In mammalian cells transcription factors of the AP-1 family are activated by either stress signals such as UV radiation, or mitogenic signals such as growth factors. Here we show that a similar situation exists in the yeast *Saccharomyces cerevisiae*. The AP-1 transcriptional activator Gcn4, known to be activated by stress signals such as UV radiation and amino acids starvation, is also induced by growth stimulation such as glucose. We show that glucose-dependent Gcn4 activation is mediated through the Ras/cAMP pathway. This pathway is also responsible for UV-dependent Gcn4 activation but is not involved in Gcn4 activation by amino acid starvation. Thus, the unusual phenomenon of activation of mitogenic pathways and AP-1 factors by contradictory stimuli through Ras is conserved from yeast to mammals. We also show that activation of Gcn4 by glucose and UV requires Gcn2 activity. However, in contrast to its role in amino acid starvation, Gcn2 does not increase eIF2α phosphorylation or translation of *GCN4* mRNA in response to glucose or UV. These findings suggest a novel mechanism of action for Gcn2. The finding that Gcn4 is activated in response to glucose via the Ras/cAMP pathway suggests that this cascade coordinates glucose metabolism with amino acids and purine biosynthesis and thereby ensures availability of both energy and essential building blocks for continuation of the cell cycle.

Exposure of cells to UV light results in dramatic changes in the spectrum and levels of gene expression. In prokaryotes most of the genes induced encode DNA repair enzymes, but some encode proteins involved in growth control (1, 2). Similarly, in eukaryotes, UV radiation evokes expression of the DNA repair system (3–5) as well as the cell cycle checkpoint machinery (6–8). Mammalian cells, in addition to inducing those responses, also activate a battery of transcription factors whose target genes are not directly involved in DNA repair or cell cycle arrest. Many of these factors are members of the AP-1 family, e.g. c-Jun, ATF2, and cAMP-response element-binding protein (9–12). Their activation is obtained through phosphorylation and is controlled by UV-responsive signal transduction pathways (9, 10, 12). In addition to activation of AP-1 proteins, UV radiation induces a dramatic increase in mRNA levels of *c-jun*, *c-fos*, and ATP2 (9, 10, 12). Thus, both AP-1 transcriptional activity and expression of AP-1 components are elevated in response to UV radiation. The increase in mRNA levels of AP-1 genes is mediated at least at two levels: through increased transcription rate (9–12) and through an increase in mRNA stability (19).

The biological role of UV-induced AP-1 activation is not entirely clear. In some cell types it seems to be essential for apoptosis induction in damaged cells, whereas in other systems AP-1 activation plays a protective role (20–22). Paradoxically, AP-1 activity and expression are also induced by mitogenic signals (9, 12, 13, 15, 18, 23). Similar signal transduction components, including tyrosine kinase receptors, Ras proteins, and mitogen-activated protein kinase cascades activate AP-1 in response to both UV and growth signals (9, 10, 12, 13, 15, 18, 20, 23). It is still a puzzle how the same signal transduction pathways and the AP-1 transcription activators respond to both UV and growth factors and induce the appropriate but diverse biological responses.

Many aspects of the Ras signaling pathway are similar in yeast and mammals. In the yeast *Saccharomyces cerevisiae* UV irradiation stimulates the Ras signaling pathway and leads to increased transcriptional activity of the yeast AP-1 factor Gcn4 (24). Gcn4 is a functional homolog of c-jun (25). In yeast Ras proteins are involved in regulation of intracellular cAMP, which is essential for entering the “start” at G1 phase of cell cycle (26–28). Glucose response in yeast is also regulated by Ras signaling. Addition of glucose to glucose-starved cells causes a rapid and dramatic increase in the intracellular cAMP concentration (26, 29, 30). The increase in cAMP levels is transient and last 1–2 min. The physiological role of this rapid and transient production of cAMP is not fully understood because cells defective in this response are fully viable and show normal growth. Yet, a recent report suggested that the transient induction in cAMP levels is important for efficient activation of glycolysis and reentry of cell cycle from stationary phase (30).

