein secretory pathway as well as with the nucleus, and these subcellular localizations are critical for Tor function [7*, 8*]. The Tor pathway often works in parallel with the cAMP-PKA cascade to control common targets and also intersects with other signaling networks, such as the general amino acid control (GAAC) response, [9-13*]. Several newly identified Tor substrates, Sch9, Ypk1, and Slm1,2, further link Tor function to ammonium sensing, actin organization, control of cell integrity, and stress response [14*-20*]. Here we discuss the latest developments in the mechanisms of signaling by the Tor kinase cascade in the budding yeast *S. cerevisiae*.

Rapamycin sensitive and insensitive Tor complexes

In *S. cerevisiae*, the highly homologous Tor1 and Tor2 proteins associate with Kog1, Tco89, and Lst8 in the protein complex TORC1. A separate pool of Tor2 associates with Lst8, Avo1, Avo2, Avo3, Bit61, and Bit2 in a distinct complex called TORC2 [2-6*]. It has generally been accepted that each complex mediates distinct physiological processes in response to nutrient cues. TORC1, which is sensitive to rapamycin, regulates temporal processes of growth while the rapamycin-insensitive TORC2 is thought to control spatial aspects of growth such as actin polarization [1*].

While this notion has prevailed to date, three independent reports suggest the division of labor associated with each Tor complex is not as clearly delineated as previously thought. A comprehensive study has provided evidence for genetic interactions between TORC1 and a network of genes involved in actin polarization and cell wall integrity [7*], processes which were thought to be regulated by TORC2. Such evidence supports previous reports of actin polarization regulation by Kog1, an exclusive TORC1 component, as well as rapamycin-induced changes in actin organization [6*,21*].

Recent studies demonstrated that the AGC kinase Ypk2 is a direct substrate of TORC2 (20*). Both TORC2 and Ypk2 regulate actin polarization and are thought to act upstream of the Rho1/Pkc1/ MAPK cell integrity pathway. This provides a likely route by which TORC2 controls actin cytoskeleton dynamics. Together, these findings show further evidence of functional overlap between TORC1 and TORC2, blurring the distinction between rapamycin-sensitive and insensitive pathways.

Signaling branches downstream of TORC1

The advent of genome-wide transcriptional analysis, as well as classical genetic approaches, revealed a remarkably robust transcriptional profile of TORC1 controlled genes [22]. This, in turn, identified a number of transcriptional regulators that act at the promoters of rapamycin-sensitive genes. Subsequent work pinpointed the nuclear exclusion of relevant transcription factors as a common theme in the regulation of TORC1 sensitive genes [23]. A continuing challenge is the elucidation of the mechanisms by which signals emanating from TORC1 exert appropriate transcriptional responses.

The first link between TORC1 signaling and a downstream component, the PP2A-like phosphatase Sit4 and its regulatory subunit Tap42 derived from studies with *tap42* alleles that blunted many rapamycin-induced phenotypes [24]. Subsequently, Tap42 was shown to be a direct target of Tor phosphorylation [25]. Analysis of additional *tap42* rapamycin-insensitive alleles and *sit4* mutants demonstrated that Tap42 represents a major branch of TORC1 signaling that is responsible for repression of stress regulated (STRE), nitrogen catabolite repressed (NCR), and retrograde signaling (RTG) genes, as well as for crosstalk between TORC1 and the GAAC response [26-28]. Remarkably, the effects of TORC1 signaling on RP (ribosomal protein) and Ribi (ribosome biogenesis) regulons are not Tap42-mediated. For some targets (NCR and RTG genes) Tap42 functions in concert with PP2A, whereas for other targets

(STRE genes) Tap42 inhibits PP2A [26,29]. Likewise, Tip41, a Tap42-interacting protein that regulates Tap42-phosphatase interactions, appears to play both negative and positive roles in Tap42 signaling [30,29]. Interestingly, a pool of Tap42 is localized to membranes in complex with TORC1 in actively growing cells (Figure 1A) [31*]. In response to TORC1 inactivation, Tap42 is released from membranes coincident with Tap42-dependent activation of target genes (Figure 1B). In addition, rapamycin-resistant mutants of Tap42 largely fail to dissociate from TORC1 associated membranes. Whether this mode of regulation governs all TORC1-Tap42 signaling, or if Tip41 plays a role in this process, is unknown.

