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TOR Signaling in Fission Yeast

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Fission yeast has two TOR kinases, Tor1 and Tor2. Recent studies have indicated that this microbe has a TSC/Rheb/TOR pathway like higher eukaryotes. Two TOR complexes, namely TORC1 and TORC2, have been identified in this yeast, as in budding yeast and mammals. Fission yeast TORC1, which contains Tor2, and TORC2, which contains Tor1, apparently have opposite functions with regard to the promotion of G1 arrest and sexual development. Rapamycin does not inhibit growth of wild-type fission yeast cells, unlike other eukaryotic cells, but precise analyses have revealed that rapamycin affects certain cellular functions involving TOR in this yeast. It appears that fission yeast has a potential to be an ideal model system to investigate the TOR signaling pathways.

Keywords TORC, rapamycin, TSC, Rheb, cell growth, sexual development

INTRODUCTION

The TOR (target of rapamycin) protein is a highly conserved serine/threonine kinase, which is thought to regulate cell growth in response to environmental changes, as in nutrient availability or the cellular energy status. In 1991, two TOR isoforms named TOR1 and TOR2 were identified in budding yeast *Saccharomyces cervisiae* through analysis of resistant mutants to rapamycin, an immunosuppressive and potential anticancer drug (Heitman *et al*., 1991). Rapamycin was originally isolated from a soil bacterium and found to inhibit growth of mammalian and budding yeast cells. This antibiotic forms a complex with FKBP12 (FK506-binding protein) and the complex inhibits TOR by binding to its FRB domain (Fig. 1). Soon after the identification of budding yeast TOR, the single mammalian target of rapamycin (mTOR) was identified and cloned by using an FKBP12-rapamycin affinity purification (Brown *et al*., 1994; Chiu *et al*., 1994; Sabatini *et al*., 1994; Sabers *et al*., 1995). Following the pioneering work in budding yeast and mammalian cells, TOR proteins have been identified and analyzed in a number of eukaryotic organisms, notably in fruit flies. While budding yeast has two TOR genes, higher organisms generally have only one TOR gene (reviewed in Wullschleger *et al*., 2006).

TOR bears several conserved domains besides the FRB domain. The N-terminus of TOR carries extended tandem HEAT repeats, which are supposed to mediate protein–protein interactions (Andrade and Bork, 1995). The C-terminus of TOR carries a FAT domain, an FRB domain, a kinase domain, and a FATC domain. These domains are related to the phosphatidylinositol kinase (Fig. 1) (Abraham, 2004).

It has been shown that TOR proteins exist as two distinct multiprotein complexes, called TORC1 and TORC2, in both mammalian and budding yeast cells (Hara *et al*., 2002; Kim *et al*., 2002; Loewith *et al*., 2002; Sarbassov *et al*., 2004; Jacinto *et al*., 2004). These complexes regulate different aspects of cell growth in response to environmental cues. TORC1 is sensitive to rapamycin, and regulates various aspects of cell growth such as translation, ribosome biogenesis, autophagy, transcription and metabolism. The best-studied substrates of TORC1 in mammalian cells are 4EBP1 and an AGC family protein kinase S6K1, which are both involved in the regulation of translation. TORC2 is not sensitive to rapamycin, and has been shown to regulate actin organization in budding yeast (Schmidt *et al*., 1996). Mammalian TORC2 (mTORC2) is also likely to regulate actin organization (Jacinto *et al*., 2004), though the overall evidence may not yet be as convincing as that in budding yeast. mTORC2 is known to regulate Akt/PKB, another member of the AGC protein kinase family (reviewed in Wullschleger *et al*., 2006; Guertin and Sabatini, 2007).