Addition of glucose to glucose-starved cells has a dramatic effect on gene expression. The most prominent effect is rapid suppression of stress-related genes whose expression is elevated under glucose starvation. Among these genes are some that encode stress-related proteins, e.g. heat shock proteins, enzymes that scavenge free oxygen radicals, and enzymes involved in glycerol synthesis (31–33) and others that encode G1 cyclins (34, 35). Expression of both stress genes and G1 cyclins is elevated under glucose starvation and rapidly suppressed upon addition of glucose (32, 34, 35). Suppression of both stress genes and G1 cyclins is regulated by the Ras/cAMP pathway. Another effect of glucose is the induction of expression of ribosomal genes (36, 37). Expression of these genes is elevated following glucose induction and remains high. So far, genes whose pattern of expression reflects directly the pattern of the
cAMP response have not been reported. Here we show that addition of glucose to glucose-starved cells leads to a transient activation of the AP-1 factor Gcn4. This pattern of expression reflects faithfully the cAMP response. We show that this transient activation is indeed dependent on the Ras/cAMP pathway. Namely, both the UV signal (24) and the growth signal are mediated through the same pathway. Activation of Gcn4 in response to glucose is unexpected because Gcn4 is not known to play any role in cell proliferation. Gcn4 is known in fact to be activated by stress signals such as UV radiation and amino acid starvation (24, 38). Thus, it seems that the paradox of AP-1 activation by both stress and growth signals is evolutionarily conserved and may be addressed in yeast.

We also show that activation of Gcn4 target genes by either UV radiation or glucose requires the Gcn2 gene. Gcn2 encodes a serine kinase whose sole known substrate is the translation initiation factor eIF2α (39–41). Gcn2 phosphorylates eIF2α under conditions of amino acid starvation (38–40). Phosphorylation of eIF2α suppresses its activity and consequently reduces the cellular translation activity. The resulting cease in translation is a protective response that provides ample time for the cell to activate endogenous biosynthesis of amino acids. Those biosynthetic pathways are not active in media supplemented with amino acids and are activated under amino acid starvation following induction of Gcn4 expression (38, 40). Most if not all Gcn4 target genes encode amino acids and purine biosynthetic enzymes. Increased expression of Gcn4 during the period of amino acid starvation is achieved through an unusual mechanism that increases translation of Gcn4 mRNA when eIF2α is phosphorylated (40, 41). Thus, when translational activity in the cell is mostly suppressed, Gcn4 translation is specifically increased. As we show in this study however, activation of Gcn4 in response to UV radiation or glucose is not mediated through an increase in Gcn4 translation, suggesting that Gcn2 functions in these responses through another, novel mechanism.

Based on our results we suggest that cAMP coordinates activation of carbohydrate metabolism and energy production with synthesis of amino acids and nucleotides prior to re-start of cell proliferation.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions**—Yeast strains used are described in Table I. YPD medium is composed of 2% glucose, 1% yeast extract, and 2% Bacto Peptone. YNB minimal medium is composed of 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 0.5% NH₄SO₄, 2% glucose, and the required amino acids or nitrogenous bases. For glucose-response experiments cultures were grown to logarithmic phase (A₅₆₀ = 0.5) on YPD medium, collected, and resuspended in the same volume of YPD medium containing 0.01% glucose instead of 2% (42). Cultures were further grown for about 17 h. Then glucose (or other sugars as indicated in particular experiments) were added from a stock solution of 20% to reach concentration of 2%. Samples were collected by centrifugation at the indicated time points, frozen immediately in liquid nitrogen, and stored at –70 °C prior to RNA or lysate preparations or frozen at –20 °C prior to β-galactosidase assay. Yeast cultures were exposed to UV radiation as previously described (24).

**RNA Preparation and Analysis**—Frozen pellets were thawed on ice, and total RNA was prepared and analyzed by primer extension as previously described (43). Specific primers for the genes analyzed are described in Stanhill et al. (44).

