Published in final edited form as:

Enzymes. 2010 ; 28: 167–187. doi:10.1016/S1874-6047(10)28008-3.

Conservation of the Tsc/Rheb/TORC1/S6K/S6 Signaling in Fission Yeast

AKIO NAKASHIMA¹ and FUYUHIKO TAMANOI

Department of Microbiology, Immunology and Molecular Genetics Molecular Biology Institute, Jonsson Comprehensive Cancer Center University of California, Los Angeles, California, USA

Abstract

The TSC/Rheb/TORC1/S6K/S6 signaling pathway plays critical roles in regulating protein synthesis and growth in eukaryotes. Our recent work using fission yeast *Schizosaccharomyces pombe* revealed that this signaling pathway is conserved from humans to fission yeast. In addition to target of rapamycin (TOR) homologs and tuberous sclerosis complex (TSC) homologs, fission yeast but not budding yeast, has a functional homolog of Rheb, a small G-protein acting as an activator of TOR complex 1 (TORC1). Several lines of genetic evidence suggest that the Tsc1–Tsc2 complex and Rheb act as upstream players of TORC1 in fission yeast. We have recently demonstrated that TORC1, but not TORC2, regulates phosphorylation of ribosomal protein S6 in response to nutrient availability. Candidate S6 kinase (S6K) protein has been identified. In addition, we find that rapamycin prevents a subset of TORC1 activity to regulate S6 phosphorylation in fission yeast.

II. Introduction

Evolutionarily conserved mechanisms by which protein phosphorylation mediates numerous cellular activities, such as cell cycle progression, differentiation, and metabolism, in response to alteration of environmental conditions operate in eukaryotic cells. TOR is a highly conserved serine/threonine kinase of the phosphatidylinositol kinase-related kinase family in eukaryotes. TOR has pivotal roles in cell proliferation, cell growth, and metabolism in response to environmental stimuli, such as nutrients, growth factors, and stresses. TOR exists as the catalytic subunit in two distinct multiprotein complexes, TORC1 and TORC2. The TORC1 signaling promotes anabolic processes, such as protein synthesis and ribosome biogenesis, and inhibits catabolic processes such as autophagy. The immunosuppressant and anticancer drug rapamycin preferentially inhibits TORC1 activity but not TORC2 through the binding to immunophilin FKBP12 [1, 2].

In mammalian cells, mammalian TORC1 (mTORC1) contributes to cell growth by directly phosphorylating translation regulators, S6K and eIF4E-binding protein (4E-BP) [3]. A member of the Ras superfamily G-protein, Rheb, positively regulates mTORC1 but not mTORC2 when bound to GTP. On the other hand, the tumor suppressor proteins, tuberous

sclerosis complex 1 (TSC1, also known as hamartin) and TSC2 (also known as tuberin), associate with each other and negatively control mTORC1 functions through inactivation of Rheb. In particular, TSC2 contains a domain that shares homology with the catalytic domain of GTPase-activating proteins (GAPs). The TSC–Rheb pathway mediates inputs from numerous signaling, such as growth factor, hypoxia, or energy status, to mTORC1 to control cell growth and other cellular processes [4, 5].

The fission yeast *S. pombe* has a similar TOR signaling system as described below. First, fission yeast possesses two TOR genes, *tor1*⁺ and *tor2*⁺. Second, the microbe also has TORC1, which consists of Tor2, a raptor homolog Mip1, an mLST8 homolog Wat1 (also known as Pop3), Toc1, and Tco89, as well as TORC2, which consists of Tor1, a rictor homolog Ste20, Sin1, Wat1, and Bit61. TORC1 controls the switch between cell cycle and cell differentiation by sensing nitrogen availability, whereas TORC2 participates in cell proliferation under stress conditions and in sexual differentiation under nitrogen starvation. Third, unlike budding yeast, the structural and functional homologs of Rheb and the TSC1–TSC2 complex are conserved as Rhb1 and the Tsc1–Tsc2 complex, respectively. It is believed that Rhb1 positively and the TSC complex negatively modulate TORC1 function. Therefore, fission yeast has emerged as an ideal system to study functions of the TOR signaling [6, 7].

Our study in fission yeast led first to the idea that the function of the TSC/Rheb/TORC1 in fission yeast is very similar to that in mammalian cells. Our further characterization of this model system led to the identification of S6 homolog in fission yeast. These results reveal that the entire TSC/Rheb/TORC1/S6K/S6 signaling pathway is conserved from human to fission yeast. We also found that this pathway in fission yeast is inhibited by rapamycin [8]. This is interesting, as, unlike some mammalian cell lines and budding yeast, rapamycin does not prevent the proliferation of the prototrophic cells in fission yeast [9, 10]. In this review, we will focus on fission yeast and describe the results obtained concerning the TSC/Rheb/TORC1/S6K/S6 signaling pathway.

III. Overview of the TSC/Rheb/TORC1 Signaling in Fission Yeast

A. TSC1–TSC2

The *TSC1* and *TSC2* genes were first identified as the genetic loci mutated in the autosomal dominant disorder TSC in human genome [11, 12]. In fission yeast, the *tsc* genes, *tsc1*⁺ and *tsc2*⁺, were identified as structural homologs of human, *TSC1* and *TSC2*, respectively [13]. The gene products Tsc1 and Tsc2 form a heterodimer and promote nutrient uptake, such as amino acids and nucleotides through regulation of localization and expression of transporters, such as amino acid transporter [13–17]. Disruption of either the *tsc1* or the *tsc2* gene markedly decreases uptake of leucine and basic amino acids, such as arginine, lysine, and histidine. Therefore, null mutant of either the *tsc1* or *tsc2* gene exhibits poor growth in media containing low concentration of leucine when the cells possess a leucine auxotroph. Conversely, decrease in uptake of the basic amino acids in the *tsc* null mutants confers resistance to canavanine and thialysine, which are toxic analogs of arginine and lysine, respectively [14, 15, 17]. It is noteworthy that the *tsc2*^{N1191K} mutant (the mutation

corresponds to human TSC2 mutation derived from patients of TSC) loses function in amino acid uptake, suggesting the use of *S. pombe* TSC as a model system for human TSC [14].

We have demonstrated that mislocalization of Cat1, a cationic amino acid transporter, is responsible for resistance to canavanine in *tsc2* cells. Cat1 mostly localizes to the golgi-like punctates in the cytoplasm in *tsc2* cells, whereas in the wild-type cells it is predominantly found on the periphery of cell tips with minor internal staining. Although it is not known how the TSC complex regulates localization of amino acid transporters, loss of Pub1, an E3 ubiquitin ligase, restores proper localization of Cat1 and sensitivity to canavanine in *tsc2*, suggesting that the Tsc complex might affect localization of Cat1 through regulation of Pub1 function [17]. In budding yeast, RSP5, a homolog of Pub1, is also involved in endocytotic downregulation of several amino acid transporters via RSP5-mediated ubiquitination [18–20].