Gln3 regulation underscores the complexity in the control of TORC1-regulated phosphatases. During growth in preferred nitrogen sources, Gln3 is cytoplasmic and translocates to the nucleus when cells are shifted to poor nitrogen sources or upon rapamycin treatment. Gln3 nuclear import induced by rapamycin is prevented by inactivation of Tap42, or deletion of Sit4 [23]. However, the nature of the signal generated by nitrogen quality may be different from that of rapamycin-induced Sit4 activity since nitrogen quality still controls Gln3 localization in *sit4* deleted cells [32].

Another target for PP2A signaling is Maf1, a regulator of ribosome biogenesis that inhibits Pol III in response to nutrient depletion or rapamycin treatment. Maf1 nuclear localization is prevented by PKA-dependent phosphorylation and translocation to the nucleus is triggered by rapamycin treatment [33]. A recent report demonstrated that rapid dephosphorylation of Maf1 in response to unfavorable environmental conditions is mediated by PP2A signaling; however, the involvement of Tap42 in this process has not been examined (Figure 1B) [34].

Many Tap42-independent TORC1 signaling events can be explained by the recent identification of the AGC kinase Sch9 as a direct TORC1 kinase substrate. Earlier, a genome-wide screen for new regulators of start uncovered a strong connection between ribosome synthesis and cell size [35]. Deletions of *SFP1* or *SCH9* were identified as conferring dramatic small cell size phenotypes. Sfp1 controls the Ribi regulon and the RP genes. Artificial activation of Sfp1 or Sch9 upregulates the Ribi and RP genes resulting in a large cell phenotype. In addition, carbon starvation or rapamycin treatment leads to dephosphorylation and inactivation of Sch9 [36]. Either rapamycin treatment or *sch9* mutation triggers nuclear localization of the Rim15 kinase, which in turn regulates a transcriptional program for entry into G₀ and increased chronologic lifespan [37,38].

These and other effects indicated a close relationship between Tor and Sch9 signaling. The TORC1-sensitive phosphorylation sites on Sch9 were identified and mutation of these sites to non-phosphorylatable amino acids was shown to block all known functions of Sch9 with little effect on TORC1-regulated programs of NCR and RTG signaling [14*]. In mammalian cells mTor directly phosphorylates and activates the AGC kinase S6 kinase [39]. These results establish a signaling branch downstream of TORC1 that is distinct from the Tap42-mediated branch (Figure 1), and this brings TORC1 signaling in line with cell growth regulation in mammalian cells with Sch9 fulfilling a role analogous to S6 kinase. Importantly, some TORC1 controlled effects, most notably Msn2 and Msn4 localization, appear to be regulated by both branches [26,29,14*]. The Tap42 and Sch9 branches of TORC1 signaling explain most TORC1 cellular roles. Finally, Tor1 itself has been shown to translocate to the nucleus where directly regulates Pol I and Pol III transcription (discussed below) (Figure 1A) [8*]. Whether Tor1 nuclear localization is subject to control by Sch9 and/or Tap42 or represents a third independent TORC1 signaling branch is currently unknown.

Tor signals from internal membranes

Recent studies have forged an intimate relationship between Tor signaling and internal membranes of the secretory pathway. Several components of TORC1, including Tor1, Tor2,

Lst8, Kog1, and Tco89, have been localized to endosomal, Golgi, prevacuolar, and vacuolar compartments [4,40,41,3,6*,5]. Similarly, the Gse/Ego complex, which control plasma membrane targeting of the general amino acid permease Gap1 in response to amino acids, resides in prevacuolar compartments [42**]. TORC1 and the Gse/Ego complex are also necessary for vacuolar membrane recycling via a process known as microautophagy, which occurs during recovery from rapamycin-induced growth arrest [43**]. Based on these findings an exciting role has been hypothesized for the Gse/Ego complex as a functional component of the mechanism involved in relaying amino acid signals from the vacuole to TORC1 [1*]. This model is particularly attractive given that the vacuole is a major cellular reservoir for amino acids.

