Protein Kinase A, TOR, and Glucose Transport Control the Response to Nutrient Repletion in *Saccharomyces cerevisiae* 

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Nutrient repletion leads to substantial restructuring of the transcriptome in *Saccharomyces cerevisiae*. The expression levels of approximately one-third of all *S. cerevisiae* genes are altered at least twofold when a nutrient-depleted culture is transferred to fresh medium. Several nutrient-sensing pathways are known to play a role in this process, but the relative contribution that each pathway makes to the total response has not been determined. To better understand this, we used a chemical-genetic approach to block the protein kinase A (PKA), TOR (target of rapamycin), and glucose transport pathways, alone and in combination. Of the three pathways, we found that loss of PKA produced the largest effect on the transcriptional response; however, many genes required both PKA and TOR for proper nutrient regulation. Those genes that did not require PKA or TOR for nutrient regulation were dependent on glucose transport for either nutrient induction or repression. Therefore, loss of these three pathways is sufficient to prevent virtually the entire transcriptional response to fresh medium. In the absence of fresh medium, activation of the cyclic AMP/PKA pathway does not induce cellular growth; nevertheless, PKA activation induced a substantial fraction of the PKA-dependent genes. In contrast, the absence of fresh medium strongly limited gene repression by PKA. These results account for the signals needed to generate the transcriptional responses to glucose, including induction of growth genes required for protein synthesis and repression of stress genes, as well as the classical glucose repression and hexose transporter responses.

The transition from quiescence to proliferative growth is an important biological process for organisms of every variety, a process that, when misregulated, can have serious implications. The laboratory yeast *Saccharomyces cerevisiae* regularly makes this transition when nutrient-starved, quiescent cultures are resuspended in fresh glucose medium. Clonal expansion in response to favorable nutrient conditions requires both an increase in cellular mass (cell growth) and an increase in cell number (cell proliferation). Accordingly, the cell that initiates growth and division soonest is at a selective advantage, as it will generate the most progeny as resources are depleted.

Refeeding of quiescent yeast leads to a robust transcriptional response in which thousands of genes are induced or repressed within minutes. Early microarray work demonstrated that approximately a third of the yeast genome is regulated as cells growing in rich medium deplete glucose and shift to slow growth on ethanol (7). More recent work has focused on the transition from slow growth or stationary phase to resumption of growth in glucose media (27, 34, 49). The general response to nutrient repletion consists of a rapid induction of genes involved in mass accumulation and cell division along with repression of genes necessary for respiration, gluconeogenesis, and stress resistance (42).

Yeast cells have multiple pathways for sensing the presence of nutrients. The TOR (17, 26, 51) and cyclic AMP (cAMP)-dependent protein kinase A (PKA) (40, 41, 49) signaling pathways have both been implicated in regulating genes that are induced during nutrient repletion, and there is evidence for signals generated by the transport of glucose into the cell and subsequent aerobic fermentation (16, 30).

Transcriptional profiling and phenotypic evidence both suggest that the TOR pathway is a primary carrier of nutrient signals (4, 9). Treatment of logarithmically growing yeast with the small molecule rapamycin, a potent inhibitor of the Tor proteins, leads to downregulation of many nutrient-sensitive protein synthesis genes and arrests cells in the G1 phase of the cell cycle (4, 9, 12). While these experiments demonstrate a role for the TOR pathway in maintaining the expression of genes related to protein synthesis and mass accumulation during exponential growth, the effect of TOR blockade on the massive changes in transcript levels caused by refeeding has not been tested.

Activation of the cAMP/PKA pathway by either the Ras GTPases or the G-protein-coupled receptor Gpr1 promotes protein synthesis and cell division, while repressing stress responses (40, 41). A recent study by Broach and colleagues demonstrated that artificial induction of the cAMP pathway by either Ras2 or the Gα homolog Gpa2 mimics the transcriptional response to glucose repletion (49). However, the researchers also found that this large-scale response to glucose occurs in cells containing a cAMP-insensitive PKA mutant, indicating a significant role for one or more unidentified cAMP-independent pathways.