It is reported that aberrant amino acid uptake in *tsc2* is suppressed by downregulation of Rhb1 (Rheb) function, as described below. Expression of a dominant negative *rhb1* mutant such as *rhb1D60K* but not the wild-type gene suppresses mislocalization of Cat1 and canavanine resistance in *tsc2* [14, 17, 21, 22]. Cpp1 encodes the β -subunit of protein farnesyltransferase that catalyzes farnesylation of Rhb1. This lipid modification is important for the function of the small G-protein [15, 23, 24]. Mutation of the *cpp1* gene, leading to downregulation of Rhb1 function, causes hypersensitivity to canavanine and suppressed decreased amino acid uptake in *tsc2*. By contrast, these phenotypes of the *cpp1* mutation are suppressed by the expression of a geranylgeranylated mutant, *rhb1^{CVIL}*, which can bypass farnesylation [15, 23]. These genetic interactions suggest that Rhb1 is a downstream player of the Tsc complex in amino acid uptake (other relationships between Tsc and Rhb1 are discussed below).

Tsc1 and Tsc2 are also involved in cell response to nitrogen starvation. Fission yeast cells arrest in G1 phase under nitrogen starvation and subsequently proceed to mating, meiosis, and sporulation if different mating type cells exist. These events are known as sexual differentiation. Disruption of either the *tsc1* or the *tsc2* gene results in a delay in G1 arrest and decrease in mating efficiency. Microarray analysis has shown that disruption of either of the *tsc* genes causes reduction of gene expression, such as *fnx1⁺*, *mei2⁺*, and *sxa2⁺*, that are involved in nitrogen starvation response and sexual differentiation under nitrogen starvation [13, 15, 25]. van Slegtenhorst et al. have shown that Pas1, a G1 cyclin, contributes to a delay in G1 phase arrest in the *tsc* mutants under nitrogen starvation [22]. However, it has not been determined how the activity of the Tsc complex is regulated when cells perceive alteration of nutrient conditions such as nitrogen source and amino acids.

B. Rheb

Fission yeast *rhb1⁺* was first identified as a homolog of human Rheb by genome sequencing [26, 27]. Cells disrupted in *rhb1⁺* do not grow, indicating that *rhb1⁺* is essential for cell proliferation [24, 27]. Interestingly, expression of the human *Rheb* gene, but not the budding yeast *RHB1*, restores cell proliferation in *rhb1* null mutant, suggesting a functional relationship between the human Rheb and the fission yeast Rhb1 [24]. Furthermore, decrease in *rhb1⁺* expression or overexpression of dominant negative *rhb1* mutations causes

growth defect, cell cycle arrest in G1 phase and small round cell [24, 27, 28]. These terminal phenotypes are similar to those of nitrogen-starved cells. Indeed, expression of *fnx1*⁺ and *mei2*⁺, whose transcription is induced by nitrogen starvation, is elevated by repressing *rhb1* expression [27]. In addition, downregulation of Rhb1 function by the failure of its farnesylation in the *cpl1* mutant results in the accumulation of G0/G1 phase cells under stationary phase and suppressing the defect of gene expression in *tsc2* cells in response to nitrogen starvation [15, 24]. These lines of evidence suggest that Rhb1 mediates nutrient signaling to regulation of cell cycle progression and gene expression.

We and others have identified hyperactivating mutations in the *rhb1* gene. Most *rhb1* hyperactivating mutations except for *rhb1-DA8* are caused by a single amino acid change that occurs at conserved amino acids in G1 and G4 boxes or near to G5, whose boxes are implicated in the recognition of guanine nucleotides [21, 25]. Mutations of Rhb1^{V17G} and Rhb1^{S21G} in G1 box are located close to the phosphates of GTP, whereas those of Rhb1^{K120R} and Rhb1^{N153T} that are in or around G4 and G5 boxes, respectively, are located close to the guanine ring. Biochemical characterization of these *Rhb1* mutants has revealed that the four mutants exhibit decrease in GDP binding, and GTPase activity is decreased with Rhb1^{V17G} and Rhb1^{S21G} mutants, but not in Rhb1^{K120R} and Rhb1^{N153T} [21]. On the other hand, the dominant negative mutation, *rhb1-D60V*, occurs at a conserved aspartic acid in G3 box. The Rhb1-D60V mutant loses GTP-binding ability and exhibits preferential GDP binding [28].

The *rhb1* hyperactivating mutations confer resistance to canavanine and thialysine and cause decrease in leucine uptake, similar to the disruption of the *tsc* genes [21, 25]. Murai et al. have shown that two distinct *rhb1* hyperactivating mutants, *rhb1-DA4* and *rhb1-DA8*, exhibit distinct phenotypes under nitrogen starvation. Although both these mutants exhibit canavanine resistance, only *rhb1-DA4* shows reduced cell response to nitrogen starvation, namely, the phenotypes of *rhb1-DA4* are largely consistent with those of *tsc2* [25]. Taken together, these molecular genetic studies indicate that Rhb1 acts as a downstream factor of the TSC complex and that Rhb1 and the TSC complex have opposite effects on amino acid uptake and nitrogen starvation response. These opposite functions are also seen in the TSC–Rheb pathway in higher eukaryotes. Interestingly, the *rhb1-DA8* cells are able to respond to nitrogen starvation similar to the wild-type cells. The *rhb1-DA8* mutation further suppresses defect of cell response of *tsc2* in the *rhb1-DA8 tsc2* double mutant [25].

C. TORC1 Signaling

Several groups have independently demonstrated that Tor2, a catalytic subunit of TORC1, monitors nitrogen conditions to determine cell fate between cell proliferation (and growth) and sexual differentiation (or quiescence). The conditional knockdown of *tor2* function by temperature-sensitive (ts) *tor2* mutations or by inhibition of *tor2*⁺ expression results in mimicking nitrogen starvation, such as cell cycle arrest in G1 phase and entry to sexual differentiation even in nitrogen-rich conditions [16, 29–32]. Microarray analysis of a *tor2-ts* mutant has revealed that downregulation of *tor2* function leads to alteration of expression of a large number but not all of nitrogen-starvation responsive genes [31]. These phenotypes are similar to those caused by the downregulation of *rhb1* function and are opposite to those

of the *rhb1* active mutants or the *tsc* mutants as described above. On the other hand, the *tor2-ts* mutations suppress defect of adenine uptake in the *tsc2* background [31]; however, it is reported that a *tor2-ts* mutation does not suppress canavanine resistance in the *rhb1* active mutant or *tsc2* [25].

In screening for downstream factors of *rhb1*⁺, we have identified a point mutation in *tor2* that is able to suppress growth defect of the *rhb1* null mutant. Further study identified a total of 20 single amino acid mutations at 18 positions located in the *tor2* gene. It is noteworthy that those mutations are clustered mainly in the FAT and the kinase domains [33]. This may suggest that the FAT and the kinase domains are probably involved in the regulation of Tor2 activity by Rhb1. Cells expressing these mutations also exhibited a delay of cell cycle arrest in G1 phase, maintained cell size, and exhibited lower sporulation efficiency under nitrogen depletion conditions [33]. Similar phenomena upon nitrogen starvation are observed in cells carrying the *rhb1* activating mutations and the *tsc* disruptions [13, 25, 33]. In addition to these genetic relationships, we and others have shown that Rhb1 binds to Tor2 [21, 30].

