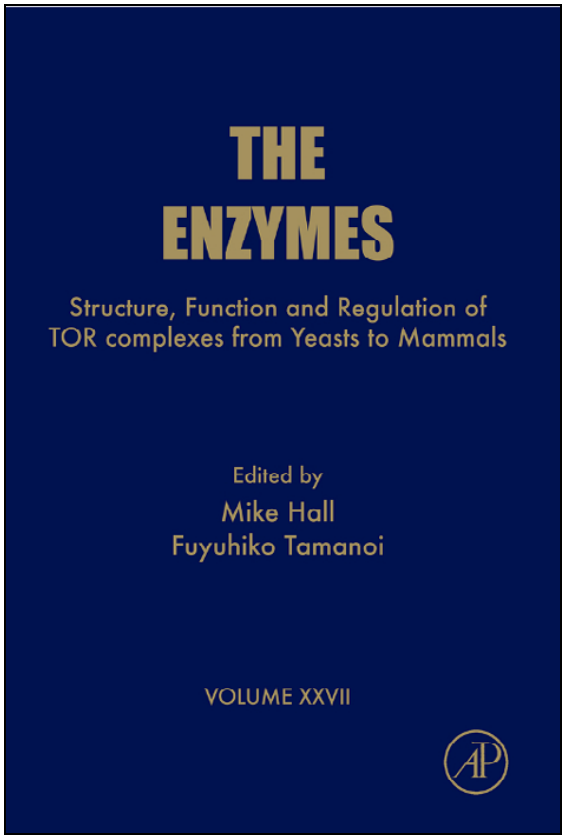

From: Ronit Weisman,
Fission Yeast TOR and Rapamycin,
In Mike Hall and Fuyuhiko Tamanoi: ***The Enzymes, Vol. 27,***
Burlington: Academic Press, 2010, pp.251-269.
ISBN: 978-0-12-381539-2,

13

Fission Yeast TOR and Rapamycin

RONIT WEISMAN[a,b]

[a]*Department of Natural and Life Sciences*
The Open University of Israel
Raanana
Israel

[b]*Department of Molecular Microbiology and Biotechnology*
Tel Aviv University
Tel Aviv
Israel

I. Abstract

Recent studies of target of rapamycin (TOR) in fission yeast revealed that in this organism, like in budding yeast and human cells, the TORC1 complex containing TOR together with a raptor-like protein, plays a central role in regulating growth, while inhibiting starvation responses. Disruption of TORC1 in fission yeast results in a phenotype very similar to that of wild-type cells starved for nitrogen, suggesting that TORC1 may regulate growth in response to nitrogen availability. The TORC2 complex in fission yeast contains TOR together with a rictor-like protein. In fission yeast, this complex is not essential under normal growth conditions but is required for survival under stress and for starvation responses. More recent studies demonstrate that TORC2 in fission yeast also has a profound role in gene silencing, telomere length maintenance and DNA damage response. Most interestingly, rapamycin does not inhibit the essential role of TORC1 or most of the cellular functions of TORC2. Yet, accumulation of data suggests that rapamycin can inhibit either TORC1- or TORC2-dependent functions under certain nutritional growth conditions and/or in the presence of loss-of-function mutations in TORC1 or its regulators. Understanding

ISSN NO: 1874-6047
DOI: 10.1016/S1874-6047(10)27013-0

the determinants that render cells sensitive to rapamycin is critical for understanding the mode of action of rapamycin and may also extend our understanding of potential interactions between TOR containing complexes.

II. Introduction

One of my first observations, which incited me into exploring the target of rapamycin (TOR) pathway in fission yeast, was that rapamycin did not inhibit vegetative growth in this organism [1]. This was in sharp contrast to the growth inhibitory effect of rapamycin on budding yeast [2]. The question that immediately comes to mind is: what renders fission yeast resistant to rapamycin?

Resistance to rapamycin in fission yeast appears less of a surprise considering the response to the drug of many higher eukaryotic cell types. A common use of rapamycin is as an immunosuppressive drug following transplant surgeries. However, rapamycin does not inhibit proliferation of T lymphocytes that have already entered the cell cycle, but rather blocks resting T cells from entering the cell cycle [3]. The proliferation of certain cancerous cells is also highly sensitive to rapamycin, a finding that led to the use of rapamycin as an anticancer drug (reviewed in Ref. [4]). Which molecular mechanisms underlie rapamycin resistance/sensitivity in various cell types? Can we learn from fission yeast about mechanisms that render cells resistant to the drug?

The budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* are distantly related. Indeed, the evolutionary distance between these two yeasts is as far apart from each other as from higher eukaryotes [5]. Thus, processes that are conserved between budding and fission yeasts are often also conserved in higher eukaryotes. Previous studies of the two yeasts have taught us that cellular processes that at first sight appeared substantially different, later proved to share common and conserved molecular mechanisms. For example, the regulation of the cell cycle first appeared fundamentally dissimilar in the two yeasts, partly owing to the fact that under optimal growth conditions, most of the control over cell cycle progression occurs at the G_1/S transition in budding yeast, while the G_2/M transition is the major control point in fission yeast. Further studies revealed that the same key regulators, in particular the master regulator CDK (cyclin-dependent kinase) are shared between the two yeasts and are conserved in higher eukaryotes [6, 7]. Indeed, it was the

difference between the two yeasts that propelled many cell cycle studies, leading to a better understanding of the evolutionary conserved mechanisms [8]. Would this also be the case for the studies of TOR pathways and rapamycin response?

Recent progress in understanding TOR signaling suggests that this may well be the case. At first, the TOR signaling pathway appeared very different in the two yeasts. One of the reasons for this apparent difference was that the first TOR homolog characterized in fission yeast was Tor1, which was subsequently identified as part of TORC2. Later, identification and characterization of Tor2 (which forms TORC1) revealed that in fission yeast, like in budding yeast, TOR plays a major role in regulating growth. Studies of the FRB (FKBP12-rapamycin binding) domains of either Tor1 or Tor2 in fission yeast demonstrated that these domains can bind rapamycin in the presence of FKBP12, indicating that the basic structural requirements for rapamycin-mediated inhibition exist. We and others identified several genetic backgrounds, in which rapamycin inhibits the growth of fission yeast cells. Although currently we understand little about the determinants that affect rapamycin sensitivity, characterization of rapamycin-sensitive mutants is likely to help us understand the rapamycin response.