In cells of higher eukaryotes, a heterodimer of the tuberous sclerosis proteins TSC1 and TSC2 regulates TOR signaling negatively (Li *et al*., 2004). TSC2 functions as a GTPase activating protein (GAP) for the small GTPase Rheb, which is necessary to elevate the kinase activity of TORC1. Curiously, however, budding yeast does not have homologs to the TSC genes, and the

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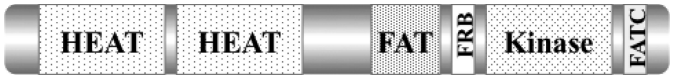

FIG. 1. Schematic illustration of the structure of TOR. See the text for explanation.

loss of RHEB, the *Saccharomyces* counterpart of mammalian Rheb, has no obvious effect on the cell cycle progression in this microbe (Urano *et al*., 2000).

Analysis of TOR in fission yeast *Schizosaccharomyces pombe* was rather incomplete until recently, although it is an established model system to study the cell cycle control in eukaryotic cells. However, dramatic advances have been made on the studies of fission yeast TOR kinases in the last few years and wealth of knowledge has been accumulated on their structure and function. This short review aims to summarize the current knowledge about fission yeast TOR, comparing it with budding yeast and mammalian TOR.

BRIEF OVERVIEW OF THE FISSION YEAST LIFECYCLE

Fission yeast is distantly related to budding yeast in phylogeny and in general is closer to higher eukaryotes than budding yeast. Fission yeast normally proliferates in the haploid state. Haploid cells bear one of the two mating types, designated h^+ (P) and h^- (M). Under rich nutrition, fission yeast cells undergo the mitotic cell cycle. Upon starvation, they arrest the cell cycle in G1 phase and, if there are opposite mating-type cells in the neighborhood, they conjugate and enter meiosis. Fission yeast is one of the best model organisms for the study of not only cell cycle control but also regulation of meiosis (Yamamoto *et al*., 1997).

TWO TOR KINASES IN FISSION YEAST, Tor1 AND Tor2

Analysis of TOR in fission yeast stems from early observation of the effect of rapamycin more than a decade ago (Weisman *et al*., 1997). In contrast to budding yeast and mammalian cells, however, rapamycin was found not to inhibit vegetative growth in this microbe. Instead, it appeared to inhibit sexual development at an early stage prior to conjugation under starved conditions. It has been concluded that in fission yeast rapamycin does not affect entry to the stationary phase or arrest in G1 phase, but severely impairs mating function (Weisman *et al*., 1997).

Subsequently, two genes encoding TOR homologs were identified from database homology search and designated as *tor1* and *tor2* (Kawai *et al*., 2001; Weisman and Choder, 2001). The two homologs show about 52% amino acid identity and they both carry the aforementioned domains conserved in the TOR family proteins. Although rapamycin does not inhibit growth of wild-type fission yeast cells, both Tor1 and Tor2 contain a highly conserved FRB domain, which may potentially interact with FKBP12-rapamycin complex (Kawai *et al*., 2001; Weisman and Choder, 2001).

Tor1 IS REQUIRED FOR GROWTH UNDER STRESSES AND AMINO ACID UPTAKE

Fission yeast *tor1* is not essential for cell growth. However, *tor1*-deletion (*tor1*Δ) cells cannot arrest properly in G1 phase under nutrient starvation and are sterile, i.e. deficient in mating. They also exhibit an elongated abnormal morphology and are unable to survive under various stressed conditions, such as high and low temperatures, high osmotic pressures, high pH, and oxidative stresses (Kawai *et al*., 2001; Weisman and Choder, 2001). The *tor1* gene was identified also as the gene responsible for the *gad2-1* mutation, which caused a defect in G1 arrest under nitrogen starvation and sterility (Matsuo *et al*., 2003). In a screening for high-copy suppressors of the sterility of the *gad2-1* mutant, the *gad8* gene was isolated. The gene product, Gad8, is a Ser/Thr kinase of the AGC family, which includes budding yeast Ypk1 and Ypk2, human PKB/Akt, PKC and S6K1, and many others. The phenotypes of *gad8*Δ cells are very similar to those of *tor1*Δ cells. The kinase activity of Gad8 and its phosphorylation state depend on the function of Tor1. Thus, it has been inferred that Tor1 regulates kinase activity of Gad8 via phosphorylation and that Gad8 is largely responsible for the phenotypes of the *tor1*-defective mutant (Matsuo *et al*., 2003). Moreover, Gad8 is regulated by another kinase Ksg1, which is a PDK1-like kinase. These findings led the authors to propose the existence of a conserved TOR-PDK1-AGC kinase signaling module (Matsuo *et al*., 2003), which was indeed demonstrated subsequently in mammalian and budding yeast cells (reviewed in Jacinto and Lorberg, 2008). The three kinase system, involving TOR, PDK and AGC kinases, may be a fundamental module for signal transduction in eukaryotic cells (Fig. 2).