**Preparation of Protein Lysates and Western Blot Analysis**—Protein lysates were prepared using a modified trichloroacetic acid precipitation protocol (45). Frozen pellets were washed with 20% trichloroacetic acid and resuspended in 200 μl of trichloroacetic acid 20%. 600 μg of glass beads were added, and the cells were vortexed two times for 4 min. After vortexing the supernatant was transferred to a new Eppendorf tube, and the beads were washed twice with 200 μl of trichloroacetic acid 5%. The supernatants were combined and centrifuged for 10 min at 3000 rpm. The pellet was suspended in 200 μl of 2x sample buffer followed by an addition of 100 μl of Tris base 1 M. The samples were vortexed for 30 s, boiled for 3 min, and cleared by centrifugation (10 min at 3000 rpm). The lysates were stored in aliquots at –20 °C

**Preparation of Plasmids and β-Galactosidase Assay**—The Gcn4-LacZ plasmid used is the p180I, which is an integrated version of p180 (46). The construction of p180I was described previously (24). p180I was digested with Smal prior to transfection of yeast cells. Disruption of GCN2 was obtained using a gcn2::LEU2 construct obtained from A. Hiinebusch (National Institutes of Health). Disruption of GPA2 was obtained by using a gpa2-1::TRP1 construct obtained from J. P. Hirsch. SP1 yeast culture was transformed with a 1.4-kilobase BamHI fragment of the gpa2-1::TRP1 plasmid. β-Galactosidase assays were performed following lysis of cells in SDS/chloroform as described (47). Each assay was repeated at least two times in duplicate.

**RESULTS**

**Transcription of HIS4 Is Activated by Growth Signals**—Activity and expression of the mammalian c-Jun transcriptional activator are induced in response to either stress signals such as UV radiation or in response to growth stimulation (9, 15, 20). The yeast Gcn4 activity is induced in response to stresses such as amino acid starvation or UV radiation (24, 38). To test if,

### Table I

<table>
<thead>
<tr>
<th>Yeast strains used in this study</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
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<tr>
<td>SP1</td>
<td>MATA hist3 leu2 ura3 trp1 ade8 can’</td>
<td>M. Wigler (24)</td>
</tr>
<tr>
<td>SP1::Gcn4Δ</td>
<td>Isogenic to SP1 but gcn4::URA3</td>
<td>(24)</td>
</tr>
<tr>
<td>SP1ras2Δ</td>
<td>Isogenic to SP1 but ras2::LEU2</td>
<td>M. Wigler (24)</td>
</tr>
<tr>
<td>TK1612R2V</td>
<td>Isogenic to SP1 but ras2::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>SP1-gen2Δ</td>
<td>Isogenic to SP1 but gcn2::LEU2</td>
<td>This study</td>
</tr>
<tr>
<td>SP1::gpa2Δ</td>
<td>Isogenic to SP1 but gpa2::TRP</td>
<td>This study</td>
</tr>
<tr>
<td>GCN4::</td>
<td>Isogenic to SP1 but GCN4::orf::URA3</td>
<td>(24)</td>
</tr>
<tr>
<td>W303</td>
<td>MATA can1–100 ade2::1 his3–11,15 leu2–3 trp1–1 ura3–1</td>
<td>Yeast genetic Stock Center (Berkeley, CA)</td>
</tr>
<tr>
<td>H4</td>
<td>MATA leu2–3,115, ura3–52</td>
<td>A. Hiinebusch (44)</td>
</tr>
<tr>
<td>Σ5227LH</td>
<td>MATA his3 leu2 ura3 trp1</td>
<td>R. Wek (49)</td>
</tr>
</tbody>
</table>

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1 The abbreviations used are: YPD, yeast extract/peptone/dextrose; YNB, yeast nitrogen base minimal medium; Mbf1, multiprotein bridging factor 1; 3-AT, 3-amino triazole.
The transient mode of *HIS4* expression is somewhat unusual and reveals a novel pattern of gene expression in response to glucose. Previous studies described glucose-dependent suppression of transcription of stress genes and G1 cyclins (26, 32–35). We also observed rapid suppression of stress genes following addition of glucose in our system. The level of *HIS26* mRNA for example that is increased upon glucose starvation decreases rapidly after addition of glucose (Figs. 1 and 2). Another type of glucose response is the slow, but continuous increase in expression of many genes. This type of response is manifested here by the steady increase in the levels of *ACTIN* mRNA (Fig. 1A and 2). Thus, it seems that at least three responses could be measured at the level of gene expression following addition of glucose to starved cells (Fig. 1C); (i) rapid suppression of stress genes and G1 cyclins, (ii) slow induction of structural and proliferative genes, and (iii) rapid and transient increase in *Gcn4* target genes.