III. TORC1 is a Major Regulator of Cellular Growth

A. Disruption of TORC1 Mimics Response to Nitrogen Starvation

Fission yeast contains two TOR homologs, Tor1 and Tor2 [9]. These were identified through sequence comparison analyses of data originating from the fission yeast genome sequencing project [10]. Tor2 mainly associates with Mip1 (similar to mammalian raptor and budding yeast Kog1) to form TOR complex 1 (TORC1). Tor1 mainly associates with Ste20 (similar to mammalian rictor and budding yeast Avo3) and with Sin1 (similar to mammalian Sin1 and budding yeast Avo1) to form TOR complex 2 (TORC2) [11, 12]. TORC1 and TORC2 in fission yeast include additional conserved components. The detailed composition of the TOR complexes is discussed by M. Yanagida in chapter 14.

tor2$^+$ is an essential gene. Depletion of *tor2*$^+$ using a heterologous repressible promoter [13] or temperature sensitive alleles (*tor2-ts*) [11, 12, 14, 15] resulted in a phenotype that highly resembled wild-type cells starved for nitrogen. This was the first indication that TOR in fission yeast plays a major role in controlling cellular growth in response to nutrient availability.

Since Tor2 physically associates with a raptor-like protein, Mip1, the structure and function of the TORC1 complex is conserved in fission yeast.

Fission yeast cells grow normally as haploid, rod-shaped cells, which divide by medial fission, giving rise to two daughter cells of equal size. Upon either nitrogen, or carbon starvation, cells exit the logarithmic phase and can opt for two alternative pathways: entrance into quiescence (stationary phase) or sexual development. Either option results in cells (or spores) that can better maintain viability for long periods and/or under harsh conditions, compared with vegetative growing cells. Entrance into sexual development requires the presence of the two opposite mating-type cells, h^+ and h^-, which can mate to form zygotes that subsequently undergo meiosis and sporulation. The nitrogen and carbon starvation responses differ in several aspects. Suffice here to say that the nitrogen-starvation response induces cells to enter mitosis at a reduced cell size, resulting in small-sized rounded stationary cells that accumulate at the G_1 phase of the cell cycle. In contrast, no mitosis advancement occurs when cells are starved for carbon, and under such conditions cells arrest their growth at the G_2 phase of the cell cycle [16]. Whether starved for nitrogen or carbon, mating occurs between two opposite mating-type cells at the G_1 phase of the cell cycle.

Like nitrogen-starved cells, cells depleted of Tor2 divide a few times, accumulate at the G_1 phase of the cell cycle and arrest their growth as small and round cells [11–15]. Since yeast cells normally grow in nature in the presence of abundant carbon but under nitrogen-limiting conditions, nitrogen availability is likely to be a major cue for growth control. Thus, Tor2 may play a major role under natural growth conditions [17].

Cells depleted of Tor2 showed a reduction in gene expression of ribosomal proteins and increased expression of nitrogen-starvation specific genes, such as regulators of sexual development, autophagy, membrane transporters, and amino acid permeases [12–15]. In accordance with this transcription profile, depletion of Tor2 induced autophagy [15] and sexual development [12–15]. Overexpression of *tor2*$^+$ or hyperactive *tor2* alleles inhibited mating and delayed cell-size adaptation in response to nitrogen starvation [14, 22]. Thus, Tor2 has a dual role as a positive regulator of growth and as a negative regulator of starvation responses (Figure 13.1). Overexpression of *tor2*$^+$ also rendered cells resistant to canavanine, a toxic analog of arginine, and was deleterious when combined with an auxotrophic mutation to leucine [13]. These findings suggest that overexpression of Tor2 inhibits amino acid uptake. The role of Tor2 in regulating amino acid uptake is particularly relevant for our discussion of the response to rapamycin, and will be further discussed in the next section.

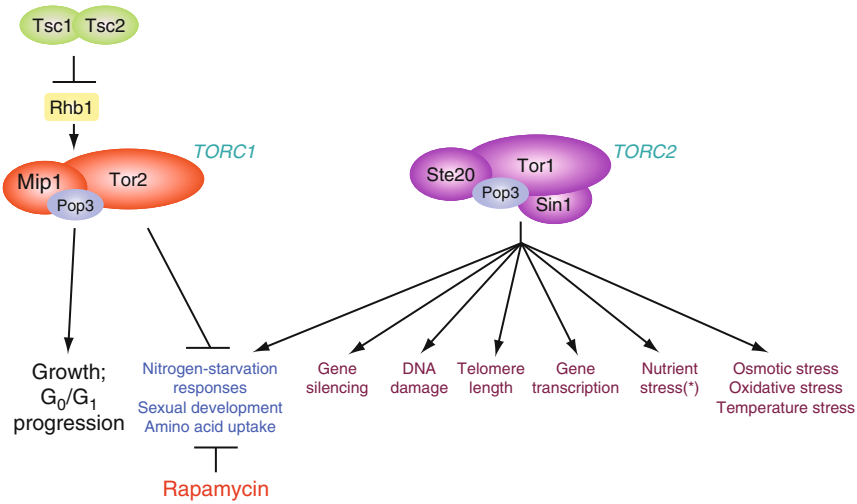

FIG. 13.1. Schematic model for the mode of action of TORC1, TORC2, and rapamycin in fission yeast. Only TORC1 complexes containing Tor2 and TORC2 complexes containing Tor1 are described, however, this may not represent the full repertoire of TOR containing complexes. It is notable that the cellular functions that are oppositely regulated by the two TOR complexes are sensitive to rapamycin. (*): Response to nutrient stress refers to the ability of the cells to properly enter and maintain stationary phase physiology following nutrient depletion.

B. REGULATION OF NITROGEN STARVATION RESPONSES AND AMINO ACID UPTAKE BY THE TSC–RHB1–TORC1 MODULE

Fission yeast provides a particularly valuable system for studying TOR pathways, since the regulation of TORC1 by the TSC–Rheb module is conserved in this organism [18]. Here, I will focus on the features of the TSC–Rheb module which are relevant for our discussion of the rapamycin response.

The fission yeast Tsc1 and Tsc2 proteins form a complex [19, 20], in which Tsc2 acts as GTPase activating protein (GAP) for the small GTPase Rhb1 (Rheb homolog) [21–23]. Rhb1 associates with Tor2 and positively regulates its activity [15, 22]. Accordingly, depletion of Rhb1 displays a nitrogen-starvation phenotype [24, 25] and activated Tor2 mutant alleles can compensate for the loss of function of Rhb1 [26].

While loss of function of Tor2 or Rhb1 mimic nitrogen starvation, deletion of *tsc1*$^+$ or *tsc2*$^+$ resulted in viable cells that exhibited reduced amino acid uptake, low concentration of inner amino acid pools, inability to induce several nitrogen-starvation response genes and partial sterility [19–21]. The defect in amino acid uptake was demonstrated in *tsc* mutant

cells by several methods: direct measurement of uptake of radioactively labeled leucine or arginine [19, 20], resistance to canavanine, thialysine or ethionine, which are the toxic analogs of arginine, lysine, and methionine, respectively [20, 22, 23] or slow growth when combined with auxotrophic mutations, in particular, auxotrophy for leucine [19, 20].