A rapamycin-sensitive TOR-dependent function in fission yeast was first reported by Weisman *et al*. (2005). It is a Tor1-dependent amino acid uptake, especially an uptake of leucine. The authors showed that growth of auxotrophic cells, particularly of leucine-requiring cells, was sensitive to rapamycin. This rapamycin sensitivity was suppressed by deletion of the *fkh1* gene encoding an FKBP12 homolog, or by replacement of wild-type *tor1* with a putative rapamycin binding-defective allele, which was identified by a two-hybrid assay. These observations suggest that the Fkh1-rapamycin complex is responsible for the inhibition of the Tor1-dependent amino acid uptake (Weisman *et al*., 2005).

Tor2 FUNCTION AND ITS REGULATION

In contrast to Tor1, fission yeast Tor2 is essential for vegetative growth. Deletion of *tor2* resulted in cell death (Kawai *et al*., 2001; Weisman and Choder, 2001). In 2006 and 2007, four groups independently reported physiological roles of Tor2, constructing several temperature-sensitive *tor2* (*tor2-ts*) mutants (Alvarez and Moreno, 2006; Uritani *et al*., 2006; Weisman *et al*., 2007; Matsuo *et al*., 2007). These mutants arrested in G1 phase at the restrictive temperature even on medium rich in nutrition. Furthermore, homothallic *tor2-ts* mutants initiated sexual

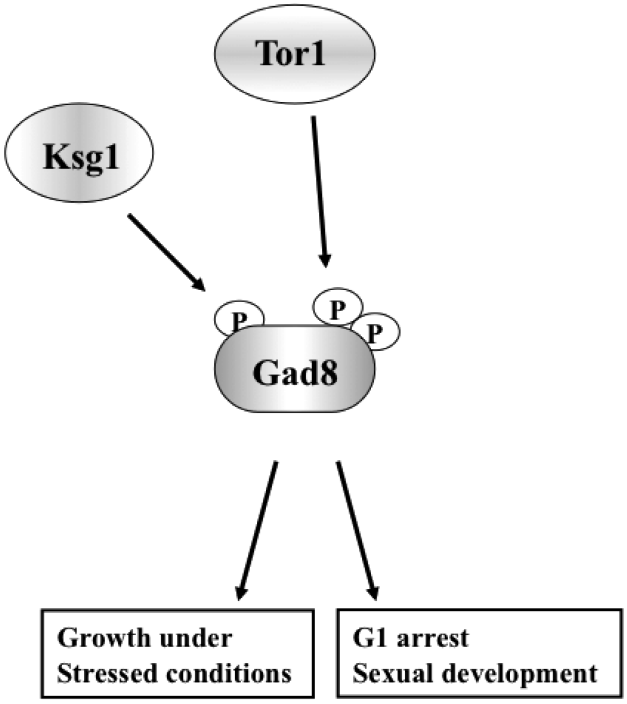

FIG. 2. A possible tripartite signaling module composed of TOR, PDK and AGC kinases. Fission yeast Tor1 (TOR) and Ksg1 (PDK) regulate the kinase activity of Gad8 (AGC) via phosphorylation, which is required for growth under stressed conditions and sexual development.

development on rich medium if shifted to the restrictive temperature. In these mutant cells expression of cell-cycle inhibitors and nitrogen starvation-responsive genes was upregulated by the temperature shift, but transcription of glucose starvation-responsive gene was not affected (Alvarez and Moreno, 2006; Uritani *et al*., 2006; Matsuo *et al*., 2007). In addition, by microarray analysis, it has been demonstrated that expression of nearly all nitrogen starvation-responsive genes, including *mei2* and *ste11* critical for sexual development, is induced by the loss of Tor2 function (Matsuo *et al*., 2007). These results suggest that inactivation of Tor2 mimics starvation of nitrogen.

