Tor and Cyclic AMP-Protein Kinase A: Two Parallel Pathways Regulating Expression of Genes Required for Cell Growth

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Received 22 October 2004/Accepted 10 November 2004

In the budding yeast Saccharomyces cerevisiae, the Tor and cyclic AMP-protein kinase A (cAMP-PKA) signaling cascades respond to nutrients and regulate coordinately the expression of genes required for cell growth, including ribosomal protein (RP) and stress-responsive (STRE) genes. The inhibition of Tor signaling by rapamycin results in repression of the RP genes and induction of the STRE genes. Mutations that hyperactivate PKA signaling confer resistance to rapamycin and suppress the repression of RP genes imposed by rapamycin. By contrast, partial inactivation of PKA confers rapamycin hypersensitivity but only modestly affects RP gene expression. Complete inactivation of PKA impairs RP gene expression and concomitantly enhances STRE gene expression; remarkably, this altered transcriptional pattern is still sensitive to rapamycin and thus subject to Tor control. These findings illustrate how the Tor and cAMP-PKA signaling pathways respond to nutrient signals to govern gene expression required for cell growth via two parallel routes, and they have broad implication for our understanding of analogous regulatory networks in normal and neoplastic mammalian cells.

The Tor kinases are central components of a signal transduction cascade primarily associated with linking amino acid and growth factor availability to translational control in organisms as diverse as flies, nematodes, and humans (reviewed in references 18, 28, and 38). Recent studies have shown that this is also the case for the yeast Saccharomyces cerevisiae, in which Tor interacts, via TAP42 and the type 2A related protein phosphatase (PP2A) Sit4, with the general amino acid control response to regulate translation (9, 26, 42, 59).

The Tor kinases are also the targets of the antiproliferative drug rapamycin. The high specificity of rapamycin to inhibit Tor activity has facilitated the dissection of the transcriptional programs regulated by Tor signaling. Inhibition of Tor by rapamycin induces genes required for utilization of poor-quality nutrients and adaptation to environmental stress and concomitantly represses genes that promote growth, such as those involved in ribosome biogenesis (2, 7, 20, 25, 37). Under rich nutrient conditions, Tor activity represses the expression of the nitrogen catabolite-repressed (NCR), retrograde response (RTG), and stress-responsive (STRE) genes. Concomitantly, Tor activity sustains the expression of genes required for ribosome biogenesis, including the ribosomal protein (RP) genes (reviewed in references 41 and 43). Control of Tor-repressed, rapamycin-inducible genes is exerted by alterations in the phosphorylation state and thereby the nuclear import of the corresponding transcription factors.

Less is known about the mechanisms by which Tor regulates RP gene expression; however, it is known that this mechanism does not involve Sit4 (11, 42). Coordinate expression of RP genes is associated with recruitment of the NuA4 histone acetylase catalytic subunit Esa1 to the promoters of these genes (39). RP gene expression is thought to be largely controlled by the Rap1 and Abf1 transcription factors (for reviews, see references 61 and 62). We have shown previously that Tor signaling recruits the Esa1 histone acetylase to RP gene promoters without affecting Rap1 or Abf1 promoter occupancy (44).

The extraordinary diversity of genes that are affected by Tor signaling implies that, in almost every case, these genes are also regulated by other signal transduction cascades. A major question, then, is how the Tor pathway interacts with these other pathways to regulate common gene targets. Tor and cyclic AMP-protein kinase A (cAMP-PKA) are two prominent, evolutionarily conserved signal transduction cascades that couple nitrogen and carbon source availability, respectively, to regulate diverse cell responses that ultimately drive cell growth and proliferation. Remarkably, Tor and the cAMP-PKA pathways control a number of common functions, including ribosome biogenesis and responses to stress. In addition, Tor and PKA signaling converge on the Rim15 kinase to control distinct transcriptional and metabolic traits required for proper entry into G₀, a key developmental transition in yeast (36). Given these myriad functions, it is not surprising that these pathways converge to regulate the expression of a panoply of genes, such as the RP and STRE genes and the Gcn4-regulated genes that are involved in amino acid metabolism (2, 7, 16, 17, 24, 32, 34, 37). Evidence has been presented recently to suggest that the Tor pathway might even signal via the PKA pathway to control several functions, including RP gene expression (47).