Glucose entry into the yeast cell also generates signals that regulate transcription. This mechanism is most evident in the case of carbon catabolite repression, in which glucose transport ultimately leads to Mig1-mediated repression of gluconeogen-
eresis and respiration genes (8, 40, 41). Glucose transport and glycolysis also appear to be necessary for the induction of certain cell cycle transcripts (30). Nevertheless, the role of glucose import in the response to nutrient repletion has not been studied at the whole-genome level, and its overall contribution to this response is unclear.

In this report, we compare the contributions made by TOR, PKA, and glucose transport to the overall transcriptional response to nutrient repletion in yeast. While much of the response is dependent on PKA, signals generated by TOR and glucose internalization also play important roles in the total response. We find that simultaneous inhibition of PKA and TOR is sufficient to prevent a majority of the transcriptional responses. Those responses that are not blocked by loss of PKA and TOR are abolished when glucose transport is blocked. Taken together, our results demonstrate that PKA, TOR, and glucose uptake are sufficient to account for the entire transcriptional response to glucose repletion.

**MATERIALS AND METHODS**

**Yeast strains and growth conditions.** The media used were YPD (1% yeast extract, 2% peptone, 2% glucose) and YP-glycerol-lactate (1% yeast extract, 2% peptone, 2% glycerol, 2% lactate). Nutrient-depleted YPD was created by filtering media from a 9-h wild-type culture. The strains used in this study were TC41 (MATa leu2-3,112 ura3-52 nap1-1 his3-524 cys4 hist1::URA3 cam) (11), MC969A (MATa his3-11,15 leu2-3,112 ura3-52) (36), and KY73 (MATa his3-11,15 leu2-3,112 ura3-52 his1::HIS3 hist1::URA3 cam1::LEU2 hist2::HIS3 hist3::LEU2 hist4::HIS3 cam1::LEU2 hist2::HIS3 gal2::HIS3 (20)). Rapamycin (10-μg/ml stock in ethanol; LC Laboratories) and CAMP (1 mM stock in water; pH 7; Sigma) treatments were as described previously (30, 38).

**Experimental setup.** TC41 (cyr1Δ) cells were grown to glucose exhaustion (48 h; optical density at 600 nm [OD600] = 4) in YPD containing 1 mM CAMP. The cyr1Δ cells were then transferred to spent YPD medium, harvested from a 4-day-old wild-type culture that had been grown in YPD lacking cAMP (final OD600 = 5); the cells were incubated in this nutrient-depleted YPD for 24 h. This produced a quiescent culture of viable cyr1Δ cells in nutrient-depleted medium lacking CAMP. We challenged quiescent cyr1Δ cells with fresh YPD medium, either with cAMP to allow the normal activation of PKA or without cAMP to prevent PKA activation. The TOR pathway was manipulated by adding the TOR inhibitor rapamycin (Fig. 1).

Specifically, the quiescent culture was then split in two; TOR signaling was inhibited with rapamycin in one half while the other half was untreated. After 15 min, aliquots from each culture were collected for microarray analysis (the baseline samples). Cells from the untreated culture were then diluted (final OD600 = 0.5) in fresh YPD supplemented with 5 mM CAMP (pka’, TOR’), or CAMP lacking CAMP (pka’/TOR’). Concurrently, cells from the rapamycin-treated quiescent culture were diluted in fresh YPD containing 5 mM CAMP and 200 mM rapamycin (pka’/TOR’), or CAMP lacking CAMP but containing 200 mM rapamycin (pka’/TOR’). Cells were collected after 1 h for microarray analysis.

To assess the transcriptional response to fresh medium in a strain that is unable to take up and metabolize glucose, we used a strain derived from MC969A (KY73) that lacks the HXT1-7 and GAL1-2 hexose transporter genes (20, 30). Because KY73 (henceforth referred to as hxt’ cells) cannot import and metabolize glucose, it is necessary to grow these cells to starvation (3 days) in glycerol-lactate rather than in glucose prior to the shift to fresh YPD. We also performed the glycerol-lactate-toYPD shift experiment in a wild-type strain (MC969A) that is isogenic to the hxt’ strain.