The fission yeast Rheb ortholog, Rhb1, is essential for growth. Rhb1-deficiency causes G1 arrest like nitrogen starvation, and expression of *fnx1* and *mei2*, which is induced under nitrogen starvation, is upregulated in *rhb1*-defective cells (Mach *et al*., 2000; Yang *et al*., 2001). It has been shown that human Rheb can replace fission yeast Rhb1 in function (Yang *et al*., 2001). Fission yeast also contains both TSC1 and TSC2 orthologs, encoded by *tsc1* and *tsc2*, respectively (Matsumoto *et al*., 2002). Like human TSC proteins, Tsc1 and Tsc2 physically interact with each other. Tsc2 protein carrying mutations found in human patients has been shown to lose function. Disruption of *tsc1* or *tsc2* causes inefficient mating, deficiency in the uptake of amino acids, and defects in gene expression upon nitrogen starvation (Matsumoto *et al*., 2002; van Slegtenhorst *et al*., 2004; Nakase *et al*., 2006).

A decrease in the Rhb1 activity elevates the sensitivity to canavanine, a toxic analogue of arginine, by enhancing its uptake (Yang *et al*., 2000). In contrast, mutants defective in *tsc1* or *tsc2* are resistant to canavanine. A dominant negative form of *rhb1* can suppress the defective arginine uptake in *tsc2*Δ cells (van Slegtenhorst *et al*., 2004). Furthermore, the phenotypes of hyperactive *rhb1* mutants are similar to those of the *tsc1/2* mutants (Urano *et al*., 2005). From these findings, it has been proposed that the Tsc1/Tsc2 complex negatively regulates Rhb1.

The phenotypes of *rhb1*-defective mutants are highly similar to those of *tor2-ts* mutants. It has been reported that Rhb1 associates with Tor2 physically, likely in a GTP-dependent manner (Urano *et al*., 2005; Uritani *et al*., 2006), and that overexpression of *tor2* in wild-type cells induces canavanine resistance (Weisman *et al*., 2007). Furthermore, the *tsc2*Δ mutant shows a defect in adenine uptake. The defective adenine uptake in *tsc2*Δ cells can be rescued by down-regulation of Tor2 activity (Matsuo *et al*., 2007). Altogether, these observations indicate that a pathway including TSC, Rheb and TOR(Tor2) exists and functions in fission yeast as in higher eukaryotes, which may rationalize the use of fission yeast as a model system to study the TSC1/TSC2-Rheb-TOR pathway (Fig. 3).

Constitutively activated mutants of *tor2* were identified recently by Urano *et al*. (Urano *et al*., 2007). These *tor2*-active mutants can bypass the requirement of Rheb for cell growth, exhibit delayed responses to nitrogen starvation, and show deficiency in sexual development. However, it has been noted that *rhb1*Δ *tor2*-active double mutants can still respond to nitrogen starvation and eventually initiate sexual development, raising the possibility that Tor2 may also be regulated by an Rhb1-independent nitrogen-sensing mechanism. Furthermore, these mutants are sensitive to stresses like the *tor1*Δ mutant (Urano *et al*., 2007; Aspuria *et al*., 2007). Thus, Rhb1 may regulate Tor1 too. Further studies are awaited to confirm this idea.

A recent study has shown that Rheb regulates mTOR through FKBP38, a protein structurally related to FKBP12, which binds to mTOR and inhibits kinase activity. Rheb is reported to interact with FKBP38 in a GTP-dependent manner and prevent its association with mTOR (Bai *et al*., 2007). This novel scheme apparently declines previous models that implicate direct interaction of Rheb with TOR, and further work might be necessary to establish it unequivocally. Interestingly, some of fission yeast *tor2*-active (Rhb1-independent) mutations have been found in the N-terminal region of the kinase domain (Urano *et al*., 2007; Aspuria *et al*., 2007), which appears to correspond to a part of the FKBP38-binding (FKB) domain of mTOR reported by Bai *et al*. Thus, further analysis of these *tor2*-active mutants may help to explore the relationship between Rheb and FKBP38.