In S. cerevisiae, the PKA pathway responds to glucose and regulates resistance to stress, glycolysis, and gluconeogenesis. PKA is composed of a regulatory subunit encoded by the BCY1 gene and three catalytic subunits encoded by the TPK1, TPK2, and TPK3 genes (6, 55, 56). In resting cells, PKA is an inactive tetramer consisting of two regulatory subunits in complex with
two catalytic subunits. In response to external signals, such as glucose, adenyl cyclase is activated, cAMP levels rise, and cAMP binding to the PKA regulatory subunit triggers conformational changes that release the PKA catalytic subunits in an active form. Adenyl cyclase is activated via two independent glucose-sensing modules. In the first, the glucose receptor Gpr1 is coupled to the Go protein Gpa2 and functions to activate cAMP production in response to ligand binding (for a review, see reference 19). In the second module, Ras1 and Ras2 function in conjunction with their positive regulators, the guanine nucleotide exchange factors Cdc25 and Scd25, to activate adenyl cyclase in response to glucose and other unknown stimuli (33). The overexpression of a dominant active RAS2V19 allele results in increased levels of cAMP (57). Ras is also negatively regulated by the GTPase-activating proteins (GAP) Ira1 and Ira2 (52), and the levels of cAMP and PKA activity are negatively controlled by the cAMP phosphodiesterases Pde1 and Pde2.

In this study, we have further investigated the interplay between the Tor and cAMP signaling cascades that regulate gene expression required for cell growth. Mutations that hyperactivate the cAMP-PKA pathway were found to render growth resistant to rapamycin and, moreover, to prevent rapamycin-induced repression of RP genes. However, RP gene expression is still sensitive to Tor signaling in strains that completely lack PKA activity or the PKA-related kinases Yak1 and Sch9. We conclude that the Tor and cAMP-PKA cascades function coordinately but independently of one another to govern appropriate RP gene expression. This regulatory network likely functions to provide cells flexibility in fine tuning translational capacity in response to distinct inputs from two global nutrient sensors.

**MATERIALS AND METHODS**

**Yeast strains and media.** Strains used in this study are listed in Table 1. With the exceptions indicated below, all strains are isogenic derivatives of MLY41 (S288c background) (30). Strains SGY73, SGY77, and ASY63 are derived from M28 and were kindly provided by Stephen Garrett. Yeast media were prepared as described previously (14, 48). Rapamycin was added to the media from concentrated stock solutions in 90% ethanol—10% Tween 20. Yeast transformations were performed by the lithium acetate method (46). Unless noted otherwise, mutant yeast strains were constructed by PCR-mediated gene disruption, replacing the entire open reading frame of the targeted gene with the G418 resistance gene cassette derived from template plasmid pPAAkanMX2, the nourseothricin (nat) resistance gene cassette derived from plasmid pAG25, or the hygromycin B (hygB) resistance gene cassette derived from plasmid pAG32 (15, 29). All gene deletions were confirmed by PCR. Strain SZY9 was constructed by crossing SZY6 to XPY14-1. The resulting diploid was sporulated, and the progeny were dissected and genotypes were verified by PCR analysis. Loss of plasmid pXP1 (containing the BCA1 gene) from strain XPY26 was selected on 5-fluoroorotic acid medium. Plasmids pRS316 (CEN URA3) and pMW2 (CEN URA3 RAS2HIS3) were previously described (49, 60).

**Northern blotting.** RNA isolation and Northern blot analysis were performed as described previously (7). DNA probes hybridizing to RPS26, RPS26, ACTI, MEF2, GAP1, CIT2, and SOD2 genes were PCR amplified from yeast genomic DNA with specific primers. Signals were quantified with a Typhoon 9200 variable mode imager by using Image Quantifier 5.2 software (Molecular Dynamics).