Several studies indicated that the *tsc* genes regulated amino acid uptake via negatively regulating Rhb1, including the finding that partial loss of function of *rhb1*$^{+}$ (*rhb1*$^{G63D/S165N}$) reversed the canavanine-resistant phenotype of *tsc* mutant cells (49), while hyperactive *rhb1* mutants conferred resistance to canavanine [22]. Overexpression of *tor2*$^{+}$ conferred canavanine and thialysine resistance and inhibited the growth of cells auxotrophic for leucine [13]. In contrast, downregulation of Tor2, using *tor2-ts* alleles, rescued the adenine uptake defect in Δ*tsc2* mutant cells [12]. Collectively, these findings are in line with the suggestion that the defect in amino acid uptake in *tsc* mutant cells results from overactivation of Tor2 via Rhb1 (Figure 13.1). It is possible that hyperactivation of Tor2 mimics conditions in which nitrogen is abundant, leading to the downregulation of amino acid import. However, the cue that is sensed by TOR or TSC–Rhb1 is currently unknown.

The phenotype of inefficient amino acid uptake in *tsc* mutant cells is associated with the downregulation of the expression of a group of amino acid permeases, *isp5*$^{+}$, 7G5.06, and c869.10 [20]. The transcripts of these permeases are strongly upregulated by a shift to medium with a poor nitrogen source (proline), suggesting that they may act as general amino acid permeases [27]. *isp5*$^{+}$ and c869.10 were also identified by global transcriptional profiling as genes whose transcripts were upregulated by depletion of Tor2 [12]. We found that overexpression of Tor2 downregulated the expression of 7G5.06 and c869.10 (M. Schonbrun and R. Weisman, unpublished data). Thus, the TSC complex and Tor2 oppositely regulate the transcription of the same amino acid permeases. The TSC–Rheb module also regulates amino acid uptake posttranscriptionally, as mislocalization of amino acid permeases was demonstrated in *tsc* mutant cells [19, 28].

Intriguingly, a recent study demonstrated that a *tor2-ts* mutation conferred resistance to canavanine [29], suggesting that downregulation of Tor2, as well as its upregulation, may lead to reduced uptake of amino acids. It is currently not clear whether a common underlying mechanism underlies canavanine resistance in *tor2-ts* or overexpression of *tor2*$^{+}$. In this respect, it is interesting to note that either upregulation of TORC1 by mutations in TSC1/2 or downregulation of TORC1 by mutations in the Birt-Hogg-Dube protein (folliculin) may lead to similar development of tumors [30].

Finally, although a clear role for Rhb1 was identified in controlling Tor2, Rhb1 may also exert Tor2-independent functions. Accordingly, hyperactivated *tor2* alleles, which rescued the lethal phenotype of deletion of *rhb1*$^{+}$, did not

compensate for the sensitivity to osmotic stress, high temperature or thialysine in the double mutant Δ*rhb1 tor2*^{act} cells. Thus, Rhb1 may also regulate amino acid uptake or survival under stress conditions in a Tor2-independent manner [26]. The possibility that Rhb1 may regulate other proteins beside Tor2 has also been suggested by [29], who isolated the *rhb1*^{Q52RI76F} allele that conferred canavanine resistance but, unlike other constitutive activated *rhb1* allele (*rhb1*^{V17A}), did not show a defect in response to nitrogen starvation. No other targets for such Rhb1 activity are known at present. An intriguing possibility is Tor1. Tor1 is required for cell survival under osmotic stress and positively regulates amino acid uptake (see below). However, so far, no clear link has been identified between TSC–Rhb1 and Tor1/TORC2.

C. Rapamycin Reduces Amino Acid Uptake in Wild-Type Fission Yeast Cells

Rapamycin does not inhibit vegetative growth in fission yeast; therefore, it is evident that under optimal growth conditions, the drug does not inhibit the essential role of Tor2. Yet, rapamycin inhibited the growth of auxotrophic mutant cells that are dependent on amino acid import for their survival [27]. Like the effect described for *tsc* mutant cells, leucine auxotrophs were particularly sensitive to the growth inhibitory effect of rapamycin. Rapamycin also reduced the efficiency of radioactively labeled leucine uptake [27] and conferred resistance to canavanine or thialysine [13]. Thus, surprisingly, introduction of rapamycin leads to an effect which is similar to activation, rather than inhibition of Tor2 (TORC1).

The slow growth of leucine auxotrophs in the presence of rapamycin was completely rescued by deletion of *fkh1*$^{+}$, the FKBP12 homolog in fission yeast, indicating that rapamycin inhibits a TOR-dependent function [27]. Further studies suggested that reduction of amino acid uptake is the result of inhibition of Tor1. First, deletion of *tor1*$^{+}$ resulted in reduced uptake of radioactive-labeled leucine and reduced expression of the *isp5*$^{+}$, 7G5.06, and c869.10 amino acid permeases [27]. Second, a rapamycin-binding defective allele, *tor1*^{S1834E}, conferred rapamycin resistance to leucine auxotrophs [27]. The sensitivity of auxotroph mutant cells to rapamycin was rescued by replacement of ammonia, which is normally used as the nitrogen source in minimal medium, with proline. Since the presence of proline leads to induction of amino acid uptake [27, 31], the rescue of the rapamycin-sensitive phenotype by using proline as the nitrogen source further supports a defect in amino acid uptake.

We also detected a reduction in the expression of the transcripts of *isp5*$^{+}$, 7G5.06, and c869.10 when cells were grown in the presence of rapamycin in minimal or proline medium [27]. In contrast to our findings, it was reported

that 3 hours after a shift into rapamycin-containing medium, the transcription of *isp5*$^+$, 7G5.06, and c869.10 was upregulated [32]. This discrepancy may be reconciled if there is a dynamic change in expression of amino acid permeases in response to rapamycin, in which there is first an induction in expression, followed by downregulation to levels that are lower compared to untreated cells. A tempting explanation is that rapamycin first inhibits TORC1, leading to upregulation of expression of amino acid permeases. Following a long period of exposure to the drug, the TORC2 complex is also inhibited, resulting in a decrease in expression of amino acid permeases. In mammalian cells, rapamycin inhibits TORC1 upon short exposure to the drug, but upon long exposure also causes disassembly and inhibition of TORC2 [33]. A similar mechanism may operate in fission yeast.

Combining the deletion of *tor1*$^+$ (Δ*tor1*) with the Δ*tsc1* or Δ*tsc2* mutations resulted in a further reduction in amino acid uptake and amino acid permease gene expression, suggesting that Tor1 acts in parallel with Tsc1/2 in regulating amino acid uptake [13]. However, *tor1*$^+$ and *tsc1/2*$^+$ show a complex genetic interaction pattern. Cell size is oppositely regulated by Tor1 and Tsc1/2. Δ*tor1* cells are elongated; in contrast, *tsc1* or *tsc2* mutants are slightly smaller than wild-type cells [13]. The Δ*tor1* mutation reversed the short cell size phenotype of *tsc1/2* mutants [13]. Also, mutation in *tsc* partially suppressed the sensitivity of Δ*tor1* to a variety of stresses [15]. Further studies are required to determine functional interactions between the TSC complex, Rhb1 and Tor1.