Taken together, these studies on *tor2*⁺, *tsc1*⁺–*tsc2*⁺, and *rhb1*⁺ show that Tor2 in TORC1 functions as a downstream player of the TSC–Rhb1 pathway, and this signaling is conserved between fission yeast and higher eukaryotes (Figure 8.1). In contrast to fission yeast, the counterparts of the TSC complex in budding yeast have not been identified so far. Furthermore, although a Rheb homolog has been found as RHB1 in budding yeast, this small G-protein appears to have no effect on the TOR signaling [34]. Therefore, fission yeast has provided an ideal system to study the TORC1 signaling. We have recently demonstrated that TORC1 activity assessed by the phosphorylation of ribosomal protein S6 is indeed influenced by the TSC–Rhb1 pathway as described in detail below [8].

IV. PAS Assay and Detection of S6 in Fission Yeast

A. Ribosomal Protein S6

In contrast to upstream factors of TORC1, its downstream factor(s) had not been identified in fission yeast. We have identified downstream molecules of TORC1 by using the anti-phospho-Akt-substrate (PAS) antibody that can recognize the consensus phosphorylation motif, R/K–X–R/K–X–X–pS/pT, where X represents any amino acid, and pS and pT is phosphorylated serine and threonine, respectively [8]. The consensus recognition motif of the PAS antibody is roughly consistent with a site phosphorylated by Akt and p70S6K [35–37]. Akt and S6K are well-known substrates of mTORC2 and mTORC1, respectively [38]; thus, substrates of Akt and S6K are predicted to be located in downstream of the TOR complexes. In mammalian cells, the PAS antibody indeed recognizes phosphorylation of ribosomal protein S6 that is directly phosphorylated by S6K regulated by mTORC1 in a nutrient-dependent manner [35, 39]. In the experiments using the PAS antibody, we find several nitrogen source (ammonium)-dependent phospho-proteins in fission yeast (Figure 8.2). Among these, phosphorylation of p27 is quickly and completely abolished under nitrogen starvation. Our analysis has further revealed that p27 corresponds to ribosomal protein (Rp) S6s, which are encoded by two distinct genes, *rps601*⁺ and *rps602*⁺. We also showed that phosphorylation of Rps6 is regulated by nitrogen availability. Downregulation of Rps6 phosphorylation by nitrogen depletion is suppressed by the *tor2* activating

mutations, which confer an *rhb1*-independent growth as described above (Figure 8.3A). Conversely, the *tor2-ts* mutations at nonpermissive temperature diminish the increase of Rps6 phosphorylation in response to the addition of ammonium (Figure 8.3B). By contrast, TORC2 and other nutritional-stress-related pathways, such as the cyclic AMP–PKA and the stress-activated protein kinases signaling cascades, are not mainly involved in the regulation of Rps6 phosphorylation [8]. Accordingly, phosphorylation of Rps6 regulated by nitrogen status is mediated by TORC1 that contains Tor2.

The TSC–Rhb1 pathway is also involved in the regulation of the Tor2-mediated Rps6 phosphorylation, namely, a *tsc2* deletion mutation or an *rhb1* constitutively active mutation leads to attenuation of Rps6 dephosphorylation after the shift to nitrogen starvation (Figure 8.3C and D). These observations provide a model in which the TSC complex negatively and Rhb1 positively regulate TORC1 activity in fission yeast (Figure 8.1). It is of interest that there is a difference in the strength of effects on Rps6 phosphorylation between different *rhb1* active mutants. To confer a partial resistance of Rps6 phosphorylation to nitrogen depletion, *rhb1*^{V17A} but not *rhb1*^{K120R} is sufficient when expressed from its own promoter (lower expression), whereas *rhb1*^{K120R} and *rhb1*^{N153T} require overexpression using multicopy plasmid (higher expression). It is noteworthy that Tor2 activity in the *tor2* activating mutants, for example in *rhb1*-independent *tor2* mutants, a *tsc2* deletion mutant, or a *rhb1* constitutive activation mutant, is eventually abolished under nitrogen starvation [8]. Similar to this finding, it appears that in the mammalian system, mTORC1 activity in fibroblast cells lacking *TSC2* is also inhibited by amino acid depletion even though a high proportion of GTP-bound Rheb in the mutant fibroblasts is not affected [40, 41]. These imply the existence of other pathway(s) that mediates nutrient signaling to TORC1 other than the TSC–Rheb pathway. It has been reported that Rag/Gtr, heterodimeric small GTPases, mediate nutrient signaling to TORC1 in mammalian cells and budding yeast [42–45]. Fission yeast also possesses the predicted homologs of Rag/Gtr, suggesting that the fission yeast Rag/Gtr proteins may participate in the nutrient-TORC1 signaling.

B. Phosphorylation Sites in Rps6

Ribosomal protein S6 is highly conserved from yeast to higher eukaryotes including plants (Figure 8.4). It has been known that the mammalian S6 has five phosphorylatable serine residues (Ser235, Ser236, Ser240, Ser244, and Ser247) in the region close to the carboxy terminus. Among the serine residues, Ser235 and Ser236 are directly phosphorylated by S6K that is subjected to regulation by the nutrient-mTORC1 signaling [36]. In yeast Rps6, the two serine residues (Ser235 and Ser236 in fission yeast and Ser232 and Ser233 in budding yeast) and their adjacent regions are conserved, whereas the three residues, which extend to the C-terminus in mammalian S6, are missing in the S6 yeast proteins (Figure 8.4). In budding yeast, the conserved serine residues (Ser232 and Ser233) are phosphorylated *in vivo*, and Sch9, which is a functional S6K homolog, can catalyze the phosphorylation of those residues *in vitro* [46, 47]. In fission yeast Ser235, Ser236, and the flanking regions in Rps6 are very similar to the consensus recognition motif of the PAS antibody (Figure 8.4). We have observed in fission yeast that Ser235 and Ser236 are phosphorylated in a nutrient-TORC1-dependent manner, similar to other eukaryotes [8].

It is known that TORC1 signaling controls translation through phosphorylation of S6K and 4E-BP [3], whereas it is possible that Rps6 phosphorylation is dispensable for protein synthesis [46, 48]. In animal model, knock-in mice, in which Rps6 contains alanine substitutions at all five phosphorylatable serine residues, are viable and fertile. In the adult Rps6-mutation mice, interestingly, pancreatic β -cells are smaller than those in normal mice, and the cellular phenotype causes insufficient insulin secretion and impaired glucose homeostasis. Mouse embryo fibroblasts (MEF) generated from the Rps6-mutation knock-in mice are significantly smaller than control cells and unexpectedly exhibit an increased rate of protein synthesis [48]. In fission yeast, although the *rps6* genes themselves are essential for cell survival, its phosphorylation is not necessary for cell proliferation and cell size control [8]. So far, physiological role of Rps6 phosphorylation via TORC1 has not been fully understood, in spite of the conserved event among eukaryotes.

