How *Saccharomyces* Responds to Nutrients

Shadia Zaman, Soyeon Im Lippman,
Xin Zhao, and James R. Broach

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544;
email: jbroach@princeton.edu

Key Words

glucose regulation, nitrogen regulation, amino acid regulation,
protein kinase A, TOR, Sch9, Snf1, ribosome biogenesis, cell size
control, stress response, filamentous growth, stationary phase, meiosis

Abstract

Yeast cells sense the amount and quality of external nutrients through
multiple interconnected signaling networks, which allow them to ad-
just their metabolism, transcriptional profile and developmental pro-
gram to adapt readily and appropriately to changing nutritional states.
We present our current understanding of the nutritional sensing net-
works yeast cells rely on for perceiving the nutritional landscape, with
particular emphasis on those sensitive to carbon and nitrogen sources.
We describe the means by which these networks inform the cell's deci-
sion among the different developmental programs available to them—
growth, quiescence, filamentous development, or meiosis/sporulation.
We conclude that the highly interconnected signaling networks pro-
vide the cell with a highly nuanced view of the environment and that
the cell can interpret that information through a sophisticated calculus
to achieve optimum responses to any nutritional condition.

INTRODUCTION

Saccharomyces, like other unicellular microorganisms, has evolved to make optimum use of accessible nutrients and to adapt to nutritional deficiencies in a manner that maximizes survival. These behaviors require that yeast cells have the ability to assess the amount and nature of available nutrients and to modify their transcriptional, metabolic, and developmental programs in response to that assessment. This review focuses predominantly on how yeast cells assess the availability of various nutrients—primarily carbon and nitrogen sources—and how cells respond to that assessment. Particularly, we emphasize the means by which yeast cells coordinate their transcriptional and developmental program to optimize their growth and survival not only to the quantity and quality of the carbon and nitrogen sources but also in the amounts of each relative to the other. Previous reviews on carbon catabolite repression (123, 238, 249), nitrogen regulation (52, 53, 166) and general amino acid control (105) in *Saccharomyces* and nutrient sensing in fungi in general (10) provide a valuable backdrop to this article.

NUTRIENT SENSING

Glucose Signaling

Yeast cells obtain energy through fermentation of sugars—glucose, fructose, sucrose, galactose, melibiose, maltose, etc., or through oxidation of a variety of fermentation products, such as glycerol, ethanol, and lactate (122). Yeast cells ferment glucose or fructose in preference to other mono-, di-, and trisaccharides, even for those di- or trisaccharides, such as sucrose, raffinose, and trehalose, that can be converted directly into glucose or fructose. Moreover, yeast cells prefer any fermentable carbon source to any carbon source that has to be metabolized by oxidation. This hierarchical arrangement is established by allosteric regulation of various key enzymes in such metabolic processes as glycolysis and gluconeogenesis and by an extensive transcriptional regulatory network. Moreover,

since yeast readily ferment glucose, both to extract energy rapidly and to generate precursors for all anabolic processes, the availability of glucose presents an opportunity for increased mass accumulation, which is realized by increased ribosome production.

Reflecting these opportunities and preferences, addition of glucose to cells growing on a nonfermentable carbon source results in a rapid change in the pattern of protein phosphorylation as well as a massive restructuring of the transcriptional state of the genome. More than 40% of the genes in yeast alter their expression by more than twofold within minutes following addition of glucose to cells growing on glycerol (281). These transcriptional changes involve increased expression of genes involved in ribosome biogenesis and repression of genes required for oxidative phosphorylation and other mitochondrial functions and of genes required for initial metabolism of sugars other than glucose and fructose. The inverse changes in gene expression and metabolism occur upon depletion of glucose growing in batch culture (55). Cells growing in the presence of glucose metabolize it solely by fermentation to ethanol and then, at the diauxic shift, convert their metabolic process to consume the ethanol by oxidative phosphorylation. These transcriptional and metabolic changes attendant on the transition to or from glucose are mediated by several complex regulatory networks that organize interconnected and overlapping processes. The nature of these individual networks and their relative contributions to the overall glucose regulatory process are discussed below.

The Ras/Protein Kinase A Network

Components of the network. Protein kinase A (PKA) plays critical roles in growth, in response of cells to glucose and in coupling cell cycle progression to mass accumulation. As in all eukaryotes, yeast protein kinase A comprises a heterotetramer composed of two catalytic subunits and two regulatory subunits. Three closely related genes, *TPK1*, *TPK2*, and *TPK3*, redundantly encode the catalytic subunits, with
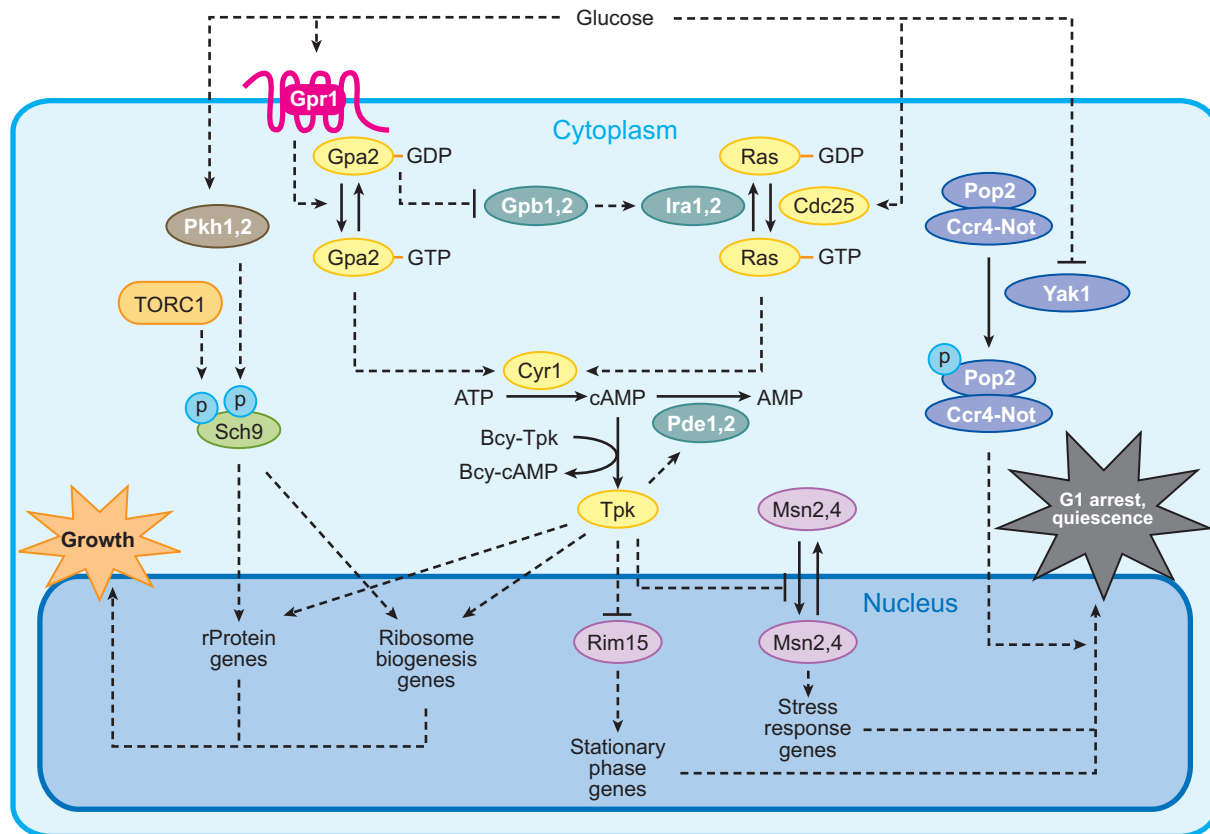
Figure 1

G-protein based glucose signaling. Glucose signaling mediated by the small G-proteins Ras and Gpa2 funnels through protein kinase A (PKA) to induce ribosome biogenesis and suppress the general stress response controlled by Msn2/Msn4 and Rim15. The kinases Sch9 and Yak1 function in parallel to PKA, the former reinforcing the PKA response and the latter antagonizing it. Dashed lines represent regulatory interactions, which may not be direct and in some cases are only surmised. See text for details.

each gene product capable of phosphorylating distinct but somewhat overlapping sets of target proteins (217). Some of the well-characterized substrates for these kinase subunits include proteins involved in metabolism of the storage carbohydrates, enzymes in glycolysis and gluconeogenesis, and transcription factors regulating stress response, ribosomal biogenesis, and carbohydrate metabolism (28, 217). Thus, PKA serves as a central regulator of the metabolic and transcriptional status of the yeast cell (**Figure 1**).

The PKA regulatory subunit is encoded by *BCY1*. This subunit acts as a pseudo substrate for the catalytic subunits to bind and restrict their activity. Cyclic AMP (cAMP) binding to

Bcy1 alleviates this inhibitory activity, releasing the catalytic subunits to perform their essential functions in the cell. *bcy1* mutants exhibit a number of phenotypes resulting from the unfettered activity of the catalytic subunit, including heat shock sensitivity, sensitivity to nutritional deprivation, and failure to arrest in G1 in response to starvation (265). These phenotypes derive in part from an inability to mount a stress response but also from a lack of stored nutrients needed to weather adverse conditions or to complete a cell cycle upon starvation (169).

A balance between cAMP synthesis catalyzed by adenylyl cyclase, encoded by *CYR1*, and cAMP degradation catalyzed by phosphodiesterases, encoded by *PDE1* and *PDE2*,

establishes the level of cAMP in the cell (**Figure 1**). The small GTP-binding proteins, Ras1 and Ras2, stimulate adenylyl cyclase through direct interaction with the enzyme. Ras1 and Ras2 cycle between a GTP-bound and a GDP-bound state and can stimulate adenylyl cyclase only in the GTP-bound state. The level of Ras-GTP in the cell results from a balance between GTP loading and GTP hydrolysis, the former catalyzed by the guanine nucleotide exchange factor, Cdc25, and the latter catalyzed by the intrinsic GTPase activity of Ras, which can be dramatically stimulated by redundant GTPase activating proteins (GAPs), Ira1 and Ira2. Glucose addition to starved cells results in a rapid but transient increase in intracellular cAMP through a process dependent on Ras. The rapid increase in cAMP production matches the increase in glucose-stimulated Ras-GTP levels in the cell and the subsequent decrease in cAMP levels likely results from feedback inhibition of synthesis and perhaps stimulation of PDE activity. Despite the clear effect of glucose on Ras activation and subsequent cAMP production and PKA activation, the means by which glucose increases Ras-GTP levels in the cell remains elusive, although not from lack of effort. Nonetheless, we know that Ras is required to maintain an essential basal level of cAMP and PKA activity in the cell and that addition of glucose stimulates Ras to produce a transient burst and subsequent long-term increase in PKA activity.

Transcriptional effects of the Ras/PKA pathway. The Ras/PKA network plays the primary role in the cell's response to glucose. Recent studies have shown that induction of an activated allele of *RAS2* (*RAS2*^V19) in yeast cells growing in glycerol medium leads to the identical qualitative and quantitative changes in expression of 90% of all the genes whose expressions are altered by addition of glucose to wild-type cells (281). Moreover, all the Ras-induced changes in gene expression depend entirely on PKA, indicating that Ras affects transcription in response to glucose solely through modulation of PKA. Ras activation results in in-

duction of genes that are involved in ribosomal protein synthesis, ribosome biogenesis, and glycolysis and repression of genes that are involved in stress response, in gluconeogenesis, and in metabolism of storage carbohydrates. Significantly, this study showed that a large fraction of the genes that are induced by glucose in a Ras2-dependent manner are involved in ribosome production. Thus, glucose stimulation of expression of genes involved in mass accumulation is mediated through Ras. This is discussed in more detail in subsequent sections.

Yak1

Yak1 is a protein kinase that works in parallel to the Ras/PKA pathway but inhibits rather than stimulates cell growth (**Figure 1**). *YAK1* was identified as a gene whose deletion suppressed complete loss of function of the Ras/PKA pathway (86). Yak1 can be phosphorylated in vitro by bovine PKA (87). However, Yak1 may not be an in vivo substrate of PKA because Yak1 is still phosphorylated in vivo in a *tpk1Δ tpk2Δ tpk3Δ* strain. Moreover, in vitro incubation with bovine PKA does not inhibit Yak1 kinase activity. Rather, glucose likely regulates Yak1 localization rather than its activity and does so through a PKA independent route (184). Several targets of Yak1 have been identified. Bcy1 is phosphorylated and restricted to the cytoplasm in a Yak1-dependent manner upon glucose starvation (95, 287), although the phenotypic consequence of this is unclear. Yak1 also directly phosphorylates Pop2 (aka Caf1), a member of the Ccr4-Caf1-Not deadenylation complex that likely controls the stability and/or translation of a wide variety of mRNAs involved in stress response and use of alternate carbon sources (184). Pop2 is necessary for glucose derepression, growth at high temperature, sporulation, and establishment of reserve carbohydrates (40). Preventing Yak1-induced phosphorylation of Pop2 results in failure to arrest in G1 at the end of postdiauxic growth prior to entering stationary phase. Msi1, isolated as a multicopy suppressor of *ira1Δ* and identified as a member of the CAK chromatin deposition

complex, also appears to function downstream of, or at least in parallel with, Yak1. Msi1 overexpression suppresses the heat shock sensitivity of *yak1*Δ (214), and *msi1*Δ suppresses the growth inhibitory effects of Yak1 overexpression. Yak1 also regulates the localization of Msi1. Msi1 accumulates in the nucleus during growth on a nonfermentable carbon source such as glycerol, and this accumulation increases upon deletion of *YAK1*.

In sum, Yak1 appears to function in parallel to PKA in response to glucose but with the opposite effect: PKA suppresses the stress response and stimulates growth whereas Yak1 stimulates the stress response and inhibits growth. Yak1 may accomplish these functions by inhibiting PKA—via Bcy1 relocalization, for instance—by stabilizing or promoting translation of growth-inhibitory, stationary-phase-inducing mRNAs, by regulating the ill-defined activity of Msi1, or by some combination of these. Finally, the means by which glucose alters Yak1 localization is not clear but appears to be independent of the PKA pathway, suggesting that a separate glucose-responsive pathway impinges on Yak1 function. Thus, Yak represents an additional, but poorly defined, channel in the glucose regulatory network.

The Gpr1/Gpa2 Circuit

Gpr1 and Gpa2 define a nutrient-sensing pathway that works in parallel with Ras to activate PKA (**Figure 1**). Gpa2 is a small GTP-binding protein that is homologous to the mammalian Gα subunit of the heterotrimeric G proteins (191). *GPR1* encodes a seven-transmembrane receptor that is homologous to G protein–coupled receptors and that physically interacts with Gpa2 (138, 293). By homology to other GPCR signaling systems, these observations suggest that Gpa2 functions as the Gα subunit coupling ligand activation of Gpr1 to an internal cellular response. *ras2*Δ is synthetically lethal with *gpr1*Δ as well as *gpa2*Δ, a phenotype that is suppressed by deletion of *PDE2* (140, 293). Moreover, diploids homozygous for *gpr1*Δ or for *gpa2*Δ are defective in initiating

pseudohyphal growth (140, 261). This defect in *gpr1*Δ/*gpr1*Δ and *gpa2*Δ/*gpa2*Δ strains can be suppressed by feeding exogenous cAMP. These phenotypes are consistent with the model that Gpr1 and Gpa2 activate PKA, perhaps through activation of adenylate cyclase (191, 229). Consistent with a role for Gpa2 in adenylyl cyclase activation, Cyr1 binds to bacterially purified Gpa2 loaded with GTPγS but not with GDP (208). Moreover, recent microarray data showed that induction of *GPA2*[Q300L] resulted in expression changes in the same sets of genes as did induction of an activated allele of *RAS2*. All of the transcriptional changes attendant on induction of the activated *GPA2* allele depended completely on PKA (281). However, this study also showed that the magnitude of Gpa2-dependent gene expression changes was much weaker than that seen using an activated allele of *RAS2*. These observations provide strong evidence that Ras2 plays the major role in mediating glucose-induced gene expression changes while Gpa2 plays a more auxiliary role in the glucose response, and both do so solely through modulation of PKA.

To date, we do not know how Gpr1 senses nutrients. No direct binding of glucose or any other ligand to Gpr1 has been demonstrated. Rather, indirect evidence implicates various sugars as Gpr1 ligands, with sucrose apparently binding more tightly than glucose (155). At face value, this would suggest that the physiological ligand for Gpr1 could be sucrose, which is a less favored nutrient for yeast than glucose. This observation is at odds with the apparent role of Gpr1 in mediating a glucose response. Moreover, deletion of *GPR1* from wild-type strains does not affect in any measurable way the changes in transcription following glucose addition, whereas induction of a dominant negative *RAS2* allele essentially eliminates almost all of the glucose response (281). Thus, whatever role the Gpr1/Gpa2 network might play in glucose signaling, it mediates only a negligible part of the rapid glucose-induced transcriptional restructuring.

Like mammalian heterotrimeric G proteins, yeast Gpa2 functions in conjunction with β and

γ subunits. Although a recent report suggests that *ASC1* encodes the β subunit (300a), substantial evidence posits Gpb1/Krh2 and Gpb2/Krh1 in this role, with Gpg1 serving as γ subunit (13, 102). Unlike mammalian Gβγ subunits, Gpb1 and Gpb2 do not have WD40 repeats but instead contain seven kelch repeat domains that, like WD40 repeats, fold into a β propeller structure and mediate protein-protein interactions. *gpb1Δ gpb2Δ* cells exhibit a hyperactive PKA phenotype: heat shock sensitivity in stationary phase and reduced sporulation efficiency. In addition, such cells have elevated *FLO11* expression and show increased haploid invasive and diploid pseudo-hyphal growth (see below). These observations suggest that Gpb1 and Gpb2 are negative regulators of PKA. *gpg1Δ* mutants display phenotypes opposite to those of *gpb1Δ gpb2Δ* cells, a unusual result since Gβ and Gγ subunits normally function as a complex. This result suggests that Gpg1 functions independently as an activator of the PKA network. Harashima et al. (101) recently showed that Gpb1/Gpb2 bind to and stabilize Ira1 and Ira2, the Ras-GAP proteins. Thus, the Gpa2/Gpb/Gpg heterotrimer appears to be able to modulate the Ras/PKA network in at least two opposing modes—direct activation of adenylyl cyclase by Gpa2 and inhibition of Ras activity by increasing Ras-GAP levels. These two opposing modes function on different time scales, consistent with an initial Gpa2-dependent stimulation followed by longer-term Gpb-dependent attenuation/habituation. In sum, most of the mutants defective in components of the Gpr1/Gpa2 network affect long-term developmental programs in response to glucose but not short-term response patterns. Thus, this system may serve as an overlay on the Ras/PKA network designed to effect long-term responses to glucose availability.

The Sch9 Pathway

Sch9 is an AGC family kinase and the closest yeast homolog to the mammalian prosurvival Akt/PKB as well as to the TOR-regulated S6 kinase. *SCH9* was initially identified as a multicopy suppressor of *cdc25-1*, and at high copy it can also suppress lethality caused by *cyr1Δ*, *ras1Δ ras2Δ*, and *tpk1Δ tpk2Δ tpk3Δ* mutations (264). Selective inhibition of an ATP analog-sensitive allele of *SCH9* (*sch9^{as}*) in cells growing in glucose media inhibits expression of genes involved in ribosome biogenesis, whereas overexpression of *SCH9* from a *GAL1* promoter leads to induction of essentially the same set of genes as does expression of *RAS2*^{V19} from the *GAL10* promoter (125). In particular, *SCH9* overexpression induces ribosome biogenesis genes and represses genes involved in carboxylic acid metabolism. Thus, the ability of Sch9 to suppress mutations in the Ras/PKA pathway likely results from the fact that Sch9 regulates a similar set of functions as does Ras/PKA.

Recent biochemical studies of Sch9 place it in both nutrient- and stress-sensing networks. The nutrient-responsive TORC1 kinase directly phosphorylates Sch9 at multiple sites at its C-terminal domain, and phosphorylation of these sites is required for Sch9 kinase activity (273). Moreover, strains with Sch9 carrying phosphomimetic substitutions at these sites exhibit significant resistance to repression of the ribosome biogenesis genes in response to rapamycin treatment (273). Glucose also affects Sch9 function, both by increasing the level of Sch9 in the cell and by inducing phosphorylation of Sch9, although the kinase(s) and phosphatase(s) responsible for the phosphorylation levels of these glucose-dependent sites is not known (125, 273). Accordingly, the means by which glucose availability is coupled to Sch9 phosphorylation is unknown. While inactivation of Sch9 in cells growing in rich medium diminishes expression of ribosome biogenesis genes, inactivation of Sch9 does not measurably diminish the massive transcriptional changes that occur following glucose addition to glycerol grown cells. In sum, Sch9 acts in parallel to the Ras/PKA pathway but seems to serve as a minor conduit for glucose-mediated changes in transcription. However, Sch9 does seem to play a significant role in connecting TOR-dependent nutrient sensing to ribosome

biogenesis. Moreover, as noted below, Sch9 plays a major role in coupling cell size to cell division.

The Snf1 Network

The Snf1 network is essential for growth on less preferred fermentable carbon sources such as sucrose, galactose, and maltose and for growth on nonfermentable carbon sources such as glycerol and ethanol. Besides its requirement for growth in the absence of glucose, the Snf1 complex affects a number of cellular processes, in-

cluding aging, meiosis, glycogen accumulation, growth on inositol, and pseudohyphal growth (9, 109, 144, 253) (**Figure 2**).

The yeast Snf1 protein kinase complex is homologous to mammalian AMP-activated protein kinase (AMPK). Like its mammalian counterpart, the yeast Snf1 protein kinase complex is a heterotrimer composed of the Snf1 catalytic subunit, the γ-like Snf4 regulatory subunit, and one of three β-subunit, encoded by *GAL83*, *SIP1*, and *SIP2*. Snf4 binds to Snf1 and, during growth in the absence of glucose, alleviates the autoinhibition of the Snf1 catalytic
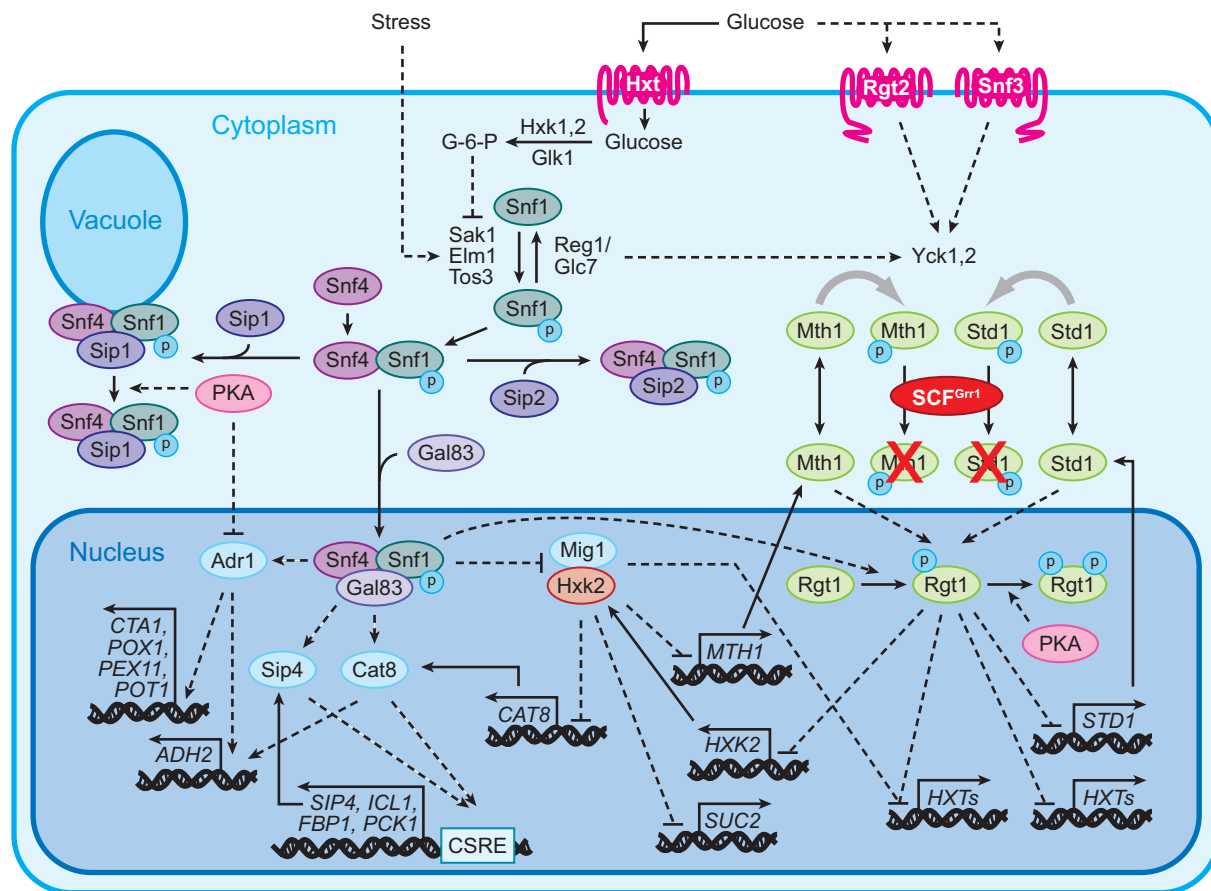

Figure 2

The interlocking Snf and Rgt glucose signaling networks. Glucose regulates genes involved in carboxylic acid metabolism and fatty acid β-oxidation by inhibiting the Snf1 kinase and regulates the hexose transporter and hexokinase genes by inactivating the Rgt1 co-repressors Mth1 and Std1. These two networks are interconnected at various points, including Snf1 activation of the Rgt1 repressor and indirect induction of the Mth1 corepressors and, inversely, Rgt1 represses hexokinase 2, which serves as a corepressor of the Snf1-regulated Mig1 repressor. These pathways are also influenced by glucose-regulated PKA at the points indicated.

domain by the C-terminal regulatory domain. Deletion of either *SNF1* or *SNF4* results in inability to grow on carbon sources other than glucose, referred to as a sucrose nonfermenting (Snf⁻) phenotype. Only deletion of all three β-subunits yields a Snf⁻ phenotype, suggesting that the β-subunits are redundant for growth on alternative carbon sources.

Regulation of Snf1 activity. The Snf1 complex is activated by growth in the absence of glucose. In mammalian cells, AMPK maintains cellular energy homeostasis when the energy level is low both by stimulating glucose uptake and lipid oxidation and by inhibiting ATP-consuming processes (163). The complex is activated by increases in the cellular AMP:ATP ratio by direct allosteric activation of the γ-subunit by AMP (267). The homology between the yeast Snf1 complex and the mammalian AMPK complex suggests that, like the mammalian system, yeast Snf1 would also be activated by AMP. However, studies to date have failed to reveal any activation of Snf1 by AMP (179, 288, 289). Rather, glucose depletion activates the Snf1 complex by promoting phosphorylation of T210 in the activation loop of Snf1 by any one of three Snf1 kinases, encoded by *SAK1* (for Snf1 Activating Kinase, previously Pak1), *ELM1*, and *TOS3* (108, 193) (**Figure 2**). These three kinases are redundant in their Snf1-activating capacity. Even though *elm1* mutants have distinct phenotypes in cell cycle progression, septin localization, and cytokinesis and *SAK1* deletion mutants have defects in glycogen accumulation, all three upstream kinases must be deleted for cells to display a Snf⁻ phenotype (103, 108, 176, 256, 257). However, Sak1 plays the primary role in mediating Snf1 functions. Deleting *SAK1* leads to the greatest decrease in Snf1 kinase activity and Sak1 plays the major role in activation of Snf1-Gal83. In cells lacking Sak1 activity, Gal83 complexes remain in the cytoplasm upon depletion of glucose (103). Furthermore, *sak1* or *gal83* mutants exhibit diminished accumulation of glycogen to the same extent as do *snf1* mutants. Tos3 and Elm1 have more variable ability to acti-

vate the Snf1 complex depending upon the carbon stress (176). Deletion of *ELM1* reduces the catalytic activity of the Snf1 complex but not as effectively as *SAK1* deletion. On the other hand, deletion of *TOS3* has very little effect on Snf1 catalytic activity. Tos3 has a more active role during long-term growth in glycerol-ethanol media (131). Deletion of *TOS3* reduces the growth rate, Snf1 catalytic activity, and expression from a carbon source response element (CSRE) reporter only during long-term growth on glycerol-ethanol. Tos3 is also phosphorylated in a Snf1-dependent manner.

The type 1 protein phosphatase, comprising the Glc7 catalytic subunit and the Reg1 regulatory subunit, counteracts Snf1/4's activation by the upstream kinases. Reg1 interacts with the kinase domain of activated Snf1 and directs Glc7 to the kinase activation loop of Snf1, resulting in the dephosphorylation and inactivation of Snf1 (269). Active Snf1 phosphorylates and inhibits Reg1-Glc7, providing a positive feedback loop during transition to an active form. Reg1 expression, localization, and interaction with Glc7 do not appear to be regulated by carbon source, but the activity of the Reg1-Glc7 complex might be regulated by posttranslational phosphorylation of Reg1, since Reg1 is phosphorylated in a carbon source-dependent manner. The fact that isolated kinase domain of Snf1 can confer glucose regulation of gene expression in a *reg1Δ* mutant suggests that glucose regulates Snf1 through a pathway independent of Reg1 (165). However, glucose can regulate Snf1 activity even in a *sak1Δ elm1Δ tos3Δ* carrying either of the mammalian AMPK kinases, LKB1 or CaMKKα, suggesting that glucose impinges on Snf1 through a process independent of its upstream kinases (108a). Thus, Glc7/Reg1 phosphatase may provide a second route for glucose regulation of Snf1.

Glucose also regulates the Snf1 complex in part through a β-subunit–dependent redistribution of its subcellular location. All the Snf1 complexes, regardless of β-subunit, reside in the cytoplasm during growth in glucose media. Upon shift to media lacking glucose, Gal83-containing complexes relocalize to the nucleus,

Sip1-containing complexes relocalize to the vacuole, and Sip2-containing complexes remain in the cytoplasm. This difference in localization suggests that the subunits are not entirely overlapping in function (277). In the absence of Gal83, Snf1 distributes relatively uniformly between nucleus and cytoplasm in a pattern that is essentially unaffected by carbon source (277). This suggests that the glucose regulates localization of Gal83-containing complexes at least in part by promoting nuclear export. Moreover, glucose has to be phosphorylated in order to stimulate nuclear export, because glucose addition to *hxk1Δ hxk2Δ glk1Δ* cells fails to induce relocalization of Gal83-containing complexes from the cytoplasm to the nucleus. However, metabolism of glucose is not required: Addition of 2-deoxyglucose, a glucose analog that is phosphorylated but not metabolized, suffices to localize Gal83-containing complexes to the cytoplasm, whereas 6-deoxyglucose, which cannot be phosphorylated, cannot induce cytoplasmic localization of Gal83-containing complexes. Thus, glucose restricts nuclear localization of the Snf1 kinase complex by promoting export of Gal83 from the nucleus, although the mechanism connecting glucose to nuclear export is unknown.

Relocalization of Snf1 in complex with Sip1 to the vacuole depends on a putative myristoylation site (MGNSPS) on the N terminus of *SIP1* (104). Mutation of the conserved glycine residue in the consensus sequence prevents localization of Sip1 to the vacuole in the absence of glucose. However, unlike in the case of Gal83-containing complexes, addition of 2-deoxyglucose does not induce Sip1 complexes to relocalize to the cytoplasm, suggesting that Sip1 might be responding to a signal other than glucose phosphorylation. Rather, cytoplasmic localization of Sip1 complexes in glucose media requires the activity of PKA: Sip1 fails to localize to the cytoplasm upon glucose addition to *tpk1Δ tpk2Δ tpk3Δ* strain and, conversely, deletion of *BCY1* prevents the vacuolar localization of Sip1-containing complexes in glycerol-ethanol media. The function of Sip1 and the significance of the vacuolar localization of Sip1-Snf complexes are unknown.

In sum, glucose regulation of the Snf1 kinase complex does not derive from changes in the AMP:ATP ratios attendant on metabolism of glucose but rather is achieved through phosphorylation of the Snf1 catalytic domain, either by regulation of three redundant upstream kinases or the Reg1/Glc7 phosphatase or both. Moreover, glucose depletion redistributes the Snf1 kinase complexes from the cytoplasm to the vacuole and the nucleus, where it can effect subsequent metabolic and transcriptional alterations. While glucose modulates phosphorylation of Snf1 to regulate its activity, the means by which glucose suppresses Snf1 activation through modulation of its kinases and/or phosphatases remains elusive, as does the means by which glucose induces redistribution of Snf1 complexes in the cell.

Downstream effects of the Snf1 complex. Snf1 regulates the expression of genes involved in use of alternate carbon sources through a variety of transcription factors (**Figure 2**). Genes required for metabolism of alternative carbon sources, such sucrose, galactose, and maltose, respond to Snf1 through the activity of the Mig1 transcription repressor. Mig1 is a C_2H_2 zinc finger protein that binds to a GC-rich consensus sequence [reviewed in (249)]. In cells grown in the absence of glucose, Snf1 phosphorylates Mig1 to inhibit Mig1's repressor activity. In the presence of glucose, Mig1 becomes dephosphorylated and localizes to the nucleus, repressing the expression of target genes such as *SUC2*. Recent evidence suggests that Mig1 acts as a repressor in association with Hxk2, one of the two yeast hexokinases (3, 4). Gel retardation analysis shows that Hxk2 forms a complex with a *SUC2* DNA-Mig1 complex, suggesting that Hxk2 interacts with Mig1 as part of the repressor complex on the *SUC2* promoter. Moreover, Hxk2 interacts specifically through the S311 residue of Mig1, mutation to a nonphosphorylatable form of which results in constitutive localization of Mig1 to the nucleus and constitutive inhibition of *SUC2* expression.

The Adr1 transcription factor activates expression of genes involved in ethanol metabolism and β-oxidation of fatty acids. Microarray studies comparing the expression of wild-type cells to *adr1* cells showed that deletion of *ADR1* reduced the expression of ca. 100 genes in low glucose media (299). This study showed that, besides regulating nonfermentable carbon metabolism and fatty acid oxidation, Adr1 affected expression of genes in other functions, such as amino acid transport and metabolism, meiosis, and sporulation. However, since only 30 genes are bound by Adr1 in cells grown in glucose-free media, altered regulation of most genes in an *adr1* mutant may well be the consequence of secondary regulatory or metabolic effects (260).

Adr1 is negatively regulated by PKA in glucose growing cells and activated in a Snf1-dependent manner in cells growing in the absence of glucose [reviewed in (249)]. The exact mechanism of Adr1 suppression by PKA or activation by Snf1 is not well understood. Adr1 is also under negative regulation of Reg1, as deletion of *REG1* increases the protein level of Adr1 and leads to induction of Adr1-regulated genes, such as *ADH2* (63). Moreover, the yeast 14-3-3 proteins, Bmh1 and Bmh2, likely act in a pathway parallel to Reg1 to inhibit expression of Adr1-regulated genes: Expression of *ADH2* under repressed conditions is increased in a *bmh1 bmh2* strain and even further increased in a *reg1 bmh1 bmh2* strain. Thus, Adr1 is sensitive to a number of glucose-dependent inputs.

Cat8 and Sip4, two unrelated transcription factors, activate expression of genes required for gluconeogenesis during growth in the absence of glucose by binding carbon source response elements (CSRE). Derepression of genes having CSRE motifs is completely abolished in *cat8 sip4* mutants, suggesting that these two proteins are the only two activators required for full activation [reviewed in (249)]. However, Cat8 and Sip4 do not equally contribute toward activation of genes in nonglucose media: *cat8* cells cannot grow on nonfermentable carbon source, whereas *sip4* mutants can. This discrepancy is further supported by the fact that Sip4 has the much stricter requirement for the consensus CSRE motifs while Cat8 binds to more variant types of CSRE (230). Microarray studies have identified 255 genes whose expression is reduced in the *cat8* relative to *CAT8* in low glucose media, only 48 of which are bound by Cat8 in vivo, again suggesting a large contribution of secondary events in the microarray studies. Sip4 responds to glucose starvation through a Gal83-mediated interaction with and phosphorylation by Snf1 (276). *CAT8* transcription is inhibited by Mig1 and activated by Hap2/3/4/5.

Snf1 protein kinase complex regulates certain stress response genes during carbon source downshift. Hsf1, a transcription factor that regulates expression of heat-inducible genes by binding to HSE (heat shock element), is phosphorylated in response to carbon stress, dependent in part on Snf1, in a pattern distinct from heat-induced phosphorylation (98, 240). Furthermore, Hsf1 binding to target promoters in vivo, such as *HSP82*, *CUP1*, *HSP30*, and *SSA3*, is Snf1 dependent, as is expression of these target genes in response to carbon stress. Snf1 also negatively regulates Msn2 in response to carbon stress. Msn2 is dephosphorylated by Reg1-Glc7 immediately following glucose depletion and localizes to the nucleus to induce expression of target genes such as *CTT1* (54). However, long-term carbon stress induces rephosphorylation of Msn2 in a Snf1-dependent manner, leading to relocalization of Msn2 to the cytoplasm and inhibition of *CTT1* expression. This suggests that Snf1 is involved in long-term adaptation to carbon stress by negatively regulating Msn2 transcriptional activity.

Snf1 also affects target genes by stimulating chromatin remodeling. Glucose depletion yields Snf1-dependent phosphorylation of S10 on histone H3 at the *INO1* promoter (161, 162). This phosphorylation leads to recruitment of the SAGA complex, resulting in acetylation of histone H3 K14. Glucose depletion results in a similar Snf1-dependent recruitment of the SAGA complex to the *HXT2* and *HXT4* promoters under glucose limitation (275). Thus, Snf1 promotes transcriptional activation through both mobilization of

transcription factors and remodeling of chromatin structure of target promoters.

In sum, Snf1 couples the absence of glucose and other stresses to the induction of a limited number of genes required for metabolism of carbon sources other than glucose as well as activation of genes required for gluconeogenesis and fatty acid oxidation. In the absence of *SNF1* function, approximately 400 genes normally induced by glucose depletion show diminished induction, although only 10% of these are direct targets of transcription factors regulated by Snf1. Unlike mammalian cells, yeast cells regulate Snf1 activity not in response to energy charge but rather through phosphorylation of the activation loop catalyzed redundantly by several upstream kinases. It is not unreasonable to speculate that glucose depletion activates these upstream kinases to facilitate various metabolic and developmental programs, one aspect of which involves activation of the Snf1 regulon. We still do not understand how glucose alters the activity of these upstream kinases, although glucose has to be phosphorylated, albeit not metabolized, in order to affect Snf1 function. The apparent role of hexokinase as a transcriptional corepressor suggests at least one connection between glucose phosphorylation and transcriptional activation.

The Rgt Network

The Rgt1 network couples expression of the hexose transporter genes to the level of available glucose (**Figure 2**). Rgt1 represses expression of all hexose transporter genes in cells grown in the absence of glucose, a function that depends on the presence of two corepressors Mth1 and Std1. Glucose binding to two membrane-spanning sensors, Snf3 and Rgt2, induces them to bind Mth1 and Std1, thereby recruiting the corepressors to the plasma membrane, where they are phosphorylated by the casein kinases Yck1 and Yck2. Once phosphorylated, the corepressors are targeted by the SCF^{Grr1} E2/E3 ubiquitin conjugating complex for degradation by the proteasome (75, 183, 246, 255). Elimination of these corepressors by

proteolysis exposes Rgt1 to phosphorylation, likely by PKA, and alleviates its repressive activity through eviction from regulated promoters (202). This Rgt network, in conjunction with Snf1/Mig1 (200), provides a graded derepression of the different hexose transporters in response to different glucose levels, such that cells express only those transporters with the appropriate affinity for the available glucose (123).

Rgt1 is a C6 zinc cluster DNA-binding protein that represses hexose transporter genes, such as *HXT1–4*, as well as the hexokinase gene, *HXK2*, through recruitment of the general repression complex Ssn6/Tup1 to the respective promoters. This repression activity likely requires phosphorylation by Snf1, or a Snf1-dependent kinase, as well as association with corepressors, Mth1 and Std1 (151, 187, 202). Besides acting to repress gene expression, Rgt1 is required for full expression of some genes, such as *HXT1*, under high glucose conditions. However, Rgt1 is not bound to the *HXT1* promoter under these conditions, suggesting that the role of Rgt1 in activation is indirect (210). The corepressors, Mth1 and Std1, play partially redundant roles in regulation: They each bind to a common site on Rgt1 to suppress transcriptional activation and promote DNA binding by blocking hyperphosphorylation. Moreover, both are degraded in response to glucose addition (210). However, *STD1* expression is auto-regulated by the Rgt1 network, and thus induced by high glucose, whereas *MTH1* expression is repressed at high glucose by the Snf1-regulated Mig1 repressor. These observations prompt a model in which Mth1 serves primarily to maintain repression while Std1 functions predominantly in the establishment of repression during transition to the absence of glucose (130).

Besides regulating hexose transporter gene expression, the glucose sensor Rgt2 also mediates glucose-induced endocytosis and degradation of the maltose permease (117). Moreover, *yck1*Δ *yck2*^{ts} mutants also block glucose-induced internalization and vacuolar localization of the permease and decrease the level of permease phosphorylation (81). Although an

economical model would postulate a glucose-dependent Rgt2 recruitment of the maltose premease to the vicinity of membrane-restricted casein kinase, resulting in phosphorylation and subsequent internalization of the permease, the system is somewhat more complex. Glucose-induced degradation of the permease by casein kinase requires the activity of the Reg1/Glc7 protein phosphatase 1, acting upstream of the kinase. Moreover, Reg1/Glc7 is also required for efficient phosphorylation of Std1 and Mth1 by casein kinase. Reg1/Glc7 activation depends on glucose transport and initial metabolism as is required for glucose inactivation of Snf1 (see above). Thus in the updated model, casein kinase responds to glucose signals from both Rgt2 and Glc7/Reg1 to induce degradation of Mth1 and Std1 with the resultant expression of hexokinase and the hexose transporters. Since hexokinase participates in glucose metabolism necessary for activation of Glc7/Reg1, these observations highlight an intriguing interplay between the Rgt network on the one hand and the Snf1/PP1 network on the other.

In sum, the Rgt network comprises a complex set of interconnecting components to insure that the appropriate glucose transporters are produced to provide the most efficient import of available glucose, regardless of its external concentration. Moreover, recent results have highlighted a number of connections between the Rgt network and both the Snf1/PP1 and the PKA networks. These interactions likely coordinate and cross-regulate the glucose metabolic and regulatory processes in ways we are just beginning to appreciate.

NITROGEN SIGNALING

Yeast cells evaluate and respond to the nature and amount of available nitrogen-containing compounds. Although *Saccharomyces* can use a variety of amino acids or other organic amines and amides as sole nitrogen sources, the common laboratory yeast strains, those derived from S288C, prefer glutamine to other nitrogen sources, including ammonia. Strains derived from Σ1278b—commonly used for stud-

ies on nitrogen catabolite repression—use glutamine or ammonia equally well. This preference in nitrogen source is manifested either quantitatively by an enhanced growth rate in media containing the preferred source relative to that containing other nitrogen sources or qualitatively by the ability of the preferred source to induce repression of genes required for catabolism of other nitrogen sources. Accordingly, addition of glutamine to cells growing on a less preferred nitrogen source results in repression of a large collection of genes involved in nitrogen catabolism as well as induction of the cohort of ribosomal biogenesis and ribosomal protein genes described below. Moreover, the quality and amount of available nitrogen source inform metabolic processes and developmental decisions. Growth in the absence of a high-quality nitrogen source induces autophagy, stabilizes the general amino acid permease Gap1, and induces turnover of a variety of specific amino acid permeases. In addition, the presence of high-quality nitrogen sources prevents pseudohyphal or invasive growth while the presence of any nitrogen source prevents meiosis and sporulation. These regulatory processes are mediated by a number of signaling networks discussed below and have been described in detail in several recent reviews (41, 166, 248). In the following, we summarize recent results regarding this regulatory process, with particular emphasis on the intersections with other nutrient-sensing pathways.

Nitrogen Discrimination Pathway

The addition of glutamine or ammonia to cells growing on a poor nitrogen source results in a number of transcriptional changes. The most thoroughly studied is repression of genes involved in metabolism of alternate nitrogen sources, designated the nitrogen discrimination pathway (NDP) (**Figure 3**). Expression of approximately 90 NDP genes is regulated by an interplay of four GATA family zinc-finger transcription factors: two transcriptional activators, Gln3 and Gat1 (Nil1, Mep80), and two repressors, Dal80 and Gzf3 (Deh1, Nil2) (41, 166,
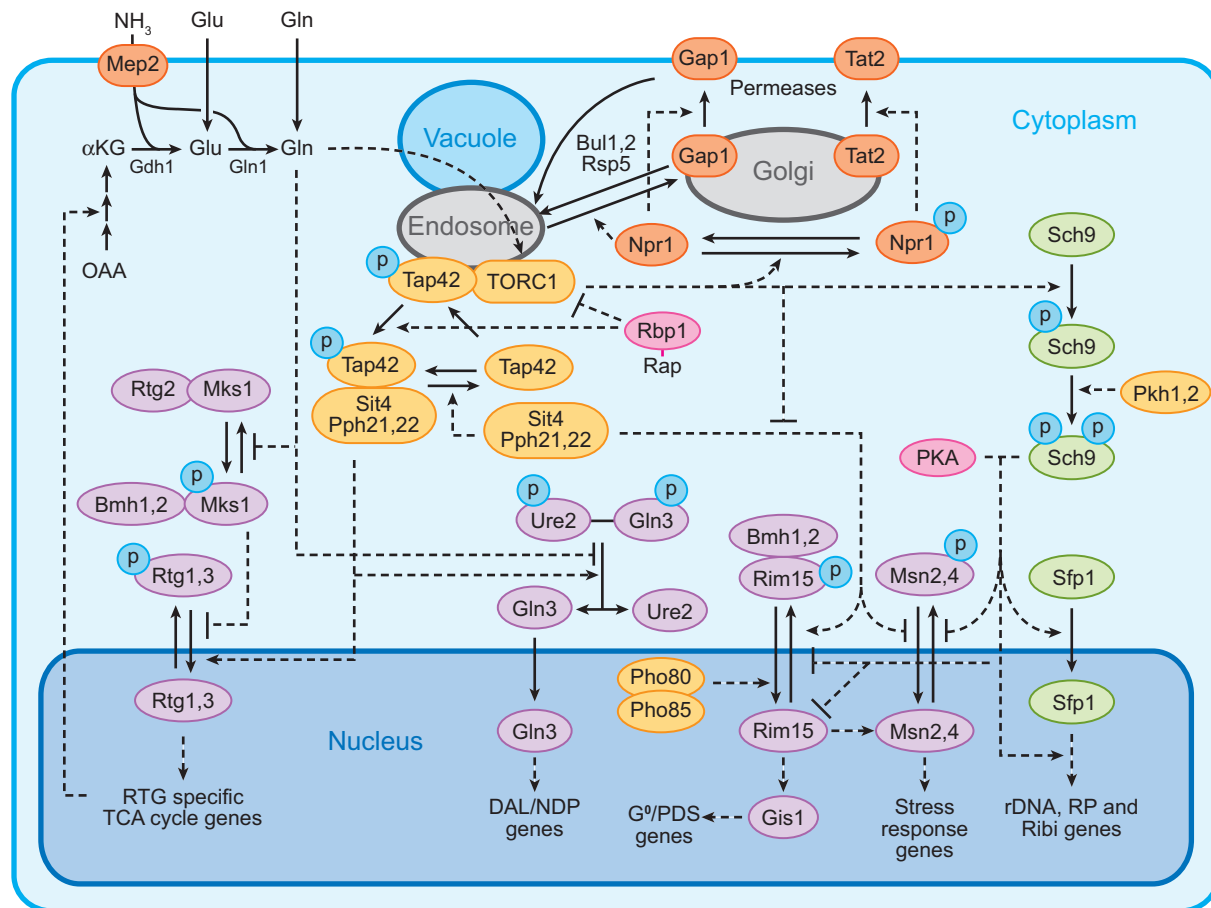
Figure 3

Nitrogen regulation and the TORC1 network. Nitrogen availability, manifested through intracellular glutamate (Glu) and glutamine (Gln) levels, affects the activity of the Tor Complex 1 (TORC1) as well as expression of the nitrogen discrimination pathway (DAL/NDP) and retrograde signaling (RTG) genes. TORC1 also regulates these sets of genes through modulation of Tap42-PP2A phosphatase activity in response to, and likely in parallel with, nitrogen availability. TORC1 also modulates stress-responsive and post diauxic shift (PDS) genes both through PP2A and through activation of the Sch9 kinase, which functions in parallel with PKA. The general amino acid (GAP) permease and a collection of amino-acid-specific permeases, including Tat2, are inversely regulated by TORC1, at least in part through control of vesicular trafficking by the Npr1 kinase.

245). Cells regulate NDP genes primarily by modulating subcellular localization of the transcriptional activators: During growth on poor nitrogen sources, Gln3 and Gat1 localize to the nucleus where they bind to GATA sequences in promoters of NDP genes, whereas during growth on ammonium or glutamine, the transcription factors reside in the cytoplasm. Both Gat1 and Dal80 are induced by Gln3, providing both positive and negative feedback loops on induction. Ure2 serves as an anchor to se-

quester Gln3 in the cytoplasm: Gln3 resides in the nucleus and fully activates NDP transcription in a *ure2* mutant, regardless of nitrogen source. This observation demonstrates not only that Ure2 serves as a cytoplasmic anchor for Gln3 but also that nitrogen deprivation acts on Gln3 solely to liberate it from sequestration by Ure2. Gat1 does not localize to the nucleus in a *ure2* mutant. This suggests that a separate as yet unidentified protein likely anchors Gat1 in the cytoplasm in cells grown on glutamine

or ammonia. Thus, the cell regulates NDP genes by promoting association of transcription factors with cytoplasmic anchor proteins when growing in a high-quality nitrogen source and inducing their release and nuclear import during growth on poor nitrogen sources.

Several experiments suggest that the metric by which the cell recognizes the quality of nitrogen source with regard to regulating NDP genes is the levels of cytoplasmic glutamine and/or glutamate. Cells carrying a leaky mutation in *GLN1*, encoding glutamine synthetase, are derepressed for NDP genes when growing on ammonia but not on glutamine, and treatment of cells growing on ammonia with methyl sulfoximine, an inhibitor of glutamine synthetase, induces NDP genes (46, 180). This would suggest that glutamine promotes association of Gln3 with Ure2. In contrast, methyl sulfoximine treatment does not promote translocation of Gat1 into the nucleus, an observation consistent with the hypothesis that Gat1 responds to cytoplasmic glutamate levels (146, 166). These results suggest a graded response of the cell to nitrogen depletion. Conditions resulting in low levels of cytoplasmic glutamine would induce dissociation of Gln3 from Ure2, dephosphorylation of Gln3, and importation of Gln3 into the nucleus (20). Conditions that reduced glutamate levels as well would further mobilize Gat1 to allow enhanced activation of NDP genes.

While cytoplasmic glutamine and glutamate levels provide the signal to which the cells respond in assessing the quality and quantity of available nitrogen, the means by which the levels of these compounds influence Gln3/Gat1 nuclear localization and subsequent NDP gene activation is less clear. A prevailing model has postulated that the level of these compounds affects the activity of the Tor1,2 containing complex, TORC1 (see below), which in turn controls Gln3 localization by regulating the phosphorylation state of Gln3 and/or Ure2. This model is consistent with the fact that inactivation of TORC1 (see below) by treatment with rapamycin induces dephosphorylation and nuclear localization of Gln3 and that Srp1, the

karyopherin responsible for nuclear importation of Gln3, binds to nonphosphorylated but not to phosphorylated Gln3 (33). However, recent evidence has shown that nuclear localization of Gln3 in response to nitrogen deprivation requires an intact actin cytoskeleton but that such localization in response to rapamycin treatment does not (44). Moreover, growth on poor nitrogen sources or treatment with methyl sulfoximine results in nuclear localization of Gln3, as previously reported, but with little dephosphorylation of the protein (43, 262). While these studies are confounded by the inability to examine individual phosphorylation sites on Gln3, and thereby monitor only those that might be crucial to Ure2 association, they do rule out the simple hypothesis that TORC1 mediates the effects of nitrogen source on Gln3 localization. Rather, current evidence suggests that TORC1 and glutamine function in parallel to affect Gln3 localization.

If nitrogen deprivation does not regulate NDP transcription through TORC1, what is the pathway connecting glutamine/glutamate levels with NDP activation? One recent model posited a role of the kinase Npr1 in regulating Gln3 nuclear localization in response to nitrogen deprivation, based on the observation that NDP genes are derepressed in *npr1* mutants grown on ammonium (45). However, subsequent studies have demonstrated that the effect of Npr1 on NDP activation is an indirect consequence of Npr1's function in regulating permeases required for nutrient uptake (72, 263). Rather, an economical, albeit untested, model based on the fact that Gln3 fully activates target NDP genes in a *ure2* mutant even in the presence of glutamine would be that Ure2 serves as the locus through which glutamine affects NDP regulation. Glutamine might stimulate Ure2's function as an anchor by direct interaction or by influencing the phosphorylation state of the protein, either directly or indirectly. In this context, Ure2 as well as Gln3 is dephosphorylated on treatment of cells with rapamycin. Whether phosphorylation affects Ure2 function as an anchor and whether the quality of

nitrogen source affects Ure2 phosphorylation have not been determined. In short, we know the signal on which the cell relies to assess nitrogen quality, but not the pathway through which that information is processed.

Retrograde Regulation

Yeast cells assimilate nitrogen from sources other than glutamate and glutamine by conversion to ammonium and then condensation with α-ketoglutarate to form glutamate. In cells growing on glucose, in which the citric acid cycle is repressed, adequate reserves of α-ketoglutarate are generated from pyruvate and acetyl-CoA by an anapleurotic pathway catalyzed by the first three enzymes of the citric acid cycle. Accordingly, to meet this demand, expression of those genes encoding this portion of the citric acid cycle enzymes are upregulated during growth on certain poor nitrogen sources. These genes constitute one of three sets of genes that are regulated in response to mitochondrial dysfunction through the retrograde (RTG) response pathway [recently reviewed in (160)]. The enzymes of the citric acid cycle are generally responsive to the heme-dependent Hap1 and heme-independent Hap2-5 activator complexes that modulate the respiratory capacity of the cell in response to carbon source. However, during growth on glucose, when cells normally repress all the citric cycle genes, the first three genes can be induced by activators of the RTG pathway in response to mitochondrial dysfunction or by growth on nitrogen sources requiring α-ketoglutarate for assimilation (159). In this manner, the RTG pathway provides a means of ammonium assimilation from poor nitrogen sources and a source of glutamate in the absence of mitochondrial function. The RTG pathway also regulates a small set of genes comprising the glyoxylate cycle and those involved in β-oxidation of fatty acids. Finally, activation of the RTG pathway results in induction of lysine biosynthetic enzymes, perhaps as an indirect consequence of a buildup by mass action of α-ketoglutarate, an inducer of lysine biosynthesis (61).

The RTG regulatory pathway consists of four positive regulators, Rtg1, Rtg3, Rtg2 and Grr1, and four negative regulators, Mks1, Bmh1, Bmh2, and Lst8 (160) (**Figure 3**). Rtg1 and Rtg3 form a heterodimeric transcriptional activator whose nuclear localization is regulated by the other components of the pathway in response to mitochondrial integrity. When mitochondria are functional, the transcription factors are cytoplasmic; disruption of mitochondrial function results in nuclear localization of the factors and subsequent transcriptional activation of target genes. Regulation of the nuclear/cytoplasmic trafficking of Rtg1/Rtg3 involves complex interactions among Mks1, Rtg2, and Bmh1/2 (60). In particular, Mks1 exhibits mutually exclusive binding to Bmh1/2 and to Rtg2, which correlates with its phosphorylation state. When phosphorylated, Mks1 complexes with Bmh1/2 to form an anchor that sequesters Rtg3/Rtg1 in the cytoplasm. Rtg2 can compete for Bmh1/2 binding to Mks1 and thereby relieve the cytoplasmic sequestration and promote nuclear entry and transcriptional activation by Rtg1/3. Release of Mks1 from Bmh1/2 is associated with reduced phosphorylation of Mks1. Grr1, the SCF targeting subunit, promotes ubiquitination and subsequent degradation of Mks1, providing a long-term modulation of the pathway, while Lst8, a subunit of the TOR complexes, renders the RTG pathway sensitive to Tor inhibition.

The primary signal initiating RTG activation appears to be glutamate and/or glutamine deficiency, although the point in the regulatory circuitry at which this signal impinges is not known. While rapamycin inhibition of TORC1 (see below) also activates the RTG pathway, accumulating evidence suggests that TORC1 does not mediate nutrient regulation of the pathway. The most telling is the fact that the growth of cells on glutamate renders the pathway resistant to activation upon rapamycin treatment (60). Thus, TORC1 does not lie downstream of the nutrient status of the cell but rather appears to function in a parallel fashion to regulate the pathway.

The TOR Network

Yeast, like all eukaryotes examined to date, elaborate two essential complexes containing the PI3-like protein kinase, Tor, designated as TORC1 and TORC2 [reviewed in (52, 290)]. While in most eukaryotes a single Tor protein suffices for both complexes, *Saccharomyces* expresses two distinct Tor kinases, Tor1 and Tor2. Tor2 and five other proteins—Avo1, Avo2, Avo3, Bit61, and Lst8—comprise TORC2 and regulate the organization of the actin cytoskeleton and cell polarity (52, 291). Tor1, or in its absence, Tor2, along with Kog1, Lst8, and Tco89 comprise TORC1, which regulates cell proliferation and the transition between growth and quiescence. TORC1, but not TORC2, is inhibited by the macrolide drug rapamycin, which in a complex with the prolyl isomerase FKBP12 binds to the complex and suppresses its interaction with target substrates.

Regulation of TORC1. Substantial evidence has accumulated to suggest that TORC1 activity responds to nutrient status, primarily the quality of the nitrogen source. The most compelling evidence for this connection is that treatment of cells with rapamycin, which inhibits TORC1, mimics the effects of nitrogen deprivation on transcription, development and metabolism. For instance, both nitrogen deprivation and rapamycin treatment induce autophagy and similarly reconfigure the constellation of amino acid permeases. Moreover, both rapamycin treatment and nitrogen starvation induce cells to exit the cell cycle and enter a G_0 state. Finally, nitrogen starvation induces significant restructuring of the transcriptome, with approximately several hundred genes changing expression. This pattern correlates remarkably well with that obtained by treatment of cells with rapamycin. In particular, both rapamycin and nitrogen deprivation yield induction of NDP, RTG, and stress response genes and repression of the Ribi and RP regulons (251). Accordingly, these observations have been invoked to place TORC1 downstream of the nitrogen sensing mechanism in regulation of the cell's metabolic, developmental, and transcriptional programs in response to that sensing.

Several recent observations have suggested that, while rapamycin treatment and nitrogen signaling have similar responses and TORC1 may respond to the quality of the nitrogen source, TORC1 is not the sole mediator of the nitrogen response pathway. First, nitrogen starvation is a readily reversible process, requiring no more than an hour of refeeding to restore normal growth. On the other hand, even short-term treatment with rapamycin induces a state from which the cell can recover only with extended time (65). Second, while both nitrogen starvation and rapamycin treatment elicit induction of NDP, RTG, and stress response genes, these two stimuli impinge on these pathways by distinct mechanisms. For instance, although both rapamycin and nitrogen starvation induce nuclear localization of Gln3 to induce activation of NDP genes, the Gln3 phosphorylation patterns induced by these two treatments are distinct (43). Similarly, although both retrograde and TORC1 pathways control RTG gene expression by targeting Mks1 and hence Rtg1/3 cytoplasm-nuclear localization, these two pathways elicit distinct Rtg3 phosphorylation patterns (60, 136, 250, 285). Moreover, Butow and coworkers demonstrated that the retrograde response is separable from TOR regulation of RTG and NDP gene expression by comparing rapamycin sensitivity between respiratory-competent (ρ^+) and respiratory-deficient (ρ°) yeast cells (89). Finally, relocalization of the stress response transcription factor shows different responses to nitrogen starvation and rapamycin treatment. Thus, the precise role of TORC1 in nitrogen sensing remains unknown. Moreover, while the complex clearly controls cell proliferation, metabolism, and transcription in a manner consistent with a nutritional response pathway, it does not solely mediate the cell's response to nitrogen availability.

If TORC1 does not mediate nitrogen availability, to what does TORC1 activity respond? One intriguing possibility is that TORC1 responds to vesicular trafficking, particularly with

regard to endocytic pathways (194). Growing evidence places TORC1 at least in part on an internal membrane structure that is located near, but distinct from, the plasma membrane and that resembles the endosome by ultrastructural analysis and biochemical fractionation (8, 285). Moreover, TORC1 interacts with and regulates downstream signaling components by sequestering them on a subcellular membrane (294). The GSE (Gap1 sorting in the endosome) complex, also known as the EGO (exit from G_0) complex, localizes to the perivacuolar late endosomal compartment. Mutants lacking any of the components of this complex fail to direct Gap1 to the plasma membrane and exhibit diminished recovery from rapamycin treatment, perhaps due to reduced microautophagy (65, 83). Such mutants also fail to reverse a variety of rapamycin-induced events, such as macroautophagy or eIF2a phosphorylation, which would suggest that this complex functions upstream of TORC1, at least insofar as reactivating it after its inactivation by rapamycin. Finally, Tor1 mutations are synthetically lethal with mutations in members of the class C vacuolar protein sorting complex, which plays a critical role in vesicle docking and fusion at the endosome and between the endosome and the vacuole (292, 303). Since mobilization of nutrients from the vacuole provides an early response to nutrient deprivation, TORC1's location on and interaction with the endosome places it in a position to sense intracellular nutrient balance. This may serve as the means of modulating TORC1 function.

What TORC1 regulates. Despite uncertainty in the position of TORC1 within the nutrient signaling pathway, TORC1 certainly plays a major role in the coherent transition between growth and quiescence, regulating processes that provide various means of survival under conditions of deprivation. Accordingly, we examine below the processes directly regulated by TORC1, namely the PP2A and Sch9 pathways (**Figure 3**).

PP2A and Tap42/Tip41. A number of TORC1 functions are mediated by protein phosphatase 2A, specifically in conjunction with Tap42. TORC1 directly phosphorylates the essential protein Tap42 to promote its direct binding to the catalytic subunits of the Ser/Thr protein phosphatase 2A (PP2A) and PP2A-like phosphatases (57, 66, 118). PP2A holoenzyme normally exists as a heterotrimeric complex, consisting of a catalytic (C) subunit, encoded by redundant genes *PPH21*, *PPH22*, and *PPH3*, a scaffold subunit (A), encoded by *TPD3* and a specificity subunit (B or B′), encoded by functionally distinct *CDC55* and *RTS1* genes. Similarly, the PP2A-like phosphatase consists of a catalytic subunit, Sit4, bound to one of three regulatory subunits, Sap155, Sap190, or Sap185. When phosphorylated by TORC1, Tap42 binds to a phosphatase catalytic subunit—Pph21, Pph22, or Sit4—to the exclusion of the other subunits of the phosphatase holoenzyme. Upon treatment with rapamycin or upon starvation for nitrogen, Tap42 becomes dephosphorylated and dissociates from the catalytic subunit (57, 118).

These observations have prompted models in which Tap42 in its phosphorylated form inhibits PP2A activity to promote growth, whereas dissolution of the complex following rapamycin treatment releases PP2A to cause growth inhibition. However, the effects of rapamycin on gene expression are much more rapid than the observed dephosphorylation and dissociation of Tap42 from PP2A. Moreover, because the cell contains five- to tenfold more PP2A catalytic subunits than Tap42, Tap42 can complex with no more than 20% of the total PP2A protein in the cell. Accordingly, a straightforward model positing Tap42 as a stoichiometric inhibitor of PP2A is not tenable. Recent results from Yan et al. provide a resolution to the kinetic conundrum (294). This group demonstrated that the Tap42-phosphatase heterodimer normally resides on a membranous structure in the cell in association with TORC1. Starvation or treatment of cells with rapamycin immediately liberates the Tap42-phosphatase complex into the cytoplasm, where it slowly decays to the unphosphorylated, dissociated state. Thus, rapamycin treatment or starvation

elicits a rapid response by immediately delivering the Tap42-phosphatase complex to the cytosol, where it can persist in the dimeric state to catalyze Tap42-directed dephorphorylation. This observation not only accounts for the kinetics of rapamycin action but also places Tap42 as an active participant in the rapamycin response.

Tap42 appears to function as a specificity factor for the catalytic phosphatase subunits, directing the phosphatase to certain substrates and inhibiting its activity to others. As such, Tap42 plays different roles in the transcriptional responses of different sets of genes regulated by TORC1. For instance, rapamycin induces a Sit4-dependent dephosphorylation of the transcription factor Gln3 and subsequent translocation of the factor to the nucleus, where it induces transcription of NDP target genes (14, 32). Inactivation of Tap42 has no effect on NDP gene expression under normal growth conditions but significantly attenuates induction of these genes by rapamycin (67). These results suggest that Tap42 is required for dephosphorylation of Gln3 following rapamycin treatment, an event catalyzed by Sit4 (14). In fact, loss of Tap42 has essentially the identical effect on rapamycin induction of NDP target genes as does inactivation of Sit4 (67). Thus, Sit4 and Tap42 act in concert to dephosphorylate downstream targets in response to rapamycin treatment, placing Tap42 as a positive regulator of phosphatase activity. Tap42 plays a similar role in rapamycin induction of RTG target genes (67).

Rapamycin also induces nuclear localization of Msn2/Msn4 and subsequent activation of stress-inducible genes. Inactivation of PP2A blocks rapamycin-induced accumulation of Msn2 in the nucleus but inactivation of Tap42, rather than blocking the effect of rapamycin, actually mimics the effects and induces nuclear localization. These and other data suggest that TORC1 promotes efficient export of Msn2/4 from the nucleus and that rapamycin treatment impedes that process, leading to nuclear accumulation of the transcription factor (91, 239). The fact that Tap42 inactivation mimics rapamycin treatment with regard to stress response gene activation is consistent with the hypothesis that PP2A inhibits nuclear export of Msn2 and TORC1-phosphorylated Tap42 inhibits this PP2A. Accordingly, either inactivation of Tap42 or inhibition of TORC1 by rapamycin liberates PP2A to inhibit nuclear export. How Tap42 normally inhibits PP2A is not known, particularly since the cell contains insufficient Tap42 to complex with all the PP2A.

Jacinto et al. (113) identified Tip41 as a Tap42 interacting protein also involved in coupling TORC1 activity to PP2A function. Although their initial studies suggested that Tip41 serves as an inhibitor of Tap42 function, subsequent work and modeling indicate that Tip41 and Tap42 collaborate in redirecting PP2A and PP2A-like activity in response to TORC1 activity. Inactivation of *TIP41* exhibits a synthetic deficiency with mutation of *TAP42* (239). Moreover, both Tap42 and Tip41 are required for rapamycin-induced activation of Gln3 (66, 113). Finally, modeling this system to fit existing detailed kinetic data suggests that Tip41 and Tap42 have essentially the same role in TOR signaling and, moreover, supports the proposal that a key aspect of Tor regulation of PP2A activity is rapid release of the Tap42-PP2A complex from association with TORC1, followed by a slow decay of the Tap42 complex (145).

Microarray analysis of *TAP42* and PP2A mutant strains has shown that phosphatases do not mediate all of TORC1 signaling. For instance, genes encoding ribosomal proteins and components of translational apparatus are induced by rapamycin, but that induction is neither abrogated, nor recapitulated, by inactivation of either Tap42 or any of the PP2A catalytic subunits (67). Rather, recent results suggest that Tor regulation of downstream kinases affects many of the Tor-induced processes.

Sch9

As noted above, Sch9 overexpression suppresses deficiencies in the PKA pathway and its inactivation results in diminished growth and

reduced expression of genes in ribosomal biogenesis (124, 125). Sch9 is a phosphoprotein whose phosphorylation is sensitive to physiological stresses—Sch9 is rapidly dephosphorylated in response to rapamycin treatment, carbon or nitrogen starvation, as well as shifting the nitrogen source from ammonium to urea. Addition of the missing nutrient quickly restored Sch9 phosphorylation. Urban et al. (273) have recently shown that TORC1 directly regulates Sch9 by phosphorylating six serine/threonine sites on its C terminus. Elimination of these phosphorylation sites eliminates the kinase activity of the protein and conversion of the sites into phosphomimetic amino acids renders the in vivo activity independent of upstream activation by TORC1. The sites for carbon-source–mediated phosphorylation have not been identified but are likely distinct from those recognized by TORC1. Recent in vitro studies have shown that Pkh1 and Pkh2, homologues of mammalian 3-phosphoinositide-dependent kinase (PDK1), also phosphorylate Sch9 (158, 226). Mutation of the putative PDK1 phosphorylation site on Sch9 diminishes its activity in vivo, as noted by a reduced ability of mutant Sch9 to suppress the temperature sensitivity of the $cdc25^{ts}$ mutant and by reduced heat shock sensitivity upon overexpression of the mutant Sch9. Whereas TORC1 targets Sch9's C terminus, Pkh1/2 phosphorylates Thr570 in the activation loop in a rapamycin-insensitive manner. Phosphorylation at the activation loop and C terminus by Pkh1/2 and TORC1, respectively, are both required for Sch9 activity (273).

TORC1 regulates the expression of ribosome biogenesis genes in part through Sch9. Cells expressing a TOR-independent *SCH9* allele (Sch9[2D3E]) displayed attenuated repression of ribosome biogenesis after rapamycin treatment (273). Results from this study suggest that TOR may also regulate activity of the stress-responsive transcription factor, Msn2/4, in part through Sch9. Induction of a group of Msn2/4-regulated genes were diminished in cells expressing Sch9[2D3E] in comparison to those expressing wild-type Sch9. Importantly,

this study showed that Sch9 does not mediate all of TOR signaling. Cells expressing either wild-type Sch9 or Sch9[2D3E] displayed comparable levels of NDP (Gln3-driven) and RTG (Rtg1/3-driven) gene induction in response to rapamycin.

Finally, TORC1 seems to prevent G_0 entry in part, but not exclusively, via Sch9. When TORC1 and Sch9 are active, Rim15 is inhibited from entering the nucleus and thus is inactive (see below). When TORC1 is inactivated by rapamycin, Rim15 enters the nucleus, cells arrest with 1N DNA content and accumulate carbon reserve carbohydrates. These readouts are partially blocked in cells expressing Sch9[2D3E]. Conversely, cells expressing constitutively inactive Sch9 display constitutive nuclear localization of Rim15 and accumulate glycogen in the absence of rapamycin. However, transcriptional reprogramming associated with G_0 entry seems to require signals in addition to Sch9, since microarray analyses did not detect differences in the expression of G_0 specific genes, such as *GRE1*, in cells expressing Sch9[2D3E] from those expressing wild-type Sch9.

In sum, Sch9 appears to serve as a major conduit by which TORC1 influences growth and mass accumulation. As such, Sch9 impinges on many of the same downstream targets as does PKA, which may account for the ability of excess Sch9 to compensate for loss of PKA activity.

AMINO ACID SIGNALING

Yeast cells import and use external amino acids for translation and, in some cases, as alternative nitrogen sources. Different permeases, subject to different regulatory processes, mediate amino acid uptake for the two different purposes. The high-capacity general amino acid permease, Gap1, and the proline-specific permease, Put4, provide the major route for uptake of amino acids for catabolic metabolism and, as such, are subject to transcriptional repression through the NDP pathway. Gap1 is also subject to nitrogen inactivation, which biases trafficking of the permease to the vacuole rather

than the plasma membrane in the presence of a preferred nitrogen source and, in addition, promotes endocytosis and vacuolar targeting of existing Gap1 in the plasma membrane (166). An additional collection of amino acid permeases with relatively restricted specificities provides uptake of amino acids for use in translation. These amino acid permeases are induced in concert by the presence of amino acids in the medium through the SPS system (see below), even during growth on a high-quality nitrogen source. Moreover, the level of the Tat2 tyrosine permease in the plasma membrane actually declines during growth on poor nitrogen sources, i.e., in an inverse fashion relative to Gap1. Whether this nitrogen source regulation of Tat2 or other SPS permeases is direct or indirect has not been fully resolved.