In conclusion, rapamycin appears to inhibit amino acid uptake via inhibiting a Tor1-dependent activity. It remains to be determined whether Tor1 exerts this function as part of TORC2. It will also be important to find out whether the apparent opposite roles of Tor1 and Tor2 in regulating amino acid uptake reflects an interdependent regulation between these two complexes, which may be disrupted by rapamycin.

IV. TORC2 is Required for Responses to Starvation, Survival Under Stress Conditions, Chromatin-Mediated Functions, DNA Damage Response and Maintenance of Telomere Length

A. Tor1 and Tor2 Oppositely Regulate Responses to Nitrogen Starvation

The first TOR homolog to be characterized in fission yeast was Tor1. This TOR homolog was subsequently shown to mainly associate with Ste20, Sin1, and Wat1 to form a TORC2-like complex [11, 12]. *tor1*$^+$ is not

essential for cell viability; however, disruption of *tor1*$^+$ resulted in pleiotropic defects. Δ*tor1* mutant cells show slightly elongated cell morphology, sensitivity to osmotic, oxidative or temperature stress conditions, inability to enter sexual development or acquire stationary phase physiology, and decrease in amino acid uptake [9, 27, 34, 35]. More recently, we found that Tor1 is also required for cell survival under DNA-damaging conditions, gene silencing and regulation of telomere length [36]. The elongated cell morphology, stress sensitivity, defects in response to starvation and sensitivity to DNA damages are shared between Δ*tor1* and deletion of other components of TORC2, supporting the notion that Tor1 mainly acts as part of a TORC2 complex [12, 34, 36]. These defects are also shared with mutant cells lacking the AGC kinase Gad8 [12, 36]. TORC2 activates Gad8 via its phosphorylation, in line with the suggestion that Gad8 is a major downstream target of TORC2 in fission yeast [34, 37]. Whether the cellular roles of Tor1 in regulating amino acid uptake or gene silencing are also regulated when Tor1 associates with the subunits of TORC2 and/or via Gad8, is yet to be established. One notable cellular function which is not attributed to TORC2 in fission yeast is the regulation of the actin cytoskeleton [12, 15, 34], a function that is attributed to TORC2 in budding yeast and mammalian cells [38–40].

The wide variety of cellular functions in which Tor1 is involved is perplexing. Unlike the ''classical'' role of TOR as a major regulator of cellular growth, Tor1 (TORC2) is not required under optimal conditions. Moreover, in certain aspects, Tor1 and Tor2 oppositely regulate the same cellular functions (Figure 13.1). Thus, while deletion of *tor2*$^+$ leads to an ''always starved for nitrogen'' phenotype, deletion of *tor1*$^+$ results in a ''never starved for nitrogen'' phenotype; cells depleted for Tor2 get shorter and are induced into sexual development on rich medium, while Δ*tor1* cells do not adapt their cell-size in response to nitrogen starvation conditions and are highly sterile. Loss-of-function mutants of *tor1*$^+$ or *tor2*$^+$ also show opposite phenotypes at the molecular level. Disruption of Tor1 leads to reduced expression of nitrogen-starvation induced genes, while disruption of Tor2 results in the induction of these genes. These include genes encoding amino acid permeases and genes required for sexual development [12, 27, 36]. The CDK inhibitor, *rum1*$^+$, which is required for G_1 arrest in response to nitrogen starvation, is also oppositely regulated by Tor2 and Tor1 [14, 34].

Combining the Δ*tor1* and Δ*tor2* or *tor2-ts* mutations resulted in nonviable double mutant cells. The double mutant cells arrested their growth at the G_2 phase and were sterile, similar to single Δ*tor1* mutant cells [13, 15]. Thus, Tor1 may act either downstream of Tor2 or independently. The Δ*tor1* Δ*tor2* double mutant cells arrested their growth at an intermediate cell-size,

compared with the respective single mutants, which suggests that Tor2 requires Tor1 for divisions at a small cell size. The finding that Tor1 and Tor2 regulate the same cellular processes argues for a close functional relationship.

B. TORC2 Affects Chromatin-Mediated Functions and DNA Damage or Stress Responses

A genome-wide expression profiling of Δ*tor1* cells revealed changes in the expression of many genes. An extensive overlap with genes that were upregulated in chromatin-structure mutants was observed [36]. These included mutations in the histone deacetylases *clr3*$^+$ (Δ*clr3*) or *clr6*$^+$ (*clr6-1*) [41] or in the subunit of the chromatin-remodeling RSC complex, *rsc58*$^+$ (Δ*rsc58*) [42]. Genes that are upregulated in Δ*tor1* cells included repeated genes and genes that were clustered at subtelomeric regions, suggesting that Tor1 affects gene silencing at heterochromatic regions. Consistent with this suggestion, cells deleted for *tor1*$^+$ derepressed the expression of a reporter gene (*ade6*$^+$) that was inserted into the mating-type locus, a region that is characterized by a heterochromatic structure [36].

clr6$^+$ is an essential gene, and the *clr6-1* hypomorphic allele has been extensively analyzed. Single *clr6-1* mutants or *clr6-1* Δ*clr3* double mutant cells show a variety of defects that include elongated telomeres and sensitivity to osmotic stress, DNA damaging condition or drugs that destabilize microtubules [41, 43]. These defects are also shared by Δ*tor1* mutant cells or deletion of other components of the TORC2, or deletion of *gad8*$^+$ [36]. Telomere length analysis showed that the telomeres of Δ*tor1*, Δ*ste20*, or Δ*gad8* mutant cells are elongated by ~150 bp compared to wild type. This elongation is similar to that observed in *clr6-1* single mutant cells [41]. Mutations in specific components of TORC2 or deletion of *gad8*$^+$ also rendered the cells sensitive to thiabendazole, a drug that destabilizes microtubules [36]. Taken together, these findings suggest that TORC2 has a profound effect on heterochromatic regions: the mating-type locus, the telomeres, and possibly also the centromeres.

TORC2–Gad8 is also required for cell survival under DNA damaging conditions. Cells deleted for *tor1*$^+$, *sin1*$^+$, *ste20*$^+$, or *gad8*$^+$ showed great sensitivity to hydroxyurea (HU), which induces replication stress by depleting dNTP pools, or to methyl-methane sulfonate (MMS), a DNA alkylating agent [36]. Recently, we also found that mutations in TORC2 rendered cells highly sensitive to camptothecin (M. Schonbrun and R. Weisman, unpublished data), further supporting a general role for TORC2 in survival under DNA damaging conditions. Cells disrupted for TORC2 showed greater

sensitivity to DNA damaging conditions compared with histone deacetylase mutants, indicating that the putative defect in chromatin structure alone cannot explain the strong sensitivity to DNA damaging conditions in TORC2 mutants.