V. S6 Kinase in Fission Yeast

It is predicted that fission yeast possesses a number of potential AGC kinases. In mammalian cells, members in the AGC kinase family include S6K and Akt, which are substrates of mTORC1 and mTORC2, respectively [38]. Based on a search using the BLAST program for p70S6K1 homolog in fission yeast, this microbe has four homologs of S6K: Psk1, Gad8, Sck1, and Sck2 [8, 49–52]. Of these, Gad8 is established as a target of TORC2; therefore, it seems to be a functional homolog of Akt [52, 53]. Our recent analysis has revealed that phosphorylation levels of Psk1, Sck1, and Sck2, but not of Gad8 are altered in response to nitrogen availability [8]. In particular, phosphorylation of Psk1 in response to nitrogen availability is highly dependent on TORC1 activity. A part of Sck1 phosphorylation is also likely to be regulated by TORC1 in response to nitrogen availability (Nakashima and Tamanoi, unpublished results). Importantly, Rps6 phosphorylation in the *psk1* deletion mutant, but not the *sck1* or *sck2* mutants is abolished even in nutrient-rich conditions. Deletion of *psk1* also abolishes Rps6 phosphorylation in a *tor2* active mutant or in a *tsc2* deletion mutant; however, the disruption has no detectable effect on cell proliferation and cell growth, similar to that observed with a strain carrying phosphorylation-defective Rps6 ([49], Nakashima and Tamanoi).

Human S6K is phosphorylated at several sites by different kinases, such as PDK1 and mTOR. For example, mTORC1 phosphorylates serine and threonine residues in the turn motif and the hydrophobic motif, respectively, and PDK1 phosphorylates a threonine in the activation loop. It is known that those phosphorylations are important or critical for the kinase activity of S6K [37, 38]. Our analysis has revealed that a threonine residue in the hydrophobic motif in Psk1 is phosphorylated in response to nitrogen conditions, and TORC1 contributes to its phosphorylation. Analysis of alanine substitutions at phosphorylatable sites has shown that phosphorylation in the hydrophobic motif or the activation loop in Psk1 is critical for its full activation to phosphorylate Rps6 and that phosphorylation in the turn motif is likely to be important in phosphorylation of the hydrophobic motif (Nakashima and Tamanoi). These observations demonstrate that Psk1 is the most functional counterpart of S6K in fission yeast. Thus, the TORC1–S6K–S6 signaling would be conserved in fission yeast like other eukaryotes.

VI. Regulation of theTORC1Signaling

As described above, TORC1 activity in fission yeast responds to environmental ammonium conditions. We have observed that the TORC1–S6 signaling is inhibited by glucose starvation [8]. When 2-deoxyglucose, a nonmetabolizable glucose analog that blocks glucose–hexokinase activity thereby inhibiting glycolysis, is added to medium instead of glucose, the TORC1 signaling is also abolished (Nakashima and Tamanoi), suggesting that TORC1 might be regulated in response to intracellular energy status, similar to the AMPK–mTORC1 pathway in mammalian cells [54]. Homologs of AMPK are conserved in fission yeast: Ppk9 and Ssp2 are catalytic subunits, Amk2 is a β -subunit, and Cbs2 is a γ -subunit [55]. These AMPKs might mediate energy status to TORC1. On the other hand, cycloheximide, which is a protein synthesis inhibitor, attenuates downregulation of TORC1–S6 signaling upon nitrogen or glucose starvation (Nakashima and Tamanoi).

It has been reported that like other eukaryotes, TORC1 is implicated in the regulation of autophagy and expression of an autophagy-related gene, that is, TORC1 disturbs autophagy induction [30, 31]. In fission yeast, nitrogen depletion becomes a trigger of autophagy induction, whereas glucose starvation does not do so even though both inhibit the TORC1–S6 signaling [8, 56, 57]. Therefore, glucose starvation might interfere with only a part of TORC1 function.

In higher eukaryotes, it has been known that amino acids, especially leucine, acts as a priming cue to activate the mTORC1 signaling, although it has not been established how the amino acid signaling is transduced to mTORC1 [58, 59]. In contrast to mammalian cells requiring supplement of essential amino acids, prototrophic yeast cells do not need any exogenous amino acids for cell proliferation. In budding yeast, it seems that alteration of intracellular glutamine affects TORC1 activity. Furthermore, TORC1 activity in cells carrying a leucine auxotroph is diminished by leucine depletion [44, 47, 60]. In fission yeast, our analysis indicates that refeeding of glutamine following nitrogen starvation can activate the TORC1–S6 signaling instead of ammonium and, conversely, that inhibition of intracellular glutamine synthesis by treatment with L-methionine sulfoximine, a glutamine synthetase inhibitor, results in a decrease in TORC1 activity. Effect of refeeding leucine, proline, or glutamate on the TORC1 signaling is much less than that of glutamine [Nakashima and Tamanoi]. As mentioned above, inhibition of protein synthesis with cycloheximide under nitrogen starvation attenuated inactivation of TORC1. This might result from maintained intracellular amino acid pool. Taken together, TORC1 signaling might monitor intracellular amino acid level.

In mammalian cells and budding yeast, TORC1 signaling is inhibited by several stress conditions in addition to nutrient depletion [47, 61]. We have observed that the TORC1–S6 signaling is inhibited by oxidative and osmotic stresses in fission yeast. In the cases of both stresses, the inhibition occurs slowly (Rps6 phosphorylation disappears by 60 min), compared with the inhibition by nutrient depletion (15 min) [8]. The Wis1–Sty1 (also known as Phh1 and Spc1) signaling in fission yeast is a major stress-activated protein kinases (SAPKs) cascade, which is activated by several stresses, such as nitrogen starvation, oxidative and osmotic stresses, etc., to maintain cell survival under those stress conditions

[62–64]. It has not been clarified yet whether the SAPK signaling is involved in the regulation of the TORC1 pathway under the stress conditions.

VII. Effect of Rapamycin on theTORC1Signaling

A TOR-specific inhibitor, rapamycin, forms an intracellular drug–protein complex with FKBP12, and the complex interacts with the FRB domain in TOR, thereby inhibiting TOR activity [65, 66]. In mammalian cells, this macrolide preferentially and quickly prevent phosphorylation of S6K and 4E-BP via inhibition of mTORC1 [1]. By contrast, prolonged treatment of the macrolide is needed to reduce the phosphorylation of Akt by mTORC2 [67]. Rapamycin also decreases cell volume through inhibition of mTORC1 signaling [68]. Recently, the existence of rapamycin-resistant mTORC1 function that includes autophagy regulation, a part of regulation of 4E-BP phosphorylation and cell size, has been reported [69–72]. In budding yeast, rapamycin blocks cell proliferation via inhibition of TORC1 function and mimics nitrogen starvation including induction of autophagy, whereas it appears to have no effect on TORC2 activity [73].