**cAMP assay.** Cell cultures were grown to exponential phase in yeast extract-peptone (YP)-glucose medium, harvested, washed three times in YP medium, transferred to YP medium, and incubated for 2 h to deplete glucose. Cell cultures were divided in two, the cultures were treated with 50 nM rapamycin or drug vehicle alone, and incubation was continued for 15 min. Cell aliquots were removed at 0 (no glucose), 0.5, 1, and 3 min after the addition of 2% glucose, and cAMP was determined as described earlier (25). Glycogen staining. Exponentially growing cells were treated with 100 nM rapamycin and incubated at 30°C. After 4 h of treatment, samples containing 5 optical densities of cells were harvested on Millipore HA filters and exposed to iodine vapor for 2 min.

**RESULTS**

Mutations that activate the PKA pathway confer resistance to rapamycin and largely prevent rapamycin-induced inactivation of RP genes. The Tor and cAMP-PKA signaling cascades are known to regulate the expression of RP genes in response to nutrients; in this study, we sought to determine whether the two pathways signal via independent or interdependent mechanisms to execute this function. To establish if the PKA pathway is linked to Tor signaling, we investigated whether mutations that activate PKA alter the sensitivity of cells to rapamycin. As shown in Fig. 1A, mutant strains lacking...
the Ras negative regulator Ira1, Ira1 and Ira2, or the PKA negative regulatory subunit Bcy1, are all more resistant to rapamycin than the wild-type strain. Similarly, the expression of the dominant activated RAS2V19 allele leads to increased rapamycin resistance.

We have previously shown that the Rpd3 histone deacetylase operates at RP gene promoters and enables repression of RP genes in response to rapamycin. Accordingly, we found that Rpd3 was bound to RP genes, and mutations affecting three different Rpd3 subunits conferred resistance to rapamycin and partially prevented RP gene repression induced by rapamycin (44). Based on these observations and the known action of PKA in regulating RP genes, we reasoned that the PKA-activating mutations that increase rapamycin resistance do so by preventing RP gene repression. To test this hypothesis, we examined the expression of the RP genes and compared it to that of other Tor-regulated genes in the different PKA-activating mutant strains.

As shown previously, in wild-type cells RP gene transcripts decreased to almost undetectable levels within 1 h of treatment with rapamycin (Fig. 1B) (7). Concomitantly, the expression of the NCR genes MEP2 and GAP1, and of the retrograde response gene CIT2 also known to be under Tor control, was rapidly induced after exposure to rapamycin (Fig. 1B). It is known that both Tor and PKA activity antagonize induction of the general stress response genes regulated by Msn2 and Msn4 (2, 16, 17). Therefore, we examined the transcription levels of the SOD2 gene, which is induced by oxidative stress and nutrient deprivation. As expected, SOD2 was strongly induced in response to rapamycin (Fig. 1B).

In accord with previous studies, we found that expression of the RP genes was slightly increased in the different PKA-activated mutants compared to the wild-type strain (24). Interestingly, PKA activation by the bcy1 or ira1 ira2 mutations, and more prominently by expression of RAS2V19, partially prevented rapamycin-induced repression of the RP genes (Fig. 1B and C). The fact that in the ira1 ira2 and bcy1 mutants some RP gene expression was still responsive to Tor inhibition by rapamycin, although to a lesser magnitude than that observed with the wild-type strain, argues that the two pathways must be at least partially independent. The PKA-activating mutations impaired expression of the STRE gene SOD2 seen in response to

FIG. 1. Hyperactivating mutations of the PKA pathway confer resistance to rapamycin and prevent rapamycin-induced RP gene repression. (A) Isogenic wild-type (MLY41a), ira1 (THY337), ira2 (THY336), ira1 ira2 (THY345), and bcy1 (XPY26) mutants, wild-type strain transformed with empty vector, and a plasmid carrying the RAS2V19 allele were grown overnight at 30°C in YP-glucose medium. Equivalent numbers of cells were serially diluted and aliquots were spotted onto plates of YP-glucose medium with and without 50 nM rapamycin. After 3 days of incubation at 30°C, plates were photographed. (B) Actively growing cultures of the strains indicated in panel A were treated with 50 nM rapamycin for 0, 30, 60, and 120 min. Total RNA was isolated, and 15 μg of RNA was analyzed by Northern blotting. Individual RNAs were detected by using radioactive oligonucleotide probes corresponding to the genes indicated at the left. (C) The graph shows the quantification of Northern blot signal for the RSP26 gene normalized to the signal for the ACT1 loading control. Northern blot results presented in all figures are representative of at least two independent experiments. WT, wild type; ND, not determined.
rapamycin and did not have significant effects on the rapamycin-induced expression of the NCR or RTG-regulated genes analyzed in this study (Fig. 1B).