In response to DNA replication stress or DNA damage, cells inhibit cell cycle progression, repair the damage and then reenter the cell cycle. This set of actions is orchestrated by the checkpoint kinase Rad3 (similar to mammalian ATR). Like TOR, Rad3 is a member of the phosphatydilinositol kinase-related kinases. Rad3 is responsible for the activation of Chk1 in response to DNA damage or Cds1 (similar to mammalian Chk2) in response to replication stress. Upon activation by Rad3, Chk1 or Cds1 inhibit entrance into mitosis by regulating the activity of the cyclin-dependent kinase Cdc2 (CDK). This is achieved by determining the status of tyrosine-15 phophorylation on Cdc2 via dual regulation of the phophatase Cdc25 and the kinase Wee1 (reviewed in Ref. [44]). The sensitivity of TORC2 mutant cells to either HU or MMS was comparable to that of Δ*rad3* mutant cells; however, the mechanism that underlies this sensitivity is distinct. Cells lacking Rad3 failed to activate Chk1 or Cds1; consequently, cells fail to inhibit entrance into mitosis and rapidly lose their viability in the presence of the DNA damage [44]. In contrast, cells lacking Tor1 activated Chk1 in response to MMS and delayed progression into mitosis in the presence of DNA replication stress [36]. In response to HU, Δ*tor1* cells accumulated with highly phosphorylated (inactive) Cdc2 kinase, consistent with our suggestion that Tor1 is not required for mitotic inhibition in the presence of unreplicated chromosomes. In wild-type cells, Cdc2 is dephosphorylated ~100 min after release from HU arrest [36, 45]. In contrast, upon release of Δ*tor1* mutant cells from HU, Cdc2 remained in its phosphorylated (inactive) form and cells failed to reenter the cell cycle [36]. Our data thus suggest that unlike Rad3, Tor1 is required for a later stage in response to DNA damage, either for repair (or the stabilization of the stalled replication forks in the presence of HU) or for reentering the cell cycle upon completion of the repair. In this respect, it is interesting to note that cells defective in histone deacetylase activity (*clr6-1*) also failed to reestablish growth following removal of HU, possibly due to failure to inactivate Cds1 [46]. Whether a similar mechanism occurs in Δ*tor1* mutant cell is currently being investigated in our laboratory.

Treatment of wild-type cells with rapamycin did not affect survival under osmotic, oxidative, or temperature stress conditions. Also, rapamycin did not affect telomere length, gene silencing, or survival in the presence of HU or MMS. Thus, most of the cellular functions of TORC2–Gad8 are not inhibited by rapamycin [36].

C. Tor1 Regulates Mitotic Entry

Cells deleted for *tor1*$^+$, *ste20*$^+$, *sin1*$^+$, or *gad8*$^+$ are slightly elongated [9, 12, 34, 35]. Recently, it was also reported that overexpression of *tor1*$^+$ can lead to a short cell phenotype [47]. Since the timing of entrance into mitosis in fission yeast is the main transition point that operates to coordinate cell growth with cell division under optimal growth conditions, the elongated morphology of TORC2 mutant cells implies a positive role for TORC2 in regulating entrance into mitosis [34, 36]. Results obtained from combining the Δ*tor1* mutation with a set of other cell cycle mutants suggest that Tor1 positively regulates entrance into mitosis by regulating Tyr-15 phosphorylation of Cdc2, possibly by controlling both the activity of Wee1 and Cdc25 [36].

Surprisingly, decrease in the level of Tor1, rather than its complete deletion, led to advancement of entry into mitosis and to a reduced cell-size phenotype [48], rather than the elongated cells observed in Δ*tor1* [36]. Further studies suggested that Tor1 can act as a negative regulator of mitotic entry via inhibition of the stress activated MAPK Sty1 (also known as Spc1), which is required for Plo1 (Polo-like)-dependent activation of Cdc2 [48]. The role of Tor1 as a modulator of Sty1/MAPK signaling has been recently reviewed [49] and will not be detailed here. More recently, it was suggested that Tor1 negatively regulates entrance into mitosis in response to a shift to poor nitrogen source but not in response to complete withdrawal of nitrogen [47]. The negative regulation of mitosis by Tor1 occurs when Tor1 is associated with Mip1, as part of TORC1, but not when it acts as part of TORC2 [47].

Does Tor2 also regulate entrance into mitosis? Depletion of Tor2 mimicked a complete withdrawal of the nitrogen source. Under such conditions, a transient inhibition of entrance into mitosis occurs, followed by two to three divisions at a reduced cell size. Depletion of Tor2 was also found to induce the levels of Rum1, which is a negative regulator of CDK [14]. Thus, Tor2 (as part of the essential TORC1 complex) may negatively regulate entrance into mitosis, in contrast to the positive role of TORC2 in regulating mitosis [34, 36]. A role for TORC1 as a negative regulator of entrance into mitosis in fission yeast is in line with findings in budding yeast, *Drosophila* and mammalian cells that demonstrate that TORC1 inhibits entrance into mitosis [50–52].

V. The Response to Rapamycin in Fission Yeast

A. The Effects of Rapamycin on Sexual Development

One of our very first observations was that rapamycin reduced the efficiency of the sexual development process [1]. Rapamycin reduced the mating between haploid fission yeast cells, one of the early steps in

the sexual development process, under either no-nitrogen or low glucose conditions [1]. Thus, rapamycin does not interfere with the sensing of the particular nutrient which is depleted. If the starvation conditions prevail, the diploid zygotes that are formed by the mating of two haploid cells undergo meiosis and sporulation. Introduction of rapamycin to diploid cells under starvation conditions only slightly reduced the efficiency of meiosis and sporulation [1], indicating that rapamycin is more effective at inhibiting early stages of the sexual development pathway. Rapamycin did not affect entrance into stationary phase, and cells that exit the logarithmic phase in the presence of rapamycin arrested properly and maintained viability comparable to untreated cells [1]. In addition, rapamycin did not interfere with resumption of vegetative growth following nitrogen or carbon starvation (S. Pur and R. Weisman, unpublished data).

If rapamycin inhibited a TOR-dependent function, leading to reduced efficiency of sexual development, then deletion of the fission yeast FKBP12 would be expected to result in a rapamycin-resistance phenotype. However, deletion of *fkh1*$^+$ resulted in partial sterility and did not affect entrance into stationary phase, similar to treatment with rapamycin [53]. Thus, rapamycin most likely directly inhibits the function of Fkh1. The partial sterile phenotype induced by rapamycin was used to isolate *fkh1* mutant genes that conferred resistance to the inhibitory effect of rapamycin. This screen identified five amino acids that are located in the rapamycin-binding pocket of Fkh1 and are critical for the effect of rapamycin on sexual development [53].