In fission yeast, rapamycin treatment has no effect on cell proliferation in normal cells and does not mimic nitrogen starvation [9, 10]. Microarray analysis has revealed that rapamycin has little impact on alteration of gene expression involved in response to nitrogen depletion [31]. On the other hand, the macrolide prevents leucine uptake owing to downregulation of gene expression of amino acid transporters, as well as in a *tor1* deletion mutant; therefore, when cells have a mutation on the *leu1* gene, the cells exhibit growth defect on a minimal medium containing rapamycin and low concentration of leucine [74]. Tor1 is a catalytic subunit of TORC2 in fission yeast and this effect of rapamycin is likely to be caused by the inhibition of Tor1, because deletion of *fkh1*, a FKBP12 homolog in fission yeast, or expression of a rapamycin-resistant *tor1* allele can suppress the growth defect. Yeast two-hybrid analysis has also shown that the macrolide interacts with the FRB domain of Tor1 [74]. Furthermore, it has been reported that mutations of the *tor* genes in fission yeast confer sensitivity to rapamycin. Deletion of *tor1* results in growth defect in the presence of the macrolide independently of decreased leucine uptake [10], whereas this defect in the *tor1* mutant is suppressed by an *fkh1* disruption [74]. A point mutation (L2048S) in *tor2* gene also causes rapamycin sensitivity [32]. Interestingly, if yeast cells were cultured in medium containing glutamate as sole nitrogen source instead of ammonium, rapamycin induced mitotic onset because of inhibition of Tor1 and facilitated entry into sexual differentiation (until 10 h after addition of rapamycin) [75]. It appears that Tor1 as part of TORC1 regulates mitotic entry through the SAPK following reduced nitrogen availability [76]. However, we observed that the macrolide in an ammonium medium as a nitrogen source did not induce cell cycle arrest in G1 phase and sexual differentiation even when the cells were cultured at least for 17 h (Nakashima and Tamanoi).

We have demonstrated that S6 phosphorylation is inhibited by rapamycin [8]. Phosphorylation of Rps6 is markedly reduced by 30 min and subsequently disappears by 60 min, whereas its inhibition requires high dose of the macrolide (Figure 8.5A). Either disruption of *fkh1* or mutation of the rapamycin-unbinding *tor2* suppresses this inhibition by rapamycin (Figure 8.5B). In addition, phosphorylation of Psk1 is also prevented by

rapamycin in a Tor2-dependent manner (Nakashima and Tamanoi), suggesting that like other eukaryotes, rapamycin is targeting the TORC1–S6K–S6 signaling in fission yeast. Furthermore, prolonged rapamycin treatment (14 h) significantly reduces cell size in a TORC1-dependent manner [8]. By contrast, we also observed that the macrolide does not mimic nitrogen starvation, consistent with previous reports. Accordingly, cell behaviors upon rapamycin treatment are markedly different from those in *tor2-ts* mutant at nonpermissive temperature including cell cycle arrest in G1 phase, small round cell, and entry into sexual differentiation; namely the effects of rapamycin are more limited than those of loss of Tor2 function. These observations suggest that rapamycin inhibits a subset of TORC1 activity and that rapamycin-resistant function of TORC1 exists in fission yeast. Consistent with the findings in mutants of Rps6 or Psk1, rapamycin-sensitive functions of TORC1, at least the TORC1–S6K–S6 signaling is not important for cell growth, whereas rapamycin-resistant ones would contribute control of the switch between cell proliferation and cell differentiation. Further investigation is needed to determine why rapamycin prevents only a part of TORC1 functions.

VIII. Future Prospects

Nutrient signaling that senses nutrient cues and then transduces the signal to regulate most cellular processes, such as cell proliferation, differentiation, and metabolism, is the most fundamental machinery among organisms. The TORC1 signaling has been established as one of the major nutrient signaling pathway. We have summarized here the TORC1–S6K–S6 signaling, which is regulated by nutrient stimulations, such as ammonium, amino acid, and glucose, in fission yeast. Therefore, it is established that this signaling is conserved between yeasts and higher eukaryotes. In addition, like in higher eukaryotes, the TSC–Rheb pathway is involved in the regulation of the TORC1 pathway in fission yeast. However, a mechanism for sensing nutrients to regulate the TORC1 signaling has not been elucidated. It is also unknown what the input signaling to regulate the TSC–Rheb pathway in fission yeast is. We have shown that phosphorylation of Rps6 and Psk1 reflects downstream activity of TORC1. Thus, phosphorylation of these proteins should serve as convenient markers to examine the status of TORC1 activity and may provide a powerful tool to identify regulators and mediators of nutrient signaling to TORC1. Furthermore, we have observed that rapamycin inhibits the TORC1–S6K–S6 pathway, suggesting that there exists rapamycin-resistant function of TORC1. In addition to downstream factors in the rapamycin-sensitive TORC1 pathway besides S6K–S6, further investigation into downstreams of rapamycin-resistant TORC1 should provide insights into physiological roles of TORC1 not only in fission yeast, but also in other eukaryotes including animals.

Acknowledgments

We thank Dr. Tatsuhiro Sato for discussion. We also thank members of the Tamanoi laboratory for comments. This work was supported by the NIH grant CA41996.

References

1. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell*. 2006; 124:471–484. [PubMed: 16469695]