Our results show that mutations that activate the PKA pathway confer resistance to rapamycin, and this resistance correlates with the ability of these mutations to prevent rapamycin-induced repression of the RP genes. These results are in agreement with a recent study demonstrating that PKA activation overcomes rapamycin hypersensitivity conferred by mutation of Gln3 and prevents RP gene repression (47).

Partial inactivation of the cAMP-PKA pathway confers rapamycin-hypersensitive growth and has modest effects on RP gene expression. Next, we tested the effect of mutations that inactivate PKA signaling on Tor-regulated gene expression. Interestingly, deletion of any of the three PKA catalytic subunits resulted in hypersensitivity to rapamycin, and this effect was more marked in the tpk1 tpk2 double mutant strain. In addition, deletion of Ras2 or Gpa2, which leads to defects in PKA activation, also conferred rapamycin hypersensitivity (Fig. 2A and data not shown).

We next analyzed whether the increased sensitivity to rapamycin of the PKA defective strains is correlated with changes in RP gene expression. Deletion of either Ras2 or Gpa2 did not alter RP gene expression; however, strains with a deletion of the TPK1 gene alone or in combination with TPK2 or TPK3 showed decreased levels of RP gene expression compared to the wild-type strain (Fig. 2B and C; data not shown for GPA2). Moreover, the PKA-inactivating mutations had little impact on the rapamycin-induced repression of RP genes or on the induction of the GAP1, MEP2, CIT2, and SOD2 genes.

Ras-cAMP PKA and Sch9 appear to stimulate growth via parallel pathways, as hyperactivation of either signaling cascade suppresses loss of the other (54). To test if the Sch9 kinase might be involved in the PKA pathway, we tested whether Tor signals via Sch9 to regulate RP gene expression. We found that mutation of Sch9 had no effect on Tor-regulated RP gene expression (data not shown). These results do not support models in which Tor regulates RP gene expression via Ras2, Gpa2, a specific PKA catalytic subunit, or the Sch9 kinase. However, our results do not rule out a redundant role for the three TPK subunits in regulating RP gene expression downstream from Tor.

Tor signaling regulates RP gene expression by a PKA-independent mechanism. To test whether there is redundancy between the three TPK subunits to regulate RP gene expression, we constructed a strain lacking all three subunits. Simulta-

FIG. 2. Mutations that partially inactivate the cAMP-PKA pathway confer rapamycin-hypersensitive growth and impair RP gene expression. (A) Isogenic wild-type (MLY41a), ras2 (MLY187), tpk1 (XPY4-1), tpk2 (XPY5-1), tpk3 (XPY6-1), and tpk1 tpk3 (XPY14-1) mutant strains were grown, serially diluted, and spotted onto plates of YP-glucose medium with and without 50 nM rapamycin as indicated in legend to Fig. 1A. Cultures were photographed after 3 days of incubation at 30°C. (B) Exponentially growing cultures of isogenic wild-type (MLY41a), ras2 (MLY187), tpk1 (XPY4-1), tpk1 tpk2 (XPY12-1), and tpk1 tpk3 (XPY14-1) mutant strains were treated with 50 nM of rapamycin for 0, 30, 60, and 120 min. RNA was prepared and analyzed by Northern blotting with radioactive probes that hybridize to the genes indicated at the left. (C) The graph shows the quantification of the Northern blot-specific signal for the RPS26 gene normalized to the ACT1 loading control signal. WT, wild type.
nous deletion of all three PKA catalytic subunits is lethal (5). However, suppression of the stress response, either by mutation of Msn2 and Msn4 or by mutation of the PKA-related kinase Yak1, restores viability of \textit{tpk1 tpk2 tpk3 yak1} mutant cells (50). The \textit{tpk1 tpk2 tpk3 yak1} deletion strain exhibited slow growth when compared to either wild-type or the \textit{yak1} strain. In addition, the \textit{tpk1 tpk2 tpk3 yak1} mutant strain exhibited a dramatic hypersensitivity to rapamycin; the MIC at which 100% of the isolates are inhibited was 10 nM for this mutant strain, compared to >100 nM for the wild type (Fig. 3A). For comparison, we also tested the effects of these PKA mutations in a different strain background. As shown in Fig. 4A, the \textit{yak1} single, \textit{tpk1 tpk2 tpk3 yak1} quadruple, and \textit{tpk1 tpk2 tpk3 msn2 msn4} quintuple mutant strains in the S288c genetic background showed rapamycin sensitivity patterns similar to those observed with the PKA mutant strains of the \textit{Σ1278b} background (Fig. 3A).