TOR COMPLEXES (TORCS) IN FISSION YEAST

The presence of two distinct TOR complexes has been demonstrated in fission yeast by analyses of physical and genetic interactions of the possible components, including a massspectroscopic analysis (Alvarez and Moreno, 2006; Hayashi

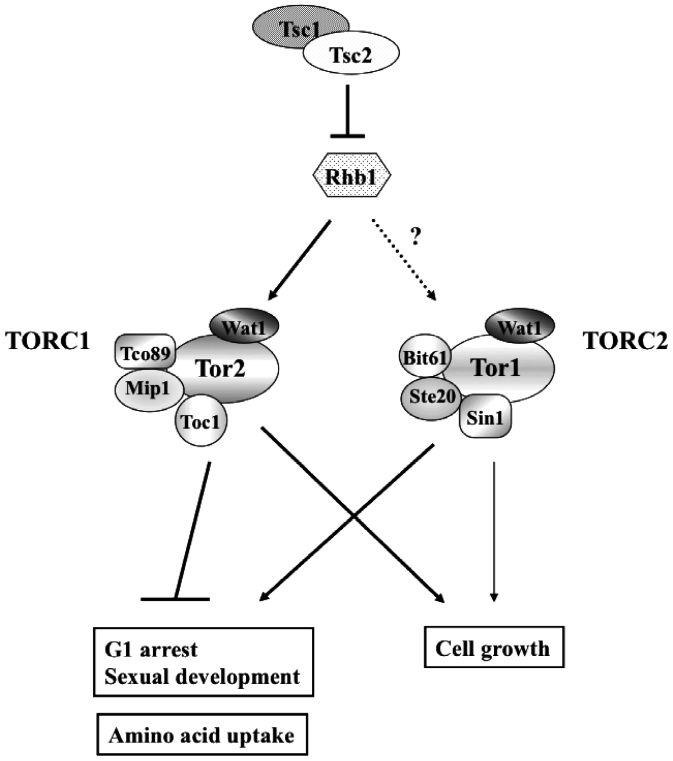

FIG. 3. TORC1 and TORC2 signaling pathways in fission yeast. The TSC-Rheb-TORC1 (Tor2) pathway regulates cell growth in fission yeast, as in higher eukaryotes, though it may remain controversial whether Rhb1 activates Tor2 in a similar manner to mammalian Rheb. Fission yeast TORC1 contains Mip1, Wat1/Pop3, Tco89, Toc1 and Tor2. It controls cell growth positively, and regulates G1 arrest, sexual development and amino acid uptake negatively. TORC2 contains Ste20, Wat1/Pop3, Sin1, Bit61 and Tor1. It regulates G1 arrest, sexual development and amino acid uptake positively. It may also positively control cell growth.

et al., 2007; Matsuo *et al*., 2007). The putative protein components of fission yeast TORC1 and TORC2 are listed in Table 1, together with budding yeast and mammal counterparts. In fission yeast, Tor2 forms a complex with Mip1 (Raptor homolog), Wat1/Pop3 (Lst8 homolog), Tco89 (budding yeast Tco89 homolog), and Toc1 (SPBP18G5.03), composing TORC1 together. By contrast, Tor1 forms a complex with Ste20 (Rictor/Avo3 homolog), Wat1/Pop3, Sin1 (budding yeast Avo1 homolog), and Bit61 (budding yeast Bit61 homolog), composing TORC2 (Table 1) (Alvarez and Moreno, 2006; Hayashi *et al*., 2007; Matsuo *et al*., 2007). Consistent with the physical interaction of the gene products, the *ste20*Δ strain phenotypically resembles *tor1*Δ. Furthermore, *ste20*Δ can be partially suppressed by an activated form of Gad8, which functions downstream of Tor1, reinforcing that Tor1 and Ste20 are likely to function in the same pathway (Matsuo *et al*., 2007).