2. Chiang GG, Abraham RT. Targeting the mTOR signaling network in cancer. *Trends Mol Med.* 2007; 13:433–442. [PubMed: 17905659]
3. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004; 18:1926–1945. [PubMed: 15314020]
4. Corradetti MN, Guan KL. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? *Oncogene.* 2006; 25:6347–6360. [PubMed: 17041621]
5. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J.* 2008; 412:179–190. [PubMed: 18466115]
6. Aspuria PJ, Sato T, Tamanoi F. The TSC/Rheb/TOR signaling pathway in fission yeast and mammalian cells: temperature sensitive and constitutive active mutants of TOR. *Cell Cycle.* 2007; 6:1692–1695. [PubMed: 17637564]
7. Otsubo Y, Yamamoto M. TOR signaling in fission yeast. *Crit Rev Biochem Mol Biol.* 2008; 43:277–283. [PubMed: 18756382]
8. Nakashima A, Sato T, Tamanoi F. Fission yeast TORC1 regulates phosphorylation of ribosomal S6 proteins in response to nutrients and its activity is inhibited by rapamycin. *J Cell Sci.* 2010; 123:777–786. [PubMed: 20144990]
9. Weisman R, Choder M, Koltin Y. Rapamycin specifically interferes with the developmental response of fission yeast to starvation. *J Bacteriol.* 1997; 179:6325–6334. [PubMed: 9335279]
10. Kawai M, Nakashima A, Ueno M, Ushimaru T, Aiba K, Doi H, Uritani M. Fission yeast Tor1 functions in response to various stresses including nitrogen starvation, high osmolarity, and high temperature. *Curr Genet.* 2001; 39:166–174. [PubMed: 11409178]
11. European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell.* 1993; 75:1305–1315. [PubMed: 8269512]
12. van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, Burley M, Jeremiah S, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science.* 1997; 277:805–808. [PubMed: 9242607]
13. Matsumoto S, Bandyopadhyay A, Kwiatkowski DJ, Maitra U, Matsumoto T. Role of the Tsc1-Tsc2 complex in signaling and transport across the cell membrane in the fission yeast *Schizosaccharomyces pombe*. *Genetics.* 2002; 161:1053–1063. [PubMed: 12136010]
14. van Slegtenhorst M, Carr E, Stoyanova R, Kruger WD, Henske EP. *tsc1*⁺ and *tsc2*⁺ regulate arginine uptake and metabolism in *Schizosaccharomyces pombe*. *J Biol Chem.* 2004; 279:12706–12713. [PubMed: 14718525]
15. Nakase Y, Fukuda K, Chikashige Y, Tsutsumi C, Morita D, Kawamoto S, Ohnuki M, Hiraoka Y, Matsumoto T. A defect in protein farnesylation suppresses a loss of *Schizosaccharomyces pombe tsc2*⁺, a homolog of the human gene predisposing to tuberous sclerosis complex. *Genetics.* 2006; 173:569–578. [PubMed: 16624901]
16. Weisman R, Roitburg I, Schonbrun M, Harari R, Kupiec M. Opposite effects of Tor1 and Tor2 on nitrogen starvation responses in fission yeast. *Genetics.* 2007; 175:1153–1162. [PubMed: 17179073]
17. Aspuria PJ, Tamanoi F. The Tsc/Rheb signaling pathway controls basic amino acid uptake via the Cat1 permease in fission yeast. *Mol Genet Genomics.* 2008; 279:441–450. [PubMed: 18219492]
18. Hein C, Springael JY, Volland C, Haguenaue-Tsapis R, André B. NPI1, an essential yeast gene involved in induced degradation of Gap1 and Fur4 permeases, encodes the Rsp5 ubiquitin-protein ligase. *Mol Microbiol.* 1995; 18:77–87. [PubMed: 8596462]
19. Soetens O, De Craene JO, André B. Ubiquitin is required for sorting to the vacuole of the yeast general amino acid permease, Gap1. *J Biol Chem.* 2001; 276:43949–43957. [PubMed: 11500494]
20. Blondel MO, Morvan J, Dupré S, Urban-Grimal D, Haguenaue-Tsapis R, Volland C. Direct sorting of the yeast uracil permease to the endosomal system is controlled by uracil binding and Rsp5p-dependent ubiquitylation. *Mol Biol Cell.* 2004; 15:883–895. [PubMed: 14657252]
21. Urano J, Comiso MJ, Guo L, Aspuria PJ, Deniskin R, Tabancay AP, Kato-Stankiewicz J, Tamanoi F. Identification of novel single amino acid changes that result in hyperactivation of the unique GTPase, Rheb, in fission yeast. *Mol Microbiol.* 2005; 58:1074–1086. [PubMed: 16262791]

22. van Slegtenhorst M, Mustafa A, Henske EP. Pas1, a G1 cyclin, regulates amino acid uptake and rescues a delay in G1 arrest in Tsc1 and Tsc2 mutants in *Schizosaccharomyces pombe*. *Hum Mol Genet.* 2005; 14:2851–2858. [PubMed: 16115814]
23. Yang W, Urano J, Tamanoi F. Protein farnesylation is critical for maintaining normal cell morphology and canavanine resistance in *Schizosaccharomyces pombe*. *J Biol Chem.* 2000; 275:429–438. [PubMed: 10617635]
24. Yang W, Tabancay AP, Urano J, Tamanoi F. Failure to farnesylate Rheb protein contributes to the enrichment of G0/G1 phase cells in the *Schizosaccharomyces pombe* farnesyltransferase mutant. *Mol Microbiol.* 2001; 41:1339–1347. [PubMed: 11580838]
25. Murai T, Nakase Y, Fukuda K, Chikashige Y, Tsutsumi C, Hiraoka Y, Matsumoto T. Distinctive responses to nitrogen starvation in the dominant active mutants of the fission yeast Rheb GTPase. *Genetics.* 2009; 183:517–527. [PubMed: 19620394]
26. Urano J, Tabancay AP, Yang W, Tamanoi F. The *Saccharomyces cerevisiae* Rheb G-protein is involved in regulating canavanine resistance and arginine uptake. *J Biol Chem.* 2000; 275:11198–11206. [PubMed: 10753927]
27. Mach KE, Furge KA, Albright CF. Loss of Rbh1, a Rheb-related GTPase in fission yeast, causes growth arrest with a terminal phenotype similar to that caused by nitrogen starvation. *Genetics.* 2000; 155:611–622. [PubMed: 10835385]
28. Tabancay AP, Gau CL, Machado IM, Uhlmann EJ, Gutmann DH, Guo L, Tamanoi F. Identification of dominant negative mutants of Rheb GTPase and their use to implicate the involvement of human Rheb in the activation of p70S6K. *J Biol Chem.* 2003; 278:39921–39930. [PubMed: 12869548]
29. Alvarez B, Moreno S. Fission yeast Tor2 promotes cell growth and represses cell differentiation. *J Cell Sci.* 2006; 119:4475–4485. [PubMed: 17046992]
30. Uritani M, Hidaka H, Hotta Y, Ueno M, Ushimaru T, Toda T. Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. *Genes Cells.* 2006; 11:1367–1379. [PubMed: 17121544]
31. Matsuo T, Otsubo Y, Urano J, Tamanoi F, Yamamoto M. Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast. *Mol Cell Biol.* 2007; 27:3154–3164. [PubMed: 17261596]
32. Hayashi T, Hatanaka M, Nagao K, Nakaseko Y, Kanoh J, Kokubu A, Ebe M, Yanagida M. Rapamycin sensitivity of the *Schizosaccharomyces pombe* tor2 mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. *Genes Cells.* 2007; 12:1357–1370. [PubMed: 18076573]
33. Urano J, Sato T, Matsuo T, Otsubo Y, Yamamoto M, Tamanoi F. Point mutations in TOR confer Rheb-independent growth in fission yeast and nutrient-independent mammalian TOR signaling in mammalian cells. *Proc Natl Acad Sci USA.* 2007; 104:3514–3519. [PubMed: 17360675]
34. Aspuria PJ, Tamanoi F. The Rheb family of GTP-binding proteins. *Cell Signal.* 2004; 16:1105–1112. [PubMed: 15240005]
35. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell.* 2002; 10:151–162. [PubMed: 12150915]
36. Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem Sci.* 2006; 31:342–348. [PubMed: 16679021]
37. Pearce LR, Komander D, Alessi DR. The nuts and bolts of AGC protein kinases. *Nat Rev Mol Cell Biol.* 2010; 11:9–22. [PubMed: 20027184]
38. Jacinto E, Lorberg A. TOR regulation of AGC kinases in yeast and mammals. *Biochem J.* 2008; 410:19–37. [PubMed: 18215152]
39. Kane S, Sano H, Liu SC, Asara JM, Lane WS, Garner CC, Lienhard GE. A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. *J Biol Chem.* 2002; 277:22115–22118. [PubMed: 11994271]
40. Smith EM, Finn SG, Tee AR, Browne GJ, Proud CG. The tuberous sclerosis protein TSC2 is not required for the regulation of the mammalian target of rapamycin by amino acids and certain cellular stresses. *J Biol Chem.* 2005; 280:18717–18727. [PubMed: 15772076]