**Labeled cDNA preparation and microarray hybridization.** Total yeast RNA was isolated using an Epicenter MasterPure yeast RNA purification kit. cDNA and labeled cDNA were generated from total yeast RNA by using a GeneChip one-cycle target labeling kit (Affymetrix) according to the manufacturer’s protocol. Briefly, first-strand cDNA was generated using a T7-oligo(dT) primer and SuperScript II reverse transcriptase. Second-strand cDNA synthesis was performed using Escherichia coli DNA ligase, E. coli DNA polymerase I, and RNase H, followed by incubation with T4 DNA polymerase. After cleanup of cDNA, biotin-labeled antisense cRNA was generated using an IVT labeling kit. Cleanup and fragmentation of labeled cRNA was performed using a GeneChip cleanup module. Labeled cRNAs were then mixed with hybridization controls and hybridized to a yeast genome S98 array (Affymetrix) at 45°C with rotation (60 rpm) for 16 h. Microarrays were then washed and stained with streptavidin-polymerase in a model 400 GeneChip fluids station.

**Microarray data analysis.** Affymetrix yeast genome S98 arrays were scanned using an Agilent GeneArray scanner and Microarray Suite 5.0. The MAS-generated CEL files were analyzed using DCHIP 1.3 (22). Intensity values were normalized across all 24 microarrays by using DCHIP’s invariant set normalization method (21). Model-based analysis, including log2 transformation of expression indexes by use of the perfect match-mismatch difference model, was performed using values from duplicate microarrays for each time point (21). Clustering was performed using the k-means algorithm in TIGR Multiexperiment Viewer, version 3.1 (39). Array data for each transcript, as well as lists of the transcripts contained in each cluster, are included in the supplemental material.

**Verification of experimental design.** First, we compared the transcriptional response of quiescent cyr1Δ cells shifted to YPD-5 mM CAMP with the response to YPD reported for wild-type cells (42). We found that the addition of YPD-5 mM CAMP produced an expression profile in the cyr1Δ strain that was consistent with the normal response to YPD previously described (not shown). In addition, since we needed to grow the hxt’ cells in the absence of glucose, we were also concerned that cells grown to quiescence in YP-glycerol-lactate medium might not respond to fresh medium in the same way as cells grown to post-log phase in YPD. Again, we found that the starting medium did not produce an appreciable difference in the response (not shown). Of the YPD-responsive transcripts described in the legends to Fig. 3 and 4, more than 85% (P < 1 x 10−19) responded in the same direction (at least 1.5-fold as reported for the 60’ point) in a previously published nutrient repletion experiment (42).

Change in response to nutrients is dependent upon expression levels at starvation and expression levels after nutrient addition. To ensure that the effect of blocking a given pathway is due to expression differences after nutrient addition—not differences in expression during starvation—we had to confirm that our starvation expression profiles were similar. In other words, starved cyr1Δ cells

**FIG. 1.** Experimental design..cyrlΔ (TC-41) cells were grown for 48 h in YPD supplemented with 1 mM cAMP, centrifuged, and resuspended in nutrient-depleted YPD without CAMP (final OD600 ~ 5; see Materials and Methods). After 24 h, the culture was split and TOR signaling was inhibited with rapamycin (200 ng/ml, final concentration) in one half, while the other half was untreated. After 15 min, aliquots from each starved culture were collected for microarray analysis. Immediately afterward, the two cultures were diluted (final OD600 ~ 0.5) into fresh YPD maintaining the original concentration of rapamycin, and each culture was split to yield a total of four cultures, two with rapamycin and two without. CAMP was added to one culture of each pair to yield the following conditions: 5 mM CAMP and no rapamycin (PKA’/TOR’), no CAMP or rapamycin (pka’/TOR’), 5 mM CAMP and 200 mM rapamycin (PKA’/TOR’), and no CAMP and 200 mM rapamycin (pka’/TOR’). Cells were then incubated for 1 h, and aliquots were collected for microarray analysis. **Wild-type (WT; MC969A) and isogenic hxt1Δ gal2Δ (KY73) cells were grown for 3 days in YP-glycerol-lactate and transferred to fresh YPD at an OD600 of 0.5. Samples were collected before and after the shift to YPD for microarray analysis. Each experiment was done in duplicate.**
had to have an expression profile similar to that of starved cyr1A that had been treated with rapamycin, and the starved htr+ cells had to be comparable to the corresponding wild-type control. We found that our starting quiescent strains had very similar expression profiles. Though the addition of rapamycin mimics nutrient starvation in growing cells (4, 9), rapamycin did not alter the expression profile of starved cyr1A cells ($R^2 = 0.97$). Similarly, the nutrient-starved wild-type and htr+ profiles were also consistent with each other ($R^2 = 0.93$).