Three current studies have indicated that the TORC1 link to intracellular membranes is not just physical but also functional. First, a novel role for the Golgi $\text{Ca}^{2+}/\text{Mn}^{2+}$ ATPase (Pmr1) in negatively regulating TORC1 signaling has been proposed [44*]. The relevant activity of Pmr1 was determined to be its ability to transport Mn^{2+} (rather than Ca^{2+}) and Mn^{2+} supplemented growth medium restores rapamycin sensitivity to *pmr1* cells [45]. The role of manganese remains unclear but it has been suggested that Mn^{2+} is required for mannosylation of proteins and lipids required for proper protein trafficking.

Second, Tor complexes are bound to detergent-resistant membranes (DRMs) and proteomic analysis reveal that several proteins involved in actin organization are colocalized with Tor1 and Tor2 there [4,7*]. Interestingly, when any of nine genes encoding these proteins are mutated in cells lacking the TORC1 non-essential components Tor1 and Tco89, synthetic lethality or reduced fitness defects are observed, indicating the function of these gene products may be linked to a TORC1-related role (see below). Third, a systematic genome-wide screen yielded over 200 genes that, when mutated in combination with the *tor1* mutation, result in synthetically lethal or reduced fitness phenotypes [46*]. These genes represent diverse functional categories (Figure 2A), illustrating further the complexity of TORC1 signaling. In particular, a striking link was evident between TORC1 and the class C Vps, the Ego/Gse, and the preautophagosome (PAS) complexes which function in vesicle docking and fusion, protein sorting, and autophagy, as well as with other genes involved in vacuolar segregation (Figure 2B). A previous study that examined the rapamycin sensitivity of the yeast gene deletion collection also reported a class C Vps complex connection unique to Tor1 and not shared with Tor2 [47]. These findings were surprising since it had been thought that Tor2 was capable of providing all of the cellular functions associated with Tor signaling. Instead we now appreciate that there are Tor1/2-shared functions as well as both Tor1- and Tor2-unique functions.

These results prompted studies to assess in greater detail the impact of TORC1 signaling in protein sorting. Mutation of Tor1 or rapamycin exposure did not have a detectable effect on maturation of vacuolar hydrolases sorted via the endosomal, the Cvt, or the non-endosomal pathways. Similarly, experiments examining alpha factor processing revealed that forward and retrograde transport between the Golgi complex and endosomal compartments is not affected by loss of Tor1 function [46*]. In contrast, while TORC1 activity was dispensable for receptor mediated endocytosis or endocytosis of the Mep2 ammonium permease, rapamycin treatment caused a modest delay in a late step of fluid phase endocytosis [48,46*,7*]. Whether this delay is of sufficient magnitude to be physiologically relevant remains to be explored and one possibility is that it is a secondary consequence of the actin polarization defect caused by rapamycin.

Remarkably, a role for the class C Vps complex function in mediating amino acid homeostasis for proper Tor signaling was also revealed and suggested that Tor1 is more efficient than Tor2 in supporting growth under conditions of amino acid limitation [46*]. Collectively, these findings suggest a model whereby localization of TORC1 to membranes of the protein transport

apparatus is important for reception of amino acid-derived signals and, in turn, to relay these signals to TORC1 effectors. These findings further underscore the role of the protein transport membranous network and its components as a prominent and strategic platform that functionally influences a growing number of signaling processes, including the Rim101-mediated pH-response, MAP kinase activation via the G α subunit Gpa1 and the PI-3 kinase Vps34, and (as discussed here) nutrient sensing [49,50].

Tor signaling in the nucleus

One major role of Tor signaling is the control of ribosome biogenesis, which requires the coordinated action of the three nuclear RNA polymerases: Pol I, Pol II, and Pol III, resulting in transcription of 35S rRNA, RP, and 5S rRNA and tRNA genes.