SPS System

The SPS (Ssy1-Ptr3-Ssy5) signaling system regulates amino acid permease gene expression in response to the presence of external amino acids (**Figure 4**). Ssy1, an integral membrane protein with homology to other amino acid
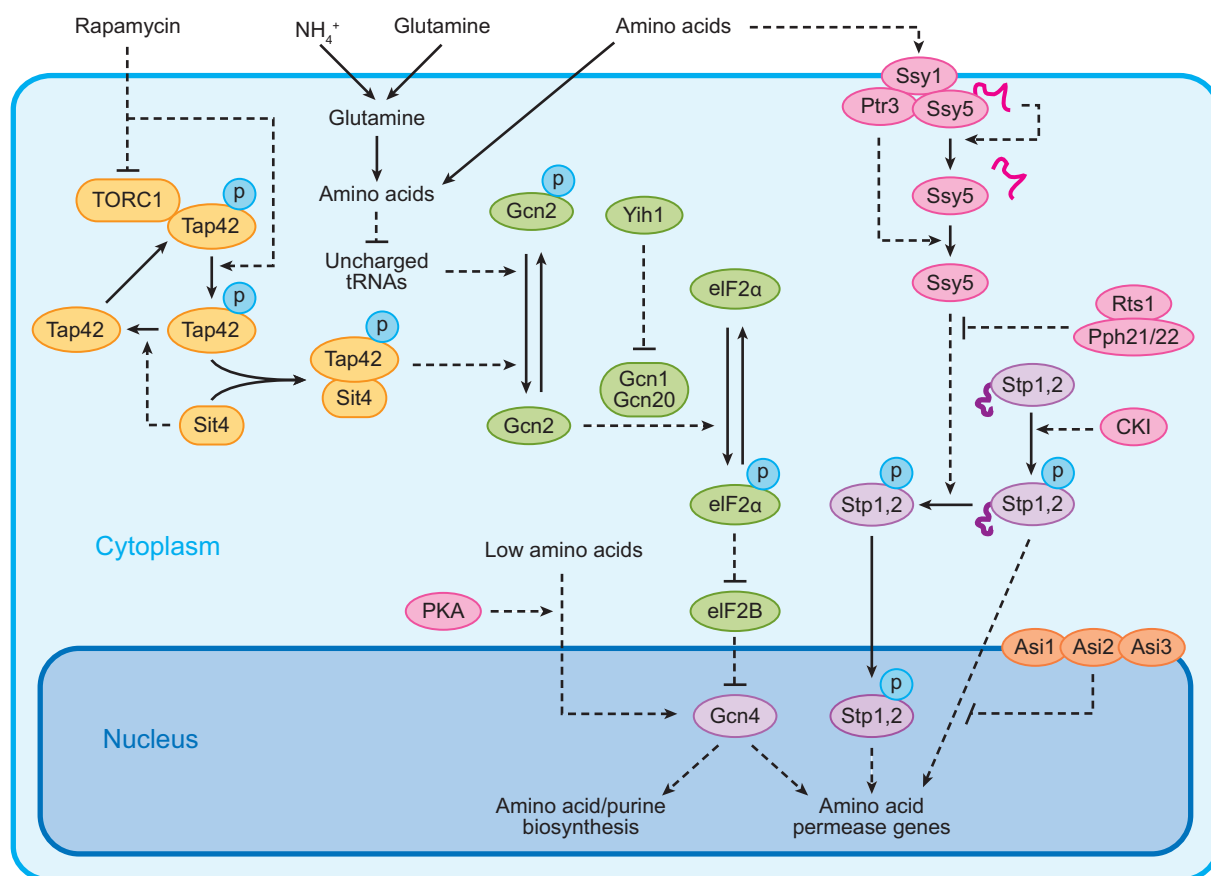
Figure 4

Regulation by amino acids. The presence of external amino acids induces expression of a collection of amino acid permease genes through a signaling network that results in activation of the Stp1/Stp2 transcription factors via proteolytic cleavage of an amino terminal inhibitory domain. Diminished intracellular levels of any amino acid results in reduced levels of uncharged tRNAs, which initiates a cascade of events yielding increased translation of the Gcn4 general amino acid transcriptional activator. This cascade can also be initiated by reduced TORC1 function resulting in a Tap42-Sit4 dependent activation of the Gcn2 kinase.

permeases, does not transport amino acids but rather functions as a sensor by directly interacting with extracellular amino acids (59, 80, 133). Different amino acids show differing capacity for initiating the signal, with leucine being most effective and arginine showing little effect. Binding of amino acids to Ssy1 initiates a signal that is transduced to the peripherally associated plasma membrane proteins Ptr3 and Ssy5 (17, 59, 78, 111, 133). Ssy5 is a serine endoprotease consisting of a Pro-domain and a catalytic domain. The Pro-domain is autolytically cleaved from the catalytic domain but remains associated, forming an inactive protease complex that binds Stp1 (1, 5, 7, 212). Ssy1, together with Ptr3, transduces amino acid-initiated signals, resulting in the release of Pro-domain inhibition and activation of the Ssy5 protease activity. The targets of the protease are two transcription factors, Stp1 and Stp2, expressed as latent cytoplasmic precursors. Ssy5-catalyzed endoproteolysis of Stp1 and Stp2 liberates their DNA-binding and transactivation domains from an approximately 10-kDa N-terminal regulatory fragment. These truncated forms of Stp1 and Stp2 accumulate in the nucleus where they induce the expression of SPS sensor-regulated genes (1, 6, 7, 50, 58, 59, 111, 133).

Other factors involved in SPS signaling have been identified recently. Asi1, Asi2, and Asi3 are integral components of the inner nuclear membrane that restrict any full-length unprocessed forms of Stp1 and Stp2 that leak into the nucleus from binding SPS sensor-regulated promoters (7, 21, 77, 300). In addition, signaling through the SPS pathway requires casein kinase I (CKI) activity and is inhibited by protein phosphatase 2A, particularly via the Rts1 regulatory subunit (68). CKI phosphorylates Stp1 as a prerequisite to Stp1 activation by endoproteolytic processing (1). PP2A likely serves to inactivate the system in the absence of external amino acids but its target component in the pathway is not known. The SCF^{Grr1} ubiquitin E3 ligase complex is also required for Stp1 processing, although which component is subject to ubiquitination is not known (7). Finally,

some of the genes subject to activation by SPS, such as the broad-specificity amino acid permease Agp1 gene, are also upregulated by growth on a poor nitrogen source through the Gln3 system, suggesting that these permeases also contribute to acquisition of amino acids for nitrogen catabolism (2).

The global importance of amino acids sensing has been investigated using genome-wide expression analysis (69, 76, 135). The results indicate that permease genes represent only a subset of the full spectrum of Ssy1-dependent genes. For example, besides the effects on amino acid permease genes, an *ssy1* and an *stp1 stp2* mutant exhibit a number of other transcriptional phenotypes, such as increased expression of nitrogen catabolite-sensitive genes and genes involved in stress response (69).

GCN Pathway

Internal amino acid concentration is sensed by the general control system (GCN) for amino acid biosynthesis (**Figure 4**). The transcriptional activator Gcn4 is induced at the translational level by limitation for any amino acid, and it activates transcription of more than 500 genes, including most of those involved in amino acid biosynthesis (116, 192). The signal for activation of the pathway is uncharged tRNAs, which are bound by, and presumably activate, the kinase Gcn2. The key substrate of Gcn2 is the α subunit of translation initiation factor 2 (eIF2) (37, 105, 106). eIF2 forms a ternary complex (TC), along with charged methionyl initiator tRNA and GTP, which associates with the 40S ribosomal subunit to facilitate scanning of the 5′ region of mRNAs to locate the appropriate initiation codon. The guanine nucleotide exchange factor eIF2B recycles inactive eIF2-GDP to active eIF2-GTP. Phosphorylation of eIF2α by Gcn2 converts eIF2-GDP from substrate to inhibitor of eIF2B, which reduces the GTP-bound form of eIF2 and impedes TC formation. Given the stoichiometric excess of eIF2 over eIF2B, phosphorylation of a small proportion of phosphorylated eIF2 is sufficient to inactivate essentially

all eIF2B activity. Although the reduction in TC levels leads to a decrease in the rate of general translation initiation, it specifically stimulates translation of Gcn4 mRNA.

Translational induction of *GCN4* is mediated by four short upstream open reading frames (uORFs) in the *GCN4* mRNA leader. However, uORF1 and uORF4 alone are sufficient to confer nearly wild-type translational control of *GCN4* (188). Under nonstarvation conditions, about 50% of 40S subunits fail to dissociate from the mRNA after translating uORF1 and continue scanning. However, virtually all of these reinitiating 40S ribosomes rebind the TC before reaching uORF4, translate uORF4, and dissociate from the mRNA. Under starvation conditions, the moderate reduction in TC level allows about 50% of rescanning 40S ribosomes to rebind TC only after bypassing uORF4 and then reinitiate at Gcn4 instead to yield increased translational initiation of the *GCN4* coding region (105, 106).

The kinase activity of Gcn2 is stimulated in amino acid–starved cells by binding of uncharged tRNA that accumulates under these conditions. C-terminal to the kinase domain Gcn2 contains a 500-residues domain homologous to histidyl-tRNA synthetase (HisRS) (286). Binding of any uncharged tRNA to the HisRS domain likely produces a conformational change in Gcn2 that stimulates kinase activity [reviewed in (64, 105, 219)]. The activation of Gcn2 by uncharged tRNA also requires interactions between the N terminus of Gcn2 and the Gcn1-Gcn20 regulatory complex through a C-terminal segment of Gcn1 (84, 142, 143, 241). Gcn1-Gcn20 likely facilitates binding of uncharged tRNA to the ribosomal A site or its transfer from the A site to the HisRS-like domain in Gcn2 for kinase activation (106). Gcn1 associates with polyribosomes in cell extracts, and this interaction is essential for Gcn2 activation (173, 242). An inhibitor of Gcn2, Yih1, has been identified recently. It competes with Gcn2 for binding to Gcn1. Overexpression of Yih1 dampens the GCN response by reducing Gcn1-Gcn2 complex formation (243).

Besides the regulation of Gcn4 translation, the cellular level of Gcn4 is also controlled at the level of protein degradation (105, 112, 137, 178). Under normal growth conditions, Gcn4 is a highly unstable protein with a half-life of only 2 to 3 min. It is rapidly degraded by the ubiquitin/proteasome system, which recognizes specifically phosphorylated substrates. Two cyclin-dependent protein kinases, Pho85 and Srb10, have crucial functions in regulating Gcn4 phosphorylation and degradation. The increased stability of Gcn4 in starvation conditions results from decreased Pho85-dependent phosphorylation of Gcn4 that can be attributed to the dissociation of the Pho85/Pcl5 complex (24).

Transcriptional activation by Gcn4 is a rapid process with the immediate occupancy of the regulatory sequence by Gcn4 on amino acid starvation, followed by nearly simultaneous recruitment of coactivators. Gcn4 requires a multiple of coactivators at individual promoters. Recent studies on the requirements of Gcn4 coactivators provide a detailed picture of the activation mechanism for Gcn4 (92, 132, 220, 258, 295, 296, 301).

The GCN pathway is linked to other signal pathways in yeast. Gcn4 translation is transiently induced independent of Gcn2 during a shift-down from amino acid–rich medium to minimal medium in a manner requiring activation of PKA (70, 270). The TORC1 pathway also influences GCN activity in that rapamycin treatment induces Gcn4 synthesis. A potential cross-talk mechanism involves rapamycin-induced dephosphorylation of Gcn2^{S577}, causing increased phosphorylation of eIF2α and a subsequent increase in Gcn4 protein levels. TORC1 promotes Ser-577 phosphorylation partly by inhibiting the PP2A-like Sit4 through the regulatory protein Tap42 (37, 141, 227, 274). Finally, the TORC1-regulated eIF4-associated protein, Eap1, was found to function downstream of Gcn2 to attenuate Gcn4 translation in a novel mechanism independent of eIF4E-binding (174).

RESPONSES TO NUTRIENT AVAILABILITY

Growth Control

Cells respond to nutrient availability by setting the rate of mass accumulation and adjusting the generation time, or growth rate. Studies in nutrient-limited chemostats have documented a linear relation between the rate of nutrient flux and generation time over a wide range of nutrient flow. How cells adjust their growth rate to match nutrient availability remains a mystery. As noted below, the growth rate or generation time depends on, and thus is set by, the rate of mass accumulation, and nutrients influence mass accumulation in two ways: in determining the amount of material and energy available for anabolic processes, particularly translation, and in regulating the rate of ribosome biogenesis. The latter process determines the biosynthetic capacity of the cell, i.e., the number of ribosomes per cell, and the former process determines the overall translation rate of the cell, i.e., the rate at which each ribosome catalyzes peptide elongation. The interplay of these two aspects of cell growth affects cell cycle progression and cell size, as discussed below. Recent studies in yeast on the regulatory circuits connecting nutrient availability to ribosome biogenesis suggest that nutrient control of ribosome biogenesis is an active process, not simply the passive consequence of availability of anabolic starting material.

Ribosome biogenesis. The budding yeast ribosome is composed of 79 ribosomal proteins, encoded by 138 genes (the RP regulon), and 4 rRNAs (5S, 5.8S, 18S, and 25S) encoded by ~150 rDNA repeats existing as a tandem array. Moreover, another 236 genes encoding nonribosomal proteins are involved in various aspects of ribosome assembly and translational capacity (the Ribi regulon): RNA polymerases I and III, tRNA synthetases, rRNA processing and modifying enzymes, translation factors, etc. Synthesis of the translation machinery is very energetically expensive to the cell. For instance, in an exponentially growing cell, ribosome synthesis utilizes ~90% of the total cellular energy (284). Not surprisingly, the cell carefully adjusts its ribosome biogenesis in response to changes in nutrient availability.

Control of rRNA synthesis. Yeast contain ca. 150 tandemly repeated rDNA copies encoding 35S precursor rRNA and 5S RNA. During growth in rich medium, half of the repeats are actively transcribed while the other half are transcriptionally inactive, or "closed." During carbon source downshift in the post diauxic phase, Pol I transcription of rDNA decreases by two mechanisms (**Figure 5**): The number of active repeats decreases and the transcriptional activity at each active repeat also decreases. Transition from "open" to "closed" complex during the carbon downshift requires the histone deacetylase Rpd3, although the mechanism connecting carbon availability to Rpd3 recruitment is not known. In contrast, although with some conflicting data, initiation of quiescence by nitrogen starvation or rapamycin treatment does not reduce the number of active repeats and Rpd3 does not play a role in reduced rDNA expression under these conditions (39, 197, 268).

Reduction in the transcriptional activity of Pol I during carbon downshift, nitrogen starvation, and rapamycin treatment all involve reduction in the initiation-competent form of Pol I. At least four general transcription factors promote initiation of polymerase I transcription at rDNA genes: the upstream activation factor (UAF), core factor (CF), TATA binding protein (TBP), and the monomeric factor Rrn3, which forms a complex with Pol I (**Figure 5**). The formation and/or stability of the initiation-competent Rrn3-Pol I complex likely serves as a key regulatory process affecting the rate of rRNA synthesis (39, 96, 175). Specifically, glucose addition increases synthesis of Rrn3 whereas TORC1-dependent signaling stabilizes the Rrn3-Pol I complex, providing at least two links between nutritional status and Pol I activity. Li et al. showed that Tor1 directly binds to the 35S rDNA promoter in a nutrient- and
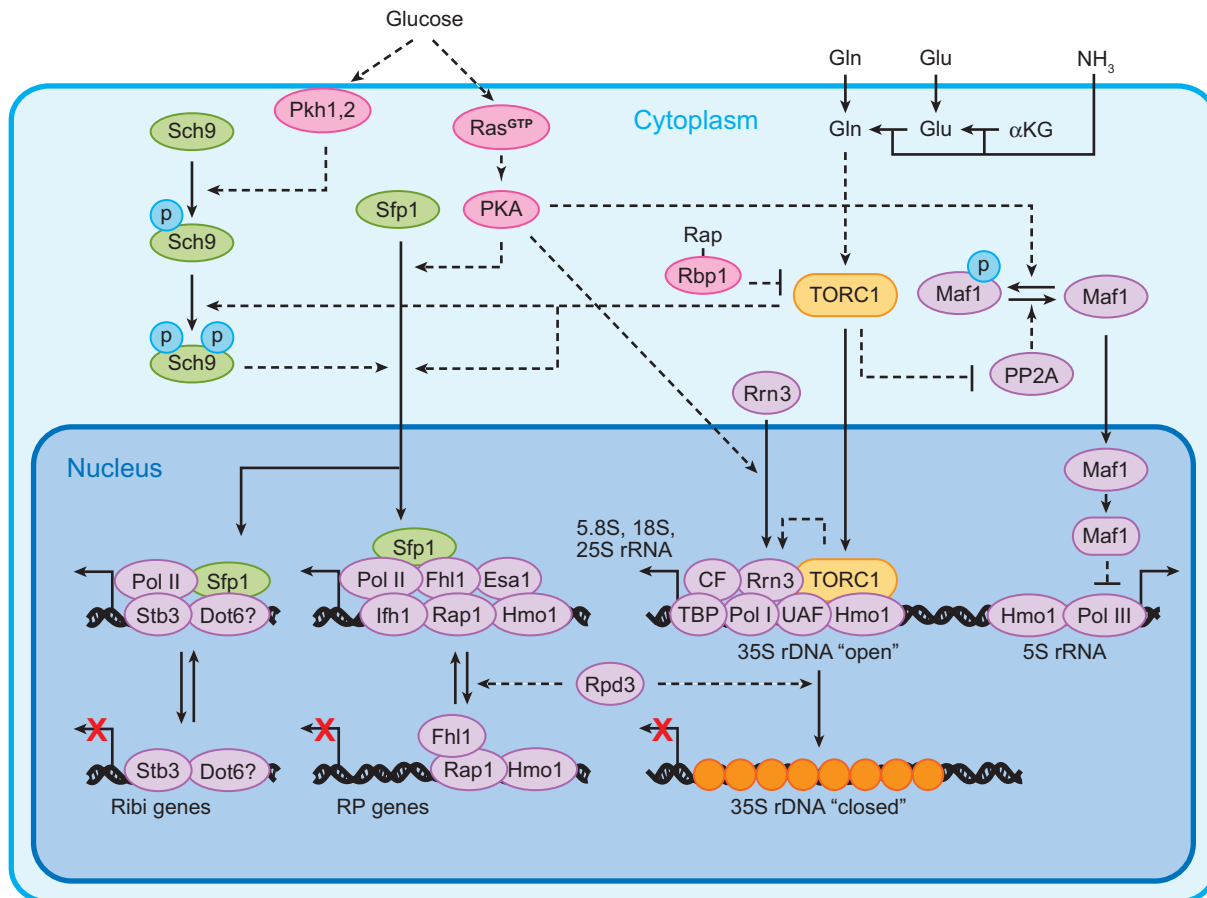
Figure 5

Nutritional control of ribosome biogenesis. Nutrient availability regulates expression of all components of the ribosome, including the RNA polymerase II-dependent ribosomal protein (RP) and ribosomal biogenesis (Ribi) genes, the RNA polymerase I-dependent ribosomal RNA genes and the RNA polymerase III-dependent 5S RNA genes. The major participants in this regulation and their likely interactions are indicated. See text for details.

rapamycin-sensitive manner and that this physical interaction is necessary for 35S rRNA synthesis (157). Finally, artificially stabilizing the Rrn3-Pol I complex by physically tethering Rrn3 to the polymerase renders rDNA expression resistant to repression by nutritional downshift or rapamycin treatment (150). In sum, these observations demonstrate that Pol I activity responds to nutritional levels via modulation of its interaction with Rrn3 and, additionally for carbon starvation, via chromatin modification.

5S RNA, along with tRNAs and a number of small nuclear RNAs are transcribed by Pol III, whose activity is regulated in response

to nutritional status by the highly conserved Maf1 repressor (**Figure 5**). In the absence of Maf1, yeast cells fail to repress Pol III transcription under nutrient limitation, environmental stress, exit from active growth or treatment with rapamycin (56, 272). Recent work has demonstrated that Maf1 function is regulated by nutrients through control of its subcellular localization. In particular, phosphorylated Maf1 is retained in the cytoplasm while dephosphorylated protein is imported into the nucleus where it inhibits pol III transcription. The phosphorylation state of Maf1 is regulated by the competing activities of PKA, Tpk1 in particular,

and protein phosphatase 2A (182, 198, 224). As noted above, PP2A activity is inhibited by TORC1, although surprisingly in this case apparently not through the action of Tap42. Thus, glucose availability through PKA and other nutrient availability through TORC1 maintain Maf1 in the cytoplasm while diminished nutritional status induces dephosphorylation, entry into the nucleus, and inhibition of 5S and tRNA synthesis.

Control of ribosomal protein synthesis. RP gene transcription is quite sensitive to the growth potential of the cell, rapidly increasing in nutrient upshifts and rapidly decreasing in nutrient downshifts or in response to a wide variety of stresses. Most of the RP genes are regulated by transcriptional factors Rap1 or Abf1 bound at their promoters (**Figure 5**). The Ras-dependent activation domain of several ribosomal protein genes maps to Rap1 binding sites (134, 195), and induction of $RAS2^{V19}$ increases expression of a reporter gene driven solely by Rap1 binding sites (281). However, Rap1 association with RP promoters in vivo is invariant to growth conditions, suggesting that glucose or other nutrients do not modulate Rap1 binding. Rap1 appears to execute two functions at the RP: generating a nucleosome free domain and recruiting other transcription factors (23, 153, 185). Deletion of Rap1 binding sites from RP gene promoters severely reduces transcription and binding of the forkhead-like transcription factor, Fhl1, which is needed to recruit Ifh1 to drive RP gene expression. Fhl1 in the absence of Ifh1 acts as a repressor (302). TORC1 controls RP gene expression by regulating the interaction between Fhl1 and Ifhl via the forkhead (FH)-associated (FHA) domain of Fhl1 (171, 235, 244, 280). Disruption of this domain or TORC1 inactivation by rapamycin results in loss of Ifhl from RP gene promoters and severe reduction in RP gene expression (171, 235, 302). Deletion of Rap1 binding sites from RP gene promoters severely reduces transcription and binding of Fhl1 and Ifh1. Just the presence of Rap1 (by LexA-Rap1 fusion) is insufficient for Fhl1 and Ifh1 to bind DNA (302).

This observation suggests that the direct binding of Rap1 to DNA is critical for RP gene expression.

In certain strains of *Saccharomyces*, primarily the Σ-derived strains (see below), the recruitment of Ifh1 is regulated by Crf1, a corepressor of transcription. When Crf1 is cytoplasmic, Ifh1 binds to Fhl1 and promotes transcription. The Yak1 kinase, an antagonist of PKA that is active under conditions of reduced growth (see above), phosphorylates Crf1 to promote its nuclear entry to displace Ifh1 from Fhl1 and thereby inhibit RP gene transcription. Thus, in these strains the competition between Ifh1 and Crf1 for binding Fhl1 controls RP gene expression (170, 171). However, Crf1 does not play a role in the transcription of RP genes in W303 (S288C-derived) strains. Deletion of *CRF1* has no effect on the repression of representative RP genes in a W303 background nor is Crf1 bound to RP promoters in vivo (302). This suggests the existence of a regulatory process independent of Crf1 and, from other data, even of Ifh1 recruitment itself.

Several other regulatory agents participate in nutrient regulation of RP gene expression. Sfp1 is a putative transcription factor containing a split zinc-finger binding domain essential to its function. In exponentially growing cells Sfp1 is located in the nucleus, but is cytoplasmically localized under TORC1 inactivation, oxidative stress, as well as carbon and nitrogen starvation. Upon reintroduction of the missing nutrient, Sfp1 rapidly returns to the nucleus. Fhl1 and Ifh1 remain bound to RP promoters under glucose limitation or in *sfp1*Δ cells but in both cases Fhl1 and Ifh1 relocalize to the nucleolus. Thus, a model consistent with existing data suggests that Sfp1 is required in the nucleus to extract Fhl1/Ifh1-bound RP promoters from the repressive domain of the nucleolus (125). In sum, Sfp1 is a prime candidate for a master nutrient-responsive regulator of ribosome production: It functions as an activator for RP and Ribi gene expression, albeit through an as-yet undefined mechanism, and its nuclear localization is rapidly and robustly responsive to the nutritional status of the cell.

Chromatin modification of the RP genes via histone acetylation also varies as a function of growth. In actively growing cells, histone acetylase Esa1 resides at RP gene promoters through association with Rap1 (222, 228). Upon inhibition of growth, Esa1 is released from the promoter and histone deacetylase Rpd3 represses transcription. Whether Rpd3 binding to RP gene promoters is constitutive or induced by growth inhibition is not clear and the precise mechanism that links nutrient availability to histone acetylation in regulating RP gene expression remains to be elucidated (18, 148, 149, 153).

Several factors connect rRNA production to RP gene expression. Hmo1, one of the ten HMG proteins in yeast, associates both with rDNA promoters, where it stimulates rRNA synthesis, and with RP gene promoters. Hmo1 binding to RP promoters requires Rap1 and, to a lesser extent, Fhl1 (99, 125). Loss of Hmo1 reduces Fhl1 and Ifh1 binding to RP promoters, although without obvious effects on RP transcription. Hmo1 interacts physically and genetically with FKBP12; however, whether FKBP12 regulates Hmo1-DNA binding is unknown (62). More recently, Rudra et al. (233) have shown that Ifh1 forms a complex with Utp22 and Rtp7, two proteins required for maturation of pre-rRNA. Depletion of either Utp22 or Rtp7, but not other pre-rRNA processing proteins, increases RP gene transcription. These observations prompt a model suggesting competition between pre-rRNA and Ifh1 for the Utp22/Rtp7 complex such that engagement of Utp22/Rtp7 in rRNA processing frees Ifh1 for transcriptional activation of RP genes. This may provide a critical coordination between rRNA and r-protein synthesis.

Ribi gene regulation. Genes in the Ribi regulon are induced quite rapidly and robustly following glucose addition or addition of glutamine to cells growing on a poor nitrogen source and, in fact, precede induction of RP gene expression. This affect is mimicked by PKA or Sch9 activation and, conversely, expression of these genes is repressed by rapamycin addition or by inactivation of Sch9 or PKA. Ribi gene promoters are enriched for PAC [RNA polymerases A (I) and C (III)], and RRPE (ribosomal RNA processing element) motifs in specific orientation (15, 125, 279). The RRPE site, but not the PAC site, can mediate Ras2^{V19}-induced activation of a reporter gene (281). Surprisingly, given its central role in cell growth, the transcription factor acting on RRPE/PAC sites has not been identified. The split finger transcription factor Sfp1 has been implicated in this process (125): Inactivation of Sfp1 results in reduced expression of Ribi genes, while induction of Sfp1 results in a rapid activation of the Ribi regulon. Despite the correlation of Sfp1 nuclear localization with Ribi gene expression, Sfp1 does not bind in vitro or in vivo to RRPE/PAC containing promoters (124, 125, 154, 168). Thus, the mechanism of regulation of RP and Ribi gene expression remains elusive.

In sum, the key processes in stimulating ribosome biogenesis in response to nutritional availability are Rrn3-Pol I formation for rRNA synthesis, Maf1 cytoplasmic localization to alleviate Pol III inhibition, Sfp1 nuclear localization, and Sch9 activation for promoting RP and Ribi gene expression. Carbon and nitrogen regulatory pathways impinge independently on each of these processes through PKA and perhaps TORC1, respectively. This is most clearly evident in the regulation of Maf1, in which PKA and TORC1 independently regulate the nuclear localization of this repressor. This summary of nutrient regulation sidesteps the question of how the cell coordinates all the subsidiary processes to insure the proper stoichiometric amounts of the vast number of components of the ribosome, in which recent studies have posited Pol I as a central player (34, 150). This observation along with the possible participation of nucleolar organization in RP gene transcription factor function raises the possibility that nutrient control of ribosome biogenesis may converge on nucleolar dynamics.

Nutritional Control of Cell Cycle Progression

G1 arrest. Since cells in a culture remain the same size over multiple generations, the duration of all the sequential events directing duplication and segregation of the cell's genetic material (the cell cycle) must in general match the amount of time required for the continuous increase in cell mass to double the nongenetic components of the cell (the growth cycle). Since the minimal amount of time required to complete all the events in the cell cycle is less than that needed to duplicate the mass of the cell, cells can maintain a constant size by requiring that progress through the cell cycle be dependent on duplicating the cell's mass. Classic studies showed that yeast cells accomplish this feat by making the transition from G1 to S, a step termed "Start," dependent on the cell attaining an appropriate "size" (121). Thus, budding yeast cells growing slowly due to limited nutrient availability extend their G1 phase to allow time for growth to this appropriate size. More recent studies using nutrient-limited chemostats reinforced these observations by documenting that the fraction of unbudded cells, i.e., those in G1, in cultures limited for glucose, ammonia, sulfate, or phosphate is proportional to the doubling time of the culture (26a). The same correlation is true for leucine auxotrophs limited for leucine, consistent with the notion that nutritional control of cell cycle initiation may well occur indirectly through effects on the rate of translation. This hypothesis resonates with the fact that low doses of cycloheximide extend G1 and with early studies on regulation of cell cycle by sulfate limitation, which showed that the cell recognizes sulfate limitation at a step in sulfate assimilation beyond charged methionyl-tRNA (271). However, a primary unresolved question is how cells sense they have attained the appropriate size and, as a subsidiary issue discussed below, how cells set the threshold size limit in response to nutritional status.

The molecular events associated with "Start" have become well defined. Commit-ment to a new round of the cell cycle occurs upon activation of the S-phase transcription factors SBF and MBF. Whi5, the yeast's functional equivalent of the mammalian retinoblastoma protein, directly binds to these transcription factors to hold them in check during G1. Cdc28 in conjunction with G1 cyclins—Clns 1, 2, and 3—phosphorylate Whi5 to alleviate its inhibition of SBF and MBF (42, 51). Thus, current models propose accumulation of sufficient levels of G1 cyclins as the rate-limiting step for executing "Start" (126). Furthermore, the level of G1 cyclins can serve as a reliable surrogate of cell size as long as the following are true: (*i*) G1 cyclins are unstable, such that the level of G1 cyclin in the cell is proportional to its synthesis rate, (*ii*) cyclins are deposited into the nucleus (or other subcellular localization), (*iii*) the concentration of cyclin in this subcellular organelle is the operative component with regard to inactivating Whi5, and (*iv*) the subcellular organelle remains relatively constant in size during G1 (or at least expands slower than does the cell volume). Whereas experimental evidence supports conclusions (*i*) and (*ii*), the status of (*iii*) is unknown and recent data suggest that (*iv*) may not be true (123a). So, more complex models for executing "Start" may be required. Nonetheless, all available genetic evidence points to synthesis of adequate G1 cyclins as the rate-limiting step in completing "Start."

Nutrients impinge on "Start" through a subset of the signaling networks described in the previous section. Inactivation of an essential component of the Ras/PKA pathway induces first cycle arrest in G1, even in the presence of adequate nutrients. This suggests that cells require adequate PKA activity to execute "Start" and that "Start" is the most sensitive step in the cell cycle to depletion of PKA activity. While PKA affects ribosome biogenesis, which may impinge on cell size determination as discussed below, the effect on cell cycle progression of inactivating PKA is too rapid to be a result of reduced biosynthetic capacity. Moreover, growth defects from loss of PKA can be suppressed by eliminating the stress response transcription factors Msn2 and Msn4 or by eliminating

the kinase Yak1 (254). As noted below, inactivation of PKA activates a stress response through Msn2/4 and various stresses induce at least a transient cessation in cell cycle progression. Moreover, activation of Msn2/4 induces Yak1 expression, whose activity stimulates the Pop2 polyA binding complex required for G1 arrest in post diauxic cells (184, 254) and *XBP1*, a repressor of G1 cyclins (167). Thus, an economical model would posit that specific translation required for "Start" may be under indirect control of the Msn2/4 stress response, which itself is sensitive to PKA activity. However, the process is likely to be more complex than this, perhaps through undefined effects of PKA activity on translation initiation, particularly as this impinges on translation of G1 cyclins.

Hyperactivation of the PKA pathway, in a *bcy1* or *RAS2*[G19V] strain, elicits the opposite cell cycle phenotype to that following inactivation of the pathway, namely failure of cells to arrest at G1 under carbon starvation. Moreover, such cells starved for carbon rapidly lose viability. One interpretation of this observation is that hyperactivation of the pathway catalyzes constitutive execution of "Start," rendering it unresponsive to nutrient signal. However, hyperactive PKA signaling prevents accumulation of storage carbohydrates, such as glycogen and trehalose. Thus, an alternative view of the failure of *bcy1* cells to accumulate in G1 following carbon starvation is that such cells are unable to complete the cell cycle, owing to an absence of reserves. In this model, cells fail to arrest at G1 because the cells simply stop wherever they are in the cell cycle at the time of nutrient deprivation (169). *bcy1* mutants also fail to survive nitrogen starvation likely because of a failure to accumulate nitrogen reserves (169). This suggests that the PKA pathway affects accumulation of nutrient reserves generally.

Futcher has recently proposed an additional layer of regulation of "Start" as a function of carbon source availability (79). He cites evidence that cells growing on nonfermentable carbon sources accumulate internal stores of glucose in the form of glycogen and trehalose during the cell cycle. When and only when a cell

has accumulated sufficient reserves does the cell metabolize the glucose via glycolysis to provide a short burst of energy to increase translational capacity and push the cell through "Start." This still raises the question of how cells would know they have accumulated sufficient stores of carbohydrates. Nonetheless, this model might account for the inability of *bcy1* cells, which cannot accumulate storage carbohydrates, to grow on nonfermentable carbon sources. Such cells would be expected to accumulate as unbudded cells, since they would not be able to execute "Start," an untested prediction. Recent experiments suggest this burst of fermentation at G1 in otherwise respiring cells may have the more important role of temporally segregating DNA replication from respiration (36). Thus, the burst of fermentation would not only provide a kick-start for "Start" but also extend into S phase so as to minimize the possibility of oxidative damage during replication.

Size control. Besides regulating execution of "Start," nutrient availability controls the size at which cells initiate the cell cycle. In rich media, cells execute "Start" at a larger cell volume than do cells growing on poor nutrients. This is true even though cells growing in poor nutrient conditions have lower levels of G1 cyclins than do cells growing in rich media. Thus, cells in poor nutrients might be expected to be larger, since they would require more time in G1 to execute "Start" (247). This conundrum is resolved by the fact that the threshold level of G1 cyclins required to execute "Start" is significantly lower in slower-growing cells than in rapidly growing cells. This modulation of the size threshold as a function of nutrient status occurs quite rapidly: Addition of glucose to glycerol-grown cells causes an immediate delay in the execution of "Start," yielding a transient rise in the percent of unbudded cells (120).

Mutational analysis of size control suggests a mechanism by which cells establish a size threshold for "Start." Inactivation of either Sfp1 or Sch9 reduces the size at which cells initiate the cell cycle and renders that size insensitive to nutrient control (124, 125). Moreover,

mutations that attenuate PKA activity also affect the size threshold such that reduced activity results in small cells and hyperactivation results in large cells (11, 12, 266). Finally, carbon source effects on cell size are manifested even in $cln3\Delta$ $bck2\Delta$ $whi5\Delta$ cells, which lack all the upstream regulators of "Start" (125).

As noted above, those components that affect the size threshold—Ras/PKA, Sch9 and Sfp1—all converge on ribosome biogenesis. This convergence has prompted a model in which the rate of ribosome biogenesis sets the size threshold by an as-yet undefined mechanism such that high biogenesis rates results in a high size threshold (125, 126, 234). Consistent with this notion, recent studies assessing the effect of inactivating ribosome biogenesis on initiation of the cell cycle confirm that the rate of ribosome biogenesis can affect execution of "Start" long before any changes in overall protein synthesis rates are observed (19). However, in this study the effect was opposite from that proposed by the model, increasing cell size in a Whi5-dependent manner upon lower biogenesis rates. These observations suggest that ribosome biogenesis can both negatively regulate "Start" by increasing the threshold size level and positively regulate "Start" by inhibiting Whi5. In sum, nutrients influence initiation of the cell cycle by taking into account both the current translation capacity of the cell, through the instantaneous translation rate, and the commitment to the future translational capacity of the cell, through the rate of ribosome biogenesis. We presume these two factors are balanced to optimize the competitive advantage of cells under a fluctuating and uncertain nutritional environment.

STRESS RESPONSE

Stress response, nutritional availability, and growth rate are intimately interconnected. Deprivation for any essential nutrient not only slows cell growth and elicits a nutrient-specific transcriptional response but also provokes an environmental stress response that is common to all nutrients (88). In fact, many of these environmental stress response transcriptional changes are proportional to the doubling time of the culture under the limiting nutritional conditions. However, the causal connection between growth rate and stress response is ambiguous: Does slow growth resulting from nutrient deprivation elicit the stress response, does the stress response resulting from nutrient deprivation cause slow growth, or are both growth and stress response independently regulated by nutritional status? Part of the answer, as noted below, is that a significant fraction of the stress response system is directly responsive to nutrient signaling.

Msn2 and Msn4 are Cys_2His_2 Zn-finger DNA-binding proteins that induce transcription of a number of environmental stress response genes, notably those containing stress response elements (STRE) in their promoters. Several nutrient signaling networks impinge on these factors to regulate their subcellular localization and thus their access to STRE sites (90). PKA directly phosphorylates the Msn2 nuclear localization signal (NLS) and this phosphorylation inhibits its function. As a consequence, Msn2 and Msn4 reside in the nucleus when PKA activity is diminished and localize to the cytoplasm when PKA is active, a process that also requires the nuclear export factor Msn5 (91). Nitrogen starvation and stresses such as heat shock or osmotic shock do not affect the NLS function of Msn2 but rather control subcellular localization through modification of a nuclear export signal (NES) localized in the amino terminal domain of the protein (91). In this case, these stimuli act through PP2A to dephosphorylate the NES domain in order to retain the protein in the nucleus. Accordingly, Msn2, or the domain containing the Msn2 NES plus a constitutive NLS, localizes to the nucleus following stress or nitrogen starvation but fails to do so in $cdc55$, $tpd3$, or $pph21$ $pph22$ strains, i.e., strains lacking any of the components of the PP2A holoenzyme (239). The PP2A-like phosphatase, Sit4, does not play a role in Msn2 localization or function. Inactivation of the PP2A associated protein Tap42 mimics this stress/starvation

response, suggesting that Tap42 serves as an inhibitor of PP2A activity with regard to Msn2 NES function (67, 239). Rapamycin treatment also induces nuclear localization of Msn2 via the NES through the activation of PP2A, although with a slower kinetics than that seen with stress or nitrogen starvation. This observation reinforces the idea noted above that nitrogen starvation may be signaled in parallel with, rather than through, TORC1. Finally, PKA also phosphorylates the NES site, so the subcellular localization specified by the NES is determined by a competition between PP2A activity and PKA activity. In sum, a number of nutrient pathways impinge on Msn2 and Msn4 to affect their nuclear localization and induction of stress response genes. The glucose and nitrogen pathways affect localization by distinct mechanisms while TORC1, stress, and nitrogen availability all act through PP2A, possibly by parallel pathways.

Recent observations suggest that in cells subject to mild stress, Msn2 cycles into and out of the nucleus completely in concert in each cell, with a periodicity of about ten minutes at 30° (115). The fact that this cycling is not observed in strains carrying a PKA mutant insensitive to regulation by cAMP suggests that Msn2 cycling results from waves of increasing and decreasing cellular cAMP levels, propagated through a dynamic negative feedback loop mediated by PKA (85).

Several other stress-responsive pathways are sensitive to nutritional input. PKA regulates stress responsive genes such as *HSP12* and *HSP26* in an Msn2/4-independent manner through modulation of Hsf1 (73). Hsf1 induces expression of heat shock responsive genes by binding to HSE in the promoters of these genes. Diminished PKA function activates Hsf1 at some, albeit not all, HSE promoters. Whether the effect of PKA on Hsf1 is direct or indirect has not been resolved.

The Hog1 MAPK pathway promotes adaptation to hyperosmotic stress by stimulating transcriptional and metabolic changes as well as cell cycle arrest (71, 107). Approximately 7%

of yeast genes change expression upon hyperosmotic stress, much of which results from Hog1 modulation of several transcription factors, including the activators Hot1 and Smp1 and the repressor Sko1. Phosphorylation of Sko1 by Hog1 converts it from a repressor to an activator. Moreover, both PKA and Sch9 also phosphorylate Sko1. The phenotypic consequence of PKA phosphorylation is minimal under normal conditions, but Sch9 is required for Sko1 activation of target genes and effective response of cells to hyperosmolarity (206, 216). These results would suggest that nutrient starvation or rapamycin treatment might diminish the response of yeast cells to hyperosmolarity, an experiment that has not been reported.

Returning to the issue of causality between nutrient deprivation, growth arrest and the environmental stress response, we note that deletion of both *MSN2* and *MSN4* suppresses the lethality resulting from loss-of-function mutations in the Ras/PKA pathway (254). In addition, rapamycin treatment both arrests cell growth and activates Msn2/4 in a PP2A-dependent mechanism (239). Mutations inactivating PP2A render the cell resistant to growth inhibition by rapamycin (118). Thus, genetics and biochemical data consistently place Msn2 and Msn4 downstream of both PKA and TORC1. Moreover, these observations suggest that growth arrest attendant on rapamycin treatment or loss of Ras/PKA function results substantially from unfettered Msn2/4 activity. In contrast to this view is the fact that starvation of a leucine auxotroph for leucine induces an environmental stress response essentially identical to that following starvation of cells for carbon or nitrogen sources (237). Thus, in this case growth arrest and the stress response appear to result from attenuation of translation or some subsequent metabolic alteration, rather than a direct signaling process. In sum, a simple causal connection among nutritional availability, stress response, and growth arrest does not readily emerge from current data. This may, in fact, reflect a more complex interrelation among these processes.

Metabolic Effects

Several studies have begun to examine the global metabolic changes attendant on transition from poor to rich nutrient state and vice versa. Studies on glucose starvation or refeeding of yeast cells document the expected changes attendant on transition between fully respiratory and fermentative modes of growth as well as an unexpected depletion in adenine nucleotides upon glucose addition (27, 139). For instance, levels of fructose-1,6-bisphosphate (FBP) drop significantly and phosphoenol pyruvate (PEP) levels rise upon carbon starvation, reflecting the reduced flow through glycolysis (27). The rationale for adenine depletion on glucose addition is not clear, but one consequence is rapid upregulation of expression of genes in purine biosynthesis. In addition, cells to which glucose is added suffer an increased drain on sources of one-carbon metabolites to fulfill the methylation requirements from increased rRNA biogenesis. Accordingly, such cells upregulate genes encoding enzymes in methionine biosynthesis and tetrahydrofolate metabolism.

The observed metabolic consequences of nitrogen starvation conform to prior expectations, although they also identify unexpected metabolic responses to starvation (27). As anticipated, cells starved for ammonia show a significant decrease in intracellular glutamine and a corresponding increase in α-ketoglutarate. However, glutamate levels, which might have been expected to drop under these conditions, remain constant. A consistent corollary to this observation is that levels of tryptophan, which obtains its amide group from glutamine, drop under these conditions but levels of phenylalanine and tyrosine, which obtain their amide group from glutamate, remain constant.

The metabolic changes observed upon nutrient transitions result in part from the change in metabolite availability but also from posttranslational modification of metabolic enzymes by nutrient-sensing pathways and, in a longer timeframe, from changes in transcription of key metabolic enzymes. The above metabolic data on glucose transitions highlight several well-established cases of enzyme modification by PKA. For instance, PKA stimulates proteolysis of fructose bisphosphatase (FBPase) and stimulation of Pfk2, which converts phosphofructose to fructose-2,6-bisphosphate, an allosteric activator of Pfk1 (28). Thus, loss of PKA activity on glucose starvation would be expected to enhance FBPase activity and diminish Pfk1 activity, yielding the observed decrease in FBP levels on glucose starvation. Similarly, pyruvate kinase activity is stimulated by PKA-dependent phosphorylation (211). This may account in part for the increase in PEP on carbon downshift. How much of the additional dynamics of metabolite concentrations results from signaling processes remains to be resolved. Future global phosphoproteome studies in conjunction with continued global metabolome studies should prove quite informative in this regard.

Glucose sparing. Yeast cells starved for nitrogen or some nutrient other than glucose arrest as unbudded cells in a metabolically quiescent state in which fermentation of glucose is suppressed. Accordingly, excess external glucose is not depleted and ethanol does not accumulate. In contrast, auxotrophs starved for the required amino acid arrest growth but continue to ferment glucose, thereby depleting external glucose and accumulating ethanol in the medium (26a). This observation points to the existence of cross control in metabolism such that "natural" starvation for an essential nutrient elicits a coherent growth cessation in which the cell's metabolic activity toward other nutrients is suppressed. Moreover, this cross-metabolic control is not simply the consequence of growth arrest, since "unnatural" starvation does not elicit this cross-metabolic regulation. The sensing, signaling, and response pathways responsible for this cross-metabolic regulation are unknown. Nonetheless, the failure of cells subject to "unnatural" starvation to perform this task is redolent of the Warburg effect, in which cancer cells excessively ferment glucose into lactose.

Autophagy. Autophagy is a vacuolar degradative pathway for bulk proteins, which provides a survival mechanism for cells deprived of nutrients. Upon nutrient starvation, cells elaborate a double-membrane vesicle, termed an autophagosome, that sequesters cytoplasmic material and that subsequently fuses with the vacuole, resulting in the breakdown of the contents. The resulting small molecules are released back into the cytosol and reused for the synthesis of new proteins that are required for cells to survive during these conditions (181, 297).

Although the mechanism by which nutrient starvation induces autophagy is not fully understood, TORC1, PKA, and Sch9 all participate in the process. Inactivation of TORC1 induces autophagy (196) while constitutive activation of PKA effectively prevents induction of autophagy by rapamycin or nutrient depletion (30). Inactivation of PKA with a dominant negative Ras2^{G22A} mutation or by drug addition to strains carrying analog-sensitive alleles of all three Tpk's can induce autophagy in nutrient-rich conditions without rapamycin (30, 298). However, the induction of autophagy in this situation is less efficient and slower than that seen with inactivation of TORC1. Inactivation of Sch9 alone has little effect on autophagy, but its inactivation in conjunction with inactivation of PKA enhances the response seen by inactivating PKA alone. Moreover, the inductive effect of loss of PKA/Sch9 is additive with that from rapamycin addition, suggesting that PKA/Sch9 and TORC1 comprise independent modes of regulating autophagy.

The locus through which nutrient signaling pathways impinge on the autophagic process is not entirely clear. In yeast, nearly 30 proteins function specifically in autophagy-related pathways. Most of these Atg proteins localize at a perivacuolar site, termed the preautophagosomal structure (PAS), where they likely function in the formation of the autophagosome. Of the Atg proteins, Atg1, Atg13, Atg17, and Atg18 are candidates to receive the signal from nutrient pathways (127, 129). Rapamycin treatment or nitrogen starvation leads to a rapid dephospho-

rylation of Atg13. This conversion apparently facilitates the interaction of Atg13 with Atg1 and Atg17. Atg1 is a protein kinase and its activity is stimulated by formation of the complex during autophagy, although the role of Atg1 kinase activity in autophagy remains unclear (190). Atg1, Atg13, and Atg18 all contain PKA consensus phosphorylation sites, but whether phosphorylation of these Atg proteins by PKA is functionally linked to autophagy remains unresolved. Atg1 is mislocalized in cells expressing a hyperactive Ras mutation, Ras2^{G19V}, whereas a mutant Atg1 protein lacking PKA phosphorylation sites is properly localized at the PAS in the presence of this mutation (29). This altered Atg1, however, does not display constitutive autophagy activity in the presence of the Ras2^{G19V} mutant protein, so it is not the sole locus through which PKA regulates autophagy. Finally, depletion of Msn2/4 or Rim15 blocks autophagy that is induced by PKA and Sch9 inactivation. Thus, nutrient regulation of autophagy may prove to be an outcome of the stress response.

Extended autophagy results in expansion of the vacuole, which becomes engorged from extensive fusion with autophagic vesicles. Removal of this accumulated material can occur by retrograde trafficking out of the vacuole or by a process termed microautophagy, in which vesicles form in the vacuole from direct invagination of the vacuolar membrane. Microautophagy in yeast occurs only after extended macroautophagy induced by starvation or treatment with rapamycin, so the direct regulation of this process is difficult to pinpoint. However, this process requires the GRE/EGO complex discussed above, suggesting a connection between TORC1 signaling and microautophagy (65).

DEVELOPMENT

Filamentous Growth

Yeast cells, particularly those derived from Σ1278b, pursue in response to partial starvation a developmental program that one might
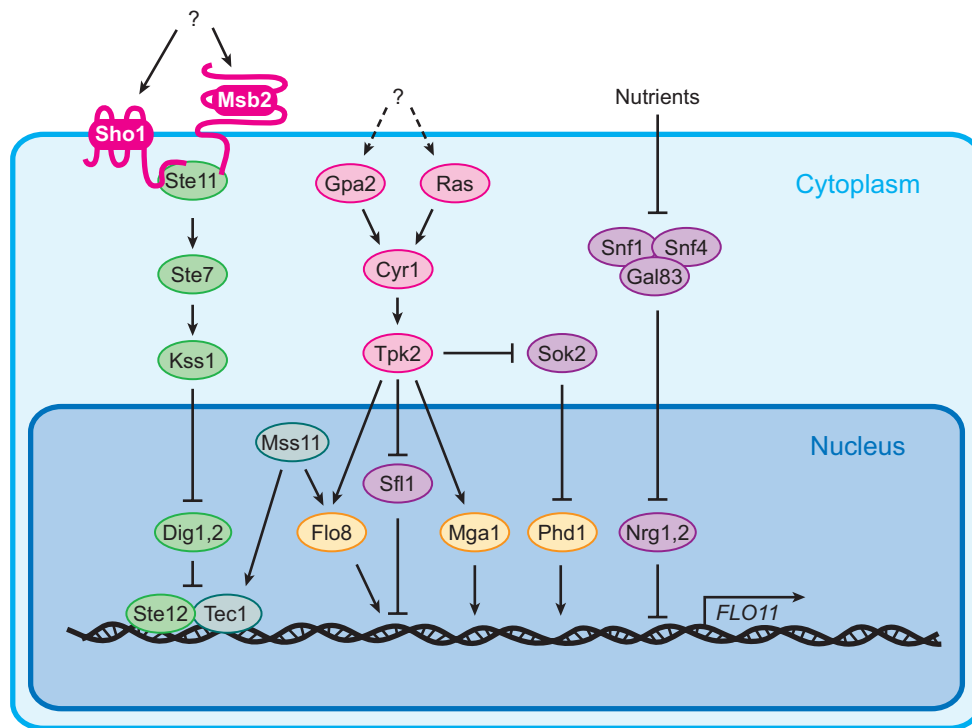
Figure 6

Nutrient regulation of filamentous development. A collection of transcriptional activators (Ash1, Flo8, Mga1, Mss11, Phd1, Ste12, and Tec1) and repressors (Ngr1, Ngr2, Sok2, and Sfl1) control expression of FLO11, encoding the mucin-like protein, Muc1, and a large collection of additional genes required for diploid pseudohyphal growth and haploid invasive growth in response to partial nutrient starvation and other less well defined conditions. The network of interactions modulating these factors in response to external conditions is shown.

interpret as a foraging mechanism. Diploid cells subjected to limiting nitrogen or haploid cells subjected to limiting glucose become elongated, exhibit polar budding, suppress budding in mother cells, undergo cytokinesis, but fail to separate and elaborate extracellular glucanases (82, 201). This cohort of features yields chains of cells, referred to as pseudohyphae in diploids or filaments in haploids, capable of invading the underlying substratum. Although these two filamentous growth programs have been viewed as distinct processes responding to different nutritional cues, recent studies have shown that the signaling pathways responsible for the two programs overlap significantly (**Figure 6**).

Activation of PKA—through use of activating alleles of *RAS2* or *GPA2* or through addition

of exogenous cAMP—stimulates diploid pseudohyphal growth and haploid invasive growth. Individual PKA catalytic subunits play distinct roles in pseudohyphal growth with Tpk2 stimulating filamentation and Tpk1 and Tpk3 inhibiting the process, the latter likely through feedback inhibition of PKA activation (203, 225). The fact that PKA stimulates filamentation is unexpected since filamentation, particularly haploid invasive growth, is stimulated by glucose limitation and PKA activity is generally associated with increased glucose availability. However, filamentous growth resulting from constitutive PKA activity can be further stimulated by glucose limitation, suggesting that glucose levels influence filamentation through a mechanism other than PKA and, furthermore,

that PKA is activated under conditions promoting filamentation by a mechanism distinct from glucose activation. Although initial studies suggested that Ras might stimulate filamentation through activation of the filamentous growth (FG) MAPK pathway, more recent results indicate that Ras influences filamentation solely through modulation of PKA (97, 186). This likely applies to the parallel Gpr1/Gpa2 branch upstream of PKA as well. Chen & Fink have recently shown that filamentation is stimulated by phenylethanol and tryptophol, excreted metabolites of phenylalanine and tryptophan whose production is stimulated by nitrogen limitation, suggesting that yeast may employ a form of quorum sensing to initiate filamentation (35). This observation likely explains prior reports noting that various excreted alcohol metabolites of yeast stimulate pseudohyphal growth. Stimulation of filamentation by phenylethanol and tryptophol requires Tpk2, suggesting that the Ras/PKA pathway in this context may be responsive to these autoinducers, rather than to glucose. How the presence of these autoinducers influences PKA and whether these are the only inputs into PKA remain to be resolved.

Snf1 serves as the primary locus coupling nutrient limitation to haploid invasive growth and diploid pseudohyphal growth (48, 144). This role in filamentation requires phosphorylation of T210 in the activating loop of Snf1, although the particular upstream kinase(s) required for this phosphorylation under conditions promoting filamentation has not been identified. Haploid invasive growth also requires the Gal83 β-subunit of the Snf1 complex and, to a lesser extent, Sip2; *gal83*Δ strains are deficient in invasive growth and haploid *gal83*Δ *sip2*Δ cells are as defective in filamentation as are *snf1*Δ cells (278). In contrast, any of the β-subunits can support diploid pseudohyphal growth (199). The primary function of Snf1 in stimulating filamentation is inactivation of the transcriptional repressors, Nrg1 and Nrg2, in the promoters of *FLO11* and a myriad other genes whose induction is required for the developmental program (144). While the partic-

ipation of Snf1 in mediating induction of haploid invasive growth is consistent with its role in signaling limited glucose availability, the participation of Snf1 in mediating diploid pseudohyphal growth in response to nitrogen limitation is less intuitive. However, recent results suggest that Snf1 T210 phosphorylation is stimulated not only by glucose limitation but also by nitrogen limitation as well as by rapamycin treatment, even in the presence of high levels of glucose (199). Thus, Snf1 appears to regulate filamentation in response to a variety of nutritional cues.

Several additional systems influence diploid filamentation in response to nitrogen limitation, although no coherent model for nitrogen sensing emerges from these anecdotal observations. First, inactivation of the high-affinity Mep2 ammonia permease blocks pseudohyphal differentiation in response to low ammonium (164). The fact that such strains grow normally at low levels of ammonium and do not exhibit transcriptional responses associated with nitrogen starvation has been taken as evidence that Mep2 influences pseudohyphal growth directly as an ammonia sensor rather than indirectly through metabolic effects. However, no signaling pathway has been associated with this putative sensor (22). Second, *ure2* and *gln3* mutants, which have opposing effects on transcription of nitrogen discrimination pathway genes, both fail to exhibit pseudohyphal growth in response to nitrogen starvation (164). This suggests that either constitutive activation or uninducibility of the NDP leads to filamentation. Third, *ptr3* and *ssy1* mutants, defective in induction of amino acid permeases, exhibit enhanced haploid invasive growth, suggesting that diminished internal amino acid levels may serve as a signal for filamentous growth (133). Moreover, a mutation in glutamine tRNA[CUG] promotes enhanced pseudohyphal growth at high nitrogen levels without affecting apparent translation rates or NDP gene expression (16, 189). Finally, as noted above, nitrogen depletion or rapamycin treatment induces Snf1 activation, establishing a direct link between nitrogen deprivation and the regulation of

pseudohyphal development. However, rapamycin, even at sublethal doses, inhibits rather than activates filamentous growth (49). This provides further evidence that TORC1 inactivation does not fully mimic nitrogen depletion. In sum, all these observations are difficult to reconcile into a simple model for nitrogen regulation of filamentous growth. The most compelling observations—that Snf1 both responds to multiple forms of nutritional deprivation and is required for filamentation—posit Snf1 as the primary route for connecting nutritional state to this developmental switch.

A nutrient-independent pathway comprising components of the pheromone-responsive MAP kinase pathway also regulates filamentous growth. Mutations ablating any component in this pathway—the MAPKKK Ste11, the MAPKK Ste7 or the MAPK Kss1—suppress filamentous growth both in haploids and diploids while a hyperactive allele of Ste11 elicits precocious filamentous growth (186). Initial studies suggested that this FG MAPK pathway responded directly to Ras stimulation, much in the way the ERK MAP kinase pathway in mammalian cells transmits signals emanating from Ras activation induced by upstream transmembrane receptors. However, more recent results indicate that Ras affects filamentation solely through PKA (97). Rather, the FG MAPK pathway responds to activation from the Sho1 osmolarity receptor as well as the transmembrane Msb2 mucin receptor (47). Besides hyperosmolarity, the extracellular signals to which the FG MAPK pathway responds are not well defined, although this pathway may sense whether cells are on a solid surface rather than in liquid suspension. Remarkably, despite the fact that the FG MAPK pathway and the pheromone MAPK pathway share a number of components, the pheromone MAPK pathway faithfully elicits a pheromone response without activating a filamentous response and filamentation-inducing conditions elicit a filamentation response without inducing a pheromone response. This specificity is achieved in part by pheromone-induced degradation of the FG-specific transcription factor Tec1, by subtle aspects of signal channeling achieved by the Ste5 scaffold protein and perhaps by lateral inhibition between the FG and pheromone MAPKs, Fus3, and Kss1 (38, 74, 221).

The complex collection of cell cycle, metabolic and morphological features that underlie the filamentous phenotypes results from alterations in activity of a number of enzymes and changes in expression of a substantial collection of genes. In fact, more than 800 genes exhibit a statistically significant change in expression in transition from yeast to pseudohyphal growth, and mutation of any of more than 180 genes affects this transition (215). The transcriptional changes associated with filamentation are coordinated by a collection of transcription factors responsive to the various nutrient signaling pathways described above (**Figure 6**). These include the transcriptional activators Ste12, Tec1, Ash1, Flo8, Phd1, Mss11, and Mga1 and the transcriptional repressors Sok2, Nrg1, Nrg2, and Sfl1. These factors comprise a highly connected and complex network with Mga1 and Phd1 serving as the predominant modulators of the filamentous transitions (25). In some cases, these factors all converge on the promoter of a gene, such as *FLO11*, whose expression is required for pseudohyphal growth, but more often these factors regulate distinct but overlapping suites of genes whose concerted action orchestrates the filamentous program. Moreover, the patterns of genes regulated by these factors highlight two distinct signaling pathways that together regulate this developmental program—Ras/PKA and the FG-MAPK pathway. In many cases direct connections between signaling pathways and transcriptional regulators have been established. For instance, the competitive binding of the Flo8 activator and Sfl1 repressor to the same promoter sites is regulated directly by PKA phosphorylation of the two factors (205, 225). Moreover, the recruitment of the Mga1 activator to promoters requires prior binding of Flo8 (25). Sok2, Ash1, and Phd1 comprise a regulatory module whose activity may be directly responsive to PKA (204, 252, 283). The Ste12/Tec1 activator complex is regulated by

the FG MAPK pathway through the Dig1,2 repressors. Finally, as noted above, Snf1 regulates expression of a number of filamentous specific genes through inactivation of the Nrg1/Nrg2 repressors. Thus, the transcriptional network regulating filamentous development responds directly to the signaling pathway sensing conditions appropriate for this response.

As evident from above, haploid cells initiate invasive growth and diploid cells initiate pseudohyphal growth in response to nutritional cues through essentially the same signaling networks and transcription factors. Mutation of a signaling or transcriptional component that affects one process almost invariably similarly affects the other. This is true even of the FG MAPK pathway, whose components normally play quite distinct roles in haploid and diploid cells. Thus, these two filamentous programs likely respond to the same environmental cues, and the use of different nutritional conditions for induction of the two programs likely reflects historical bias rather than fundamental differences in the biology of the two processes. The Snf1 pathway likely plays the predominant role in nutritional sensing in both processes, responding both to glucose and nitrogen limitations. Thus, while there are likely subtle distinctions in the physiology and regulation of the two programs, they are essentially two sides of the same coin.

Starvation, Stationary Phase, and Meiosis

Quiescence. Yeast cells, like all other living cells, spend most of their time in a quiescent state induced by starvation for one or more nutrients. Haploid or diploid yeast cells starved for carbon, nitrogen, phosphate, or sulfur cease accumulating mass, arrest cell cycle progression prior to "Start," and enter a poorly defined G_0 state. G_0 cells do have a number of distinguishing characteristics, including a thickened cell wall, increased storage carbohydrates, enhanced resistance to heat and high osmolarity, substantially reduced translation, a specific transcriptional profile, and, most important, the ability to maintain viability under the starvation condition (94). This last characteristic distinguishes cells suffering from "natural" starvation, for a carbon source, for instance, from those subject to an "unnatural starvation" such as auxotrophic cells deprived of the required amino acid. In the latter case, cells fail to arrest uniformly as unbudded cells and rapidly lose viability (237). Thus, the quiescent G_0 state requires the coordinated and deliberate activity of cells sensing impending depletion of a core nutrient and not simply the cessation of growth attendant on abrogation of protein or RNA synthesis.

Several studies have addressed the genetic underpinnings of quiescence, primarily by identifying those genes whose deletion enhances or diminishes survival in stationary phase, a process occasionally referred to as "chronological life span" (172, 213). Such mutants highlight the signaling pathways responsible for entry into stationary phase, such as TOR and Ras/PKA, as well as those genes required for persistence during quiescence and those required for exit from stationary phase (94). Mutants defective in signaling through the Ras/PKA pathway exhibit many of the characteristics of stationary phase cells, whereas hyperactive mutants, such as *bcy1*, fail to assume stationary phase characteristics and rapidly lose viability upon starvation. However, cells exhibiting low-level constitutive PKA activity enter stationary phase normally and maintain normal stability, indicating that signaling through PKA may participate in, but is not absolutely required for, entering quiescence. Other nutrient-sensing pathways contribute to quiescence, even upon glucose starvation. A number of mutants associated with diminished Tor function have enhanced persistence during stationary phase. This is consistent with the fact that rapamycin treatment induces many of the characteristics associated with stationary phase cells (114). However, unlike quiescent cells obtained by starvation, rapamycin treated cells show substantial delay in resuming growth, so rapamycin treatment does not precisely

phenocopy starvation (65). The large collection of additional mutants with either enhanced or diminished survival in stationary phase highlights processes other than nutrient signaling, such as mitochondrial function, that are required for normal entry into or survival during stationary phase. However, most of these mutants do not comprise clearly identifiable functional categories and their specific roles in stationary phase survival have not been determined (213).

The signaling pathways regulating quiescence converge on several regulatory proteins responsible for executing various aspects of the quiescence program. Induction of stress resistance upon starvation results in part from activation of the Msn2/Msn4 stress-responsive transcription factors, whose nuclear localization is independently regulated by the PKA and TORC1 signaling networks, as discussed above.

A second critical regulator of entry into quiescence is the Rim15 kinase (259). *rim15* mutants exhibit many stationary phase defects, such as diminished trehalose and glycogen accumulation, reduced expression of stress response genes, decreased thermotolerance, and failure to arrest prior to S phase. Moreover, deletion of *RIM15* suppresses the lethality of *tpk1Δ tpk2Δ tpk3Δ* strains, suggesting that Rim15 inhibits expression of genes required for growth. Reciprocally, overexpression of Rim15 during exponential growth inappropriately elicits some stationary phase responses and causes a synthetic growth defect with mutations that partially attenuate PKA signaling. Consistent with these genetic epistasis studies, Rim15 kinase activity is negatively regulated by PKA-dependent phosphorylation (223). Moreover, TORC1, Sch9, and the phosphate-responsive signaling complex Pho80/Pho85 all regulate Rim15's nuclear/cytoplasmic distribution. The cyclin/CDK complex Pho80/Pho85 phosphorylates Rim15^{T1075} to promote association of Rim15 with the cytoplasmic 14-3-3 protein Bmh2 and Sch9 likely phosphorylates a second 14-3-3 binding site on Rim15. TORC1 inhibits dephosphorylation of T1075, and perhaps the Sch9 sites as well, presumably through

inhibition of one or more of its effector PP2A phosphatases (207, 282). Thus, three distinct nutrient-responsive pathways converge on Rim15 through distinct mechanisms. How Rim15 integrates input from these three pathways is not clear: Starvation for any one of the three nutrients is sufficient to induce quiescence and yet full Rim15 function should require both nuclear localization as well as activation of its kinase activity, necessitating input from at least two nutrient pathways. Thus, this aspect of Rim15 function remains to be resolved.

Rim15's effect on quiescence derives in part through changes in the transcriptional spectrum of the cell, mediated through the stress response transcription factors Msn2/Msn4 and the related post diauxic shift transcription factor Gis1 (31). How Rim15 affects the activity of these transcription factors is not known. While it may affect nuclear localization and/or activity of the factors, another intriguing possibility is that Rim15 may stimulate Msn2/4's interaction with TFIID through a novel PP1 phosphatase regulated by the Ccr4/Caf1/Not complex (156). As noted above, components of this complex also participate in the deadenylation complex that regulates mRNA stability under starvation conditions, a process influenced by the Yak1 kinase, suggesting that it may play a multifaceted role in coordinating entry into quiescence. Rim15 also binds to the Tps1 component of the trehalose synthase complex, suggesting that part of its role in quiescence involves direct regulation of key enzymatic activities. Finally, while overexpression of Rim15 induces some of the phenotypes of quiescence, it by no means induces a full-blown stationary phase response. Accordingly, other as-yet unidentified factors participate in the execution of the stationary phase program.

Most of the studies on quiescence in yeast examine stationary phase cells, that is, cells that were first depleted for glucose and then for the residual ethanol in the culture generated from the prior fermentation of glucose. Less information is available on the nature of cells that have entered quiescence owing to depletion of other nutrients, such as nitrogen or sulfur. In

fact, most of the studies on stationary phase cells are conducted with auxotrophic strains, precluding cross-studies of these strains on nitrogen starvation. Accordingly, we do not know whether cells starved for one nutrient enter the same G_0 state as those starved for a different nutrient. Transcriptional profiling indicates that, as noted above, starved cells induce a common set of stress-responsive genes regardless of which nutrient is limiting. However, cells also exhibit nutrient-specific alterations in gene expression upon starvation (88). Glucose addition alone, but not any other nutrient, stimulates growth-related events of stationary phase cells (93). Moreover, mutations that eliminate signaling through the PKA pathway cause a significant delay in exiting stationary phase upon glucose addition (119). However, the presence of glucose clearly does not stimulate cells in quiescence from nitrogen starvation to resume growth. So, from these perspectives, all quiescent states are not the same. Cross mutational studies of quiescence should help resolve the extent of the differences among cells starved for different nutrients. For example, what are the effects on survival during nitrogen starvation of mutants identified as defective in surviving stationary phase, i.e., carbon starvation? Such studies would not only identify differences among different quiescent cells but also define the core constellation of processes that permit survival during starvation.

Meiosis. MATa/MATα diploid cells can embark on meiosis and sporulation in response to a nutritional environment that meets three criteria: the absence of one essential growth nutrient such that cells arrest in G1; the absence of glucose; and the presence of a nonfermentable carbon source. Although nitrogen starvation is the normal laboratory condition for sporulation, starvation for phosphate or sulfur can also induce sporulation even in the presence of an adequate nitrogen source. The critical regulatory role of starvation in eliciting meiosis and sporulation is to lower the level of Cln/Cdk activity. In addition, glucose addition, even in the presence of a nonfermentable carbon source,

inhibits sporulation. Finally, these specific starvation conditions are required not only to initiate meiosis and sporulation but also to maintain the developmental program. Refeeding cells in the early stages of meiosis, up to approximately the pachytene stage, will abort the program and return cells to mitotic growth.

The mechanism by which nutritional cues elicit initiation of meiosis has been substantially explored and several useful reviews on this topic provide additional details (110, 147). Nutrient signals impinge on the expression and function of two key regulators of initiation of meiosis, the transcription factor Ime1 and the S/T kinase Ime2 (**Figure 7**). Ime1 activates transcription of Ime2 as well as other early meiosis genes (EMGs) and transcription initiation of *IME1* responds to mating-type, glucose and nonfermentable carbon sources through distinct elements in the *IME1* promoter region. Glucose represses *IME1* expression through a 32 bp IREu element, which serves as an activator binding site for the stress response factors Msn2/4. As noted above, these factors are inhibited by glucose through PKA. Moreover, IREu serves as a binding site for Sok2, whose phosphorylation by PKA converts it into a repressor of *IME1* expression. Thus, glucose affects expression of *IME1* both by activating a repressor and inactivating an activator of the gene.

The presence of a nonfermentable carbon source is perceived by the cell as a consequence of its metabolism to CO_2 and resultant alkalization of the medium. High external pH activates a highly conserved fungal pH sensing pathway comprising cell surface receptors and the Rim101 transcription factor (formerly Rim1) that is activated by proteolytic cleavage catalyzed by the Rim13 protease. Remarkably, cleavage occurs on the surface of endosomes through interaction with components of the ESCRT III complex, particularly Snf7, whose primary function is in endocytic trafficking (26). These observations provide further evidence of a connection between endosome metabolism and nutrient signaling. Rim101 regulates initiation of meiosis and adaptation of external alkalization through its activity as a
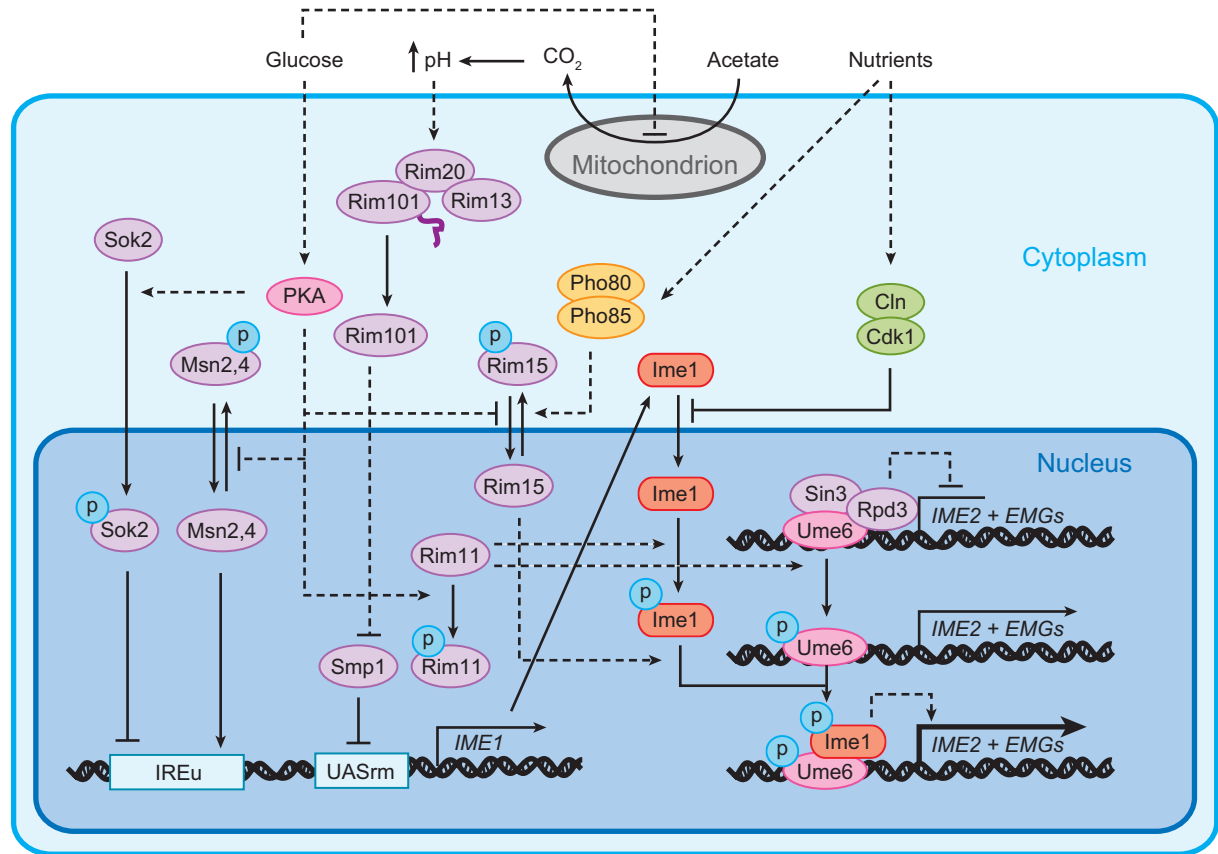
Figure 7

Nutrient control of initiation of meiosis. The presence of a nonfermentable carbon source and the absence of glucose and one or more essential nutrients induces meiosis and sporulation in *MAT*a/*MAT*α diploid cells through a complex regulatory network sequentially controlling expression of *IME1*, encoding a transcriptional activator, and *IME2* and other early meiotic genes (EMGs). Glucose impinges on expression of *IME1* through activation of the Sok2 repressor and inactivation of the Msn2/4 activators as well as by inhibiting respiration, needed to increase external pH to yield proteolytic activation of the Rim101 and subsequent inactivation of the Smp1 repressor. Nutrient starvation reduces Cln/Cdk activity, allowing nuclear import of Ime1 for activation of *IME2* and other EMGs. Mating type controls expression of *IME1* by a circuit not shown. See text for details.

transcriptional repressor, two of whose targets are the transcriptional repressors Smp1 and Nrg1 (152). The effect of Rim101 on *IME1* expression is mediated by a site called UASrm in the promoter (236). Although untested to date, UASrm may serve as the binding site for Smp1 and/or Nrg1, whose repressive activity would be alleviated by Rim101. In fact, Rim101 and Nrg1 act as corepressors by simultaneous binding to promoters of mid- to late sporulation genes (231). However, the fact that deletion of *SMP1*, but not *NRG1*, alleviates the sporulation defect in *rim101Δ* strains would

indicate that Smp1 is the immediate regulator of *IME1* (152). Finally, we note that glucose also likely influences this signaling pathway through repression of respiration, thereby blocking alkalization by blocking metabolism of nonfermentable carbon sources.