**Analysis of gene sets.** Overrepresented DNA motifs were extracted using the oligonucleotide analysis pattern discovery program in the Regulatory Sequence Analysis Tools resource (46). Each gene set was tested for overrepresented 6- through 8-mers, and when multiple motifs were deemed significant (Regulatory Sequence Analysis Tools significance index greater than 1), only the top three dissimilar, nonoverlapping motifs were chosen. Overrepresented functional categories were determined using the MIPS functional catalogue database (37). The three most significant nonredundant functional categories ($P < 0.005$; each included at least 10 genes) are given for each gene set. The significance of the overlap between two gene sets was calculated using the exact hypergeometric distribution calculator at http://www.alewandr.de/stattab/tabdiske.htm.

**RESULTS AND DISCUSSION**

**Experimental approach.** It has long been known that several signal transduction pathways allow yeast to sense the presence of glucose in the medium and that these signals affect transcription. However, it is not known how many signal transduction pathways are needed to produce the total response to nutrient repletion, nor is it known how much each individual pathway contributes to the overall response. To test this, we blocked these pathways in nutrient-depleted, quiescent cells. We then followed global changes in transcript levels after addition of fresh medium. A schematic of this approach is shown in Fig. 1.

A detailed description of the experimental setup is described in Materials and Methods. Briefly, we used a strain (TC41) lacking CYR1, encoding adenylyl cyclase, to manipulate the cAMP/PKA pathway. This strain is completely dependent on exogenous cAMP for growth (38). The small molecule rapamycin was used to block the TOR pathway, and a strain (KY73) lacking the hexose transporters HXT1,7 and GAL2 was used to block glucose entry into the cell. Ultimately, this experimental approach allowed us to test the response of quiescent, nutrient-depleted cells to fresh YPD under conditions in which PKA and TOR were blocked (PKA+/TOR+, pka-/TOR+, PKA+/tor−, and pka-/tor−) and when glucose transport was blocked (hxt−).

**Overall effect of pathway blockade.** The dot plots in Fig. 2A show how the loss of specific pathways affects the response to fresh YPD for each of ~6,000 transcripts. In these graphs, each transcript is represented as a dot. The position on the x axis represents the change (n-fold) in response to fresh YPD in normal cells. The position on the y axis represents the change (n-fold) in cells in which specific pathways are blocked. The changes are expressed as log2 values. When the response is unchanged by pathway blockade, then each point has the same value on the x and y axes and falls on a diagonal line with a slope of 1 (indicated by the dotted lines). While blockade of each pathway had an effect, it can be seen that rapamycin blockade of the TOR pathway had a relatively small effect, while loss of PKA and TOR together produced the greatest effect.

The R-squared values for each of the four dot plots are shown in Fig. 2B. Individual loss of the PKA, TOR, or glucose transport pathways affected the genome-wide nutrient repletion response to various degrees. PKA signaling played the largest role of the three individual pathways; blockade of hexose transport or TOR produced smaller changes. Simultaneous inhibition of PKA and TOR eliminated a substantial portion of the overall response and produced the lowest correlation with the normal response.

**Nutrient-induced genes.** While the dot plots show involvement of the different pathways in the response to nutrients, the plots do not tell us which genes are regulated by the different pathways, nor do they show a clear picture of the pattern of overlap between the genes sets regulated by the different pathways. To get a better picture of this, we generated heat maps by k-means clustering of the transcripts that are normally altered by YPD (changed 1.5-fold or more in PKA+/TOR+ or wild-type cells). This identified distinct groups of genes regulated by different pathway combinations. These groups tended to have distinct functional and regulatory characteristics. Furthermore,
the patterns seen with induced genes were distinct from those seen with the repressed genes.

Figure 3 shows the heat map generated by clustering of the 1,011 transcripts normally induced by YPD. Each transcript is shown as a horizontal line, with YPD induction indicated by the degree of red color saturation. The normal YPD induction responses are shown in the PKA/TOR column for the PKA/TOR experiments and in the wild-type column, indicating the isogenic wild-type control strain for the hxt mutant. Some clusters with obvious similarities were grouped together, with the 11 clusters organized into groups A through E.