An interesting aspect in Tor control of ribosome biogenesis was recently brought to light by the discovery that a significant fraction of Tor1 localizes to the nucleus (Figure 1A) [8**]. This nuclear localization is dynamic and can be prevented by either nutrient starvation or rapamycin exposure (Figure 1B). Tor1 nucleocytoplasmic shuttling is assisted by the alternate action of the importin Srp1 and the exportin Crm1 and by nuclear localization (NLS) and nuclear export sequences (NES) within the Tor1 protein. Nuclear Tor1 was found to bind the 35S rDNA promoter via a helix turn helix motif (HTH). Either mutation of the Tor1 NLS or HTH motifs, which prevent nuclear entry and promoter binding respectively, impaired the ability of Tor1 to regulate Pol I-directed expression of 35S rRNA and Pol III-driven expression of 5S rRNA. These mutations however, had no effect on Pol II-regulated expression of the NCR genes or the RP genes. This later event is tightly coordinated with rRNA expression to ensure a balanced supply of building blocks for ribosome biogenesis. In contrast, the Tor1 NES mutation, which prevented nuclear export, did not impair TORC1 control, thought to occur in the cytoplasm, of Pol II-driven NCR and RP gene expression. Interestingly, mTor has also been previously reported to shuttle between the nucleus and the cytoplasm, and thus this may be an evolutionary conserved feature of the Tor kinases [51].

These exciting findings challenge current models that TORC1 regulation of Pol II-directed genes is initiated in the cytoplasm by promoting the nuclear translocation of the transcriptional activators. However, several outstanding questions remain to be addressed. First, which are the direct targets of TORC1 at the rRNA promoters and within the nucleocytoplasmic trafficking machinery? An obvious candidate for a nuclear TORC1 target is the ubiquitously conserved Pol I transcriptional activator Rrn3/TIF-1A, which recruits Pol I to the 35S rDNA promoters. In human cells TIF-1A is phosphorylated and thereby activated by TORC1 [52]. Although this phosphorylation has not as yet been demonstrated in yeast cells, TORC1 signaling does positively regulate Rrn3 interaction with RNA Pol I (53). Other possible Tor substrates are the Rpd3 histone deacetylase, which is thought to be recruited to the rDNA repeats in a TORC1-dependent fashion under conditions of active transcription, and the RNA Pol III repressor Maf1 (discussed above) [54,34]. Because Zheng and coworkers showed that TORC1 regulates its own nuclear–cytoplasmic shuttling, karyopherins and accessory factors could also be potential targets for TORC1 action. Second, by which mechanisms is TORC1 recruited to the rDNA promoters? While it is possible that Tor1 might directly bind the rDNA promoters via its HTH motif, this remains to be tested.

The answers to these questions should clarify the extent of the impact of TORC1 nuclear signaling and will thereby advance our understanding of ribosome biogenesis and cell growth control.

Integration of Tor with other pathways

In response to nutrient cues the TOR pathway intersects with other signaling cascades to orchestrate cell growth (summarized in Table 1). Both Tor/Sch9 and the cAMP-PKA pathways often function in parallel to regulate common targets, including expression of the RP, Ribi and STRE genes as well as genes required for entry into the G₀ phase regulated by Rim15, and converge on downstream effectors (Table 1) [10-14*].

Similarly, the NCR pathway that controls cellular responses to nitrogen quality and the retrograde pathway that responds to mitochondrial dysfunction are intimately related to TORC1 activity. This relation is underscored by the fact that Lst8, a regulator of retrograde signaling, is a component of both Tor complexes [55]. Tor also negatively regulates two signaling programs that are activated by nitrogen source limitation: the GAAC response and autophagy [27,28,56,57].

Finally, two branches downstream of TORC2 act on signaling programs that promote polarization of the actin cytoskeleton. In one branch, TORC2 phosphorylates Ypk2 to activate Rho1-PKC, as discussed above. In the second branch, the newly identified TORC2 effectors Slm1 and Slm2 (PH domain proteins subject to control by PI4, 5P (2) and sphingolipid signaling) drive actin polarization and are required to cope with heat and oxidative stress [15*-19]. TORC2-Slm signaling antagonizes the Ca²⁺ and calmodulin-dependent phosphatase calcineurin; Slm1 and Slm2 negatively influence calcineurin signaling and Slm1,2-depleted or *slm* mutant cells exhibit actin depolarization and stress hypersensitivity phenotypes, which are suppressed by calcineurin defects [19,18*].