As described above, Tor1 and Tor2 are also strongly implicated in regulating sexual development. Cells disrupted for *tor1*$^+$ are highly sterile and thus show a far more severe phenotype compared with Δ*fkh1* cells or rapamycin treatment. Cells depleted for Tor2 show derepression of sexual development. Unlike our observation which indicated inhibitory effect of rapamycin on sexual development, it was reported that addition of rapamycin to wild-type cells grown in glutamate medium induced sexual development [48], mimicking a shift from ‘‘good’’ to ‘‘poor’’ nitrogen medium. It is thus possible that rapamycin also affects TOR-dependent regulation of sexual development; however, further studies are required to determine the specific conditions that are required for such an effect and the underlying molecular mechanism.

B. Mutations that Render the Growth of Fission Yeast Sensitive to Rapamycin

The FRB domain of TOR is highly conserved. The FRB of Tor1 [9] or Tor2 (S. Finkelstein and R. Weisman, unpublished data) binds FKBP12 in the presence of rapamycin, as determined by two hybrid assays. Yet, wild-type

cells are not sensitive to rapamycin during the growth phase or under stress conditions. As described above, the growth of auxotrophic mutant cells, particularly those auxotrophic for leucine, is sensitive to rapamycin. This sensitivity is associated with reduction in amino acid permeases gene expression, and is mediated by inhibition of Tor1 [27].

Cells auxotrophic for leucine (e.g., *leu1-32* strains) were sensitive to rapamycin only when grown in a minimal (ammonia-based) medium supplemented with leucine (50–75 μg/ml). No growth inhibition by rapamycin was observed when auxotrophic mutant cells were grown in a rich (YE) or minimal medium in which the nitrogen source is proline. In contrast, deletion of *tor1*$^{+}$ rendered cells sensitive to rapamycin in a rich as well as minimal medium [35, 54]. This rapamycin sensitivity was dependent on the presence of Fkh1 (FKBP12) suggesting that Tor2 is inhibited in this genetic background. Reduction in Tor2 activity by *tor2-ts* mutant alleles [11] also rendered cells sensitive to rapamycin, in rich as well as in minimal medium. These findings may suggest that Tor1 and Tor2 share a common function which is sensitive to rapamycin. Alternatively, the absence of Tor1 may cause a reduction in the level of TORC1 and consequently render cells sensitive to rapamycin. Fragmentary data also suggest a reduction in cell-size following treatment with rapamycin. Either wild-type cells grown in glutamate medium [48] or *tsc* mutant cells [13] show reduction in cell size following rapamycin treatment. This rapamycin-induced reduction in cell size is somewhat reminiscent of the reduction in cell size in mammalian cells following rapamycin treatment [55] and may reflect acceleration of entrance into mitosis and/or inhibition of TORC1 complexes.

Two other mutations that reduce TORC1-dependent activities: deletion of *bhd*$^{+}$ (the homolog of the Birt-Hogg-Dube disease gene) or the hypomorphic allele of *rhb1*$^{+}$ (*rhb1*$^{G63D/S165N}$), also rendered cells sensitive to rapamycin during vegetative growth. Growth of the double mutant Δ*bhd rhb1*$^{G63D/S165N}$ cells was completely inhibited by rapamycin, and expression of genes encoding amino acid permeases was elevated, compared with each of the single mutants. These findings suggest that Bhd and Rhb1 regulate TORC1 by separate mechanisms [32] and that in the Δ*bhd rhb1*$^{G63D/S165N}$ the levels of TORC1 activity is low, rendering cell sensitive to rapamycin.

Most surprisingly, we noted that rapamycin completely inhibited growth of prototrophic Δ*tsc1* or Δ*tsc*2 mutant cells, when the nitrogen source in the medium is proline [13]. The sensitivity of Δ*tsc1* or Δ*tsc*2 cells to rapamycin is rescued by deletion of *fkh1*$^{+}$, indicating that a TOR-dependent function is inhibited. Δ*tsc1* or Δ*tsc*2 mutant cells did not show rapamycin-sensitivity when the nitrogen source in the medium was ammonia or glutamate [13]. The finding that cells lacking a negative inhibitor of TORC1 are sensitive to rapamycin is surprising, since in such genetic background the activity of

TORC1 is expected to be high. Further studies using *tor1*- and *tor2*-rapamycin-binding defective alleles are required to ascertain this possibility.

VI. Conclusion and Future Prospective

Fission yeast, like budding yeast has two TOR homologs. In budding yeast either Tor1 or Tor2 can form TORC1, whereas TORC2 contains Tor2 only. In fission yeast Tor1 mainly associates with partner proteins to form TORC2, while Tor2 forms TORC1 [11, 12]. However, Tor1 may also be part of TORC1 [47], while Tor2 may also form complexes with TORC2 subunits [12]. It should be stressed that the major cellular functions of Tor1 and Tor2 are not interchangeable.

The cellular function of TORC1 as a central growth regulator is conserved in fission yeast. Yet, unlike budding yeast and certain mammalian cell types, the essential role of TORC1 is not inhibited by rapamycin under normal growth conditions. The TORC2 complex is required under various stress conditions but is not essential for growth under normal conditions. Our recent results, suggesting that TORC2 affects chromatin structure and participates in DNA damage response, are most intriguing. A defect in chromatin structure may explain several defects associated with disruption of TORC2, including derepression in gene silencing, elongated telomeres and possibly also defects in regulating the cell cycle progression and growth under stress conditions. In budding yeast, TORC1 has been suggested to regulate chromatin structure in a rapamycin-sensitive manner *via* the histone deacetylase Rpd3 [56]. TORC1 in budding yeast has also been suggested to positively regulate ribosomal gene expression via a chromatin mediated mechanism [57]. Our studies in fission yeast suggest that TORC2 is required for gene silencing. Thus, yet again, TORC1 and TORC2 appear to have opposite effects. TORC1 has also been implicated in regulating the response to DNA damage in budding yeast and higher eukaryotes (e.g., see Refs. [58, 59]). However, the relevance of these studies to the role of TORC2 under DNA damage conditions in fission yeast is not clear [60, 62].

Finally TORC1 and TORC2 oppositely regulate response to nitrogen starvation, regulation of amino acid uptake and possibly also entrance into mitosis. Interestingly, amino acid uptake and sexual development are two cellular processes that are inhibited by rapamycin. At least with respect to amino acid uptake, rapamycin appears to have an immediate inhibitory effect on TORC1 and a long-term inhibitory effect on TORC2. These findings together with the observation that sensitivity of *tsc* mutant cells are sensitive to rapamycin suggest a close relationship between TORC1 and TORC2 that awaits further characterization.