**Glucose-dependent HIS4 Induction Is Mediated via the Ras/CAMP Cascade—**Addition of glucose to glucose-starved cells is known to induce rapid activation of the Ras/CAMP pathway that is manifested in a transient increase in cAMP levels (29, 33). As the kinetic pattern of *HIS4* expression following addition of glucose is strikingly similar to the pattern of changes in cAMP concentrations, it could be that the Ras/CAMP system is involved in glucose-induced *Gcn4* activation. To test this possibility we measured glucose-dependent induction of *HIS4* transcription in isogenic strains deleted in either *RAS2* or *GPA2* (Fig. 2, A and B). *GPA2* encodes the α subunit of a heterotrimeric G protein that has been shown recently to function either upstream or parallel to Ras2 (33). The results presented in Fig. 2. A and B clearly show that the mutants are unable to induce *HIS4* transcription in response to addition of glucose. Thus, induction of *HIS4* expression in response to glucose is mediated via the Ras pathway. The unexpected observation that *HIS4* is activated in response to a growth stimulus in the presence of amino acids raises the possibility that...
under these conditions it is not induced by Gcn4. To test this idea we measured HIS4 induction in gcn4Δ cells. In these cells the low levels of HIS4 mRNA did not change when glucose was added to glucose-starved cells (Fig. 2C). Notably, the RAS2, GPA2, and GCN4 genes are also essential for HIS4 induction in response to UV radiation (Ref. 24 and data not shown).

In contrast to their effect on HIS4 activation, the ras2Δ, gpa2Δ, and gcn4Δ mutations had no effect on the other glucose-mediated responses. The reduction in HSP26 mRNA and elevation of ACTIN mRNA following addition of glucose were intact in all strains analyzed (Fig. 2, A–C), suggesting that the ras2Δ, gpa2Δ, and gcn4Δ cells are specifically defective in only one of the three measured glucose responses.

Activation of Gcn4 by a mitogen signal through the mitogenic Ras/cAMP cascade is unexpected because this transcriptional activator is known to be activated by stresses such as amino acid starvation. To test whether the Ras signaling pathway is also involved in Gcn4 activation in response to amino acid starvation, we monitored HIS4 induction in the various mutants in response to amino acid starvation (Fig. 3, A–D). As expected, the gcn4Δ strain was not able to induce HIS4 under these conditions, similar to its inability to induce HIS4 in response to glucose (Fig. 3D). The response of gpa2Δ cells, however, was indistinguishable from the wild-type response (compare Fig. 3C to 3D). The response of ras2Δ cells to amino acid starvation was somewhat delayed but reached wild-type levels of HIS4 mRNA (Fig. 3B). Thus, unlike the case of the glucose response, amino acid starvation-mediated Gcn4 activation is independent of the Ras pathway.

Glucose-dependent HIS4 Induction Is Not Mediated through GCN4 Translation—Having verified that glucose-dependent Gcn4 activation requires the Ras/cAMP pathway, we sought more components of the Ras/Gcn4 pathway in particular those that function between the Ras/cAMP cascade and Gcn4. The main mechanism known to induce Gcn4 activation/expression is activated by amino acid starvation and involves Gcn2-mediated increase in translation of GCN4 mRNA (40, 41). Yet, there must be other mechanisms because in the RAS2Δ and bcy1Δ strains, the constitutive activity of Gcn4 is explained by a cooperation of two mechanisms: 1) a moderate increase (2.5-fold) in GCN4 translation and 2) another, unknown mechanism that operates posttranslationally (24). We tested if the increase in HIS4 mRNA following addition of glucose may be a consequence of elevated translation of GCN4. To this end we used a strain harboring an integrated copy of the GCN4-LacZ fusion gene (46) (provided by A. Hinnebusch, NIH). This construct contains the promoter 5'UTR and 153 base pairs of the GCN4 coding sequence fused to β-galactosidase. As GCN4 transcription is constitutive, β-galactosidase activity derived from this construct reflects GCN4 translation (41, 46). Surprisingly, the activity of this construct that was very low on YPD not only was not reduced, as was expected from the decrease in HIS4 mRNA (Fig. 1A), but was even somewhat increased upon glucose starvation (Fig. 4A). An increase in GCN4-LacZ activity under glucose starvation was also observed recently by Yang et al. (49). Namely, GCN4 translation increased under conditions that caused suppression of HIS4 transcription (Figs. 1 and 2). Furthermore β-galactosidase activity of this construct did not change when glucose was provided and Gcn4 was activated (Fig. 4A), suggesting that GCN4 translation was not correlated to HIS4 transcription (Fig. 1). To verify that the GCN4-lacZ construct that was integrated in the cells is intact and responsive, the same culture was starved not for glucose but for amino acids. Fig. 4B shows that under these conditions GCN4-LacZ activity, reflecting GCN4 translation, was induced 10-fold. This increase in GCN4 translation is well correlated with the increase in HIS4 transcription (Fig. 3A).