Concluding remarks

Recent years have witnessed considerable advances in the understanding of nutrient sensing and control of cell growth via Tor signaling in *S. cerevisiae*. In this review we sought to take a “reductionist view” of these advances to draw attention to three important, emerging themes. First, signaling events downstream of Tor complexes occur in separate branches. Second, nutrient sensing and Tor signaling are intimately linked with internal membranes. Third, TORC1 responsive phenomenon involves direct nuclear shuttling of Tor1 itself to Pol I and Pol III promoters. Whether this represents a third TORC1 branch or is subject to control by one or both of the other TORC1 signaling branches is unknown.

The challenges ahead are to elucidate the mechanisms by which nutrient and stress signals are transmitted to Tor, to identify the remaining Tor substrates, and to understand the precise mechanisms by which Tor and its substrates orchestrate cell growth.

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Figure 1. TORC1 dependent signaling events regulating synthesis of ribosome components

A, when nutrients are abundant Tap42 is localized to the membrane and is associated with TORC1. Under these conditions, TORC1 phosphorylates Sch9, which promotes expression of the Ribi and RP genes required for ribosome biogenesis. The RP and Ribi genes are controlled by the localization and activity of specific transactivators (including Abf1, Rap1, Sfp1, Ifh1, Fhl1, Hmo1, and Crf1) as well as the histone acetylase and deacetylase Esa1 and Rpd3, respectively. The precise mechanisms that regulate the activities and localization of these factors, as well as the signaling events that link Sch9 to RP and Ribi promoters, remain to be elucidated. Tor1 translocates to the nucleus in nutrient replete conditions and associates with Pol I and Pol III promoters. **B**, in response to nutrient deprivation or rapamycin treatment, Sch9 phosphorylation is inhibited and ribosome biogenesis is turned off. Tor1 is exported from the nucleus and Tap42 is displaced from the membrane and, in concert with the catalytic subunits of PP2A (and PP2A-like phosphatases), dephosphorylates specific targets, such as the Pol III repressor Maf1. The signaling events that govern Tor1 nuclear shuttling, and whether these are influenced by the activities of Tap42 or Sch9, are unknown.

Figure 2. Genetic synthetic interaction network of *Tor1*

A, graphical representation of genes (grouped in functional categories) that when mutated in combination with *tor1* result in synthetic lethal or reduced fitness defects. **B**, schematic representation of the distinct functional categories involved in protein sorting and vacuolar function, that exhibit synthetic interactions with *tor1*. Mutation of the genes shown in bold confer rapamycin hypersensitivity as demonstrated by two recent studies [46*,47].

Table 1**Summary of the major pathways that interact with Tor signaling**

Pathway	Cellular cue	Major outputs	Intersection point(s) with TOR signaling
Calcineurin	Ca ²⁺	Negative regulation of STRE genes, Ion homeostasis	Slm1, Slm2, Fpr1
GCN	Amino acid deprivation (uncharged tRNA)	Upregulation of amino acid anabolism and scavenging	Gcn2, eIF2 α
Snf1	Glucose depletion	Activation of alternative carbon source utilization	Snf1, Gln3
Nitrogen discrimination	Quality of N source	Activation of non-preferred nitrogen source utilization	Ure2, Gln3, Gat1
Retrograde	Mitochondrial dysfunction	Replenishing of TCA cycle components	Lst8, Mks1, Rtg1, Rtg2, Rtg3
PKC	Osmotic homeostasis	Cell wall integrity	Rom2
PKA	Fermentable carbon source	Promotion of ribosome biogenesis, repression of stress response and G ₀ entrance	Fhl1, Ihf1, Sfp1, Maf1, Yak1, Msn2, Msn4, Rim15,
PHG (diploid specific)	Poor N source	Foraging behavior-filamentous growth	Snf1, Mep2
Autophagy	N starvation	Autophagy	Apq1, Apq13