Deletion of the three \textit{TPK} genes resulted in a marked reduction in RP gene expression compared with the wild-type strain or the \textit{yak1} mutant strain. Moreover, in the \textit{tpk1 tpk2 tpk3 yak1} mutant cells from the two different strain backgrounds, RP gene expression was still repressed by rapamycin and thus was responsive to Tor signaling (compare Fig. 3B and C with Fig. 4B and C). PKA mutation did not have any significant effect on the rapamycin-induced expression of the NCR gene \textit{MEP2}. As expected, PKA deletion resulted in derepression of the stress response gene \textit{SOD2}, and this derepressed level of expression was further increased by rapamycin treatment (Fig. 3B; quantified in Fig. 3D). These results lend further support to an earlier model proposed by two previous reports indicating that while PKA activity regulates the nuclear

FIG. 3. PKA catalytic activity is not required for Tor regulation of RP genes. (A) Isogenic \textit{Σ1278b} wild-type (MLY41), \textit{yak1} (SZY2), and \textit{tpk1 tpk2 tpk3 yak1} (SZY9) strains were serially diluted onto YP-glucose medium with or without 50 nM rapamycin, grown for 3 days at 30°C, and photographed. (B) Strains were grown to exponential phase and treated with 50 nM rapamycin for 0, 30, 60, and 120 min as indicated. RNA was prepared and analyzed by Northern blotting with radioactive probes that hybridize to the genes indicated at the left. The \textit{RPS26} (C) and \textit{SOD2} (D) gene-specific Northern blot signals were quantified and normalized to the \textit{ACT1} gene-specific signal. The graphs show the \textit{RPS26/ACT1} and \textit{SOD2/ACT1} ratios, respectively. WT, wild type.

FIG. 4. PKA catalytic activity is not required for Tor regulation of RP genes. (A) Isogenic wild-type, \textit{yak1} (SGY73), \textit{tpk1 tpk2 tpk3 yak1} (SGY77), and \textit{tpk1 tpk2 tpk3 msn2 msn4} (ASY63) strains from the S288c genetic background were serially diluted onto YP-glucose medium with or without 50 nM rapamycin, grown, and photographed as indicated in the legend to Fig. 1A. (B) The strains indicated in panel A were grown to exponential phase and treated with 50 nM rapamycin for 0, 30, 60, and 120 min as indicated. RNA was prepared and analyzed by Northern blotting with radioactive probes that hybridize to the genes indicated at the left. (C) The \textit{RPS26} gene-specific Northern blot signal was quantified and normalized to the \textit{ACT1} gene-specific signal. The graph shows the \textit{RPS26/ACT1} ratio.
import of Msn2 and Msn4, Tor signaling regulates their nuclear export in a PKA-independent fashion (11, 17). We conclude that the PKA catalytic subunits are not required for Tor regulation of either RP or STRE gene expression. Importantly, these studies also indicate that if Tor signaling acts via the cAMP-PKA pathway, the point of cross talk between these two pathways is not at the level of any of the PKA catalytic subunits.

Activated Ras signals via the PKA catalytic subunits to overcome RP gene repression in response to rapamycin. To address whether Tor signaling intercepts the PKA pathway at a component upstream from the three TPK subunits, we first examined the possibility that Tor regulates AMP levels. We found that rapamycin treatment did not have any effect on the increase of cAMP levels produced in response to glucose addition to glucose-starved, nitrogen-replete cells (Fig. 5A).