Under mitotic growth conditions, expression of *IME2* and other EMGs is repressed by Ume6-mediated recruitment of the histone deacetylases Sin3 and Rpd1 and the chromatin remodeling complex Isw2 to the URS1 sequence in the promoters of the regulated genes. Under meiotic induction conditions, Sin3

and Rpd1 dissociate from Ume6, alleviating repression, while Ime1 associates with Ume6 to recruit the histone acetyl transferase Gcn5 and activate transcription. The GSK3-β homolog Rim11 and the stationary phase kinase Rim15 mediate this transition from repression to activation in response to a variety of nutritional inputs. Both Rim11 and Rim15 are inactivated by phosphorylation by PKA and so are active only in the absence of PKA signaling (232, 259). In addition, as noted above, TORC1 blocks nuclear import of Rim15 and phosphate through Pho80/Pho85 to stimulate nuclear export of the protein. Rim15, perhaps through phosphorylation of Ume6, promotes dissociation of Sin3 and Rpd1, whereas Rim11 phosphorylation of Ime1 stimulates interaction of Ime1 with Ume6 (209). Finally, Cln/Cdk blocks nuclear import of Ime1, an impediment to EMG activation that is alleviated by nutrient starvation–induced arrest in G1. Thus, nutritional cues inform initiation of meiosis through a variety of routes.

As noted above, addition of nutrients to cells undergoing meiosis can abrogate the developmental program and restore cells to mitotic growth. The targets for this nutritional control of meiotic progression have not been fully defined, although some information has emerged. For instance, Ime2, which is required at multiple stages during meiotic progression, is destabilized by glucose addition through degradation targeted by the SCF[Grr1] ubiquitin ligase (218). However, while expression of a degradation-resistant version of Ime2 renders cells resistant to a glucose-induced block to meiotic DNA replication, it does not render later steps in meiosis resistant to glucose. Thus, additional nutrition-sensitive processes have yet to be identified.

Replicative Aging

Replicative aging in yeast is defined as the number of daughters to which a cell can give rise before ceasing to bud. Remarkably, this replicative lifespan of yeast is influenced by both genetic and nutritional factors similar to those that influence the aging processes in larger eukaryotes, including mammals. In particular, caloric restriction extends replicative aging in yeast in a manner reminiscent of the same effect in worms, flies, and mice. Consistent with the contribution of nutrient status to replicative lifespan, mutants defective in Ras/PKA, Sch9, and TORC1 signaling all have extended lifespan (128). Part of the effects of these pathways on aging results from their regulation of stress response, as noted above. However, whether other aspects of nutrient control also influence aging and how activation of the stress response extends replication lifespan remain unresolved.

CONCLUSIONS

Substantial progress has been made in defining the networks in yeast that detect and signal the nature and quantity of available nutrients. One emerging theme from these studies has been the extent of interconnections among the different sensing pathways. For the case of Snf1 and Rgt regulation of hexose transporters, this interconnection provides a graded response such that the transporters with the appropriate affinity and capacity are produced in response to the specific level of external glucose. Similarly, PKA and TORC1 signaling converge on several "response regulators" such as Msn2/4 and Rim15 to promote a unified output from multiple inputs. We anticipate that other interconnections among pathways also provide nuanced responses to different levels or combinations of nutrients. However, more sophisticated analyses and analytical tools need to be applied to tease out these responses. As the complexity of these pathways becomes more apparent, our need increases for quantitative, dynamic models, both to organize and represent the pathways and to predict their behavior. However, even with the substantial amount of information on yeast signaling networks currently available, the uncertainties in the topologies and the lack of knowledge about specific kinetic parameters render straightforward pathway modeling infeasible. Accordingly, we need new computational tools capable of predicting network behavior even in the face of limited data. These have begun to emerge.

FUTURE ISSUES

Remarkable gaps still exist even in the most well studied signaling networks. For instance, we still do not know the means by which glucose activates the Ras/PKA or the means by which TORC1 activity is connected to nutritional status. Moreover, the means by which these pathways control the biological responses of the cells remains unclear. A small sampling of the unresolved questions includes, What is the causal relationship between stress response and growth control? What is the mechanistic basis of the difference between "natural" and "unnatural" starvation? Do yeast cells enter different quiescent states depending on which nutrient is depleted? How is size controlled by nutrient availability and how is size connected to initiation of "Start"? What is the basis for the connection between nutritional status and aging? How does the cell balance input from multiple nutrient pathways to make developmental decisions?

Finally, most of the studies on yeast signaling and the biological consequences of nutrient availability have examined only the aggregate behavior of populations of cells. As a consequence, potentially important differences in the responses of individual cells are ignored. For instance, in a population of cells subjected to conditions inducing filamentous growth, some cells stably assume characteristics of filamentous cells whereas others stably retain their yeast-like morphology and behavior (100). Thus, nutrient input can induce transitions between distinct epigenetic states rather than shifting cells along a continuum between one state and another. Moreover, signaling circuits in yeast appear to be wired to promote different responses among individuals experiencing identical conditions (177). In this manner, individual cells in a population can explore different developmental programs in response to a complex environment, thereby enhancing overall survival of the colony. Thus, a rich area for future studies of nutrient signaling in yeast can be single-cell analysis of behavioral responses. This will likely reveal a much more complex relationship of yeast cells with their nutritional environment than we currently appreciate.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Abdel-Sater F, El Bakkoury M, Urrestarazu A, Vissers S, Andre B. 2004. Amino acid signaling in yeast: Casein kinase I and the Ssy5 endoprotease are key determinants of endoproteolytic activation of the membrane-bound Stp1 transcription factor. *Mol. Cell Biol.* 24:9771–85

2. Abdel-Sater F, Iraqui I, Urrestarazu A, Andre B. 2004. The external amino acid signaling pathway promotes activation of Stp1 and Uga35/Dal81 transcription factors for induction of the AGP1 gene in *Saccharomyces cerevisiae*. *Genetics* 166:1727–39

3. Ahuatzi D, Herrero P, de la Cera T, Moreno F. 2004. The glucose-regulated nuclear localization of hexokinase 2 in *Saccharomyces cerevisiae* is Mig1-dependent. *J. Biol. Chem.* 279:14440–46

4. Ahuatzi D, Riera A, Pelaez R, Herrero P, Moreno F. 2007. Hxk2 regulates the phosphorylation state of Mig1 and therefore its nucleocytoplasmic distribution. *J. Biol. Chem.* 282:4485–93

5. Andreasson C, Heessen S, Ljungdahl PO. 2006. Regulation of transcription factor latency by receptor-activated proteolysis. *Genes Dev.* 20:1563–68

6. Andreasson C, Ljungdahl PO. 2002. Receptor-mediated endoproteolytic activation of two transcription factors in yeast. *Genes Dev.* 16:3158–72

7. Andreasson C, Ljungdahl PO. 2004. The N-terminal regulatory domain of Stp1p is modular and, fused to an artificial transcription factor, confers full Ssy1p-Ptr3p-Ssy5p sensor control. *Mol. Cell Biol.* 24:7503–13

8. Aronova S, Wedaman K, Anderson S, Yates J 3rd, Powers T. 2007. Probing the membrane environment of the TOR kinases reveals functional interactions between TORC1, actin, and membrane trafficking in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 18:2779–94

9. Ashrafi K, Lin SS, Manchester JK, Gordon JI. 2000. Sip2p and its partner snf1p kinase affect aging in *S. cerevisiae*. *Genes Dev.* 14:1872–85

10. Bahn YS, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME. 2007. Sensing the environment: lessons from fungi. *Nat. Rev. Microbiol.* 5:57–69

11. Baroni MD, Martegani E, Monti P, Alberghina L. 1989. Cell size modulation by CDC25 and RAS2 genes in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 9:2715–23

12. Baroni MD, Monti P, Alberghina L. 1994. Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast. *Nature* 371:339–42

13. Batlle M, Lu A, Green DA, Xue Y, Hirsch JP. 2003. Krh1p and Krh2p act downstream of the Gpa2p G(alpha) subunit to negatively regulate haploid invasive growth. *J. Cell Sci.* 116:701–10

14. Beck T, Hall MN. 1999. The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402:689–92

15. Beer MA, Tavazoie S. 2004. Predicting gene expression from sequence. *Cell* 117:185–98

16. Beeser AE, Cooper TG. 1999. Control of nitrogen catabolite repression is not affected by the tRNAGln-CUU mutation, which results in constitutive pseudohyphal growth of *Saccharomyces cerevisiae*. *J. Bacteriol.* 181:2472–76

17. Bernard F, Andre B. 2001. Genetic analysis of the signalling pathway activated by external amino acids in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 41:489–502

18. Bernstein BE, Liu CL, Humphrey EL, Perlstein EO, Schreiber SL. 2004. Global nucleosome occupancy in yeast. *Genome Biol.* 5:R62

19. Bernstein KA, Bleichert F, Bean JM, Cross FR, Baserga SJ. 2007. Ribosome biogenesis is sensed at the Start cell cycle checkpoint. *Mol. Biol. Cell* 18:953–64

20. Bertram PG, Choi JH, Carvalho J, Ai W, Zeng C, et al. 2000. Tripartite regulation of Gln3p by TOR, Ure2p, and phosphatases. *J. Biol. Chem.* 275:35727–33

21. Boban M, Zargari A, Andreasson C, Heessen S, Thyberg J, Ljungdahl PO. 2006. Asi1 is an inner nuclear membrane protein that restricts promoter access of two latent transcription factors. *J. Cell Biol.* 173:695–707

22. Boeckstaens M, Andre B, Marini AM. 2007. The yeast ammonium transport protein Mep2 and its positive regulator, the Npr1 kinase, play an important role in normal and pseudohyphal growth on various nitrogen media through retrieval of excreted ammonium. *Mol. Microbiol.* 64:534–46

23. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD. 2003. Nucleosomes unfold completely at a transcriptionally active promoter. *Mol. Cell* 11:1587–98

24. Bomeke K, Pries R, Korte V, Scholz E, Herzog B, et al. 2006. Yeast Gcn4p stabilization is initiated by the dissociation of the nuclear Pho85p/Pcl5p complex. *Mol. Biol. Cell* 17:2952–62

25. Borneman AR, Leigh-Bell JA, Yu H, Bertone P, Gerstein M, Snyder M. 2006. Target hub proteins serve as master regulators of development in yeast. *Genes Dev.* 20:435–48

26. Boysen JH, Mitchell AP. 2006. Control of Bro1-domain protein Rim20 localization by external pH, ESCRT machinery, and the *Saccharomyces cerevisiae* Rim101 pathway. *Mol. Biol. Cell* 17:1344–53

26a. Brauer MJ, Huttenhower C, Airoldi EM, Rosenstein R, Matese JC, et al. 2008. Coordination of growth rate, cell cycle, stress response, and metabolic activity in yeast. *Mol. Biol. Cell* 19:352–67

27. Brauer MJ, Yuan J, Bennett BD, Lu W, Kimball E, et al. 2006. Conservation of the metabolomic response to starvation across two divergent microbes. *Proc. Natl. Acad. Sci. USA* 103:19302–7

28. Broach JR, Deschenes RJ. 1990. The function of ras genes in *Saccharomyces cerevisiae*. *Adv. Cancer Res.* 54:79–139

28a. Broach JR, Pringle JR, Jones EW, eds. 1992. *Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press

29. Budovskaya YV, Stephan JS, Deminoff SJ, Herman PK. 2005. An evolutionary proteomics approach identifies substrates of the cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* 102:13933–38

30. Budovskaya YV, Stephan JS, Reggiori F, Klionsky DJ, Herman PK. 2004. The Ras/cAMP-dependent protein kinase signaling pathway regulates an early step of the autophagy process in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:20663–71

31. Cameroni E, Hulo N, Roosen J, Winderickx J, De Virgilio C. 2004. The novel yeast PAS kinase Rim15 orchestrates G0-associated antioxidant defense mechanisms. *Cell Cycle* 3:462–68

32. Cardenas ME, Cutler NS, Lorenz MC, Di Como CJ, Heitman J. 1999. The TOR signaling cascade regulates gene expression in response to nutrients. *Genes Dev.* 13:3271–79

33. Carvalho J, Bertram PG, Wente SR, Zheng XF. 2001. Phosphorylation regulates the interaction between Gln3p and the nuclear import factor Srp1p. *J. Biol. Chem.* 276:25359–65

34. Chedin S, Laferte A, Hoang T, Lafontaine DL, Riva M, Carles C. 2007. Is ribosome synthesis controlled by pol I transcription? *Cell Cycle* 6:11–15

35. Chen H, Fink GR. 2006. Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes Dev.* 20:1150–61

36. Chen Z, Odstrcil EA, Tu BP, McKnight SL. 2007. Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. *Science* 316:1916–19

37. Cherkasova VA, Hinnebusch AG. 2003. Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. *Genes Dev.* 17:859–72

38. Chou S, Huang L, Liu H. 2004. Fus3-regulated Tec1 degradation through SCFCdc4 determines MAPK signaling specificity during mating in yeast. *Cell* 119:981–90

39. Claypool JA, French SL, Johzuka K, Eliason K, Vu L, et al. 2004. Tor pathway regulates Rrn3p-dependent recruitment of yeast RNA polymerase I to the promoter but does not participate in alteration of the number of active genes. *Mol. Biol. Cell* 15:946–56

40. Collart MA. 2003. Global control of gene expression in yeast by the Ccr4-Not complex. *Gene* 313:1–16

41. Cooper TG. 2002. Transmitting the signal of excess nitrogen in *Saccharomyces cerevisiae* from the Tor proteins to the GATA factors: connecting the dots. *FEMS Microbiol. Rev.* 26:223–38

42. Costanzo M, Nishikawa JL, Tang X, Millman JS, Schub O, et al. 2004. CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. *Cell* 117:899–913

43. Cox KH, Kulkarni A, Tate JJ, Cooper TG. 2004. Gln3 phosphorylation and intracellular localization in nutrient limitation and starvation differ from those generated by rapamycin inhibition of Tor1/2 in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:10270–78

44. Cox KH, Tate JJ, Cooper TG. 2004. Actin cytoskeleton is required for nuclear accumulation of Gln3 in response to nitrogen limitation but not rapamycin treatment in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:19294–301

45. Crespo JL, Helliwell SB, Wiederkehr C, Demougin P, Fowler B, et al. 2004. NPR1 kinase and RSP5-BUL1/2 ubiquitin ligase control GLN3-dependent transcription in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:37512–17

46. Crespo JL, Powers T, Fowler B, Hall MN. 2002. The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. *Proc. Natl. Acad. Sci. USA* 99:6784–89

47. Cullen PJ, Sabbagh W Jr, Graham E, Irick MM, van Olden EK, et al. 2004. A signaling mucin at the head of the Cdc42- and MAPK-dependent filamentous growth pathway in yeast. *Genes Dev.* 18:1695–708

48. Cullen PJ, Sprague GF Jr. 2000. Glucose depletion causes haploid invasive growth in yeast. *Proc. Natl. Acad. Sci. USA* 97:13619–24

49. Cutler NS, Pan X, Heitman J, Cardenas ME. 2001. The TOR signal transduction cascade controls cellular differentiation in response to nutrients. *Mol. Biol. Cell* 12:4103–13

50. de Boer M, Nielsen PS, Bebelman JP, Heerikhuizen H, Andersen HA, Planta RJ. 2000. Stp1p, Stp2p and Abf1p are involved in regulation of expression of the amino acid transporter gene BAP3 of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 28:974–81

51. de Bruin RA, McDonald WH, Kalashnikova TI, Yates J 3rd, Wittenberg C. 2004. Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. *Cell* 117:887–98

52. De Virgilio C, Loewith R. 2006. Cell growth control: little eukaryotes make big contributions. *Oncogene* 25:6392–415

53. De Virgilio C, Loewith R. 2006. The TOR signalling network from yeast to man. *Int. J. Biochem. Cell Biol.* 38:1476–81

54. De Wever V, Reiter W, Ballarini A, Ammerer G, Brocard C. 2005. A dual role for PP1 in shaping the Msn2-dependent transcriptional response to glucose starvation. *EMBO J.* 24:4115–23

55. DeRisi JL, Iyer VR, Brown PO. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–86

56. Desai N, Lee J, Upadhya R, Chu Y, Moir RD, Willis IM. 2005. Two steps in Maf1-dependent repression of transcription by RNA polymerase III. *J. Biol. Chem.* 280:6455–62

57. Di Como CJ, Arndt KT. 1996. Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. *Genes Dev.* 10:1904–16

58. Didion T, Grausland M, Kielland-Brandt C, Andersen HA. 1996. Amino acids induce expression of BAP2, a branched-chain amino acid permease gene in *Saccharomyces cerevisiae*. *J. Bacteriol.* 178:2025–29

59. Didion T, Regenberg B, Jorgensen MU, Kielland-Brandt MC, Andersen HA. 1998. The permease homologue Ssy1p controls the expression of amino acid and peptide transporter genes in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 27:643–50

60. Dilova I, Aronova S, Chen JC, Powers T. 2004. Tor signaling and nutrient-based signals converge on Mks1p phosphorylation to regulate expression of Rtg1.Rtg3p-dependent target genes. *J. Biol. Chem.* 279:46527–35

61. Dilova I, Chen CY, Powers T. 2002. Mks1 in concert with TOR signaling negatively regulates RTG target gene expression in *S. cerevisiae*. *Curr. Biol.* 12:389–95

62. Dolinski KJ, Heitman J. 1999. Hmo1p, a high mobility group 1/2 homolog, genetically and physically interacts with the yeast FKBP12 prolyl isomerase. *Genetics* 151:935–44

63. Dombek KM, Kacherovsky N, Young ET. 2004. The Reg1-interacting proteins, Bmh1, Bmh2, Ssb1, and Ssb2, have roles in maintaining glucose repression in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:39165–74

64. Dong J, Qiu H, Garcia-Barrio M, Anderson J, Hinnebusch AG. 2000. Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain. *Mol. Cell* 6:269–79

65. Dubouloz F, Deloche O, Wanke V, Cameroni E, De Virgilio C. 2005. The TOR and EGO protein complexes orchestrate microautophagy in yeast. *Mol. Cell* 19:15–26

66. Duvel K, Broach JR. 2004. The role of phosphatases in TOR signaling in yeast. *Curr. Top. Microbiol. Immunol.* 279:19–38

67. Duvel K, Santhanam A, Garrett S, Schneper L, Broach JR. 2003. Multiple roles of Tap42 in mediating rapamycin-induced transcriptional changes in yeast. *Mol. Cell* 11:1467–78

68. Eckert-Boulet N, Larsson K, Wu B, Poulsen P, Regenberg B, et al. 2006. Deletion of RTS1, encoding a regulatory subunit of protein phosphatase 2A, results in constitutive amino acid signaling via increased Stp1p processing. *Eukaryot. Cell* 5:174–79

69. Eckert-Boulet N, Nielsen PS, Friis C, dos Santos MM, Nielsen J, et al. 2004. Transcriptional profiling of extracellular amino acid sensing in *Saccharomyces cerevisiae* and the role of Stp1p and Stp2p. *Yeast* 21:635–48

70. Engelberg D, Klein C, Martinetto H, Struhl K, Karin M. 1994. The UV response involving the Ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. *Cell* 77:381–90

71. Escote X, Zapater M, Clotet J, Posas F. 2004. Hog1 mediates cell-cycle arrest in G1 phase by the dual targeting of Sic1. *Nat. Cell Biol.* 6:997–1002

72. Feller A, Boeckstaens M, Marini AM, Dubois E. 2006. Transduction of the nitrogen signal activating Gln3-mediated transcription is independent of Npr1 kinase and Rsp5-Bul1/2 ubiquitin ligase in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 281:28546–54

73. Ferguson SB, Anderson ES, Harshaw RB, Thate T, Craig NL, Nelson HC. 2005. Protein kinase A regulates constitutive expression of small heat-shock genes in an Msn2/4p-independent and Hsf1p-dependent manner in *Saccharomyces cerevisiae*. *Genetics* 169:1203–14

74. Flatauer LJ, Zadeh SF, Bardwell L. 2005. Mitogen-activated protein kinases with distinct requirements for Ste5 scaffolding influence signaling specificity in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 25:1793–803

75. Flick KM, Spielewoy N, Kalashnikova TI, Guaderrama M, Zhu Q, et al. 2003. Grr1-dependent inactivation of Mth1 mediates glucose-induced dissociation of Rgt1 from HXT gene promoters. *Mol. Biol. Cell* 14:3230–41

76. Forsberg H, Gilstring CF, Zargari A, Martinez P, Ljungdahl PO. 2001. The role of the yeast plasma membrane SPS nutrient sensor in the metabolic response to extracellular amino acids. *Mol. Microbiol.* 42:215–28

77. Forsberg H, Hammar M, Andreasson C, Moliner A, Ljungdahl PO. 2001. Suppressors of ssy1 and ptr3 null mutations define novel amino acid sensor-independent genes in *Saccharomyces cerevisiae*. *Genetics* 158:973–88

78. Forsberg H, Ljungdahl PO. 2001. Genetic and biochemical analysis of the yeast plasma membrane Ssy1p-Ptr3p-Ssy5p sensor of extracellular amino acids. *Mol. Cell Biol.* 21:814–26

79. Futcher B. 2006. Metabolic cycle, cell cycle, and the finishing kick to Start. *Genome Biol.* 7:107

80. Gaber RF, Ottow K, Andersen HA, Kielland-Brandt MC. 2003. Constitutive and hyperresponsive signaling by mutant forms of *Saccharomyces cerevisiae* amino acid sensor Ssy1. *Eukaryot. Cell* 2:922–29

81. Gadura N, Robinson LC, Michels CA. 2006. Glc7-Reg1 phosphatase signals to Yck1,2 casein kinase 1 to regulate transport activity and glucose-induced inactivation of *Saccharomyces* maltose permease. *Genetics* 172:1427–39

82. Gancedo JM. 2001. Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 25:107–23

83. Gao M, Kaiser CA. 2006. A conserved GTPase-containing complex is required for intracellular sorting of the general amino-acid permease in yeast. *Nat. Cell Biol.* 8:657–67

84. Garcia-Barrio M, Dong J, Ufano S, Hinnebusch AG. 2000. Association of GCN1-GCN20 regulatory complex with the N-terminus of eIF2alpha kinase GCN2 is required for GCN2 activation. *EMBO J.* 19:1887–99

85. Garmendia-Torres C, Goldbeter A, Jacquet M. 2007. Nucleocytoplasmic oscillations of the yeast transcription factor Msn2: evidence for periodic PKA activation. *Curr. Biol.* 17:1044–49

86. Garrett S, Broach J. 1989. Loss of Ras activity in *Saccharomyces cerevisiae* is suppressed by disruptions of a new kinase gene, YAK1, whose product may act downstream of the cAMP-dependent protein kinase. *Genes Dev.* 3:1336–48

87. Garrett S, Menold MM, Broach JR. 1991. The *Saccharomyces cerevisiae* YAK1 gene encodes a protein kinase that is induced by arrest early in the cell cycle. *Mol. Cell Biol.* 11:4045–52

88. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, et al. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* 11:4241–57

89. Giannattasio S, Liu Z, Thornton J, Butow RA. 2005. Retrograde response to mitochondrial dysfunction is separable from TOR1/2 regulation of retrograde gene expression. *J. Biol. Chem.* 280:42528–35

90. Gorner W, Durchschlag E, Martinez-Pastor MT, Estruch F, Ammerer G, et al. 1998. Nuclear localization of the C_2H_2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev.* 12:586–97

91. Gorner W, Durchschlag E, Wolf J, Brown EL, Ammerer G, et al. 2002. Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *EMBO J.* 21:135–44

92. Govind CK, Yoon S, Qiu H, Govind S, Hinnebusch AG. 2005. Simultaneous recruitment of coactivators by Gcn4p stimulates multiple steps of transcription in vivo. *Mol. Cell Biol.* 25:5626–38

93. Granot D, Snyder M. 1991. Glucose induces cAMP-independent growth-related changes in stationary-phase cells of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 88:5724–28

94. Gray JV, Petsko GA, Johnston GC, Ringe D, Singer RA, Werner-Washburne M. 2004. "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 68:187–206

95. Griffioen G, Branduardi P, Ballarini A, Anghileri P, Norbeck J, et al. 2001. Nucleocytoplasmic distribution of budding yeast protein kinase A regulatory subunit Bcy1 requires Zds1 and is regulated by Yak1-dependent phosphorylation of its targeting domain. *Mol. Cell Biol.* 21:511–23

96. Grummt I. 2003. Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. *Genes Dev.* 17:1691–702

97. Guldal CG, Koblish HK, Schneper L, Wang Y, Davis C, et al. 2007. Ras affects filamentous growth in *Saccharomyces cerevisiae* solely through regulation of protein kinase A. *Proc. Natl. Acad. Sci. USA.* In press

98. Hahn JS, Thiele DJ. 2004. Activation of the *Saccharomyces cerevisiae* heat shock transcription factor under glucose starvation conditions by Snf1 protein kinase. *J. Biol. Chem.* 279:5169–76

99. Hall DB, Wade JT, Struhl K. 2006. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 26:3672–79

100. Halme A, Bumgarner S, Styles C, Fink GR. 2004. Genetic and epigenetic regulation of the FLO gene family generates cell-surface variation in yeast. *Cell* 116:405–15

101. Harashima T, Anderson S, Yates JR 3rd, Heitman J. 2006. The kelch proteins Gpb1 and Gpb2 inhibit Ras activity via association with the yeast RasGAP neurofibromin homologs Ira1 and Ira2. *Mol. Cell* 22:819–30

102. Harashima T, Heitman J. 2002. The Galpha protein Gpa2 controls yeast differentiation by interacting with kelch repeat proteins that mimic Gbeta subunits. *Mol. Cell* 10:163–73

103. Hedbacker K, Hong SP, Carlson M. 2004. Pak1 protein kinase regulates activation and nuclear localization of Snf1-Gal83 protein kinase. *Mol. Cell Biol.* 24:8255–63

104. Hedbacker K, Townley R, Carlson M. 2004. Cyclic AMP-dependent protein kinase regulates the subcellular localization of Snf1-Sip1 protein kinase. *Mol. Cell Biol.* 24:1836–43

105. Hinnebusch AG. 2005. Translational regulation of GCN4 and the general amino acid control of yeast. *Annu. Rev. Microbiol.* 59:407–50

106. Hinnebusch AG, Natarajan K. 2002. Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress. *Eukaryot. Cell* 1:22–32

107. Hohmann S. 2002. Osmotic stress signaling and osmoadaptation in yeasts. *Microbiol. Mol. Biol. Rev.* 66:300–72

108. Hong SP, Leiper FC, Woods A, Carling D, Carlson M. 2003. Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proc. Natl. Acad. Sci. USA* 100:8839–43

108a. Hong SP, Momcilovic M, Carlson M. 2005. Function of mammalian LKB1 and Ca^{2+}/calmodulin-dependent protein kinase kinase α as Snf1-activting kinases in yeast. *J. Biol. Chem.* 280:21804–8

109. Honigberg SM, Lee RH. 1998. Snf1 kinase connects nutritional pathways controlling meiosis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 18:4548–55

110. Honigberg SM, Purnapatre K. 2003. Signal pathway integration in the switch from the mitotic cell cycle to meiosis in yeast. *J. Cell Sci.* 116:2137–47

111. Iraqui I, Vissers S, Bernard F, de Craene JO, Boles E, et al. 1999. Amino acid signaling in *Saccharomyces cerevisiae*: a permease-like sensor of external amino acids and F-Box protein Grr1p are required for transcriptional induction of the AGP1 gene, which encodes a broad-specificity amino acid permease. *Mol. Cell Biol.* 19:989–1001

112. Irniger S, Braus GH. 2003. Controlling transcription by destruction: the regulation of yeast Gcn4p stability. *Curr. Genet.* 44:8–18

113. Jacinto E, Guo B, Arndt KT, Schmelzle T, Hall MN. 2001. TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. *Mol. Cell* 8:1017–26

114. Jacinto E, Hall MN. 2003. Tor signalling in bugs, brain and brawn. *Nat. Rev. Mol. Cell Biol.* 4:117–26

115. Jacquet M, Renault G, Lallet S, De Mey J, Goldbeter A. 2003. Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in *Saccharomyces cerevisiae*. *J. Cell Biol.* 161:497–505

116. Jia MH, Larossa RA, Lee JM, Rafalski A, Derose E, et al. 2000. Global expression profiling of yeast treated with an inhibitor of amino acid biosynthesis, sulfometuron methyl. *Physiol. Genomics* 3:83–92

117. Jiang H, Medintz I, Michels CA. 1997. Two glucose sensing/signaling pathways stimulate glucose-induced inactivation of maltose permease in *Saccharomyces*. *Mol. Biol. Cell* 8:1293–304

118. Jiang Y, Broach JR. 1999. Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. *EMBO J.* 18:2782–92

119. Jiang Y, Davis C, Broach JR. 1998. Efficient transition to growth on fermentable carbon sources in *Saccharomyces cerevisiae* requires signaling through the Ras pathway. *EMBO J.* 17:6942–51

120. Johnston GC, Ehrhardt CW, Lorincz A, Carter BL. 1979. Regulation of cell size in the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 137:1–5

121. Johnston GC, Pringle JR, Hartwell LH. 1977. Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* 105:79–98

122. Johnston M, Carlson M. 1992. Carbon regulation in *Saccharomyces*. In *The Molecular Biology of the Yeast* Saccharomyces*: Gene Expression*, ed. EW Jones, JR Pringle, JR Broach, pp. 198–281. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press

123. Johnston M, Kim JH. 2005. Glucose as a hormone: receptor-mediated glucose sensing in the yeast *Saccharomyces cerevisiae*. *Biochem. Soc. Trans.* 33:247–52

123a. Jorgensen P, Edgington NP, Schneider BL, Rupes I, Tyers M, Futcher B. 2007. The size of the nucleus increases as yeast cells grow. *Mol. Biol. Cell* 18:3523–32

124. Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M. 2002. Systematic identification of pathways that couple cell growth and division in yeast. *Science* 297:395–400

125. Jorgensen P, Rupes I, Sharom JR, Schneper L, Broach JR, Tyers M. 2004. A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes Dev.* 18:2491–505

126. Jorgensen P, Tyers M. 2004. How cells coordinate growth and division. *Curr. Biol.* 14:R1014–27

127. Kabeya Y, Kamada Y, Baba M, Takikawa H, Sasaki M, Ohsumi Y. 2005. Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. *Mol. Biol. Cell* 16:2544–53

128. Kaeberlein M, Burtner CR, Kennedy BK. 2007. Recent developments in yeast aging. *PLoS Genet.* 3:e84

129. Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y. 2000. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J. Cell Biol.* 150:1507–13

130. Kim JH, Brachet V, Moriya H, Johnston M. 2006. Integration of transcriptional and posttranslational regulation in a glucose signal transduction pathway in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 5:167–73

131. Kim MD, Hong SP, Carlson M. 2005. Role of Tos3, a Snf1 protein kinase kinase, during growth of *Saccharomyces cerevisiae* on nonfermentable carbon sources. *Eukaryot. Cell* 4:861–66

132. Kim SJ, Swanson MJ, Qiu H, Govind CK, Hinnebusch AG. 2005. Activator Gcn4p and Cyc8p/Tup1p are interdependent for promoter occupancy at ARG1 in vivo. *Mol. Cell Biol.* 25:11171–83

133. Klasson H, Fink GR, Ljungdahl PO. 1999. Ssy1p and Ptr3p are plasma membrane components of a yeast system that senses extracellular amino acids. *Mol. Cell Biol.* 19:5405–16

134. Klein C, Struhl K. 1994. Protein kinase A mediates growth-regulated expression of yeast ribosomal protein genes by modulating RAP1 transcriptional activity. *Mol. Cell Biol.* 14:1920–28

135. Kodama Y, Omura F, Takahashi K, Shirahige K, Ashikari T. 2002. Genome-wide expression analysis of genes affected by amino acid sensor Ssy1p in *Saccharomyces cerevisiae*. *Curr. Genet.* 41:63–72

136. Komeili A, Wedaman KP, O'Shea EK, Powers T. 2000. Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. *J. Cell Biol.* 151:863–78

137. Kornitzer D, Raboy B, Kulka RG, Fink GR. 1994. Regulated degradation of the transcription factor Gcn4. *EMBO J.* 13:6021–30

138. Kraakman L, Lemaire K, Ma P, Teunissen AW, Donaton MC, et al. 1999. A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Mol. Microbiol.* 32:1002–12

139. Kresnowati MT, van Winden WA, Almering MJ, ten Pierick A, Ras C, et al. 2006. When transcriptome meets metabolome: fast cellular responses of yeast to sudden relief of glucose limitation. *Mol. Syst. Biol.* 2:49

140. Kubler E, Mosch HU, Rupp S, Lisanti MP. 1997. Gpa2p, a G-protein alpha-subunit, regulates growth and pseudohyphal development in *Saccharomyces cerevisiae* via a cAMP-dependent mechanism. *J. Biol. Chem.* 272:20321–23

141. Kubota H, Obata T, Ota K, Sasaki T, Ito T. 2003. Rapamycin-induced translational derepression of GCN4 mRNA involves a novel mechanism for activation of the eIF2 alpha kinase GCN2. *J. Biol. Chem.* 278:20457–60

142. Kubota H, Ota K, Sakaki Y, Ito T. 2001. Budding yeast GCN1 binds the GI domain to activate the eIF2alpha kinase GCN2. *J. Biol. Chem.* 276:17591–96

143. Kubota H, Sakaki Y, Ito T. 2000. GI domain-mediated association of the eukaryotic initiation factor 2alpha kinase GCN2 with its activator GCN1 is required for general amino acid control in budding yeast. *J. Biol. Chem.* 275:20243–46

144. Kuchin S, Vyas VK, Carlson M. 2002. Snf1 protein kinase and the repressors Nrg1 and Nrg2 regulate FLO11, haploid invasive growth, and diploid pseudohyphal differentiation. *Mol. Cell Biol.* 22:3994–4000

145. Kuepfer L, Peter M, Sauer U, Stelling J. 2007. Ensemble modeling as a novel concept to analyze cell signaling dynamics. *Nat. Biotechnol.* 25:1001–6

146. Kulkarni A, Buford TD, Rai R, Cooper TG. 2006. Differing responses of Gat1 and Gln3 phosphorylation and localization to rapamycin and methionine sulfoximine treatment in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 6:218–29

147. Kupiec M, Byers B, Esposito RE, Mitchell AP. 1997. Meiosis and sporulation in *Saccharomyces cerevisiae*. See Ref. 28a, pp. 889–1036

148. Kurdistani SK, Grunstein M. 2003. Histone acetylation and deacetylation in yeast. *Nat. Rev. Mol. Cell Biol.* 4:276–84

149. Kurdistani SK, Robyr D, Tavazoie S, Grunstein M. 2002. Genome-wide binding map of the histone deacetylase Rpd3 in yeast. *Nat. Genet.* 31:248–54

150. Laferte A, Favry E, Sentenac A, Riva M, Carles C, Chedin S. 2006. The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. *Genes Dev.* 20:2030–40

151. Lakshmanan J, Mosley AL, Ozcan S. 2003. Repression of transcription by Rgt1 in the absence of glucose requires Std1 and Mth1. *Curr. Genet.* 44:19–25

152. Lamb TM, Mitchell AP. 2003. The transcription factor Rim101p governs ion tolerance and cell differentiation by direct repression of the regulatory genes NRG1 and SMP1 in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 23:677–86

153. Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* 36:900–5

154. Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, et al. 2002. Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science* 298:799–804

155. Lemaire K, Van de Velde S, Van Dijck P, Thevelein JM. 2004. Glucose and sucrose act as agonist and mannose as antagonist ligands of the G protein-coupled receptor Gpr1 in the yeast *Saccharomyces cerevisiae*. *Mol. Cell* 16:293–99

156. Lenssen E, James N, Pedruzzi I, Dubouloz F, Cameroni E, et al. 2005. The Ccr4-Not complex independently controls both Msn2-dependent transcriptional activation—via a newly identified Glc7/Bud14 type I protein phosphatase module—and TFIID promoter distribution. *Mol. Cell Biol.* 25:488–98

157. Li H, Tsang CK, Watkins M, Bertram PG, Zheng XF. 2006. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature* 442:1058–61

158. Liu K, Zhang X, Lester RL, Dickson RC. 2005. The sphingoid long chain base phytosphingosine activates AGC-type protein kinases in *Saccharomyces cerevisiae* including Ypk1, Ypk2, and Sch9. *J. Biol. Chem.* 280:22679–87

159. Liu Z, Butow RA. 1999. A transcriptional switch in the expression of yeast tricarboxylic acid cycle genes in response to a reduction or loss of respiratory function. *Mol. Cell Biol.* 19:6720–28

160. Liu Z, Butow RA. 2006. Mitochondrial retrograde signaling. *Annu. Rev. Genet.* 40:159–85

161. Lo WS, Duggan L, Emre NC, Belotserkovskaya R, Lane WS, et al. 2001. Snf1—a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. *Science* 293:1142–46

162. Lo WS, Gamache ER, Henry KW, Yang D, Pillus L, Berger SL. 2005. Histone H3 phosphorylation can promote TBP recruitment through distinct promoter-specific mechanisms. *EMBO J.* 24:997–1008

163. Long YC, Zierath JR. 2006. AMP-activated protein kinase signaling in metabolic regulation. *J. Clin. Invest.* 116:1776–83

164. Lorenz MC, Heitman J. 1998. The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *EMBO J.* 17:1236–47

165. Ludin K, Jiang R, Carlson M. 1998. Glucose-regulated interaction of a regulatory subunit of protein phosphatase 1 with the Snf1 protein kinase in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 95:6245–50

166. Magasanik B, Kaiser CA. 2002. Nitrogen regulation in *Saccharomyces cerevisiae*. *Gene* 290:1–18

167. Mai B, Breeden L. 1997. Xbp1, a stress-induced transcriptional repressor of the *Saccharomyces cerevisiae* Swi4/Mbp1 family. *Mol. Cell Biol.* 17:6491–501

168. Marion RM, Regev A, Segal E, Barash Y, Koller D, et al. 2004. Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proc. Natl. Acad. Sci. USA* 101:14315–22

169. Markwardt DD, Garrett JM, Eberhardy S, Heideman W. 1995. Activation of the Ras/cyclic AMP pathway in the yeast *Saccharomyces cerevisiae* does not prevent G1 arrest in response to nitrogen starvation. *J. Bacteriol.* 177:6761–65

170. Martin DE, Powers T, Hall MN. 2006. Regulation of ribosome biogenesis: Where is TOR? *Cell Metab.* 4:259–60

171. Martin DE, Soulard A, Hall MN. 2004. TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. *Cell* 119:969–79

172. Martinez MJ, Roy S, Archuletta AB, Wentzell PD, Anna-Arriola SS, et al. 2004. Genomic analysis of stationary-phase and exit in *Saccharomyces cerevisiae*: gene expression and identification of novel essential genes. *Mol. Biol. Cell* 15:5295–305

173. Marton MJ, Vazquez de Aldana CR, Qiu H, Chakraburtty K, Hinnebusch AG. 1997. Evidence that GCN1 and GCN20, translational regulators of GCN4, function on elongating ribosomes in activation of eIF2alpha kinase GCN2. *Mol. Cell Biol.* 17:4474–89

174. Matsuo R, Kubota H, Obata T, Kito K, Ota K, et al. 2005. The yeast eIF4E-associated protein Eap1p attenuates GCN4 translation upon TOR-inactivation. *FEBS Lett.* 579:2433–38

175. Mayer C, Zhao J, Yuan X, Grummt I. 2004. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes Dev.* 18:423–34

176. McCartney RR, Rubenstein EM, Schmidt MC. 2005. Snf1 kinase complexes with different beta subunits display stress-dependent preferences for the three Snf1-activating kinases. *Curr. Genet.* 47:335–44

177. McClean MN, Mody A, Broach JR, Ramanathan S. 2007. Cross-talk and decision making in MAP kinase pathways. *Nat. Genet.* 39:409–14

178. Meimoun A, Holtzman T, Weissman Z, McBride HJ, Stillman DJ, et al. 2000. Degradation of the transcription factor Gcn4 requires the kinase Pho85 and the SCF(CDC4) ubiquitin-ligase complex. *Mol. Biol. Cell* 11:915–27

179. Mitchelhill KI, Stapleton D, Gao G, House C, Michell B, et al. 1994. Mammalian AMP-activated protein kinase shares structural and functional homology with the catalytic domain of yeast Snf1 protein kinase. *J. Biol. Chem.* 269:2361–64

180. Mitchell AP, Magasanik B. 1984. Regulation of glutamine-repressible gene products by the GLN3 function in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 4:2758–66

181. Mizushima N, Klionsky DJ. 2007. Protein turnover via autophagy: implications for metabolism. *Annu. Rev. Nutr.* 27:19–40

182. Moir RD, Lee J, Haeusler RA, Desai N, Engelke DR, Willis IM. 2006. Protein kinase A regulates RNA polymerase III transcription through the nuclear localization of Maf1. *Proc. Natl. Acad. Sci. USA* 103:15044–49

183. Moriya H, Johnston M. 2004. Glucose sensing and signaling in *Saccharomyces cerevisiae* through the Rgt2 glucose sensor and casein kinase I. *Proc. Natl. Acad. Sci. USA* 101:1572–77

184. Moriya H, Shimizu-Yoshida Y, Omori A, Iwashita S, Katoh M, Sakai A. 2001. Yak1p, a DYRK family kinase, translocates to the nucleus and phosphorylates yeast Pop2p in response to a glucose signal. *Genes Dev.* 15:1217–28

185. Morse RH. 2000. RAP, RAP, open up! New wrinkles for RAP1 in yeast. *Trends Genet.* 16:51–53

186. Mosch HU, Roberts RL, Fink GR. 1996. Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 93:5352–56

187. Mosley AL, Lakshmanan J, Aryal BK, Ozcan S. 2003. Glucose-mediated phosphorylation converts the transcription factor Rgt1 from a repressor to an activator. *J. Biol. Chem.* 278:10322–27

188. Mueller PP, Hinnebusch AG. 1986. Multiple upstream AUG codons mediate translational control of GCN4. *Cell* 45:201–7

189. Murray LE, Rowley N, Dawes IW, Johnston GC, Singer RA. 1998. A yeast glutamine tRNA signals nitrogen status for regulation of dimorphic growth and sporulation. *Proc. Natl. Acad. Sci. USA* 95:8619–24

190. Nair U, Klionsky DJ. 2005. Molecular mechanisms and regulation of specific and nonspecific autophagy pathways in yeast. *J. Biol. Chem.* 280:41785–88

191. Nakafuku M, Obara T, Kaibuchi K, Miyajima I, Miyajima A, et al. 1988. Isolation of a second yeast *Saccharomyces cerevisiae* gene (GPA2) coding for guanine nucleotide-binding regulatory protein: studies on its structure and possible functions. *Proc. Natl. Acad. Sci. USA* 85:1374–78

192. Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, et al. 2001. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol. Cell Biol.* 21:4347–68

193. Nath N, McCartney RR, Schmidt MC. 2003. Yeast Pak1 kinase associates with and activates Snf1. *Mol. Cell Biol.* 23:3909–17

194. Neufeld TP. 2007. TOR regulation: sorting out the answers. *Cell Metab.* 5:3–5

195. Neuman-Silberberg FS, Bhattacharya S, Broach JR. 1995. Nutrient availability and the RAS/cyclic AMP pathway both induce expression of ribosomal protein genes in *Saccharomyces cerevisiae* but by different mechanisms. *Mol. Cell Biol.* 15:3187–96

196. Noda T, Ohsumi Y. 1998. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J. Biol. Chem.* 273:3963–66

197. Oakes ML, Siddiqi I, French SL, Vu L, Sato M, et al. 2006. Role of histone deacetylase Rpd3 in regulating rRNA gene transcription and nucleolar structure in yeast. *Mol. Cell Biol.* 26:3889–901

198. Oficjalska-Pham D, Harismendy O, Smagowicz WJ, Gonzalez de Peredo A, Boguta M, et al. 2006. General repression of RNA polymerase III transcription is triggered by protein phosphatase type 2A-mediated dephosphorylation of Maf1. *Mol. Cell* 22:623–32

199. Orlova M, Kanter E, Krakovich D, Kuchin S. 2006. Nitrogen availability and TOR regulate the Snf1 protein kinase in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 5:1831–37

200. Ozcan S, Johnston M. 1996. Two different repressors collaborate to restrict expression of the yeast glucose transporter genes HXT2 and HXT4 to low levels of glucose. *Mol. Cell Biol.* 16:5536–45

201. Palecek SP, Parikh AS, Kron SJ. 2002. Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth. *Microbiology* 148:893–907

202. Palomino A, Herrero P, Moreno F. 2006. Tpk3 and Snf1 protein kinases regulate Rgt1 association with *Saccharomyces cerevisiae* HXK2 promoter. *Nucleic Acids Res.* 34:1427–38

203. Pan X, Heitman J. 1999. Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 19:4874–87

204. Pan X, Heitman J. 2000. Sok2 regulates yeast pseudohyphal differentiation via a transcription factor cascade that regulates cell-cell adhesion. *Mol. Cell Biol.* 20:8364–72

205. Pan X, Heitman J. 2002. Protein kinase A operates a molecular switch that governs yeast pseudohyphal differentiation. *Mol. Cell Biol.* 22:3981–93

206. Pascual-Ahuir A, Proft M. 2007. The Sch9 kinase is a chromatin-associated transcriptional activator of osmostress-responsive genes. *EMBO J.* 26(13):3098–108

207. Pedruzzi I, Dubouloz F, Cameroni E, Wanke V, Roosen J, et al. 2003. TOR and PKA signaling pathways converge on the protein kinase Rim15 to control entry into G0. *Mol. Cell* 12:1607–13

208. Peeters T, Louwet W, Gelade R, Nauwelaers D, Thevelein JM, Versele M. 2006. Kelch-repeat proteins interacting with the Galpha protein Gpa2 bypass adenylate cyclase for direct regulation of protein kinase A in yeast. *Proc. Natl. Acad. Sci. USA* 103:13034–39

209. Pnueli L, Edry I, Cohen M, Kassir Y. 2004. Glucose and nitrogen regulate the switch from histone deacetylation to acetylation for expression of early meiosis-specific genes in budding yeast. *Mol. Cell Biol.* 24:5197–208

210. Polish JA, Kim JH, Johnston M. 2005. How the Rgt1 transcription factor of *Saccharomyces cerevisiae* is regulated by glucose. *Genetics* 169:583–94

211. Portela P, Howell S, Moreno S, Rossi S. 2002. In vivo and in vitro phosphorylation of two isoforms of yeast pyruvate kinase by protein kinase A. *J. Biol. Chem.* 277:30477–87

212. Poulsen P, Lo Leggio L, Kielland-Brandt MC. 2006. Mapping of an internal protease cleavage site in the Ssy5p component of the amino acid sensor of *Saccharomyces cerevisiae* and functional characterization of the resulting pro- and protease domains by gain-of-function genetics. *Eukaryot. Cell* 5:601–8

213. Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S. 2006. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 20:174–84

214. Pratt ZL, Drehman BJ, Miller ME, Johnston SD. 2007. Mutual interdependence of MSI1 (CAC3) and YAK1 in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 368(1):30–43

215. Prinz S, Avila-Campillo I, Aldridge C, Srinivasan A, Dimitrov K, et al. 2004. Control of yeast filamentous-form growth by modules in an integrated molecular network. *Genome Res.* 14:380–90

216. Proft M, Pascual-Ahuir A, de Nadal E, Arino J, Serrano R, Posas F. 2001. Regulation of the Sko1 transcriptional repressor by the Hog1 MAP kinase in response to osmotic stress. *EMBO J.* 20:1123–33

217. Ptacek J, Devgan G, Michaud G, Zhu H, Zhu X, et al. 2005. Global analysis of protein phosphorylation in yeast. *Nature* 438:679–84

218. Purnapatre K, Gray M, Piccirillo S, Honigberg SM. 2005. Glucose inhibits meiotic DNA replication through SCFGrr1p-dependent destruction of Ime2p kinase. *Mol. Cell Biol.* 25:440–50

219. Qiu H, Dong J, Hu C, Francklyn CS, Hinnebusch AG. 2001. The tRNA-binding moiety in GCN2 contains a dimerization domain that interacts with the kinase domain and is required for tRNA binding and kinase activation. *EMBO J.* 20:1425–38

220. Qiu H, Hu C, Zhang F, Hwang GJ, Swanson MJ, et al. 2005. Interdependent recruitment of SAGA and Srb mediator by transcriptional activator Gcn4p. *Mol. Cell Biol.* 25:3461–74

221. Ramanathan S, Broach JR. 2007. Do cells think? *Cell Mol. Life Sci.* 64:1801–4

222. Reid JL, Iyer VR, Brown PO, Struhl K. 2000. Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol. Cell* 6:1297–307

223. Reinders A, Burckert N, Boller T, Wiemken A, De Virgilio C. 1998. *Saccharomyces cerevisiae* cAMP-dependent protein kinase controls entry into stationary phase through the Rim15p protein kinase. *Genes Dev.* 12:2943–55

224. Roberts DN, Wilson B, Huff JT, Stewart AJ, Cairns BR. 2006. Dephosphorylation and genome-wide association of Maf1 with Pol III-transcribed genes during repression. *Mol. Cell* 22:633–44

225. Robertson LS, Fink GR. 1998. The three yeast A kinases have specific signaling functions in pseudo-hyphal growth. *Proc. Natl. Acad. Sci. USA* 95:13783–87

226. Roelants FM, Torrance PD, Thorner J. 2004. Differential roles of PDK1- and PDK2-phosphorylation sites in the yeast AGC kinases Ypk1, Pkc1 and Sch9. *Microbiology* 150:3289–304

227. Rohde JR, Campbell S, Zurita-Martinez SA, Cutler NS, Ashe M, Cardenas ME. 2004. TOR controls transcriptional and translational programs via Sap-Sit4 protein phosphatase signaling effectors. *Mol. Cell Biol.* 24:8332–41

228. Rohde JR, Cardenas ME. 2003. The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. *Mol. Cell Biol.* 23:629–35

229. Rolland F, De Winde JH, Lemaire K, Boles E, Thevelein JM, Winderickx J. 2000. Glucose-induced cAMP signalling in yeast requires both a G-protein coupled receptor system for extracellular glucose detection and a separable hexose kinase-dependent sensing process. *Mol. Microbiol.* 38:348–58

230. Roth S, Kumme J, Schuller HJ. 2004. Transcriptional activators Cat8 and Sip4 discriminate between sequence variants of the carbon source-responsive promoter element in the yeast *Saccharomyces cerevisiae*. *Curr. Genet.* 45:121–28

231. Rothfels K, Tanny JC, Molnar E, Friesen H, Commisso C, Segall J. 2005. Components of the ESCRT pathway, DFG16, and YGR122w are required for Rim101 to act as a corepressor with Nrg1 at the negative regulatory element of the DIT1 gene of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 25:6772–88

232. Rubin-Bejerano I, Sagee S, Friedman O, Pnueli L, Kassir Y. 2004. The in vivo activity of Ime1, the key transcriptional activator of meiosis-specific genes in *Saccharomyces cerevisiae*, is inhibited by the cyclic AMP/protein kinase A signal pathway through the glycogen synthase kinase 3-beta homolog Rim11. *Mol. Cell Biol.* 24:6967–79

233. Rudra D, Mallick J, Zhao Y, Warner JR. 2007. Potential interface between ribosomal protein production and pre-rRNA processing. *Mol. Cell Biol.* 27:4815–24

234. Rudra D, Warner JR. 2004. What better measure than ribosome synthesis? *Genes Dev.* 18:2431–36

235. Rudra D, Zhao Y, Warner JR. 2005. Central role of Ifh1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. *EMBO J.* 24:533–42

236. Sagee S, Sherman A, Shenhar G, Robzyk K, Ben-Doy N, et al. 1998. Multiple and distinct activation and repression sequences mediate the regulated transcription of IME1, a transcriptional activator of meiosis-specific genes in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 18:1985–95

237. Saldanha AJ, Brauer MJ, Botstein D. 2004. Nutritional homeostasis in batch and steady-state culture of yeast. *Mol. Biol. Cell* 15:4089–104

238. Santangelo GM. 2006. Glucose signaling in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 70:253–82

239. Santhanam A, Hartley A, Duvel K, Broach JR, Garrett S. 2004. PP2A phosphatase activity is required for stress and Tor kinase regulation of yeast stress response factor Msn2p. *Eukaryot. Cell* 3:1261–71

240. Sanz P. 2003. Snf1 protein kinase: a key player in the response to cellular stress in yeast. *Biochem. Soc. Trans.* 31:178–81

241. Sattlegger E, Hinnebusch AG. 2000. Separate domains in GCN1 for binding protein kinase GCN2 and ribosomes are required for GCN2 activation in amino acid-starved cells. *EMBO J.* 19:6622–33

242. Sattlegger E, Hinnebusch AG. 2005. Polyribosome binding by GCN1 is required for full activation of eukaryotic translation initiation factor 2α kinase GCN2 during amino acid starvation. *J. Biol. Chem.* 280:16514–21

243. Sattlegger E, Swanson MJ, Ashcraft EA, Jennings JL, Fekete RA, et al. 2004. YIH1 is an actin-binding protein that inhibits protein kinase GCN2 and impairs general amino acid control when overexpressed. *J. Biol. Chem.* 279:29952–62

244. Schawalder SB, Kabani M, Howald I, Choudhury U, Werner M, Shore D. 2004. Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. *Nature* 432:1058–61

245. Scherens B, Feller A, Vierendeels F, Messenguy F, Dubois E. 2006. Identification of direct and indirect targets of the Gln3 and Gat1 activators by transcriptional profiling in response to nitrogen availability in the short and long-term. *FEMS Yeast Res.* 6:777–91

246. Schmidt MC, McCartney RR, Zhang X, Tillman TS, Solimeo H, et al. 1999. Std1 and Mth1 proteins interact with the glucose sensors to control glucose-regulated gene expression in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 19:4561–71

247. Schneider BL, Zhang J, Markwardt J, Tokiwa G, Volpe T, et al. 2004. Growth rate and cell size modulate the synthesis of, and requirement for, G1-phase cyclins at start. *Mol. Cell Biol.* 24:10802–13

248. Schneper L, Duvel K, Broach JR. 2004. Sense and sensibility: nutritional response and signal integration in yeast. *Curr. Opin. Microbiol.* 7:624–30

249. Schuller HJ. 2003. Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. *Curr. Genet.* 43:139–60

250. Sekito T, Liu Z, Thornton J, Butow RA. 2002. RTG-dependent mitochondria-to-nucleus signaling is regulated by MKS1 and is linked to formation of yeast prion [URE3]. *Mol. Biol. Cell* 13:795–804

251. Shamji AF, Kuruvilla FG, Schreiber SL. 2000. Partitioning the transcriptional program induced by rapamycin among the effectors of the Tor proteins. *Curr. Biol.* 10:1574–81

252. Shenhar G, Kassir Y. 2001. A positive regulator of mitosis, Sok2, functions as a negative regulator of meiosis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 21:1603–12

253. Shirra MK, Arndt KM. 1999. Evidence for the involvement of the Glc7-Reg1 phosphatase and the Snf1-Snf4 kinase in the regulation of INO1 transcription in *Saccharomyces cerevisiae*. *Genetics* 152:73–87

254. Smith A, Ward MP, Garrett S. 1998. Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation. *EMBO J.* 17:3556–64

255. Spielewoy N, Flick K, Kalashnikova TI, Walker JR, Wittenberg C. 2004. Regulation and recognition of SCFGrr1 targets in the glucose and amino acid signaling pathways. *Mol. Cell Biol.* 24:8994–9005

256. Sreenivasan A, Bishop AC, Shokat KM, Kellogg DR. 2003. Specific inhibition of Elm1 kinase activity reveals functions required for early G1 events. *Mol. Cell Biol.* 23:6327–37

257. Sutherland CM, Hawley SA, McCartney RR, Leech A, Stark MJ, et al. 2003. Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. *Curr. Biol.* 13:1299–305

258. Swanson MJ, Qiu H, Sumibcay L, Krueger A, Kim SJ, et al. 2003. A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. *Mol. Cell Biol.* 23:2800–20

259. Swinnen E, Wanke V, Roosen J, Smets B, Dubouloz F, et al. 2006. Rim15 and the crossroads of nutrient signalling pathways in *Saccharomyces cerevisiae*. *Cell Div.* 1:3

260. Tachibana C, Yoo JY, Tagne JB, Kacherovsky N, Lee TI, Young ET. 2005. Combined global localization analysis and transcriptome data identify genes that are directly coregulated by Adr1 and Cat8. *Mol. Cell Biol.* 25:2138–46

261. Tamaki H, Miwa T, Shinozaki M, Saito M, Yun CW, et al. 2000. GPR1 regulates filamentous growth through FLO11 in yeast *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 267:164–68

262. Tate JJ, Rai R, Cooper TG. 2005. Methionine sulfoximine treatment and carbon starvation elicit Snf1-independent phosphorylation of the transcription activator Gln3 in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 280:27195–204

263. Tate JJ, Rai R, Cooper TG. 2006. Ammonia-specific regulation of Gln3 localization in *Saccharomyces cerevisiae* by protein kinase Npr1. *J. Biol. Chem.* 281:28460–69

264. Toda T, Cameron S, Sass P, Wigler M. 1988. SCH9, a gene of *Saccharomyces cerevisiae* that encodes a protein distinct from, but functionally and structurally related to, cAMP-dependent protein kinase catalytic subunits. *Genes Dev.* 2:517–27

265. Toda T, Cameron S, Sass P, Zoller M, Scott JD, et al. 1987. Cloning and characterization of BCY1, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 7:1371–77

266. Tokiwa G, Tyers M, Volpe T, Futcher B. 1994. Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. *Nature* 371:342–45

267. Townley R, Shapiro L. 2007. Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase. *Science* 315:1726–29

268. Tsang CK, Bertram PG, Ai W, Drenan R, Zheng XF. 2003. Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. *EMBO J.* 22:6045–56

269. Tu J, Carlson M. 1995. REG1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*. *EMBO J.* 14:5939–46

270. Tzamarias D, Roussou I, Thireos G. 1989. Coupling of GCN4 mRNA translational activation with decreased rates of polypeptide chain initiation. *Cell* 57:947–54

271. Unger MW, Hartwell LH. 1976. Control of cell division in *Saccharomyces cerevisiae* by methionyl-tRNA. *Proc. Natl. Acad. Sci. USA* 73:1664–68

272. Upadhya R, Lee J, Willis IM. 2002. Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. *Mol. Cell* 10:1489–94

273. Urban J, Soulard A, Huber A, Lippman S, Mukhopadhyay D, et al. 2007. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell* 26:663–74

274. Valenzuela L, Aranda C, Gonzalez A. 2001. TOR modulates GCN4-dependent expression of genes turned on by nitrogen limitation. *J. Bacteriol.* 183:2331–34

275. van Oevelen CJ, van Teeffelen HA, van Werven FJ, Timmers HT. 2006. Snf1p-dependent Spt-Ada-Gcn5-acetyltransferase (SAGA) recruitment and chromatin remodeling activities on the HXT2 and HXT4 promoters. *J. Biol. Chem.* 281:4523–31

276. Vincent O, Carlson M. 1999. Gal83 mediates the interaction of the Snf1 kinase complex with the transcription activator Sip4. *EMBO J.* 18:6672–81

277. Vincent O, Townley R, Kuchin S, Carlson M. 2001. Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism. *Genes Dev.* 15:1104–14

278. Vyas VK, Kuchin S, Berkey CD, Carlson M. 2003. Snf1 kinases with different beta-subunit isoforms play distinct roles in regulating haploid invasive growth. *Mol. Cell Biol.* 23:1341–48

279. Wade CH, Umbarger MA, McAlear MA. 2006. The budding yeast rRNA and ribosome biosynthesis (RRB) regulon contains over 200 genes. *Yeast* 23:293–306

280. Wade JT, Hall DB, Struhl K. 2004. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. *Nature* 432:1054–58

281. Wang Y, Pierce M, Schneper L, Guldal CG, Zhang X, et al. 2004. Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *PLoS Biol.* 2:E128

282. Wanke V, Pedruzzi I, Cameroni E, Dubouloz F, De Virgilio C. 2005. Regulation of G0 entry by the Pho80-Pho85 cyclin-CDK complex. *EMBO J.* 24:4271–78

283. Ward MP, Gimeno CJ, Fink GR, Garrett S. 1995. SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. *Mol. Cell Biol.* 15:6854–63

284. Warner JR, Vilardell J, Sohn JH. 2001. Economics of ribosome biosynthesis. *Cold Spring Harbor Symp. Quant. Biol.* 66:567–74

285. Wedaman KP, Reinke A, Anderson S, Yates J 3rd, McCaffery JM, Powers T. 2003. Tor kinases are in distinct membrane-associated protein complexes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 14:1204–20

286. Wek SA, Zhu S, Wek RC. 1995. The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. *Mol. Cell Biol.* 15:4497–506

287. Werner-Washburne M, Brown D, Braun E. 1991. Bcy1, the regulatory subunit of cAMP-dependent protein kinase in yeast, is differentially modified in response to the physiological status of the cell. *J. Biol. Chem.* 266:19704–9

288. Wilson WA, Hawley SA, Hardie DG. 1996. Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Curr. Biol.* 6:1426–34

289. Woods A, Munday MR, Scott J, Yang X, Carlson M, Carling D. 1994. Yeast SNF1 is functionally related to mammalian AMP-activated protein kinase and regulates acetyl-CoA carboxylase in vivo. *J. Biol. Chem.* 269:19509–15

290. Wullschleger S, Loewith R, Hall MN. 2006. TOR signaling in growth and metabolism. *Cell* 124:471–84

291. Wullschleger S, Loewith R, Oppliger W, Hall MN. 2005. Molecular organization of target of rapamycin complex 2. *J. Biol. Chem.* 280:30697–704

292. Xie MW, Jin F, Hwang H, Hwang S, Anand V, et al. 2005. Insights into TOR function and rapamycin response: chemical genomic profiling by using a high-density cell array method. *Proc. Natl. Acad. Sci. USA* 102:7215–20

293. Xue Y, Batlle M, Hirsch JP. 1998. GPR1 encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway. *EMBO J.* 17:1996–2007

294. Yan G, Shen X, Jiang Y. 2006. Rapamycin activates Tap42-associated phosphatases by abrogating their association with Tor complex 1. *EMBO J.* 25:3546–55

295. Yoon S, Govind CK, Qiu H, Kim SJ, Dong J, Hinnebusch AG. 2004. Recruitment of the ArgR/Mcm1p repressor is stimulated by the activator Gcn4p: a self-checking activation mechanism. *Proc. Natl. Acad. Sci. USA* 101:11713–18

296. Yoon S, Qiu H, Swanson MJ, Hinnebusch AG. 2003. Recruitment of SWI/SNF by Gcn4p does not require Snf2p or Gcn5p but depends strongly on SWI/SNF integrity, SRB mediator, and SAGA. *Mol. Cell Biol.* 23:8829–45

297. Yorimitsu T, Klionsky DJ. 2005. Autophagy: molecular machinery for self-eating. *Cell Death Differ.* 12(Suppl 2):1542–52

298. Yorimitsu T, Zaman S, Broach JR, Klionsky DJ. 2007. Protein kinase A and Sch9 cooperatively regulate induction of autophagy in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 18:4180–89

299. Young ET, Dombek KM, Tachibana C, Ideker T. 2003. Multiple pathways are coregulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. *J. Biol. Chem.* 278:26146–58

300. Zargari A, Boban M, Heessen S, Andreasson C, Thyberg J, Ljungdahl PO. 2007. Inner nuclear membrane proteins Asi1, Asi2, and Asi3 function in concert to maintain the latent properties of transcription factors Stp1 and Stp2. *J. Biol. Chem.* 282:594–605

300a. Zeller CE, Parnell SC, Dohlman HG. 2007. The RACK1 ortholog Asc1 functions as a G-protein b subunit coupled to glucose responsiveness in yeast. *J. Biol. Chem*. 282:25168–76

301. Zhang F, Sumibcay L, Hinnebusch AG, Swanson MJ. 2004. A triad of subunits from the Gal11/tail domain of Srb mediator is an in vivo target of transcriptional activator Gcn4p. *Mol. Cell Biol.* 24:6871–86

302. Zhao Y, McIntosh KB, Rudra D, Schawalder S, Shore D, Warner JR. 2006. Fine-structure analysis of ribosomal protein gene transcription. *Mol. Cell Biol.* 26:4853–62

303. Zurita-Martinez SA, Puria R, Pan X, Boeke JD, Cardenas ME. 2007. Efficient Tor signaling requires a functional Class C Vps protein complex in *Saccharomyces cerevisiae*. *Genetics* doi:10.1534/genetics.107.072835

Contents

Errata

An online log of corrections to *Annual Review of Genetics* articles may be found at http://genet.annualreviews.org/errata.shtml

Target of Rapamycin (TOR) Regulates Growth in Response to Nutritional Signals

RONIT WEISMAN

Department of Natural and Life Sciences, The Open University of Israel, Raanana, 435379, Israel

ABSTRACT All organisms can respond to the availability of nutrients by regulating their metabolism, growth, and cell division. Central to the regulation of growth in response to nutrient availability is the target of rapamycin (TOR) signaling that is composed of two structurally distinct complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2). The TOR genes were first identified in yeast as target of rapamycin, a natural product of a soil bacterium, which proved beneficial as an immunosuppressive and anticancer drug and is currently being tested for a handful of other pathological conditions including diabetes, neurodegeneration, and age-related diseases. Studies of the TOR pathway unraveled a complex growth-regulating network. TOR regulates nutrient uptake, transcription, protein synthesis and degradation, as well as metabolic pathways, in a coordinated manner that ensures that cells grow or cease growth in response to nutrient availability. The identification of specific signals and mechanisms that stimulate TOR signaling is an active and exciting field of research that has already identified nitrogen and amino acids as key regulators of TORC1 activity. The signals, as well as the cellular functions of TORC2, are far less well understood. Additional open questions in the field concern the relationships between TORC1 and TORC2, as well as the links with other nutrient-responsive pathways. Here I review the main features of TORC1 and TORC2, with a particular focus on yeasts as model organisms.

INTRODUCTION

A universal feature of all organisms is their ability to respond to nutrient availability by regulating growth and developmental programs. The identification of the target of rapamycin (TOR) pathway was a seminal discovery in the quest to understand the molecular mechanisms that govern such processes. TOR is an evolutionarily conserved serine/threonine kinase belonging to the family of phosphatidylinositol kinase-related kinases. Other members of this family include the mammalian DNA damage checkpoint kinases ATM and ATR, which are conserved from yeast to human, and DNA-PK and SMG1, which are not found in yeasts. TOR regulates growth (accumulation of mass), proliferation (accumulation in cell number), and survival in response to nutritional changes by diverse mechanisms that include regulation of anabolic and catabolic metabolism, nutrient uptake, protein translation and turnover, gene transcription, and the epigenome (reviewed in [1]–[3]).

The first TOR genes were isolated in the budding yeast *Saccharomyces cerevisiae* as point mutations that conferred resistance to the growth-inhibiting effect of rapamycin ([4]–[6]). Rapamycin is a small hydrophobic molecule, a product of the soil bacterium *Streptomyces hygroscopicus* that was first isolated as an antifungal antibiotic but later proved to be a potent inhibitor of cellular growth in humans via inhibition of mammalian TOR (mTOR). The antiproliferative effect of rapamycin has proved beneficial for several clinical applications, including immunosuppression for the prevention of allograft rejection, treatment of cancer, and elution of stents to prevent restenosis after angioplasty ([7], [8]).

Rapamycin induces cell growth arrest by mimicking a starvation-like response, which is characterized, among other features, by the induction of autophagy. This feature of rapamycin and its derivatives, or second-generation ATP-competitive TOR-specific inhibitors, may prove useful for the treatment of neurodegenerative and metabolic diseases (7, 8). Rapamycin, somewhat similar to calorie restriction, extends the life span in several model systems, including yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, and mice, making it the first drug with the potential to treat aging (7, 8).

TOR proteins can be found in two distinct evolutionarily conserved complexes termed TOR complex 1 (TORC1) and TOR complex 2 (TORC2) (see Fig. 1). TOR provides the catalytic subunit in these complexes and is accompanied by TORC1-specific and TORC2-specific subunits, as well as protein subunits that are shared by the two complexes. TORC1 and TORC2 are controlled by distinct upstream and downstream regulators but share common features, such as the phosphorylation and activation of AGC kinases (a family of

protein kinases named after protein kinases A, G, and C) at their C terminal hydrophobic and turn motifs. Since distinct AGC kinases are phosphorylated and activated by TORC1 and TORC2, monitoring the phosphorylation status of these kinases at specific sites is most useful for determining TORC1- or TORC2-specific activation (1).

In higher eukaryotes (nematodes, flies, mice, and humans) there is a single gene for TOR. In contrast, two TOR genes are found in several fungal groups as the result of independent events of whole genome or segmental duplications (9). The two best-studied unicellular organisms for TOR signaling are the budding yeast *S. cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, which contain two TOR genes. In *S. cerevisiae*, TORC1 (*Sc*TORC1) mainly contains *Sc*Tor1 as the catalytic subunit, but a minor *Sc*TORC1 variant that contains *Sc*Tor2 also exists; ScTORC2 contains *Sc*Tor2 as the catalytic subunit (10). In *S. pombe*, *Sp*Tor2 mainly acts as the catalytic subunit of *S. pombe* TORC1 (*Sp*TORC1), while *Sp*Tor1 mainly acts as the catalytic subunit of *Sp*TORC2 (11, 12). This awkward naming

FIGURE 1 TORC1 and TORC2 subunits and downstream AGC kinases in mammalian, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* cells. TORC1 and TORC2 have shared and unique components. The human protein Raptor is TORC1-specific and is conserved in both yeast species. The human proteins Rictor and Sin1 are TORC2-specific subunits and are conserved in both yeast species. The target kinases of TORC1 and TORC2 are shown as green parallelograms.
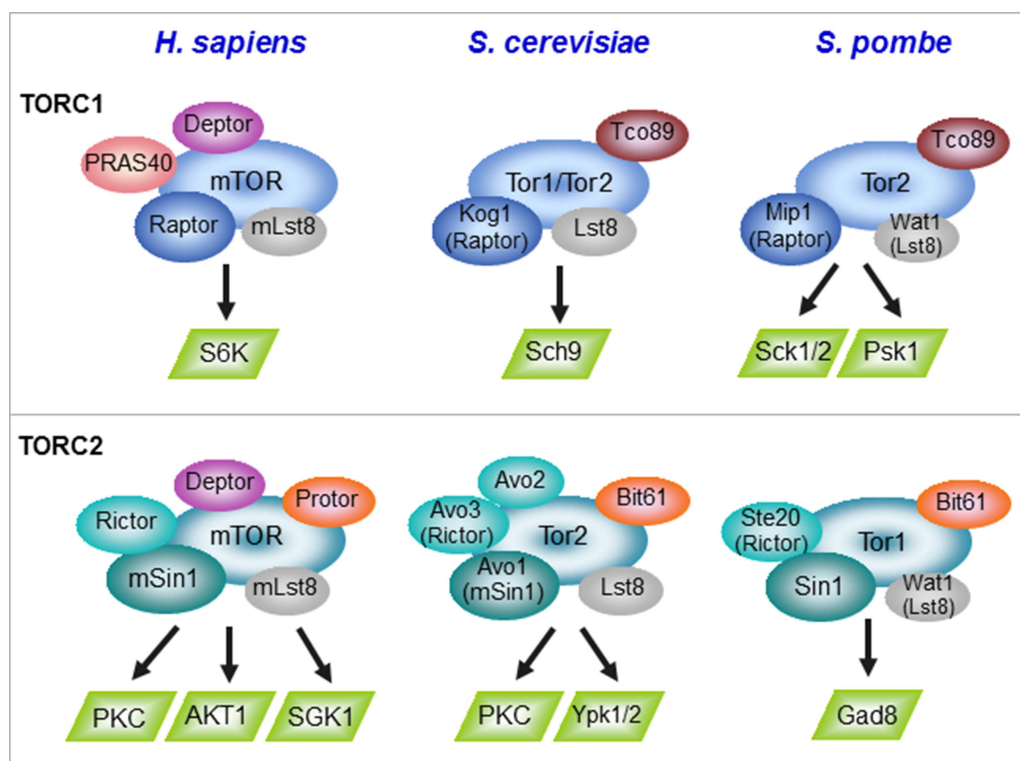
of the *S. pombe* TOR genes is the result of their identification prior to the isolation of the TOR complexes in *S. cerevisiae* ([13](#)) and reflects the lack of known specific sequences for the TOR catalytic subunit that can predict its association with TORC1 or TORC2 subunits. A single TOR gene is found in *Candida albicans* and filamentous fungi, and TOR inhibition plays an important role in the pathogenicity of these organisms ([14](#)–[16](#)) (see further discussion below).

TORC1: A MASTER REGULATOR OF GROWTH AND STARVATION RESPONSES

TORC1 Architecture, Rapamycin Sensitivity, and Downstream Signaling

TORC1 is defined by the essential subunit known as Raptor in metazoans, Kog1 in *S. cerevisiae*, and Mip1 in *S. pombe*. A highly conserved but nonspecific subunit is Lst8 (also known as GβL in metazoans and Wat1 or Pop3 in *S. pombe*), which is found both in TORC1 and TORC2. Other components of TORC1 are not as highly conserved; Tco89 is found in *S. cerevisiae*, *S. pombe*, and *C. albicans*, but no homologues have been identified in higher eukaryotes. In contrast, PRAS40 and Deptor are found in higher eukaryotes, but no homologues have been identified in fungi ([1](#)) (see [Fig. 1](#)).