The 436 transcripts in group A were dependent primarily on PKA signaling. A significant fraction of these genes (32%; $P < 10^{-18}$) are involved in posttranscriptional RNA-related processes such as the rRNA and tRNA processing/modification needed for increased protein translation (classified as “transcription” by MIPS). The promoters in these two clusters are enriched in the nutrient-responsive RRPE (rRNA process-
ing element: AAAWTTTT) (15, 42), PAC (polymerase A and C; GATGAG) (6), and GRE (glucose response element; A\textsubscript{2}GA\textsubscript{3}) motifs (31, 33, 43). This is consistent with the idea that transcriptional regulation via these cis-acting elements must involve factors that are downstream of PKA (49).

Group B consists of 299 genes that are dependent upon both PKA and TOR for full YPD induction. For these genes, the TOR input was more apparent in the absence of PKA signaling. Protein synthesis genes make up a significant fraction of this set (29%; \( P = 2.4 \times 10^{-3}\)). The RRPE, PAC, and GRE motifs were also overrepresented in these promoters.

For the 276 genes in the remaining clusters (groups C to E), YPD induction was dependent at least in part on glucose transport. Interestingly, groups C and E contain a significant percentage of cell cycle genes (30% and 26%, respectively). Consistent with this, the cell cycle-regulated and glucose-responsive MCB (ACCGG) and/or SCB (CRCGAAA) regulatory motifs (2, 32, 49) are overrepresented in the promoters of these three groups.

The fact that PKA and TOR are required for the induction of genes required for protein synthesis is not surprising. However, these results show that the PKA-dependent genes can be divided into TOR-dependent and TOR-independent subgroups. Furthermore, it is clear that PKA is not required for a significant group of YPD-induced genes. These PKA-independent genes, however, are almost all dependent on glucose transport.

**Nutrient-repressed genes.** Nutrient-repressed transcripts displayed regulatory patterns similar to those seen with nutrient-induced transcripts (Fig. 4). As with induction, blocking signals generated by PKA, TOR, and glucose transport was sufficient to prevent virtually the entire response. However, in this case almost all genes displayed some degree of dependence on all three pathways, although for some groups a single pathway often predominated. Glucose transport played a larger role, but PKA and TOR were still the major regulators. Concurrent inhibition of both PKA and TOR prevented the repression of almost three-quarters of the nutrient-responsive genes (groups A to C). For the remaining genes that showed nutrient repression despite the double PKA and TOR block, this repression was dependent on glucose transport.

Over half of the nutrient-repressed genes fell into group A and were primarily PKA dependent. This group includes many genes that are necessary for generating and using reserve carbohydrates, recycling of cellular machinery, and responding to stressful environmental conditions. The Msn2/4-regulated STRE (AGGGG) motif is highly represented in the promoters of these genes, consistent with the finding that Msn2/4 activity is negatively regulated by PKA activity (23, 28).

Nutrient repression of the 148 genes in groups B and C was dependent on the TOR pathway. Group C includes a number of genes that are involved in amino acid metabolism and are part of the nitrogen catabolite repression response. Nitrogen catabolite repression genes are normally repressed in the presence of a preferred nitrogen source, a condition that would be provided by fresh YPD. The sequence GATAA is found upstream of genes in this set at a higher than expected frequency; this DNA motif is known to bind the GATA transcription factor Gln3, a well-characterized downstream effector of the TOR pathway (5).

The two clusters in group D were largely dependent on glucose transport for nutrient repression. This group contains a number of transcripts encoding proteins that are necessary for oxidative metabolism, such as enzymes of the TCA cycle and electron transport chain. The promoters of this gene set are enriched in the sequences GGGGTA and CCAAT, motifs that are bound by the transcriptional repressor Mig1 and the transactivating HAP complex, respectively (29, 47). It is worth noting that the presence of these two promoter motifs in this highly glucose transport-dependent gene set is consistent with current models of glucose repression. The repressive activity of the Mig1 repressor is positively regulated by intracellular glucose via the Hxk2 and Snf1 kinases (1). This group of transcripts that are primarily dependent on glucose entry into the cell accounts for the classical glucose repression genes that are among the longest-studied set of glucose-sensitive genes. Similarly, the expression of HAP4, the transactivator subunit of the HAP complex, is negatively regulated by glucose (35).

