How *Saccharomyces* Responds to Nutrients

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Abstract

Yeast cells sense the amount and quality of external nutrients through multiple interconnected signaling networks, which allow them to adjust their metabolism, transcriptional profile and developmental program to adapt readily and appropriately to changing nutritional states. We present our current understanding of the nutritional sensing networks 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 decision among the different developmental programs available to them—growth, quiescence, filamentous development, or meiosis/sporulation. We conclude that the highly interconnected signaling networks provide 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

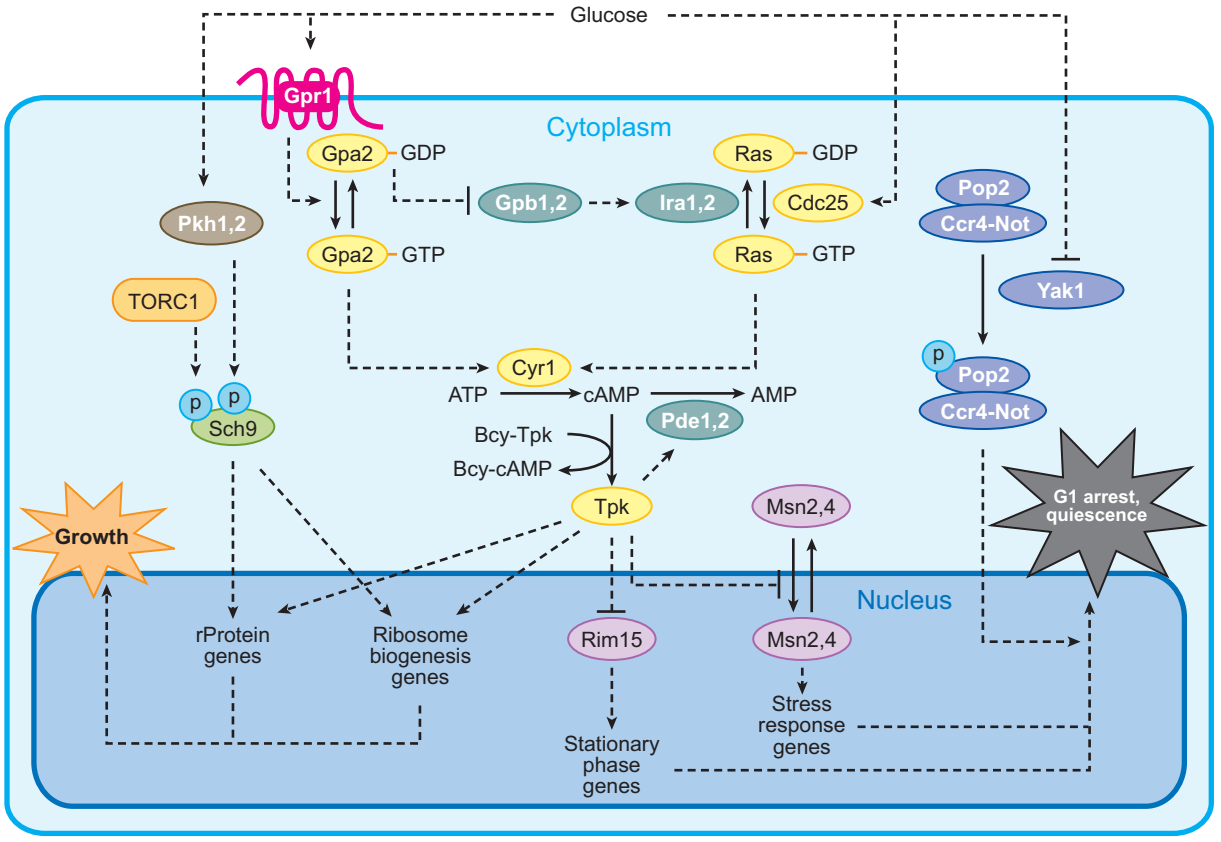

Figure 1

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 induction 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 *tpk1*Δ *tpk2*Δ *tpk3*Δ 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 *ira1*Δ 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. *gpb1*Δ *gpb2*Δ 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 pseudohyphal growth (see below). These observations suggest that Gpb1 and Gpb2 are negative regulators of PKA. *gpg1*Δ mutants display phenotypes opposite to those of *gpb1*Δ *gpb2*Δ 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 *cyr1*Δ, *ras1*Δ *ras2*Δ, and *tpk1*Δ *tpk2*Δ *tpk3*Δ 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, including 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