The TOR catalytic subunit in TORC1 binds rapamycin when the drug is in complex with the prolyl isomerase FKBP12. The FKBP12-rapamycin complex (hereafter referred to simply as rapamycin) binds the FKBP12-rapamycin binding (FRB) domain in TOR, which is located in close proximity to the kinase domain. As a result, most (but not all) TORC1-dependent activities are inhibited. Studies of *S. cerevisiae* played a central role in understanding the mode of action of rapamycin, including the identification of FKBP12 and the FKBP12-rapamycin binding (FKBP12/rapamycin-binding) domain and the characterization of the differential sensitivity to rapamycin of TORC1 and TORC2 ([1](#), [4](#)–[6](#), [10](#)). Still, the mechanism by which rapamycin inhibits TOR signaling and the determinants that establish rapamycin sensitivity are not fully understood. Several mechanisms of action have been suggested, including rapamycin-mediated disruption of TOR complexes and inhibition of the access of specific substrates to the kinase domain of TOR (reviewed in [17](#)). TORC2 is less sensitive to inhibition by rapamycin compared with TORC1 or is not sensitive at all. Recent structural studies of *Sc*TORC2 suggest that the *Sc*TORC2-specific subunit Avo3 (Rictor in metazoans) masks the

rapamycin-FKBP12 binding site of Tor2, thus preventing the inhibition of *Sc*TORC2 signaling ([18](#)).

The specificity of rapamycin for TORC1 proved most useful in deciphering cellular functions of this complex. Disruption of TORC1 in *S. cerevisiae*, either through genetic manipulations or inhibition by rapamycin, results in a phenotype that resembles starved cells, including arrest at the G1 phase of the cell cycle, G0-like cellular morphology and physiology, rapid drop in protein synthesis, repression of ribosomal gene expression, induction of stress- and nutrient-starvation genes, and stimulation of autophagy ([19](#)–[22](#)). Further studies demonstrated a conserved role of TORC1 in growth regulation through stimulating nutrient uptake, ribosome biogenesis, and the synthesis of proteins, lipids, and nucleotides ([23](#)–[25](#); reviewed in [1](#), [26](#)). To methodically screen for conditions that activate *Sc*TORC1, the phosphorylation status of Sch9, the AGC kinase downstream of *Sc*TORC1, was monitored. These studies demonstrated that Sch9 becomes dephosphorylated and inactivated in response to nitrogen, carbon, or phosphate starvation; high temperature; salt or redox conditions ([27](#)); and amino acid starvation or low quality of the nitrogen source ([28](#)). However, the signal or signals that are transduced to *Sc*TORC1 under these conditions are only partially understood.

Somewhat curiously, the growth of wild-type *S. pombe* cells is resistant to treatment with rapamycin ([29](#)). Yet rapamycin inhibits the phosphorylation of Psk1, the AGC kinase that lies downstream of *Sp*TORC1, or the phosphorylation of the Rps6 protein (S6 in human), the substrate of Psk1 ([30](#), [31](#)). S6 is a well-established target for mTORC1-S6K in humans and is often used to screen for hyperactivation of mTOR in cancer cells ([32](#)). Since rapamycin does not inhibit the essential function of *Sp*TORC1, conditional mutants of the catalytic subunit of *Sp*TORC1, *tor2*, or *mip1* (Raptor homologue) are used to study the cellular functions of *Sp*TORC1. Disruption of *Sp*TORC1 resulted in a phenotype that specifically resembles nitrogen-starved cells: growth arrest at the G1 phase of the cell cycle (unlike growth arrest at the G2 phase, characteristic for carbon starvation in *S. pombe*), small and rounded cell morphology, induction of nitrogen-starvation-induced genes, and activation of the sexual development pathway ([11](#), [12](#), [33](#), [34](#)). Complete withdrawal of the nitrogen source from the medium or a shift to a poor-quality nitrogen source (proline instead of ammonium) resulted in downregulation of *Sp*TOC1 activity as measured by decreased Rps6 phosphorylation ([30](#), [35](#)), leading to division at a reduced size and

advancement into mitosis ([36], [37]). In *C. albicans*, rapamycin inhibits growth via inhibition of the single TOR gene, *Tor1*, while nitrogen and carbon starvation both result in reduced *Ca*S6 phosphorylation ([15], [38]), suggesting that similar to *S. cerevisiae*, *Ca*TORC1 is sensitive to rapamycin and responds to both carbon and nitrogen limitation.

At the transcriptional level, inhibition of *Sc*TORC1 or rapamycin treatment in *S. cerevisiae* ([20], [21], [39], [40]), disruption of *Sp*TORC1 ([12], [33]), or rapamycin treatment in *C. albicans* ([41]) led to the reduction of ribosomal gene expression and induction of starvation- or stress-responsive transcription. Of note is the regulation of the response to changes in quantity or quality of the nitrogen source by the conserved TORC1-protein phosphatase 2A (PP2A) module, which regulates the activity of GATA-transcription factors (a family of transcription factors that are characterized by their ability to bind DNA sequences that contain the GATA motif). Early studies identified the zinc-finger GATA transcription factors Gln3 and Gat1 as targets for *Sc*TORC1 ([20], [21], [40]). Further studies unraveled a pathway in which *Sc*TORC1 regulates the activity of PP2A and PP2A-like phosphatases such as Sit4 by regulating their association with the inhibitory regulatory subunit Tap42. Upon reduction of the quantity or quality of the nitrogen source, *Sc*TORC1 is inactivated, promoting the disassociation of Tap42 and the activation of the phosphatases. In turn, the Ure2 protein that sequesters Gln3 and Gat1 is dephosphorylated, and Gln3 and Gat1 are dephosphorylated and translocate into the nucleus ([20], [21], [42]–[44]). Gln3 and Gat1 are required for transcriptional activation of genes that are normally repressed in the presence of high quantity and quality of the nitrogen source and are critical regulators of nitrogen catabolite repression, the processes by which high-quality nitrogen sources are imported and assimilated in preference to poor-quality nitrogen sources.

The *C. albicans* *GLN3* and *GAT1* homologues are also required for regulation of nitrogen metabolism, and their disruption resulted in rapamycin resistance, similar to the effect of disrupting *GLN3* and *GAT1* in *S. cerevisiae* ([45]). More recently, *Sp*TORC1 was demonstrated to regulate the phosphorylation and nuclear localization of the GATA transcription factor Gaf1. Gaf1 becomes dephosphorylated and inactivated in a manner dependent on the PP2A phosphatase Ppe1 in response to nitrogen (but not carbon) starvation ([46], [47]). The conservation of the TORC1-PP2A-GATA-transcription signaling in the distantly related *S. cerevisiae* and *S. pombe*, two yeasts that diverged in evolu-

tion more than 300 million years ago, suggests that this pathway may also be conserved in higher eukaryotes.

Early studies showed that treatment of *S. cerevisiae* with rapamycin led to a rapid drop in protein synthesis ([19]). Further studies revealed that *Sc*TORC1-Sch9 and *Sc*TORC1-Tap42-PP2A play prominent roles in the regulation of ribosome biogenesis, one of the most energetically demanding processes and thus a focal point for the coordination of growth with nutritional changes. *Sc*TORC1 regulates ribosome biogenesis at many levels, including transcriptional induction of rRNA, ribosomal proteins, and the ribosome biogenesis (Ribi) regulon. Several transcription factors were identified as targets of *Sc*TORC1 in the regulation of ribosome biogenesis, including Maf1, the repressor of RNA pol III ([23], [48]), Sfp1 ([49]), the forkhead transcription factor Fhl1 ([50]), and the transcriptional repressors Stb3, Dot6, and Tod6 ([24]). *Sc*TORC1 also directly binds 35S rDNA promoters to promote rRNA synthesis ([51]). Similar to *S. cerevisiae*, *Sp*TORC1 also regulates transcription of ribosomal proteins ([33]) and phosphorylates the repressor of RNA pol III Maf1 ([52]). At the posttranscriptional level, *Sc*TORC1 regulates protein synthesis through regulation of the translation-initiation factor eIF2α, in a manner that is at least partially conserved in *S. pombe* ([53], [54]). In both *S. cerevisiae* and *S. pombe* eIF2α is phosphorylated in response to amino acid starvation or rapamycin treatment. The phosphorylation of eIF2α is mediated via the *S. cerevisiae* or *S. pombe* Gcn2 kinases; however, *Sc*TORC1 regulates the phosphorylation of Gcn2 at sites that are not conserved in *S. pombe*.

Autophagy is a general name for catabolic processes mediated by membrane trafficking pathways that lead cytoplasmic material from the cytosol into the lumen of the lysosome or vacuole, where massive degradation takes place. *Sc*TORC1 inhibits autophagy under favorable growth conditions through the phosphorylation of Atg13 that inhibits the assembly of the Atg1 protein kinase complex ([55]). Autophagy has been linked to several human pathologies, including cancer and neurodegenerative diseases and is also implicated in the process of aging. Most interestingly, rapamycin treatment or reduced TORC1 activity in *S. cerevisiae*, in *S. pombe*, or in higher eukaryotes extended the life span ([56]–[58]). TORC1 controls aging through Sch9 in *S. cerevisiae* ([56]), in a process that involves the induction of autophagy and stress genes, as reviewed in more detail in reference [8]. Additional downstream effects of TORC1 have been studied in detail, including amino acid uptake, regulation of cell size, and developmental responses, including entrance into quiescence or regu-

lation of filamentous growth. These are not detailed here due to limited space but are reviewed elsewhere ([1], [59]).

TORC1 Upstream Signaling and the Response to Nitrogen Quantity or Quality or the Presence of Amino Acids

In yeast cells, the activity of TORC1 is sensitive to nutritional starvation and a variety of stresses, while mTORC1 is also sensitive to energy levels (ATP) and growth factors ([3]). The direct signals or mechanisms for activation of TORC1 are as yet unknown; however, a large body of evidence suggests that the nitrogen source and/or amino acids (which may also serve as a nitrogen source) play a key role in TORC1 activation.

Nitrogen is an essential element required for synthesis of amino acids, nucleotides, and other cellular components. Yeast cells can sense, take up, and assimilate several nitrogen sources. A high-quality nitrogen source (glutamine or ammonium) is defined by its ability to promote rapid growth and suppress the nitrogen catabolite repression genes ([60]). As described above, *Sc*TORC1 and *Sp*TORC1 are major regulators of the transcriptional control in response to the quality and quantity of the nitrogen source, and the activity of these complexes drops in response to low levels or poor quality of the nitrogen source ([28], [30], [35]). *Sc*TORC1 and *Sp*TORC1 are also activated in response to specific amino acids such as leucine, glutamine, asparagine, arginine, aspartate, methionine, and cysteine ([28], [61]–[64]), raising the intriguing possibility that the nitrogenous compounds critical for TORC1 activation are amino acids. This possibility is particularly tempting in view that in higher eukaryotes, amino acids (particularly leucine, glutamine, and arginine) are potent activators of mTOR signaling ([65]–[67]). Whether amino acid levels are directly sensed by TORC1 and, if so, by what mechanisms is still an open question. Moreover, the mechanism of activation of TORC1 by the quantity and quality of the nitrogen source appears distinct from the mechanism of response to amino acids ([28], [36]). Moreover, different amino acids are thought to activate TORC1 through different mechanisms, including glutaminolysis and leucyl-tRNA synthetase ([61], [68]).

TORC1 and several remarkably conserved upstream regulators of TORC1 are localized to the vacuole in yeast cells and to its equivalent compartment, the lysosome, in higher eukaryotes (reviewed in [17]). At the vacuolar or lysosomal surface TORC1 is activated by two distinct guanosine triphosphate GTPases: the Gtr (Rag in higher eukaryotes) and the Rhb1 (Rheb in higher eukaryotes). The Rag/Gtr-TORC1 axis is conserved in *S. cerevisiae* and *S. pombe*, while the Rhb1/Rheb-TORC1 axis is conserved in *S. pombe* but is absent in *S. cerevisiae* (see Fig. 2). The vacuole and lysosome act as major sites for protein degradation and reservoirs of amino acids; however, whether TORC1 responds to amino acids in the vacuole/lysosome or in the cytoplasm is not known yet.

In *S. cerevisiae*, the Gtr complex is composed of Gtr1 and Gtr2. Gtr1 is analogous to the mammalian RagA or RagB, while Gtr2 is analogous to RagC or RagD. Only when Gtr1 or RagA/B is bound to GTP and Gtr2 or RagC/D is bound to GDP is the heterodimer active and able to lead to activation of TORC1 ([69]–[71]). The Gtr1/2 GTPases associate with the EGO complex, which contains Ego1, Ego2, and Ego3 to form a complex that tethers Gtr1/2 and *Sc*TORC1 to the vacuolar membrane ([71]–[73]). The EGO equivalent complex in higher eukaryotes is the pentameric Ragulator complex, which is required for lysosmal localization and also acts as a guanine exchange factor toward RagA/B. In *S. cerevisiae* the guanine exchange factor activity toward Gtr1 is provided by Vam6 (vacuolar morphogenesis protein), which apparently has no mammalian equivalent (reviewed in [17]). The vacuolar Vam6-Gtr1/2 module is conserved in *S. pombe* and activates *Sp*TORC1 in response to the presence of amino acids ([62]). The interaction and activation of either *Sc*TORC1 or *Sp*TORC1 by the Vam6-Gtr1/2 module are sensitive to the presence of amino acids, but *Sc*TORC1 and *Sp*TORC1 are localized to the vacuole irrespective of the nutritional status ([62], [71]). In contrast, mTORC1 dissociates from the lysosome upon amino acid starvation ([69], [70]). The Gtr and Rag complexes are negatively regulated by conserved GAP (GTPase activating protein) complexes that are named SEACIT and GATOR1 in *S. cerevisiae* and humans, respectively (Fig. 2). SEACIT and GATOR1 are in turn subjected to negative regulation by additional conserved complexes, SEACAT and GATOR2 ([74]; reviewed in [17]) (Fig. 2). More recently, Lst4-Lst7 was identified as the complex that acts as a GAP toward Gtr2, promoting the binding of GDP to Gtr2 and thus activating *Sc*TORC1 ([64]), similar to the FNIP-folliculin complex in mammalian cells (reviewed in [17]).

The *S. cerevisiae* Gtr1/2 GTPases regulate TORC1 activity but are not essential genes, suggesting that they play only a limited role in activation of *Sc*TORC1. Similarly, disruption of *gtr1/2* in *S. pombe* leads to a reduced level of *Sp*TORC1 activity and a hyper-mating phenotype, characteristic of conditional loss-of-function *Sp*TORC1 mutants; still, *S. pombe* cells lacking Gtr1/2 or Vam6 can respond to changes in the nitrogen quan-
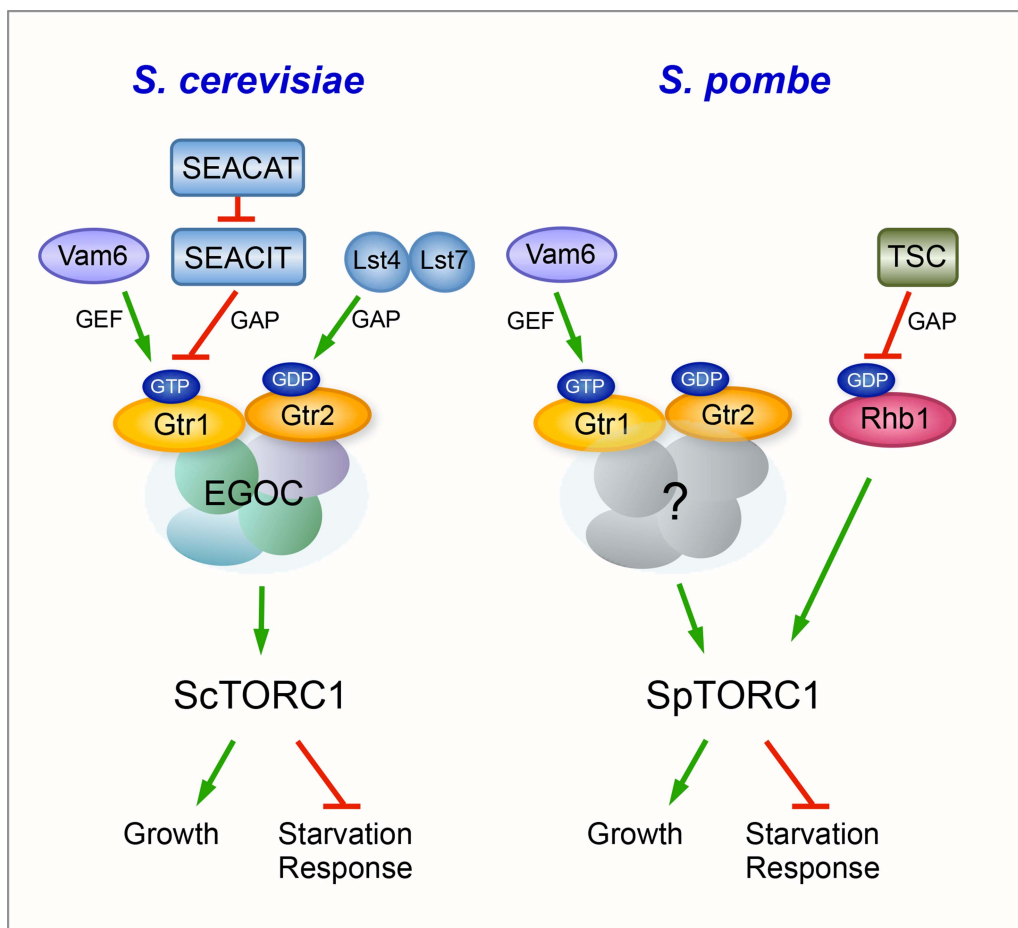
FIGURE 2 TORC1 is activated by GTPases to promote growth and inhibit starvation responses. *Saccharomyces cerevisiae* TORC1 (*Sc*TORC1) and *Schizosaccharomyces pombe* TORC1 (*Sp*TORC1) are activated by the GTPase complex Gtr1/Gtr2. The complex is active when Gtr1 is bound to GTP and Gtr2 is bound to GDP. Vam6 is a guanine exchange factor (GEF) for Gtr1 that is conserved between *S. cerevisiae* and *S. pombe*. In *S. cerevisiae*, the Gtr1/Gtr2 complex is associated with the EGO complex and is controlled by the SEACIT (which acts as GTPase activating protein, GAP), SEACAT, and Lst4-Lst7 complexes. These complexes have as yet unidentified equivalents in *S. pombe*. *Sp*TORC1 is also regulated by the Rhb1 GTPase (Rheb in mammals) and the TSC (tuberous sclerosis complex, a tumor suppressor complex in mammals), which acts as a GAP towards Rhb1.

tity or quality. These findings argue that the Gtr-TOR axis plays only a relatively limited role in activation of TORC1 ([62](#)).

While the Gtr1/2 complex is dispensable for activation of TORC1 in yeast cells, the *S. pombe* Rhb1 GTPase (Rheb in human) is essential for *Sp*TORC1 activation ([75](#)–[78](#)). Accordingly, loss of function of Rhb1 results in a phenotype that mimics nitrogen starvation and is highly similar to disruption of *Sp*TORC1, including the G1 arrest and activation of nitrogen-starvation gene expression. Rhb1 is negatively regulated by the TSC1-TSC2 complex, which is conserved in humans and functions as a GAP toward Rhb1. The TSC complex functions as a tumor suppressor complex, and mutations in either TSC1 or TSC2 can lead to the tuberous sclerosis syndrome, which is characterized by rapid development of tumors and severe neurological defects ([79](#)). Disruption of the TSC complex in *S. pombe* results in prolonged phosphorylation of ribosomal S6 under nitrogen starvation ([31](#)), reduced transcription of nitrogen-starvation-induced amino acid permeases, and reduced amino acid uptake ([80](#), [81](#)). These findings suggest that deletion of TSC in *S. pombe* leads to hyperactivation of *Sp*TORC1 that shuts down transcriptional programs dedicated to scavenging for nitrogen sources. *C. albicans* homologues for Rheb and TSC2 were also identified.

Disruption of *Ca*Rhb1 resulted in hypersensitivity to rapamycin, while *Ca*Rhb1 overexpression and *Ca*Tsc2 deletion are defective in filamentous growth under low or poor nitrogen, suggesting that Rhb1 activates *Ca*TORC1 and prevents normal responses to nitrogen starvation ([82]).

The TSC-Rheb axis acts as a hub for several signaling pathways, among them the AMPK, a serine/threonine kinase that coordinates cell growth and metabolism with available energy resources (reviewed in [83]). AMPK is a heterotrimeric complex composed of a catalytic (α) subunit and two regulatory (β and γ) subunits. In humans, the tumor suppressors LKB1 and CaMKK (calcium/calmodulin-dependent protein kinase kinase) activate mammalian AMPK, which in turn directly phosphorylates TSC2 and activates its GAP activity, leading to conversion of Rheb to its inactive form and inhibition of mTORC1 ([84]; reviewed in reference [83]). It was recently demonstrated that in *S. pombe*, the α subunit of AMPK is required to respond to changes in the quality of the nitrogen source. Under nitrogen stress conditions (shift from a high-quality to poor-quality nitrogen source), a specific homologue of CaMKK (Ppk34) stimulates *Sp*AMPK activity, which leads to downregulation of *Sp*TORC1 in a manner dependent on the TSC-Rhb1 ([36]). How TORC1 may integrate the complex signals from different pathways and coordinate these with the nutritional status awaits further studies.

TORC2: ROLES IN CELLULAR METABOLISM, GROWTH, AND SURVIVAL

TORC2 Architecture and Downstream Signaling

TORC2 contains the TOR catalytic subunit together with two additional highly conserved proteins that are essential for the function of the complex: Rictor in metazoans (Avo3 in *S. cerevisiae* and Ste20 in *S. pombe*) and mSin1 in metazoans (Avo1 in *S. cerevisiae* and Sin1 in *S. pombe*) (see [Fig. 1]). TORC2 contains the Lst8 subunit, which is also found in TORC1. The Protor subunit in mammals has orthologues in *S. cerevisiae* and *S. pombe* (Bit61). Avo2 is specific for *S. cerevisiae* and *C. albicans*, while Deptor (which is also part of mTORC1) has been identified only in mammals (reviewed in [1]).

The cellular functions of TORC2 are less well understood compared with TORC1. This is partly due to the lack of TORC2-specific inhibitors. In addition, in contrast to the starvation-like phenotypes observed upon disruption of TORC1, the loss of TORC2 results in diverse effects (reviewed in [85]), making it difficult

to attribute a primary cellular function to TORC2. Recent findings suggest that TORC2 plays significant roles in cellular metabolism, including cancer metabolism, growth control, and survival, encouraging further research of TORC2 and the search for TORC2-specific inhibitors ([85], [86]). As in TORC1 signaling, AGC kinases downstream of TORC2 play important roles in mediating downstream effects (see [Fig. 1]). The best-studied AGC kinases downstream of *Sc*TORC2 are Ypk1 and Ypk2 ([87]), while most of *Sp*TORC2 functions are mediated via Gad8 ([88]).

*Sc*TORC2 is essential for growth, while *Sp*TORC2 becomes essential only under starvation and a variety of other stress conditions. So far, only a limited overlap exists between the functions attributed to *Sc*TORC2 and *Sp*TORC2. It is not clear whether this reflects a true divergence in function or our incomplete understanding of the cellular roles of TORC2. *Sc*TORC2 was first implicated in the regulation of the polarization of the actin cytoskeleton, suggesting that it mainly regulates spatial aspects of cell growth ([89], [90]). Further studies revealed that *Sc*TORC2-Ypk1/2 also controls endocytosis ([91]) and sphingolipid biosynthesis ([92]–[95]), in a manner that is antagonized by the calcium- and calmodulin-dependent protein phosphatase calcineurin, which acts either directly downstream of *Sc*TORC2 or in a parallel pathway ([92], [93]). *Sc*TORC2 regulates actin polarization and endocytosis via flipase protein kinases ([96]–[99]), in a manner that was also suggested to be linked to the control of reactive oxygen species accumulation ([97], [98]). Interestingly, the level of reactive oxygen species is regulated by *Sc*TORC2 and can act as a signal to activate *Sc*TORC2 ([97], [98]). The Slm proteins, which were first identified downstream of *Sc*TORC2, can also function upstream of *Sc*TORC2 to promote activation of this complex in response to plasma membrane stress, resulting in a homeostatic loop in which *Sc*TORC2 regulates several aspects of membrane biogenesis and is also affected by changes in the membrane ([100]). Recently, it was demonstrated that *Sc*TORC2-Ypk1 positively regulates autophagy in response to amino acid starvation ([101]). This finding is interesting because it suggests that *Sc*TORC1 and *Sc*TORC2 oppositely regulate autophagy, albeit by different mechanisms. It may also suggest that *Sc*TORC2 is linked to nutritional changes more closely than has previously been recognized.

The most pronounced effects of loss of function of *Sp*TORC2-Gad8 become apparent under starvation and stress conditions. *Sp*TORC2 is essential to execute the two main responses to starvation: sexual development

and entrance into stationary phase. Thus, cells that lack *Sp*TORC2 or Gad8 are highly sterile and quickly die once they exit the logarithmic phase ([13](#), [102](#)). Loss of *Sp*TORC2-Gad8 also renders cells sensitive to a variety of stress conditions, including low or high temperature, osmotic or oxidative stress, and DNA damage or replication stress conditions ([13](#), [103](#), [104](#)). Under normal growth conditions, *Sp*TORC2-Gad8 is required for the G2/M transition, and cells disrupted for *Sp*TORC2 show an elongated phenotype characteristic of a delay in entrance into mitosis ([13](#), [103](#), [105](#), [106](#)). *Sp*TORC2 is also required for amino acid uptake ([107](#)) and for the localization of Ght5, a high-affinity glucose transporter, to the cell surface under low-glucose conditions ([108](#)). An abnormal distribution of actin cortical dots and excess actin polymerization at the cell equator occur in cells lacking functional *Sp*TORC2 ([106](#)), as do defects in reorganizing the actin observed during new end take-off, the phase during which the new cell end starts to grow ([109](#)).

Unexpectedly, transcriptional profiles of *S. pombe* cells lacking the catalytic subunit of *Sp*TORC2 resembled those of cells lacking histone deacetylases or chromatin remodeling subunits ([104](#)). This finding led to the identification of additional defective phenotypes in *Sp*TORC2 mutant cells, including elongation of telomeres and loss of gene silencing ([104](#)). Of interest are the similarities observed upon loss of function of *Sc*TORC2 or *Sp*TORC2 with respect to DNA damage sensitivity ([104](#), [110](#)). In both cases, TORC2 plays a role in survival of DNA damage in a manner that is independent of DNA checkpoint activation but involves suppression of accumulation of DNA damage sites. However, in *S. cerevisiae*, elevated levels of DNA damage are thought to be the result of the defect in the actin cytoskeleton, whereas in *S. pombe* no such link is known ([85](#)). Interestingly, the inability of cells that lack *Sp*TORC2 or Gad8 to execute nitrogen starvation responses is opposite to the "always starved for nitrogen" phenotype of cells lacking *Sp*TORC1 ([35](#), [106](#)). This is somewhat reminiscent of the opposite effects of *Sc*TORC1 and *Sc*TORC2 in the regulation of autophagy and may suggest regulatory links between *Sp*TORC1 and *Sp*TORC2.

TORC2 Upstream Signaling and the Response to Glucose Availability

The upstream regulation of TORC2 is poorly characterized. *Sc*TORC2 has been localized to membrane structure organelles, primarily to the plasma membrane, but has also been detected throughout the cytoplasm ([111](#), [112](#)). Visualization of GFP tagged *Sp*TORC2 components also indicated cytoplasmic as well as corti-

cal localization ([113](#)), but a biochemical approach has suggested that Tor1, the catalytic subunit of *Sp*TORC2, and Gad8 are also found in the nucleus ([114](#)). *Sc*TORC2 is activated by direct association with the ribosome ([115](#), [116](#)), in a manner that is conserved in mammals and that may link TORC2 to growth capacity. In accord with the localization of *Sc*TORC2 to the plasma membrane, *Sc*TORC2 is activated by plasma membrane stress, which stems from cell surface expansion or stress on the plasma membrane ([100](#)). This mode of activation is thought to reflect a feedback loop mechanism in which *Sc*TORC2 regulates membrane biosynthesis but is also affected by membrane growth ([17](#)). In *S. pombe*, glucose but not nitrogen is required for activation of *Sp*TORC2 ([117](#), [118](#)). The activity of *Sp*TORC2-Gad8 in response to glucose requires the activation of Rhy1, a Rab family GTPase that lies upstream of *Sp*TORC2-Gad8 ([118](#)). The regulation of *Sp*TORC2-Gad8 in response to glucose availability is fast and does not require protein translation, suggesting a close link between a nutritional change and TORC2 activation, although the nature of the glucose signal that is sensed by *Sp*TORC2 is as yet unknown.

TOR IN PATHOGENIC FUNGI

Virulence in plant or human pathogenic fungi requires several cellular traits, including invasive growth, morphogenetic yeast-to-hyphal transition (e.g., in *C. albicans*), vegetative hyphal fusion, and expression of adhesion and stress response genes. As discussed above, TOR signaling regulates many aspects of the stress response as well as the switch from vegetative growth to developmental programs in response to environmental changes. Accordingly, accumulation of data indicates that TOR signaling is critical for virulence in several pathogenic fungi and may thus serve as a target for antifungal treatment. Early studies demonstrated that rapamycin and several less immunosuppressive analogues inhibited growth in *S. cerevisiae*, *C. albicans*, and *Cryptococcus neoformans*, suggesting that the use of rapamycin analogues may be beneficial as antifungal agents ([15](#); reviewed in [16](#)). Further support of the role of TOR in *C. albicans* pathogenicity is evident from studies that demonstrate the role of *Ca*Tor1 in regulating the expression of cell wall- and hyphal-specific genes, including adhesins ([41](#)). Moreover, genetic manipulation of *Ca*TOR signaling, including disruption of *Ca*Sch9 ([119](#)), *Ca*Rhb1 or *Ca*Tsc2 ([82](#)), or *Ca*Sit4 ([120](#)), supports a role for *Ca*Tor1 in promoting hyphal formation and virulence in response to nutritional changes, as well as in response to changes in pH ([121](#)).

Interestingly, reduced *Ca*Tor1 signaling during hyphal initiation leads to the expression of the GATA transcription factor Brg1, which competes for promoter binding with Nrg1, the major transcription repressor of hyphal development ([122]). Thus, similar to *S. cerevisiae* or *S. pombe*, TOR is found to regulate a family member of the GATA transcription factors in *C. albicans*, but unlike *S. cerevisiae* or *S. pombe*, *Ca*Tor1 regulates the level of Brg1 indirectly via the Hog1 MAP kinase pathway ([122]). A study of fluconazole-sensitive mutants in *C. neoformans* also suggests that TORC2 signaling is conserved and may play an important role in recurrent infections of *C. neoformans* ([123]). Thus, for example, similar to equivalent mutations in *S. cerevisiae*, the deletion of *C. neoformans* Sin1 or Ypk1 resulted in hypersensitivity to inhibition of sphingolipid synthesis, and the mutant cells contained low levels of complex sphingolipids ([123]).

Rapamycin also induced growth inhibition in filamentous fungi, including the human pathogens *Aspergillus fumigatus* ([124]) and *Aspergillus nidulans* ([125]). The single gene encoding the TOR kinase in *A. fumigatus* (*Af*Tor1) is essential for growth. However, the use of a conditionally repressible version of *Af*Tor1 in combination with proteomic analysis identified potential protein targets of *Af*Tor1 that are involved in cell cycle regulation, nutrient sensing, and stress response ([124]). *Af*Tor1 also regulates siderophore biosynthesis and the adaptation to iron starvation, a response that is critical for the virulence of *A. fumigatus* ([124]). TOR signaling may play an essential role in *Aspergillus* growth via its function as part of TORC2. Thus, for example, the Sin1 homologue in *Aspergillus niger*, RmsA, is required for hyphal elongation and branching ([126]) and for viability and stress responses ([127]), reminiscent of the roles identified for TORC2 in cell polarity and stress response in yeasts.

Conservation in the architecture of TOR signaling and the response to rapamycin also exists in phytopathogenic fungi. Rapamycin inhibits the growth and virulence of phytopathogenic fungi, including several species of *Fusarium* ([128], [129]). Moreover, it was demonstrated that TOR participates in nitrogen metabolite repression (e.g., in *Fusarium fujikuroi* [[128]]). In addition, the Tap42-type 2A phosphatase module ([129]) and Sch9 homologues ([130]) were identified as downstream targets of TOR signaling in *Fusarium graminearum*. Still, better understanding of the response of pathogenic and filamentous fungi to rapamycin or other TOR inhibitors awaits further identification of TOR components, especially the characterization of the distinct cellular roles of TORC1 and TORC2 and the identification of additional components of these pathways.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Since its early discovery as the target for action of rapamycin, TOR has emerged as a central signaling pathway that regulates many different aspects of growth in response to environmental changes, in particular nutritional changes. Regulation of TORC1 in response to nitrogen availability and/or amino acids is the most well-established nutritional response for the TOR pathway. TORC2 may also respond to nutritional changes, but the mechanisms involved are far less well understood. How TOR signaling mediates response to stress conditions, other than nutritional stress, also remains to be determined. The finding that TOR complexes often act as part of feedback loops has suggested that TOR may function primarily as a mediator of cellular homeostasis ([17]).

The identification of the two TOR complexes marked a breakthrough in our understanding of TOR signaling pathways and rapamycin response. TORC1 and TORC2 are structurally and functionally distinct, and the search for specific TORC1 and TORC2 downstream and upstream effectors is still the focus of many studies. More recent studies suggest that understanding the regulatory links between TORC1 and TORC2 is also critical for understanding the role of TOR in growth control. *Sp*TORC1 and *Sp*TORC2 oppositely regulate several responses to nitrogen starvation including sexual development and transcriptional reprogramming ([131]). mTORC1 negatively regulates mTORC2 through phosphorylation of several mTORC2-specific components, while mTORC1 and mTORC2 also converge to control certain aspects of metabolism ([86]). Future studies are expected to unravel the links between TORC1 and TORC2 in more detail.

Finally, *S. cerevisiae* has played a key role in deciphering the mode of action of rapamycin, a drug that has already proved most valuable for the treatment of several human pathologies. The powerful tools of yeast genetics are expected to contribute to further understanding of the mode of action of rapamycin and second-generation ATP-competitive TOR inhibitors. TORC2-specific inhibitors have been much sought after. Whether yeast will also contribute to finding such valuable research tools and possible drugs is an exciting option that at present remains an open question.

REFERENCES

1. **Loewith R, Hall MN.** 2011. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **189:**1177–1201. http://dx.doi.org /10.1534/genetics.111.133363.

2. **Workman JJ, Chen H, Laribee RN.** 2014. Environmental signaling through the mechanistic target of rapamycin complex 1: mTORC1 goes nuclear. *Cell Cycle* **13:**714–725. http://dx.doi.org/10.4161/cc.28112.

3. **Chantranupong L, Wolfson RL, Sabatini DM.** 2015. Nutrient-sensing mechanisms across evolution. *Cell* **161:**67–83. http://dx.doi.org/10.1016 /j.cell.2015.02.041.

4. **Heitman J, Movva NR, Hall MN.** 1991. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253:**905–909. http://dx.doi.org/10.1126/science.1715094.

5. **Cafferkey R, Young PR, McLaughlin MM, Bergsma DJ, Koltin Y, Sathe GM, Faucette L, Eng WK, Johnson RK, Livi GP.** 1993. Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin cytotoxicity. *Mol Cell Biol* **13:**6012–6023. http://dx.doi.org/10.1128/MCB.13 .10.6012.

6. **Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN.** 1993. Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* **73:**585–596. http://dx.doi.org/10.1016/0092-8674(93)90144-F.

7. **Laplante M, Sabatini DM.** 2012. mTOR signaling in growth control and disease. *Cell* **149:**274–293. http://dx.doi.org/10.1016/j.cell.2012.03.017.

8. **Cornu M, Albert V, Hall MN.** 2013. mTOR in aging, metabolism, and cancer. *Curr Opin Genet Dev* **23:**53–62. http://dx.doi.org/10.1016/j.gde .2012.12.005.

9. **Shertz CA, Bastidas RJ, Li W, Heitman J, Cardenas ME.** 2010. Conservation, duplication, and loss of the Tor signaling pathway in the fungal kingdom. *BMC Genomics* **11:**510. doi:10.1186/1471-2164-11-510 http://dx.doi.org/10.1186/1471-2164-11-510.

10. **Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN.** 2002. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* **10:**457–468. http://dx.doi.org/10.1016/S1097 -2765(02)00636-6.

11. **Hayashi T, Hatanaka M, Nagao K, Nakaseko Y, Kanoh J, Kokubu A, Ebe M, 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. http://dx.doi.org/10.1111/j.1365-2443.2007.01141.x.

12. **Matsuo T, Otsubo Y, Urano J, Tamanoi F, Yamamoto M.** 2007. Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast. *Mol Cell Biol* **27:**3154–3164. http://dx.doi.org/10.1128/MCB.01039-06.

13. **Weisman R, 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. http://dx.doi.org/10.1074/jbc .M010446200.

14. **Cruz MC, Cavallo LM, Görlach JM, Cox G, Perfect JR, Cardenas ME, Heitman J.** 1999. Rapamycin antifungal action is mediated via conserved complexes with FKBP12 and TOR kinase homologs in *Cryptococcus neoformans*. *Mol Cell Biol* **19:**4101–4112. http://dx.doi.org/10.1128 /MCB.19.6.4101.

15. **Cruz MC, Goldstein AL, Blankenship J, Del Poeta M, Perfect JR, McCusker JH, Bennani YL, Cardenas ME, Heitman J.** 2001. Rapamycin and less immunosuppressive analogs are toxic to *Candida albicans* and *Cryptococcus neoformans* via FKBP12-dependent inhibition of TOR. *Antimicrob Agents Chemother* **45:**3162–3170. http://dx.doi.org/10.1128 /AAC.45.11.3162-3170.2001.

16. **Bastidas RJ, Reedy JL, Morales-Johansson H, Heitman J, Cardenas ME.** 2008. Signaling cascades as drug targets in model and pathogenic fungi. *Curr Opin Investig Drugs* **9:**856–864.

17. **Eltschinger S, Loewith R.** 2016. TOR complexes and the maintenance of cellular homeostasis. *Trends Cell Biol* **26:**148–159. http://dx.doi.org /10.1016/j.tcb.2015.10.003.

18. **Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G, Rispal D, Eltschinger S, Robinson GC, Thore S, Aebersold R, Schaffitzel C, Loewith R.** 2015. Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. *Mol Cell* **58:**977–988. http://dx.doi.org/10.1016/j.molcel.2015.04.031.

19. **Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN.** 1996. TOR controls translation initiation and early G1 progression in yeast. *Mol Biol Cell* **7:**25–42. http://dx.doi.org/10.1091/mbc.7.1.25.

20. **Beck T, Hall MN.** 1999. The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* **402:**689–692. http://dx.doi.org/10.1038/45287.

21. **Cardenas ME, Cutler NS, Lorenz MC, Di Como CJ, Heitman J.** 1999. The TOR signaling cascade regulates gene expression in response to nutrients. *Genes Dev* **13:**3271–3279. http://dx.doi.org/10.1101/gad.13 .24.3271.

22. **Noda T, Ohsumi Y.** 1998. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J Biol Chem* **273:**3963–3966. http://dx.doi.org/10.1074/jbc.273.7.3963.

23. **Huber A, Bodenmiller B, Uotila A, Stahl M, Wanka S, Gerrits B, Aebersold R, Loewith R.** 2009. Characterization of the rapamycin-sensitive phosphoproteome reveals that Sch9 is a central coordinator of protein synthesis. *Genes Dev* **23:**1929–1943. http://dx.doi.org/10.1101 /gad.532109.

24. **Huber A, French SL, Tekotte H, Yerlikaya S, Stahl M, Perepelkina MP, Tyers M, Rougemont J, Beyer AL, Loewith R.** 2011. Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L. *EMBO J* **30:**3052–3064. http://dx.doi.org/10.1038 /emboj.2011.221.

25. **Oliveira AP, Ludwig C, Zampieri M, Weisser H, Aebersold R, Sauer U.** 2015. Dynamic phosphoproteomics reveals TORC1-dependent regulation of yeast nucleotide and amino acid biosynthesis. *Sci Signal* **8:** rs4. http://dx.doi.org/10.1126/scisignal.2005768.

26. **Albert V, Hall MN.** 2015. mTOR signaling in cellular and organismal energetics. *Curr Opin Cell Biol* **33:**55–66. http://dx.doi.org/10.1016/j.ceb .2014.12.001.

27. **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, Hall MN, Loewith R.** 2007. Sch9 is a major target of TORC1 in *Saccharomyces cerevisiae*. *Mol Cell* **26:**663–674. http://dx.doi .org/10.1016/j.molcel.2007.04.020.

28. **Stracka D, Jozefczuk S, Rudroff F, Sauer U, Hall MN.** 2014. Nitrogen source activates TOR (target of rapamycin) complex 1 via glutamine and independently of Gtr/Rag proteins. *J Biol Chem* **289:**25010–25020. http://dx.doi.org/10.1074/jbc.M114.574335.

29. **Weisman R, Choder M, Koltin Y.** 1997. Rapamycin specifically interferes with the developmental response of fission yeast to starvation. *J Bacteriol* **179:**6325–6334.

30. **Nakashima A, Sato T, Tamanoi F.** 2010. Fission yeast TORC1 regulates phosphorylation of ribosomal S6 proteins in response to nutrients and its activity is inhibited by rapamycin. *J Cell Sci* **123:**777–786. http://dx.doi.org/10.1242/jcs.060319.

31. **Nakashima A, Otsubo Y, Yamashita A, Sato T, Yamamoto M, Tamanoi F.** 2012. Psk1, an AGC kinase family member in fission yeast, is directly phosphorylated and controlled by TORC1 and functions as S6 kinase. *J Cell Sci* **125:**5840–5849. http://dx.doi.org/10.1242/jcs.111146.

32. **Tee AR, Blenis J.** 2005. mTOR, translational control and human disease. *Semin Cell Dev Biol* **16:**29–37. http://dx.doi.org/10.1016/j.semcdb .2004.11.005.

33. **Alvarez B, Moreno S.** 2006. Fission yeast Tor2 promotes cell growth and represses cell differentiation. *J Cell Sci* **119:**4475–4485. http://dx.doi .org/10.1242/jcs.03241.

34. **Weisman R, Roitburg I, Schonbrun M, Harari R, Kupiec M.** 2007. Opposite effects of tor1 and tor2 on nitrogen starvation responses in fission yeast. *Genetics* **175:**1153–1162. http://dx.doi.org/10.1534/genetics.106.064170.

35. **Laor D, Cohen A, Pasmanik-Chor M, Oron-Karni V, Kupiec M, Weisman R.** 2014. Isp7 is a novel regulator of amino acid uptake in the TOR signaling pathway. *Mol Cell Biol* **34:**794–806. http://dx.doi.org/10.1128/MCB.01473-13.

36. **Davie E, Forte GM, Petersen J.** 2015. Nitrogen regulates AMPK to control TORC1 signaling. *Curr Biol* **25:**445–454. http://dx.doi.org/10.1016/j.cub.2014.12.034.

37. **Chica N, Rozalén AE, Pérez-Hidalgo L, Rubio A, Novak B, Moreno S.** 2016. Nutritional control of cell size by the greatwall-endosulfine-PP2A·B55 pathway. *Curr Biol* **26:**319–330. http://dx.doi.org/10.1016/j.cub.2015.12.035.

38. **Chowdhury T, Köhler JR.** 2015. Ribosomal protein S6 phosphorylation is controlled by TOR and modulated by PKA in *Candida albicans*. *Mol Microbiol* **98:**384–402. http://dx.doi.org/10.1111/mmi.13130.

39. **Hardwick JS, Kuruvilla FG, Tong JK, Shamji AF, Schreiber SL.** 1999. Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. *Proc Natl Acad Sci USA* **96:**14866–14870. http://dx.doi.org/10.1073/pnas.96.26.14866.

40. **Bertram PG, Choi JH, Carvalho J, Ai W, Zeng C, Chan TF, Zheng XF.** 2000. Tripartite regulation of Gln3p by TOR, Ure2p, and phosphatases. *J Biol Chem* **275:**35727–35733. http://dx.doi.org/10.1074/jbc.M004235200.

41. **Bastidas RJ, Heitman J, Cardenas ME.** 2009. The protein kinase Tor1 regulates adhesin gene expression in *Candida albicans*. *PLoS Pathog* **5:**e1000294. doi:10.1371/journal.ppat.1000294 http://dx.doi.org/10.1371/journal.ppat.1000294.

42. **Di Como CJ, Arndt KT.** 1996. Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. *Genes Dev* **10:**1904–1916. http://dx.doi.org/10.1101/gad.10.15.1904.

43. **Jiang Y, Broach JR.** 1999. Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. *EMBO J* **18:**2782–2792. http://dx.doi.org/10.1093/emboj/18.10.2782.

44. **Zheng Y, Jiang Y.** 2005. The yeast phosphotyrosyl phosphatase activator is part of the Tap42-phosphatase complexes. *Mol Biol Cell* **16:**2119–2127. http://dx.doi.org/10.1091/mbc.E04-09-0797.

45. **Liao WL, Ramón AM, Fonzi WA.** 2008. GLN3 encodes a global regulator of nitrogen metabolism and virulence of *C. albicans*. *Fungal Genet Biol* **45:**514–526. http://dx.doi.org/10.1016/j.fgb.2007.08.006.

46. **Laor D, Cohen A, Kupiec M, Weisman R.** 2015. TORC1 regulates developmental responses to nitrogen stress via regulation of the GATA transcription factor Gaf1. *MBio* **6:**e00959-15. doi:10.1128/mBio.00959-15 http://dx.doi.org/10.1128/mBio.00959-15.

47. **Kim L, Hoe KL, Yu YM, Yeon JH, Maeng PJ.** 2012. The fission yeast GATA factor, Gaf1, modulates sexual development via direct down-regulation of ste11+ expression in response to nitrogen starvation. *PLoS One* **7:**e42409. doi:10.1371/journal.pone.0042409 http://dx.doi.org/10.1371/journal.pone.0042409.

48. **Lee J, Moir RD, Willis IM.** 2009. Regulation of RNA polymerase III transcription involves SCH9-dependent and SCH9-independent branches of the target of rapamycin (TOR) pathway. *J Biol Chem* **284:**12604–12608. http://dx.doi.org/10.1074/jbc.C900020200.

49. **Lempiäinen H, Uotila A, Urban J, Dohnal I, Ammerer G, Loewith R, Shore D.** 2009. Sfp1 interaction with TORC1 and Mrs6 reveals feedback regulation on TOR signaling. *Mol Cell* **33:**704–716. http://dx.doi.org/10.1016/j.molcel.2009.01.034.

50. **Martin DE, Soulard A, Hall MN.** 2004. TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. *Cell* **119:**969–979. http://dx.doi.org/10.1016/j.cell.2004.11.047.

51. **Li H, Tsang CK, Watkins M, Bertram PG, Zheng XF.** 2006. Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature* **442:**1058–1061 http://dx.doi.org/10.1038/nature05020.

52. **Du W, Hálová L, Kirkham S, Atkin J, Petersen J.** 2012. TORC2 and the AGC kinase Gad8 regulate phosphorylation of the ribosomal protein S6 in fission yeast. *Biol Open* **1:**884–888. http://dx.doi.org/10.1242/bio.20122022.

53. **Cherkasova VA, Hinnebusch AG.** 2003. Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. *Genes Dev* **17:**859–872. http://dx.doi.org/10.1101/gad.1069003.

54. **Valbuena N, Rozalén AE, Moreno S.** 2012. Fission yeast TORC1 prevents eIF2α phosphorylation in response to nitrogen and amino acids via Gcn2 kinase. *J Cell Sci* **125:**5955–5959. http://dx.doi.org/10.1242/jcs.105395.

55. **Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y.** 2010. Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol* **30:**1049–1058. http://dx.doi.org/10.1128/MCB.01344-09.

56. **Kaeberlein M, Powers RW III, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK.** 2005. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* **310:**1193–1196. http://dx.doi.org/10.1126/science.1115535.

57. **Powers RW III, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S.** 2006. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev* **20:**174–184. http://dx.doi.org/10.1101/gad.1381406.

58. **Rallis C, Codlin S, Bähler J.** 2013. TORC1 signaling inhibition by rapamycin and caffeine affect lifespan, global gene expression, and cell proliferation of fission yeast. *Aging Cell* **12:**563–573 http://dx.doi.org/10.1111/acel.12080.

59. **Rohde JR, Cardenas ME.** 2004. Nutrient signaling through TOR kinases controls gene expression and cellular differentiation in fungi. *Curr Top Microbiol Immunol* **279:**53–72. http://dx.doi.org/10.1007/978-3-642-18930-2_4.

60. **Cooper TG.** 2002. Transmitting the signal of excess nitrogen in *Saccharomyces cerevisiae* from the Tor proteins to the GATA factors: connecting the dots. *FEMS Microbiol Rev* **26:**223–238. http://dx.doi.org/10.1111/j.1574-6976.2002.tb00612.x.

61. **Bonfils G, Jaquenoud M, Bontron S, Ostrowicz C, Ungermann C, De Virgilio C.** 2012. Leucyl-tRNA synthetase controls TORC1 via the EGO complex. *Mol Cell* **46:**105–110. http://dx.doi.org/10.1016/j.molcel.2012.02.009.

62. **Valbuena N, Guan KL, Moreno S.** 2012. The Vam6 and Gtr1-Gtr2 pathway activates TORC1 in response to amino acids in fission yeast. *J Cell Sci* **125:**1920–1928. http://dx.doi.org/10.1242/jcs.094219.

63. **Kingsbury JM, Sen ND, Cardenas ME.** 2015. Branched-chain aminotransferases control TORC1 signaling in *Saccharomyces cerevisiae*. *PLoS Genet* **11:**e1005714. doi:10.1371/journal.pgen.1005714 http://dx.doi.org/10.1371/journal.pgen.1005714.

64. **Péli-Gulli MP, Sardu A, Panchaud N, Raucci S, De Virgilio C.** 2015. Amino acids stimulate TORC1 through Lst4-Lst7, a GTPase-activating protein complex for the Rag family GTPase Gtr2. *Cell Rep* **13:**1–7. http://dx.doi.org/10.1016/j.celrep.2015.08.059.

65. **Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J.** 1998. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J Biol Chem* **273:**14484–14494. http://dx.doi.org/10.1074/jbc.273.23.14484.

66. **Wang X, Campbell LE, Miller CM, Proud CG.** 1998. Amino acid availability regulates p70 S6 kinase and multiple translation factors. *Biochem J* **334:**261–267. http://dx.doi.org/10.1042/bj3340261.

67. **Jewell JL, Kim YC, Russell RC, Yu FX, Park HW, Plouffe SW, Tagliabracci VS, Guan KL.** 2015. Differential regulation of mTORC1 by leucine and glutamine. *Science* **347:**194–198. http://dx.doi.org/10.1126/science.1259472.

68. **Durán RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, Hall MN.** 2012. Glutaminolysis activates Rag-mTORC1 signaling. *Mol Cell* **47:**349–358. http://dx.doi.org/10.1016/j.molcel.2012.05.043.

69. **Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM.** 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320:**1496–1501. http://dx.doi.org/10.1126/science.1157535.

70. **Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL.** 2008. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* **10:**935–945. http://dx.doi.org/10.1038/ncb1753.

71. **Binda M, Péli-Gulli MP, Bonfils G, Panchaud N, Urban J, Sturgill TW, Loewith R, De Virgilio C.** 2009. The Vam6 GEF controls TORC1 by activating the EGO complex. *Mol Cell* **35:**563–573. http://dx.doi.org/10.1016/j.molcel.2009.06.033.

72. **Dubouloz F, Deloche O, Wanke V, Cameroni E, De Virgilio C.** 2005. The TOR and EGO protein complexes orchestrate microautophagy in yeast. *Mol Cell* **19:**15–26. http://dx.doi.org/10.1016/j.molcel.2005.05.020.

73. **Kira S, Kumano Y, Ukai H, Takeda E, Matsuura A, Noda T.** 2016. Dynamic relocation of the TORC1-Gtr1/2-Ego1/2/3 complex is regulated by Gtr1 and Gtr2. *Mol Biol Cell* **27:**382–396. http://dx.doi.org/10.1091/mbc.E15-07-0470.

74. **Panchaud N, Péli-Gulli MP, De Virgilio C.** 2013. SEACing the GAP that nEGOCiates TORC1 activation: evolutionary conservation of Rag GTPase regulation. *Cell Cycle* **12:**2948–2952. http://dx.doi.org/10.4161/cc.26000.

75. **Mach KE, Furge KA, Albright CF.** 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.

76. **Urano J, Comiso MJ, Guo L, Aspuria PJ, Deniskin R, Tabancay AP Jr, Kato-Stankiewicz J, 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. http://dx.doi.org/10.1111/j.1365-2958.2005.04877.x.

77. **Uritani M, Hidaka H, Hotta Y, Ueno M, Ushimaru T, Toda T.** 2006. Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. *Genes Cells* **11:**1367–1379. http://dx.doi.org/10.1111/j.1365-2443.2006.01025.x.

78. **Urano J, Sato T, Matsuo T, Otsubo Y, Yamamoto M, 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. http://dx.doi.org/10.1073/pnas.0608510104.

79. **Manning BD, Cantley LC.** 2003. Rheb fills a GAP between TSC and TOR. *Trends Biochem Sci* **28:**573–576. http://dx.doi.org/10.1016/j.tibs.2003.09.003.

80. **Matsumoto S, Bandyopadhyay A, Kwiatkowski DJ, Maitra U, 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.

81. **Van Slegtenhorst M, Carr E, Stoyanova R, Kruger W, Henske EP.** 2004. Tsc1+ and tsc2+ regulate arginine uptake and metabolism in *Schizosaccharomyces pombe*. *J Biol Chem* **279:**12706–12713.

82. **Tsao CC, Chen YT, Lan CY.** 2009. A small G protein Rhb1 and a GTPase-activating protein Tsc2 involved in nitrogen starvation-induced morphogenesis and cell wall integrity of *Candida albicans*. *Fungal Genet Biol* **46:**126–136. http://dx.doi.org/10.1016/j.fgb.2008.11.008.

83. **Jewell JL, Russell RC, Guan KL.** 2013. Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* **14:**133–139. http://dx.doi.org/10.1038/nrm3522.

84. **Inoki K, Zhu T, Guan KL.** 2003. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115:**577–590. http://dx.doi.org/10.1016/S0092-8674(03)00929-2.

85. **Weisman R, Cohen A, Gasser SM.** 2014. TORC2-a new player in genome stability. *EMBO Mol Med* **6:**995–1002. http://dx.doi.org/10.15252/emmm.201403959.

86. **Masui K, Cavenee WK, Mischel PS.** 2014. mTORC2 in the center of cancer metabolic reprogramming. *Trends Endocrinol Metab* **25:**364–373. http://dx.doi.org/10.1016/j.tem.2014.04.002.

87. **Kamada Y, Fujioka Y, Suzuki NN, Inagaki F, Wullschleger S, Loewith R, Hall MN, Ohsumi Y.** 2005. Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. *Mol Cell Biol* **25:**7239–7248. http://dx.doi.org/10.1128/MCB.25.16.7239-7248.2005.

88. **Matsuo T, Kubo Y, Watanabe Y, 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. http://dx.doi.org/10.1093/emboj/cdg298.

89. **Schmidt A, Kunz J, Hall MN.** 1996. TOR2 is required for organization of the actin cytoskeleton in yeast. *Proc Natl Acad Sci USA* **93:**13780–13785. http://dx.doi.org/10.1073/pnas.93.24.13780.

90. **Helliwell SB, Schmidt A, Ohya Y, Hall MN.** 1998. The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. *Curr Biol* **8:**1211–1214. http://dx.doi.org/10.1016/S0960-9822(07)00511-8.

91. **deHart AK, Schnell JD, Allen DA, Tsai JY, Hicke L.** 2003. Receptor internalization in yeast requires the Tor2-Rho1 signaling pathway. *Mol Biol Cell* **14:**4676–4684. http://dx.doi.org/10.1091/mbc.E03-05-0323.

92. **Tabuchi M, Audhya A, Parsons AB, Boone C, Emr SD.** 2006. The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. *Mol Cell Biol* **26:**5861–5875. http://dx.doi.org/10.1128/MCB.02403-05.

93. **Aronova S, Wedaman K, Aronov PA, Fontes K, Ramos K, Hammock BD, Powers T.** 2008. Regulation of ceramide biosynthesis by TOR complex 2. *Cell Metab* **7:**148–158. http://dx.doi.org/10.1016/j.cmet.2007.11.015.

94. **Roelants FM, Breslow DK, Muir A, Weissman JS, Thorner J.** 2011. Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **108:**19222–19227. http://dx.doi.org/10.1073/pnas.1116948108.

95. **Muir A, Ramachandran S, Roelants FM, Timmons G, Thorner J.** 2014. TORC2-dependent protein kinase Ypk1 phosphorylates ceramide synthase to stimulate synthesis of complex sphingolipids. *eLife* **3:**e03779. doi:http://dx.doi.org/10.7554/eLife.03779 http://dx.doi.org/10.7554/eLife.03779.

96. **Roelants FM, Baltz AG, Trott AE, Fereres S, Thorner J.** 2010. A protein kinase network regulates the function of aminophospholipid flippases. *Proc Natl Acad Sci USA* **107:**34–39. http://dx.doi.org/10.1073/pnas.0912497106.

97. **Niles BJ, Powers T.** 2014. TOR complex 2-Ypk1 signaling regulates actin polarization via reactive oxygen species. *Mol Biol Cell* **25:**3962–3972. http://dx.doi.org/10.1091/mbc.E14-06-1122.

98. **Niles BJ, Joslin AC, Fresques T, Powers T.** 2014. TOR complex 2-Ypk1 signaling maintains sphingolipid homeostasis by sensing and regulating ROS accumulation. *Cell Rep* **6:**541–552. http://dx.doi.org/10.1016/j.celrep.2013.12.040. (Erratum, **6:**592.)

99. **Rispal D, Eltschinger S, Stahl M, Vaga S, Bodenmiller B, Abraham Y, Filipuzzi I, Movva NR, Aebersold R, Helliwell SB, Loewith R.** 2015. Target of rapamycin complex 2 regulates actin polarization and endocytosis via multiple pathways. *J Biol Chem* **290:**14963–14978. http://dx.doi.org/10.1074/jbc.M114.627794.

100. **Berchtold D, Piccolis M, Chiaruttini N, Riezman I, Riezman H, Roux A, Walther TC, Loewith R.** 2012. Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat Cell Biol* **14:**542–547. http://dx.doi.org/10.1038/ncb2480.

101. **Vlahakis A, Graef M, Nunnari J, Powers T.** 2014. TOR complex 2-Ypk1 signaling is an essential positive regulator of the general amino acid control response and autophagy. *Proc Natl Acad Sci USA* **111:**10586–10591. http://dx.doi.org/10.1073/pnas.1406305111.

102. **Kawai M, Nakashima A, Ueno M, Ushimaru T, Aiba K, Doi H, 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 http://dx.doi.org/10.1007/s002940100198.

103. **Ikeda K, Morigasaki S, Tatebe H, Tamanoi F, 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. http://dx.doi.org/10.4161/cc.7.3.5245.

104. **Schonbrun M, Laor D, López-Maury L, Bähler J, Kupiec M, Weisman R.** 2009. TOR complex 2 controls gene silencing, telomere length maintenance, and survival under DNA-damaging conditions. *Mol Cell Biol* **29:**4584–4594. http://dx.doi.org/10.1128/MCB.01879-08.

105. **Petersen J, 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. http://dx.doi.org/10.1038/ncb1646.

106. **Ikai N, Nakazawa N, Hayashi T, Yanagida M.** 2011. The reverse, but coordinated, roles of Tor2 (TORC1) and Tor1 (TORC2) kinases for growth, cell cycle and separase-mediated mitosis in *Schizosaccharomyces pombe*. *Open Biol* **1:**110007. doi:10.1098/rsob.110007 http://dx.doi.org/10.1098/rsob.110007.

107. **Weisman R, Roitburg I, Nahari T, Kupiec M.** 2005. Regulation of leucine uptake by tor1+ in *Schizosaccharomyces pombe* is sensitive to rapamycin. *Genetics* **169:**539–550. http://dx.doi.org/10.1534/genetics.104.034983.

108. **Saitoh S, Mori A, Uehara L, Masuda F, Soejima S, Yanagida M.** 2015. Mechanisms of expression and translocation of major fission yeast glucose transporters regulated by CaMKK/phosphatases, nuclear shuttling, and TOR. *Mol Biol Cell* **26:**373–386. http://dx.doi.org/10.1091/mbc.E14-11-1503.

109. **Du W, Forte GM, Smith D, Petersen J.** 2016. Phosphorylation of the amino-terminus of the AGC kinase Gad8 prevents its interaction with TORC2. *Open Biol* **6:**6. doi:10.1098/rsob.150189 http://dx.doi.org/10.1098/rsob.150189.

110. **Shimada K, Filipuzzi I, Stahl M, Helliwell SB, Studer C, Hoepfner D, Seeber A, Loewith R, Movva NR, Gasser SM.** 2013. TORC2 signaling pathway guarantees genome stability in the face of DNA strand breaks. *Mol Cell* **51:**829–839. http://dx.doi.org/10.1016/j.molcel.2013.08.019.

111. **Sturgill TW, Cohen A, Diefenbacher M, Trautwein M, Martin DE, Hall MN.** 2008. TOR1 and TOR2 have distinct locations in live cells. *Eukaryot Cell* **7:**1819–1830. http://dx.doi.org/10.1128/EC.00088-08.

112. **Kunz J, Schneider U, Howald I, Schmidt A, Hall MN.** 2000. HEAT repeats mediate plasma membrane localization of Tor2p in yeast. *J Biol Chem* **275:**37011–37020. http://dx.doi.org/10.1074/jbc.M007296200.

113. **Tatebe H, Morigasaki S, Murayama S, Zeng CT, Shiozaki K.** 2010. Rab-family GTPase regulates TOR complex 2 signaling in fission yeast. *Curr Biol* **20:**1975–1982. http://dx.doi.org/10.1016/j.cub.2010.10.026.

114. **Cohen A, Kupiec M, Weisman R.** 2016. Gad8 is found in the nucleus where it interacts with the MluI cell cycle box-binding factor (MBF) transcriptional complex to regulate the response to DNA replication stress. *J Biol Chem* **291:**9371–9381. http://dx.doi.org/10.1074/jbc.M115.705251.

115. **Zinzalla V, Stracka D, Oppliger W, Hall MN.** 2011. Activation of mTORC2 by association with the ribosome. *Cell* **144:**757–768. http://dx.doi.org/10.1016/j.cell.2011.02.014.

116. **Oh WJ, Wu CC, Kim SJ, Facchinetti V, Julien LA, Finlan M, Roux PP, Su B, Jacinto E.** 2010. mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. *EMBO J* **29:**3939–3951. http://dx.doi.org/10.1038/emboj.2010.271.

117. **Cohen A, Kupiec M, Weisman R.** 2014. Glucose activates TORC2-Gad8 protein via positive regulation of the cAMP/cAMP-dependent protein kinase A (PKA) pathway and negative regulation of the Pmk1 protein-mitogen-activated protein kinase pathway. *J Biol Chem* **289:**21727–21737. http://dx.doi.org/10.1074/jbc.M114.573824.

118. **Hatano T, Morigasaki S, Tatebe H, Ikeda K, Shiozaki K.** 2015. Fission yeast Ryh1 GTPase activates TOR complex 2 in response to glucose. *Cell Cycle* **14:**848–856. http://dx.doi.org/10.1080/15384101.2014.1000215.

119. **Liu W, Zhao J, Li X, Li Y, Jiang L.** 2010. The protein kinase CaSch9p is required for the cell growth, filamentation and virulence in the human fungal pathogen *Candida albicans*. *FEMS Yeast Res* **10:**462–470. http://dx.doi.org/10.1111/j.1567-1364.2010.00617.x.

120. **Lee CM, Nantel A, Jiang L, Whiteway M, Shen SH.** 2004. The serine/threonine protein phosphatase SIT4 modulates yeast-to-hypha morphogenesis and virulence in *Candida albicans*. *Mol Microbiol* **51:**691–709. http://dx.doi.org/10.1111/j.1365-2958.2003.03879.x.

121. **Zacchi LF, Gomez-Raja J, Davis DA.** 2010. Mds3 regulates morphogenesis in *Candida albicans* through the TOR pathway. *Mol Cell Biol* **30:**3695–3710. http://dx.doi.org/10.1128/MCB.01540-09.

122. **Su C, Lu Y, Liu H.** 2013. Reduced TOR signaling sustains hyphal development in *Candida albicans* by lowering Hog1 basal activity. *Mol Biol Cell* **24:**385–397. http://dx.doi.org/10.1091/mbc.E12-06-0477.

123. **Lee H, Khanal Lamichhane A, Garraffo HM, Kwon-Chung KJ, Chang YC.** 2012. Involvement of PDK1, PKC and TOR signalling pathways in basal fluconazole tolerance in *Cryptococcus neoformans*. *Mol Microbiol* **84:**130–146. http://dx.doi.org/10.1111/j.1365-2958.2012.08016.x.

124. **Baldin C, Valiante V, Krüger T, Schafferer L, Haas H, Kniemeyer O, Brakhage AA.** 2015. Comparative proteomics of a Tor inducible *Aspergillus fumigatus* mutant reveals involvement of the Tor kinase in iron regulation. *Proteomics* **15:**2230–2243. http://dx.doi.org/10.1002/pmic.201400584.

125. **Fitzgibbon GJ, Morozov IY, Jones MG, Caddick MX.** 2005. Genetic analysis of the TOR pathway in *Aspergillus nidulans*. *Eukaryot Cell* **4:**1595–1598. http://dx.doi.org/10.1128/EC.4.9.1595-1598.2005.

126. **Meyer V, Arentshorst M, Flitter SJ, Nitsche BM, Kwon MJ, Reynaga-Peña CG, Bartnicki-Garcia S, van den Hondel CA, Ram AF.** 2009. Reconstruction of signaling networks regulating fungal morphogenesis by transcriptomics. *Eukaryot Cell* **8:**1677–1691. http://dx.doi.org/10.1128/EC.00050-09.

127. **Meyer V, Minkwitz S, Schütze T, van den Hondel CA, Ram AF.** 2010. The *Aspergillus niger* RmsA protein: a node in a genetic network? *Commun Integr Biol* **3:**195–197. http://dx.doi.org/10.4161/cib.3.2.10983.

128. **Teichert S, Wottawa M, Schönig B, Tudzynski B.** 2006. Role of the *Fusarium fujikuroi* TOR kinase in nitrogen regulation and secondary metabolism. *Eukaryot Cell* **5:**1807–1819. http://dx.doi.org/10.1128/EC.00039-06.

129. **Yu F, Gu Q, Yun Y, Yin Y, Xu JR, Shim WB, Ma Z.** 2014. The TOR signaling pathway regulates vegetative development and virulence in *Fusarium graminearum*. *New Phytol* **203:**219–232. http://dx.doi.org/10.1111/nph.12776.

130. **Chen D, Wang Y, Zhou X, Wang Y, Xu JR.** 2014. The Sch9 kinase regulates conidium size, stress responses, and pathogenesis in *Fusarium graminearum*. *PLoS One* **9:**e105811. doi:10.1371/journal.pone.0105811 http://dx.doi.org/10.1371/journal.pone.0105811.

131. **Weisman R.** 2010. Fission yeast TOR and rapamycin, p 251–269. *In* Hall MN, Tamanoi F (ed), *The Enzymes: Structure, Function and Regulation of TOR Complexes from Yeast to Mammals. Part A*, vol 27. Elsevier, London, United Kingdom.

TOR Controls Translation Initiation and Early G1 Progression in Yeast

Nik C. Barbet,* Ulrich Schneider,* Stephen B. Helliwell,* Ian Stansfield,[†] Michael F. Tuite,[†] and Michael N. Hall*[‡]

*Department of Biochemistry, Biozentrum, University of Basel, CH-4056 Basel, Switzerland; and [†]Research School of Biosciences, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ, United Kingdom

Saccharomyces cerevisiae cells treated with the immunosuppressant rapamycin or depleted for the targets of rapamycin TOR1 and TOR2 arrest growth in the early G1 phase of the cell cycle. Loss of TOR function also causes an early inhibition of translation initiation and induces several other physiological changes characteristic of starved cells entering stationary phase (G0). A G1 cyclin mRNA whose translational control is altered by substitution of the UBI4 5' leader region (UBI4 is normally translated under starvation conditions) suppresses the rapamycin-induced G1 arrest and confers starvation sensitivity. These results suggest that the block in translation initiation is a direct consequence of loss of TOR function and the cause of the G1 arrest. We propose that the TORs, two related phosphatidylinositol kinase homologues, are part of a novel signaling pathway that activates eIF-4E-dependent protein synthesis and, thereby, G1 progression in response to nutrient availability. Such a pathway may constitute a checkpoint that prevents early G1 progression and growth in the absence of nutrients.

INTRODUCTION

The immunosuppressant rapamycin and the related compound FK506 exert their immunosuppressive effects by inhibiting intermediate steps in signal transduction that lead to T cell activation and proliferation (Heitman et al., 1991; Schreiber and Crabtree, 1992; Sigal and Dumont, 1992; Liu, 1993; Fruman et al., 1994). FK506 in complex with its intracellular receptor FKBP inhibits the Ca^{2+}/calmodulin-dependent phosphatase calcineurin. As a downstream effector of the T cell receptor (TCR), calcineurin normally triggers nuclear import of a subunit of the transcription factor NF-AT which, in turn, activates 50–100 genes, including the gene encoding the lymphokine interleukin-2 (IL-2) (Weiss and Littman, 1994). Rapamycin also forms a toxic complex with FKBP, but instead of inhibiting the TCR signaling pathway, inhibits a subsequent signal transduction cascade that is stimulated by IL-2 (Bierer et al., 1990; Dumont et al., 1990). The IL-2 signaling pathway mediates G1 progression (pro-

liferation) of a T cell. Rapamycin prevents the phosphorylation and activation of p70 S6 kinase, a downstream effector of IL-2 and several other growth factors, including insulin, EGF, PDGF, IL-3, and erythropoietin (Calvo et al., 1992, 1994; Chung et al., 1992; Kuo et al., 1992; Price et al., 1992; Terada et al., 1992; Ferrari et al., 1993; Lane et al., 1993). Although best known for its inhibition of IL-2–dependent p70 S6 kinase activation, rapamycin also inhibits p70 S6 kinase activation in response to these other mitogens (Calvo et al., 1992; Chung et al., 1992, 1994; Price et al., 1992). The p70 S6 kinase phosphorylates the ribosomal protein S6 which, in turn, leads to the activation of translation initiation (Kuo et al., 1992; Thomas, 1992; Jefferies et al., 1994; Terada et al., 1994). The p70 S6 kinase thus links mitogenic stimulation and the initiation of protein synthesis. A homologue of the yeast TOR proteins (FRAP/RAFT1/RAPT1/mTOR) (see below) has recently been identified in mammalian cells as a direct target of the rapamycin-FKBP complex (Brown et al., 1994; Chiu et al., 1994; Sabatini et al., 1994; Sabers et al., 1995), suggesting that FRAP/RAFT1/RAPT1/mTOR is required for p70 S6 kinase activation and is part of a general mitogenic signaling

[‡] Corresponding author.

pathway (for a figure that summarizes the pathway, see Downward, 1994).

In the yeast *Saccharomyces cerevisiae*, rapamycin-FKBP inhibits the *TOR1* and *TOR2* gene products and arrests cells with an unbudded morphology, indicative of a G1 cell cycle arrest similar to that observed in T cells (Heitman *et al.*, 1991; Cafferkey *et al.*, 1993; Kunz and Hall, 1993; Kunz *et al.*, 1993; Helliwell *et al.*, 1994; Stan *et al.*, 1994; Zheng *et al.*, 1995). A dominant point mutation in either *TOR* gene renders a cell resistant to rapamycin, whereas disruption of both genes results in an unbudded morphology, similar to that seen when wild-type cells are treated with rapamycin, and a 1n DNA content (Heitman *et al.*, 1991; Cafferkey *et al.*, 1993; Kunz *et al.*, 1993; Helliwell *et al.*, 1994). TOR1 and TOR2 are large (~280 kDa), functionally homologous proteins that are structurally related to phosphatidylinositol kinases (PI kinases) (Kunz *et al.*, 1993; Garcia-Bustos *et al.*, 1994; Helliwell *et al.*, 1994; Yoshida *et al.*, 1994). PI kinases are required for production of phosphatidylinositol-derived second messengers (Carpenter and Cantley, 1990). Thus, TOR1 and TOR2, like FRAP/RAFT1/RAPT1/mTOR, may be components of a rapamycin-sensitive signaling pathway required for cell cycle progression in G1.