Acknowledgments

I thank Martin Kupiec, Miriam Schonbrun, Dana Laor, and Shiri Pur for critical reading of the manuscript and for enthusiastic and fruitful discussions.

References

1. Weisman, R., Choder, M., and Koltin, Y. (1997). Rapamycin specifically interferes with the developmental response of fission yeast to starvation. *J Bacteriol* 179:6325–6334.
2. Heitman, J., Movva, N.R., and Hall, M.N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253:905–909.
3. Terada, N., Franklin, R.A., Lucas, J.J., Blenis, J., and Gelfand, E.W. (1993). Failure of rapamycin to block proliferation once resting cells have entered the cell cycle despite inactivation of p70 S6 kinase. *J Biol Chem* 268:12062–12068.
4. Sabatini, D.M. (2006). mTOR and cancer: insights into a complex relationship. *Nat Rev Cancer* 6:729–734.
5. Sipiczki, M. (2000). Where does fission yeast sit on the tree of life? *Genome Biol* 1: REVIEWS 1011.
6. Moser, B.A., and Russell, P. (2000). Cell cycle regulation in *Schizosaccharomyces pombe*. *Curr Opin Microbiol* 3:631–636.
7. Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* 344:503–508.
8. Forsburg, S.L. (1999). The best yeast? *Trends Genet* 15:340–344.
9. Weisman, R., and Choder, M. (2001). The fission yeast TOR homolog, tor1^{+}, is required for the response to starvation and other stresses via a conserved serine. *J Biol Chem* 276:7027–7032.
10. Wood, V., Gwilliam, R., Rajandream, M.A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., *et al.* (2002). The genome sequence of *Schizosaccharomyces pombe*. *Nature* 415:871–880.
11. Hayashi, T., Hatanaka, M., Nagao, K., Nakaseko, Y., Kanoh, J., Kokubu, A., Ebe, M., and Yanagida, M. (2007). Rapamycin sensitivity of the *Schizosaccharomyces pombe* tor2 mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. *Genes Cells* 12:1357–1370.
12. Matsuo, T., Otsubo, Y., Urano, J., Tamanoi, F., and Yamamoto, M. (2007). Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast. *Mol Cell Biol* 8:3154–3164.
13. Weisman, R., Roitburg, I., Schonbrun, M., Harari, R., and Kupiec, M. (2007). Opposite effects of tor1 and tor2 on nitrogen starvation responses in fission yeast. *Genetics* 175:1153–1162.
14. Alvarez, B., and Moreno, S. (2006). Fission yeast Tor2 promotes cell growth and represses cell differentiation. *J Cell Sci* 119:4475–4485.
15. Uritani, M., Hidaka, H., Hotta, Y., Ueno, M., Ushimaru, T., and Toda, T. (2006). Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. *Genes Cells* 11:1367–1379.
16. Su, S.S., Tanaka, Y., Samejima, I., Tanaka, K., and Yanagida, M. (1996). A nitrogen starvation-induced dormant G0 state in fission yeast: the establishment from uncommitted G1 state and its delay for return to proliferation. *J Cell Sci* 109(Pt 6):1347–1357.

17. Yanagida, M. (2009). Cellular quiescence: are controlling genes conserved? *Trends in Cell Biol* 12:705–715.
18. Aspuria, P.J., Sato, T., and Tamanoi, F. (2007). The TSC/Rheb/TOR signaling pathway in fission yeast and mammalian cells: temperature sensitive and constitutive active mutants of TOR. *Cell Cycle* 6:1692–1695.
19. Matsumoto, S., Bandyopadhyay, A., Kwiatkowski, D.J., Maitra, U., and Matsumoto, T. (2002). Role of the Tsc1-Tsc2 complex in signaling and transport across the cell membrane in the fission yeast *Schizosaccharomyces pombe*. *Genetics* 161:1053–1063.
20. Van Slegtenhorst, M., Carr, E., Stoyanova, R., Kruger, W., and Henske, E.P. (2004). $Tsc1^{+}$ and $tsc2^{+}$ regulate arginine uptake and metabolism in *Schizosaccharomyces pombe*. *J Biol Chem* 279:12706–12713.
21. Nakase, Y., Fukuda, K., Chikashige, Y., Tsutsumi, C., Morita, D., Kawamoto, S., Ohnuki, M., Hiraoka, Y., and Matsumoto, T. (2006). A defect in protein farnesylation suppresses a loss of *Schizosaccharomyces pombe* $tsc2^{+}$, a homolog of the human gene predisposing tuberous sclerosis complex (TSC). *Genetics* 173:569–578.
22. Urano, J., Comiso, M.J., Guo, L., Aspuria, P.J., Deniskin, R., Tabancay, A.P., Jr., Kato-Stankiewicz, J., and Tamanoi, F. (2005). Identification of novel single amino acid changes that result in hyperactivation of the unique GTPase, Rheb, in fission yeast. *Mol Microbiol* 58:1074–1086.
23. van Slegtenhorst, M., Mustafa, A., and Henske, E.P. (2005). 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* 14:2851–2858.
24. Mach, K.E., Furge, K.A., and Albright, C.F. (2000). Loss of Rhb1, a Rheb-related GTPase in fission yeast, causes growth arrest with a terminal phenotype similar to that caused by nitrogen starvation. *Genetics* 155:611–622.
25. Yang, W., Tabancay, A.P., Jr., Urano, J., and Tamanoi, F. (2001). Failure to farnesylate Rheb protein contributes to the enrichment of G0/G1 phase cells in the *Schizosaccharomyces pombe* farnesyltransferase mutant. *Mol Microbiol* 41:1339–1347.
26. Urano, J., Sato, T., Matsuo, T., Otsubo, Y., Yamamoto, M., and Tamanoi, F. (2007). 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* 104:3514–3519.
27. Weisman, R., Roitburg, I., Nahari, T., and Kupiec, M. (2005). Regulation of leucine uptake by $tor1^{+}$ in *Schizosaccharomyces pombe* is sensitive to rapamycin. *Genetics* 169:539–550.
28. Aspuria, P.J., and Tamanoi, F. (2008). The Tsc/Rheb signaling pathway controls basic amino acid uptake via the Cat1 permease in fission yeast. *Mol Genet Genomics* 279:441–450.
29. Murai, T., Nakase, Y., Fukuda, K., Chikashige, Y., Tsutsumi, C., Hiraoka, Y., and Matsumoto, T. (2009). Distinctive responses to nitrogen starvation in the dominant active mutants of the fission yeast Rheb GTPase. *Genetics* 183:517–527.
30. Hartman, T.R., Nicolas, E., Klein-Szanto, A., Al-Saleem, T., Cash, T.P., Simon, M.C., and Henske, E.P. (2009). The role of the Birt-Hogg-Dube protein in mTOR activation and renal tumorigenesis. *Oncogene* 28:1594–1604.
31. Karagiannis, J., Saleki, R., and Young, P.G. (1999). The pub1 E3 ubiquitin ligase negatively regulates leucine uptake in response to NH(4)(+) in fission yeast. *Curr Genet* 35:593–601.
32. van Slegtenhorst, M., Khabibullin, D., Hartman, T.R., Nicolas, E., Kruger, W.D., and Henske, E.P. (2007). The Birt-Hogg-Dube and tuberous sclerosis complex homologs have opposing roles in amino acid homeostasis in *Schizosaccharomyces pombe*. *J Biol Chem* 282:24583–24590.