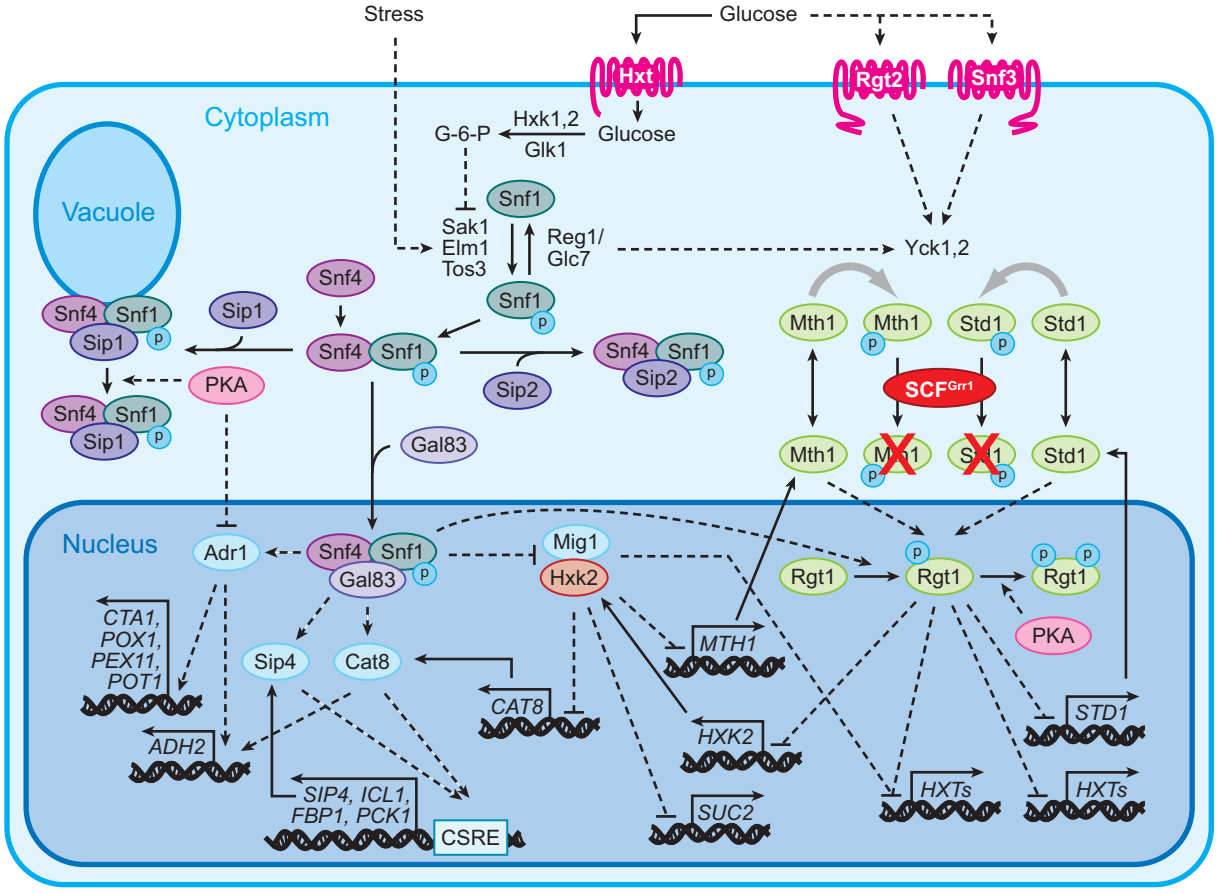

Figure 2

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 activate 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 *reg1*Δ mutant suggests that glucose regulates Snf1 through a pathway independent of Reg1 (165). However, glucose can regulate Snf1 activity even in a *sak1*Δ *elm1*Δ *tos3*Δ 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 *hxk1*Δ *hxk2*Δ *glk1*Δ 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 *tpk1*Δ *tpk2*Δ *tpk3*Δ 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 permease 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 studies 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,