In *S. cerevisiae*, the decision to commit to a cell cycle is made at a point in late G1 termed START. Traversal of START and entry into S phase is regulated by the activity of the cyclin-dependent kinase encoded by *CDC28* (Reed, 1992; Nasmyth, 1993). Three G1-specific cyclin genes were originally identified and named *CLN1*, *CLN2*, and *CLN3* (Cross, 1988; Nash *et al.*, 1988; Richardson *et al.*, 1989; Wittenberg *et al.*, 1990); additional candidate G1 cyclin genes have subsequently been identified and named *HCS26*, *ORFD*, *CLB5*, and *CLB6* (Frohlich *et al.*, 1991; Ogas *et al.*, 1991; Epstein and Cross, 1992; Kuehne and Linder, 1993; Schwob and Nasmyth, 1993). All except *CLN3* are transcribed only in late G1 with *CLN1*, *CLN2*, and *HCS26*, and possibly *ORFD*, under control of the transcription factor SBF. CLN3, whose transcript is present throughout the cell cycle, is regulated post-transcriptionally and acts as an upstream activator of other G1 cyclins (Nasmyth and Dirick, 1991; Ogas *et al.*, 1991; Tyers *et al.*, 1992, 1993; Cvrckova and Nasmyth, 1993).

When nutrients are limiting, haploid yeast cells do not proceed to START in late G1, but instead exit the mitotic cell cycle in early G1 and enter a stationary or G0 phase (for review see Werner-Washburne *et al.*, 1993). Stationary phase enables a cell to maintain viability for long periods when nutrients are not available, and is characterized by several physiological properties including 1n DNA content, failure to reach START, reduced protein synthesis, accumulation of glycogen, acquisition of thermotolerance, and changes in the pattern of transcription (Werner-Washburne *et al.*, 1993). Nutrient sensing and the regulation of entry

into stationary phase are poorly understood, but are generally thought to involve the RAS/cAMP pathway (Broach, 1991; Thevelein, 1994). However, this is not the sole nutrient-sensing pathway, as mutants in the RAS/cAMP pathway have been isolated that exhibit a normal response to starvation independently of intracellular cAMP levels (Cameron *et al.*, 1988).

Here we report that cells lacking TOR function (cells treated with rapamycin or depleted of TOR) arrest growth and rapidly exhibit, by all criteria examined, properties diagnostic of G0 or stationary phase, including a reduction in translation initiation. The cell cycle arrest upon loss of TOR function is suppressed by altering the translational control of the G1 cyclin CLN3. Our results and analogy with mammalian cells suggest that TOR is part of a novel signal transduction pathway required for translation initiation and G1 progression, perhaps in response to nutrients.

MATERIALS AND METHODS

Strains, Plasmids, and Media

The parental strain in this study was JK9–3da (*MATa leu2-3, 112 ura3-52 trp1 his4 rme1 HMLa*). Isogenic derivatives with only the changes indicated are shown in Table 1. The composition of rich medium (YPD), synthetic galactose/glycerol medium (SGal/Gly), and synthetic glucose medium (SD) supplemented with the appropriate nutrients was as described (Sherman, 1991). All cultures were incubated at 30°C unless otherwise indicated. Rapamycin (provided by Sandoz Pharma, Basel, Switzerland) was added to the medium to a final concentration of 0.2 μg/ml. Rapamycin was diluted into media from a stock solution of 1 mg/ml in 10% Tween-20/90% ethanol (Heitman *et al.*, 1993). Plasmid pJK5 contains the entire *TOR2* gene under control of the *GAL1* promoter (Kunz *et al.*, 1993). YEplac181::*tor2–61*^{ts} (amp^r 2 μ *LEU2*) contains the entire *TOR2* gene and was isolated by hydroxylamine mutagenesis as a temperature-sensitive *TOR2* allele (Barbet and Hall, unpublished data). The *RAS2*^{val19} allele on plasmid YEp213 (amp^r 2 μ *URA3*) (Broek *et al.*, 1987) was transformed into JK9–3da. YCplac111 is amp^r *CEN4 LEU2* (Gietz and Sugino, 1988). The *BCY1* gene was disrupted (*bcy1::URA3*) as described using the one-step gene replacement technique (Toda *et al.*, 1987; Rothstein, 1991). Integration of *ADH-*

Table 1. Strains used in this study

Strain	Genotype
JK9-3da	*MATa leu2-3,112 ura3-42 trp1 his4 rme1 HMLa*
JK350-21a	JK9-3da *tor1:LEU2-4 tor2::ADE2-3*/pJK5
JH11-1c	JK9-3da *TOR1-1*
JH12-17b	JK9-3da *TOR2-1*
NB17-3d	JK9-3da *his3 HIS4 tor1::HIS3*
NB30	JK9-3da *bcy1::URA3*
NB32	JK9-3da *ura3::[URA3 ADH-CLN2]*
NB33	JK9-3da *ura3::[URA3 CLN3-1]*
NB34	JK9-3da/YEp213::*RAS2*^{val19}
NB35	JK9-3da *ade2 his3 HIS4 tor1::HIS3 tor2::ADE2*// YEplac181::*tor2-61*^{ts}
NB36	JK9-3da/YCplac111::*UB14*$^{5'}$*-CLN3*
NB37	JK9-3da/YCplac111::*UB14*$^{5'}$
NB38	JK9-3da/YEpURA::*CLN3*

CLN2 (Nasmyth and Dirick, 1991) at the *ura3* locus was achieved by linearizing the plasmid containing the *CLN2* construct with *Eco*RV. Disruptions and integrations were confirmed by Southern blot analysis. All transformations were performed using the lithium acetate procedure (Ito *et al.*, 1983).

Flow Cytometry

Overnight cultures of yeast in SD complete medium were diluted to OD_{600} <0.05 and allowed to grow before the addition of rapamycin at OD_{600} = 0.2. Three hundred-microliter samples were taken from these cultures at hourly intervals, sonicated for 2 min, and immediately fixed by addition of 700 μl absolute ethanol. Samples were incubated overnight at 4°C, washed, and resuspended in 50 mM sodium citrate, pH 7.4, and treated with RNAse (0.25 mg/ml) for 1 h at 37°C. DNA was stained by the addition of 500 μl citrate buffer containing 16 μg/ml propidium iodide. For each timepoint taken, 10,000 events were analyzed for DNA content using a Becton Dickinson FACScan (Mountain View, CA) and data was processed using Lysys II software (Lincoln Park, NJ).

Order-of-Function Mapping

A reciprocal shift experiment was performed with the temperature-sensitive *tor2* strain NB35 and α-factor as described (Hereford and Hartwell, 1974) and also with the modification of a 1.5-h overlap in which both blocks were imposed. Because the effects of rapamycin are irreversible (presumably because the drug cannot be washed out) we were unable to perform a standard reciprocal shift experiment with α-factor and rapamycin. To circumvent this problem, we performed a double block experiment and an α-factor to rapamycin shift experiment. For the double block experiment, logarithmically growing cultures of JK9–3da in SD medium were treated with nocodazole (10 μg/ml; Sigma, St. Louis, MO) for 2.5 h to arrest the cells in mitosis. Cells were harvested by filtration and nocodazole was washed out of the cells with 10 volumes of sterile water followed by 10 volumes of SD medium. Cells were then incubated in fresh SD medium either with no addition, with 10 μg/ml mating pheromone (α-factor), with 0.2 μg/ml rapamycin, or with both mating pheromone and rapamycin. Samples were taken at 30-min intervals, sonicated for 2 min to separate cells, and scored for emergence of buds and appearance of the shmoo phenotype (Sprague, 1991). For the α-factor to rapamycin shift experiment, logarithmically growing cultures of JK9–3da in SD medium were treated with 10 μg/ml α-factor for 2.5 h. Cells were harvested by filtration and washed with 10 volumes of sterile water followed by 10 volumes of SD medium. The culture was then split; one half of the culture received 0.2 μg/ml rapamycin and the other half received drug vehicle alone. Samples were removed at 30 min intervals, sonicated for 2 min, and scored for the emergence of buds.

Northern Analysis

Extraction of total cellular RNA was performed as previously described (Jensen *et al.*, 1983). For Northern analysis, 10 μg of total RNA was separated on 1% agarose gels containing 6% formaldehyde, and transferred overnight to Hybond-N+ nylon membrane (Amersham, Arlington Heights, IL) in 20× SSC. The *HCS26, ORFD, CTT1, SSA3, UBI4, CLB5,* and *CLN2* DNA probes were amplified from genomic DNA by the polymerase chain reaction (PCR). The primers used for PCR were as follows, with the 5′ primer listed first and the fragment size generated given in parentheses: *HCS26*, 5′-ATGTGTGAATACAGCAAG-3′ and 5′-AAACCCATGTTGACT-CAT-3′ (963 bp); *ORFD*, 5′-ATGTCAAACTACGAAGCC-3′ and 5′-CCTGTGTTCTTCCGCCTT-3′ (998 bp); *CTT1*, 5′-ATGAACGTG-TTCGGTAAA-3′ and 5′-TGGCACTTGCAATGGACC-3′ (1686 bp); *SSA3*, 5′-ATGTCTAGAGCAGTTGGT-3′ and 5′-ATCAACCTCTTC-CACTGT-3′ (1947 bp); *UBI4*, 5′-ATGCAGATTTTCGTCAAG-3′ and 5′-GTTACCACCCCTCAACCT-3′ (1142 bp); *CLB5*, 5′-ATGG-

GAGAGAACCACGAC-3′ and 5′-TGCTATGCATTTCGGATG-3′ (1278 bp); and *CLN2*, 5′-ATGGCTAGTGCTGAACCA-3′ and 5′-TATTACTTGGGTATTGCC-3′ (1634 bp). The *SWI4* probe was a 2.2-kbp *Bam*HI fragment from the plasmid YCplac33::SWI4 (gift of K. Nasmyth). The *SWI6* probe was a 2.2-kbp *Xho*I/*Cla*I fragment from 1941 (gift of K. Nasmyth). The *CLN3* probe was a 500-bp *Hin*dIII/*Eco*RI fragment from pBF30 (Nash *et al.*, 1988). The probe for *CLN1* was a 2-kbp *Hin*dIII fragment from pcln1::URA3 (Hadwiger *et al.*, 1989). The probe for *CDC28* was a 1.2-kbp *Xho*I/*Xba*I fragment from YEp13::CDC28 (gift of K. Nasmyth). The probe for *TOR1* was a 4.3-kbp *Hin*dIII fragment from pPW20 (Helliwell *et al.*, 1994). The *TOR2* probe was a 5.3-kbp *Bgl*II fragment from pJK3–3 (Kunz *et al.*, 1993). The *HSP26* probe was a 800-bp *Bgl*II/*Nde*I fragment from pHSP26 (gift of S. Lindquist). The probe for *SSB1* was a 2.2-kbp *Hin*dIII fragment from pFKR15. The probe for *SSA1* was a 2-kbp *Sal*I fragment from EC551 (gift of E. Craig). The *ACT1* probe was a 1-kbp *Eco*RI/*Pst*I fragment from pUC18::ACT1 (gift of P. Linder). *SSA1* and *SSA2* transcripts are indistinguishable, as are *SSB1* and *SSB2* transcripts, because the DNA sequences of these pairs of genes are 97% and 94% identical, respectively (Werner-Washburne *et al.*, 1989). Probes were labeled with [^{32}P]dATP using the random-primed DNA labeling kit (United States Biochemical, Cleveland, OH). Filters were exposed to x-ray film (Kodak X-OMAT) AR at −70°C with intensifying screens (Dupont Cronex). Signals were quantitated by scanning appropriately exposed films using a Molecular Dynamics densitometer (Sunnyvale, CA). In the experiment shown in Figure 7B, the total cellular RNAs of strains NB36 and NB38 were prepared identically, run on the same gel, transferred to the same filter, and hybridized to the same probe at the same time.

Incorporation of [^{35}S]Methionine into Total Yeast Protein

For analysis of gross protein synthesis, tricholoracetic acid (TCA)-precipitable counts were quantitated from pulse-labeled cultures at the indicated times after treatment. For rapamycin treatment, exponentially growing cultures of JK9–3da in SD medium minus methionine were treated with 0.2 μg/ml rapamycin, 100 μg/ml cycloheximide, or with drug vehicle alone (10% Tween-20/90% ethanol). For TOR depletion, exponentially growing cultures of the *tor*^{ts} strain NB35 and the control strain NB17–3d in SD minus methionine were resuspended in prewarmed medium at 37°C. For each timepoint, 0.01 OD_{600} equivalents were removed and labeled at 30°C for 7 min with 2 μCi [^{35}S]methionine (Amersham). Aliquots of the pulse-labeled cells were lysed on Whatman filters presoaked in 50% TCA, and deacylated by boiling for 10 min in 5% TCA. Filters were washed in acetone, air dried, and TCA-precipitable counts were quantitated by scintillation counting using a Canberra Packard 1900TR liquid scintillation analyzer.

Polysome Gradient Analysis

Strains JK9–3da and NB35 were grown in YPD to a cell density of 10^7 cells ml^{-1}. Following harvesting, polysomes were prepared as described (Stansfield *et al.*, 1992), except that polysomes were resolved on a 15–50% w/v sucrose gradient by centrifuging for 2.1 h at 17,000 × g using a Beckman SW40 Ti rotor. Cycloheximide (200 μg/ml) and rapamycin (0.2 μg/ml) were added to cultures at the indicated times before harvest. Drugs used in this way to inhibit yeast cultures were also included at the same concentration in the lysis buffer (Stansfield *et al.*, 1992).

Glycogen Staining

Logarithmically growing cultures in SD medium were treated with 0.2 μg/ml rapamycin and incubated at 30°C in the presence of the drug. At hourly intervals up to 5 h after rapamycin addition, 5 OD equivalents of cells were harvested onto Millipore HA filters (Bed-

ford, MA), placed upon a solid agar matrix, and exposed to iodine vapor for 1 min.

Construction and Analysis of the UBI4-CLN3 Fusion

The 5' region (containing the untranslated leader and promoter sequences) of the *UBI4* polyubiquitin gene and a sequence containing the open reading frame of the *CLN3* gene were amplified from *S. cerevisiae* genomic DNA using the polymerase chain reaction. Oligonucleotides were designed to produce a 752-bp *UBI4* 5' region fragment flanked by a 5' *Hind*III and a 3' *Sal*I restriction site, and a 1821-bp *CLN3* fragment flanked by 5' *Sal*I and 3' *Sma*I sites. The oligonucleotides were as follows: *UBI4* 5' end, 5'-GCAAAGCTTC-CCACCACCAGCACTAGCTTAGAT-3'; *UBI4* 3' end, 5'-AATGTC-GACCTATTAGTTAAAGTAAAGTGGGTG-3'; *CLN3* 5' end, 5'-TACGTCGACTGTACGATGGCCATATTGAAGGAT-3'; and *CLN3* 3' end, 5'-GTACCCGGGACGTATTTGCTTTGCAAATTTTA-3'. The *UBI4-CLN3* construct was obtained by first introducing the *Hind*III/*Sal*I-cut *UBI4* 5' region fragment into a *Hind*III/*Sal*I cut YCplac111 vector (*CEN4 LEU2*). Following transformation and amplification in *E. coli*, this "parent plasmid" was digested with *Sal*I and *Sma*I and the *Sal*I/*Sma*I-cut *CLN3* fragment was introduced. The *UBI4* 5' region was fused 7 bp upstream of the *CLN3* start codon. The resultant plasmid (YCplac111::UBI4[5']-CLN3) and its parent plasmid (YCplac111::UBI4[5']) were transformed into the wild-type haploid yeast strain JK9–3da to yield strains NB36 and NB37, respectively. Strain NB38 is JK9–3da containing the plasmid YEpURA::CLN3 (gift of K. Nasmyth), which consists of a 7-kb genomic *Bgl*II fragment containing the *CLN3* gene inserted into YEp352. For the asynchronous flow cytometry experiments, strains were grown in SD medium minus leucine to early log phase, and treated with 0.2 μg/ml rapamycin. Cell number and DNA content were analyzed hourly for 5 h following rapamycin treatment. For the synchrony experiments, NB36 and NB37 were grown to early log phase, then treated with 10 μg/ml α-factor for 2.5 h to arrest cells at start. α-Factor was removed by filtration and washing with water, followed by SD medium minus leucine, and cells were resuspended in fresh SD medium minus leucine. Samples were removed at 20-min intervals, washed, sonicated to separate cells, and assessed for emergence of buds and DNA content (flow cytometry). At maximal budding (generally 60 min after release from α-factor), the cultures were split; half received 0.2 μg/ml rapamycin, the remaining half received drug vehicle alone. Flow cytometry was performed as described above.

Assay of Starvation Sensitivity

Strain NB36 containing the *UBI4-CLN3* fusion and control strain NB37 containing the *UBI4* 5' region without the *CLN3* open reading frame, on a *LEU2* plasmid, were grown in SD medium minus leucine for 6 days. Samples were removed daily and assessed for cell number/milliliter of culture, cell viability, and percentage of budded cells. For viability determination, 10^3 cells were plated on rich medium (YPD) in duplicate, and the number of cells able to form colonies was determined as a percentage of total number of cells plated. Replica plating to SD medium minus leucine showed that over 80% of the cells retained their respective plasmid, even after prolonged incubation.

RESULTS

Rapamycin Blocks G1 Progression

We have shown previously that rapamycin treatment causes yeast cells to arrest with an unbudded morphology (Heitman *et al.*, 1991; Kunz *et al.*, 1993). Such a phenotype, although suggestive of, is not necessarily

indicative of a G1 arrest, as mutants have been isolated that are perturbed in budding but not in the onset of DNA synthesis (Adams *et al.*, 1990; Johnson and Pringle, 1990; Bender and Pringle, 1991; Cvrckova and Nasmyth, 1993). We therefore examined whether yeast cells treated with rapamycin arrest with a 1n DNA content, and are thus indeed impaired in G1 progression. An exponentially growing asynchronous culture of the haploid strain JK9–3da was treated with 0.2 μg/ml rapamycin, and at hourly intervals samples were removed for flow cytometry. As shown in Figure 1, a shift to a 1n DNA content was observed after 1 h of rapamycin treatment, and after 2–3 h, ~85% of the cells contained a 1n DNA complement (Figure 1D). The shift to 1n DNA content paralleled growth arrest; rapamycin-treated cells never completed more than one doubling, as determined by direct counting of the cells in the treated culture at the different time intervals. A control culture treated with the drug vehicle alone (10% Tween/90% ethanol) continued to grow normally, doubling in cell number every 125 min for the duration of the experiment. Thus, rapamycin causes a G1 arrest within one generation. As shown previously, TOR depletion also causes cells to arrest growth with a 1n DNA content (Helliwell *et al.*, 1994).

When the size distribution of cells was analyzed, we observed two subpopulations in the rapamycin-treated cells (Figure 1E). The major subpopulation of cells increased in size throughout the experiment, whereas the minor subpopulation of cells appeared to remain as small cells. Although the two subpopulations became more evident at later time points as the larger cells continued to increase in volume, two discrete populations could already be discerned after 2 h. The small cells most likely represent newly formed, starved daughter cells (see below) (Johnston *et al.*, 1977). The increased size of the larger cells can be accounted for by the observation that they contain an exceptionally large vacuole (Heitman *et al.*, 1991). Because an enlarged vacuole is also symptomatic of starvation (Granot and Snyder, 1991), these cells might also be starved (in G0) despite the presence of nutrients. The reason for the biphasic size distribution is unclear.

The TOR Restriction Point Is in Early G1 Before START

To determine the TOR restriction point within G1, we performed an order-of-function (reciprocal shift) analysis using a temperature-sensitive *tor* mutant and the mating pheromone α-factor (Hereford and Hartwell, 1974). This maps the TOR restriction point relative to START, the α-factor arrest point. The mutant strain (NB35) used in this experiment contained a temperature-sensitive *tor2* allele on a plasmid and chromosomal disruptions of both *TOR1* and *TOR2*. NB35
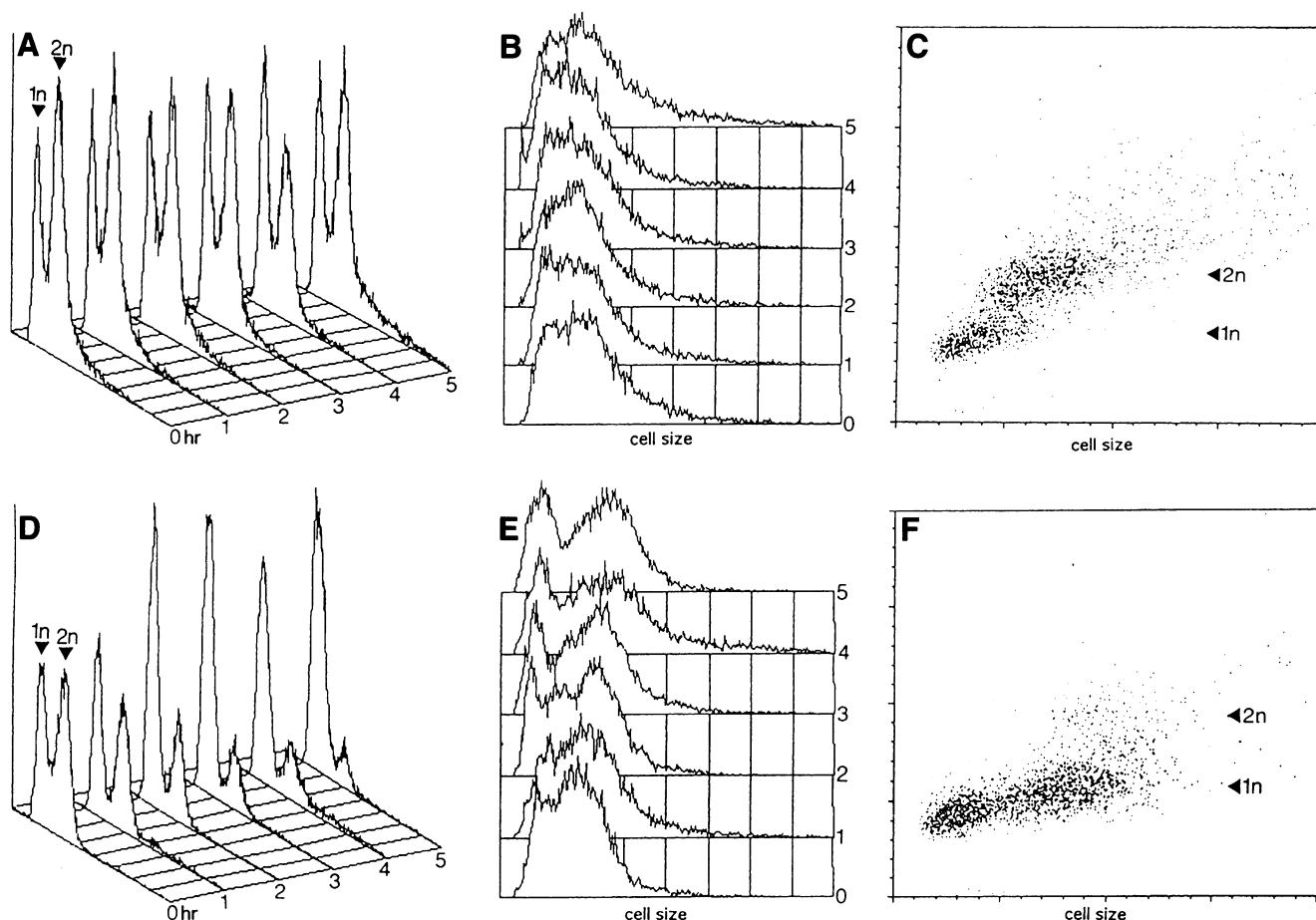
Figure 1. Rapamycin causes wild-type yeast cells (JK9–3da) to arrest with a 1n DNA content. Exponentially growing cells were treated with 0.2 μg/ml rapamycin and sampled for flow cytometry at hourly intervals up to 5 h. (A and D) DNA content, (B and E) cell size, and (C and F) a two-dimensional plot of cell size distribution (x-axis) versus DNA content (y-axis) for rapamycin-untreated (A–C) and -treated cells (D–F). The two-dimensional plot corresponds to the 5-h time point. 1n and 2n refer to DNA content.

(*tor*^{ts}) arrests growth with a 1n DNA content after shift to the nonpermissive temperature, and resumes growth upon return to the permissive temperature. The growth arrest of NB35 (*tor*^{ts}) occurs within one generation; this strain fails to complete more than one doubling after shift to the nonpermissive temperature, as determined by cell counting. Following release from a mating pheromone block and a simultaneous shift from the permissive temperature (24°C) to the nonpermissive temperature (37°C), cells synchronously entered S phase as determined by emergence of new buds (Figure 2A); cells maintained at 24°C behaved similarly. In contrast, when cells were arrested at the TOR restriction point, then released by resuspending in fresh medium at 24°C and treated with mating pheromone, they formed shmoos and did not initiate a new round of budding for the duration of the experiment (Figure 2B). Budding after shift from α-factor to the restrictive temperature was not due to a slow inactivation of temperature-sensitive TOR.

First, NB35 (*tor*^{ts}) arrests within one generation. Second, shifting cells to the nonpermissive temperature 1.5 h before release from the α-factor block did not prevent budding (Figure 2C). Third, wild-type cells released from an α-factor block into medium containing rapamycin also resumed budding (Figure 2D). The results of a double block experiment performed with α-factor and rapamycin (see MATERIALS AND METHODS) were also consistent with a TOR restriction point in early G1; rapamycin prevented nocodazole-synchronized cells from forming shmoos in response to α-factor (our unpublished results). Thus, the TOR restriction point is in early G1 before START.

As further evidence that loss of TOR function causes an early G1 arrest, we observed that rapamycin-treated cells lack START-specific transcripts encoding the G1 cyclins (Figure 3) (see below), and that providing *CLN2* under control of the rapamycin-unresponsive, constitutive *Schizosaccharomyces*
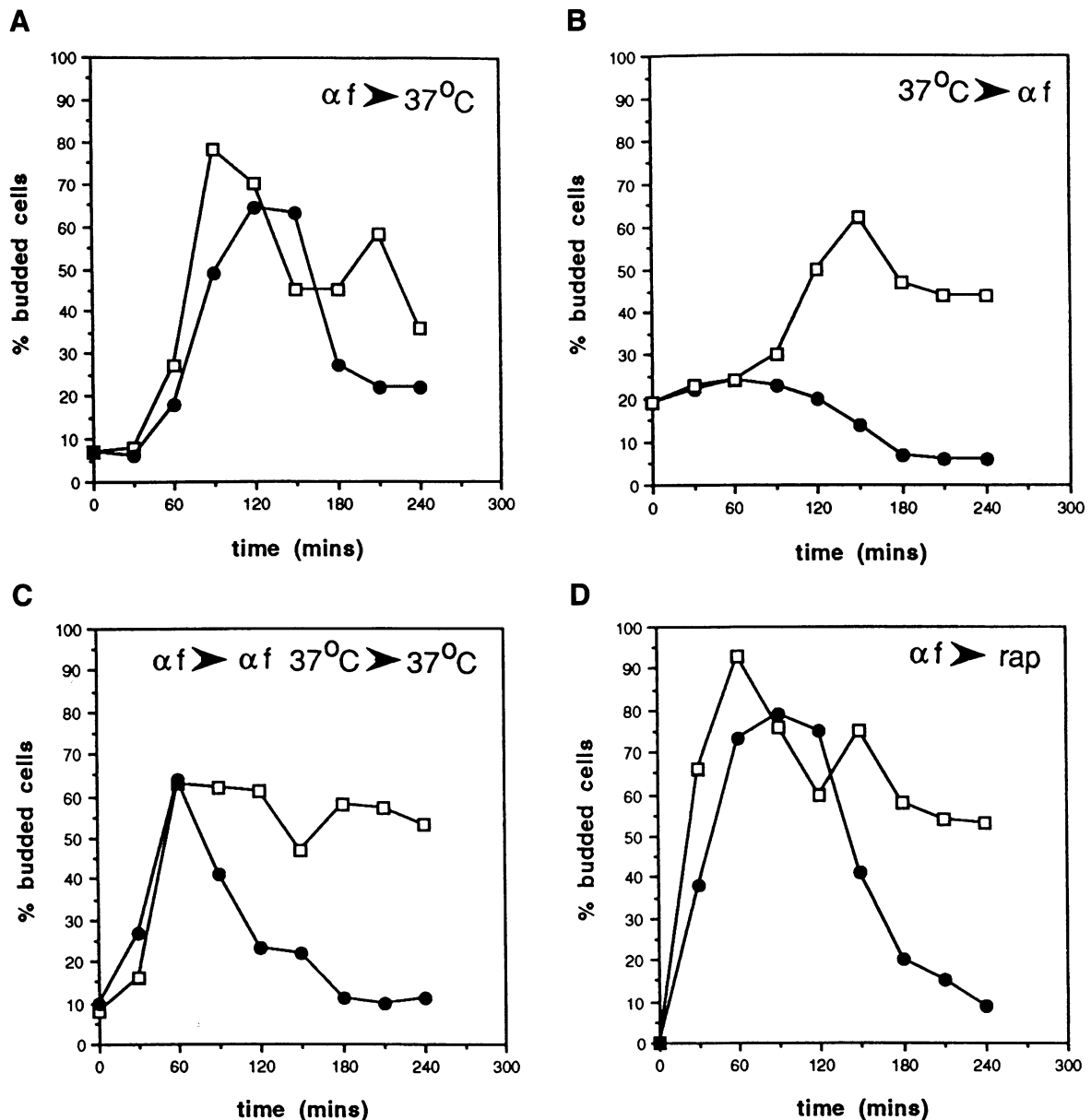
Figure 2. TOR depletion arrests cells in early G1 before START. (A–C) Order-of-function determination by an α-factor (αf) and *tor*[ts] reciprocal shift. The percentage of budded cells was monitored at the indicated time points after release from the first block. (A) Strain NB35 (*tor*[ts]) was arrested by pretreatment with α-factor at the permissive temperature, washed, and resuspended in fresh medium without α-factor at 24°C (open squares) or 37°C (closed circles). (B) Strain NB35 (*tor*[ts]) was arrested by preincubation for 5 h at the nonpermissive temperature (37°C), washed, and resuspended in fresh medium at the permissive temperature (24°C) containing vehicle alone (open squares) or α-factor (closed circles). (C) Strain NB35 (*tor*[ts]) was treated as in panel A with the modification that cells were shifted to the nonpermissive temperature 1.5 h before release from the α-factor block. (D) Order-of-function determination by an α-factor to rapamycin shift. Wild-type strain JK9–3da was arrested at START by α-factor treatment, washed, and resuspended in medium containing vehicle alone (open squares) or 0.2 μg/ml rapamycin (closed circles). The percentage of budded cells was determined at the indicated times after release from α-factor.

pombe ADH promoter (Nasmyth and Dirick, 1991) does not abrogate the rapamycin-induced cell cycle arrest (our unpublished results). Thus, TOR is not directly (or solely) required for *CLN* gene transcription, and the loss of START-specific transcripts is a downstream effect rather than the direct cause of the

cell cycle arrest. A constitutively expressed *CLN2* transcript does not suppress the rapamycin-induced cell cycle arrest presumably because it is not translated (see below).

The effects of rapamycin treatment on START-specific transcripts were as follows. The mRNAs for *CLN1*

and *CLN2* (Figure 3 and our unpublished results for *CLN2*) were no longer detectable after 2 h of rapamycin treatment. Surprisingly, the normally constitutively expressed *CLN3* transcript was also reduced with similar kinetics as seen for *CLN1* and *CLN2*, but was not completely eliminated. As determined by densitometry of appropriately exposed autoradiographs and normalization to *ACT1* transcript levels, the *CLN3* mRNA level was maximally reduced by ~60%. The mRNAs for the three additional genes, *HCS26*, *ORFD*, and *CLB5*, which bear limited homology to the *CLN* genes and are also expressed only in late G1 also disappeared upon rapamycin treatment, with kinetics identical to those seen for the *CLN1* and *CLN2* transcripts (Figure 3 for *HCS26* and *ORFD*).

Expression of the *CLN1*, *CLN2*, and *HCS26* genes (and possibly *ORFD*) is under control of the transcription factor SBF, which is composed of the DNA binding moiety SWI4 and its regulatory subunit SWI6 (Nasmyth and Dirick, 1991; Ogas *et al.*, 1991). We therefore assessed the levels of *SWI4* and *SWI6* transcripts in rapamycin-treated cells. Normally, the mRNA for SWI6 is constitutively expressed whereas the mRNA for SWI4 oscillates, peaking in late G1 and falling to a low but detectable basal level elsewhere in the cell cycle (Breeden and Mikesell, 1991). Like the *CLN3* transcript, the mRNA for SWI6 was depleted by ~60% (Figure 3). The transcript for SWI4 fell to basal levels 2 h after rapamycin treatment, thus behaving like other START-specific mRNAs.
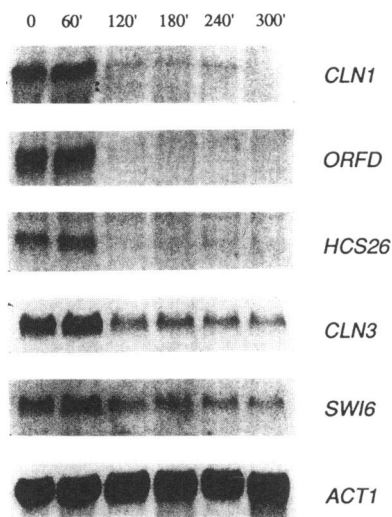
Figure 3. START-specific transcripts are depleted upon rapamycin treatment. Northern blot analysis of RNA isolated from cells (JK9–3da) treated with 0.2 µg/ml rapamycin for 0, 1, 2, 3, 4, and 5 h (indicated in minutes). The mRNAs for *CLN1*, *ORFD*, and *HCS26* are abolished, and mRNAs for *CLN3* and *SWI6* are reduced by ~60% relative to *ACT1* levels. *ACT1* encodes actin and is a control for a message that is not START specific. The level of *ACT1* message is not affected by rapamycin. See text for additional information.

These observations are not due to a global repression of transcription as the transcripts for actin (*ACT1*) and *CDC28* and also the previously identified targets of rapamycin *TOR1* and *TOR2* were not depleted throughout the time course of these experiments (Figure 3 and our unpublished results). Furthermore, some transcripts are actually induced upon rapamycin treatment (see below). As mentioned above, the absence of START-specific transcripts upon rapamycin treatment is presumably an indirect consequence of a cell cycle arrest before START (Hubler *et al.*, 1993). The reduction in the normally constitutive messages could reflect the inherent instability of untranslated (see below) mRNAs.

TOR Is Required for Translation Initiation

Because rapamycin blocks activation of protein synthesis in mammalian cells (Jefferies *et al.*, 1994; Terada *et al.*, 1994) and because inhibition of protein synthesis in yeast causes an early G1 arrest (Hartwell and Unger, 1977; Pringle and Hartwell, 1981; Brenner *et al.*, 1988), we investigated whether rapamycin blocks protein synthesis in yeast by assaying incorporation of [^{35}S]methionine at intervals after addition of rapamycin. We observed an early decrease in incorporation upon rapamycin treatment (Figure 4A). Protein synthesis fell to a low (~10% of normal levels) but detectable level after 120 min, and remained at this low level throughout the course of the experiment. The low level of protein synthesis was greater than that observed in cells treated with cycloheximide (100 µg/ml), which reduced protein synthesis to undetectable levels. Up to 100-fold higher concentrations of rapamycin did not have a more severe effect on incorporation. Protein synthesis was not affected in a rapamycin-resistant *TOR1-1* (JH11–1c) or *TOR2-1* (JH12–17b) mutant, as assayed by [^{35}S]methionine incorporation in the presence of rapamycin. Thus, rapamycin is an effective inhibitor of protein synthesis acting through TOR.

To confirm that TOR is required for protein synthesis, as suggested by the above observation, we examined the effect of TOR depletion on protein synthesis. The *tor*^{ts} strain NB35 was shifted to the nonpermissive temperature and levels of protein synthesis were determined at time intervals after the temperature shift. At the nonpermissive temperature, we observed a progressive decrease in the levels of [^{35}S]methionine incorporation (Figure 4B). Incorporation levels fell to a minimum of ~10% after 6 h of incubation at the nonpermissive temperature. Levels of incorporation in NB35 (*tor*^{ts}) at the permissive temperature were less than those in wild type, indicating that there is a protein synthesis defect in this mutant even at the permissive temperature. Thus, TOR is required for protein synthesis. Furthermore, because an inhibi-
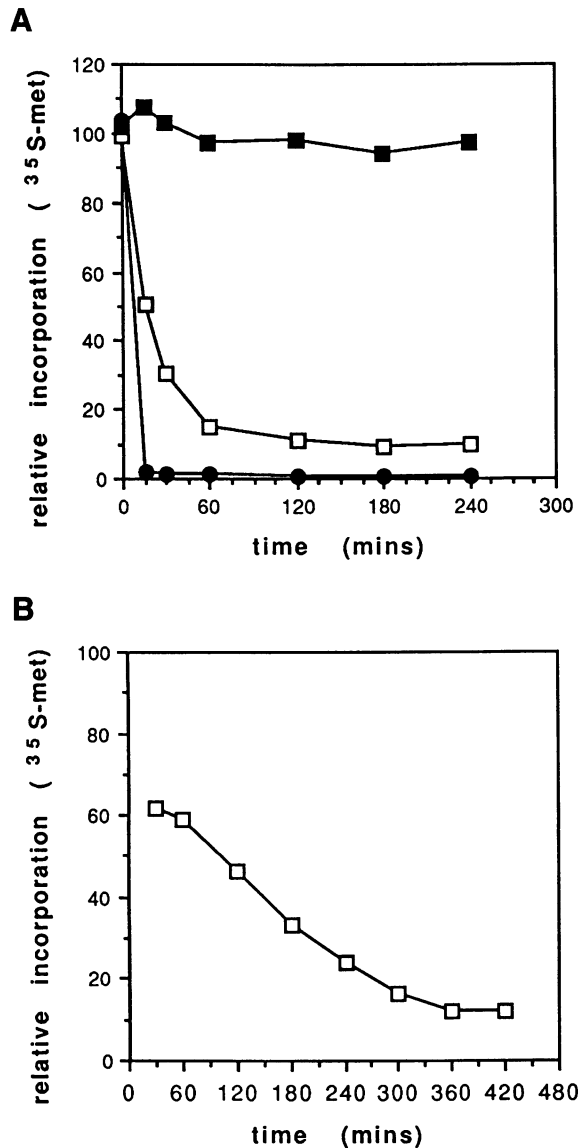
A

B

Figure 4. Rapamycin treatment inhibits protein synthesis. (A) Cells were assessed for incorporation of [^{35}S]methionine by labeling for 7 min at intervals (0, 15, 30, 60, 120, 180, and 240 min) after addition of 0.2 μg/ml rapamycin. Cells treated were wild-type JK9–3da (open squares) and rapamycin-resistant *TOR1–1* mutant JH11–1c (closed squares). Also plotted is JK9–3da treated with 100 μg/ml cycloheximide (closed circles). Incorporation (relative incorporation) is plotted as a percentage of the control, wild-type strain JK9–3da treated with drug vehicle alone. (B) Inhibition of protein synthesis upon TOR depletion. Strain NB35 (*tor^{ts}*) was incubated at the restrictive temperature, and samples were removed at the indicated time intervals for determination of [^{35}S]methionine incorporation. Values are plotted as a percentage of [^{35}S]methionine incorporation in NB17–3d at the restrictive temperature. An early time point is not included because a reliable value could not be obtained for either the temperature-sensitive mutant NB35 or NB17–3d immediately after shift to the nonpermissive temperature. Shown (A and B) are representative curves of three or more independent experiments.

tion of protein synthesis causes an early G1 arrest (Hartwell and Unger, 1977; Pringle and Hartwell, 1981; Brenner *et al.*, 1988), the protein synthesis defect may be the cause of the cell cycle arrest; the relatively slow inhibition of incorporation in NB35 (*tor^{ts}*) at the nonpermissive temperature, compared with rapamycin-treated cells at the permissive temperature, is not necessarily inconsistent with the first cycle arrest of NB35 (*tor^{ts}*) because this strain has a translation defect even at the permissive temperature and because cells have a longer cell cycle at the higher temperature.

To determine whether the inhibition of protein synthesis was at the level of initiation or elongation, polysome profiles of wild-type cells treated with rapamycin for 1 and 2 h were analyzed. This experiment was performed in the absence of the translation elongation inhibitor cycloheximide so that a block in elongation, if imposed, could be observed. Such a block is characterized by an accumulation of polysomes. No polysomes were present in either extract, only a single peak corresponding to 80S monosomes and ribosomes (our unpublished results). Rapamycin does not, therefore, cause a translation elongation block; however, a mild defect in the rate of elongation that is not sufficiently stringent to prevent ribosome "run-off" during the time needed to harvest, wash, and lyse cells in preparation for sucrose gradients would not be detected. To investigate whether rapamycin causes a block in translation initiation, wild-type cells were treated with drug vehicle alone or with rapamycin for 1 and 2 h followed by a 10-min treatment with cycloheximide to prevent run-off of any polysomes present (Figure 5, A and B; our unpublished result for 1-h timepoint). Rapamycin treatment caused a progressive decay of polysomes with a coincident increase in the 80S peak, indicating an initiation block. The apparent discrepancy between the observed inhibition of [^{35}S]methionine incorporation (~90%) and the inhibition of polysomes (~60%) after 2 h of rapamycin treatment may reflect a difference in the sensitivities of the two assays or a mild elongation defect in addition to a block in initiation.

We next examined the polysome profiles of TOR-depleted cells using the *tor^{ts}* strain NB35. Again, a severe reduction in the number of polysomes and a coincident increase in the 80S peak were evident after incubation for 5 h at the nonpermissive temperature (Figure 5C). A similar but less pronounced effect was observed after 3 h at the nonpermissive temperature. Thus, TOR is required for translation initiation.

Loss of TOR Causes a Starvation Response, but TOR Is Not Part of the RAS/cAMP Pathway

Starved yeast cells exit the cell cycle (stop dividing) and enter G0. Cells entering G0 are characterized by

several distinct properties (Werner-Washburne *et al.,* 1993) including 1n DNA content, failure to reach START (Pringle and Hartwell, 1981), a reduction in protein synthesis to ~10% of normal levels, down-regulation of *CLN3* message (Hubler *et al.,* 1993), and enlargement of the vacuole (Granot and Snyder, 1991). As described above, rapamycin-treated or TOR-depleted cells display all these characteristics. Additionally, rapamycin-treated or TOR-depleted cells are still alive (metabolically active) despite the observed reduction in protein synthesis; rapamycin-treated cells exclude the vital dye phloxin B even 24 h after treatment, and all temperature-sensitive *tor2* alleles isolated to date are reversible (Barbet and Hall, unpublished data). This led us to consider that rapamycin might be causing a starvation response despite the presence of nutrients, and inducing cells to enter G0. To test this, we examined by Northern analysis the effect of rapamycin on the transcription of genes whose mRNA levels are known to change upon entry into G0. The heat shock genes *SSA3* and *HSP26* and the ubiquitin gene *UBI4* are transcriptionally induced upon entry into G0 (Werner-Washburne *et al.,* 1993). The catalase T gene *CTT1* is also transcriptionally induced upon entry into G0, with enzymatic activity peaking and then declining 3 h after cells enter stationary phase (Werner-Washburne *et al.,* 1993). In contrast, the mRNA level of the heat shock genes *SSA1* and *SSA2* (*SSA1/2*) fluctuates in different ways depending on the starvation regimen but can remain largely unchanged, and transcription of the cold-inducible "heat shock" genes *SSB1* and *SSB2* (*SSB1/2*) is severely repressed upon entry into G0 (Werner-Washburne *et al.,* 1993). As shown in Figure 6A, we observed these same changes in transcription upon rapamycin treatment. The mRNAs for *SSA3, HSP26,* and *UBI4* were induced upon rapamycin treatment; maximal induction occurred 2 h after rapamycin addition for *SSA3* and *HSP26,* and after 30 min for *UBI4.* The *CTT1* transcript was also induced upon rapamycin treatment, and transcript levels remained high for 2 h before falling. In contrast, the *SSB1/2* transcripts decreased to almost undetectable levels within 1 h of treatment. The level of *SSA1/2* transcripts fluctuated but remained largely unchanged. Thus, it appears that rapamycin causes a starvation response and induces entry into G0.
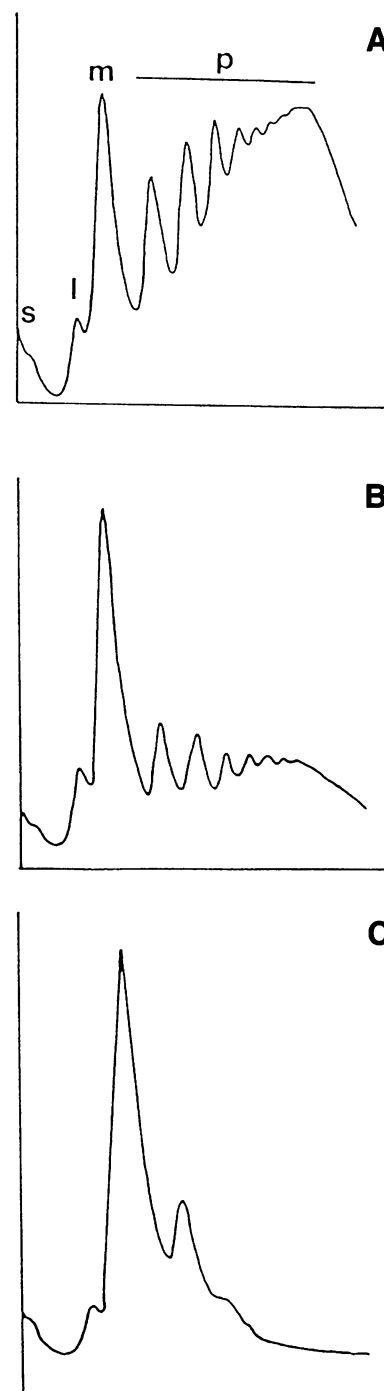

Figure 5. Rapamycin treatment or TOR depletion blocks translation initiation. (A and B) Polysome profiles of wild-type cells (JK9–3da) treated with (A) vehicle alone, and (B) 0.2 μg/ml rapamycin for 2 h. (C) Polysome profile of the *tor*[ts] strain NB35 after 5 h at the nonpermissive temperature (37°C). In all the above cases, cycloheximide was added 10 min before harvest, to prevent "run off." Wild-type strain JK9–3da grown at the nonpermissive temperature is slightly stimulated for polysome accumulation. The positions of 40S ribosomal subunits (s), 60S ribosomal subunits (l), 80S monosomes (m), and polysomal ribosomes (p) are indicated.

A

B

C
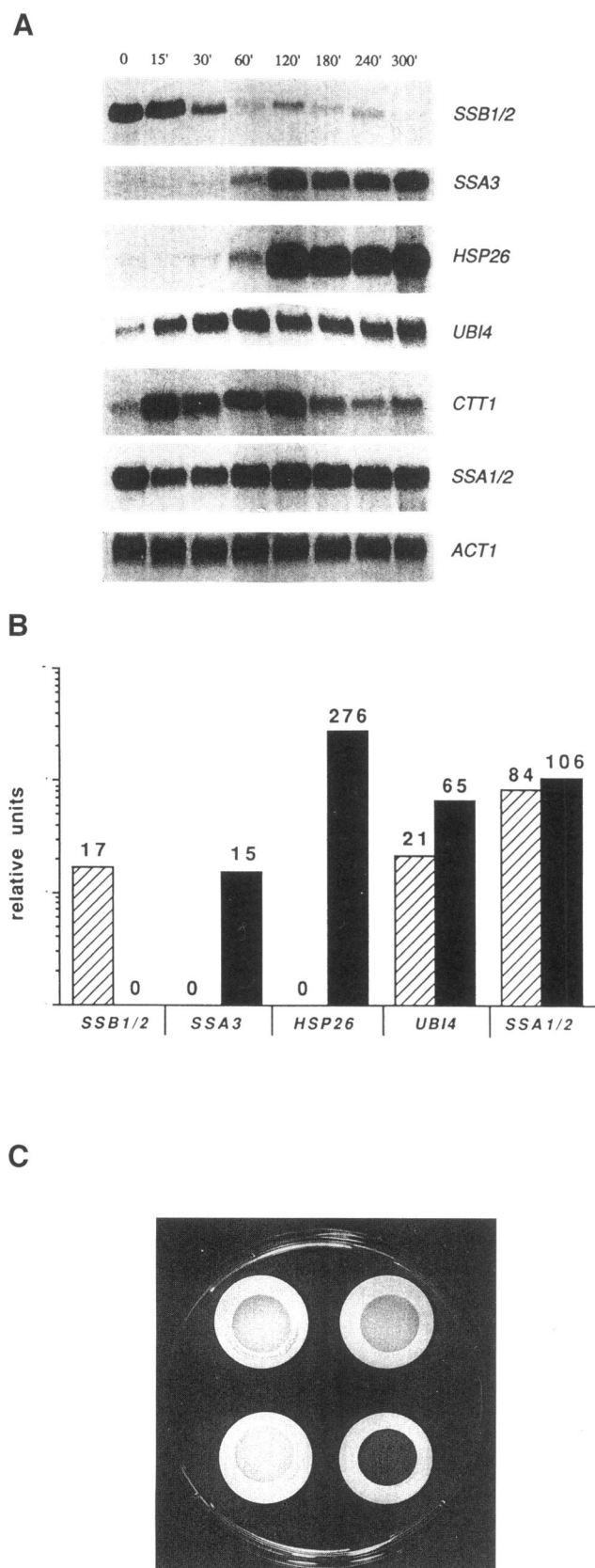
We next examined whether TOR depletion elicits the same starvation-induced changes in transcript levels. We could not utilize the *tor*[ts] strain for these experiments, because many of the same changes in transcript levels occur normally at high temperature (the nonpermissive temperature of our *tor*[ts] mutant) independently of starvation. Therefore, we used a strain containing *TOR2* under control of the regulatable *GAL1* promoter and chromosomal disruptions of both *TOR1* and *TOR2* (JK350–21a) to deplete the cells of TOR (Kunz *et al.*, 1993; Helliwell *et al.*, 1994). After shifting from galactose- (SGal/Gly) to glucose-containing (SD) medium (TOR-depletion conditions), we observed changes in the pattern of transcription similar to those seen when wild-type cells are treated with rapamycin (Figure 6B). Thus, TOR depletion also induces a starvation response.

Additional indicators of stationary phase are the accumulation of the storage carbohydrate glycogen and acquisition of thermotolerance. We examined whether cells treated with rapamycin accumulate glycogen. Cultures were treated for 5 h with rapamycin, harvested by filtration at hourly intervals, and stained for glycogen using iodine vapor, which stains glycogen-containing cells dark brown (Chester, 1968). As shown in Figure 6C for the 5-h time point, cells treated with rapamycin did indeed stain darkly when exposed to iodine. Accumulation of glycogen was weakly detectable after 1 h of treatment. Also confirming that loss of TOR function induces a starvation response, we observed that cells depleted for TOR exhibit increased resistance to the killing effects of high temperatures when compared with wild-type cells (our unpublished results).

The RAS/cAMP signal transduction pathway acts in early G1 (before the mating pheromone arrest point) and may be involved in the controlled entry into G0 (Broach, 1991; Thevelein, 1994). To investigate

Figure 6. Rapamycin treatment or TOR depletion induces a starvation response. (A) RNA was isolated from cells (JK9–3da) treated with 0.2 µg/ml rapamycin for 0, 15, 30, 60, 120, 180, 240, and 300 min, and probed by Northern analysis with the indicated genes (see MATERIALS AND METHODS). *SSA1/2* refers to *SSA1* and *SSA2*. *SSB1/2* refers to *SSB1* and *SSB2*. The observed changes in transcript levels are characteristic of cells entering G0. (B) Histogram showing level changes for the indicated transcripts upon depletion of TOR by galactose to glucose shift. Conditions of the galactose to glucose shift were as described (Helliwell *et al.*, 1994). Hatched bars correspond to a wild-type (JK9–3da) strain; solid bars correspond to a TOR-depleted strain (JK350–21a). Transcript levels were normalized to *ACT1* mRNA levels. Transcript level values in relative units are given above each bar. A value of 0 indicates an undetectable mRNA level. (C) Rapamycin treatment causes accumulation of glycogen. (Top left) Rapamycin-resistant strain JH12–17b treated with drug vehicle alone. (Top right) JH12–17b treated for 5 h with 0.2 µg/ml rapamycin. (Bottom left) Wild-type strain JK9–3da treated with drug vehicle alone. (Bottom right) JK9–3da treated for 5 h with 0.2 µg/ml rapamycin. Filters were exposed to iodine vapor for 1 min to stain for glycogen.

whether loss of TOR function induces entry into G0 by inhibiting the RAS/cAMP cascade, we constitutively activated this pathway, and then tested for abrogation of the rapamycin-induced cell cycle arrest. Two methods were used to constitutively activate the pathway. First, we disrupted the *BCY1* gene (Toda *et al.*, 1987). A *BCY1* disruption activates the RAS/cAMP pathway by eliminating the negative regulatory subunit of the cAMP-dependent protein kinase A (Cannon and Tatchell, 1987; Toda *et al.*, 1987). Second, we introduced the dominant, activated *RAS2* allele *RAS2*^{val19}. The *RAS2*^{val19} mutation hyperactivates the RAS/cAMP pathway by maintaining RAS2 in its active, GTP-bound state (Kataoka *et al.*, 1984). Both *bcy1* (NB30) and *RAS2*^{val19} (NB34) cells were as sensitive as wild-type cells to rapamycin, based upon growth arrest in the presence of drug. Flow cytometry on these strains indicated that greater than 85% of the cells arrested with a 1n DNA content after 3 h of rapamycin treatment, as observed with wild-type cells (see Figure 1 for wild-type cells). Rapamycin-treated *bcy1* and *RAS2*^{val19} cells also accumulated glycogen, as determined by iodine staining. Therefore, activation of the RAS/cAMP pathway does not abrogate the rapamycin-induced cell cycle arrest, indicating that TOR is not part of the RAS/cAMP pathway.

Our data do not rule out the possibility that TOR lies in the RAS/cAMP pathway downstream of BCY1, but we consider this very unlikely. First, subcellular localization studies (Kunz, Stevenson, Schneider, and Hall, unpublished data) and their homology to lipid kinases indicate that the TORs are membrane-associated proteins, whereas BCY1 is a membrane-distal component of the RAS/cAMP pathway. Second, diploid cells lacking TOR function arrest in G1 (2n DNA content) but do not sporulate, whereas diploids compromised in the RAS/cAMP pathway do sporulate. Third, activation of p70 S6 kinase, a presumed downstream component of TOR in mammalian cells, is independent of p21^{ras} (Downward, 1994; Ming *et al.*, 1994). Fourth, there is no example of, or need for, a lipid kinase in a signaling pathway that utilizes cAMP as a second messenger; the lipid kinases mediate production of the fundamentally different, phosphatidylinositol-derived second messengers. Thus, TOR1 and TOR2 appear to define a novel nutrient-related process mediating progression through early G1. This would be in agreement with the observations of Cameron *et al.* (1988), who described mutants that express low-level, constitutive cAMP-dependent protein kinase A activity but that still respond appropriately to nutrient conditions, even in the absence of essential upstream components of the RAS/cAMP pathway.

Expression of CLN3 under Altered Translational Control Confers TOR-independent G1 Progression

The finding that loss of TOR function causes an early reduction in protein synthesis and a G1 arrest within one generation suggested that TOR might be controlling translation of an unstable protein(s) required for G1 progression. Good candidates for such proteins were the G1 cyclins, as these proteins are unstable and limiting for G1 progression (Cross, 1988; Nash *et al.*, 1988; Hubler *et al.*, 1993; Tyers *et al.*, 1993). To test whether cells lacking TOR function arrest in early G1 (G0) because they do not synthesize G1 cyclins, we devised a situation in which one of these, CLN3, would be synthesized upon rapamycin treatment, and asked whether this would be sufficient to drive rapamycin-treated cells through G1. CLN3 was chosen because the transcript for this cyclin is normally present under conditions of rapamycin treatment (Figure 3). We fused the *CLN3* open reading frame to the 5' region (untranslated leader and promoter) of the *UBI4* gene. The *UBI4* 5' region was chosen because it is both transcriptionally and translationally active in G0 and would therefore express CLN3 upon rapamycin treatment (Finley *et al.*, 1987; Brenner *et al.*, 1988; Werner-Washburne *et al.*, 1993) (Figure 6A). We then examined whether the *UBI4-CLN3* fusion suppresses the rapamycin-induced cell cycle arrest.

An asynchronously growing wild-type yeast strain containing the *UBI4-CLN3* fusion on a centromeric plasmid (NB36) was treated with rapamycin, and at hourly intervals the DNA content of the cells was analyzed by flow cytometry. Like a control strain (NB37) containing a plasmid-borne *UBI4* 5' region without the *CLN3* open reading frame, NB36 cells arrested growth after approximately 2 h of rapamycin treatment. This was expected because rapamycin causes a general inhibition of protein synthesis (Figure 4), and TOR has an essential non-cell cycle function in addition to its essential role in G1 (Kunz *et al.*, 1993). Analysis of DNA content of the arrested cells, however, indicated that NB36 (*UBI4-CLN3*) arrested throughout the cell cycle, whereas the control strain arrested in G1 (Figure 7). Thus, cells containing the *UBI4-CLN3* fusion no longer arrest in G1 upon rapamycin treatment.

Northern analysis of the strain (NB36) containing the *UBI4-CLN3* fusion indicated that it produces approximately 20-fold more *CLN3* mRNA upon rapamycin treatment than an isogenic strain lacking the fusion. To determine whether the suppression of the cell cycle arrest in strain NB36 was due to altered control of CLN3 translation or merely to the increased dosage of the *CLN3* transcript, we examined whether cells containing the wild-type *CLN3* gene on a high-copy-number plasmid (NB38) also arrested outside of G1 upon rapamycin treatment. After 2 h of treatment,
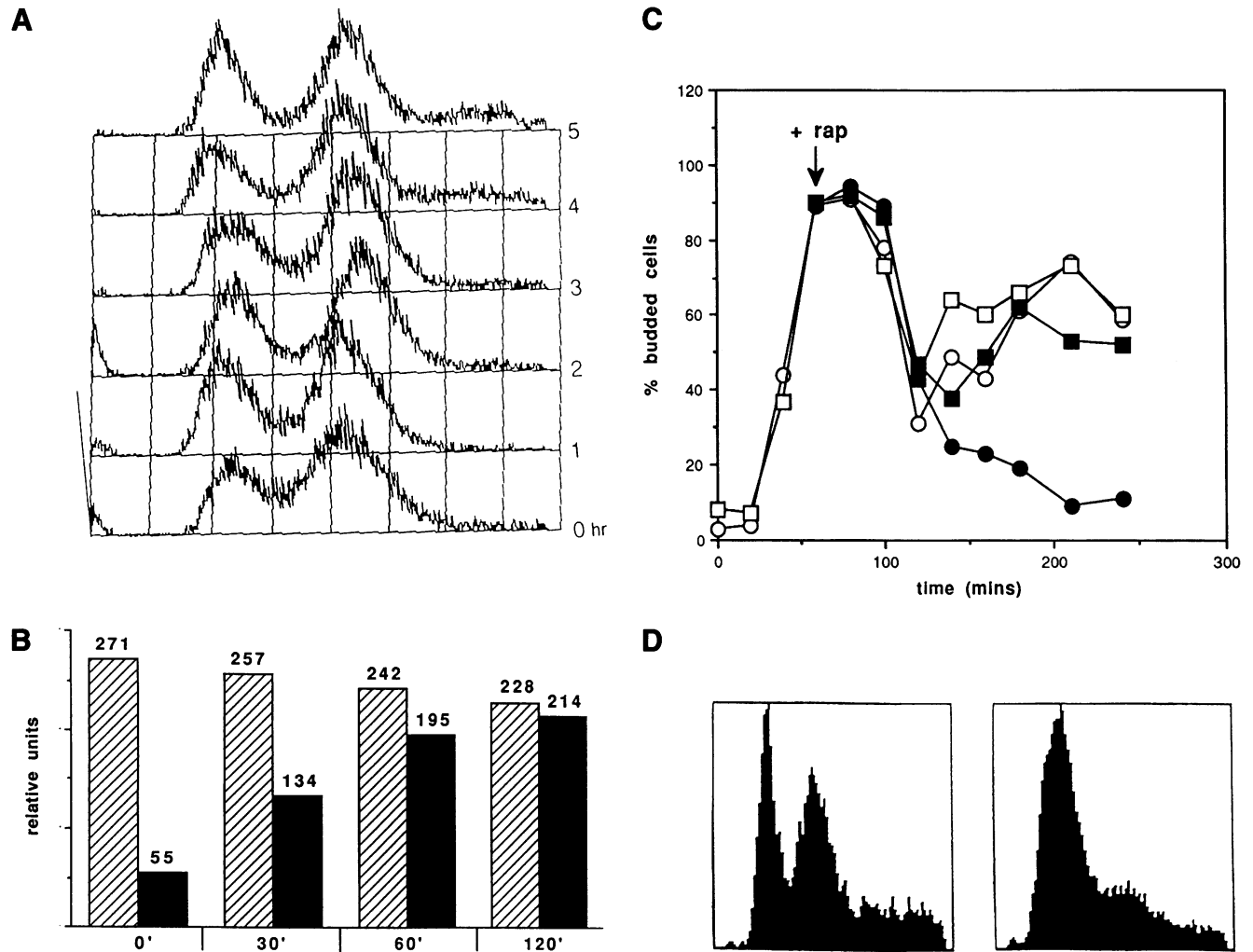
A

B

C

D

Figure 7. Altered translational control of CLN3 suppresses the cell cycle–specific arrest of rapamycin-treated cells. (A) Exponentially growing cells containing the *UBI4-CLN3* fusion (NB36) were treated with 0.2 μg/ml rapamycin and sampled for flow cytometry at hourly intervals up to 5 h. Rapamycin-treated NB36 arrested throughout the cell cycle, as indicated by a roughly even distribution of cells with a 1n and 2n DNA content. Results of flow cytometry on control strain NB37 treated with rapamycin were indistinguishable from the results in Figure 1D. (B) Northern analysis of strains treated with rapamycin and assessed for levels of *CLN3* transcript. Shown are the levels of *CLN3* transcript in strain NB38 (hatched bars) containing the wild-type *CLN3* gene in a high copy number plasmid and strain NB36 (solid bars) containing the *UBI4-CLN3* fusion, at the indicated times (in minutes) following rapamycin treatment. All values were normalized to the levels of actin transcript. At 120 min following treatment, cells had arrested growth. (C and D) A *UBI4-CLN3* strain treated with rapamycin is able to traverse G1. (C) Percentage of budded cells of rapamycin-treated (closed squares) or -untreated (open squares) NB36 (*UBI4-CLN3*) compared with rapamycin-treated (closed circles) or -untreated (open circles) control strain NB37. Percentage of budded cells was determined at 20-min intervals following release from the α-factor block at START. Rapamycin (rap) was added 60 min following release from α-factor. (D) Flow cytometry of NB36 (*UBI4-CLN3*) and NB37 control cells released from an α-factor block at time 0 and treated with rapamycin 60 min following release from α-factor. Shown is the DNA content of the rapamycin-treated cells at the end of the experiment (240 min). The rapamycin-treated cells arrested growth approximately 150 min after rapamycin addition. The left panel shows the DNA content for strain NB36, the right panel shows the DNA content for NB37 (G1 arrest). DNA content of untreated cells at 240 min was indistinguishable from that shown in the left panel. Data shown is representative of three independently performed experiments.

NB38 cells arrested growth, with ~85% of cells containing a 1n DNA content. Northern analysis of the rapamycin-treated NB36 (*UBI4-CLN3*) and NB38 (high copy *CLN3*) cells indicated that the level of CLN3 transcripts in NB38 was greater than that in NB36

(Figure 7B). In addition, high level overexpression of *CLN3* from the inducible *GAL1* promoter was also unable to overcome a rapamycin-induced G1 arrest, and plasmid-borne *UBI4-CLN3* still caused a random arrest despite disruption of the chromosomal copy of

CLN3 (our unpublished results). Furthermore, an integrated copy of the *CLN3–1* allele (strain NB33), which bears a mutation that stabilizes the CLN3 protein but does not otherwise affect its cyclin function (Cross, 1988; Nash *et al.*, 1988), had the same effect as *UBI4-CLN3* in causing a random arrest upon rapamycin treatment. This confirms that the *UBI4-CLN3* fusion does not promote G1 progression simply because of an elevated level of *CLN3* transcripts.

To determine more directly whether rapamycin-treated cells containing the *UBI4-CLN3* fusion are able to traverse the G1 phase of the cell cycle, we examined the effect of rapamycin on synchronized cells. Strain NB36 (*UBI4-CLN3*) and the control strain NB37 were synchronized at START by addition of α-factor. Following release from the pheromone block, the cultures were split into two and rapamycin was added to one half, the remaining halves receiving drug vehicle alone. As shown in Figure 7C, NB37 control cells treated with rapamycin entered G1 and arrested as unbudded cells. Rapamycin-treated NB36 (*UBI4-CLN3*), however, entered G1 but then began to produce new buds before arresting growth, indicating that cells were traversing G1 and beginning a new cycle. Analysis of the arrested cells by flow cytometry confirmed that the NB36 (*UBI4-CLN3*) cells had traversed G1 whereas the NB37 control cells had not (Figure 7D). Thus, *UBI4* leader-dependent expression of CLN3 causes rapamycin-treated cells to traverse G1.

Expression of UBI4-CLN3 Confers Starvation Sensitivity

The finding that TOR may normally modulate synthesis of CLN3 (among other proteins) as part of a starvation response suggested that cells containing the *UBI4-CLN3* fusion might be sensitive to starvation. To test this suggestion, *UBI4-CLN3* strain NB36 and control strain NB37 were grown to stationary phase (starved) and samples were removed daily for assessment of cell viability and the percentage of budded cells. As NB36 (*UBI4-CLN3*) cells entered stationary phase (cell number no longer increased) (Figure 8A), their ability to form colonies on rich medium rapidly decreased (Figure 8B). In contrast, starved NB37 control cells retained high viability for the duration of the experiment. The starvation sensitivity of NB36 (*UBI4-CLN3*) was most likely due to this strain's inability to arrest in G1 (G0), as suggested by the observations that it stopped dividing at a higher cell density (~1.5-fold) than the control strain (Figure 8A) and with a high percentage of budded cells (Figure 8C). Strain NB38 containing the wild-type *CLN3* gene in high dosage behaved in this experiment like control strain NB37. These findings support the involvement of TOR in nutrient sensing, and also confirm that modulating

the level of translation is part of the regulated entry into stationary phase (G0).

DISCUSSION

We have shown that loss of TOR function (rapamycin treatment or TOR depletion) causes yeast cells to arrest in early G1 and to exhibit, by all criteria examined, characteristics of starved cells entering stationary phase, or G0. We have also demonstrated that loss of TOR function causes a general inhibition of translation initiation. Providing the transcript for the G1 cyclin CLN3 under the translational control of the *UBI4* 5′ region suppresses the rapamycin-induced G1 arrest and confers starvation sensitivity. These results suggest the following model for the role of TOR in cell cycle control (Figure 9). In response to nutrient availability, TOR stimulates general translation initiation, including translation of G1-regulatory transcripts such as those for CLN3 and other G1 cyclins. This then drives cells through G1 and into S phase. In the converse situation, the absence of nutrients causes inactivation of TOR, which leads to loss of translation and a subsequent early G1 arrest and entry into G0. It is important to emphasize that TOR is required for general translation and that the role of TOR in cell cycle control, as proposed here, is just part of a greater role in general growth control. We would also like to stress that, although it accounts for all our data, the model is largely speculative and is intended only as a framework to bring together our and other findings.

Several lines of evidence suggest that TOR is part of a signaling pathway. First, the TORs are homologous to PI kinases, enzymes implicated in signaling. Second, because loss of TOR rapidly causes a starvation response, TOR is likely involved in sensing and relaying the availability of nutrients. Indeed, constitutively activating the proposed pathway by providing CLN3 independently of upstream components (*CLN3* under the translational control of the *UBI4* untranslated leader) causes starvation sensitivity. Third, the mammalian counterpart of TOR (FRAP/RAFT1/RAPT1/mTOR) appears to mediate an intermediate step in a defined, rapamycin-sensitive signal transduction pathway required for cell proliferation (Brown *et al.*, 1994; Chiu *et al.*, 1994; Downward, 1994; Sabatini *et al.*, 1994; Sabers *et al.*, 1995). The putative TOR pathway is novel because it acts in early G1, and TOR is not part of the RAS/cAMP pathway.

The observed inhibition of translation initiation is likely a direct consequence of loss of TOR function and the cause (rather than an effect) of the cell cycle arrest, for the following reasons. First, the reduction in translation is the earliest effect observed upon loss of TOR function. Second, a specific block in translation initiation, either by mutation of an initiation factor or by treatment with a low concentration of cyclo-

A

B

C
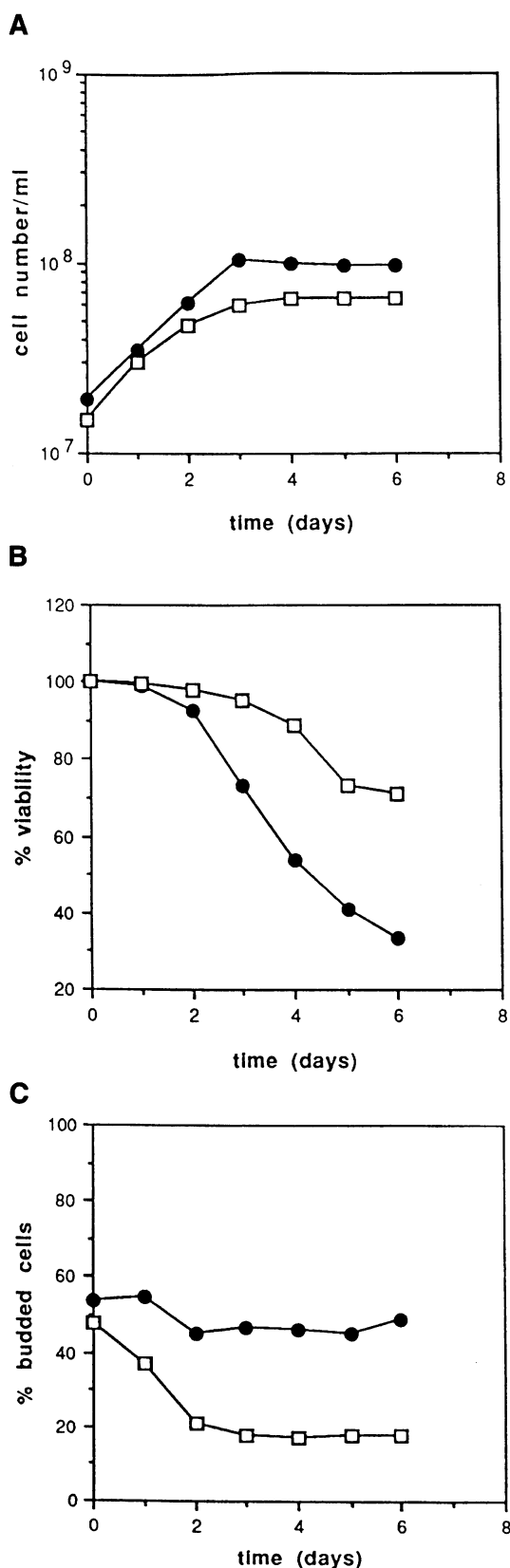
heximide, causes yeast cells to arrest in early G1 (Hartwell and Unger, 1977; Johnston *et al.*, 1977; Pringle and Hartwell, 1981; Hanic-Joyce *et al.*, 1987; Brenner *et al.*, 1988; Hubler *et al.*, 1993; Barnes *et al.*, 1995). Third, and most important, allowing translation initiation of an appropriate, cell cycle–controlling transcript is sufficient to suppress the rapamycin-induced G1 arrest. Fourth, TOR in mammalian cells probably activates translation initiation and G1 progression in response to mitogens (Downward, 1994; see INTRODUCTION). Thus, the TOR pathway in yeast appears to control translation initiation and, thereby, early G1 progression.