41. Roccio M, Bos JL, Zwartkruis FJ. Regulation of the small GTPase Rheb by amino acids. *Oncogene*. 2006; 25:657–664. [PubMed: 16170341]
42. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 2008; 320:1496–14501. [PubMed: 18497260]
43. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol*. 2008; 10:935–945. [PubMed: 18604198]
44. Binda M, Péli-Gulli MP, Bonfils G, Panchaud N, Urban J, Sturgill TW, Loewith R, De Virgilio C. The Vam6 GEF controls TORC1 by activating the EGO complex. *Mol Cell*. 2009; 35:563–573. [PubMed: 19748353]
45. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 2010; 141:290–303. [PubMed: 20381137]
46. Johnson SP, Warner JR. Phosphorylation of the *Saccharomyces cerevisiae* equivalent of ribosomal protein S6 has no detectable effect on growth. *Mol Cell Biol*. 1987; 7:1338–1345. [PubMed: 3299046]
47. Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, Deloche O, Wanke V, Anrather D, Ammerer G, Riezman H, Broach JR, De Virgilio C, et al. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell*. 2007; 26:663–674. [PubMed: 17560372]
48. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, Dor Y, Zisman P, Meyuhos O. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev*. 2005; 19:2199–2211. [PubMed: 16166381]
49. Mukai H, Miyahara M, Takanaga H, Kitagawa M, Shibata H, Shimakawa M, Ono Y. Identification of *Schizosaccharomyces pombe* gene *psk1+*, encoding a novel putative serine/threonine protein kinase, whose mutation conferred resistance to phenylarsine oxide. *Gene*. 1995; 166:155–159. [PubMed: 8529881]
50. Jin M, Fujita M, Culley BM, Apolinario E, Yamamoto M, Maundrell K, Hoffman CS. *sch1*, a high copy number suppressor of defects in the cAMP-dependent protein kinase pathway in fission yeast, encodes a protein homologous to the *Saccharomyces cerevisiae* SCH9 kinase. *Genetics*. 1995; 140:457–467. [PubMed: 7498728]
51. Fujita M, Yamamoto M. *S. pombe sch2+*, a second homologue of *S. cerevisiae* SCH9 in fission yeast, encodes a putative protein kinase closely related to PKA in function. *Curr Genet*. 1998; 33:248–254. [PubMed: 9560431]
52. Matsuo T, Kubo Y, Watanabe Y, Yamamoto M. *Schizosaccharomyces pombe* AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. *EMBO J*. 2003; 22:3073–3083. [PubMed: 12805221]
53. Ikeda K, Morigasaki S, Tatebe H, Tamanoi F, Shiozaki K. Fission yeast TOR complex 2 activates the AGC-family Gad8 kinase essential for stress resistance and cell cycle control. *Cell Cycle*. 2008; 7:358–364. [PubMed: 18235227]
54. Kimball SR. Interaction between the AMP-activated protein kinase and mTOR signaling pathways. *Med Sci Sports Exerc*. 2006; 38:1958–1964. [PubMed: 17095930]
55. Hanyu Y, Imai KK, Kawasaki Y, Nakamura T, Nakaseko Y, Nagao K, Kokubu A, Ebe M, Fujisawa A, Hayashi T, Obuse C, Yanagida M. *Schizosaccharomyces pombe* cell division cycle under limited glucose requires Ssp1 kinase, the putative CaMKK, and Sds23, a PP2A-related phosphatase inhibitor. *Genes Cells*. 2009; 14:539–554. [PubMed: 19371376]
56. Nakashima A, Hasegawa T, Mori S, Ueno M, Tanaka S, Ushimaru T, Sato S, Uritani M. A starvation-specific serine protease gene, *isp6+*, is involved in both autophagy and sexual development in *Schizosaccharomyces pombe*. *Curr Genet*. 2006; 49:403–413. [PubMed: 16550352]
57. Kohda TA, Tanaka K, Konomi M, Sato M, Osumi M, Yamamoto M. Fission yeast autophagy induced by nitrogen starvation generates a nitrogen source that drives adaptation processes. *Genes Cells*. 2007; 12:155–170. [PubMed: 17295836]

58. Avruch J, Hara K, Lin Y, Liu M, Long X, Ortiz-Vega S, Yonezawa K. Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase. *Oncogene*. 2006; 25:6361–6372. [PubMed: 17041622]
59. Wang X, Proud CG. Nutrient control of TORC1, a cell-cycle regulator. *Trends Cell Biol*. 2009; 19:260–267. [PubMed: 19419870]
60. Crespo JL, Powers T, Fowler B, Hall MN. The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. *Proc Natl Acad Sci USA*. 2002; 99:6784–6789. [PubMed: 11997479]
61. Patel J, McLeod LE, Vries RG, Flynn A, Wang X, Proud CG. Cellular stresses profoundly inhibit protein synthesis and modulate the states of phosphorylation of multiple translation factors. *Eur J Biochem*. 2002; 269:3076–3085. [PubMed: 12071973]
62. Shiozaki K, Russell P. Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. *Genes Dev*. 1996; 10:2276–2288. [PubMed: 8824587]
63. Kato T Jr, Okazaki K, Murakami H, Stettler S, Fantes PA, Okayama H. Stress signal, mediated by a Hog1-like MAP kinase, controls sexual development in fission yeast. *FEBS Lett*. 1996; 378:207–212. [PubMed: 8557102]
64. Wilkinson MG, Samuels M, Takeda T, Toone WM, Shieh JC, Toda T, Millar JB, Jones N. The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. *Genes Dev*. 1996; 10:2289–2301. [PubMed: 8824588]
65. Chen J, Zheng XF, Brown EJ, Schreiber SL. Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue. *Proc Natl Acad Sci USA*. 1995; 92:4947–4951. [PubMed: 7539137]
66. Lorenz MC, Heitman J. TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J Biol Chem*. 1995; 270:27531–27537. [PubMed: 7499212]
67. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*. 2006; 22:159–168. [PubMed: 16603397]
68. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*. 2002; 110:163–175. [PubMed: 12150925]
69. Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl Acad Sci USA*. 2008; 105:17414–17419. [PubMed: 18955708]
70. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol*. 2009; 7:e38. [PubMed: 19209957]
71. García-Martínez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi DR. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochem J*. 2009; 421:29–42. [PubMed: 19402821]
72. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem*. 2009; 284:8023–8032. [PubMed: 19150980]
73. De Virgilio C, Loewith R. Cell growth control: little eukaryotes make big contributions. *Oncogene*. 2006; 25:6392–6415. [PubMed: 17041625]
74. Weisman R, Roitburg I, Nahari T, Kupiec M. Regulation of leucine uptake by *tor1+* in *Schizosaccharomyces pombe* is sensitive to rapamycin. *Genetics*. 2005; 169:539–550. [PubMed: 15466417]
75. Petersen J, Nurse P. TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. *Nat Cell Biol*. 2007; 9:1263–1272. [PubMed: 17952063]
76. Hartmuth S, Petersen J. Fission yeast Tor1 functions as part of TORC1 to control mitotic entry through the stress MAPK pathway following nutrient stress. *J Cell Sci*. 2009; 122:1737–1746. [PubMed: 19417002]