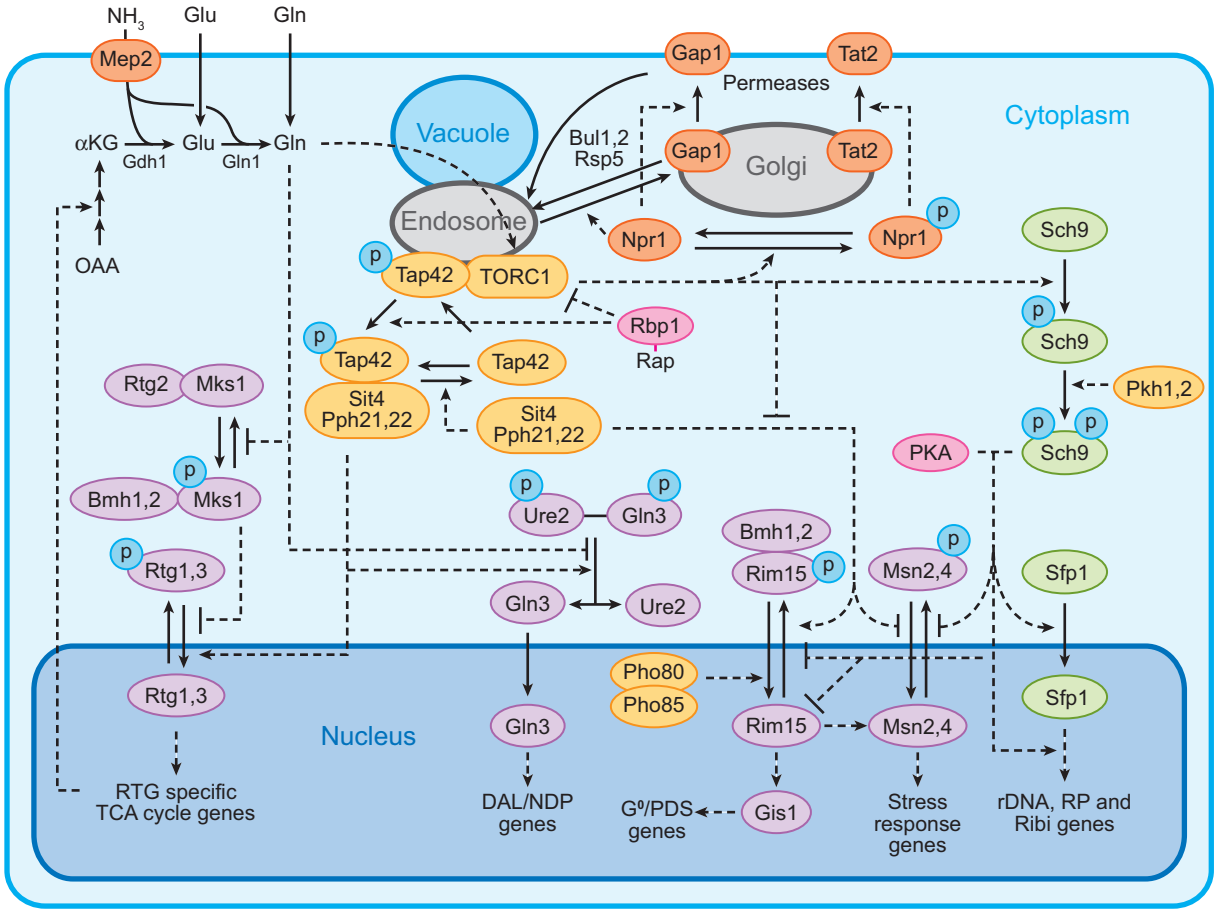

Figure 3

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 sequester 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 connundrum (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