The observation that phosphorylation of the yeast equivalent of S6 (S10) is not important for growth (Zinker and Warner, 1976; Kruse *et al.*, 1985; Johnson and Warner, 1987) suggests that TOR is not regulating translation initiation in yeast through S6 (see INTRODUCTION). One alternative possibility is that the TOR pathway controls translation initiation through the initiation factor eIF-4E (or an associated subunit). eIF-4E is the cap-binding subunit of the eIF-4F complex, which also contains eIF-4A, an RNA helicase, and eIF-4γ, a protein of unknown function (Rhoads, 1988; Lanker *et al.*, 1992; Linder, 1992; Goyer *et al.*, 1993; Redpath and Proud, 1994). eIF-4F binds to the 5' cap structure of mRNA and promotes unwinding of 5' secondary structure, facilitating binding of the 43S ribosomal preinitiation complex to the mRNA. Several observations suggest that TOR could control eIF-4E. First, analyses of *CDC33* (encodes eIF-4E) and *TOR* mutants indicate that eIF-4E and TOR have remarkably similar roles. Both have essential functions required for general translation initiation (Altmann *et al.*, 1989; Kunz *et al.*, 1993; see RESULTS). Furthermore, both have an early G1-specific function and an essential function that is not G1 specific (Johnston *et al.*, 1977; Pringle and Hartwell, 1981; Brenner *et al.*, 1988; Kunz *et al.*, 1993); protein synthesis is required at several points in the cell cycle but is most limiting in G1 (Burke and Church, 1991). Second, in mammalian cells, eIF-4E is the rate-limiting protein in translation (Duncan *et al.*, 1987) and a target for regulation. Growth factors activate protein synthesis by triggering the phosphorylation and release of the eIF-4E-

Figure 8. The *UBI4-CLN3* fusion confers starvation sensitivity and an inability to arrest in G0. (A) Growth curve of NB36 cells (closed circles) expressing *UBI4-CLN3* and NB37 cells (open squares) expressing the *UBI4* 5' region alone. (B) Viability curve of NB36 (closed circles) and NB37 (open squares) strains. Cells reached stationary phase after 3 days of growth. Strains were grown in SD medium minus leucine for the indicated times. Viability was assessed by plating 10^3 cells on YPD medium and counting colony-forming units. (C) Percentage of budded cells in cultures of NB36 (closed circles) and NB37 (open squares).
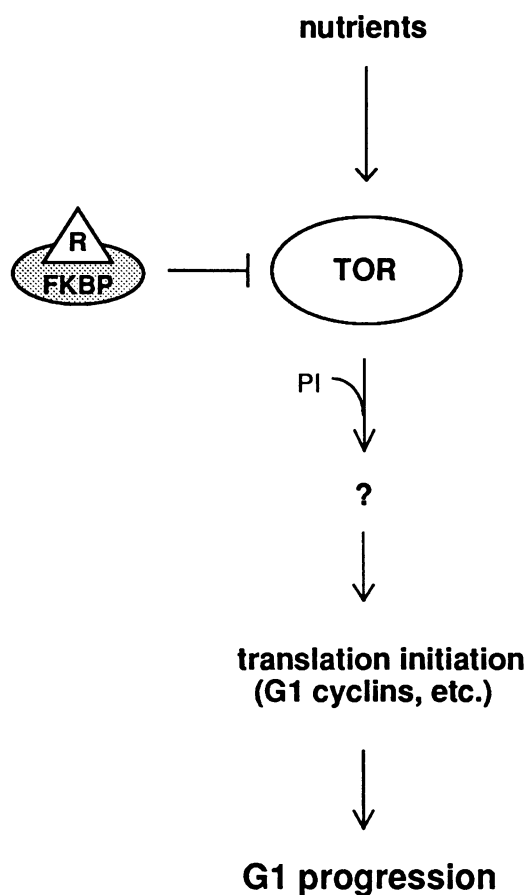
nutrients

↓

⟨R / FKBP⟩ ———⊣ (**TOR**)

PI ⌐↓

?

↓

**translation initiation
(G1 cyclins, etc.)**

↓

G1 progression

Figure 9. Model of the TOR pathway in cell cycle control. Rapamycin (R) forms a complex with FKBP to inhibit TOR (Heitman et al., 1993; Kunz et al., 1993). TOR is TOR1 and TOR2. PI is phosphatidylinositol. See DISCUSSION for further details. Because TOR is required for general translation (see RESULTS), the role of TOR in cell cycle control is just part of a greater role in general growth control; the model proposed here focuses exclusively on that part of TOR's role in general growth control that affects progression through the G1 phase of the cell cycle.

inhibiting factor 4E-BP1/PHAS-I (Haystead et al., 1994; Hu et al., 1994; Lin et al., 1994; Pause et al., 1994). Importantly, rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation (Beretta et al., 1996). Third, in proliferating yeast and mammalian cells, eIF-4E and an associated subunit are phosphorylated and therefore potentially subject to regulation by this type of modification (Duncan et al., 1987; Joshi-Barve et al., 1990; Morley et al., 1991; Rhoads et al., 1993; Redpath and Proud, 1994; Zanchin et al., 1994). Fourth, translation of UBI4 appears to have, at least, reduced dependence on eIF-4E (Brenner et al., 1988). Thus, the block in translation initiation caused by loss of TOR function may be due to a down regulation of eIF-4E.

ACKNOWLEDGMENTS

REFERENCES

Adams, A.M., Johnson, D.I., Longnecker, R.M., Sloat, B.F., and Pringle, J.R. (1990). CDC42 and CDC43: two additional genes involved in budding and the establishment of cell polarity in the yeast Saccharomyces cerevisiae. J. Cell Biol. 111, 131–142.

Altmann, M., Sonenberg, N., and Trachsel, H. (1989). Translation in Saccharomyces cerevisiae: initiation factor 4E-dependent cell-free system. Mol. Cell. Biol. 9, 4467–4472.

Barnes, C.A., MacKenzie, M.M., Johnston, G.C., and Singer, R.A. (1995). Efficient translation of an SSA1-derived heat-shock mRNA in yeast cells limited for cap-binding protein and eIF-4F. Mol. Gen. Genet. 246, 619–627.

Bender, A., and Pringle, J.R. (1991). Use of a screen for synthetic lethal and multicopy suppressor mutants to identify two new genes involved in morphogenesis in Saccharomyces cerevisiae. Mol. Cell. Biol. 11, 1295–1305.

Beretta, L., Gingras, A-C., Svitkin, Y.V. and Sonenberg, N. (1996). Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. EMBO J. (in press).

Bierer, B.E., Mattila, P.S., Standaert, R.F., Herzenberg, L.A., Burakoff, S.J., Crabtree, G., and Schreiber, S.L. (1990). Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. Proc. Natl. Acad. Sci. USA 87, 9231–9235.

Breeden, L., and Mikesell, G.E. (1991). Cell cycle-specific expression of the SWI4 transcription factor is required for the cell cycle regulation of HO transcription. Genes Dev. 5, 1183–1190.

Brenner, C., Nakayama, N., Goebl, M., Tanaka, K., Toh-e, A., and Matsumoto, K. (1988). CDC33 encodes mRNA cap-binding protein eIF-4E of Saccharomyces cerevisiae. Mol. Cell. Biol. 8, 3556–3559.

Broach, J.R. (1991). RAS genes in Saccharomyces cerevisiae: signal transduction in search of a pathway. Trends Genetics 7, 28–32.

Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S., and Wigler, M. (1987). The S. cerevisiae CDC25 gene product regulates the RAS/adenylate cyclase pathway. Cell 48, 789–799.

Brown, E.J., Albers, M.W., Shin, T.B., Ichikawa, K., Keith, C.T., Lane, W.S., and Schreiber, S.L. (1994). A mammalian protein targeted by the G1-arresting rapamycin-receptor complex. Nature 369, 756–758.

Burke, D.J., and Church, D. (1991). Protein synthesis requirement for nuclear division, cytokinesis and cell separation in Saccharomyces cerevisiae. Mol. Cell. Biol. 11, 3691–3698.

Cafferkey, R., Young, P.R., McLaughlin, M.M., Bergsma, D.J., Koltin, Y., Sathe, G.M., Faucette, L., Eng, W.K., Johnson, R.K., and Livi, G.P. (1993). Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin toxicity. Mol. Cell. Biol. 13, 6012–6023.

Calvo, V., Crews, C.M., Vik, T.A., and Bierer, B.E. (1992). Interleukin 2 stimulation of p70 S6 kinase activity is inhibited by the immuno-suppressant rapamycin. Proc. Natl. Acad. Sci. USA *89*, 7571–7575.

Calvo, V., Wood, M., Gjertson, C., Vik, T., and Bierer, B.E. (1994). Activation of 70-kDa S6 kinase, induced by the cytokines interleukin-3 and erythropoietin and inhibited by rapamycin, is not an absolute requirement for cell proliferation. Eur. J. Immunol. *24*, 2664–2671.

Cameron, S., Levin, D., Zoller, M., and Wigler, M. (1988). cAMP-independent control of sporulation, glycogen metabolism, and heat shock resistance in *S. cerevisiae*. Cell *53*, 555–566.

Cannon, J.F., and Tatchell, K. (1987). Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. *7*, 2653–2663.

Carpenter, C.L., and Cantley, L.C. (1990). Phosphoinositide kinases. Biochemistry *29*, 11147–11156.

Chester, V.A. (1968). Heritable glycogen-storage deficiency in yeast and its induction by ultraviolet light. J. Gen. Microbiol. *51*, 49–56.

Chiu, M.I., Katz, H., and Berlin, V. (1994). RAPT1, a mammalian homolog of yeast Tor, interacts with FKBP12/rapamycin complex. Proc. Natl. Acad. Sci. USA *91*, 12574–12578.

Chung, J., Grammer, T.C., Lemon, K.P., Kazlauskas, A., and Blenis, J. (1994). PDGF- and insulin-dependent Pp 70^{S6k} activation mediated by phosphatidylinositol-3-OH kinase. Nature *370*, 71–75.

Chung, J., Kuo, C.J., Crabtree, G.R., and Blenis, J. (1992). Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell *69*, 1227–1236.

Cross, F.R. (1988). *DAF1*, a mutant gene affecting size control, pheromone arrest and cell cycle kinetics of *Saccharomyces cerevisiae*. Mol. Cell. Biol. *8*, 4675–4684.

Cvrckova, F., and Nasmyth, K. (1993). Yeast G1 cyclins *CLN1* and *CLN2* and a GAP-like protein have a role in bud formation. EMBO J. *12*, 5277–5286.

Downward, J. (1994). Regulating S6 kinase. Nature *371*, 378–379.

Dumont, F.J., Staruch, M.J., Koprak, S.J., Melino, M.R., and Sigal, N.H. (1990). Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK506 and rapamycin. J. Immunol. *144*, 251–258.

Duncan, R., Milburn, S.C., and Hershey, J.W.B. (1987). Regulated phosphorylation and low abundance of HeLa cell initiation factor eIF-4F suggest a role in translational control. J. Biol. Chem. *262*, 380–388.

Epstein, C.B., and Cross, F.R. (1992). *CLB5*: a novel B cyclin from budding yeast with a role in S phase. Genes Dev. *6*. 1695–1706.

Ferrari, S., Pearson, R.B., Siegmann, M., Kozma, S.C., and Thomas, G. (1993). The immunosuppressant rapamycin induces inactivation of p70^{S6K} through dephosphorylation of a novel set of sites. J. Biol. Chem. *268*, 16091–16094.

Finley, D., Oezaynak, E., and Varshavsky, A. (1987). The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation and other stresses. Cell *48*, 1035–1046.

Frohlich, K.U., Fries, H.W., Rudiger, M., Erdmann, R., Botstein, D., and Merke, D. (1991). Yeast cell cycle protein CDC48p shows full length homology to mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation and gene expression. J. Cell Biol. *114*, 443–453.

Fruman, D.A., Burakoff, S.J., and Bierer, B.E. (1994). Immunophilins in protein folding and immunosuppression. FASEB J. *8*, 391–400.

Garcia-Bustos, J., Marini, F., Frei, C., Stevenson, I., and Hall, M.N. (1994). PIK1, an essential phosphatidylinositol 4-kinase associated with the yeast nucleus. EMBO J. *13*, 2352–2361.

Gietz, R.D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with in vitro–mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527–534.

Goyer, C., Altmann, M., Lee, H.S., Blanc, A., Deshmukh, M., Woolford, J.L., Trachsel, H., and Sonenberg, N. (1993). TIF4631 and TIF4632: two yeast genes encoding the high molecular-weight subunits of the cap-binding protein complex (eukaryotic initiation factor 4F) contain an RNA recognition motif-like sequence and carry out an essential function. Mol. Cell. Biol. *13*, 4860–4874.

Granot, D., and Snyder, M. (1991). Glucose induces cAMP-independent growth-related changes in stationary-phase cells of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *88*, 5724–5728.

Hadwiger, J.A., Wittenberg, C., Richardson, H.E., de Barros Lopes, M., and Reed, S.I. (1989). A family of cyclin homologs that control the G1 phase in yeast. Proc. Natl. Acad. Sci. USA *86*, 6255–6259.

Hanic-Joyce, P.J., Singer, R.A., and Johnston, G.C. (1987). Molecular characterization of the yeast prt1 gene in which mutations affect translation initiation and regulation of cell proliferation. J. Biol. Chem. *262*, 2845–2851.

Hartwell, L.H., and Unger, M.W. (1977). Unequal division in *Saccharomyces cerevisiae* and its implications for the control of cell division. J. Cell Biol. *75*, 422–435.

Haystead, T.A.J., Haystead, C.M.M., Hu, C., Lin, T.-A., and Lawrence, J.C., Jr. (1994). Phosphorylation of PHAS-I by mitogen-activated protein (MAP) kinase. J. Biol. Chem. *269*, 23185–23191.

Heitman, J., Koller, A., Cardenas, M.E., and Hall, M.N. (1993). Identification of immunosuppressive drug targets in yeast. In: Methods–A Companion to Methods in Enzymology, vol. 5, New York: Academic Press, 176–187.

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.

Heitman, J., Movva, N.R., and Hall, M.N. (1992). Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. *4*, 448–460.

Helliwell, S.B., Wagner, P., Kunz, J., Deuter-Reinhard, M., Henriquez, R., and Hall, M.N. (1994). TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologs in yeast. Mol. Biol. Cell *5*, 105–118.

Hereford, L.M., and Hartwell, L.H. (1974). Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. J. Mol. Biol. *84*, 445–461.

Hu, C., Pang, S., Kong, X., Velleca, M., and Lawrence, J.C. (1994). Molecular cloning and tissue distribution of PHAS-I, an intracellular target for insulin and growth factors. Proc. Natl. Acad. Sci. USA *91*, 3730–3734.

Hubler, L., Bradshaw-Rouse, J., and Heideman, W. (1993). Connections between the Ras-cyclic AMP pathway and G1 cyclin expression in the budding yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. *13*, 6274–6282.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.

Jefferies, H.B.J., Reinhard, C., Kozma, S.C., and Thomas, G. (1994). Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. Proc. Natl. Acad. Sci. USA *91*, 4441–4445.

Jensen, R., Sprague, G.F., and Herskowitz, I. (1983). Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating type locus. Proc. Natl. Acad. Sci. USA *80*, 3035–3039.

Johnson, D.I., and Pringle, J.R. (1990). Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. J. Cell Biol. *111*, 143–152.

Johnson, S.P., and Warner, J.R. (1987). Phosphorylation of the *Saccharomyces cerevisiae* equivalent of ribosomal protein S6 has no detectable effect on growth. Mol. Cell. Biol. *7*, 1338–1345.

Johnston, G.C., Pringle, J.R., and Hartwell, L.H. (1977). Coordination of growth with cell division in the yeast *Saccharomyces cerevisiae*. Exp. Cell Res. *205*, 79–98.

Joshi-Barve, S., Rychlik, W., and Rhoads, R.E. (1990). Alteration of the major phosphorylation site of eukaryotic protein synthesis initiation factor 4E prevents its association with the 48 S initiation complex. J. Biol. Chem. *265*, 2979–2983.

Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J., and Wigler, M. (1984). Genetic analysis of yeast *RAS1* and *RAS2* genes. Cell *37*, 437–445.

Kruse, C., Johnson, S.P., and Warner, J.R. (1985). Phosphorylation of the yeast equivalent of ribosomal S6 is not essential for growth. Proc. Natl. Acad. Sci. USA *82*, 7515–7519.

Kuehne, C., and Linder, P. (1993). A new pair of B-type cyclins from *Saccharomyces cerevisiae* that function early in the cell cycle. EMBO J. *12*, 3437–3447.

Kunz, J., and Hall, M.N. (1993). Cyclosporin A, FK506 and rapamycin: more than just immunosuppression. Trends Biol. Sci. *18*, 334–338.

Kunz, J., Henriquez, R., Schneider, U., Deuter-Reinhard, M., Movva, N.R., and Hall, M.N. (1993). Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. Cell *73*, 585–596.

Kuo, C., Chung, J., Fiorentino, D.F., Flanagan, J.M., Blenis, J., and Crabtree, G.R. (1992). Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. Nature *358*, 70–72.

Lane, H.A., Fernandez, A., Lamb, N.J.C., and Thomas, G. (1993). p70^{S6K} function is essential for G1 progression. Nature *363*, 170–172.

Lanker, S., Müller, P.P., Altmann, M., Goyer, C., Sonenberg, N., and Trachsel, H. (1992). Interaction of the eIF-4F subunits in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. *267*, 21167–21171.

Lin, T.-A., Kong, X., Haystead, T.A.J., Pause, A., Belsham, G., Sonenberg, N., and Lawrence, J.C., Jr. (1994). PHAS-I as a link between mitogen-activated protein kinase and translation initiation. Science *266*, 653–656.

Linder, P. (1992). Molecular biology of translation in yeast. In: Molecular Biology of *Saccharomyces*, ed. L.A. Grivell, Dordrecht, The Netherlands: Kluwer Academic Publishers, 47–62.

Liu, J. (1993). FK506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunol. Today *14*, 290–295.

Ming, X.-F., Burgering, B.M.Th., Wenström, S., Claesson-Welsh, L., Heldin, C.-H., Bos, J.L., and Thomas, G. (1994). Activation of p70/p85 S6 kinase by a pathway independent of p21^{ras}. Nature *371*, 426–429.

Morley, S.J., Dever, T.E., Etchison, D., and Traugh, J.A. (1991). Phosphorylation of eIF-4F by protein kinase C or multipotential S6 kinase stimulates protein synthesis at initiation. J. Biol. Chem. *266*, 4669–4672.

Nash, R., Tokiwa, G., Anand, S., Erikson, K., and Futcher, A.B. (1988). The *WHI1*$^+$ gene of *Saccharomyces cerevisiae* tethers cell division to cell size and is a cyclin homolog. EMBO J. *7*, 4335–4346.

Nasmyth, K. (1993). Control of the yeast cell cycle by the Cdc28 protein kinase. Curr. Opin. Cell Biol. *5*, 168–179.

Nasmyth, K., and Dirick, L. (1991). The role of *SWI4* and *SWI6* in the activity of G1 cyclins in yeast. Cell *66*, 995–1013.

Ogas, J., Andrews, B.J., and Herskowitz, I. (1991). Transcriptional activation of *CLN1*, *CLN2* and a putative new cyclin *HCS26* by *SWI4*, a positive regulator of G1-specific transcription. Cell *66*, 1015–1026.

Pause, A., Belsham, G.J., Gingras, A.-C., Donze, O., Lin, T.-A., Lawrence, J.C., and Sonenberg, N. (1994). Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. Nature *371*, 762–767.

Price, D.J., Grove, J.R., Calvo, V., Avruch, J., and Bierer, B.E. (1992). Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science *257*, 973–977.

Pringle, J.R., and Hartwell, L.H. (1981). The *Saccharomyces cerevisiae* cell cycle. In: Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance, ed. J. Broach, J. Strathern, and E. Jones, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 97–142.

Redpath, N.T., and Proud, C.G. (1994). Molecular mechanisms in the control of translation by hormones and growth factors. Biochim. Biophys. Acta *1220*, 147–162.

Reed, S.I. (1992). The role of p34 kinases in the G1 to S transition. Annu. Rev. Cell Biol. *8*, 529–562.

Rhoads, R.E. (1988). Cap recognition and the entry of mRNA into the protein synthesis initiation cycle. Trends Biol. Sci. *13*, 52–56.

Rhoads, R.E., Joshi-Barve, S., and Rinker-Schaeffer, C. (1993). Mechanism of action and regulation of protein synthesis initiation factor 4E: effects on mRNA discrimination, cellular growth rate, and oncogenesis. In: Progress in Nucleic Acid Research and Molecular Biology, vol. 46, ed. W.E. Cohn and K. Moldave, San Diego, CA: Academic Press, 183–219.

Richardson, H.E., Wittenberg, C., Cross, F., and Reed, S.I. (1989). An essential G1 function for cyclin-like proteins in yeast. Cell *59*, 1127–1133.

Rothstein, R. (1991). Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. In: Guide to Yeast Genetics and Molecular Biology, ed. J. Guthrie and G.R. Fink, San Diego, CA: Academic Press, 281–301.

Sabatini, D.M., Erdjument-Bromage, H., Lui, M., Tempst, P., and Snyder, S. (1994). RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell *78*, 35–43.

Sabers, C.J., Wiederrecht, G., Williams, J.M., Martin, M.M., Dumont, F.J., and Abraham, R.T. (1995). Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J. Biol. Chem. *270*, 815–822.

Schreiber, S.L., and Crabtree, G.R. (1992). The mechanism of action of cyclosporin A and FK506. Immunol. Today *13*, 36–141.

Schwob, E., and Nasmyth, K. (1993). *CLB5* and *CLB6*, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. Genes Dev. *7*, 1160–1175.

Sherman, F. (1991). Getting started with yeast. In: Guide to Yeast Genetics and Molecular Biology, ed. J. Guthrie and G.R. Fink, San Diego, CA: Academic Press, 3–20.

Sigal, N.H., and Dumont, F.J. (1992). Cyclosporin A, FK506 and rapamycin-pharmalogical probes of lymphocyte signal transduction. Annu. Rev. Immunol. *10*, 519–560.

Sprague, G.F., Jr. (1991). Assay of yeast mating reaction. In: Guide to Yeast Genetics and Molecular Biology, ed. J. Guthrie and G.R. Fink, San Diego, CA: Academic Press, 77–93.

Stan, R., McLaughlin, M.M., Cafferkey, R., Johnson, R.K., Rosenberg, M., and Livi, G.P. (1994). Interaction between FKBP12-rapamycin and TOR involves a conserved serine residue. J. Biol. Chem. *269*, 32027–32030.

Stansfield, I., Grant, C.M., Akhmaloka, and Tuite, M.F. (1992). Ribosomal association of the yeast *SAL11(SUP45)* gene product: implications for its role in translation fidelity and termination. Mol. Microbiol. *6*, 3469–3478.

Terada, N., Lucas, J.J., Szepesi, A., Franklin, R.A., Takase, K., and Gelfand, E.W. (1992). Rapamycin inhibits the phosphorylation of p70 S6 kinase in IL-2 and mitogen-activated human T cells. Biophys. Biochem. Res. Commun. *186*, 1315–1321.

Terada, N., Patel, H.R., Takase, K., Kohno, K., Nairns, A.C., and Gelfand, E.W. (1994). Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. Proc. Natl. Acad. Sci. USA *91*, 11477–11481.

Thevelein, J.M. (1994). Signal transduction in yeast. Yeast *10*, 1753–1790.

Thomas, G. (1992). The mitogen-activated p70^{S6k}. Biochem. Soc. Trans. *20*, 678–681.

Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J.D., McMullen, B., Hurwitz, M., Krebs, E.G., and Wigler, M. (1987). Cloning and characterization of *BCY1*, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *7*, 1371–1377.

Tyers, M., Tokiwa, G., and Futcher, B. (1993). Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. EMBO J. *12*, 1955–1968.

Tyers, M., Tokiwa, G., Nash, R., and Futcher, B. (1992). The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation. EMBO J. *11*, 1773–1784.

Weiss, A., and Littman, D.R. (1994). Signal transduction by lymphocyte antigen receptors. Cell *76*, 263–274.

Werner-Washburne, M., Becker, J., Kosic-Smithers, J., and Craig, E.A. (1989). Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. J. Bacteriol. *171*, 2680–2688.

Werner-Washburne, M., Braun, E., Johnston, G.C., and Singer, R.A. (1993). Stationary phase in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. *57*, 383–401.

Wittenberg, C., Sugimoto, K., and Reed, S.I. (1990). G1-specific cyclins of *S. cerevisiae*: cell cycle periodicity, regulation by mating pheromone, and association with p34^{cdc28} protein kinase. Cell *62*, 225–237.

Yoshida, S., Ohya, Y., Goebl, M., Nakano, A., and Anraku, Y. (1994). A novel gene, *STT4*, encodes a phosphatidylinositol 4-kinase in the PKC1 pathway of *Saccharomyces cerevisiae*. J. Biol. Chem. *269*, 1166–1171.

Zanchin, N.I.T., Proud, C., and McCarthy, J.E.G. (1994). Phosphorylation of cap-binding proteins *Saccharomyces cerevisiae*. In: Translational Control, ed. A. Jacobson, M. Mathews, and D. Steege, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 247.

Zheng, X.-F., Fiorentino, D., Chen, J., Crabtree, G.R., and Schreiber, S.L. (1995). TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. Cell *82*, 121–130.

Zinker, S., and Warner, J.R. (1976). The ribosomal proteins of *Saccharomyces cerevisiae*. J. Biol. Chem. *251*, 1799–1806.

Target of Rapamycin (TOR) in Nutrient Signaling and Growth Control

Robbie Loewith*,1 and Michael N. Hall†,1

*Department of Molecular Biology and National Centers of Competence in Research and Frontiers in Genetics and Chemical Biology, University of Geneva, Geneva, CH-1211, Switzerland, and †Biozentrum, University of Basel, Basel CH-4056, Switzerland

ABSTRACT TOR (**T**arget **O**f **R**apamycin) is a highly conserved protein kinase that is important in both fundamental and clinical biology. In fundamental biology, TOR is a nutrient-sensitive, central controller of cell growth and aging. In clinical biology, TOR is implicated in many diseases and is the target of the drug rapamycin used in three different therapeutic areas. The yeast *Saccharomyces cerevisiae* has played a prominent role in both the discovery of TOR and the elucidation of its function. Here we review the TOR signaling network in *S. cerevisiae*.

CONTENTS, *continued*

THE contributors to this GENETICS set of reviews were asked to focus on the developments in their field since 1991, the year the last yeast monographs were published. Coincidentally, **T**arget **O**f **R**apamycin (TOR) was discovered in 1991. We thus have the whole TOR story to tell, from the beginning, in a review that marks the 20th anniversary of TOR. As we review TOR signaling in *Saccharomyces cerevisiae*, the reader is referred to other reviews for descriptions of TOR in other organisms (Wullschleger *et al.* 2006; Polak and Hall 2009; Soulard *et al.* 2009; Caron *et al.* 2010; Kim and Guan 2011; Zoncu *et al.* 2011).

The story of the TOR-signaling network begins with a remarkable drug, rapamycin (Abraham and Wiederrecht 1996; Benjamin *et al.* 2011). Rapamycin is a lipophilic macrolide and a natural secondary metabolite produced by *Streptomyces hygroscopicus*, a bacterium isolated from a soil sample collected in Rapa-Nui (Easter Island) in 1965—hence the name rapamycin. Rapamycin was originally purified in the early 1970s as an antifungal agent. Although it effectively inhibits fungi, it was discarded as an antifungal agent because of its then undesirable immunosuppressive side effects. Years later, it was "rediscovered" as a T-cell inhibitor and as an immunosuppressant for the treatment of allograft rejection. Preclinical studies subsequently showed that rapamycin and its derivatives, CCI-779 (Wyeth-Ayerst) and RAD001 (Novartis), also strongly inhibit the proliferation of tumor cells. Rapamycin received clinical approval in 1999 for use in the prevention of organ rejection in kidney transplant patients, and additional applications as an immunosuppressive agent have since been developed. CCI-779 (Torisel) and RAD001 (Afinitor) were approved in 2007 and 2009, respectively, for treatment of advanced kidney cancer. Rapamycin is effective against tumors because it blocks the growth of tumor cells directly and because of the indirect effect of preventing the growth of new blood vessels (angiogenesis) that supply oxygen and nutrients to the tumor cells (Guba *et al.* 2002). Finally, rapamycin-eluting stents prevent restenosis after angioplasty. Thus, rapamycin has clinical applications in three major therapeutic areas: organ transplantation, cancer, and coronary artery disease. What do fungi and the seemingly very different conditions of transplant rejection, cancer, and restenosis have in common in their underlying biology such that all can be treated with the same drug? All three conditions (and the spread of pathogenic fungi) are due to ectopic or otherwise undesirable cell growth, suggesting that the molecular target of rapamycin is a central controller of cell growth. TOR is indeed dedicated to controlling cell growth, but what is this target and how does it control cell growth?

The Early Days

Studies to identify the cellular target of rapamycin and to elucidate the drug's mode of action were initiated in the late 1980s by several groups working with yeast (Heitman *et al.* 1991a; Cafferkey *et al.* 1993; Kunz *et al.* 1993) and mammalian cells (Brown *et al.* 1994; Chiu *et al.* 1994; Sabatini *et al.* 1994; Sabers *et al.* 1995). At that time, rapamycin was known to inhibit the vertebrate immune system by blocking a signaling pathway in helper T cells that mediates cell cycle (G1) progression in response to the lymphokine IL-2. However, the molecular mode of action of the drug was not known other than it possibly involved binding and inhibiting the cytosolic peptidyl-prolyl *cis-trans* isomerase FKBP12 (FK506-binding protein 12), also known as an immunophilin (Schreiber 1991). Furthermore, the observation that rapamycin inhibited cell cycle progression in yeast as in mammalian cells suggested that the molecular target was conserved from yeast to vertebrates and that yeast cells could thus be exploited to identify the target of rapamycin (Heitman *et al.* 1991a). It should be noted that the early

researchers were interested not only in understanding rapamycin's mechanism of action, but also in using rapamycin as a probe to identify novel proliferation-controlling signaling pathways (Kunz and Hall 1993). In the late 1980s, significantly less was known about signaling pathways than today; indeed, few and only incomplete pathways were known.

The early studies in yeast first focused on identifying an FKBP (FK506-binding protein) (Heitman *et al.* 1991b; Koltin *et al.* 1991; Tanida *et al.* 1991; Wiederrecht *et al.* 1991). FKBP12 had previously been identified in mammalian cell extracts as a rapamycin (and FK506)-binding protein. Yeast FKBP was purified to homogeneity using an FK506 column and partially sequenced. The protein sequence information was used to design degenerate oligonucleotides that were then used to isolate the FKBP-encoding gene *FPR1* (Heitman *et al.* 1991b). The predicted amino acid sequence of yeast Fpr1 was 54% identical to that of the concurrently characterized human FKBP12, providing further support that the mode of action of rapamycin was conserved from yeast to humans. Curiously, disruption of the FKBP gene in yeast (*FPR1*) revealed that FKBP is not essential for growth (Heitman *et al.* 1991b; Koltin *et al.* 1991; Tanida *et al.* 1991; Wiederrecht *et al.* 1991). Additional FKBPs and cyclophilins (also an immunophilin and proline isomerase) were subsequently discovered and cloned, and again single and multiple disruptions were constructed without consequential loss of viability (Heitman *et al.* 1991b, 1992; Davis *et al.* 1992; Kunz and Hall 1993; Dolinski *et al.* 1997). The finding that *FPR1* disruption did not affect viability was paradoxical because FKBP was believed to be the *in vivo* binding protein/target for the toxic effect of rapamycin. Why did inhibition of FKBP by rapamycin block growth whereas inhibition of FKBP by disruption of the *FPR1* gene have no effect on growth? The subsequent finding that an *FPR1* disruption confers rapamycin resistance (Heitman *et al.* 1991a,b), combined with the observation that some drug analogs are not immunosuppressive despite being able to bind and inhibit FKBP12 proline isomerase (Schreiber 1991), provided the answer to the above question and led to the well-established model of immunosuppressive drug action: an immunophilin-drug complex (*e.g.*, FKBP-rapamycin) gains a new toxic activity that acts on another target. In other words, FKBP is only a cofactor or receptor required by the drug to act on something else; FKBP itself is not the target required for viability. This mode of drug action also applies to the well-known immunosuppressants cyclosporin A and FK506 (from cyclophilin–cyclosporin A and FKBP–FK506 complexes) and is conserved from yeast to humans (Schreiber 1991). These early studies in yeast were the first of many that have since contributed to an understanding of rapamycin action and TOR signaling even in mammalian cells (Crespo and Hall 2002), illustrating that a model organism such as yeast is valuable in both basic and biomedical research.

To identify the target of the FKBP–rapamycin complex, rapamycin-resistant yeast mutants were selected (Heitman *et al.* 1991a; Cafferkey *et al.* 1993). As expected, *fpr1*

mutants defective in FKBP were recovered, but also obtained were mutants altered in either one of two novel genes termed *TOR1* and *TOR2*. The *fpr1* mutations were common and recessive. Interestingly, the *TOR1* and *TOR2* mutations were rare and dominant. The *TOR1* and *TOR2* genes were cloned, on the basis of the dominant rapamycin-resistance phenotype of the mutant alleles, and sequenced (Cafferkey *et al.* 1993; Kunz *et al.* 1993; Helliwell *et al.* 1994). Both TOR1 and TOR2 proteins are 282 kDa in size (2470 and 2474 amino acids, respectively) and 67% identical. TOR1 and TOR2 are also the founding members of the PI kinase-related protein kinase (PIKK) family of atypical Ser/Thr-specific kinases (other members include TEL1, ATM, DNA-PK, and MEC1) (Keith and Schreiber 1995). Although the catalytic domain of all members of this protein kinase family resembles the catalytic domain of lipid kinases (PI3K and PI4K), no PIKK family member has lipid kinase activity, and the significance of the resemblance to lipid kinases is unknown. Two reports in 1995—before TOR was shown to be a protein kinase—claimed that yeast and mammalian TOR had lipid kinase (PI4K) activity, but these findings were never confirmed and are now thought to have been due to a contaminating PI4K. Disruption of *TOR1* and *TOR2* in combination caused a growth arrest similar to that caused by rapamycin treatment, suggesting that TOR1 and TOR2 are indeed the targets of FKBP–rapamycin and that the FKBP–rapamycin complex inhibits TOR activity (Kunz *et al.* 1993). It was subsequently demonstrated that the FKBP–rapamycin complex binds directly to TOR1 and TOR2 (Stan *et al.* 1994; Lorenz and Heitman 1995; Zheng *et al.* 1995) and that TOR is widely conserved both structurally and as the target of FKBP–rapamycin (Schmelzle and Hall 2000). However, *S. cerevisiae* is unusual in having two *TOR* genes whereas almost all other eukaryotes, including plants, worms, flies, and mammals, have a single *TOR* gene. As described below, this additional complexity in *S. cerevisiae* helped the analysis of TOR signaling because it allowed differentiating two functionally different signaling branches on the basis of different requirements for the two TORs.

It should be noted that there is no evidence to indicate that FKBP has a role in normal TOR signaling, *i.e.*, in the absence of rapamycin. Rapamycin hijacks or corrupts FKBP to interact with TOR. In addition, some have speculated that rapamycin mimics an endogenous metabolite that normally regulates TOR with or without FKBP. Although this would provide an explanation for the evolution of the mechanism of action of rapamycin, no evidence has been reported for an endogenous rapamycin-like compound or for such a mode of TOR regulation.

All TORs have a similar domain structure (Figure 1A). The domains found in TOR—in order from the N to the C terminus of TOR—compose the so-called HEAT repeats, the FAT domain, the FRB domain, the kinase domain, and the FATC domain (Schmelzle *et al.* 2002). The HEAT repeats (originally found in **h**untingtin, **e**longation factor 3, the **A** subunit of PP2A, and **T**OR1) consist of ∼20 HEAT motifs,

each of which is ∼40 residues that form a pair of interacting antiparallel α-helices (Andrade and Bork 1995; Perry and Kleckner 2003). The HEAT repeats occupy the N-terminal half of TOR and are the binding region for subunits of the TOR complexes (Wullschleger *et al.* 2005) (see below). The central FAT domain (∼500 residues) and the extreme C-terminal FATC domain (∼35 residues), flanking the FRB and kinase domains, are always paired and found in all PIKK family members (Alarcon *et al.* 1999; Bosotti *et al.* 2000; Dames *et al.* 2005). The FRB domain (∼100 residues) is the FKBP–rapamycin-binding region. All rapamycin resistance-conferring *TOR* mutations fall within the FRB domain, thereby directly preventing the binding of FKBP–rapamycin without otherwise affecting TOR activity (Heitman *et al.* 1991a; Cafferkey *et al.* 1993; Helliwell *et al.* 1994; Stan *et al.* 1994; Chen *et al.* 1995; Lorenz and Heitman 1995; Choi *et al.* 1996). Interestingly, all the original rapamycin-resistance conferring mutations in *TOR1* and *TOR2* are missense mutations confined to a single, equivalent codon encoding a critical serine residue (Ser1972Arg or Ser1972-Asn in *TOR1* and Ser1975Ile in *TOR2*) (Cafferkey *et al.* 1993; Helliwell *et al.* 1994), which explains why the rapamycin-resistance *TOR* mutations were rare. Recreating an equivalent mutation (Ser2035Ile) in mammalian TOR (mTOR) was instrumental in demonstrating that mTOR is the target of FKBP–rapamycin in mammalian cells (Brown *et al.* 1995). Thus, the early rapamycin-resistant yeast mutants turned out to be very informative. They not only identified TOR, but also identified the FKBP–rapamycin-binding site in TOR and contributed to elucidating the mechanism of action of rapamycin. The kinase domain is the catalytic domain and resembles the kinase domain of PI3K and PI4K lipid kinases. Despite high interest in a structure of the kinase domain, no such structure exists for any TOR, which is likely due to technical difficulties in expressing this domain for structural studies. In the absence of a true model, a homology model based on the crystal structure of related PI3K has been elaborated (Sturgill and Hall 2009). A number of groups have identified activating, missense mutations in *S. cerevisiae* and *Schizosaccharomyces pombe* TORs (Reinke *et al.* 2006; Urano *et al.* 2007; Ohne *et al.* 2008). These mutations fall within the FAT, FRB, and kinase domains, and, interestingly, one of the hotspots in the kinase domain corresponds to a region for oncogenic mutations in PI3K (Sturgill and Hall 2009; Hardt *et al.* 2011).

In the mid-1990s, research in the TOR field focused on elucidating the cellular roles of TOR1 and TOR2. It was found that TOR1 and TOR2 play a central role in controlling cell growth as part of two separate signaling branches. Although structurally similar, TOR1 and TOR2 are not functionally identical (Kunz *et al.* 1993; Helliwell *et al.* 1994). Combined disruption of *TOR1* and *TOR2*, or rapamycin treatment, mimics nutrient deprivation including causing a G0 growth arrest within one generation (Barbet *et al.* 1996). Disruption of *TOR1* alone has little-to-no effect, and disruption of *TOR2* alone causes cells to arrest growth
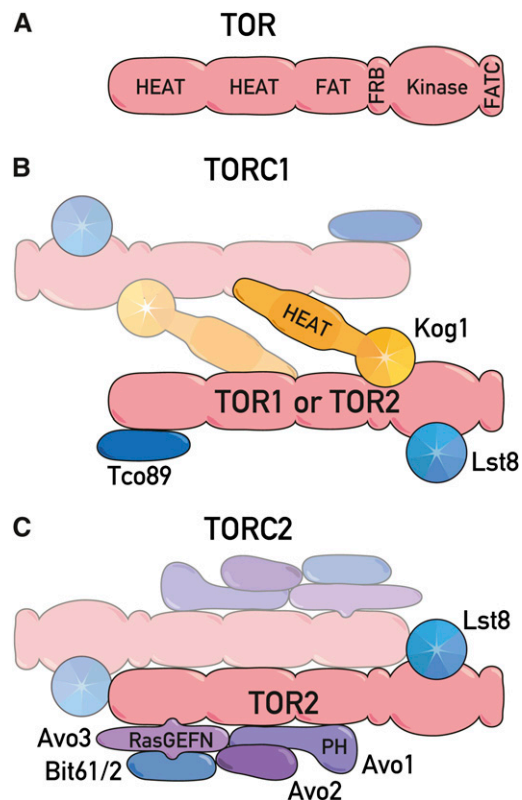

Figure 1 (A) Conserved domain structure of TOR. The N-terminal half of TOR is composed of two blocks of ∼20 HEAT repeats, 40 aa that form pairs of interacting antiparallel α-helices. The ∼500-aa FAT (FRAP-ATM-TRRAP) domain contains modified HEAT repeats. Missense mutations in the ∼100-aa FRB (FKBP12-rapamycin-binding) domain confer complete resistance to rapamycin. The kinase domain phosphorylates Ser/Thr residues in protein substrates, but at the sequence level resembles the catalytic domain of phosphatidylinositol kinases. The ∼35-aa FATC domain is always found C-terminal to the FAT domain and is essential for kinase activity. (B) Composition of TOR complex 1. TORC1 is ∼2 MDa in size and contains Kog1, Tco89, Lst8, and either TOR1 or TOR2. The HEAT repeats found in Kog1 and the seven-bladed propellers of the WD-40 repeats found in Kog1 and Lst8 are depicted. The binding of Kog1 to TOR is complex, involving multiple domains on each protein. Lst8 binds to the kinase domain of TOR. Each component is likely present in two copies. (C) Composition of TOR complex 2. TORC2 is ∼2 MDa in size and contains Avo1, Avo2, Avo3, Bit61, and/or its paralog Bit2, Lst8, and TOR2 but not TOR1. The RasGEFN domain of Avo3 and the PH domain of Avo1 are indicated. Each component is likely present in two copies.

within a few generations as small-budded cells in the G2/M phase of the cell cycle and with a randomized actin cytoskeleton (Kunz *et al.* 1993; Helliwell *et al.* 1994, 1998a; Schmidt *et al.* 1996). These and other findings led to the model that TOR2 has two essential functions: one function is redundant with TOR1 (TOR shared) and the other function is unique to TOR2 (TOR2 unique) (Hall 1996; Helliwell *et al.* 1998a). As described below, these two TOR2 functions turned out to be two separate signaling branches (each corresponding to a structurally and functionally distinct TOR complex) that control cell growth in different ways (Barbet *et al.* 1996; Schmidt *et al.* 1997, 1998; Bickle *et al.* 1998; Helliwell *et al.* 1998a; Loewith *et al.* 2002; Loewith and Hall

2004; De Virgilio and Loewith 2006; Breitkreutz *et al.* 2010; Kaizu *et al.* 2010).

The early characterization of *TOR* disruptions and rapamycin treatment led to two more important conclusions. First, as described in more detail below, the finding that TOR inhibition mimics starvation led to the notion that TOR controls cell growth in response to nutrients (Barbet *et al.* 1996; Rohde *et al.* 2001). Subsequent studies confirmed this notion and demonstrated that TOR in higher eukaryotes also controls cell growth in response to nutrients; *i.e.*, TOR is conserved in structure and function (Thomas and Hall 1997; Hara *et al.* 1998; Schmelzle and Hall 2000). Second, the observation that inhibition specifically of the TOR-shared signaling branch (disruption of both *TOR*s but not of *TOR2* alone) or rapamycin treatment mimics starvation suggested that only the TOR-shared pathway is nutrient responsive and rapamycin sensitive (Zheng *et al.* 1995; Barbet *et al.* 1996; Schmidt *et al.* 1996; Rohde *et al.* 2001). The molecular basis of these findings would remain a mystery until the discovery of the two structurally and functionally distinct TOR complexes (see below).

The realization that TOR controls growth (increase in cell size or mass) was a particularly important development (Barbet *et al.* 1996; Thomas and Hall 1997; Schmelzle *et al.* 2002). Rapamycin or loss of *TOR* function causes a cell cycle arrest, and TOR was thus originally thought to be a controller of cell division (increase in cell number). Furthermore, at that time, growth was largely thought to be controlled passively: *i.e.*, the simple availability of nutrients (building blocks) led to cell growth. As described below, the realization that TOR controls many cellular processes that collectively determine mass accumulation, combined with the fact that there was no direct role for TOR in the cell cycle machinery then being characterized, led to the notions that TOR controls growth and that growth is thus actively controlled. The originally confusing defect in cell cycle progression observed upon TOR inhibition is in fact an indirect effect of growth inhibition: a growth defect is dominant over cell cycle progression.

Since the late 1990s, many groups have been characterizing the two separate TOR2-signaling branches. It was found that the TOR-shared signaling branch is composed of various effector pathways that control a wide variety of readouts that collectively determine the mass of the cell. The readouts controlled by this branch include protein synthesis and degradation, mRNA synthesis and degradation, ribosome biogenesis, nutrient transport, and autophagy (Schmelzle and Hall 2000). This branch is viewed as mediating temporal control of cell growth. The TOR2-unique branch controls the polarized organization of the actin cytoskeleton, endocytosis, and sphingolipid synthesis. This second branch is viewed as mediating spatial control of cell growth, on the basis largely of early work showing that it controls the actin cytoskeleton. Thus, the logic of the two branches appears to be to integrate temporal and spatial control of cell growth (Loewith and Hall 2004). However,

this way of thinking about the two branches has subsided in recent years as the TOR2-unique pathway was shown to control sphingolipid synthesis and endocytosis in addition to the actin cytoskeleton (Powers *et al.* 2010).

Another major breakthrough in the TOR field occurred in 2002: the identification of the two multiprotein complexes termed TOR complex 1 (TORC1) and TORC2 (Loewith *et al.* 2002; Wedaman *et al.* 2003; Reinke *et al.* 2004; Wullschleger *et al.* 2006). The two structurally and functionally distinct TOR complexes were biochemically purified from yeast cells and subsequently shown to correspond to the two genetically defined TOR-signaling branches. TORC1, which contains either TOR1 or TOR2 and is rapamycin sensitive, mediates the TOR-shared pathway. TORC2, which specifically contains TOR2 and is rapamycin insensitive, mediates the TOR2-unique pathway. The TORCs were a major breakthrough because they provided a molecular basis for the functional complexity and selective rapamycin sensitivity of TOR signaling. The biochemical identification of the TORCs and the genetic definition of the two signaling branches also, gratifyingly, cross-validated each other such that there is a high level of confidence in the current "two branch-two complex" model of TOR signaling. The subsequent identification of TORCs in other eukaryotes, including plants, worms, flies, and mammals (Table 1), showed that the two complexes, like TOR itself, are conserved and gave further support to the above model (Hara *et al.* 2002; Kim *et al.* 2002; Loewith *et al.* 2002; Jacinto *et al.* 2004; Sarbassov *et al.* 2004). Below we focus on the structure, function, and regulation of the two TOR complexes. We discuss some downstream readouts of the TORCs that were originally described before the discovery of the TORCs but are now retroactively attributed to TORC1 or TORC2 on the basis of their TOR requirement or rapamycin sensitivity.

TOR Complex 1

Composition of TOR complex 1

TORC1 consists of Kog1, Lst8, Tco89, and either TOR1 or TOR2 (Figure 1B) (Loewith *et al.* 2002; Wedaman *et al.* 2003; Reinke *et al.* 2004). Gel filtration chromatography (R. Loewith, W. Oppliger, and M. Hall, unpublished results) indicated that TORC1 has a size of ~2 MDa, suggesting that the entire complex is likely dimeric. This would be consistent with the dimeric structures proposed for TORC2 (Wullschleger *et al.* 2005) and mTORC1 (Yip *et al.* 2010). The names of mammalian and invertebrate orthologs of TORC1 subunits and the salient features of *S. cerevisiae* TORC1 subunits are summarized in Table 1 and Table 2, respectively. Although all subunits are thought to act positively with TOR1/2 *in vivo*, by and large their functions await characterization. In the presence of rapamycin, all components of TORC1 can be coprecipitated with FKBP12 (Loewith *et al.* 2002), demonstrating that, unlike mammalian TORC1 (Yip *et al.* 2010), the structural integrity

Table 1 TORC1, TORC2, and EGO complex orthologs in various genera

S. cerevisiae	S. pombe	C. albicans	D. discoideum	A. thaliana	C. elegans	D. melanogaster	Mammals
TORC1							
TOR1 or TOR2	Tor1 or Tor2	Tor1	Tor	TOR	TOR/let-363	TOR	mTOR
Kog1/Las24	Mlp1	Kog1	Raptor	RAPTOR1A and RAPTOR1B	daf-15	Raptor	Raptor
Lst8	Wat1/Pop3	Orf19.3862	lst8?	AT2G22040 AT3G18140	lst-87?	CG3004	mLST8
Tco89	Tco89	Tco89	pcr25kl1p3887	—	—	—	—
—	Toc1	—	—	—	—	—	—
—	—	—	—	—	—	—	PRAS40
—	—	—	—	—	—	—	DEPTOR
TORC2							
TOR2	Tor1 or Tor2	Tor1	tor	TOR	TOR/let-363	TOR	mTOR
Avo1	Sin1	orf19.5221	piaA		sinh-1	Sin1	mSIN1
Avo2	—	Avo2			—	—	—
Avo3/Tsc11	Ste20	Tsc11	rip3		rict-1	Rictor	Rictor
Lst8	Wat1/Pop3	Orf19.3862	lst8	AT2G22040 AT3G18140	lst-8	CG3004	mLST8
Bit61	Bit61	—	—	—	—	—	PRR5/Protor
—	—	—	—	—	—	—	DEPTOR
EGO complex							
Gtr1	Gtr1	Gtr1	ragA	—	raga-1	RagA	RagA,B
Gtr2	Gtr2	Gtr2	ragC	—	ragc-1	RagC	RagC,D
Ego1/Meh1/Gse2	—	—	—	—	—	CG14184	LAMTOR1/p18
Ego3/Slm4/Nir1/Gse1	—	—	—	—	lamtor-2, ?	CG5189, CG5110	LAMTOR2/p14, LAMTOR3/ MP1

Orthologs listed are from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Dictyostelium discoideum*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Dictyostelium melanogaster*, and mammals. P-POD: Princeton Protein Orthology Database/BLAST. We note that TORC2 appears to be absent in plants, *e.g.*, *A. thaliana*. —, no demonstrated/obvious ortholog.

Table 2 Salient features of TORC1 components

Protein	Size	Motifs/domains	Potential function
TOR1	2470 aa	HEAT repeats, FAT domain, FRB domain, kinase domain, and FATC domain	Protein kinase, scaffold
TOR2	2474 aa	HEAT repeats, FAT domain, FRB domain, kinase domain, and FATC domain	Protein kinase, scaffold
Kog1	1557 aa	An N-terminal conserved region 4, HEAT repeats, 7 C-terminal WD-40 repeats	Present substrate to TOR
Tco89	799 aa	None obvious	Receive signals from EGO complex
Lst8	303 aa	7 WD-40 repeats	Stabilize kinase domain

of yeast TORC1 is not compromised by this macrolide. Despite recent molecular reconstructions from low resolution (25 Å) electron microscopy of a TOR1–Kog1 subcomplex (Adami *et al.* 2007), the molecular mechanism by which binding of FKBP-rapamycin inhibits TORC1 activity is enigmatic and remains a fascinating question.

Localization of TORC1

Tagging of Kog1, Tco89, Lst8, and TOR1 with GFP demonstrates that TORC1 is concentrated on the limiting membrane of the vacuole (Urban *et al.* 2007; Sturgill *et al.* 2008; Berchtold and Walther 2009; Binda *et al.* 2009). These observations are consistent with previous studies that localized TORC1 via immunogold electron microscopy and cellular fractionation (Chen and Kaiser 2003; Reinke *et al.* 2004). Artificial tethering of a TORC1 peptide substrate to the vacuole demonstrates that vacuole-localized TORC1 is catalytically competent (Urban *et al.* 2007). This localization appears to be constitutive (Binda *et al.* 2009), suggesting that changes in "geography" play no obvious role in regulating yeast TORC1-signaling output. The yeast vacuole is a major nutrient reservoir and TORC1 signaling is responsive to nutrient cues (see below). Thus, vacuolar localization of TORC1 seems logical. Although convincing, these studies do not exclude the possibility that a fraction of TORC1 may also be active elsewhere in the cell. Li *et al.* (2006), for example, have reported that TOR1 dynamically associates with the rDNA locus to regulate 35S rRNA transcription.

Upstream of TORC1

Physiological regulators (carbon, nitrogen, phosphate, stress, caffeine): A major breakthrough in the TOR field came with the observation that rapamycin treatment alters yeast physiology in much the same way as nutrient starvation (Barbet *et al.* 1996). Like starvation, exposure

of yeast cells to rapamycin results in a dramatic drop in protein synthesis, induction of autophagy, and exit from the cell cycle and entrance into a quiescent G0 state. This was the first indication that TOR, actually TORC1, might regulate growth downstream of nutrient cues. This hypothesis was strengthened when TORC1, in response to nitrogen and carbon cues, was found to promote the sequestration of several nutrient-responsive transcription factors in the cytoplasm (Beck and Hall 1999). Consistently, transcriptome profiling demonstrated a highly similar transcriptional response of yeast cells exposed to rapamycin, nutrient starvation, or noxious stressors (Cardenas *et al.* 1999; Hardwick *et al.* 1999; Komeili *et al.* 2000; Shamji *et al.* 2000; Gasch and Werner-Washburne 2002). Although suggestive, these observations provided only correlative evidence that TORC1 activity is regulated in response to environmental cues. Characterization of a *bona fide* substrate of TORC1 allowed this model to be tested directly.

As detailed below, Sch9 presently remains the best-characterized substrate of TORC1, and monitoring its phosphorylation by Western blotting serves as a convenient proxy for TORC1 activity. In addition to exposure to rapamycin, Sch9 is rapidly dephosphorylated in cells experiencing acute starvation of carbon, nitrogen, phosphate, or amino acids (Urban *et al.* 2007; Binda *et al.* 2009). These and other observations confirm that TORC1 is responsive to both the abundance and the quality of nutrients in the environment; but, with few exceptions (see *The EGO complex*), how nutrient cues are sensed and how this information is transduced to TORC1 remain unknown.

TORC1 activity is also regulated in response to noxious stressors. When cells are subjected to various stress conditions, including high salt, redox stress, a shift to a higher temperature, or caffeine, Sch9 phosphorylation is reduced dramatically (Kuranda *et al.* 2006; Urban *et al.* 2007). With the exception of caffeine, which directly inhibits TORC1

Table 3 Salient features of EGO Complex components

Protein	Size	Motifs/domains	Potential function
Gtr1	310 aa	Ras-family GTPase	GTP-bound form activates TORC1
Gtr2	341 aa	Ras-family GTPase	GDP-bound form activates TORC1
Ego1/Meh1/ Gse2	184 aa	N-terminal palmitoylation/myristolation[a]	Vacuolar recruitment
Ego3/Slm4/Nir1/Gse1	162 aa	Transmembrane domain, PtdIns(3,5)P2 binding[a]	Vacuolar recruitment

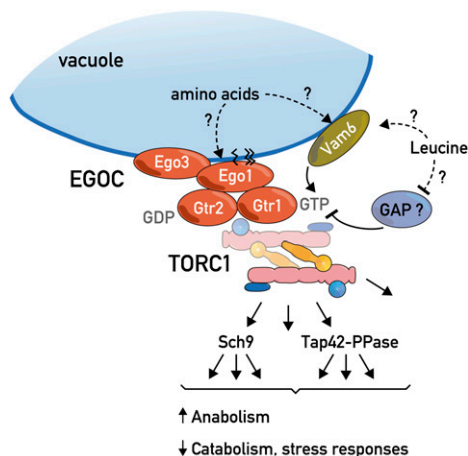[a] Described in Dubouloz *et al.* (2005), Hou *et al.* (2005), and references therein.

Figure 2 The EGO complex is a major regulator of TORC1. The EGO complex (EGOC) is composed of four proteins: the palmitoylated and myristolated protein Ego1, the transmembrane protein Ego3, and two Ras-family GTPases, Gtr1 and Gtr2. Like TORC1, the EGO complex is localized to the vacuolar membrane where it appears to sense/respond to intracellular leucine levels and potentially to intravacuolar amino acid levels. Vam6 has been identified as a guanine nucleotide exchange factor for Gtr1 but no other GEFs or GAPS for this GTPase system have been reported. In the Gtr1^{GTP} and Gtr2^{GDP} configuration, the EGO complex somehow activates TORC1; the reverse conformation inactivates TORC1. Activated TORC1, via its two main effector branches, the AGC kinase Sch9 and the Tap42-PP2a and PP2a-like protein phosphatases, stimulates growth by favoring anabolic processes and by antagonizing catabolic processes and stress-response programs.

kinase activity (Kuranda *et al.* 2006; Reinke *et al.* 2006; Wanke *et al.* 2008), how environmental stress signals are transduced to TORC1 is also unclear.

The EGO complex: When environmental conditions are inappropriate for growth, cells stop dividing, slow their metabolism, induce the expression of stress-responsive proteins, and accumulate energy stores. This nondividing but metabolically active state is known as quiescence (G0). How cells enter into quiescence is relatively well characterized. In contrast—and despite its medical relevance (inappropriate exit from quiescence can lead to cancer or reactivation of a latent infection)—how quiescent cells reinitiate growth is poorly understood. To shed light on this process, a clever screen was performed to identify mutants that are unable to **e**scape from rapamycin-induced **gro**wth arrest (*EGO*) mutants (Dubouloz *et al.* 2005). This and a follow-up study (Binda *et al.* 2009) identified the EGO complex as an important regulator of TORC1.

The EGO complex is composed of four proteins: Ego1, Ego3, Gtr1, and Gtr2 (Table 3 and Figure 2). Gtr1 and Gtr2 are Ras-family GTPases and orthologs of the metazoan Rag GTPases (Kim *et al.* 2008; Sancak *et al.* 2008) (Table 1). Although they lack obvious sequence homologies, Ego1 and Ego3 are likely the functional homologs of vertebrate p18 (LAMTOR1) and p14 + MP1 (LAMTOR2 + LAMTOR3), respectively, which function together as the "Ragulator" complex (Kogan *et al.* 2010; Sancak *et al.* 2010). Ragulator

and the Rags mediate amino acid sufficiency signals to mTORC1 (reviewed in Kim and Guan 2011). Like its mammalian counterpart, the EGO complex resides on the vacuolar/lysosomal membrane and is thought to couple amino acid signals to TORC1 (Binda *et al.* 2009). Curiously, the Gtr1^{GTP} Gtr2^{GDP} combination activates TORC1 with the nucleotide-binding status of Gtr1 seemingly dominant over the nucleotide-binding status of Gtr2.

TORC1 activity in both metazoans and yeast appears to be particularly responsive to glutamine (Crespo *et al.* 2002) and the branched-chain amino acid leucine (Binda *et al.* 2009; Cohen and Hall 2009). In yeast, leucine starvation destabilizes Gtr1-TORC1 association and causes a reduction in Sch9 phosphorylation whereas GTP-locked Gtr1^{Q65L} remains bound to TORC1 and Sch9 dephosphorylation is delayed in cells expressing this mutant (Binda *et al.* 2009). Loss of Gtr1 results in reduced Sch9 phosphorylation and slow growth whereas GDP-locked Gtr1^{S20L} is dominant negative. When Gtr1^{S20L} is expressed as the sole version of Gtr1, cells are extremely sick. This near inviability is suppressed by deletion of the *TCO89* gene encoding the TORC1 subunit Tco89. Collectively, these observations suggest that the EGO complex can both positively and negatively regulate TORC1 activity via Tco89. The fact that the EGO complex can negatively regulate TORC1 activity seems to be at odds with the current metazoan model according to which the EGO complex counterpart serves only to localize TORC1 to the vacuole. Indeed, in contrast to the results obtained in metazoans, in yeast, TORC1 appears to stably localize to the vacuolar membrane regardless of nutrient conditions. Thus, how the EGO complex influences TORC1 activity remains a mystery although the crystal structure of the Gtr1–Gtr2 complex, reported very recently, provides some mechanistic insights (Gong *et al.* 2011).

Also mysterious are the mechanisms by which amino acid sufficiency modulates Gtr1/2 guanine nucleotide loading. Given its localization, it is tempting to postulate that the EGO complex responds to levels of intravacuolar amino acids, possibly via the recently described Gtr1 guanine–nucleotide exchange factor (GEF) Vam6/Vps39 (Binda *et al.* 2009). It is equally plausible, however, that this signal is mediated by an as-yet-unidentified GTPase-activating protein (GAP) activity. Consistent with the conserved function of the EGO/Ragulator complex, and like its yeast ortholog, hVPS39 has been found to function positively upstream of mTORC1 (Flinn *et al.* 2010).

Feedback loop/ribosome biogenesis homeostasis: Although most recognized as a target of signals emanating from extracellular nutrients and noxious stresses, it is becoming increasingly apparent that TORC1 also responds to intracellular cues. In addition to the sensing of intracellular amino acids as described above, outputs from distal effectors also regulate TORC1 in apparent feedback loops. For example, in both yeast and mammalian cells, it is well documented that TORC1 activity stimulates translation initiation

(Wullschleger *et al.* 2006). Interestingly, inhibition of translation with cycloheximide causes a pronounced increase in (m)TORC1 activity presumably by triggering an increase in the concentration of free amino acids in the cytoplasm (Beugnet *et al.* 2003; Urban *et al.* 2007; Binda *et al.* 2009). Ribosome biogenesis (described in more detail below) is a second example. TORC1 regulates ribosome biogenesis in part via two substrates, Sch9 and the transcription factor Sfp1. Reduced ribosome biogenesis resulting from deletion of SCH9 or SFP1 results in a dramatic increase in TORC1 activity (Lempiainen *et al.* 2009). It is possible that blocking ribosome biogenesis, like translation inhibition, causes an increase in free amino acids that subsequently activates TORC1. Alternatively, other mechanisms could be at play. Regardless of mechanism, such feedback loops provide an elegant means by which growth homeostasis can be maintained by TORC1.

Downstream of TORC1

In general terms, when growth conditions permit, TORC1 is active and its signals promote the accumulation of cellular mass. However, as both proximal and distal TORC1 effectors continue to be described, the extent of this temporal regulation of growth control is only starting to be appreciated.

Proximal TORC1 effectors: Characterization of Sch9 as a TORC1 substrate: Arguably, the best-characterized substrates of both yeast and metazoan TOR complexes are the AGC family kinases. This rather well-studied family of kinases is so named on the basis of its mammalian members PKA, PKG, and PKC (Pearce *et al.* 2010). Typically, activation of AGC family kinases requires phosphorylation of two conserved regulatory motifs, the "T," or "activation," loop located in the catalytic domain and the "hydrophobic" motif found toward the C terminus. Phosphorylation of these motifs helps stabilize the kinase domain in an active conformation. Several AGC family kinases additionally contain a "turn" motif located between the kinase domain and the hydrophobic motif, phosphorylation of which is thought to promote protein stability. While the T loop is phosphorylated by PDK1 or its ortholog Pkh in mammalian or yeast cells, respectively, phosphorylation of the hydrophobic and possibly the turn motifs is often mediated by TORC1 or TORC2.

Analogous to S6K for mTORC1, the AGC kinase Sch9 was recently found to be a direct substrate for yeast TORC1 (Powers 2007). Six target sites in the C terminus of Sch9 are phosphorylated by TORC1: Thr737 found in a classical hydrophobic motif; Thr723 and Ser726, Ser/Thr-Pro sites found in what appears to be a turn motif; Ser758 and Ser765 found in sequences that resemble the hydrophobic motif; and Ser711 in a region that partially resembles a hydrophobic motif. TORC1-mediated phosphorylation is necessary for Sch9 activity. Replacing the target amino acids with alanine yields a nonfunctional Sch9, whereas replacing them with a phosphomimetic residue confers constitutive

kinase activity, *i.e.*, activity even in the absence of TORC1 (Urban *et al.* 2007). Presumably, phosphorylation of the turn motif helps to stabilize Sch9 while phosphorylation of the hydrophobic motif stabilizes Sch9 in an active conformation. Curiously, although their *in vivo* functions are unknown, *in vitro* TORC1 preferentially phosphorylates Ser758 and Ser765 within the hydrophobic-like motifs (R. Loewith, unpublished results). That TORC1 can phosphorylate amino acids found within such diverse sequence contexts, which is rather atypical for protein kinases, is also curious.

Characterization of Tap42-PP2A as a TORC1 effector: In addition to Sch9, TORC1 also regulates type 2A (Pph21, Pph22, and Pph3—generically PP2Ac) and 2A-related phosphatases (Sit4, Ppg1). These partially redundant yet pleiotropic enzymes are notoriously difficult to study. Analysis of Sit4 function, and therefore of TORC1 function, is further complicated by strain-dependent polymorphisms at the SSD1 (**S**uppressor of *SIT4* **D**eletion) locus (Reinke *et al.* 2004).

A role for these phosphatases downstream of TORC1 was first described by the Arndt lab (Di Como and Arndt 1996). In this work, a subpopulation of these enzymes was found to interact in a TORC1-dependent manner with a regulatory protein known as Tap42. Rrd1 and Rrd2, two peptidyl-prolyl *cis/trans* isomerases, were subsequently also found to be present in these Tap42 complexes (Zheng and Jiang 2005; Jordens *et al.* 2006). Work, done in large part by the Jiang group, posits that when TORC1 is active, Tap42 is phosphorylated and bound tightly to the phosphatase–Rrd complex (Di Como and Arndt 1996; Jiang and Broach 1999; Zheng and Jiang 2005). Inactivation of TORC1 results in Tap42 dephosphorylation and a weakened association with phosphatases that presumably results in their activation and/or change in substrate preference (Duvel *et al.* 2003; Yan *et al.* 2006). How TORC1 maintains Tap42 phosphorylation is mechanistically unclear. It may phosphorylate Tap42 directly (Jiang and Broach 1999), or it may act via the **T**ap42 **i**nteracting **p**hosphoprotein Tip41 (Jacinto *et al.* 2001). Interestingly, Tip41 has been proposed to both antagonize and cooperate with Tap42 in controlling TORC1 signaling (Jacinto *et al.* 2001; Kuepfer *et al.* 2007).

Although the mechanisms coupling TORC1 to Tap42–PPase complexes remain to be elucidated, genetic arguments clearly position Tap42 as a prominent effector of TORC1. Specifically, several alleles of TAP42 (*e.g.*, TAP42-11) that confer strong resistance to rapamycin by blocking a subset of rapamycin-induced readouts have been identified (Di Como and Arndt 1996; Duvel *et al.* 2003).

Curiously, *TAP42-11* does not provide rapamycin resistance in all strain backgrounds. However, co-expression of genetically activated Sch9 (described above) in rapamycin-sensitive *TAP42-11* backgrounds results in a very strong synthetic resistance to rapamycin (Urban *et al.* 2007). From this observation, it appears that Sch9 and Tap42-PPase complexes are major effector branches downstream of TORC1 with each branch, at least in some backgrounds, performing

one or more essential function. The readouts mediated by these two TORC1 branches are discussed below.

Other TORC1 substrates: In addition to the regulation of these two major effector branches, TORC1 has been reported to directly phosphorylate other substrates including Sfp1(Lempiainen *et al.* 2009), Gln3 (Bertram *et al.* 2000), and Atg13 (Kamada *et al.* 2010). The roles that these proteins play downstream of TORC1 are discussed below.

Tyers and colleagues have recently defined a global protein kinase and phosphatase interaction network in yeast (Breitkreutz *et al.* 2010). This study, consisting of affinity purification followed by mass spectrometry, included TOR1 and TOR2. They found and confirmed that TORC1 physically interacts with the following proteins: Mks1, a protein involved in retrograde (RTG) mitochondria-to-nucleus signaling (see below); curiously, FMP48, an uncharacterized protein presumed to localize to the mitochondria (Reinders *et al.* 2006); Npr1, a protein kinase involved in the intracellular sorting of nutrient permeases (see below); Ksp1, a protein kinase involved in nutrient-regulated haploid filamentous growth (Bharucha *et al.* 2008); Nap1, a chromatin assembly factor and a mitotic factor involved in regulation of bud formation (Calvert *et al.* 2008); Nnk1, the **n**itrogen **n**etwork **k**inase presumably involved in intermediate nitrogen metabolism (Breitkreutz *et al.* 2010); Sky1, an Ser/Arg domain kinase involved in pre-mRNA splicing (Shen and Green 2006); and Bck1 and Kdx1, which are involved in MAPK signaling (Breitkreutz *et al.* 2010). Given their physical interaction with TORC1, all of these proteins, in addition to multiple other, as-yet-unconfirmed interactors, are potential substrates (or regulators) of TORC1. These results underscore the central role that TORC1 plays in cell growth.

Distal readouts downstream of TORC1: *TORC1 promotes cell growth:* When environmental conditions are favorable, TORC1 coordinates the production and accumulation of cellular mass, *i.e.*, growth, via regulation of several processes.

Protein synthesis: The first realization that TORC1 serves to couple environmental cues to the cell growth machinery came with the observation that rapamycin treatment elicits a marked drop in protein synthesis by blocking translation initiation (Barbet *et al.* 1996). A major target for this regulation appears to be the translation initiation factor eIF2. Upon amino acid starvation or rapamycin treatment, the α-subunit of eIF2 is phosphorylated and this dominantly interferes with 5′CAP-dependent mRNA translation (reviewed in Hinnebusch 2005). TORC1 signals to eIF2α via both the Sch9 and Tap42-PPase branches. The sole eIF2α kinase is the conserved Gcn2 protein. Gcn2 binds and is activated by uncharged tRNAs that accumulate when cells are starved for an amino acid (detailed below). Gcn2 activity is also regulated by phosphorylation. Gcn2 phosphorylation on Ser577 reduces tRNA binding and, consequently, kinase activity. Treating cells with rapamycin elicits a rapid, Tap42-PPase-dependent dephosphory-

lation of Ser577 and, consequently, an increase in Gcn2 activity and a reduction in 5′CAP-dependent translation (Cherkasova and Hinnebusch 2003). It is possible that one or more Tap42-associated phosphatases directly dephosphorylates Ser577, but this has not been formally demonstrated. The nature of the kinase that phosphorylates Gcn2 Ser577 is unknown other than it is not Sch9 (M. Stahl and R. Loewith, unpublished results). Sch9 inhibition, however, also leads to eIF2α phosphorylation via an undefined pathway (Urban *et al.* 2007).

Studies with rapamycin suggest that, in addition to eIF2α, TORC1 may target additional translation factors such as the 5′CAP-binding protein (eIF4E) interacting proteins Eap1 and/or the eIF4G scaffold (Berset *et al.* 1998; Cosentino *et al.* 2000). Finally, recent phosphoproteomics studies (Huber *et al.* 2009; Loewith 2010; Soulard *et al.* 2010) have identified several translation-related proteins whose phosphorylation is altered by rapamycin treatment, suggesting that these factors could also couple TORC1 to protein synthesis.

Ribosome biogenesis: In optimal conditions, yeast cells grow and divide approximately every 100 min. Such rapid growth requires robust protein synthesis, which of course requires ribosomes. Indeed, rapidly growing yeast cells contain ~200,000 ribosomes, implying that each cell must produce and assemble ~2000 ribosomes per minute (Warner 1999). This is not a trivial feat as each ribosome contains 78 unique proteins (encoded by 137 *RP* genes) in addition to four rRNA molecules, three derived from the RNA Pol I-transcribed 35S pre-rRNA and one transcribed by RNA Pol III. Fifty percent of RNA Pol II transcription is devoted to ribosomal proteins. In addition, numerous protein and small RNA *trans*-acting factors, known as **ri**bosome **bi**ogenesis (RiBi) factors, are required for the correct processing, folding, assembly, nuclear export of pre-ribosomal particles to the cytoplasm, and final maturation events into 40S and 60S particles. The production of all these abundant molecules represents a huge energetic investment. Not surprisingly, yeast cells have developed elaborate measures to coordinate the expression of rRNA, tRNA, RPs, and RiBi factors in response to environmental conditions. Much of this regulation is mediated by TORC1 at the level of transcription. As ribosome biogenesis has clear links to diseases such as cancer, anemia, and aging, dissection of its regulation will undoubtedly have clinical ramifications (Lempiainen and Shore 2009).

In *S. cerevisiae*, the rDNA locus consists of ~150 tandemly repeated transcription units on chromosome XII, and yet rRNA production is still limiting for cell growth (Warner 1999). Each of these rDNA units comprises the RNA polymerase III transcribed 5S rRNA gene, the intergenic spacer region, and the RNA Pol I-transcribed 35S rRNA gene, encoding the 35S precursor of the mature 18S, 5.8S, and 25S rRNAs. RNA Pol III also transcribes tRNA genes as well as several additional genes encoding small noncoding RNAs. In the late 1990s, it was reported that rapamycin results in
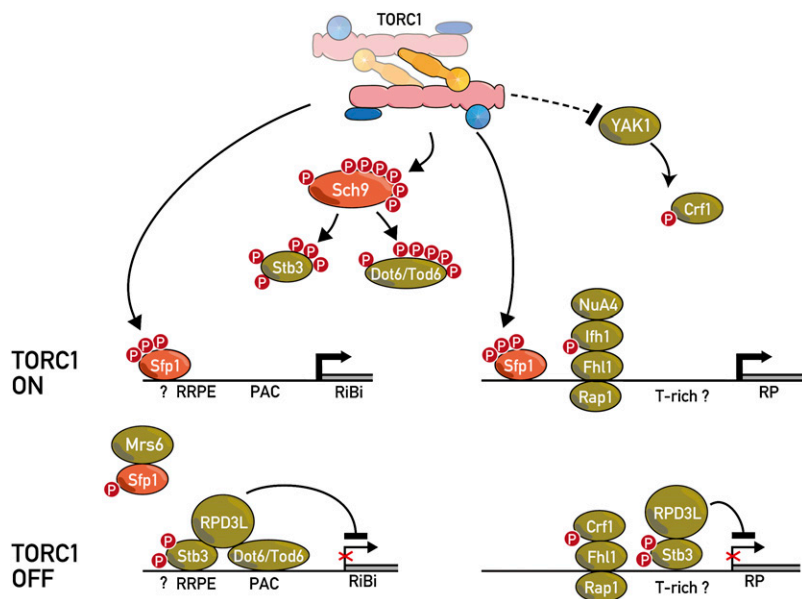
Figure 3 Control of RiBi and RP gene transcription by TORC1. RiBi factors are required for the proper expression, processing, assembly, export, and maturation of rRNA and RPs into ribosomes. This energetically costly procedure is under tight regulation, particularly at the transcription level. TORC1 regulates RiBi and RP gene transcription via multiple pathways: (1) TORC1 directly phosphorylates the Split Zn-finger transcription factor Sfp1, which presumably regulates its nuclear localization and/or binding to RP and possibly RiBi gene promoters to stimulate their expression. (2) Fhl1 and Rap1 bind constitutively to *RP* promoters. When TORC1 is active, phosphorylated Ifh1 binds to Fhl1 to stimulate transcription, possibly by recruiting the NuA4 histone acetyltransferase. When TORC1 is inactive, Yak1 phosphorylates Crf1, which subsequently outcompetes Ifh1 for binding to Fhl1. (3) Sch9 phosphorylates and thus inhibits Stb3 and the paralogs Dot6 and Tod6. Inhibition of TORC1/Sch9 results in the dephosphorylation of these three transcription repressors, which subsequently bind to *RRPE* and *PAC* elements found in RiBi promoters. Stb3 additionally binds RP promoters. Bound to promoters, these repressors recruit the RPD3L histone deacetylase complex to repress transcription.

a rapid and pronounced drop in 5S, 35S, and tRNA production (Zaragoza *et al.* 1998; Powers and Walter 1999). Recently, the relevant signaling pathways in this regulation have become clearer.