Fig. 8.1.
Conservation of the TORC1 signaling in mammal and fission yeast. Note that the TSC/
Rheb/TORC1 signaling functions similarly in both systems and that the components of
TORC1 are similar.

Fig. 8.2.

Nitrogen source-dependent phospho-proteins recognized with the PAS antibody. JUp1204 cells were grown to exponential phase and then incubated in medium with or without ammonium for the indicated times. Cell extracts were run on a SDS polyacrylamide gel and probed with the PAS antibody. Tubulin is shown as a loading control. (From Ref. [8], Fig. 1A, p. 778.)

Fig. 8.3.

The TSC–Rhb1–TORC1 signaling is involved in the regulation of Rps6 phosphorylation. (A) Cells expressing the mutant Tor2 (L1310P or E2221K), as well as the wild-type Tor2 were grown and incubated with or without ammonium. Extracts were immunoblotted with the PAS antibody. (B) Tor2 *ts* mutants, *ts-6* and *ts-10*, as well as the wild-type cells were grown to exponential phase, incubated at the permissive or nonpermissive temperature and then incubated with or without ammonium. Extracts were immunoblotted with the PAS antibody. (C) The wild-type and the *tsc2* mutant were grown and incubated with or without ammonium. Extracts were immunoblotted with the PAS antibody. (D) Cells expressing mutant Rhb1 V17A, and K120R, as well as the wild-type Rhb1 were grown and incubated with or without ammonium. Extracts were immunoblotted with the PAS antibody, as well as anti-myc antibody. Tubulin is shown as a loading control. (From Ref. [8], Figs. 2A and C and 3A and C, p. 779 and p. 780.)

```

Sprps601 1 MKLNISYPANGTQKLIIDDDRLRVFMEKRMGOEVPGDSVGPPEFAGYVFKITGGNDRQG
Sprps602 1 MKLNISYPANGTQKLIIDDDRLRVFMEKRMGOEVPGDSVGPPEFAGYVFKITGGNDRQG
Scrps6a 1 MKLNISYPANGTQKLIIDDDRLRVFMEKRMGOEVPGDSVGPPEFAGYVFKITGGNDRQG
Hss6 1 MKLNISYPANGTQKLIIDDDRLRVFMEKRMGOEVPGDSVGPPEFAGYVFKITGGNDRQG
Atrps6 1 MKFNVANPTTCCQKKLEIDDDRLRAEYDKKRISQEVSGDALGEFFKGYVFKIKGGCDKQG

Sprps601 61 FPMFQGVLLPFRVRLLLRAGHPCYR--PRRDGERKKRSVRGCIVGODLAVLALAIIVKQGE
Sprps602 61 FPMFQGVLLPFRVRLLLRAGHPCYR--PRRDGERKKRSVRGCIVGODLAVLALAIIVKQGE
Scrps6a 61 FPMKQGVLLPFRVRLLLTKNVSCYR--PRRDGERKKRSVRGCIVGODLAVLALAIIVKQGE
Hss6 61 FPMKQGVLLPFRVRLLLSKGHSCYR--PRRDGERKKRSVRGCIVGODLAVLALAIIVKQGE
Atrps6 61 FPMKQGVLLPFRVRLLLHRGTTCFRGHGRRTGERRKRSVRGCIVSPDLVLLIVKQGE

Sprps601 119 QDIPGLTDVTPKRLGPKRASKIRFFNLSKEDDVROFVIRREVVPKKEGKKPYTKAPKI
Sprps602 119 QDIPGLTDVTPKRLGPKRASKIRFFNLSKEDDVROFVIRREVVPKKEGKKPYTKAPKI
Scrps6a 119 QDEGLTDVTPKRLGPKRANKIRFFGLSKEDDVROFVIRREVTK--GEKTYTKAPKI
Hss6 119 KDIPGLTDVTPKRLGPKRASKIRFFNLSKEDDVROFVIRREVTK--GEKKKPYTKAPKI
Atrps6 121 NDIPGLTDVTPKRLGPKRASKIRFFNLSKEDDVROFVIRREVTK--GEKPYTKAPKI

Sprps601 179 ORLVTPRLQKRRRFAIKRROEENREEAABPAOLMAKRVAEAKQRE--VVKARRASS
Sprps602 179 ORLVTPRLQKRRRFAIKRROEENREEAABPAOLMAKRVAEAKQRE--VVKARRASS
Scrps6a 176 ORLVTPRLQKRRRFAIKRROEENREEAABPAOLMAKRVAEAKQRE--VVKARRASS
Hss6 177 ORLVTPRLQKRRRFAIKRROEENREEAABPAOLMAKRVAEAKQRE--VVKARRASS
Atrps6 181 ORLVTPRLQKRRRFAIKRROEENREEAABPAOLMAKRVAEAKQRE--VVKARRASS

Sprps601 237 LRK-----
Sprps602 237 MKK-----
Scrps6a 234 LKA-----
Hss6 237 LRASTKSSESSQK
Atrps6 241 SAIAKPSVTA---

```

●●: S235, S236

Fig. 8.4.

Alignment of predicted amino acid sequences of ribosomal protein S6. Red underline indicates a predicted recognition motif (Arg/Lys-X-Arg/Lys-X-X-Ser/Thr) for the PAS antibody. Circles show phosphorylated serine residues of human S6 by S6Ks. Numbers on the left indicate amino acids.

Fig. 8.5.

Rapamycin inhibits Rps6 phosphorylation by blocking TORC1 activity. (A) Cells were added with either 200 nM rapamycin or DMSO and incubated for the indicated times. Extracts were subjected to immunoblotting with the PAS antibody. (B) Null mutant of FKBP12 and *tor2* mutant resistant to rapamycin were grown and incubated with or without rapamycin. Extracts were immunoblotted with the PAS antibody. Tubulin is shown as a loading control. (From Ref. [8], Fig. 7A and C, p. 782.)