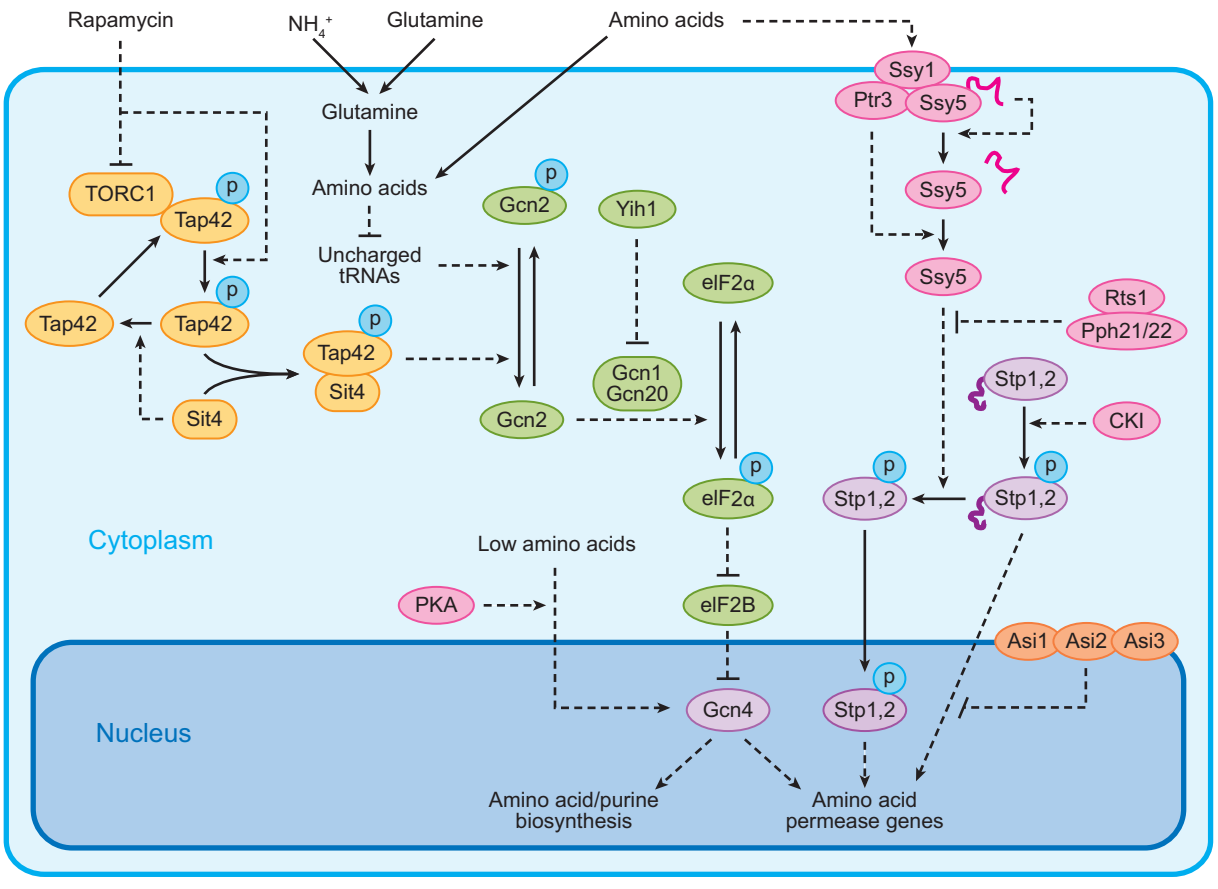

Figure 4

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

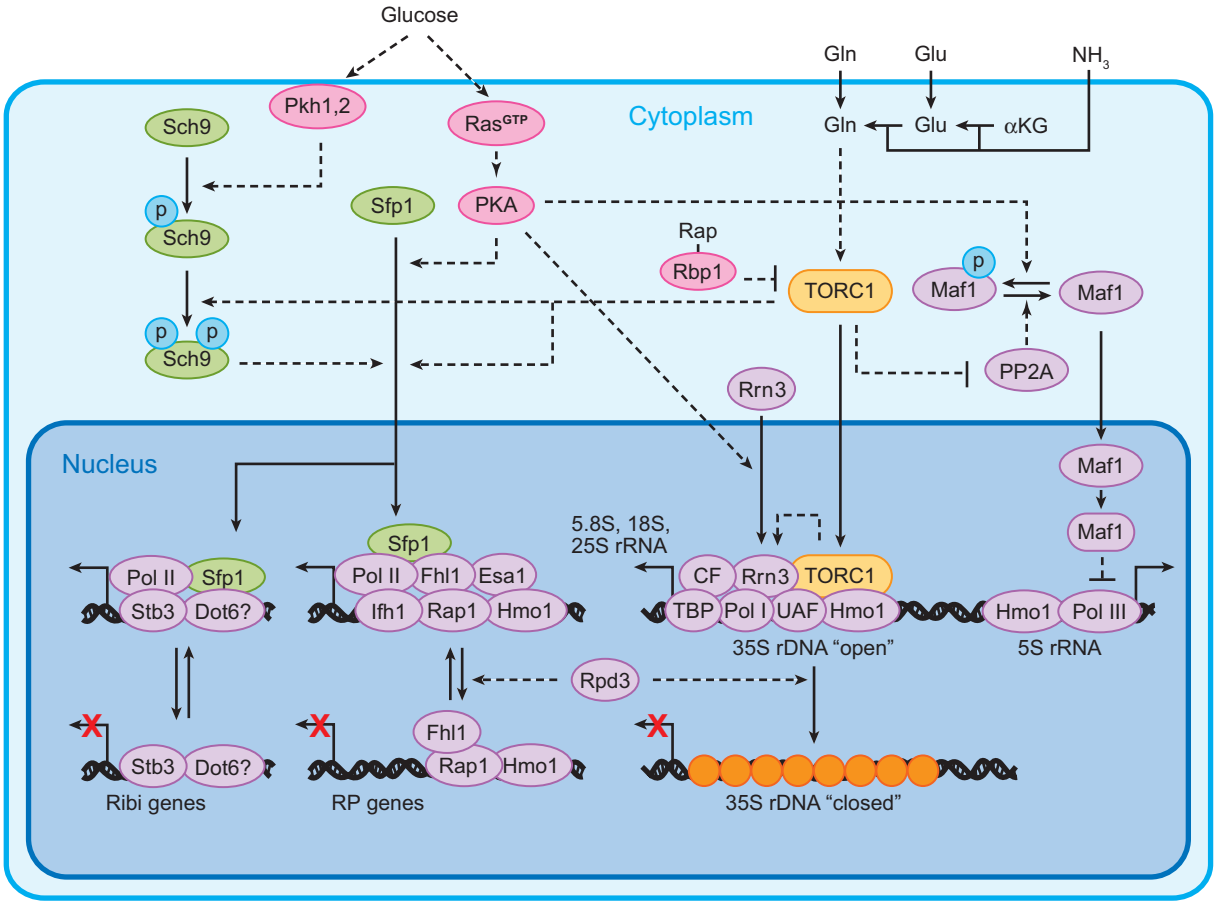

Figure 5

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. Commitment 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*Δ *bck2*Δ *whi5*Δ 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 post-translational 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 dephosphorylation 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