33. Sarbassov dos, D., Ali, S.M., Sengupta, S., Sheen, J.H., Hsu, P.P., Bagley, A.F., Markhard, A.L., and Sabatini, D.M. (2006). Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22:159–168.
34. Ikeda, K., Morigasaki, S., Tatebe, H., Tamanoi, F., and Shiozaki, K. (2008). Fission yeast TOR complex 2 activates the AGC-family Gad8 kinase essential for stress resistance and cell cycle control. *Cell Cycle* 7:358–364.
35. Kawai, M., Nakashima, A., Ueno, M., Ushimaru, T., Aiba, K., Doi, H., and Uritani, M. (2001). Fission yeast tor1 functions in response to various stresses including nitrogen starvation, high osmolarity, and high temperature. *Curr Genet* 39:166–174.
36. Schonbrun, M., Laor, D., Lopez-Maury, L., Bahler, J., Kupiec, M., and Weisman, R. (2009). TOR complex 2 controls gene silencing, telomere length maintenance, and survival under DNA-damaging conditions. *Mol Cell Biol* 29:4584–4594.
37. Matsuo, T., Kubo, Y., Watanabe, Y., and Yamamoto, M. (2003). *Schizosaccharomyces pombe* AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. *EMBO J* 22:3073–3083.
38. Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M.A., Hall, A., and Hall, M.N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 6:1122–1128.
39. Sarbassov, D.D., Ali, S.M., Kim, D.H., Guertin, D.A., Latek, R.R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D.M. (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 14:1296–1302.
40. Schmidt, A., Kunz, J., and Hall, M.N. (1996). TOR2 is required for organization of the actin cytoskeleton in yeast. *Proc Natl Acad Sci USA* 93:13780–13785.
41. Hansen, K.R., Burns, G., Mata, J., Volpe, T.A., Martienssen, R.A., Bahler, J., and Thon, G. (2005). Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Mol Cell Biol* 25:590–601.
42. Monahan, B.J., Villen, J., Marguerat, S., Bahler, J., Gygi, S.P., and Winston, F. (2008). Fission yeast SWI/SNF and RSC complexes show compositional and functional differences from budding yeast. *Nat Struct Mol Biol* 15:873–880.
43. Nicolas, E., Yamada, T., Cam, H.P., Fitzgerald, P.C., Kobayashi, R., and Grewal, S.I. (2007). Distinct roles of HDAC complexes in promoter silencing, antisense suppression and DNA damage protection. *Nat Struct Mol Biol* 14:372–380.
44. Carr, A.M. (2002). DNA structure dependent checkpoints as regulators of DNA repair. *DNA Repair (Amst)* 1:983–994.
45. Rhind, N., and Russell, P. (1998). Tyrosine phosphorylation of cdc2 is required for the replication checkpoint in *Schizosaccharomyces pombe*. *Mol Cell Biol* 18:3782–3787.
46. Kunoh, T., Habu, T., and Matsumoto, T. (2008). Involvement of fission yeast Clr6-HDAC in regulation of the checkpoint kinase Cds1. *Nucleic Acids Res* 36:3311–3319.
47. Hartmuth, S., and Petersen, J. (2009). Fission yeast Tor1 functions as part of TORC1 to control mitotic entry through the stress MAPK pathway following nutrient stress. *J Cell Sci* 122:1737–1746.
48. Petersen, J., and Nurse, P. (2007). TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. *Nat Cell Biol* 9:1263–1272.
49. Petersen, J. (2009). TOR signalling regulates mitotic commitment through stress-activated MAPK and Polo kinase in response to nutrient stress. *Biochem Soc Trans* 37:273–277.
50. Nakashima, A., Maruki, Y., Imamura, Y., Kondo, C., Kawamata, T., Kawanishi, I., Takata, H., Matsuura, A., Lee, K.S., Kikkawa, U., Ohsumi, Y., Yonezawa, K., *et al.* (2008). The yeast Tor signaling pathway is involved in G2/M transition via polo-kinase. *PLoS ONE* 3:e2223.

51. Smith, E.M., and Proud, C.G. (2008). cdc2-cyclin B regulates eEF2 kinase activity in a cell cycle- and amino acid-dependent manner. *EMBO J* 27:1005–1016.
52. Wu, M.Y., Cully, M., Andersen, D., and Leevers, S.J. (2007). Insulin delays the progression of *Drosophila* cells through G2/M by activating the dTOR/dRaptor complex. *EMBO J* 26:371–379.
53. Weisman, R., Finkelstein, S., and Choder, M. (2001). Rapamycin blocks sexual development in fission yeast through inhibition of the cellular function of an FKBP12 homolog. *J Biol Chem* 276:24736–24742.
54. Weisman, R. (2004). The fission yeast TOR proteins and the rapamycin response: an unexpected tale. *Curr Top Microbiol Immunol* 279:85–95.
55. Fingar, D.C., Salama, S., Tsou, C., Harlow, E., and Blenis, J. (2002). Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* 16:1472–1487.
56. Rohde, J.R., and Cardenas, M.E. (2003). The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. *Mol Cell Biol* 23:629–635.
57. Tsang, C.K., Bertram, P.G., Ai, W., Drenan, R., and Zheng, X.F. (2003). Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. *EMBO J* 22:6045–6056.
58. Reiling, J.H., and Sabatini, D.M. (2006). Stress and mTORture signaling. *Oncogene* 25:6373–6383.
59. Shen, C., Lancaster, C.S., Shi, B., Guo, H., Thimmaiah, P., and Bjornsti, M.A. (2007). TOR signaling is a determinant of cell survival in response to DNA damage. *Mol Cell Biol* 27:7007–7017.
60. Lee, C.H., Inoki, K., Karbowniczek, M., Petroulakis, E., Sonenberg, N., Henske, E.P., and Guan, K.L. (2007). Constitutive mTOR activation in TSC mutants sensitizes cells to energy starvation and genomic damage via p53. *EMBO J* 26:4812–4823.
61. Budanov, A.V., and Karin, M. (2008). P53 target genes sestin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* 134:451–460.
62. Bandhakavi, S., Kim, Y.M., Ro, S.H., Xie, H., Onsongo, G., Jun, C.B., Kim, D.H., and Griffin, T.J. (2010). Quantitative nuclear proteomics identifies mTOR regulation of DNA damage response. *Mol Cell Proteomics* 9:403–414.