TORC1 regulates the accumulation of RNA Pol I transcripts at multiple levels. Processing of the 35S pre-rRNA occurs cotranscriptionally and is dependent on the presence of ribosomal proteins (Tschochner and Hurt 2003). The fast drop in RNA Pol I-dependent transcripts observed upon rapamycin treatment is apparently due to decreased translation (described above) of ribosomal proteins (Reiter *et al.* 2011). The majority of mRNAs being translated in a rapidly growing cell encode ribosomal proteins (Warner 1999), and thus a drop in translation will rapidly reduce the levels of free ribosomal proteins that are themselves needed stoichiometrically for processing of rRNA into pre-ribosome particles. rRNA that is not efficiently processed is immediately degraded, presumably to prevent imbalances in structural components of the ribosome. At later time points following rapamycin treatment, RNA Pol I no longer associates with the rDNA and transcription stops. This late effect could be the result of rapamycin-induced degradation of the essential RNA Pol I transcription factor Rrn3 (Claypool *et al.* 2004; Laferte *et al.* 2006; Reiter *et al.* 2011).

TORC1 regulates RNA Pol III apparently exclusively via Sch9 and a repressor protein named Maf1 (Upadhya *et al.* 2002; Oficjalska-Pham *et al.* 2006; Reina *et al.* 2006; Huber *et al.* 2009; Lee *et al.* 2009). Sch9 directly phosphorylates seven sites in Maf1 that prevent it from interacting with and thus inhibiting RNA Pol III (Vannini *et al.* 2010). Phosphomimetic variants of Maf1 clearly fail to associate with RNA Pol III, but, curiously, Sch9 inhibition still causes a reduction in RNA Pol III activity in these strains but not in *maf1Δ* strains. This and other observations suggest that an additional Sch9 target exists that, when dephosphorylated,

represses RNA Pol III in a Maf1-dependent fashion (Huber *et al.* 2009; Michels 2011). Maf1 is conserved and also functions downstream of mTORC1 to regulate RNA Pol III activity. However, in mammalian cells, and perhaps in yeast cells too, Maf1 is directly phosphorylated by mTORC1 rather than by the Sch9 ortholog S6K1 (Wei *et al.* 2009; Wei and Zheng 2010; Michels 2011).

A total of 137 genes encode the 78 proteins that make up a yeast ribosome (most RPs are encoded by two genes yielding nearly identical proteins). TORC1 coordinately regulates the expression of these genes through several mechanisms (Figure 3) (Lempiainen and Shore 2009). A central component of this regulation is the Fhl1 protein (Lee *et al.* 2002; Martin *et al.* 2004; Schawalder *et al.* 2004; Wade *et al.* 2004; Rudra *et al.* 2005). Fhl1 has a forkhead DNA-binding domain, and its constitutive association to ribosomal protein gene (*RP*) promoters is facilitated by the DNA-binding protein Rap1 and the high mobility group protein Hmo1 (Hall *et al.* 2006; Berger *et al.* 2007). TORC1 regulates *RP* transcription by determining the association between Fhl1 and either one of two FHB-containing proteins, Ifh1 and Crf1. Both Ifh1 and Crf1 are phosphoproteins. When cells are growing and TORC1 is active, Ifh1 is phosphorylated and binds to Fhl1 to stimulate *RP* transcription. Conversely, inhibition of TORC1 results in the phosphorylation of Crf1, which displaces Ifh1 to repress *RP* transcription. The signaling events upstream of Ifh1 are not known, whereas TORC1 seems to signal to Crf1 via the Ras/PKA pathway target Yak1 (Martin *et al.* 2004). However, it should be noted that the crosstalk between TORC1 signals and Ras/PKA signals has been debated. While it is clear that hyperactivation of Ras/PKA can suppress many rapamycin-induced phenotypes (Schmelzle *et al.* 2004), suggesting that PKA is downstream of TORC1, it has also been proposed that TORC1 and PKA signal in parallel

pathways that impinge on common targets (Zurita-Martinez and Cardenas 2005; Ramachandran and Herman 2011). Recently, Soulard et al. (2010) have provided some clarification of this dilemma by proposing that TORC1 functions upstream of PKA but only for a subset of PKA targets. Thus, TORC1 may be both upstream and parallel to PKA.

TORC1-dependent regulation of RP gene transcription still occurs in the absence of the Fhl1/Ifh1/Crf1 system, suggesting the existence of additional regulatory mechanisms. One of these is the split zinc (Zn)-finger protein Sfp1 (Fingerman et al. 2003; Jorgensen et al. 2004; Marion et al. 2004; Lempiainen et al. 2009; Singh and Tyers 2009). TORC1 binds and directly phosphorylates Sfp1 to promote its binding to a subset of RP gene promoters. Curiously, unlike Sch9, TORC1-mediated Sfp1 phosphorylation appears to be insensitive to osmotic or nutritional stress, suggesting that TORC1 regulates these two substrates via very different mechanisms (Lempiainen et al. 2009). Sfp1 also interacts with the conserved Rab escort protein Mrs6, an essential protein functioning in membrane sorting (Lempiainen et al. 2009; Singh and Tyers 2009). Sfp1-Mrs6 association is important for the nuclear localization of Sfp1, but its functional implications are otherwise unclear. Intriguingly, this association may underlie the presently unexplained genetic and biochemical interactions between TORC1 and vesicular transport machineries (Aronova et al. 2007; Zurita-Martinez et al. 2007). Although physical interaction with RiBi promoters has not been reported, overexpression of Sfp1 causes a rapid upregulation of most RiBi genes, suggesting that Sfp1 also regulates this important regulon (Jorgensen et al. 2004). Better understood is the regulation of RiBi gene expression downstream of Sch9. RiBi promoters typically possess polymerase A and C (PAC) and/or ribosomal RNA processing element (RRPE) elements. PAC elements are bound by the myb-family transcription factors Dot6 and Tod6 (Freckleton et al. 2009; Zhu et al. 2009) whereas RRPE elements are bound by Stb3 (Liko et al. 2007). Stb3 seems to bind to T-rich elements in RP promoters as well (Huber et al. 2011). All three transcription factors are phosphorylated by Sch9 and thus are under TORC1 control (Lippman and Broach 2009; Liko et al. 2010; Huber et al. 2011). When TORC1 is inactivated, Dot6, Tod6, and Stb3 are dephosphorylated, allowing them to bind to their cognate promoter elements and recruit the RPD3L histone acetyltransferase complex to repress transcription.

In summary, TORC1 plays a central role in regulating ribosome biogenesis, particularly at the transcriptional level. However, it is now clear that TORC1 also influences ribosome biogenesis post-transcriptionally. Phosphoproteomics as well as more directed studies suggest that TORC1 regulates various catalytic steps of ribosome assembly per se (Honma et al. 2006; Huber et al. 2009; Loewith 2010). Phosphoproteomics and biochemical studies (Albig and Decker 2001; Grigull et al. 2004; Huber et al. 2009; Breitkreutz et al. 2010; Loewith 2010; Soulard et al. 2010) also suggest that TORC1 plays an active role in mRNA stability and, via its potential substrate Sky1, in pre-mRNA splicing. This observation is significant when one considers that 90% of all mRNA splicing events occur on RP transcripts (Warner 1999). Thus, TORC1 is well positioned to coordinate multiple aspects of ribosome biogenesis in response to growth stimuli. As introduced above, TORC1 activity is dramatically increased in sfp1 and sch9 cells (Lempiainen et al. 2009), suggesting that some aspect of ribosome biogenesis must also signal in a feedback loop to TORC1. It will be interesting to see what steps of ribosome biogenesis contribute to TORC1 regulation.

Regulation of cell cycle/cell size: Although distinct processes, cell growth and cell division are often intimately linked. Yeast cells, for example, commit to a new round of cell division only after attaining a critical size. This cell-size threshold is dictated in large part by environmental growth conditions (Cook and Tyers 2007). How cells couple environmental cues to the cell cycle machinery is fascinating but poorly understood. Interestingly, sfp1 and sch9 were the top two hits in a systematic search for mutations conferring small cell size (Jorgensen et al. 2002, 2004). This and follow-up observations demonstrated that ribosome biogenesis plays a major role in cell-size determination. These results further predict that environmental cues regulate the cell-size threshold via TORC1, i.e., that poor growth conditions reduce the activity of TORC1 and subsequently the activities of Sfp1 and Sch9. Consequently, this would decrease ribosome biogenesis, which, in mysterious ways, would lower the cell-size threshold required for cell division. In contrast, acute inhibition of TORC1 with high concentrations of rapamycin leads to an arrest in G1 due to reduced translation of the cyclin Cln3 (Barbet et al. 1996) and a paradoxical increase in cell size. This increase in cell size is actually due to swelling of the vacuole as a consequence of increased autophagy (see below; sfp1 or sch9 deletions presumably do not induce autophagy).

Although best appreciated for its role in G1 regulation, TORC1 additionally regulates the transition through other phases of the cell cycle. TORC1 promotes S phase by maintaining deoxynucleoside triphosphate pools. Deoxynucleoside triphosphates are the obligate building blocks for DNA synthesis, and a role for TORC1 in their synthesis becomes apparent under conditions of DNA replication stress or DNA damage when elevated deoxynucleoside triphosphate pools are necessary for error-prone translesion DNA polymerases (Shen et al. 2007). Via the Tap42-PPase branch, TORC1 also influences the G2/M transition (Nakashima et al. 2008). Specifically, TORC1 regulates the subcellular localization of the polo-like kinase Cdc5. Cdc5 activity destabilizes Swe1, a kinase that phosphorylates and thus inactivates the mitotic cyclin-dependent kinase Cdc28. Inhibition of TORC1 mislocalizes Cdc5, causing an inappropriate stabilization of Swe1 and, consequently, inactivation of Cdc28 and prolonged G2/M. Although TORC1 signals likely impinge upon additional nodes in the cell division cycle (Huber et al. 2009; Soulard et al. 2010), the above

observations already exemplify the intricate connections between cell growth signals and the cell division cycle. Reciprocal, but less well described, cues and/or outputs from the cell division cycle regulate cell growth, likely in part via TORC1 (Goranov and Amon 2010).

TORC1 inhibits stress responses: In addition to stimulating anabolic processes, TORC1 also promotes growth by suppressing a variety of stress-response programs. Although essential for surviving environmental insults, activation of stress-responsive pathways is incompatible with rapid growth, and constitutive activation of these pathways generally results in cell death. As described below, the best-characterized stress-response programs under the influence of TORC1 are transcriptional in nature. However, it is clear that TORC1 also regulates post-transcriptional aspects of stress responses such as mRNA stability, protein trafficking, and the activities of metabolic enzymes.

Environmental stress response: Exposure of yeast cells to noxious stressors, including nutrient limitation and entry into stationary phase, elicits a stereotypic transcriptional response known as the environmental stress response (ESR) (Gasch and Werner-Washburne 2002). This includes ∼300 upregulated genes that encode activities such as protein chaperones and oxygen radical scavengers that help cells endure stressful environments. The central components of this pathway are the Zn-finger transcription factors Msn2/4 and Gis1, the LATS family kinase Rim15, and the α-endosulfine family paralogs Igo1 and Igo2 (De Virgilio 2011). TORC1 via Sch9, and possibly also Tap42-PPase, promotes cytoplasmic anchoring of Rim15 to 14-3-3 proteins by maintaining Rim15 phosphorylated on Ser1061 and Thr1075 (Wanke *et al.* 2005, 2008). Inhibition of TORC1 results in nuclear localization of Rim15, which subsequently triggers the activation, in a poorly understood fashion, of the expression of Msn2/4- and Gis1-dependent ESR genes. However, TORC1 inhibition results in a marked turnover of mRNAs (Albig and Decker 2001), and, as noted above, in a dramatic drop in translation. Thus it would appear that increasing transcription of protein-coding genes in TORC1-inhibited cells would be futile as these mRNA would likely be degraded before ever being translated. This appears not to be the case as Rim15 phosphorylates Igo1 and its paralog Igo2, allowing them to associate with newly transcribed Msn2/4- and Gis1-regulated mRNAs to protect these transcripts from degradation via the 5′-3′ mRNA decay pathway (Talarek *et al.* 2010; Luo *et al.* 2011).

Nutrient uptake and intermediary metabolism: To best compete with other microbes in their environment, yeast have optimized the use of available nutrients to accommodate fast growth (De Virgilio and Loewith 2006). Although a wide variety of compounds can be utilized as carbon or nitrogen sources, yeast cells will exclusively assimilate preferred nutrient sources before using nonpreferred, suboptimal ones. To attain this dietary specificity, and to respond to nutritional stress, yeast cells carefully regulate the expression and sorting of their many (>270) membrane transport-

ers, enabling them to selectively import only the desired nutrients (Van Belle and Andre 2001). In general terms, in good growth conditions, many high-affinity, substrate-selective permeases are expressed and sorted to the plasma membrane to actively pump in nutrients that are used directly in ATP production and/or anabolism of nitrogenous compounds. Shift to poor growth conditions results in the replacement of high-affinity permeases, which are targeted to the vacuole for degradation with few low-affinity, broad-specificity permeases that facilitate uptake of a wide range of carbon and nitrogenous compounds that can be catabolized by the cell. For example, in response to nitrogen starvation, the high-affinity tryptophan-specific permease, Tat2, localized to the plasma membrane, is ubiquitinated, endocytosed, and ultimately degraded. In contrast, the general amino acid permease Gap1 is re-routed to the plasma membrane instead of to the vacuole/endosomes. Although details are still emerging, TORC1 appears to regulate such permease-sorting events primarily via Tap42-PPase and its (potentially direct) effector Npr1 (Schmidt *et al.* 1998; Beck *et al.* 1999; De Craene *et al.* 2001; Jacinto *et al.* 2001; Soetens *et al.* 2001; Breitkreutz *et al.* 2010). Npr1 is a heavily phosphorylated, seemingly fungal-specific, Ser/Thr kinase that upon TORC1 inactivation is rapidly dephosphorylated and activated (Gander *et al.* 2008). Although genetic studies clearly imply a role for Npr1 in protein-sorting events, the mechanisms of this regulation have remained elusive. It is possible that the permeases themselves are Npr1 substrates. Indeed, several nutrient and cation permeases have been identified as rapamycin-sensitive phosphoproteins (Huber *et al.* 2009; Soulard *et al.* 2010). Also identified in these phosphoproteomics studies were several α-arrestin-related proteins. These phosphoproteins function as clathrin adaptor molecules and have been implicated in mediating the sorting fates of a number of different permeases; and, one, Aly2, has recently been reported to be an Npr1 substrate (Lin *et al.* 2008; Nikko *et al.* 2008; Nikko and Pelham 2009; O'Donnell *et al.* 2010). Whether this observation is indicative of a more general trend in Npr1-meditated permease trafficking remains to be seen.

TORC1 regulates permease activity by regulating not only permease localization but also expression. This was shown in early transcriptomics experiments, which clearly demonstrated that TORC1 regulates the expression of a large number of permeases and other factors required for the assimilation of alternative nitrogenous sources (Cardenas *et al.* 1999; Hardwick *et al.* 1999; Komeili *et al.* 2000; Shamji *et al.* 2000). TORC1 regulates the expression of **n**itrogen **c**atabolite **r**epression (NCR)-sensitive genes via the Tap42-PPase branch. The proteins encoded by these genes (*e.g.,* Gap1) enable cells to import and metabolize poor nitrogen sources such as proline and allantoin. In the presence of preferred nitrogen sources such as glutamine, glutamate, or ammonia, active TORC1 promotes the association of the GATA-family transcription factor Gln3 with its cytoplasmic anchor Ure2. Mechanistically, this involves both TORC1-dependent

and TORC1-independent regulation of Gln3, and possibly of Ure2, phosphorylation (Beck and Hall 1999; Cardenas *et al.* 1999; Hardwick *et al.* 1999; Carvalho and Zheng 2003; Georis *et al.* 2009a; Tate *et al.* 2009, 2010). Two other less-characterized GATA factors, Gat1 and Dal81, also have roles in the regulation of NCR-sensitive genes (Georis *et al.* 2009b).

In addition to the NCR pathway, TORC1 also regulates the expression of amino acid permeases by modulating the activity of the SPS-sensing pathway. This pathway consists of a plasma-membrane-localized sensor of external amino acids, Ssy1, and two downstream factors, Ptr3 and Ssy5 (Ljungdahl 2009). Upon activation of the pathway, Ssy5 catalyzes an endoproteolytic processing event that cleaves and releases an N-terminal regulatory domain from two transcription factors, Stp1 and Stp2, the shortened forms of which translocate to the nucleus and activate the transcription of a number of amino acid permease-encoding genes. TORC1 via Tap42-PPase modulates this pathway by promoting the stability of Stp1 and thus the ability of cells to utilize external amino acids (Shin *et al.* 2009).

In contrast to the SPS-sensing pathway that is activated by amino acids, the Gcn4 transcription factor is activated upon amino acid starvation (Hinnebusch 2005). As mentioned above, rapamycin treatment or amino acid starvation results in a rapid decline in translation initiation by triggering phosphorylation of the α-subunit of eIF2. Although eIF2α phosphorylation results in the repression of bulk translation, due to the presence of four short upstream open reading frames in its leader sequence, the mRNA encoding Gcn4 is, in contrast, preferentially translated. Subsequent accumulation of Gcn4 protein leads to the transcriptional induction of nearly all genes encoding amino acid biosynthetic enzymes.

TORC1 also regulates amino acid biosynthesis, in particular glutamine and glutamate homeostasis, via the retrograde response pathway (Komeili *et al.* 2000; Crespo and Hall 2002; Crespo *et al.* 2002; Liu and Butow 2006). This signaling pathway serves to communicate mitochondrial dysfunction to the nucleus to induce an appropriate transcriptional response. In addition to hosting the aerobic energy production machinery, mitochondria are also the sites of amino acid precursor, nucleotide, and lipid production. Signals, possibly changes in glutamate or glutamine levels, emanating from dysfunctional mitochondria impinge upon a cytosolic regulatory protein, Rtg2. Thus activated, Rtg2 antagonizes the ability of Mks1 to sequester the heterodimeric bZip/HLH transcription factor complex composed of Rtg1 and Rtg3 in the cytoplasm. Allowed to enter the nucleus, Rtg1/3 activates genes encoding enzymes required for anaplerotic reactions that resupply tri-carboxylic acid cycle intermediates that have been extracted for biosynthetic reactions. Key among these intermediates is α-ketoglutarate, the precursor of glutamate and glutamine from which all nitrogen-containing metabolites evolve (Magasanik and Kaiser 2002). Both transcriptome-profiling experiments as well as genetic studies have implicated TORC1 as a negative regulator of Rtg1/3-dependent transcription (Komeili *et al.* 2000; Shamji *et al.* 2000; Chen and Kaiser 2003). However, it is presently unclear how TORC1 influences this pathway; TORC1 inhibition could indirectly influence retrograde response signaling via alterations in metabolite levels. Alternatively, the direct association between TORC1 and Mks1 observed by the Tyers group and described above and the fact that Mks1 is a rapamycin-sensitive phosphoprotein instead suggest that TORC1 could play a much more direct role in regulating this pathway (Liu *et al.* 2003; Breitkreutz *et al.* 2010). Finally, phosphoproteomics studies suggest that TORC1 regulates intermediate metabolism by directly altering the activities of metabolic enzymes, particularly those involved in the early steps of glycolysis (Loewith 2011).

Autophagy: As described above, starved cells express a suite of stress-responsive proteins to help them negotiate hostile environmental conditions. This new synthesis requires both energy and amino acids that yeast cells obtain by inducing autophagy. Autophagy refers to a variety of mechanisms by which cytosplasmic material, including proteins and lipids, is translocated to the vacuole and catabolized. Amino acids and fatty acids thus acquired are, respectively, used to synthesize new proteins and oxidized by mitochondria to produce ATP. Mechanistically, there are two different modes of autophagy in yeast. One is microautophagy, which involves the direct transfer of cytoplasm into the vacuole via invaginations of the vacuolar membrane. The other is macroautophagy, which involves the *de novo* formation of double-membrane vesicles called autophagosomes. Autophagosomes encapsulate cytoplasm and then fuse with the vacuole. Both forms of autophagy are regulated by TORC1 (De Virgilio and Loewith 2006) although, mechanistically, macroautophagy is better understood (reviewed in Cebollero and Reggiori 2009; Nakatogawa *et al.* 2009; Inoue and Klionsky 2010).

Autophagy is conserved across eukarya, and there is much interest in understanding how macroautophagy is regulated as it has been linked to several pathologies including cancer, neurological disorders, and longevity (Yang and Klionsky 2010). In yeast, many **a**utopha**g**y-related (*ATG*) genes encode proteins that participate in the induction of autophagy, the nucleation of the autophagosome, elongation and completion of the autophagosome, and, finally, in fusion of the autophagosome with the vacuole to release the autolysosome into the vacuolar lumen (Chen and Klionsky 2011; Reiter *et al.* 2011). TORC1 regulates macroautophagy by signaling to the Atg1 kinase complex that is required for the induction of macroautophagy. Specifically, when TORC1 is active, Atg13 is hyperphosphorylated, presumably directly by TORC1 (although Tap42-PPase has also been implicated in this regulation), and this prevents the association of Atg13 with Atg1, Atg17, Atg31, and Atg29 (Yorimitsu *et al.* 2009; Kamada *et al.* 2010). Inhibition of TORC1 results in dephosphorylation of Atg13, assembly of the Atg1 protein kinase complex, phosphorylation and

activation of Atg1 (Kijanska *et al.* 2010; Yeh *et al.* 2010), and, subsequently, macroautophagy mediated by as-yet-unidentified Atg1 substrates. Although metazoan homologs exist for many of the Atg1 kinase complex components, a unifying model of how TORC1 regulates this complex in different species has yet to emerge (Chen and Klionsky 2011; Reiter *et al.* 2011).

Cell-wall integrity pathway: The cell wall is essential for yeast cells to survive hostile environments and, more importantly, to prevent internal turgor pressure from rupturing the plasma membrane. Although a thickening of the cell wall helps protect stressed or stationary-phase cells, this rigid structure must also be remodelled to accommodate cell growth. Homeostasis of this structure is maintained by the cell-wall integrity (CWI) pathway (Levin 2005). Cell-wall integrity is monitored by WSC (cell-wall integrity and stress response component) family proteins. WSCs, which are integral plasma membrane proteins, function upstream of the Rho1 GTPase by modulating the activity of the GEFs Rom1 and Rom2. Rho1^{GTP} has several effectors including the yeast protein kinase C homolog, Pkc1. The best-characterized Pkc1 effector is a mitogen-activated protein kinase (MAPK) cascade composed of Bck1 (a MAPKKK), Mkk1 and -2 (redundant MAPKKs), and Slt2/Mpk1 (a MAPK). Activation of this pathway leads to the expression of many cell-wall biosynthetic enzymes, which helps to remodel the cell wall both during normal growth and in response to stress.

Both TORC1 and TORC2 (discussed below) appear to impinge upon the CWI pathway. Entry into stationary phase, carbon starvation, nitrogen starvation, and rapamycin treatment all elicit activation of the CWI pathway, demonstrating that TORC1 negatively regulates the CWI pathway (Ai *et al.* 2002; Krause and Gray 2002; Torres *et al.* 2002; Reinke *et al.* 2004; Araki *et al.* 2005; Soulard *et al.* 2010). Furthermore, *pkc1*, *bck1*, and *mpk1* mutants rapidly lose viability upon carbon or nitrogen starvation, demonstrating that the CWI pathway is required for viability in G0. Mechanistically, how TORC1 signals impinge on the CWI pathway is not clear. Soulard *et al.* (2010) have implicated the Sch9 effector branch while Torres *et al.* (2002) have postulated that signals through the Tap42-PPase branch causes membrane stress that, via WSC family members, activates downstream components of the CWI pathway.

TORC1 accelerates aging: Arguably one of the most interesting functions of TORC1 is its involvement in the regulation of life span. It is well established that, in virtually every biological system, aging, *i.e.*, the progressive deterioration of cell, tissue, and organ function, can be delayed through calorie or dietary restriction. Epistasis studies have led many to believe that this is due to reduced TORC1 signaling (reviewed in Weindruch and Walford 1988; Kapahi *et al.* 2010; Zoncu *et al.* 2010, 2011; Kaeberlein and Kennedy 2011). Indeed, genetic or chemical targeting of TORC1 has been demonstrated to increase life span in yeast, worms, flies, and mice (Vellai *et al.* 2003; Jia *et al.* 2004; Kapahi *et al.* 2004; Wanke *et al.* 2008; Harrison *et al.*

2009; Bjedov *et al.* 2010). These observations have created much excitement in that aging is now thought of as a disease, which, like other diseases, can be ameliorated through pharmaceutical intervention. These observations have also raised the important question, what are the downstream function(s) of TORC1 that modulate life span? The answer to this question is presently unclear, and it is very likely that multiple TORC1 effector pathways contribute (Blagosklonny and Hall 2009). Studies in many model systems are presently underway to address this issue. Below are some of the highlights from studies in yeast.

Yeast life span is assayed in one of two ways. Replicative life span (RLS) is a measure of the number of progeny that a single mother cell can produce before senescence. Chronological life span (CLS) is a measure of the length of time a population of yeast cells can remain in stationary phase before they lose the ability to restart growth following re-inoculation into fresh media. RLS is thought to be a paradigm for aging of mitotic cells while CLS is thought to be a paradigm for aging of quiescent cells. Consistent with bigger eukaryotes, where newborns are obviously born young, gametogenesis (*i.e.*, cells derived from meiotic cell divisions) resets RLS in yeast (Unal *et al.* 2011).

Kaeberlein *et al.* (2005) have recently attempted labor-intensive approaches to identify genes involved in both replicative and chronological life span. A random screen of 564 yeast strains, each lacking a single nonessential gene, implicated both *TOR1* and *SCH9* in RLS downstream of caloric restriction. Also identified in this screen were a number of genes encoding ribosomal proteins. Further analyses of *RP* genes subsequently demonstrated that specific depletion of 60S ribosomal protein subunits extends RLS (Steffen *et al.* 2008). Curiously, RLS extension observed upon TORC1 inhibition and 60S subunit depletion seems to be mediated by Gcn4, the TORC1-dependent transcription factor that regulates the expression of amino acid biosynthetic genes as described above. The relevant Gcn4 target genes/processes involved in RLS are not yet known, but an interesting candidate could be macroautophagy. Induction of macroautophagy, like TORC1 and Sch9 inhibition, increases both RLS and CLS (Madeo *et al.* 2010a,b; Morselli *et al.* 2011; and see below), and Gcn4 is required for amino acid-starvation-induced macroautophagy (Ecker *et al.* 2010). Furthermore, spermidine, a potent inducer of macroautophagy, potentially via Gcn4 (Teixeira *et al.* 2010), appears to promote longevity not only in yeast but also in several other model organisms (Eisenberg *et al.* 2009). Since TORC1, Sch9, and Gcn4 homologs are found in most eukaryotes, this appears to represent a conserved aging pathway (Kaeberlein and Kennedy 2011).

Sch9 was one of the first genes to be implicated in CLS (Fabrizio *et al.* 2001). A subsequent high-throughput assay involving 4800 viable single-gene yeast mutants further implicated TORC1 in CLS (Powers *et al.* 2006). These and other studies (Wanke *et al.* 2008; Wei *et al.* 2008) provided evidence that reduced TORC1-Sch9-signaling activity promotes life span by increasing the Rim15-dependent expression of environmental

stress-response genes (described above). Later, Burtner *et al.* (2009) demonstrated that acetic acid-induced mortality is the primary mechanism of chronological aging in yeast under standard conditions and that this toxicity is better tolerated when environmental stress-response genes are artificially induced, for example, upon inhibition of TORC1 or Sch9 activities. However, this model is not universally accepted. Pan *et al.* (2011) have proposed that TORC1 inhibition leads to increased mitochondrial function and a consequent increase in reactive oxygen species that elicit a Rim15-independent pro-survival signal. Furthermore, acetic acid accumulation appears not to be a contributing factor in CLS in this study. Given its apparent conservation across eukarya (Blagosklonny and Hall 2009), elucidation of the mechanisms by which TORC1 regulates life span is eagerly awaited.

Less-characterized effectors identified in phosphoproteomic studies: As alluded to above, large-scale mass spectrometry-based phosphoproteomic studies have recently been performed to identify the rapamycin-sensitive phosphoproteome (Huber *et al.* 2009; Soulard *et al.* 2010). The major limitation of these studies was their poor coverage as evidenced by their rather modest overlap, although this could be partly explained by the different growth conditions and technical approaches employed. Rapamycin exposure times were chosen such that layers of signaling events (*e.g.*, kinase/phosphatase cascades) would be observed. These events should have been triggered as a direct consequence of TORC1 inhibition and not as a secondary consequence of cell cycle delays or changes in transcription. Hundreds of rapamycin-sensitive phosphorylation sites were mapped, the majority of which are in proteins not previously implicated in TORC1 signaling. However, as sufficient time elapsed to activate entire signaling cascades, a potential TORC1 consensus target motif was not evident from the data analyses. Still, the data from these studies will be instrumental in both elucidating how TORC1 signals to its known distal readouts and discovering new TORC1 functions.

TOR Complex 2

Composition and localization of TOR complex 2

TOR complex 2 (TORC2) is rapamycin insensitive and consists of TOR2, Avo1, Avo2, Avo3, Bit61 (and/or its paralog Bit2), and Lst8 (Loewith *et al.* 2002; Wedaman

et al. 2003; Reinke *et al.* 2004; Zinzalla *et al.* 2010) (Figure 1C). The names of mammalian and invertebrate orthologs of TORC2 subunits and the salient features of *S. cerevisiae* TORC2 subunits are summarized in Table 1 and Table 4, respectively. The highly conserved, essential core subunits are TOR2, Avo1, Avo3, and Lst8. Avo1 and Avo3 bind cooperatively to the N-terminal HEAT repeat region in TOR2 and are required for TORC2 integrity (Wullschleger *et al.* 2005). TORC2 autophosphorylates sites in Avo1 and Avo3, but the purpose of this phosphorylation is unknown. Avo1 has a C-terminal PH-like domain that mediates binding to the plasma membrane (Berchtold and Walther 2009). Avo3 has a RasGEFN domain, a subdomain often found in the N-terminal part of a larger GDP/GTP exchange domain of exchange factors for Ras-like GTPases, but the function of the RasGEFN domain is unknown. Lst8 binds to the kinase domain in TOR2 and is required for TOR2 kinase activity (Wullschleger *et al.* 2005). Lst8 is a Gβ-like propeller protein consisting of seven WD40 motifs. TORC2 is rapamycin insensitive whereas TORC1 is rapamycin sensitive because FKBP-rapamycin binds only TORC1 (Loewith *et al.* 2002). This selective FKBP-rapamycin binding is presumably due to Avo1 masking the FRB domain in TOR2 in TORC2. Finally, co-immunoprecipitation and gel filtration experiments suggest that TORC2 is a multimer, likely a TORC2-TORC2 dimer (Wullschleger *et al.* 2005).

The cellular localization of TORC2 has been studied by subcellular fractionation, indirect immunofluorescence, immunogold electron microscopy, and visualization of GFP-tagged TORC2 components (Kunz *et al.* 2000; Wedaman *et al.* 2003; Aronova *et al.* 2007; Sturgill *et al.* 2008; Berchtold and Walther 2009). In considering these studies, it is important to realize that the vast majority of TOR2 (~90%) is in TORC2 (*vs.* TORC1), and thus TOR2 localization studies presumably detect mainly, if not exclusively, TORC2. All studies indicate that TORC2 is at or near the plasma membrane. Berchtold and Walther (2009) suggest that TORC2 is dynamically localized to a previously unrecognized plasma membrane domain termed the MCT (**m**embrane **c**ompartment containing **T**ORC2). Furthermore, they conclude that TORC2 plasma membrane localization is essential for viability and is mediated by the C-terminal PH domain in Avo1. Most of the localization studies have found that TORC2 is also at another, ill-defined cellular location(s). For example, Kunz *et al.*

Table 4 Salient features of TORC2 components

Protein	Size	Motifs/domains	Potential function
Tor2	2470 aa	HEAT repeats, FAT domain, FRB domain, kinase domain, and FATC domain	Protein kinase, scaffold
Avo1	1176 aa	PH	Recruit TORC2 to plasma membrane
Avo2	426 aa	None obvious	Unknown
Avo3/Tsc11	1430 aa	RasGEFN	Scaffold
Bit61	543 aa	None obvious	Paralogs with unknown function
Bit2	545 aa	None obvious	Paralogs with unknown function
Lst8	303 aa	7 WD-40 repeats	Stabilize kinase domain

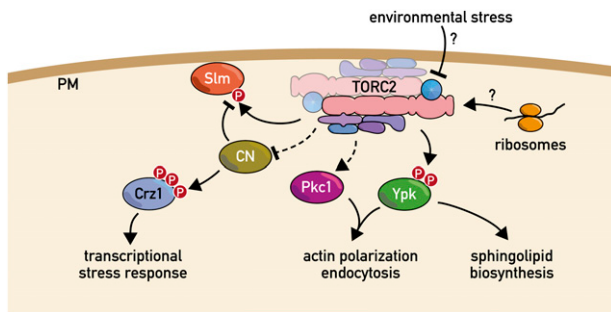Data for this table were obtained from Cybulski and Hall (2009).

Figure 4 Signaling by TORC2. TORC2 directly phosphorylates the AGC kinase family member Ypk (Ypk1 and 2) and the PH domain containing protein Slm (Slm1 and -2). Downstream effectors include the phosphatase calcineurin, the transcription factor Crz1, and Pkc1. TORC2 controls organization of the actin cytoskeleton, endocytosis, sphingolipid biosynthesis, and stress-related transcription. The effector pathways by which TORC2 controls these processes are incompletely understood (see *Distal readouts downstream of TORC2* for further details).

(2000) report that part of TOR2 is also in an unknown subcellular membrane fraction distinct from Golgi, vacuoles, mitochondria, and the nucleus. Wedaman *et al.* (2003) showed that TOR2 can be in the cell interior often in association with membrane tracks. Sturgill *et al.* (2008) detected a cytoplasmic fluorescent signal in cells expressing GFP-tagged TOR2. In conclusion, TORC2 appears to be at multiple cellular locations, the plasma membrane, and one or possibly more other sites. A plasma membrane location is consistent with the role of TORC2 in controlling the actin cytoskeleton and endocytosis (see below).

Upstream of TORC2

The upstream regulation of TORC2 is poorly characterized (Cybulski and Hall 2009). Several lines of evidence in many different organisms indicate that nutrients regulate TORC1 (see above). On the other hand, there is no reported evidence supporting the notion that TORC2 is controlled by nutrients. Knockout of TORC2 does not confer a starvation-like phenotype, and the nutrient-sensitive EGO complex appears not to be upstream of TORC2. Zinzalla *et al.* (2011) recently devised a "reverse" suppressor screen to identify upstream regulators of TORC2. This screen was based on the observation that constitutively active Ypk2 (Ypk2*) suppresses the loss of viability due to a TORC2 defect. Ypk2 is a protein kinase normally phosphorylated and activated by TORC2 (see below). Zinzalla *et al.* (2011) screened for mutants that require Ypk2* for viability. As predicted, this screen isolated several mutants defective in genes encoding essential TORC2 components, but also in the gene *NIP7*. Subsequent experiments confirmed that Nip7, a ribosome maturation factor, is required for TORC2 kinase activity. The role of Nip7 in the activation of yeast TORC2 has so far not been pursued further, but experiments in mammalian cells suggest that mNip7 is required for mTORC2 activation indirectly via its role in ribosome maturation. In mammalian cells, and presumably also in yeast cells, TORC2 is activated by direct association with the ribo-

some. As ribosomes determine the growth capacity of a cell, this mechanism ensures that TORC2 is active only in growing cells.

There are also indications that environmental stress inhibits TORC2 signaling, possibly to prevent growth in unfavorable conditions. The mechanism of this regulation and the level at which it intersects with the TORC2 pathway are poorly defined, but it may involve the Slm proteins (see below) and the stress-activated phosphatase calcineurin (Bultynck *et al.* 2006; Mulet *et al.* 2006).

TORC2 substrates

The best-characterized and possibly the major TORC2 substrate is the protein kinase Ypk. Ypk1 and Ypk2 are an essential pair of homologous kinases and members of the AGC kinase family (Roelants *et al.* 2004) (Figure 4). Kamada *et al.* (2005) linked Ypk to TORC2 signaling upon isolating *YPK2* as a multicopy suppressor of a TORC2 defect. They then showed that immunopurified TOR2 directly phosphorylates Ypk2 at Ser641 in the turn motif and Thr659 in the hydrophobic motif. TORC2 phosphorylates and activates Gad8 and SGK1, the *S. pombe* and mammalian orthologs of Ypk, respectively, in a similar manner (Matsuo *et al.* 2003; Garcia-Martinez and Alessi 2008). It is well established that TORC1 or TORC2 phosphorylates the turn and hydrophobic motifs in several kinases as a conserved mechanism of activation of AGC kinase family members (see above) (Jacinto and Lorberg 2008). Ypk/Gad8/SGK1 appears to be a major TORC2 substrate as an *ypk*, *gad8*, or *sgk1* mutation phenocopies a TORC2 defect, and overexpression of Ypk2, Gad8, or SGK1 is sufficient to suppress a TORC2 defect in *S. cerevisiae*, *S. pombe*, or *Caenorhabditis elegans*, respectively (Matsuo *et al.* 2003; Kamada *et al.* 2005; Jones *et al.* 2009; Soukas *et al.* 2009). The two homologous, TORC2- and phosphoinositide (PI4,5P$_2$)-binding proteins Slm1 and Slm2 have also been reported to be phosphorylated in a TORC2-dependent manner both *in vivo* and *in vitro* (Audhya *et al.* 2004; Fadri *et al.* 2005). However, the physiological relevance of Slm phosphorylation is unknown other than that it appears to be required for localization of Slm to the plasma membrane (Audhya *et al.* 2004; Fadri *et al.* 2005).

Distal readouts downstream of TORC2

The first described and best-characterized TORC2 readout is the actin cytoskeleton (Figure 4). TORC2 controls the cell cycle-dependent polarization of the actin cytoskeleton. As the polarized actin cytoskeleton directs the secretory pathway and thereby newly made protein and lipid to the growing daughter bud, this is a mechanism by which TORC2 mediates spatial control of cell growth. The first indication that TOR2 is linked to the actin cytoskeleton came from the isolation of *TCP20*, which encodes an actin-specific chaperone, as a dosage suppressor of a dominant-negative *TOR2* "kinase-dead" mutation (Schmidt *et al.* 1996). This, in turn, led to the discovery that *tor2* mutants display an actin

organization defect (Schmidt *et al.* 1996). The subsequent isolation of *sac7*, which encodes a Rho-GAP (GTPase-activating protein), as a second-site suppressor of a *tor2*-temperature-sensitive (ts) mutation suggested that TOR2 is linked to the actin cytoskeleton via a signaling pathway containing a Rho GTPase. It was later demonstrated that Sac7 is indeed a GAP for Rho1 and that TOR2 activates the Rho1 GTPase switch via the Rho1-GEF Rom2 (Schmidt *et al.* 1997; Bickle *et al.* 1998). Rom2 GEF activity is reduced in extracts from a *tor2*-ts mutant (Schmidt *et al.* 1997; Bickle *et al.* 1998). The finding that overexpression of Rom2 suppresses a *tor2*-ts mutation, whereas overexpression of catalytically active Rom2 lacking its lipid-binding PH domain does not suppress, suggested that TOR2 signals to Rom2 via the PH domain. It was subsequently shown that TOR2 signals to the actin cytoskeleton mainly, if not exclusively, via the Rho1 effector Pkc1 (protein kinase C) and the Pkc1-controlled cell-wall integrity MAP kinase cascade (Helliwell *et al.* 1998b).

How might TORC2 signal to Rom2 to activate the Rho1 GTPase switch? The PH domain in Rom2 suggests that it may involve a lipid intermediate. This possibility is supported by the observation that overexpression of the PI-4-P 5-kinase Mss4 suppresses a *tor2*-ts mutation (Desrivieres *et al.* 1998; Helliwell *et al.* 1998a) and that PI4,5P$_2$ at the plasma membrane is required to recruit/activate Rom2 (Audhya and Emr 2002). The mechanism by which TORC2 may activate PI4,5P$_2$ signaling or possibly a parallel pathway converging on the cell-wall integrity pathway is unknown, but likely involves the well-established TORC2 substrate Ypk (Roelants *et al.* 2002; Schmelzle *et al.* 2002; Kamada *et al.* 2005; Mulet *et al.* 2006). The phosphoinositide-binding Slm proteins and sphingolipids may also be functionally related to TORC2-mediated control of the actin cytoskeleton (Sun *et al.* 2000; Friant *et al.* 2001; Roelants *et al.* 2002; Audhya *et al.* 2004; Fadri *et al.* 2005; Liu *et al.* 2005; Tabuchi *et al.* 2006; Daquinag *et al.* 2007).

A second downstream process controlled by TORC2 is endocytosis. Efficient internalization of cell-surface components is an important aspect of cell growth control. deHart *et al.* (2003) identified a *tor2* mutation in a screen for mutants defective in ligand-stimulated internalization of a cell-surface receptor. TORC2 appears to control endocytosis via Rho1, Ypk1, and possibly the Slm proteins, but how Rho1, Ypk1, and the Slm proteins are functionally related in mediating TORC2-controlled endocytosis is unknown (deHart *et al.* 2002, 2003; Bultynck *et al.* 2006).

A third TORC2-regulated process is sphingolipid biosynthesis (Powers *et al.* 2010). Sphingolipids serve as essential structural components in lipid bilayers and as signaling molecules. The first indication that TORC2 controls sphingolipid synthesis was the finding that overexpression of *SUR1* suppresses a temperature-sensitive *tor2* mutation (Helliwell *et al.* 1998a). In a parallel study, Beeler *et al.* (1998) reported that a mutation in *TOR2* or *AVO3* (also known as *TSC11*), or mutations in genes encoding components of the sphingolipid biosynthetic pathway, suppress a *csg2* mutation. Sur1/Csg1 and Csg2 are subunits, probably the catalytic and regulatory subunits, respectively, of mannosylinositol phosphorylceramide synthase that mediates a late step in sphingolipid biosynthesis. The Slm proteins were subsequently also linked to sphingolipid metabolism (Tabuchi *et al.* 2006; Daquinag *et al.* 2007). Most recently, Aronova *et al.* (2008) profiled sphingolipids in a conditional *avo3* mutant and thereby confirmed that TORC2 plays a positive role in sphingolipid biosynthesis. Aronova *et al.* (2008) also investigated the molecular mechanism by which TORC2 controls sphingolipids. They found that TORC2 regulates sphingolipid production via Ypk2 and suggest a model wherein TORC2 signaling is coupled to sphingoid long-chain bases (early intermediates in sphingolipid synthesis) to control Ypk2 and late steps in sphingolipid synthesis. Furthermore, the biosynthetic step controlled by TORC2 and Ypk2 is antagonized by the phosphatase calcineurin that is functionally linked to the Slm proteins (Bultynck *et al.* 2006; Mulet *et al.* 2006; Aronova *et al.* 2008). Another potential target for the regulation of sphingolipid biosynthesis by TOR are the Orm1 and Orm2 proteins. The conserved Orm proteins, identified as a potential risk factor for childhood asthma, form a complex that negatively regulates the first and rate-limiting step in sphingolipid biosynthesis (Breslow *et al.* 2010; Han *et al.* 2010). Both Orm1 and Orm2 are phosphoproteins and at least Orm1 phosphorylation changes upon rapamycin treatment (Huber *et al.* 2009; Soulard *et al.* 2010). Furthermore, loss of Orm2 suppresses a Ypk deficiency (Roelants *et al.* 2002; Schmelzle *et al.* 2002; Kamada *et al.* 2005; Mulet *et al.* 2006). These findings suggest that both TORC1 and TORC2 may control sphingolipid synthesis via Orm proteins.

Future Directions

What is upstream of the two complexes?

How TORC activities are altered in response to environmental cues remains a major void in our understanding of the TOR-signaling network. The TOR complexes are regulated by nutrients, stress, or ribosomes, but the mechanisms by which these inputs are sensed and how this information is transduced, with the notable exceptions discussed above, to ultimately regulate kinase activity remain largely unknown. Genetic screens, such as the reverse suppressor screen described above, should help to further elucidate these signaling pathways. Unlike growth factor-signaling pathways, which are present only in metazoans, nutrient and stress-responsive pathways are found in all eukaryotic cells, and thus their characterization in model organisms would have far-reaching implications.

What is downstream of the TORCs?

The TORCs play a central role in the regulation of cell growth by signaling to a staggering number of distal

downstream processes. Recent phosphoproteomics studies have begun to illuminate the relevant phosphorylation cascades and, in addition, have suggested the existence of novel growth-related effectors downstream of TORC1. Similar studies describing the TORC2-dependent phosphoproteome are eagerly anticipated. Elucidating these downstream signaling events is both academically interesting and medically important; cell growth, like cell birth (division) and cell death, is a fundamental aspect of life, and pathological or pharmaceutical dysregulation of TOR pathways is clinically relevant. For example, unbridled ribosome biogenesis has been strongly implicated in cancer, and the motivation to understand the TORC1 effectors that modulate longevity is obvious. Thus, characterization of TOR pathways in yeast and mammals will identify potentially druggable factors whose targeting could yield therapeutic gain in any of several pathologies.

Acknowledgments

Literature Cited

Abraham, R. T., and G. J. Wiederrecht, 1996   Immunopharmacology of rapamycin. Annu. Rev. Immunol. 14: 483–510.

Adami, A., B. Garcia-Alvarez, E. Arias-Palomo, D. Barford, and O. Llorca, 2007   Structure of TOR and its complex with KOG1. Mol. Cell 27: 509–516.

Ai, W., P. G. Bertram, C. K. Tsang, T. F. Chan, and X. F. Zheng, 2002   Regulation of subtelomeric silencing during stress response. Mol. Cell 10: 1295–1305.

Alarcon, C. M., J. Heitman, and M. E. Cardenas, 1999   Protein kinase activity and identification of a toxic effector domain of the target of rapamycin TOR proteins in yeast. Mol. Biol. Cell 10: 2531–2546.

Albig, A. R., and C. J. Decker, 2001   The target of rapamycin signaling pathway regulates mRNA turnover in the yeast Saccharomyces cerevisiae. Mol. Biol. Cell 12: 3428–3438.

Andrade, M. A., and P. Bork, 1995   HEAT repeats in the Huntington's disease protein. Nat. Genet. 11: 115–116.

Araki, T., Y. Uesono, T. Oguchi, and E. A. Toh, 2005   LAS24/KOG1, a component of the TOR complex 1 (TORC1), is needed for resistance to local anesthetic tetracaine and normal distribution of actin cytoskeleton in yeast. Genes Genet. Syst. 80: 325–343.

Aronova, S., K. Wedaman, S. Anderson, J. Yates III. and T. Powers, 2007   Probing the membrane environment of the TOR kinases reveals functional interactions between TORC1, actin, and membrane trafficking in Saccharomyces cerevisiae. Mol. Biol. Cell 18: 2779–2794.

Aronova, S., K. Wedaman, P. A. Aronov, K. Fontes, K. Ramos et al., 2008   Regulation of ceramide biosynthesis by TOR complex 2. Cell Metab. 7: 148–158.

Audhya, A., and S. D. Emr, 2002   Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. Dev. Cell 2: 593–605.

Audhya, A., R. Loewith, A. B. Parsons, L. Gao, M. Tabuchi et al., 2004   Genome-wide lethality screen identifies new PI4,5P2 effectors that regulate the actin cytoskeleton. EMBO J. 23: 3747–3757.

Barbet, N. C., U. Schneider, S. B. Helliwell, I. Stansfield, M. F. Tuite et al., 1996   TOR controls translation initiation and early G1 progression in yeast. Mol. Biol. Cell 7: 25–42.

Beck, T., and M. N. Hall, 1999   The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature 402: 689–692.

Beck, T., A. Schmidt, and M. N. Hall, 1999   Starvation induces vacuolar targeting and degradation of the tryptophan permease in yeast. J. Cell Biol. 146: 1227–1238.

Beeler, T., D. Bacikova, K. Gable, L. Hopkins, C. Johnson et al., 1998   The Saccharomyces cerevisiae TSC10/YBR265w gene encoding 3-ketosphinganine reductase is identified in a screen for temperature-sensitive suppressors of the Ca2+-sensitive csg2Delta mutant. J. Biol. Chem. 273: 30688–30694.

Benjamin, D., M. Colombi, C. Moroni, and M. N. Hall, 2011   Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat. Rev. Drug Discov. 10: 868–880.

Berchtold, D., and T. C. Walther, 2009   TORC2 plasma membrane localization is essential for cell viability and restricted to a distinct domain. Mol. Biol. Cell 20: 1565–1575.

Berger, A. B., L. Decourty, G. Badis, U. Nehrbass, A. Jacquier et al., 2007   Hmo1 is required for TOR-dependent regulation of ribosomal protein gene transcription. Mol. Cell. Biol. 27: 8015–8026.

Berset, C., H. Trachsel, and M. Altmann, 1998   The TOR (target of rapamycin) signal transduction pathway regulates the stability of translation initiation factor eIF4G in the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 95: 4264–4269.

Bertram, P. G., J. H. Choi, J. Carvalho, W. Ai, C. Zeng et al., 2000   Tripartite regulation of Gln3p by TOR, Ure2p, and phosphatases. J. Biol. Chem. 275: 35727–35733.

Beugnet, A., A. R. Tee, P. M. Taylor, and C. G. Proud, 2003   Regulation of targets of mTOR (mammalian target of rapamycin) signalling by intracellular amino acid availability. Biochem. J. 372: 555–566.

Bharucha, N., J. Ma, C. J. Dobry, S. K. Lawson, Z. Yang et al., 2008   Analysis of the yeast kinome reveals a network of regulated protein localization during filamentous growth. Mol. Biol. Cell 19: 2708–2717.

Bickle, M., P. A. Delley, A. Schmidt, and M. N. Hall, 1998   Cell wall integrity modulates RHO1 activity via the exchange factor ROM2. EMBO J. 17: 2235–2245.

Binda, M., M. P. Peli-Gulli, G. Bonfils, N. Panchaud, J. Urban et al., 2009   The Vam6 GEF controls TORC1 by activating the EGO complex. Mol. Cell 35: 563–573.

Bjedov, I., J. M. Toivonen, F. Kerr, C. Slack, J. Jacobson et al., 2010   Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. Cell Metab. 11: 35–46.

Blagosklonny, M. V., and M. N. Hall, 2009   Growth and aging: a common molecular mechanism. Aging (Albany NY) 1: 357–362.

Bosotti, R., A. Isacchi, and E. L. Sonnhammer, 2000   FAT: a novel domain in PIK-related kinases. Trends Biochem. Sci. 25: 225–227.

Breitkreutz, A., H. Choi, J. R. Sharom, L. Boucher, V. Neduva et al., 2010   A global protein kinase and phosphatase interaction network in yeast. Science 328: 1043–1046.

Breslow, D. K., S. R. Collins, B. Bodenmiller, R. Aebersold, K. Simons et al., 2010   Orm family proteins mediate sphingolipid homeostasis. Nature 463: 1048–1053.

Brown, E. J., M. W. Albers, T. B. Shin, K. Ichikawa, C. T. Keith et al., 1994   A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369: 756–758.

Brown, E. J., P. A. Beal, C. T. Keith, J. Chen, T. B. Shin et al., 1995   Control of p70 s6 kinase by kinase activity of FRAP in vivo. Nature 377: 441–446.

Bultynck, G., V. L. Heath, A. P. Majeed, J. M. Galan, R. Haguenauer-Tsapis et al., 2006   Slm1 and slm2 are novel substrates of the calcineurin phosphatase required for heat stress-induced endocytosis of the yeast uracil permease. Mol. Cell. Biol. 26: 4729–4745.

Burtner, C. R., C. J. Murakami, B. K. Kennedy, and M. Kaeberlein, 2009   A molecular mechanism of chronological aging in yeast. Cell Cycle 8: 1256–1270.

Cafferkey, R., P. R. Young, M. M. McLaughlin, D. J. Bergsma, Y. Koltin et al., 1993   Dominant missense mutations in a novel yeast protein related to mammalian phosphatidylinositol 3-kinase and VPS34 abrogate rapamycin cytotoxicity. Mol. Cell. Biol. 13: 6012–6023.

Calvert, M. E., K. M. Keck, C. Ptak, J. Shabanowitz, D. F. Hunt et al., 2008   Phosphorylation by casein kinase 2 regulates Nap1 localization and function. Mol. Cell. Biol. 28: 1313–1325.

Cardenas, M. E., M. C. Cruz, M. Del Poeta, N. Chung, J. R. Perfect et al., 1999   Antifungal activities of antineoplastic agents: Saccharomyces cerevisiae as a model system to study drug action. Clin. Microbiol. Rev. 12: 583–611.

Caron, E., S. Ghosh, Y. Matsuoka, D. Ashton-Beaucage, M. Therrien et al., 2010   A comprehensive map of the mTOR signaling network. Mol. Syst. Biol. 6: 453.

Carvalho, J., and X. F. Zheng, 2003   Domains of Gln3p interacting with karyopherins, Ure2p, and the target of rapamycin protein. J. Biol. Chem. 278: 16878–16886.

Cebollero, E., and F. Reggiori, 2009   Regulation of autophagy in yeast Saccharomyces cerevisiae. Biochim. Biophys. Acta 1793: 1413–1421.

Chen, E. J., and C. A. Kaiser, 2003   LST8 negatively regulates amino acid biosynthesis as a component of the TOR pathway. J. Cell Biol. 161: 333–347.

Chen, J., X. F. Zheng, E. J. Brown, and S. L. Schreiber, 1995   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 92: 4947–4951.

Chen, Y., and D. J. Klionsky, 2011   The regulation of autophagy: unanswered questions. J. Cell Sci. 124: 161–170.

Cherkasova, V. A., and A. G. Hinnebusch, 2003   Translational control by TOR and TAP42 through dephosphorylation of eIF2alpha kinase GCN2. Genes Dev. 17: 859–872.

Chiu, M. I., H. Katz, and V. Berlin, 1994   RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proc. Natl. Acad. Sci. USA 91: 12574–12578.

Choi, J., J. Chen, S. L. Schreiber, and J. Clardy, 1996   Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. Science 273: 239–242.

Claypool, J. A., S. L. French, K. Johzuka, K. Eliason, L. Vu et al., 2004   Tor pathway regulates Rrn3p-dependent recruitment of yeast RNA polymerase I to the promoter but does not participate in alteration of the number of active genes. Mol. Biol. Cell 15: 946–956.

Cohen, A., and M. N. Hall, 2009   An amino acid shuffle activates mTORC1. Cell 136: 399–400.

Cook, M., and M. Tyers, 2007   Size control goes global. Curr. Opin. Biotechnol. 18: 341–350.

Cosentino, G. P., T. Schmelzle, A. Haghighat, S. B. Helliwell, M. N. Hall et al., 2000   Eap1p, a novel eukaryotic translation initiation factor 4E-associated protein in Saccharomyces cerevisiae. Mol. Cell. Biol. 20: 4604–4613.

Crespo, J. L., and M. N. Hall, 2002   Elucidating TOR signaling and rapamycin action: lessons from Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 66: 579–591.

Crespo, J. L., T. Powers, B. Fowler, and M. N. Hall, 2002   The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. Proc. Natl. Acad. Sci. USA 99: 6784–6789.

Cybulski, N., and M. N. Hall, 2009   TOR complex 2: a signaling pathway of its own. Trends Biochem. Sci. 34: 620–627.

Dames, S. A., J. M. Mulet, K. Rathgeb-Szabo, M. N. Hall, and S. Grzesiek, 2005   The solution structure of the FATC domain of the protein kinase target of rapamycin suggests a role for redox-dependent structural and cellular stability. J. Biol. Chem. 280: 20558–20564.

Daquinag, A., M. Fadri, S. Y. Jung, J. Qin, and J. Kunz, 2007   The yeast PH domain proteins Slm1 and Slm2 are targets of sphingolipid signaling during the response to heat stress. Mol. Cell. Biol. 27: 633–650.

Davis, E. S., A. Becker, J. Heitman, M. N. Hall, and M. B. Brennan, 1992   A yeast cyclophilin gene essential for lactate metabolism at high temperature. Proc. Natl. Acad. Sci. USA 89: 11169–11173.

De Craene, J. O., O. Soetens, and B. Andre, 2001   The Npr1 kinase controls biosynthetic and endocytic sorting of the yeast Gap1 permease. J. Biol. Chem. 276: 43939–43948.

deHart, A. K., J. D. Schnell, D. A. Allen, and L. Hicke, 2002   The conserved Pkh-Ypk kinase cascade is required for endocytosis in yeast. J. Cell Biol. 156: 241–248.

deHart, A. K., J. D. Schnell, D. A. Allen, J. Y. Tsai, and L. Hicke, 2003   Receptor internalization in yeast requires the Tor2-Rho1 signaling pathway. Mol. Biol. Cell 14: 4676–4684.

Desrivieres, S., F. T. Cooke, P. J. Parker, and M. N. Hall, 1998   MSS4, a phosphatidylinositol-4-phosphate 5-kinase required for organization of the actin cytoskeleton in Saccharomyces cerevisiae. J. Biol. Chem. 273: 15787–15793.

De Virgilio, C., 2011   The essence of yeast quiescence. FEMS Microbiol. Rev. (in press).

De Virgilio, C., and R. Loewith, 2006   Cell growth control: little eukaryotes make big contributions. Oncogene 25: 6392–6415.

Di Como, C. J., and K. T. Arndt, 1996   Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. Genes Dev. 10: 1904–1916.

Dolinski, K., S. Muir, M. Cardenas, and J. Heitman, 1997   All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 94: 13093–13098.

Dubouloz, F., O. Deloche, V. Wanke, E. Cameroni, and C. De Virgilio, 2005   The TOR and EGO protein complexes orchestrate microautophagy in yeast. Mol. Cell 19: 15–26.

Duvel, K., A. Santhanam, S. Garrett, L. Schneper, and J. R. Broach, 2003   Multiple roles of Tap42 in mediating rapamycin-induced transcriptional changes in yeast. Mol. Cell 11: 1467–1478.

Ecker, N., A. Mor, D. Journo, and H. Abeliovich, 2010   Induction of autophagic flux by amino acid deprivation is distinct from nitrogen starvation-induced macroautophagy. Autophagy 6: 879–890.

Eisenberg, T., H. Knauer, A. Schauer, S. Buttner, C. Ruckenstuhl et al., 2009   Induction of autophagy by spermidine promotes longevity. Nat. Cell Biol. 11: 1305–1314.

Fabrizio, P., F. Pozza, S. D. Pletcher, C. M. Gendron, and V. D. Longo, 2001   Regulation of longevity and stress resistance by Sch9 in yeast. Science 292: 288–290.

Fadri, M., A. Daquinag, S. Wang, T. Xue, and J. Kunz, 2005   The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2. Mol. Biol. Cell 16: 1883–1900.

Fingerman, I., V. Nagaraj, D. Norris, and A. K. Vershon, 2003 Sfp1 plays a key role in yeast ribosome biogenesis. Eukaryot. Cell 2: 1061–1068.

Flinn, R. J., Y. Yan, S. Goswami, P. J. Parker, and J. M. Backer, 2010 The late endosome is essential for mTORC1 signaling. Mol. Biol. Cell 21: 833–841.

Freckleton, G., S. I. Lippman, J. R. Broach, and S. Tavazoie, 2009 Microarray profiling of phage-display selections for rapid mapping of transcription factor-DNA interactions. PLoS Genet. 5: e1000449.

Friant, S., R. Lombardi, T. Schmelzle, M. N. Hall, and H. Riezman, 2001 Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast. EMBO J. 20: 6783–6792.

Gander, S., D. Bonenfant, P. Altermatt, D. E. Martin, S. Hauri et al., 2008 Identification of the rapamycin-sensitive phosphorylation sites within the Ser/Thr-rich domain of the yeast Npr1 protein kinase. Rapid Commun. Mass Spectrom. 22: 3743–3753.

Garcia-Martinez, J. M., and D. R. Alessi, 2008 mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). Biochem. J. 416: 375–385.

Gasch, A. P., and M. Werner-Washburne, 2002 The genomics of yeast responses to environmental stress and starvation. Funct. Integr. Genomics 2: 181–192.

Georis, I., A. Feller, J. J. Tate, T. G. Cooper, and E. Dubois, 2009a Nitrogen catabolite repression-sensitive transcription as a readout of Tor pathway regulation: the genetic background, reporter gene and GATA factor assayed determine the outcomes. Genetics 181: 861–874.

Georis, I., A. Feller, F. Vierendeels, and E. Dubois, 2009b The yeast GATA factor Gat1 occupies a central position in nitrogen catabolite repression-sensitive gene activation. Mol. Cell. Biol. 29: 3803–3815.

Gong, R., L. Li, Y. Liu, P. Wang, H. Yang et al., 2011 Crystal structure of the Gtr1p-Gtr2p complex reveals new insights into the amino acid-induced TORC1 activation. Genes Dev. 25: 1668–1673.

Goranov, A. I., and A. Amon, 2010 Growth and division: not a one-way road. Curr. Opin. Cell Biol. 22: 795–800.

Grigull, J., S. Mnaimneh, J. Pootoolal, M. D. Robinson, and T. R. Hughes, 2004 Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors. Mol. Cell. Biol. 24: 5534–5547.

Guba, M., P. von Breitenbuch, M. Steinbauer, G. Koehl, S. Flegel et al., 2002 Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat. Med. 8: 128–135.

Hall, D. B., J. T. Wade, and K. Struhl, 2006 An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in Saccharomyces cerevisiae. Mol. Cell. Biol. 26: 3672–3679.

Hall, M. N., 1996 The TOR signalling pathway and growth control in yeast. Biochem. Soc. Trans. 24: 234–239.

Han, S., M. A. Lone, R. Schneiter, and A. Chang, 2010 Orm1 and Orm2 are conserved endoplasmic reticulum membrane proteins regulating lipid homeostasis and protein quality control. Proc. Natl. Acad. Sci. USA 107: 5851–5856.

Hara, K., K. Yonezawa, Q. P. Weng, M. T. Kozlowski, C. Belham et al., 1998 Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J. Biol. Chem. 273: 14484–14494.

Hara, K., Y. Maruki, X. Long, K. Yoshino, N. Oshiro et al., 2002 Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell 110: 177–189.

Hardt, M., N. Chantaravisoot, and F. Tamanoi, 2011 Activating mutations of TOR (target of rapamycin). Genes Cells 16: 141–151.

Hardwick, J. S., F. G. Kuruvilla, J. K. Tong, A. F. Shamji, and S. L. Schreiber, 1999 Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. Proc. Natl. Acad. Sci. USA 96: 14866–14870.

Harrison, D. E., R. Strong, Z. D. Sharp, J. F. Nelson, C. M. Astle et al., 2009 Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460: 392–395.

Heitman, J., N. R. Movva, and M. N. Hall, 1991a Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 253: 905–909.

Heitman, J., N. R. Movva, P. C. Hiestand, and M. N. Hall, 1991b FK 506-binding protein proline rotamase is a target for the immunosuppressive agent FK 506 in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 88: 1948–1952.

Heitman, J., N. R. Movva, and M. N. Hall, 1992 Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. 4: 448–460.

Helliwell, S. B., P. Wagner, J. Kunz, M. Deuter-Reinhard, R. Henriquez et al., 1994 TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. Mol. Biol. Cell 5: 105–118.

Helliwell, S. B., I. Howald, N. Barbet, and M. N. Hall, 1998a TOR2 is part of two related signaling pathways coordinating cell growth in Saccharomyces cerevisiae. Genetics 148: 99–112.

Helliwell, S. B., A. Schmidt, Y. Ohya, and M. N. Hall, 1998b The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. Curr. Biol. 8: 1211–1214.

Hinnebusch, A. G., 2005 Translational regulation of GCN4 and the general amino acid control of yeast. Annu. Rev. Microbiol. 59: 407–450.

Honma, Y., A. Kitamura, R. Shioda, H. Maruyama, K. Ozaki et al., 2006 TOR regulates late steps of ribosome maturation in the nucleoplasm via Nog1 in response to nutrients. EMBO J. 25: 3832–3842.

Hou, H., K. Subramanian, T. J. LaGrassa, D. Markgraf, L. E. Dietrich et al., 2005 The DHHC protein Pfa3 affects vacuole-associated palmitoylation of the fusion factor Vac8. Proc. Natl. Acad. Sci. USA 102: 17366–17371.

Huber, A., B. Bodenmiller, A. Uotila, M. Stahl, S. Wanka et al., 2009 Characterization of the rapamycin-sensitive phosphoproteome reveals that Sch9 is a central coordinator of protein synthesis. Genes Dev. 23: 1929–1943.

Huber, A., S. L. French, H. Tekotte, S. Yerlikaya, M. Stahl et al., 2011 Sch9 regulates ribosome biogenesis via Stb3, Dot6 and Tod6 and the histone deacetylase complex RPD3L. EMBO J. 30: 3052–3064.

Inoue, Y., and D. J. Klionsky, 2010 Regulation of macroautophagy in Saccharomyces cerevisiae. Semin. Cell Dev. Biol. 21: 664–670.

Jacinto, E., and A. Lorberg, 2008 TOR regulation of AGC kinases in yeast and mammals. Biochem. J. 410: 19–37.

Jacinto, E., B. Guo, K. T. Arndt, T. Schmelzle, and M. N. Hall, 2001 TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. Mol. Cell 8: 1017–1026.

Jacinto, E., R. Loewith, A. Schmidt, S. Lin, M. A. Ruegg et al., 2004 Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat. Cell Biol. 6: 1122–1128.

Jia, K., D. Chen, and D. L. Riddle, 2004 The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. Development 131: 3897–3906.

Jiang, Y., and J. R. Broach, 1999 Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. EMBO J. 18: 2782–2792.

Jones, K. T., E. R. Greer, D. Pearce, and K. Ashrafi, 2009 Rictor/TORC2 regulates Caenorhabditis elegans fat storage, body size, and development through sgk-1. PLoS Biol. 7: e60.

Jordens, J., V. Janssens, S. Longin, I. Stevens, E. Martens et al., 2006 The protein phosphatase 2A phosphatase activator is a novel peptidyl-prolyl cis/trans-isomerase. J. Biol. Chem. 281: 6349–6357.

Jorgensen, P., J. L. Nishikawa, B. J. Breitkreutz, and M. Tyers, 2002 Systematic identification of pathways that couple cell growth and division in yeast. Science 297: 395–400.

Jorgensen, P., I. Rupes, J. R. Sharom, L. Schneper, J. R. Broach et al., 2004 A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. Genes Dev. 18: 2491–2505.

Kaeberlein, M., and B. K. Kennedy, 2011 Hot topics in aging research: protein translation and TOR signaling, 2010. Aging Cell 10: 185–190.

Kaeberlein, M., R. W. Powers III. K. K. Steffen, E. A. Westman, D. Hu et al., 2005 Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 310: 1193–1196.

Kaizu, K., S. Ghosh, Y. Matsuoka, H. Moriya, Y. Shimizu-Yoshida et al., 2010 A comprehensive molecular interaction map of the budding yeast cell cycle. Mol. Syst. Biol. 6: 415.

Kamada, Y., Y. Fujioka, N. N. Suzuki, F. Inagaki, S. Wullschleger et al., 2005 Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. Mol. Cell. Biol. 25: 7239–7248.

Kamada, Y., K. Yoshino, C. Kondo, T. Kawamata, N. Oshiro et al., 2010 Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol. Cell. Biol. 30: 1049–1058.

Kapahi, P., B. M. Zid, T. Harper, D. Koslover, V. Sapin et al., 2004 Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr. Biol. 14: 885–890.

Kapahi, P., D. Chen, A. N. Rogers, S. D. Katewa, P. W. Li et al., 2010 With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. Cell Metab. 11: 453–465.

Keith, C. T., and S. L. Schreiber, 1995 PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. Science 270: 50–51.

Kijanska, M., I. Dohnal, W. Reiter, S. Kaspar, I. Stoffel et al., 2010 Activation of Atg1 kinase in autophagy by regulated phosphorylation. Autophagy 6: 1168–1178.

Kim, D. H., D. D. Sarbassov, S. M. Ali, J. E. King, R. R. Latek et al., 2002 mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110: 163–175.

Kim, E., P. Goraksha-Hicks, L. Li, T. P. Neufeld, and K. L. Guan, 2008 Regulation of TORC1 by Rag GTPases in nutrient response. Nat. Cell Biol. 10: 935–945.

Kim, J., and Guan, K. L. (2011). Amino acid signaling in TOR activation. Annu. Rev. Biochem. 80: 1001–1032.

Kogan, K., E. D. Spear, C. A. Kaiser, and D. Fass, 2010 Structural conservation of components in the amino acid sensing branch of the TOR pathway in yeast and mammals. J. Mol. Biol. 402: 388–398.

Koltin, Y., L. Faucette, D. J. Bergsma, M. A. Levy, R. Cafferkey et al., 1991 Rapamycin sensitivity in Saccharomyces cerevisiae is mediated by a peptidyl-prolyl cis-trans isomerase related to human FK506-binding protein. Mol. Cell. Biol. 11: 1718–1723.

Komeili, A., K. P. Wedaman, E. K. O'Shea, and T. Powers, 2000 Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. J. Cell Biol. 151: 863–878.

Krause, S. A., and J. V. Gray, 2002 The protein kinase C pathway is required for viability in quiescence in Saccharomyces cerevisiae. Curr. Biol. 12: 588–593.

Kuepfer, L., M. Peter, U. Sauer, and J. Stelling, 2007 Ensemble modeling for analysis of cell signaling dynamics. Nat. Biotechnol. 25: 1001–1006.

Kunz, J., and M. N. Hall, 1993 Cyclosporin A, FK506 and rapamycin: more than just immunosuppression. Trends Biochem. Sci. 18: 334–338.

Kunz, J., R. Henriquez, U. Schneider, M. Deuter-Reinhard, N. R. Movva et al., 1993 Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. Cell 73: 585–596.

Kunz, J., U. Schneider, I. Howald, A. Schmidt, and M. N. Hall, 2000 HEAT repeats mediate plasma membrane localization of Tor2p in yeast. J. Biol. Chem. 275: 37011–37020.

Kuranda, K., V. Leberre, S. Sokol, G. Palamarczyk, and J. Francois, 2006 Investigating the caffeine effects in the yeast Saccharomyces cerevisiae brings new insights into the connection between TOR, PKC and Ras/cAMP signalling pathways. Mol. Microbiol. 61: 1147–1166.

Laferte, A., E. Favry, A. Sentenac, M. Riva, C. Carles et al., 2006 The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes Dev. 20: 2030–2040.

Lee, J., R. D. Moir, and I. M. Willis, 2009 Regulation of RNA polymerase III transcription involves SCH9-dependent and SCH9-independent branches of the target of rapamycin (TOR) pathway. J. Biol. Chem. 284: 12604–12608.

Lee, T. I., N. J. Rinaldi, F. Robert, D. T. Odom, Z. Bar-Joseph et al., 2002 Transcriptional regulatory networks in Saccharomyces cerevisiae. Science 298: 799–804.

Lempiainen, H., and D. Shore, 2009 Growth control and ribosome biogenesis. Curr. Opin. Cell Biol. 21: 855–863.

Lempiainen, H., A. Uotila, J. Urban, I. Dohnal, G. Ammerer et al., 2009 Sfp1 interaction with TORC1 and Mrs6 reveals feedback regulation on TOR signaling. Mol. Cell 33: 704–716.

Levin, D. E., 2005 Cell wall integrity signaling in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 69: 262–291.

Li, H., C. K. Tsang, M. Watkins, P. G. Bertram, and X. F. Zheng, 2006 Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. Nature 442: 1058–1061.

Liko, D., M. G. Slattery, and W. Heideman, 2007 Stb3 binds to ribosomal RNA processing element motifs that control transcriptional responses to growth in Saccharomyces cerevisiae. J. Biol. Chem. 282: 26623–26628.

Liko, D., M. K. Conway, D. S. Grunwald, and W. Heideman 2010 Stb3 plays a role in the glucose-induced transition from quiescence to growth in Saccharomyces cerevisiae. Genetics 185: 797–810.

Lin, C. H., J. A. MacGurn, T. Chu, C. J. Stefan, and S. D. Emr, 2008 Arrestin-related ubiquitin-ligase adaptors regulate endocytosis and protein turnover at the cell surface. Cell 135: 714–725.

Lippman, S. I., and J. R. Broach, 2009 Protein kinase A and TORC1 activate genes for ribosomal biogenesis by inactivating repressors encoded by Dot6 and its homolog Tod6. Proc. Natl. Acad. Sci. USA 106: 19928–19933.

Liu, K., X. Zhang, R. L. Lester, and R. C. Dickson, 2005 The sphingoid long chain base phytosphingosine activates AGC-type protein kinases in Saccharomyces cerevisiae including Ypk1, Ypk2, and Sch9. J. Biol. Chem. 280: 22679–22687.