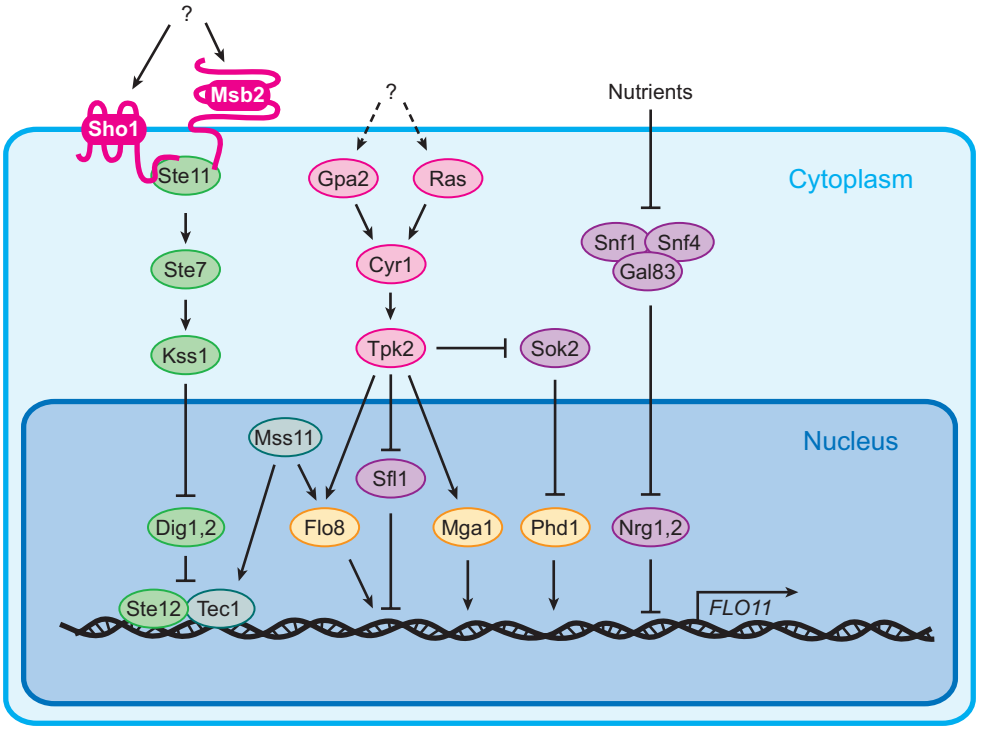

Figure 6

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 participation 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 *tpk1*Δ *tpk2*Δ *tpk3*Δ 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

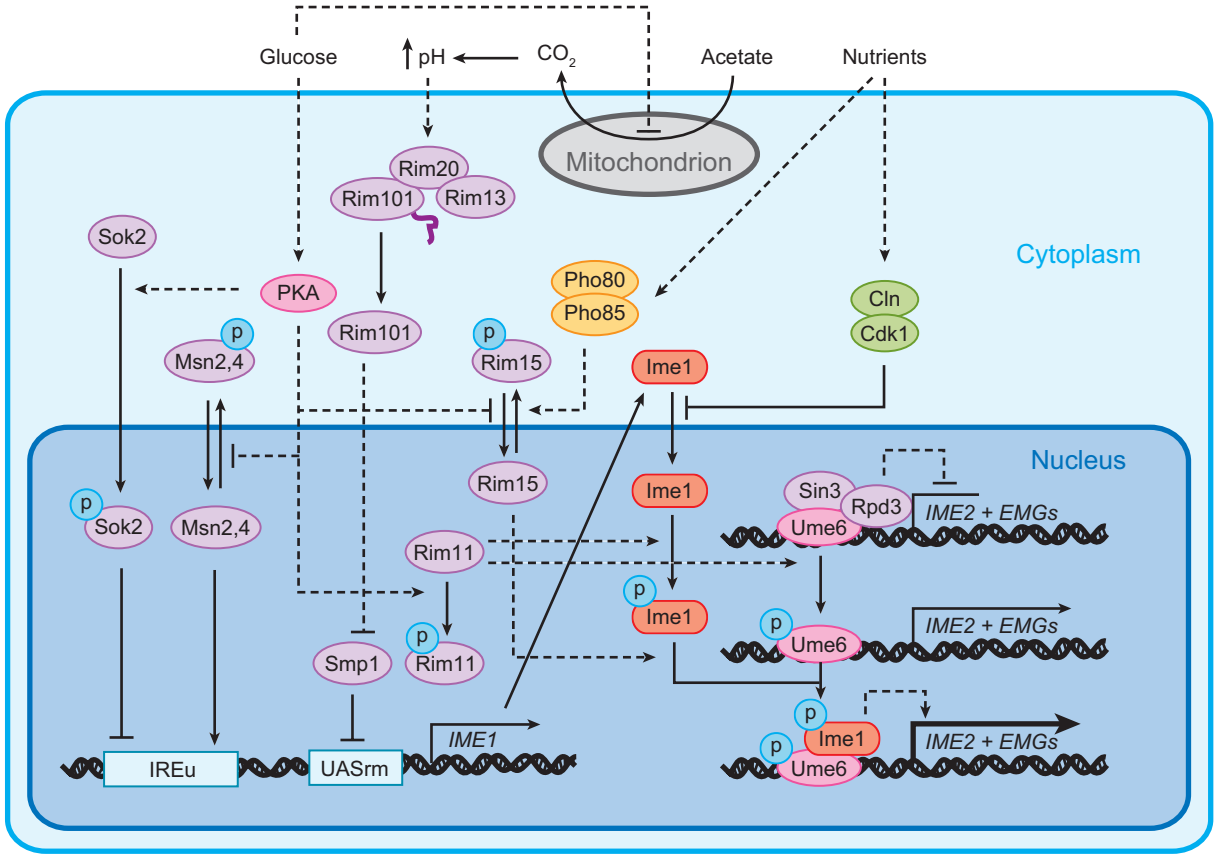

Figure 7

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 *rim101*Δ 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.

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Contents

Errata

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