As described above, fission yeast TORC1 (composed of Tor2p)[1] and TORC2 (composed of Tor1p [1]) apparently have

TABLE 1
Components of TORC1 and TORC2 in three organisms

	S. pombe	*S. cerevisiae*	Mammals
TORC1	Tor2	TOR1 or TOR2	mTOR
	Mip1	KOG1	Raptor
	Wat1/ Pop3	LST8	mLST8
	Tco89	TCO89	—
	Toc1	—	—
	—	—	PRAS40
TORC2	Tor1	TOR2	mTOR
	Sin1	AVO1	hSIN1
	—	AVO2	—
	Ste20	AVO3	Rictor
	Wat1/Pop3	LST8	mLST8
	Bit61	BIT61	PRR5

Three *S. pombe* proteins Tti1, Tel2 and Cka1/Orb5, which interact with TORC1 and TORC2, are not listed here as it is ambiguous whether they are specific components of TORCs.

opposite functions with respect to the promotion of G1 arrest and sexual development (Fig. 3). The function of TORC1 to promote cellular proliferation appears to be conserved among fission yeast, budding yeast and mammals, whereas the function of TORC2 may be more diverse. It has been noted that overproduced Tor2 can physically interact with Ste20, though weakly, which may indicate that a small portion of TORC2 is composed of Tor2 (Matsuo *et al*., 2007). However, it remains to be determined whether this weak interaction has any physiological significance. Wat1/Pop3 is a component of both TORC1 and TORC2. However, deletion of *wat1* is not lethal in fission yeast (Kemp *et al*., 1997; Ochotorena *et al*., 2001). This situation is distinct from that with the budding yeast homolog *LST8*, deletion of which results in lethality (Roberg *et al*., 1997). In addition, three novel proteins, namely Tti1 (SPCC622.13c), Tel2 and Cka1/Orb5, have been identified recently as interacting components for TORC1 and TORC2 in fission yeast (Hayashi *et al*., 2007). In mammalian cells, Tel2 has been shown to regulate the stability of PI3K-related kinases including mTOR (Takai *et al*., 2007).

POSSIBLE FUNCTIONS OF TORC1 AND TORC2

Although Tor1 and Tor2 function apparently in opposite directions with regard to sexual development, the *tor1*Δ and the *tor2-ts* mutations do not suppress each other. Rather, the *tor1*Δ *tor2-ts* double mutant appears to have additive phenotypes (Uritani *et al*., 2006; Matsuo *et al*., 2007). Thus, it appears unlikely that Tor1 and Tor2 share a common ultimate target and affect this target in opposite ways. Because the double mutant showed a lower restrictive temperature than the *tor2-ts* single mutant, Uritani *et al*. (2006) inferred that Tor1 may also be important for cell growth.

A truncated form of Mip1, the raptor homolog in fission yeast, was originally identified as a high-copy suppressor of ectopic meiosis induced by an activated form of Mei2 (Shinozaki-Yabana *et al*., 2000). Mei2 is an RNA-binding protein and is a potent regulator of meiosis in fission yeast. A large amount of information has been accumulated about its molecular function, but it is beyond the scope of this review to explain the details. Refer to a recent review for this purpose (Harigaya and Yamamoto, 2007). It has been shown that Mip1 binds to Mei2 and Ste11, the latter of which is a transcription factor that regulates a number of genes necessary for sexual development, including *mei2* (Shinozaki-Yabana *et al*., 2000). Alvarez and Moreno (2006) observed physical interaction of Tor2 with Ste11 and Mei2. Thus, it is presumable that TORC1 represses the initiation of sexual development by preventing the function of Stell and Mei2.

Budding yeast TORC2 is involved in actin organization (Schmidt *et al*., 1996). However, no obvious disorder of actin organization has been observed in fission yeast *tor1*Δ or *tor2-ts* cells, suggesting that fission yeast TOR may not be directly relevant to organization of actin. It is reported that actin cables in *tor1*Δ cells might be slightly thicker than ones in wild-type cells, though (Uritani *et al*., 2006; Matsuo *et al*., 2007).