Liu, Z., and R. A. Butow, 2006 Mitochondrial retrograde signaling. Annu. Rev. Genet. 40: 159–185.

Liu, Z., T. Sekito, M. Spirek, J. Thornton, and R. A. Butow, 2003 Retrograde signaling is regulated by the dynamic interaction between Rtg2p and Mks1p. Mol. Cell 12: 401–411.

Ljungdahl, P. O., 2009 Amino-acid-induced signalling via the SPS-sensing pathway in yeast. Biochem. Soc. Trans. 37: 242–247.

Loewith, R., 2010 TORC1 signaling in budding yeast, pp. 147–176 in *The Enzymes*, edited by M. N. Hall, and F. Tamanoi. Academic Press/Elsevier, New York.

Loewith, R., 2011 A brief history of TOR. Biochem. Soc. Trans. 39: 437–442.

Loewith, R., and M. N. Hall, 2004 TOR signaling in yeast: temporal and spatial control of cell growth, pp. 139–166 in *Cell Growth: Control of Cell Size*, edited by M. N. Hall, M. Raff, and G. Thomas. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Loewith, R., E. Jacinto, S. Wullschleger, A. Lorberg, J. L. Crespo et al., 2002 Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol. Cell 10: 457–468.

Lorenz, M. C., and J. Heitman, 1995 TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. J. Biol. Chem. 270: 27531–27537.

Luo, X., N. Talarek, and C. De Virgilio, 2011 Initiation of the yeast G 0 program requires Igo1 and Igo2, which antagonize activation of decapping of specific nutrient-regulated mRNAs. RNA Biol. 8: 14–17.

Madeo, F., T. Eisenberg, S. Buttner, C. Ruckenstuhl, and G. Kroemer, 2010a Spermidine: a novel autophagy inducer and longevity elixir. Autophagy 6: 160–162.

Madeo, F., N. Tavernarakis, and G. Kroemer, 2010b Can autophagy promote longevity? Nat. Cell Biol. 12: 842–846.

Magasanik, B., and C. A. Kaiser, 2002 Nitrogen regulation in Saccharomyces cerevisiae. Gene 290: 1–18.

Marion, R. M., A. Regev, E. Segal, Y. Barash, D. Koller et al., 2004 Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. Proc. Natl. Acad. Sci. USA 101: 14315–14322.

Martin, D. E., A. Soulard, and M. N. Hall, 2004 TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell 119: 969–979.

Matsuo, T., Y. Kubo, Y. Watanabe, and M. Yamamoto, 2003 Schizosaccharomyces pombe AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. EMBO J. 22: 3073–3083.

Michels, A. A., 2011 MAF1: a new target of mTORC1. Biochem. Soc. Trans. 39: 487–491.

Morselli, E., G. Marino, M. V. Bennetzen, T. Eisenberg, E. Megalou et al., 2011 Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J. Cell Biol. 192: 615–629.

Mulet, J. M., D. E. Martin, R. Loewith, and M. N. Hall, 2006 Mutual antagonism of target of rapamycin and calcineurin signaling. J. Biol. Chem. 281: 33000–33007.

Nakashima, A., Y. Maruki, Y. Imamura, C. Kondo, T. Kawamata et al., 2008 The yeast Tor signaling pathway is involved in G2/M transition via polo-kinase. PLoS ONE 3: e2223.

Nakatogawa, H., K. Suzuki, Y. Kamada, and Y. Ohsumi, 2009 Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol. 10: 458–467.

Nikko, E., and H. R. Pelham, 2009 Arrestin-mediated endocytosis of yeast plasma membrane transporters. Traffic 10: 1856–1867.

Nikko, E., J. A. Sullivan, and H. R. Pelham, 2008 Arrestin-like proteins mediate ubiquitination and endocytosis of the yeast metal transporter Smf1. EMBO Rep. 9: 1216–1221.

O'Donnell, A. F., A. Apffel, R. G. Gardner, and M. S. Cyert, 2010 Alpha-arrestins Aly1 and Aly2 regulate intracellular trafficking in response to nutrient signaling. Mol. Biol. Cell 21: 3552–3566.

Oficjalska-Pham, D., O. Harismendy, W. J. Smagowicz, A. Gonzalez de Peredo, M. Boguta et al., 2006 General repression of RNA polymerase III transcription is triggered by protein phosphatase type 2A-mediated dephosphorylation of Maf1. Mol. Cell 22: 623–632.

Ohne, Y., T. Takahara, R. Hatakeyama, T. Matsuzaki, M. Noda et al., 2008 Isolation of hyperactive mutants of mammalian target of rapamycin. J. Biol. Chem. 283: 31861–31870.

Pan, Y., E. A. Schroeder, A. Ocampo, A. Barrientos, and G. S. Shadel, 2011 Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. Cell Metab. 13: 668–678.

Pearce, L. R., D. Komander, and D. R. Alessi, 2010 The nuts and bolts of AGC protein kinases. Nat. Rev. Mol. Cell Biol. 11: 9–22.

Perry, J., and N. Kleckner, 2003 The ATRs, ATMs, and TORs are giant HEAT repeat proteins. Cell 112: 151–155.

Polak, P., and M. N. Hall, 2009 mTOR and the control of whole body metabolism. Curr. Opin. Cell Biol. 21: 209–218.

Powers, R. W. III. M. Kaeberlein, S. D. Caldwell, B. K. Kennedy, and S. Fields, 2006 Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. 20: 174–184.

Powers, T., 2007 TOR signaling and S6 kinase 1: yeast catches up. Cell Metab. 6: 1–2.

Powers, T., and P. Walter, 1999 Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in Saccharomyces cerevisiae. Mol. Biol. Cell 10: 987–1000.

Powers, T., S. Aranova, and B. Niles, 2010 TORC2 and spingolipid biosynthesis and signaling: lessons from budding yeast, pp. 177–197 in *The Enzymes: Structure, Function and Regulation of TOR Complexes from Yeast to Mammals*, edited by M. N. Hall and F. Tamanoi. Academic Press, San Diego.

Ramachandran, V., and P. K. Herman, 2011 Antagonistic interactions between the cAMP-dependent protein kinase and Tor signaling pathways modulate cell growth in *Saccharomyces cerevisiae*. Genetics 187: 441–454.

Reina, J. H., T. N. Azzouz, and N. Hernandez, 2006 Maf1, a new player in the regulation of human RNA polymerase III transcription. PLoS ONE 1: e134.

Reinders, J., R. P. Zahedi, N. Pfanner, C. Meisinger, and A. Sickmann, 2006 Toward the complete yeast mitochondrial proteome: multidimensional separation techniques for mitochondrial proteomics. J. Proteome Res. 5: 1543–1554.

Reinke, A., S. Anderson, J. M. McCaffery, J. Yates III. S. Aronova et al., 2004 TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in Saccharomyces cerevisiae. J. Biol. Chem. 279: 14752–14762.

Reinke, A., J. C. Chen, S. Aronova, and T. Powers, 2006 Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p. J. Biol. Chem. 281: 31616–31626.

Reiter, A., R. Steinbauer, A. Philippi, J. Gerber, H. Tschochner et al., 2011 Reduction in ribosomal protein synthesis is sufficient to explain major effects on ribosome production after short-term TOR inactivation in Saccharomyces cerevisiae. Mol. Cell. Biol. 31: 803–817.

Roelants, F. M., P. D. Torrance, N. Bezman, and J. Thorner, 2002 Pkh1 and Pkh2 differentially phosphorylate and activate Ypk1 and Ykr2 and define protein kinase modules required for maintenance of cell wall integrity. Mol. Biol. Cell 13: 3005–3028.

Roelants, F. M., P. D. Torrance, and J. Thorner, 2004 Differential roles of PDK1- and PDK2-phosphorylation sites in the yeast AGC kinases Ypk1, Pkc1 and Sch9. Microbiology 150: 3289–3304.

Rohde, J., J. Heitman, and M. E. Cardenas, 2001 The TOR kinases link nutrient sensing to cell growth. J. Biol. Chem. 276: 9583–9586.

Rudra, D., Y. Zhao, and J. R. Warner, 2005   Central role of Ifh1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. EMBO J. 24: 533–542.

Sabatini, D. M., H. Erdjument-Bromage, M. Lui, P. Tempst, and S. H. Snyder, 1994   RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78: 35–43.

Sabers, C. J., M. M. Martin, G. J. Brunn, J. M. Williams, F. J. Dumont et al., 1995   Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. J. Biol. Chem. 270: 815–822.

Sancak, Y., T. R. Peterson, Y. D. Shaul, R. A. Lindquist, C. C. Thoreen et al., 2008   The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 320: 1496–1501.

Sancak, Y., L. Bar-Peled, R. Zoncu, A. L. Markhard, S. Nada et al., 2010   Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141: 290–303.

Sarbassov, D. D., S. M. Ali, D. H. Kim, D. A. Guertin, R. R. Latek et al., 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.

Schawalder, S. B., M. Kabani, I. Howald, U. Choudhury, M. Werner et al., 2004   Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature 432: 1058–1061.

Schmelzle, T., and M. N. Hall, 2000   TOR, a central controller of cell growth. Cell 103: 253–262.

Schmelzle, T., S. B. Helliwell, and M. N. Hall, 2002   Yeast protein kinases and the RHO1 exchange factor TUS1 are novel components of the cell integrity pathway in yeast. Mol. Cell. Biol. 22: 1329–1339.

Schmelzle, T., T. Beck, D. E. Martin, and M. N. Hall, 2004   Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. Mol. Cell. Biol. 24: 338–351.

Schmidt, A., J. Kunz, and M. N. Hall, 1996   TOR2 is required for organization of the actin cytoskeleton in yeast. Proc. Natl. Acad. Sci. USA 93: 13780–13785.

Schmidt, A., M. Bickle, T. Beck, and M. N. Hall, 1997   The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. Cell 88: 531–542.

Schmidt, A., T. Beck, A. Koller, J. Kunz, and M. N. Hall, 1998   The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. EMBO J. 17: 6924–6931.

Schreiber, S. L., 1991   Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 251: 283–287.

Shamji, A. F., F. G. Kuruvilla, and S. L. Schreiber, 2000   Partitioning the transcriptional program induced by rapamycin among the effectors of the Tor proteins. Curr. Biol. 10: 1574–1581.

Shen, C., C. S. Lancaster, B. Shi, H. Guo, P. Thimmaiah et al., 2007   TOR signaling is a determinant of cell survival in response to DNA damage. Mol. Cell. Biol. 27: 7007–7017.

Shen, H., and M. R. Green, 2006   RS domains contact splicing signals and promote splicing by a common mechanism in yeast through humans. Genes Dev. 20: 1755–1765.

Shin, C. S., S. Y. Kim, and W. K. Huh, 2009   TORC1 controls degradation of the transcription factor Stp1, a key effector of the SPS amino-acid-sensing pathway in Saccharomyces cerevisiae. J. Cell Sci. 122: 2089–2099.

Singh, J., and M. Tyers, 2009   A Rab escort protein integrates the secretion system with TOR signaling and ribosome biogenesis. Genes Dev. 23: 1944–1958.

Soetens, O., J. O. De Craene, and B. Andre, 2001   Ubiquitin is required for sorting to the vacuole of the yeast general amino acid permease, Gap1. J. Biol. Chem. 276: 43949–43957.

Soukas, A. A., E. A. Kane, C. E. Carr, J. A. Melo, and G. Ruvkun, 2009   Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans. Genes Dev. 23: 496–511.

Soulard, A., A. Cohen, and M. N. Hall, 2009   TOR signaling in invertebrates. Curr. Opin. Cell Biol. 21: 825–836.

Soulard, A., A. Cremonesi, S. Moes, F. Schutz, P. Jeno et al., 2010   The rapamycin-sensitive phosphoproteome reveals that TOR controls protein kinase A toward some but not all substrates. Mol. Biol. Cell 21: 3475–3486.

Stan, R., M. M. McLaughlin, R. Cafferkey, R. K. Johnson, M. Rosenberg et al., 1994   Interaction between FKBP12-rapamycin and TOR involves a conserved serine residue. J. Biol. Chem. 269: 32027–32030.

Steffen, K. K., V. L. MacKay, E. O. Kerr, M. Tsuchiya, D. Hu et al., 2008   Yeast life span extension by depletion of 60s ribosomal subunits is mediated by Gcn4. Cell 133: 292–302.

Sturgill, T. W., and M. N. Hall, 2009   Activating mutations in TOR are in similar structures as oncogenic mutations in PI3KCalpha. ACS Chem. Biol. 4: 999–1015.

Sturgill, T. W., A. Cohen, M. Diefenbacher, M. Trautwein, D. E. Martin et al., 2008   TOR1 and TOR2 have distinct locations in live cells. Eukaryot. Cell 7: 1819–1830.

Sun, Y., R. Taniguchi, D. Tanoue, T. Yamaji, H. Takematsu et al., 2000   Sli2 (Ypk1), a homologue of mammalian protein kinase SGK, is a downstream kinase in the sphingolipid-mediated signaling pathway of yeast. Mol. Cell. Biol. 20: 4411–4419.

Tabuchi, M., A. Audhya, A. B. Parsons, C. Boone, and S. D. Emr, 2006   The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. Mol. Cell. Biol. 26: 5861–5875.

Talarek, N., E. Cameroni, M. Jaquenoud, X. Luo, S. Bontron et al., 2010   Initiation of the TORC1-regulated G0 program requires Igo1/2, which license specific mRNAs to evade degradation via the 5′-3′ mRNA decay pathway. Mol. Cell 38: 345–355.

Tanida, I., M. Yanagida, N. Maki, S. Yagi, F. Namiyama et al., 1991   Yeast cyclophilin-related gene encodes a nonessential second peptidyl-prolyl cis-trans isomerase associated with the secretory pathway. Transplant. Proc. 23: 2856–2861.

Tate, J. J., I. Georis, A. Feller, E. Dubois, and T. G. Cooper, 2009   Rapamycin-induced Gln3 dephosphorylation is insufficient for nuclear localization: Sit4 and PP2A phosphatases are regulated and function differently. J. Biol. Chem. 284: 2522–2534.

Tate, J. J., I. Georis, E. Dubois, and T. G. Cooper, 2010   Distinct phosphatase requirements and GATA factor responses to nitrogen catabolite repression and rapamycin treatment in Saccharomyces cerevisiae. J. Biol. Chem. 285: 17880–17895.

Teixeira, M. C., T. R. Cabrito, Z. M. Hanif, R. C. Vargas, S. Tenreiro et al., 2010   Yeast response and tolerance to polyamine toxicity involving the drug H+ antiporter Qdr3 and the transcription factors Yap1 and Gcn4. Microbiology 157: 945–956.

Thomas, G., and M. N. Hall, 1997   TOR signalling and control of cell growth. Curr. Opin. Cell Biol. 9: 782–787.

Torres, J., C. J. Di Como, E. Herrero, and M. A. De La Torre-Ruiz, 2002   Regulation of the cell integrity pathway by rapamycin-sensitive TOR function in budding yeast. J. Biol. Chem. 277: 43495–43504.

Tschochner, H., and E. Hurt, 2003   Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol. 13: 255–263.

Unal, E., B. Kinde, and A. Amon, 2011   Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. Science 332: 1554–1557.

Upadhya, R., J. Lee, and I. M. Willis, 2002   Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. Mol. Cell 10: 1489–1494.

Urano, J., T. Sato, T. Matsuo, Y. Otsubo, M. Yamamoto *et al.*, 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.

Urban, J., A. Soulard, A. Huber, S. Lippman, D. Mukhopadhyay *et al.*, 2007 Sch9 is a major target of TORC1 in Saccharomyces cerevisiae. Mol. Cell 26: 663–674.

Van Belle, D., and B. Andre, 2001 A genomic view of yeast membrane transporters. Curr. Opin. Cell Biol. 13: 389–398.

Vannini, A., R. Ringel, A. G. Kusser, O. Berninghausen, G. A. Kassavetis *et al.*, 2010 Molecular basis of RNA polymerase III transcription repression by Maf1. Cell 143: 59–70.

Vellai, T., K. Takacs-Vellai, Y. Zhang, A. L. Kovacs, L. Orosz *et al.*, 2003 Genetics: influence of TOR kinase on lifespan in C. elegans. Nature 426: 620.

Wade, J. T., D. B. Hall, and K. Struhl, 2004 The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 432: 1054–1058.

Wanke, V., I. Pedruzzi, E. Cameroni, F. Dubouloz, and C. De Virgilio, 2005 Regulation of G0 entry by the Pho80-Pho85 cyclin-CDK complex. EMBO J. 24: 4271–4278.

Wanke, V., E. Cameroni, A. Uotila, M. Piccolis, J. Urban *et al.*, 2008 Caffeine extends yeast lifespan by targeting TORC1. Mol. Microbiol. 69: 277–285.

Warner, J. R., 1999 The economics of ribosome biosynthesis in yeast. Trends Biochem. Sci. 24: 437–440.

Wedaman, K. P., A. Reinke, S. Anderson, J. Yates III. J. M. McCaffery *et al.*, 2003 Tor kinases are in distinct membrane-associated protein complexes in Saccharomyces cerevisiae. Mol. Biol. Cell 14: 1204–1220.

Wei, M., P. Fabrizio, J. Hu, H. Ge, C. Cheng *et al.*, 2008 Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet. 4: e13.

Wei, Y., and X. S. Zheng, 2010 Maf1 regulation: a model of signal transduction inside the nucleus. Nucleus 1: 162–165.

Wei, Y., C. K. Tsang, and X. F. Zheng, 2009 Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1. EMBO J. 28: 2220–2230.

Weindruch, R., and R. L. Walford, 1988 *The Retardation of Aging and Disease by Dietary Restriction*. Charles C Thomas, Springfield, IL.

Wiederrecht, G., L. Brizuela, K. Elliston, N. H. Sigal, and J. J. Siekierka, 1991 FKB1 encodes a nonessential FK 506-binding protein in Saccharomyces cerevisiae and contains regions suggesting homology to the cyclophilins. Proc. Natl. Acad. Sci. USA 88: 1029–1033.

Wullschleger, S., R. Loewith, W. Oppliger, and M. N. Hall, 2005 Molecular organization of target of rapamycin complex 2. J. Biol. Chem. 280: 30697–30704.

Wullschleger, S., R. Loewith, and M. N. Hall, 2006 TOR signaling in growth and metabolism. Cell 124: 471–484.

Yan, G., X. Shen, and Y. Jiang, 2006 Rapamycin activates Tap42-associated phosphatases by abrogating their association with Tor complex 1. EMBO J. 25: 3546–3555.

Yang, Z., and D. J. Klionsky, 2010 Eaten alive: a history of macroautophagy. Nat. Cell Biol. 12: 814–822.

Yeh, Y. Y., K. Wrasman, and P. K. Herman, 2010 Autophosphorylation within the Atg1 activation loop is required for both kinase activity and the induction of autophagy in *Saccharomyces cerevisiae*. Genetics 185: 871–882.

Yip, C. K., K. Murata, T. Walz, D. M. Sabatini, and S. A. Kang, 2010 Structure of the human mTOR complex I and its implications for rapamycin inhibition. Mol. Cell 38: 768–774.

Yorimitsu, T., C. He, K. Wang, and D. J. Klionsky, 2009 Tap42-associated protein phosphatase type 2A negatively regulates induction of autophagy. Autophagy 5: 616–624.

Zaragoza, D., A. Ghavidel, J. Heitman, and M. C. Schultz, 1998 Rapamycin induces the G0 program of transcriptional repression in yeast by interfering with the TOR signaling pathway. Mol. Cell. Biol. 18: 4463–4470.

Zheng, X. F., D. Florentino, J. Chen, G. R. Crabtree, and S. L. Schreiber, 1995 TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. Cell 82: 121–130.

Zheng, Y., and Y. Jiang, 2005 The yeast phosphotyrosyl phosphatase activator is part of the Tap42-phosphatase complexes. Mol. Biol. Cell 16: 2119–2127.

Zhu, C., K. J. Byers, R. P. McCord, Z. Shi, M. F. Berger *et al.*, 2009 High-resolution DNA-binding specificity analysis of yeast transcription factors. Genome Res. 19: 556–566.

Zinzalla, V., T. W. Sturgill, and M. N. Hall, 2010 TOR complexes: composition, structure and phosphorylation, pp. 1–20 in *The Enzymes: Structure, Function and Regulation of TOR Complexes from Yeast to Mammals*, edited by M. N. Hall and F. Tamanoi. Academic Press, San Diego.

Zinzalla, V., D. Stracka, W. Oppliger, and M. N. Hall, 2011 Activation of mTORC2 by association with the ribosome. Cell 144: 757–768.

Zoncu, R., A. Efeyan, and D. M. Sabatini, 2011 mTOR: from growth signal integration to cancer, diabetes and ageing. Nat. Rev. Mol. Cell Biol. 12: 21–35.

Zurita-Martinez, S. A., and M. E. Cardenas, 2005 Tor and cyclic AMP-protein kinase A: two parallel pathways regulating expression of genes required for cell growth. Eukaryot. Cell 4: 63–71.

Zurita-Martinez, S. A., R. Puria, X. Pan, J. D. Boeke, and M. E. Cardenas, 2007 Efficient Tor signaling requires a functional class C Vps protein complex in *Saccharomyces cerevisiae*. Genetics 176: 2139–2150.

Communicating editor: J. Thorner

JB Review
Evolutionarily conserved regulation of TOR signalling

Terunao Takahara[1,2] and Tatsuya Maeda[1,]*

[1]Institute of Molecular and Cellular Biosciences, The University of Tokyo; and [2]Graduate School of Bioagricultural Sciences, Nagoya University

*Tatsuya Maeda, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Tel: +81-3-5841-7820, Fax: +81-3-5841-7899, email: maeda@iam.u-tokyo.ac.jp

The target of rapamycin (TOR) is an evolutionarily conserved protein kinase that regulates cell growth in response to various environmental as well as intracellular cues through the formation of 2 distinct TOR complexes (TORC), TORC1 and TORC2. Dysregulation of TORC1 and TORC2 activity is closely associated with various diseases, including diabetes, cancer and neurodegenerative disorders. Over the past few years, new regulatory mechanisms of TORC1 and TORC2 activity have been elucidated. Furthermore, recent advances in the study of TOR inhibitors have revealed previously unrecognized cellular functions of TORC1. In this review, we briefly summarize the current understanding of the evolutionarily conserved TOR signalling from upstream regulators to downstream events.

The Structure and Complex Formation of TOR

The target of rapamycin (TOR) was originally identified as the cellular target of the immunosuppressant and anti-cancer drug rapamycin. TOR is a conserved serine/threonine kinase that belongs to the phosphatidylinositol 3-kinase (PI3K)-related kinase (PIKK) family, which includes ATR, ATM and DNA-PKcs. Structurally, TOR possesses HEAT repeats at the N-terminal region and the FAT, kinase catalytic and FATC domains at the C-terminal region (Fig. 1A). Rapamycin, together with its cellular receptor 12-kDa FK506-binding protein (FKBP12), binds to the FKBP12-rapamycin binding (FRB) domain located between the FAT and kinase catalytic domains on TOR and thus allosterically inhibits the activity of TOR (*1*) (Fig. 1A). The precise mechanism of this inhibition remains unclear.

TOR exerts its function by forming two functionally and structurally distinct complexes, called TOR complex 1 (TORC1) and TORC2. Mammalian TORC1 (mTORC1) comprises of mammalian (or mechanistic) TOR (mTOR), the regulatory-associated protein of mTOR (Raptor), mammalian LST8 (mLst8, also known as GβL), the DEP domain-containing mTOR-interacting protein (Deptor), and proline-rich Akt substrate of 40-kDa (PRAS40). On the other hand, mTORC2 contains mTOR, the rapamycin-insensitive companion of mTOR (Rictor), mLst8, mSin1, Deptor and protein observed with Rictor 1 and 2 (Protor1/2) (Fig. 1B). Both mTORC1 and mTORC2, and most likely TORC1 and TORC2 in general, function as multimers (*1*). The structural components of both these TORCs are highly conserved from yeast to mammals (Table I). A prominent difference between these two complexes is their rapamycin sensitivity. TORC1 is sensitive to rapamycin treatment, whereas TORC2 is resistant to rapamycin treatment (*1*). However, mTORC2 is sensitive to long-term (>24 h) rapamycin treatment, and this sensitivity is most likely achieved through inhibition of nascent mTOR molecules from assembling with mTORC2 components such as Rictor and mSin1 (*2*). The rapamycin sensitivity of mTORC2 is cell-type dependent, and the factors that bring about this difference are unknown.

Although it was believed that rapamycin inhibits TORC1 activity completely, development of newer ATP-competitive mTOR inhibitors (Torin1, Ku-0063794, PP242 *etc.*), which inhibit mTOR kinase activity by competing with ATP for binding to the kinase catalytic site (Fig. 1A), revealed that rapamycin only partially inhibits mTORC1 activity (*3*). In fact, even
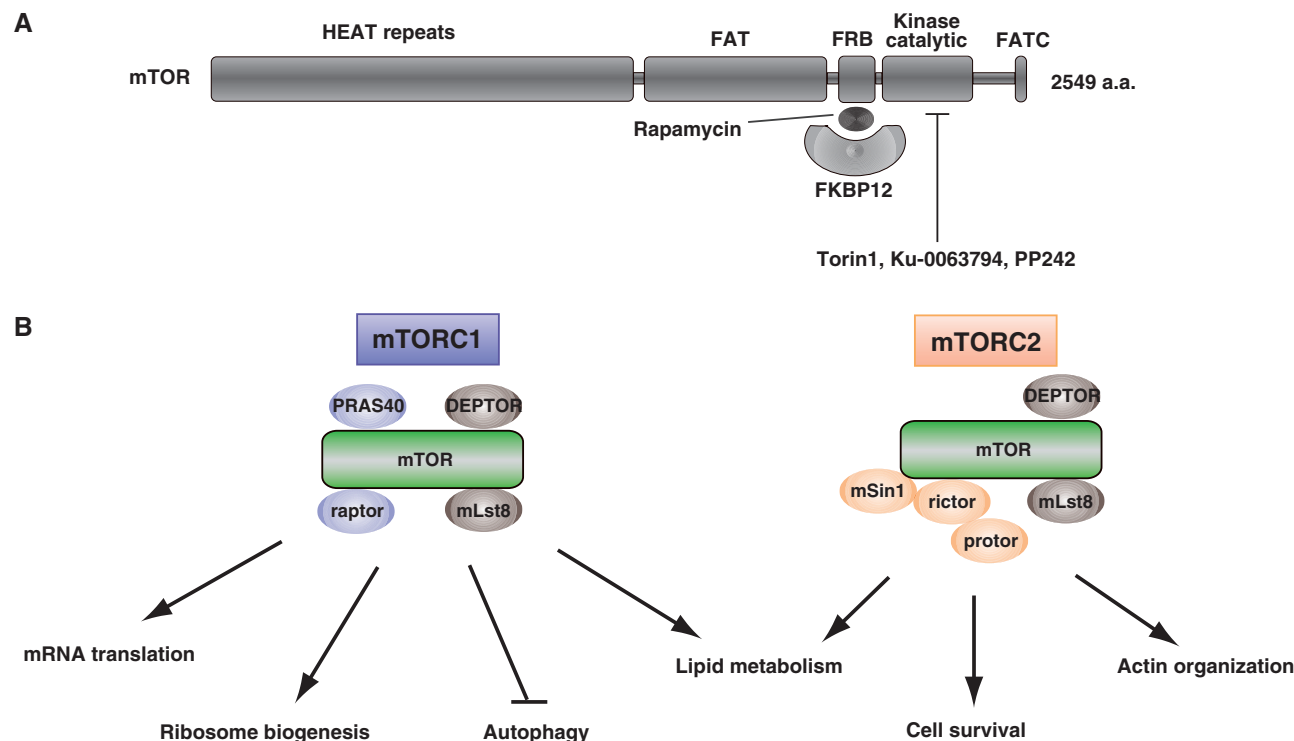
Fig. 1 Schematic representation of the structure and complex formation of mTOR. (**A**) The domain structure of mTOR. Rapamycin, together with FKBP12, binds to the FRB domain of mTOR, and inhibits mTOR (mTORC1) allosterically. ATP-competitive inhibitors (Torin1, Ku-0063794, PP242) directly inhibit kinase activity. FAT and FATC domains are conserved in PIKK family members. (**B**) Two distinct mTOR complexes (mTORC1 and mTORC2) and their functions. Specific components of mTORC1 and mTORC2 are shown in colour.

Table I. The components of TORC1 and TORC2.

	H. sapiens	*S. cerevisiae*	*S. pombe*	*D. melanogaster*	*C. elegans*
TORC1	mTOR	Tor1 (Tor2)	Tor2 (Tor1)	TOR1	TOR
	Raptor	Kog1	Mip1	Raptor	daf-15
	mLst8	Lst8	Wat1/Pop3	Lst8	lst-8
	PRAS40			Lobe	
	DEPTOR				
		Tco89	Tco89		
			Tco1		
TORC2	mTOR	Tor2	Tor1	TOR	TOR
	Rictor	Avo3/Tsc11	Ste20	Rictor	rict-1
	mSin1	Avo1	Sin1	Sin1	sinh-1
	mLst8	Lst8	Wat1/Pop3	Lst8	lst-8
	Protor/PRR5	Bit61, Bit2	Bit61		
	DEPTOR				
		Avo2			

the phosphorylation status of eIF4E-binding protein 1 (4E-BP1), one of the best-characterized substrates of mTORC1, is refractory to inhibition by rapamycin, but is sensitive to the ATP-competitive inhibitors of mTOR, indicating that mTORC1 regulates far more events than previously believed (*3*). Discovery of the two TOR complexes (TORC) and rapamycin-insensitive TORC1 activity expands the list of known cellular functions of TOR.

Regulation of TORC1 Activity

Multiple inputs such as nutrients, energy, growth factors and stress converge on TORC1 to coordinately regulate cellular responses to these environmental cues (*1, 4*) (Fig. 2). Among these, nutrients, especially amino acids (or nitrogen source for yeast), are the most fundamental inputs affecting TORC1 activity throughout evolution. In multicellular organisms, growth factors further fine-tune the regulation of TORC1 activity to achieve appropriate activation status according to the demand of cell growth and overall body growth.

The mechanism of how growth factors (e.g. insulin) signal mTORC1 regulation has been studied extensively. There are at least two mechanisms leading to activation of mTORC1: the tuberous sclerosis complex (TSC)-dependent and TSC-independent pathways (*4*). TSC1 (also known as hamartin) and TSC2 (also
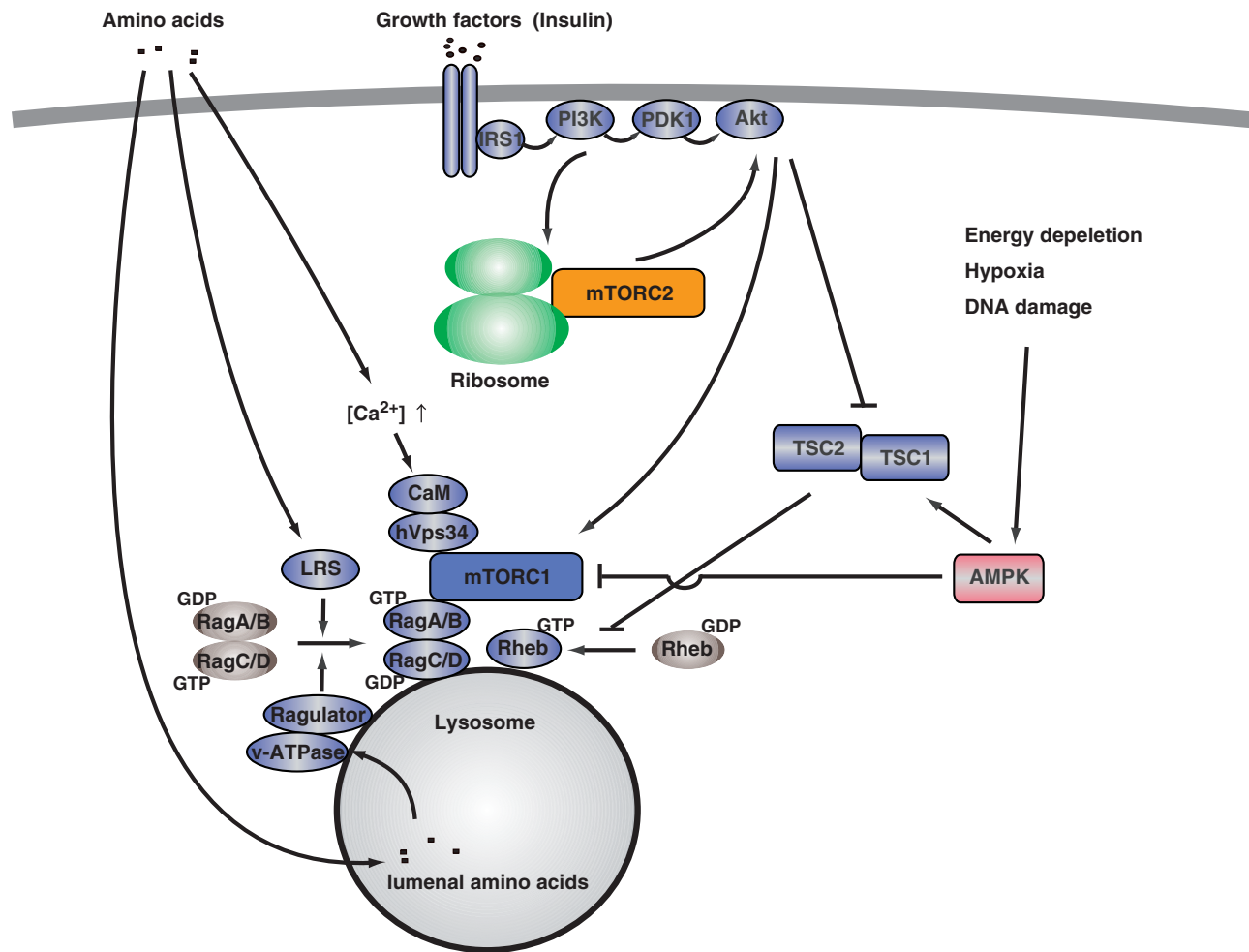
Fig. 2 mTOR signalling pathway. mTORC1 is activated by growth factors and amino acids and repressed by stressful conditions such as energy depletion, hypoxia and DNA damage. mTORC2 is activated by growth factors through association with ribosomes. Only key upstream regulators of mTORC1 and mTORC2 are shown.

known as tuberin) are gene products whose inactivation causes tuberous sclerosis. The TSC1/TSC2 complex acts as a GTPase activating protein (GAP) for the small GTPase Rheb (*1*, *4*). The active GTP-bound Rheb directly binds to mTORC1 and enhances its kinase activity. Rheb also acts as a scaffold and mediates the binding of mTORC1 to its substrates (*5*). Stimulation by growth factors such as insulin leads to activation of the PI3K-Akt pathway, and then activated Akt phosphorylates and inhibits the GAP activity of TSC2, leading to activation of Rheb. Akt phosphorylation of TSC2 also causes sequestration of TSC2 through binding to 14-3-3 protein. In the TSC1/2-independent pathway of mTORC1 activation, upon stimulation by growth factors, Akt phosphorylates and inactivates PRAS40, the inhibitory component of mTORC1, leading to mTORC1 activation (*6*).

Amino acids (especially leucine) or nitrogen sources are essential activators of the TORC1 pathway throughout evolution. However, how this amino acid signal activates TORC1 was a long-standing mystery in the TOR field. Over the past few years, there have been considerable advances in our understanding of the amino acid-induced activation of mTORC1. The identification of Rag, a family of small GTPases, was a

great step towards understanding regulation of mTORC1 activation by the amino acids (*7*). The Rag GTPase family consists of four proteins Rag A, B, C and D. They have a unique property, in that they function as heterodimers composed of two highly similar groups of proteins: RagA or RagB forms heterodimers with either RagC or RagD. Activation of mTORC1 by amino acids is regulated by GTP–GDP exchange of these proteins in a reciprocal manner (Fig. 2). The presence of amino acids leads to heterodimer formation between GTP-bound RagA/B and GDP-bound RagC/D, whereas deprivation of amino acids results in the formation of GDP-bound RagA/B and GTP-bound RagC/D (*7*). Rag GTPases localize to the surface of the lysosome through association with a heteropenta protein complex called Ragulator (*8*). The essential role of the Rag GTPases is to recruit mTORC1 to the surface of lysosome through their ability to bind to Raptor when they are in the activated heterodimer form, *i.e.* GTP-bound RagA/B-GDP-bound RagC/D (Fig. 2). Upon being recruited to the surface of the lysosome, mTORC1 is proposed to be activated by association with Rheb that resides on the lysosome (*7*). Thus, amino acid signalling mainly operates through the spatial regulation of mTORC1 in

the cells. The importance of Rag GTPases in the TORC1 activation in response to amino acids seems essentially conserved throughout evolution (9, 10), although the amino acid-dependent redistribution of yeast TORC1 has not been observed.

Recent reports by several groups suggest that multiple factors and pathways participate in the amino acid-induced activation of Rag GTPases. Sabatini's group proposed that the amino acid content inside the lysosome is the primary stimulus for activation of mTORC1 (11). In this scenario, the amino acids present in the lumen of the lysosome regulate the association between Rags and Ragulator through an undefined mechanism involving the activity of vacuolar H^+-ATPase (v-ATPase), and Ragulator acts as a guanine nucleotide exchange factor (GEF) for RagA and RagB, leading to activation of Rag heterodimers (Fig. 2). Recent results from both Kim's (in mammalian cells) and Virgilio's (in yeast) groups suggest an amino acid sensing mechanism in the cytoplasm through direct recognition of leucine by leucyl-tRNA synthetase (LRS) (12, 13) (Fig. 2). Mammalian LRS when bound to leucine specifically acts as GAP for RagD, leading to GTP-to-GDP transition of RagD and subsequent mTORC1 activation. In yeast, LRS together with leucine binds to Gtr1, which is the ortholog of mammalian RagA/B, leading to activation of TORC1 through an unknown mechanism. Furthermore, leucine and glutamine are also reported to activate mTORC1 through glutaminolysis (14). Glutaminolysis converts glutamine to α-ketogluatrate (α-KG) through two sequential steps, where leucine acts as an allosteric activator in the second step. Elevated α-KG level leads to activation of mTORC1 by enhancing GDP-to-GTP transition of RagB, although the precise mechanism remains unknown. Recent reports have further revealed that SH3-domain binding protein 4 (SH3BP4) acts as an inhibitor of Rag GTPases-mTORC1 association in an amino acid-dependent manner (15). Amino acids promote dissociation of SH3BP4 from Rag GTPases, which allows subsequent binding of mTORC1 to Rag GTPases and mTORC1 activation. In addition, MAP4K3 also appears to act upstream of Rag GTPases in the amino acid sensing pathway (16).

Although amino acid sensing through the Rag GTPase-mTORC1 axis is a key regulatory mechanism in the mTORC1 pathway not only in cultured cells but also in the physiological setting (17), another pathway seems to activate mTORC1 in response to amino acids. It is known that human vacuolar protein sorting 34 homologue (hVps34), a class III PI3K, mediates amino acid signalling through mTORC1 (18). Amino acid stimulation elevates intracellular Ca^{2+} levels, which is recognized by calmodulin (CaM). Ca^{2+}-bound CaM binds to hVps34, which in turn directly binds to mTORC1 (Fig. 2). Consistent with this finding, a recent study also demonstrated that increase of intracellular Ca^{2+} level during physical overload activates mTORC1 and promotes protein synthesis, leading to skeletal muscle hypertrophy (19). mTORC1 activation by hVps34 requires phosphatidylinositol 3-phosphate (PI3P), the lipid product of hVps34.

Interestingly, phospholipase D1 (PLD1) is an effector of PI3P generated by hVps34 (20), and has been reported to mediate Rheb action towards mTORC1 (21). Therefore, it is possible that amino acid input regulates both Rag GTPases-mediated localization of mTORC1 and hVps34-PLD1-mediated association of Rheb with mTORC1 to fully activate mTORC1 (Fig. 2).

Under many stressful conditions, TORC1 activity is repressed to stop cell growth and to shift cell physiology towards inducing cytoprotective programs (1, 22). Understanding mechanisms leading to TORC1 inactivation are also important because constitutive activation of mTORC1 is implicated in the development of many diseases (4). In mammals, adenosine monophosphate-activated protein kinase (AMPK) and TSC1/2 inhibit mTORC1 activity under a broad array of stressful conditions including hypoxia, energy depletion and DNA damage. AMPK is activated by increase in the AMP/ATP ratio under conditions such as energy deprivation and hypoxia. Activated AMPK inhibits mTORC1 via two known mechanisms (23). AMPK directly phosphorylates TSC2 at Ser1387, which results in activation of its GAP activity towards Rheb and this in turn represses mTORC1. In the second mechanism, AMPK directly phosphorylates Raptor at Ser922 and Ser972, which promotes association of Raptor with 14-3-3 protein and inactivation of mTORC1 kinase activity (Fig. 2). DNA-damaging agents inhibit mTORC1 through activation of AMPK, in which Sestrin1 and Sestrin2 induced by p53 directly bind and activate AMPK without increase in the AMP/ATP ratio (24). p53 may also repress mTORC1 activity through transcriptionally upregulating negative regulators of mTORC1 including PTEN, AMPK and TSC2 (25, 26). Furthermore, DNA damage and hypoxia inhibit mTORC1 by inducing the expression of REDD1 protein that activates TSC1/2 by dissociating 14-3-3 protein from TSC2 (27, 28).

Novel mechanisms of TORC1 regulation under stressful conditions have recently emerged in both yeast and mammals (Fig. 3). Budding yeast (Saccharomyces cerevisiae) lacks TSC1/2, but TORC1 activity is still repressed under various stressed conditions such as heat, oxidation and cell wall damage (29). Recent studies have uncovered that inactivation of TORC1 under these conditions is at least in part mediated by Rho1 small GTPase (30, 31). Rho1 (RhoA in mammalian cells) directly binds to TORC1 and thereby inhibits the TORC1 kinase activity both in yeast and in mammalian cells. Furthermore, stress granules, cytoplasmic aggregates composed of stalled translation initiation complexes formed under stressful conditions (32), play a role in the regulation of TORC1 activity both in yeast and in mammals. Under severe heat stress, yeast TORC1 dissociates from the limiting membrane of the vacuole (the lysosome counterpart in yeast) and is recruited into stress granules (33). Spatial sequestration of yeast TORC1 into stress granules maintains yeast TORC1 in an inactive state, which promotes cytoprotective effects. In mammalian cells, mTORC1 is also sequestered into oxidative stress- and osmotic stress-induced stress granules and
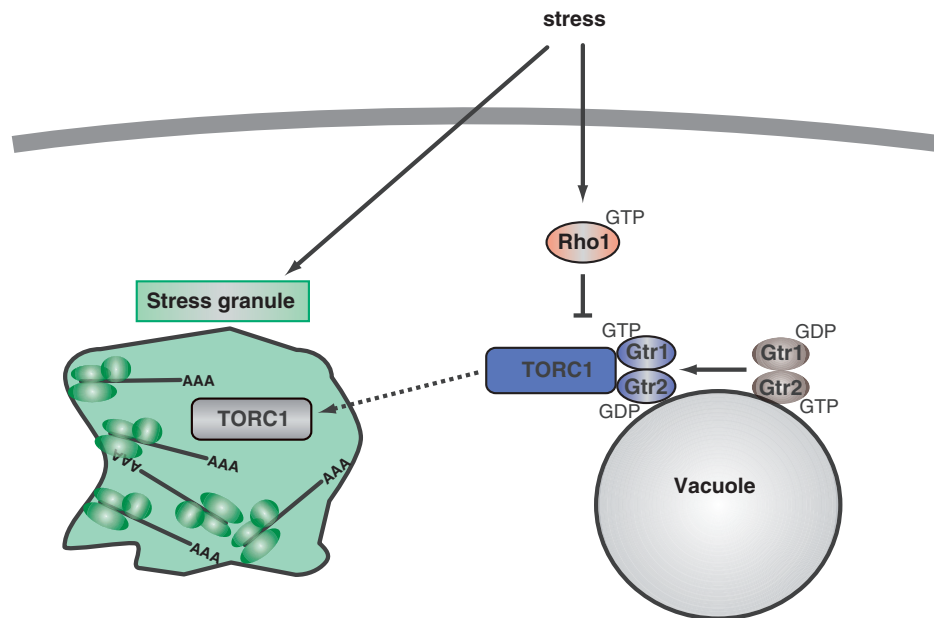
4

Fig. 3 Stress granule-mediated regulation of TORC1 in yeast. Upon certain stressful conditions (e.g. high temperature), Rho1 is activated, which in turn inhibits TORC1 and releases it from the vacuole. TORC1 is recruited into stress granules and is maintained in an inactive state. Relocalization of TORC1 into stress granules is also conserved in mammals.

inactivated (*34*). Further elucidation of new regulatory mechanisms of TORC1 will lead to a comprehensive understanding of complex regulation of TORC1 under various stressful conditions.

Regulation of TORC2 Activity

Less is known about the regulation of TORC2 than about TORC1 regulation. A well-known signal activating mTORC2 is growth factors such as insulin (*35*). mTORC2 may also be regulated by amino acids, depending on specific substrates, cellular contexts and/or conditions (*30–32*). Although the mechanism of mTORC2 regulation is poorly defined, ribosome has been reported to be a direct activator of mTORC2 in response to insulin (*36*) (Fig. 2).

Insulin promotes binding of the ribosome to mTORC2 and this interaction leads to activation of mTORC2 (*36*). Because mTORC1 is a primary regulator of ribosome biogenesis, activation status of mTORC1 may coordinately control mTORC2 activation to achieve appropriate cell growth. Although yeast lacks a growth factor signalling pathway, the requirement of ribosome for activation of TORC2 is likely conserved (*36*). Thus, ribosome–TORC2 association may be a key step in activation of TORC2 that is conserved throughout evolution. The localization of TORC2 in both mammalian cells and yeast appears to be on the plasma membrane (or at least on membranous structures) (*37*). Interestingly, in endothelial cells, localization of mTORC2 on the plasma membranes is regulated by syndecan-4 in a PKCα-dependent manner, and the localization is required for mTORC2 activation (*38*). It will be interesting to determine whether the plasma membrane-associated pool of ribosomes is specifically involved in the activation of TORC2.

In yeast *S. cerevisiae*, TORC2 is activated by plasma membrane stress, which is encountered during cell growth because of the enlargement of cell surface area (*37*). In this step, the PH-domain-containing plasma membrane proteins Slm1 and Slm2 act as upstream regulators of TORC2. Upon stress on the plasma membrane, such as inhibition of sphingolipid biosynthesis or mechanical stretching of plasma membrane, Slm proteins are relocalized away from specialized compartments within the plasma membrane called eisosomes, and then they associate with TORC2. Slm proteins likely play a role to facilitate TORC2 phosphorylation of Ypk1, a downstream effector, by directly recruiting Ypk1 to proximity of TORC2. The activated Ypk1 in turn promotes biosynthesis of sphingolipids, which eventually limits TORC2 activation, thereby building a feedback loop of regulation of sphingolipids homeostasis (*37, 39*).

Another upstream regulator of TORC2 is Ryh1 in *Schizosaccharomyces pombe* (also called fission yeast). Although the stimuli that activates TORC2 in *S. pombe* is unclear, some studies have indicated that Ryh1, the ortholog of mammalian Rab6 GTPase, directly binds to TORC2 and regulates TORC2 activity (*40*). Ryh1 has been implicated in regulation of the vesicle transport between endosomes and the Golgi apparatus; although the function of Ryh1 in TORC2 activation is likely distinct from its role in the control of vesicular trafficking.

TORC1 Downstream Events

TORC1 generally regulates anabolic processes such as protein synthesis and catabolic processes such as autophagy to make an appropriate decision of cell growth in response to environmental cues. The well-characterized cellular processes that TORC1 regulates

are mRNA translation, ribosome biogenesis, lipid metabolism and autophagy (Fig. 1B).

mRNA translation

The best-characterized substrates of mTORC1 are 4E-BP1 and p70 ribosomal protein S6 kinase 1 (S6K1), both of which are involved in the regulation of mRNA translation. Very recently, it was reported that inhibition of mTORC1 activity by Torin 1, an ATP-competitive inhibitor, resulted in decrease of translation of almost all mRNAs (99.8% of 4840 transcripts examined) to some extent (median = 60.5%), suggesting the large contribution of mTORC1 in the general control of mRNA translation (41).

4E-BP1 is an endogenous inhibitor of eIF4E, a translation initiation factor that binds to the 5′-cap structure of mRNA, which promotes translation initiation. Phosphorylation of 4E-BP1 by mTORC1 prevents its association with eIF4E, thereby allowing cap-dependent translation initiation. The mRNAs that are most suppressed upon mTORC1 inhibition possess 5′-TOP (terminal oligopyrimidine tract), which consists of cytosine at the 5′-terminus followed by a stretch of 4—15 pyrimidines, or 5′-TOP-like sequences (41). How translation of these 5′-TOP mRNAs are regulated has been controversial. However, recent studies have clearly demonstrated that translation of the 5′-TOP mRNAs is largely controlled by mTORC1-mediated phosphorylation of 4E-BP1 (41, 42). The 5′-TOP is mainly found in mRNAs that encode ribosomal proteins and other factors responsible for regulation of protein synthesis. Activation of mTORC1 affects overall mRNA translation not only by regulation of translation initiation through relieving eIF4E from inhibition by 4E-BP1, but also by upregulation of the translation capacity.

Phosphorylation of S6K1 by mTORC1 also promotes mRNA translation through various pathways by phosphorylation of multiple downstream effectors of S6K1 involved in mRNA translation and splicing processes, such as eIF4B, PDCD4, eEF2K and SKAR (43). Intriguingly, the eukaryotic translation initiation factor eIF3 complex acts as a scaffold for mTORC1 to phosphorylate S6K1 (43, 44). In the absence of nutrients or growth factors, inactive S6K1 is bound to eIF3. Upon activation of mTORC1 by nutrients or growth factors, mTORC1 is recruited to the eIF3 complex and phosphorylates S6K1. Activated S6K1 dissociates from eIF3, and in turn phosphorylates many downstream effectors as mentioned earlier. It will be interesting to determine how and if the activated mTORC1 on the lysosomal surface binds to either the general or a specific pool of eIF3.

Ribosome biogenesis

Ribosome biogenesis is a fundamental process for maintaining cell growth and proliferation. It is a highly energy-consuming task requiring coordinated regulation of all three RNA polymerases (RNA polymerase I, II and III). TORC1 is known to be involved in processes associated with all three RNA polymerases.

rRNA synthesis by RNA polymerase I requires factors such as transcription initiation factor 1A (TIF-IA), selectivity factor 1 (SL1) and upstream binding factor (UBF), which recruit RNA polymerase I onto rDNA promoters. The mTORC1-S6K1 axis activates TIF-IA and UBF, promoting the association of RNA polymerase I with rDNA promoter and enhances rRNA expression (45). TORC1 in both yeast and mammals can be recruited to the promoter region of rDNA (46, 47), indicating that mTORC1 may also regulate rRNA transcription in a more direct way.

Regulation of ribosomal protein (RP) transcription by RNA polymerase II has been elucidated in yeast. Ribosome consists of 79 different RPs, and therefore the coordinated expression and assembly of these RPs are required for efficient production of ribosomes. Yeast TORC1 regulates RP transcription and ribosome biogenesis (Ribi) regulon, the latter of which comprises a set of genes involved in ribosome synthesis and maturation, mostly through the activation of the downstream effector Sch9, the yeast ortholog of S6K1 (48).

tRNA and 5S RNA are synthesized by RNA polymerase III. TORC1 regulates their transcription through phosphorylation of Maf1, a repressor of RNA polymerase III. In yeast, Maf1 is mainly phosphorylated by Sch9, while it is directly phosphorylated by mTORC1 in mammals (49). In both systems, Maf1 phosphorylation blocks its nuclear localization, leading to induction of tRNA and 5S RNA expression.

Lipid biosynthesis

Recent studies have revealed that mTORC1 positively regulates lipid biosynthesis mainly through sterol regulatory element-binding protein-1c (SREBP-1c), a master transcription factor regulating gene required for lipids biosynthesis (50). Insulin stimulates nuclear accumulation of SREBP-1c by promoting processing and maturation of it's membrane-bound inactive precursor in the endoplasmic reticulum (ER), thereby leading to transcriptional activation of lipogenic genes. Although the exact mechanism by which mTORC1 could promote SREBP-1c activation has not been well established, it could occur through multiple pathways: mTORC1 activation increases SREBP-1c expression by an unknown mechanism, and promotes SREBP1 processing and nuclear accumulation, in part, through S6K1 (4, 50). Moreover, a recent report has demonstrated that mTORC1 phosphorylation of lipin 1, a phosphatidic acid phosphatase, inhibits its translocation into the nucleus, allowing nuclear accumulation of SREBP-1c through an uncharacterized mechanism (51). A recent study has also suggested that mTORC2 activation in addition to mTORC1 activation is required for promotion of SREBP-1c-mediated lipogenesis (52).

Autophagy

Autophagy is a major lysosomal degradation process of cytoplasmic components and organelles, which supplies nutrients to cells enabling them to survive under conditions of nutrient deprivation (53). In both yeast and mammalian cells, TORC1 regulates the initial step

of autophagy induction, although the precise regulatory mechanism differs between the two systems. In yeast, TORC1 directly phosphorylates and inhibits Atg13, an initiator of autophagy. TORC1 inhibition under nutrient starvation causes dephosphorylation of Atg13, allowing association of Atg13 with the Ser/Thr kinase Atg1 and the subsequent activation of the Atg13–Atg1 kinase complex. The activated Atg13–Atg1 complex phosphorylates downstream effectors to promote formation of autophagosomes. In mammalian cells, mTORC1 regulates the UNC-51-like kinases (ULK1) complex, which consists of ULK1, Atg13, Atg101 and a 200-kDa FAK-family interacting protein (FIP200), of these, ULK1 is the mammalian ortholog of Atg1. In the nutrient-rich condition, mTORC1 directly phosphorylates ULK1 and Atg13 through binding with ULK1, and inactivates the ULK1 complex. Upon mTORC1 inhibition, mTORC1 dissociates from the ULK1 complex, and thereby activates ULK1. Activated ULK1 is thought to phosphorylate Atg13 and FIP200, which in turn induces formation of autophagosomes through unknown mechanisms (53).

TORC2 Downstream Events

mTORC2 phosphorylates several AGC family kinases on their hydrophobic motifs, including Akt, SGK1 and protein kinase C (PKC), through which mTORC2 regulates a variety of cellular processes such as cell survival, proliferation and actin reorganization (Fig. 1B).

AGC kinase phosphorylation

mTORC2 phosphorylates Akt1 at Ser473 within the hydrophobic motif, which is required for its full activation (54). This phosphorylation appears to occur on the plasma membrane, where mTORC2 resides. Although mTORC2 kinase activity is enhanced in response to growth factors, Akt1 phosphorylation at Ser473 appears to be regulated also through the recruitment of Akt1 to the plasma membrane in response to $PI(3,4,5)P_3$ production by PI3K. Indeed, hyperactive mTORC2 is unable to maintain Akt1 phosphorylation upon growth factor starvation (55). Such a local control of Akt1 phosphorylation, *i.e.* differential phosphorylation of distinct Akt1 fractions in the cells, might affect regulation of downstream effectors. In fact, the need for this Akt1 phosphorylation by mTORC2 differs among the downstream effectors. In mTORC2-deficient cells, where Akt1 Ser473 phosphorylation is completely abolished, FOXO1/3a phosphorylation is impaired, whereas phosphorylation of other downstream effectors such as TSC2 and GSK3β is unaffected (56, 57).

mTORC2 also phosphorylates Akt1 at Thr450 within the turn motif even in the absence of growth factor. This phosphorylation is constitutive and occurs cotranslationally, which contributes to the folding and stability of Akt1 (58).

mTORC2 phosphorylates and activates SGK1, a kinase involved in regulating sodium transport and cell survival. SGK1 phosphorylation by mTORC2 is specifically mediated by binding of SGK1 to the mTORC2 component mSin1 and has been shown to be required for activation of sodium transport in epithelial kidney cells (59). The yeast counterparts of SGK1, Ypk1 and Ypk2 are also phosphorylated by TORC2 and are involved in controlling sphingolipid biosynthesis and actin organization (37, 60).

Actin organization

The link between TORC2 and actin organization was first shown in yeast and later in mammalian cells (61). Depletion of TORC2 causes defects in actin organization. In *S. cerevisiae*, although the precise mechanism by which TORC2 regulates actin organization remains to be elucidated, several effectors such as Rho1 GTPase, Ypk2 and Slm1/2 have been shown to be involved in this process (48). Upon activation, Rho1 binds to Pkc1 kinase, which in turn activates the downstream cascade, leading to actin organization in yeast. TORC2 appears to activate Rho1 through its GEF Rom2 by an unknown mechanism. It is possible that changes in the lipid composition of the plasma membrane through the TORC2-Slm1/2-Ypk1/2 axis primarily affects the ability of Rom2 to bind $PI(4,5)P_2$ on the plasma membrane, which in turn activates the Rho1-Pkc1 pathway (62).

In mammals, mTORC2 appears to regulate actin organization by promoting phosphorylation of PKCα and paxillin and by activation of RhoA and Rac1 (4, 35, 61). Interestingly, Rac1 also binds to mTOR and regulates subcellular localization of mTORC1 and mTORC2 (63), thereby acting both upstream and downstream of mTORC2. It is proposed that Rac1 translocates to the plasma membrane upon growth factor stimulation and recruits mTORC2 and P-Rex1 (a GEF towards Rac1), resulting in local activation of Rac1 and subsequent increase of $PI(3,4,5)P_3$ synthesis through promotion of PI3K activation (64), leading to both actin organization and Akt activation.

Lipid biosynthesis

As described in TORC1-mediated events, lipid biosynthesis is mainly regulated by mTORC1 in mammalian cells. However, recent studies, especially in yeast, suggest that TORC2 plays an important role in the regulation of sphingolipid biosynthesis, building a feedback loop of sphingolipid biosynthesis and TORC2 activation (37, 39).

Although mTORC1 regulates lipogenesis in mammalian cells, activation of mTORC1 alone was not sufficient for activation of SREBP-1c and lipogenesis in TSC1 knockout mice (65). Furthermore, a recent study of liver-specific Rictor knockout mice suggests that mTORC2 appears to positively regulate lipogenesis, at least in part, through Akt1-mediated activation of SREBP-1c (52). Importantly, SREBP-1c activation and subsequent expression of lipogenic genes are impaired in these mice without affecting mTORC1 activity, suggesting the mTORC2-specific function of lipogenesis. Thus, both mTORC1 and mTORC2 positively activate lipogenesis. Further studies are needed to elucidate the complex metabolic outcomes regulated via TORC1 and TORC2, which will provide valuable

data that can be used to develop targeted therapy for obesity and diabetes.

Conclusion

Much progress has been made in the understanding of TOR signalling during the past years, especially regarding amino acid-induced TORC1 activation. However, only a few of the vast array of functions of TOR are known, given that data revealing new links of TOR to unrecognized cellular activities are constantly emerging. Because TOR is involved in essentially all aspects of cellular activities owing to its fundamental ability to sense and respond to nutrients, the regulatory mechanisms of TOR must be tightly controlled by multiple mechanisms while allowing the necessary sharp and robust responses to even subtle or conflicting environmental conditions. Elucidation of the complex molecular mechanisms of TOR signalling through genetic, pharmacological and biochemical studies on various experimental models ranging from yeast to mammals will certainly provide beneficial knowledge.

Conflict of Interest
None declared.

References

1. Wullschleger, S., Loewith, R., and Hall, M.N. (2006) TOR signaling in growth and metabolism. *Cell* **124**, 471–484
2. Sarbassov, D.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
3. Dowling, R.J.O., Topisirovic, I., Fonseca, B.D., and Sonenberg, N. (2010) Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochim. Biophys. Acta* **1804**, 433–439
4. Laplante, M. and Sabatini, D.M. (2012) mTOR signaling in growth control and disease. *Cell* **149**, 274–293
5. Sato, T., Nakashima, A., Guo, L., and Tamanoi, F. (2009) Specific activation of mTORC1 by Rheb G-protein in vitro involves enhanced recruitment of its substrate protein. *J. Biol. Chem.* **284**, 12783–12791
6. Dunlop, E.A. and Tee, A.R. (2009) Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. *Cell Signal.* **21**, 827–835
7. Efeyan, A., Zoncu, R., and Sabatini, D.M. (2012) Amino acids and mTORC1: from lysosomes to disease. *Trends in Mol. Med.* **18**, 524–533
8. Bar-Peled, L., Schweitzer, L.D., Zoncu, R., and Sabatini, D.M. (2012) Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell* **150**, 1196–1208
9. Binda, M., Péli-Gulli, M.-P., Bonfils, G., Panchaud, N., Urban, J., Sturgill, T.W., Loewith, R., and De Virgilio, C. (2009) The Vam6 GEF controls TORC1 by activating the EGO complex. *Mol. Cell* **35**, 563–573
10. Valbuena, N., Guan, K.-L., and Moreno, S. (2012) The Vam6 and Gtr1-Gtr2 pathway activates TORC1 in response to amino acids in fission yeast. *J. Cell Sci.* **125**, 1920–1928
11. Zoncu, R., Bar-Peled, L., Efeyan, A., Wang, S., Sancak, Y., and Sabatini, D.M. (2011) mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H^+-ATPase. *Science* **334**, 678–683
12. Han, J.M., Jeong, S.J., Park, M.C., Kim, G., Kwon, N.H., Kim, H.K., Ha, S.H., Ryu, S.H., and Kim, S. (2012) Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. *Cell* **149**, 410–424
13. Bonfils, G., Jaquenoud, M., Bontron, S., Ostrowicz, C., Ungermann, C., and De Virgilio, C. (2012) Leucyl-tRNA synthetase controls TORC1 via the EGO complex. *Mol. Cell* **46**, 105–110
14. Durán, R.V., Oppliger, W., Robitaille, A.M., Heiserich, L., Skendaj, R., Gottlieb, E., and Hall, M.N. (2012) Glutaminolysis activates Rag-mTORC1 signaling. *Mol. Cell* **47**, 349–358
15. Kim, Y.-M., Stone, M., Hwang, T.H., Kim, Y.-G., Dunlevy, J.R., Griffin, T.J., and Kim, D.-H. (2012) SH3BP4 is a negative regulator of amino acid-Rag GTPase-mTORC1 signaling. *Mol. Cell* **46**, 833–846
16. Yan, L., Mieulet, V., Burgess, D., Findlay, G.M., Sully, K., Procter, J., Goris, J., Janssens, V., Morrice, N.A., and Lamb, R.F. (2010) PP2A$_{T61ε}$ is an inhibitor of MAP4K3 in nutrient signaling to mTOR *Mol. Cell* **37**, 633–642
17. Efeyan, A., Zoncu, R., Chang, S., Gumper, I., Snitkin, H., Wolfson, R.L., Kirak, O., Sabatini, D.D., and Sabatini, D.M. (2013) Regulation of mTORC1 by the Rag GTPases is necessary for neonatal autophagy and survival. *Nature* **493**, 679–683
18. Gulati, P. and Thomas, G. (2010) Amino acid regulation of hVps34 and mTORC1 signaling in *The Enzymes* (Hall, M.N. and Tamanoi, F., eds.) Vol. 27 pp. 89–100, Academic Press, Burlington
19. Ito, N., Ruegg, U.T., Kudo, A., Miyagoe-Suzuki, Y., and Takeda, S. (2013) Activation of calcium signaling through Trpv1 by nNOS and peroxynitrite as a key trigger of skeletal muscle hypertrophy. *Nat. Med.* **19**, 101–106
20. Yoon, M.-S., Du, G., Backer, J.M., Frohman, M.A., and Chen, J. (2011) Class III PI-3-kinase activates phospholipase D in an amino acid-sensing mTORC1 pathway. *J. Cell Biol.* **195**, 435–447
21. Sun, Y., Fang, Y., Yoon, M.-S., Zhang, C., Roccio, M., Zwartkruis, F.J., Armstrong, M., Brown, H.A., and Chen, J. (2008) Phospholipase D1 is an effector of Rheb in the mTOR pathway. *Proc. Natl Acad. Sci. USA* **105**, 8286–8291
22. Sengupta, S., Peterson, T.R., and Sabatini, D.M. (2010) Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol. Cell* **40**, 310–322
23. Shackelford, D.B. and Shaw, R.J. (2009) The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat. Rev. Cancer* **9**, 563–575
24. Lee, J.H., Budanov, A.V., Talukdar, S., Park, E.J., Park, H.L., Park, H.-W., Bandyopadhyay, G., Li, N.,

Aghajan, M., Jang, I., Wolfe, A.M., Perkins, G.A., Ellisman, M.H., Bier, E., Scadeng, M., Foretz, M., Viollet, B., Olefsky, J., and Karin, M. (2012) Maintenance of metabolic homeostasis by Sestrin2 and Sestrin3. *Cell Metab.* **16**, 311–321

25. Feng, Z., Zhang, H., Levine, A.J., and Jin, S. (2005) The coordinate regulation of the p53 and mTOR pathways in cells. *Proc. Natl Acad. Sci. USA* **102**, 8204–8209

26. Feng, Z., Hu, W., De Stanchina, E., Teresky, A.K., Jin, S., Lowe, S., and Levine, A.J. (2007) The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Res.* **67**, 3043–3053

27. DeYoung, M.P., Horak, P., Sofer, A., Sgroi, D., and Ellisen, L.W. (2008) Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev.* **22**, 239–251

28. Vadysirisack, D.D., Baenke, F., Ory, B., Lei, K., and Ellisen, L.W. (2011) Feedback control of p53 translation by REDD1 and mTORC1 limits the p53-dependent DNA damage response. *Mol. Cell. Biol.* **31**, 4356–4365

29. Loewith, R. (2010) TORC1 Signaling in budding yeast in *The Enzymes* (Hall, M.N. and Tamanoi, F., eds.) Vol. 27 pp. 147–175, Academic Press, Burlington

30. Yan, G., Lai, Y., and Jiang, Y. (2012) The TOR complex 1 is a direct target of Rho1 GTPase. *Mol. Cell* **45**, 743–753

31. Yan, G., Lai, Y., and Jiang, Y. (2012) TOR under stress: targeting TORC1 by Rho1 GTPase. *Cell Cycle* **11**, 3384–3388

32. Buchan, J.R. and Parker, R. (2009) Eukaryotic stress granules: the ins and outs of translation. *Mol. Cell* **36**, 932–941

33. Takahara, T. and Maeda, T. (2012) Transient sequestration of TORC1 into stress granules during heat stress. *Mol. Cell* **47**, 242–252

34. Wippich, F., Bodenmiller, B., Trajkovska, M.G., Wanka, S., Aebersold, R., and Pelkmans, L. (2013) Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. *Cell* **152**, 791–805

35. Oh, W.J. and Jacinto, E. (2011) mTOR complex 2 signaling and functions. *Cell Cycle* **10**, 2305–2316

36. Zinzalla, V., Stracka, D., Oppliger, W., and Hall, M.N. (2011) Activation of mTORC2 by association with the ribosome. *Cell* **144**, 757–768

37. Berchtold, D., Piccolis, M., Chiaruttini, N., Riezman, I., Riezman, H., Roux, A., Walther, T.C., and Loewith, R. (2012) Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat. Cell Biol.* **14**, 542–547

38. Partovian, C., Ju, R., Zhuang, Z.W., Martin, K.A., and Simons, M. (2008) Syndecan-4 regulates subcellular localization of mTOR complex 2 and Akt activation in a PKCα-dependent manner in endothelial cells. *Mol. Cell* **32**, 140–149

39. Niles, B.J. and Powers, T. (2012) Plasma membrane proteins Slm1 and Slm2 mediate activation of the AGC kinase Ypk1 by TORC2 and sphingolipids in *S. cerevisiae*. *Cell Cycle* **11**, 3745–3749

40. Tatebe, H. and Shiozaki, K. (2010) Rab small GTPase emerges as a regulator of TOR complex 2. *Small GTPases* **1**, 180–182

41. Thoreen, C.C., Chantranupong, L., Keys, H.R., Wang, T., Gray, N.S., and Sabatini, D.M. (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* **485**, 109–113

42. Hsieh, A.C., Liu, Y., Edlind, M.P., Ingolia, N.T., Janes, M.R., Sher, A., Shi, E.Y., Stumpf, C.R., Christensen, C., Bonham, M.J., Wang, S., Ren, P., Martin, M., Jessen, K., Feldman, M.E., Weissman, J.S., Shokat, K.M., Rommel, C., and Ruggero, D. (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature* **485**, 55–61

43. Ma, X.M. and Blenis, J. (2009) Molecular mechanisms of mTOR-mediated translational control. *Nat. Rev. Mol. Cell Biol.* **10**, 307–318

44. Holz, M.K., Ballif, B.A., Gygi, S.P., and Blenis, J. (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* **123**, 569–580

45. Mahoney, S.J., Dempsey, J.M., and Blenis, J. (2009) Cell signaling in protein synthesis: ribosome biogenesis and translation initiation and elongation. *Prog. Mol. Biol. Transl. Sci.* **90**, 53–107

46. Li, H., Tsang, C.K., Watkins, M., Bertram, P.G., and Zheng, X.F.S. (2006) Nutrient regulates Tor1 nuclear localization and association with rDNA promoter. *Nature* **442**, 1058–1061

47. Tsang, C.K., Liu, H., and Zheng, X.F.S. (2010) mTOR binds to the promoters of RNA polymerase I- and III-transcribed genes. *Cell Cycle* **9**, 953–957

48. Loewith, R. and Hall, M.N. (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **189**, 1177–1201

49. Michels, A.A. (2011) MAF1: a new target of mTORC1. *Biochem. Soc. Trans.* **39**, 487–491

50. Laplante, M. and Sabatini, D.M. (2009) An emerging role of mTOR in lipid biosynthesis. *Curr. Biol.* **19**, R1046–R1052

51. Peterson, T.R., Sengupta, S.S., Harris, T.E., Carmack, A.E., Kang, S.A., Balderas, E., Guertin, D.A., Madden, K.L., Carpenter, A.E., Finck, B.N., and Sabatini, D.M. (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* **146**, 408–420

52. Hagiwara, A., Cornu, M., Cybulski, N., Polak, P., Betz, C., Trapani, F., Terracciano, L., Heim, M.H., Rüegg, M.A., and Hall, M.N. (2012) Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. *Cell Metab.* **15**, 725–738

53. Mizushima, N. (2010) The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* **22**, 132–139

54. Sarbassov, D.D., Guertin, D.A., Ali, S.M., and Sabatini, D.M. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**, 1098–1101

55. Ohne, Y., Takahara, T., Hatakeyama, R., Matsuzaki, T., Noda, M., Mizushima, N., and Maeda, T. (2008) Isolation of hyperactive mutants of mammalian target of rapamycin. *J. Biol. Chem.* **283**, 31861–31870

56. Guertin, D.A., Stevens, D.M., Thoreen, C.C., Burds, A.A., Kalaany, N.Y., Moffat, J., Brown, M., Fitzgerald, K.J., and Sabatini, D.M. (2006) Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCα, but not S6K1. *Dev. Cell* **11**, 859–871

57. Jacinto, E., Facchinetti, V., Liu, D., Soto, N., Wei, S., Jung, S.Y., Huang, Q., Qin, J., and Su, B. (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and

regulates Akt phosphorylation and substrate specificity. *Cell* **127**, 125–137

58. Facchinetti, V., Ouyang, W., Wei, H., Soto, N., Lazorchak, A., Gould, C., Lowry, C., Newton, A.C., Mao, Y., Miao, R.Q., Sessa, W.C., Qin, J., Zhang, P., Su, B., and Jacinto, E. (2008) The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J.* **27**, 1932–1943

59. Lu, M., Wang, J., Ives, H.E., and Pearce, D. (2011) mSIN1 protein mediates SGK1 protein interaction with mTORC2 protein complex and is required for selective activation of the epithelial sodium channel. *J. Biol. Chem.* **286**, 30647–30654

60. Kamada, Y., Fujioka, Y., Suzuki, N.N., Inagaki, F., Wullschleger, S., Loewith, R., Hall, M.N., and Ohsumi, Y. (2005) Tor2 directly phosphorylates the AGC kinase Ypk2 to regulate actin polarization. *Mol. Cell. Biol.* **25**, 7239–7248

61. Malik, A.R., Urbanska, M., Macias, M., Skalecka, A., and Jaworski, J. (2013) Beyond control of protein translation: What we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). *Biochim. Biophys. Acta*, DOI: 10.1016/j.bbapap.2012.12.010

62. Audhya, A. and Emr, S.D. (2002) Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. *Dev. Cell* **2**, 593–605

63. Saci, A., Cantley, L.C., and Carpenter, C.L. (2011) Rac1 regulates the activity of mTORC1 and mTORC2 and controls cellular size. *Mol. Cell* **42**, 50–61

64. Hernández-Negrete, I., Carretero-Ortega, J., Rosenfeldt, H., Hernández-García, R., Calderón-Salinas, J.V., Reyes-Cruz, G., Gutkind, J. S., and Vázquez-Prado, J. (2007) P-Rex1 links mammalian target of rapamycin signaling to Rac activation and cell migration. *J. Biol. Chem.* **282**, 23708–23715

65. Yecies, J.L., Zhang, H.H., Menon, S., Liu, S., Yecies, D., Lipovsky, A.I., Görgün, C., Kwiatkowski, D.J., Hotamisligil, G.S., Lee, C.-H., and Manning, B.D. (2011) Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. *Cell Metab.* **14**, 21–32