To show unequivocally that Gcn4 expression is not increased upon addition of glucose, we measured directly through Western blot analysis the level of endogenous Gcn4p (antibodies were kindly provided by Daniel Kornitzer). This analysis shows that Gcn4p levels remain very low during glucose starvation and following addition of glucose (Fig. 4C). We found Gcn4 levels to increase only in response to amino acid starvation (lane 8 in Fig. 4C) in the RAS2Δ strain (lane 11 in Fig. 4C) as expected (24) and in the GCN4 strain in which GCN4 mRNA is constitutively translated (lane 9 in Fig. 4C). The results of the Western blot agree with the results obtained with the GCN4-LacZ constructs. Taken together, these results show that in response to the addition of glucose, Gcn4 increases HIS4 transcription via a mechanism that does not involve an increase in GCN4 translation.

Activation of Gcn4 by Glucose Is a General Phenomenon That Occurs in All Laboratory Strains—The results above described two unexpected findings: (i) Gcn4 is activated by growth signals and (ii) this activation is obtained via a mechanism that is independent of GCN4 translation. These unexpected observations raised the concern that the phenomenon is restricted to one genetic background that may be utilizing a peculiar mechanism for Gcn4 activation. To test whether activation of Gcn4 by glucose is a general phenomenon in yeast, we tested a battery of commonly used laboratory strains for their ability to activate HIS4 transcription in response to glucose. Cells of the W303, Σ1278b, and H4 (a derivative of the S288C strain) genetic backgrounds were grown on YPD medium, starved for glucose for 17 h before readdition of glucose. As is shown in Fig. 5, transcription of HIS4 was suppressed upon glucose starva-
and rapidly increased when glucose was added in all the strains. Thus, activation of Gcn4 target genes in response to glucose was measured in all laboratory strains tested. It is interesting to note that the pattern of HIS4 activation is different in every genetic background. In the \( \Sigma 1278b \) background (strain \( \Sigma L5527LH \)) for example, glucose-induced increase in HIS4 mRNA is sustained and not transient (Fig. 5B). This result is expected because in the \( \Sigma 1278b \) genetic background HIS4 mRNA levels are high during exponential growth even on YPD medium, similar to the situation in \( RAS2^{val19} \) mutants (44). This is a consequence of the high levels of cAMP in the \( \Sigma 1278b \) background (44). Another reflection of the high cAMP levels in \( \Sigma L5527LH \) cells is suppression of the stress response (note HSP26 levels in Fig. 5B and Ref. 44).

To test whether in all genetic background Gcn4 activation and HIS4 transcription are not correlated with GCN4 translation, we integrated the GCN4-LacZ gene into the genome of the strains shown above as well as into the EG328–1A (used by Yang et al. (49)) and ras2\( \Delta \) strains. As is shown in Fig. 6, in all genetic backgrounds GCN4-LacZ activity increased upon glucose starvation (when HIS4 transcription is almost totally suppressed, Fig. 5) and did not change when glucose was added. The observed increase in GCN4-LacZ activity upon glucose starvation is in agreement with the observation made by Yang et al. (49). Thus, induction of Gcn4 activity by glucose via a mechanism independent of GCN4 translation was measured in all yeast strains. Even the ras2\( \Delta \) strain, which does not induce HIS4 in response to glucose (Fig. 2A), showed GCN4-LacZ activity similar to that of its parental wild type strain (Fig. 6), emphasizing the lack of correlation between HIS4 induction and GCN4-LacZ activity in response to glucose.