The *tor2-51* mutant (*ts*) isolated by Alvarez and Moreno (2006) showed decreased expression of ribosomal protein genes at the restrictive temperature. This suggests that TORC1 controls cell growth by regulating transcription of ribosomal protein genes, at least in part, as in other organisms.

As discussed above, both Tsc1/2 and Tor1 are required for amino acid uptake and expression of several amino acid permeases (van Slegtenhorst *et al*., 2004; Weisman *et al*., 2005). In the *tor1*Δ *tsc2*Δ double mutant, expression of these permeases decreases more extensively than in each single mutant, suggesting that Tor1 and the Tsc1/Tsc2 complex may regulate these functions in two parallel pathways (Weisman *et al*., 2007). In addition, mutants defective in *tsc1* or *tsc2* are resistant to canavanine (van Slegtenhorst *et al*., 2004), and overexpression of *tor2* in wild-type cells induces canavanine resistance (Weisman *et al*., 2007). From these results, it has been proposed that Tor1 regulates amino acid uptake positively whereas Tor2 regulates negatively (Weisman *et al*., 2007). A microarray analysis has demonstrated that Tor2 regulates expression of several genes encoding permeases and transporters for amino acids and other nutrients (Matsuo *et al*., 2007) (Fig. 3) .

RAPAMYCIN AND FISSION YEAST TOR

Rapamycin does not affect vegetative growth of wild-type fission yeast cells. Upon starvation, it inhibits sexual development at an early stage prior to mating, but it does not appear to inhibit entry to the stationary phase or arrest in G1 phase (Weisman *et al*., 1997). Cells defective in *fkh1*, encoding a homolog of FKBP12, cannot undergo an early step of the sexual development in response to nutrient starvation. Thus, rapamycin-treated wild-type cells appear to phenocopy the *fkh1*Δ mutant (Weisman *et al*., 2001). The *tor1*Δ mutant is sensitive to rapamycin in an Fkh1-dependent manner (Kawai *et al*., 2001; Weisman, 2004; Weisman *et al*., 2005). This may mean that some of Tor2 functions are inhibited by rapamycin. Recently, Hayashi *et al*. (2007) reported that a *tor2* temperature-sensitive mutant, named *tor2-287*, is hypersensitive to rapamycin. This strain carries a mutation in the Tor2 kinase domain. Weisman *et al*. (2007) demonstrated that cells treated with rapamycin behave like *tsc1/2*Δ cells, exhibiting resistance to toxic amino acid analogs. It has been also noted that *tsc1/2*Δ cells are sensitive to rapamycin if grown in proline medium.

When cells are shifted from a good nitrogen source to a poor one, mitosis is advanced and cells divide at a smaller size. Petersen and Nurse (2007) recently reported that fission yeast TOR signaling is relevant to this nutrient-modulated control of mitotic onset. Reduction in the Tor1 activity and addition of rapamycin both advance mitotic onset, activating Cdc2 kinase through stimulation of the MAPK stress response pathway. In addition they observed that wild-type homothallic cells grown in glutamate medium initiated sexual differentiation if rapamycin was added to the medium, suggesting that Tor2 can be also downregulated by rapamycin (Petersen and Nurse, 2007). At present, however, it remains unclear why rapamycin does not affect vegetative growth of wild-type fission yeast cells as in other eukaryotes, and whether rapamycin inhibits Tor2 functions directly or indirectly.

CONCLUSIONS

How cells recognize nutritional conditions has been a long-standing unsolved problem for cell and molecular biology. Recent findings have indicated that TOR kinase plays a critical role in nutritional signaling in fission yeast as well as other eukaryotic organisms. Fission yeast TORCs regulate cell growth and sexual development in response to nutritional conditions. Further analysis of TOR functions in fission yeast will certainly help to clarify the molecular mechanisms underlying nutritional signaling.

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NOTE

1. Because naming of the TOR genes was done before their products were fully characterized, it has turned out that fission yeast Tor1 is a close counterpart of budding yeast TOR2 and that fission yeast Tor2 is a close counterpart of budding yeast TOR1.

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