While findings such as the connection between PKA and downregulation of stress response genes are not surprising, again our results map out the groups of genes for which specific pathways are needed for repression by YPD. This shows the degree of redundancy in regulation by the different pathways, and again, we find that virtually all of the gene repression produced by transfer to YPD can be abolished by blockade of some combination of the three pathways.

**Effects of PKA activation in the absence of nutrients.** Activation of PKA via cAMP can account for a large fraction of the transcriptional response to fresh medium. However, in our experiments, as in nature, glucose and other nutrients were present when cAMP was added. It seems possible that the transcriptional response to cAMP might depend on ongoing metabolism and that the response to cAMP might be dependent on other signals generated by the actions and metabolism of glucose. One reason for thinking that this could be true is the simple fact that cAMP alone is not sufficient to promote either growth or cell division when nutrients are limiting (14, 25). This is shown in Fig. 5A, in which quiescent cyr1\( \Delta \) cells were transferred to either YPD-cAMP or cAMP alone. As can be seen, the YPD-cAMP allowed the cells to reenter the cell division cycle, with thinner cell walls and reduced vacuole size. In contrast, we could see little if any difference in appearance between the cells receiving cAMP alone and the starting quiescent cells, leading one to wonder whether the massive changes in transcript abundance were also occurring in these cells.

To examine the transcriptional response to cAMP alone, we repeated the experiment from Fig. 2 of adding 5 mM cAMP to quiescent cyr1\( \Delta \) cells, but without the inclusion of fresh YPD. We found that gene induction produced by cAMP alone correlated well with the induction produced by cAMP-YPD (Fig. 5B). This shows that the metabolism afforded by nutrient repletion was not needed for the induction of these genes. As would be expected, the set of PKA-dependent genes identified in Fig. 3 (groups A and B) tended to be the ones induced by cAMP alone, while the groups from Fig. 3 that were dependent on glucose transport rather than on PKA (groups C to E) did not tend to respond to cAMP alone (Fig. 6A and B).

On the other hand, genes that were repressed by cAMP-YPD were far less likely to be repressed by cAMP alone,
producing a correlation value of only 0.13 (Fig. 5C). This indicates that in contrast to induction, PKA-dependent gene repression requires some additional signal generated by YPD that is not provided by cAMP alone. Many of the genes down-regulated by PKA are involved in stress responses; perhaps as cells reach quiescence additional PKA-independent mechanisms maintain the expression of these genes, despite PKA activation.

Wang et al. reported a related experiment in which cells growing slowly in glycerol medium were challenged by activating expression of upstream components of the PKA pathway, either constitutively activated Ras2 or Gpa2 (49). Glycerol is a much poorer nutrient source for *S. cerevisiae* than glucose; however, the cells in glycerol were steadily growing, while the cells in our experiment had reached a plateau. Nonetheless, our results with gene induction by cAMP in the cells in exhausted medium are largely in agreement with those of Wang et al., with a significant portion of the induction being independent of nutrient conditions. This indicates that for most genes, induction by PKA activation is independent of the rate
of metabolism. In contrast, our results showing a nutrient requirement for gene repression in response to PKA activation do not so well match those of Wang et al. While PKA activation produced normal gene repression in cells growing on glycerol, we found that this response was significantly reduced when starting with quiescent cells and adding no new nutrients. One obvious possible reason for this discrepancy, as mentioned above, is the fact that the cells in glycerol were growing, while the cells in our experiments were not able to.

Regulatory proteins downstream of TOR, PKA, and glucose transport. A complete picture of the transcriptional response to nutrient repletion requires identification and placement of downstream pathways. With regard to the nutrient-induced genes, it is clear that the proteins Sfp1 and Sch9 play a significant regulatory role (Fig. 7A). Sfp1 nuclear localization, and presumably Sfp1 activity, is positively regulated by the TOR and PKA pathways (17, 24), while evidence indicates that Sch9 is directly activated by TOR (17, 45). Tyers and colleagues have found that Sfp1 and Sch9 regulate essentially the same gene set, which they have termed the RP (ribosomal protein) and Ribi (ribosome biogenesis) regulons (17). We compared these genes to our YPD-induced clusters, and consistent with
the idea that PKA and TOR are upstream of these regulators, we find a highly significant fraction of Sfp1/Sch9 target genes in our PKA- and PKA/TOR-dependent clusters (Fig. 7C).