Next, we tested whether the expression of the RAS2V19 allele in the tpk1 tpk2 tpk3 yak1 mutant strain overcomes RP gene repression induced by rapamycin. As shown above, wild-type cells become resistant to rapamycin when they are transformed with the activated RAS2 allele. However, the RAS2V19 allele is unable to confer rapamycin-resistant growth to the tpk1 tpk2 tpk3 yak1 quadruple mutant strain (Fig. 5). Similarly, overexpression of RAS2V19 requires PKA catalytic activity to overcome the rapamycin-induced repression of the RP genes in the tpk1 tpk2 tpk3 yak1 mutant strain (Fig. 5C and D).

These data exclude models in which Tor controls the activity of either adenyl cyclase or cAMP phosphodiesterases. These results also demonstrate that Ras2V19 signals via the TPK subunits to overcome rapamycin toxicity. Our results are consistent with a model in which the TPK subunits are not required for Tor-regulated RP gene expression. Moreover, these results do not support models in which Tor signals to a PKA component upstream of the TPK subunits.

Tor regulates glycogen accumulation via a PKA-independent mechanism. It is well established that both the Tor and PKA pathways control glycogen homeostasis (1, 12, 21). In addition, data from our gene array expression analysis revealed that rapamycin treatment induces the expression of genes required for glycogen synthesis (Table 2) (7). This result is consistent with several reports indicating that a number of genes required for glycogen synthesis are regulated, at least in part, by the Tor-regulated Msn2 and Msn4 transactivators (45, 50).

Recently, it was proposed that rapamycin-induced accumulation of glycogen is blocked by high levels of PKA activity, suggesting that Tor signals through the PKA pathway to regulate glycogen homeostasis (47).

We tested whether Tor signaling regulates glycogen synthesis in strains lacking PKA activity. As expected, we observed that the tpk1 tpk2 tpk3 yak1 mutant strain contains higher levels of glycogen than wild-type cells. Remarkably, after exposure to rapamycin the tpk1 tpk2 tpk3 yak1 mutant strain stained darker when exposed to iodine vapors, indicating an increase in glycogen levels (Fig. 6A). We also tested glycogen accumulation in the presence of rapamycin in the PKA mutant strain from the S288c genetic background. We observed that tpk1 tpk2 tpk3 yak1 mutant cells in this background showed an increased level of glycogen and that this level increases further in response to rapamycin, as seen in the corresponding PKA mutants in S1278b (compare Fig. 6A and B). These results indicate that rapamycin the of glycogen than wild-type cells. Remarkably, after exposure to rapamycin. As expected, we observed that the Tor-regulated Msn2 and Msn4 transactivators (45, 50). Recently, it was proposed that rapamycin-induced accumulation of glycogen is blocked by high levels of PKA activity, suggesting that Tor signals through the PKA pathway to regulate glycogen homeostasis (47).

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Both the Tor and the cAMP-PKA pathways act early in G1 phase to regulate growth, cell cycle progression, entry into G0, and pseudohyphal differentiation in S. cerevisiae (1, 4, 8, 10, 22, 27, 30, 36, 40, 53, 63).

Cell growth is dependent upon ribosome biogenesis and translation. It is well established that the cAMP-PKA pathway has a positive role in regulating the expression of genes required for cell growth, including the RP genes, while simultaneously down-regulating genes required for stress responses and carbohydrate storage. The advent of DNA microarray technology and its application to analyze genome-wide gene expression allowed this view to be expanded and has resulted in the characterization of the Tor pathway as a second global nutrient-sensing signal transduction cascade that regulates growth via several of the same gene families governed by the cAMP-PKA cascade (7, 20; reviewed in references 41 and 43).

Given the myriad functions shared by the Tor and cAMP-PKA cascades, we reasoned that there could be cross talk between these pathways to execute these biological functions. Remarkably, contrary to this expectation, we find no evidence supporting models in which Tor signals via the cAMP-PKA pathway. First, complete elimination of all PKA activity by deletion of the three Tpk catalytic subunits resulted in a substantial reduction in RP gene expression, yet this reduced level of expression was still sensitive to rapamycin and thus subject to Tor signaling. Second, we found that tpk1 tpk2 tpk3 yak1 mutant cells accumulate higher levels of glycogen than wild-type cells, and this level increased further upon exposure to rapamycin. Third, the ability of the activated RAS2V19 allele to confer rapamycin-resistant growth and to overcome rapamycin-induced RP gene repression was found to be dependent upon PKA catalytic activity. This result argues against models in which Tor acts on PKA components downstream of Ras and upstream of the Tpk catalytic subunits. Fourth, because rapamycin treatment does not block the ability of the cell to increase cAMP levels in response to glucose, we can exclude models in which Tor signaling activates Ras and thereby adenylyl cyclase or those in which Tor regulates the activity of cAMP phosphodiesterases. Taken together, these results are in accord with a model in which Tor and cAMP-PKA function as two parallel pathways to independently regulate molecular factors engaged in the expression of RP and STRE genes as well as genes required for glycogen accumulation. While the Tor pathway negatively regulates STRE genes through the Tap42-PP2A effector branch and induces RP genes by a Tap42-PP2A-independent mechanism, the cAMP-PKA cascade orchestrates control of both gene families via the Tpk catalytic subunits (11, 42). This model is also consistent with the epistasis observations in this and a previous study that hyperactivation of one pathway can compensate for a block in the other (47). Our findings are in accord with a recent study in which it was demonstrated that Tor and PKA act independently of each other to regulate the Rim15 protein kinase that controls entry into G0 (36). Moreover, our model is also in line with the observation that while PKA activity prevents the nuclear import of the STRE gene transactivators Msn2 and Msn4, Tor signaling controls the nuclear export of these factors (11, 17).

Our results are in agreement with a recent study demonstrating that hyperactivation of the PKA pathway increases the rapamycin resistance of gln3 gap1 mutant cells but has no effect on nutrient-sensing gene expression as observed in the characterization of the Tor pathway as a second global nutrient-sensing signal transduction cascade that regulates growth via several of the same gene families governed by the cAMP-PKA cascade (7, 20; reviewed in references 41 and 43).

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Our results are in agreement with a recent study demonstrating that hyperactivation of the PKA pathway increases the rapamycin resistance of gln3 gap1 mutant cells but has no effect
on wild-type cells of the JK93d genetic background (47). We observed that PKA hyperactivation confers rapamycin resistance to otherwise wild-type cells of the Σ1278b genetic background. It is known that under normal conditions, the Σ1278b strain has a hyperactive PKA pathway (51). This fact may explain in part why the Σ1278b strain is more resistant to rapamycin than strains from other genetic backgrounds, including JK93d (42), and may explain why PKA hyperactivation in Σ1278b cells can overcome rapamycin toxicity in otherwise wild-type cells. Recently, it was shown that inactivation of Tor by rapamycin triggers the nuclear localization of the Tpk1 catalytic subunit and the Yak1 kinase (47). This effect was taken as evidence to propose that Tor signals via the PKA pathway to control common effector targets (47). While the functional significance of this nuclear localization event is still not understood, deletion of Tpk1 alone or in combination with Tpk2 and/or Tpk3 in the context of the tpk1 tpk2 tpk3 yak1 and the tpk1 tpk2 tpk3 msn2 msn4 multiple mutant strains did not perturb Tor-regulated expression of the RP and STRE genes or of the genes required for glycosyl formation in our studies. Our findings therefore support models in which Tor and PKA signal independently of each other and do not support a recent proposal that Tor signals via PKA (47).

A major challenge in the Tor field is to understand how Tor acts in concert with other signaling cascades. In the case of the response to amino acid deprivation, there is cross talk between the Tor and the general amino acid control response pathways from the Tap42-Sit4 effector branch to the Gcn2 kinase and eIF2α (9, 26, 42). Here, we show that in contrast, Tor and the cAMP-PKA pathways act in parallel and converge to globally control the expression of genes involved in tuning the translational capacity of the cell. This model argues that for the regulation of RP, Tor and PKA genes converge to act at a late step, closer to the transcriptional activators of these genes. This convergence provides a molecular mechanism for coordinating the sensing of both nitrogen (via Tor) and carbon (via the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity). Mol. Cell. Biol. 12:4103–4113.

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