The Snf3/Rgt2 glucose sensors are known to activate glucose transporter transcription through the Rgt1 transcription factor (16) and are therefore expected to regulate a subset of the nutrient-induced genes (Fig. 7B). Kaniak et al. have identified a core set of Snf3/Rgt2/Rgt1-dependent genes (29 genes total, from reference 18) present in each of the five gene groups from Fig. 3; approximately 10% of these genes fall into our PKA-dependent gene set, and 10% are in the PKA/TOR/glucose-dependent gene set (Fig. 7C). Though the Snf3/Rgt2 pathway is traditionally thought of as a separate glucose-sensing pathway, a PKA input is supported by the recent finding that Rgt1 is also directly targeted by PKA (19).

The Rim15 kinase regulates a set of genes that are repressed during growth on YPD. Rim15 activates these starvation-induced genes through the Msn2/4 and Gis1 transcription factors, and Rim15 activity is negatively regulated by both TOR and PKA (Fig. 8A) (44). Accordingly, we found that half of the previously identified Rim15 targets (3) are downregulated by YPD in a PKA-dependent manner (Fig. 8C). Rim15 targets were not significantly dependent on TOR for their repression. Interestingly, a small but statistically significant fraction of Rim15-dependent genes were highly dependent on glucose import (Fig. 8C). Perhaps glucose metabolism itself contributes to inhibition of Rim15 activity.

Snf1 kinase activity also regulates a number of YPD-repressed genes. Snf1 acts through the Cat8, Sip4, and Adr1 transcriptional activators and the Mig1 repressor (40, 48, 50). It has been proposed that Snf1 activity is intimately linked to glucose metabolism via readouts such as Hxk2 enzymatic activity or the AMP/ATP ratio (Fig. 8B) (8, 40). However, it now appears that Snf1 also responds to stresses unrelated to carbon source, and there is increasing evidence for glycolysis-independent input into Snf1 activity (13). When we compared a published list of Snf1-dependent genes (50) to our YPD-repressed clusters, we found significant overlap between Snf1 target genes and both PKA-dependent and glucose transport-dependent clusters (Fig. 8C). This indicates that intracellular glucose might regulate Snf1, which is consistent with traditional models (Fig. 8B), but also suggests a significant role for PKA in regulating Snf1. Hedbacker et al. demonstrated that PKA regulates Snf1 localization (10), and it is possible that this plays a larger-than-expected role in modulating Snf1 activity.
Overall, these findings support a model in which PKA and TOR promote growth gene regulation through the proteins Sfp1 and Sch9 and lend support to the idea that PKA contributes to Rgt1-mediated glucose induction. Neither Sfp1/Sch9 nor Rgt1 appears to be significantly dependent on glucose transport alone, so the regulators downstream of glucose metabolism remain to be discovered. In addition, our results suggest that PKA also promotes nutrient repression through the Snf1 and Rim15 kinases, but a signal generated by glucose transport or metabolism also feeds into these pathways.

Summary. The transfer of starved yeast to fresh medium has a dramatic, reproducible effect on gene expression (27, 34, 42, 49). Our study provides a detailed map showing which genes are regulated by the different members of a set of important nutrient-sensing pathways. As expected, the cAMP-PKA pathway appears to be the primary regulator of these gene expression changes. A subset of the PKA-dependent genes was also affected by loss of TOR. Approximately 25% of the nutrient-regulated genes are regulated by the different members of a set of important nutrient-sensing changes. A subset of the PKA-dependent genes was also affected by loss of TOR. Approximately 25% of the nutrient-regulated genes and 10% of the nutrient-induced genes are regulated by YPD even in the absence of both PKA and TOR activity, and this regulation is dependent on glucose import. Thus, virtually the entire response to YPD can be prevented by blockade of just these three pathways.

Our results show the role that PKA, TOR, and Hsk2/Snf1 play as hubs lying upstream of local regulators, such as Sch9, Sfp1, and Msn2/4. The mechanisms by which these components interact to carry signals generated at these hubs out to the genes to be regulated remain to be discovered.

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