**Gcn2 Is Essential for Glucose- and UV-dependent HIS4 Induction**—Unlike the situation described here, under conditions of amino acid starvation GCN4 expression is induced at the translational level. This induction requires Gcn2-mediated phosphorylation and inhibition of eIF2\( \alpha \) (39–41, 49). As we showed previously (24), in the bcy1\( \Delta \) strain GCN2 is not required for the high and constitutive HIS4 expression. To further verify that Gcn2 is not required for Ras/cAMP-dependent Gcn4 activation, we disrupted the GCN2 gene in the \( RAS2^{val19} \) strain and in wild type cells. Similar to the case of the bcy1\( \Delta \) strain, deletion of GCN2 in the \( RAS2^{val19} \) strain did not abolish constitutive HIS4 expression, although it was somewhat reduced (Fig. 7A). Thus, in mutants harboring a constitutively active Ras pathway, Gcn2 is not an essential mediator of the constitutive Gcn4 activity. However, deletion of GCN2 in wild type cells destroyed their capability to induce HIS4 in response to glucose or UV radiation (Fig. 7, B and C). Thus, although GCN2 is not required for HIS4 expression in \( RAS2^{val19} \) and bcy1\( \Delta \) strains, it is required for induction of the Ras/Gcn4
In all laboratory strains tested, glucose-dependent increase in HIS4 transcription is not mediated through GCN4 translation. β-Galactosidase activity was measured in cells of the laboratory strains SP1, W303, ΣL527LH, H4, and EG328–1A harboring an integrated GCN4-LacZ reporter gene. Cells of the ras2Δ strain, which is incapable of HIS4 induction in response to glucose (Fig. 2A), were also included in this experiment. Samples for β-galactosidase assay were collected from cells grown under the indicated growth conditions. Minutes refer to time after glucose addition to glucose-starved cells.

FIG. 7. GCN2 is essential for glucose and UV induction of HIS4 but not for Ras2Val19-mediated HIS4 induction. A, primer extension analysis of RNAs prepared from the RAS2Val19 and the RAS2Val19/gcn2Δ strains grown on YPD to logarithmic phase. B, analysis of HIS4 and ACTIN mRNA levels in gcn2Δ cells grown in logarithmic phase (Log. phase), exposed to glucose starvation (starvation), and at different time points after glucose was added. C, primer extension analysis of RNAs prepared from cells of the SP1 and gcn2Δ strains at various time points after exposure to UV radiation (40 joules/m²).

pathway by external signals. The requirement of GCN2 for the transmission of these signals is further intriguing because GCN4 translation is not elevated in response to glucose or UV radiation (Figs. 4 and 6 and data not shown). It seems therefore that Gcn2 is required for the UV and glucose responses but functions through a novel mechanism. Under amino acid starvation Gcn2 phosphorylates and inhibits eIF2α and thereby increases GCN4 translation (40). To verify that in response to glucose Gcn2 functions in a different way, we tested the level of phospho-eIF2α during the course of glucose response. Fig. 8 depicts the results of a Western blot analysis showing that eIF2α is somewhat phosphorylated upon starvation to glucose. This observation explains the increase in GCN4 translation under these conditions (Figs. 4A and 6) and is in agreement with previous observations (49). Yet, it is not correlated with HIS4 transcription that is suppressed under glucose starvation (Fig. 1). Furthermore, no changes in eIF2α phosphorylation were measured upon addition of glucose (Fig. 8), which caused strong induction of Gcn4-dependent HIS4 expression (Figs. 1 and 2). Thus, Gcn2 mediates Gcn4 activation in response to UV or glucose through a mechanism that does not involve eIF2α phosphorylation and GCN4 translation. This is the first indication of another mechanism of action of Gcn2.

FIG. 8. Induction of HIS4 transcription in response to glucose is not correlated with changes in eIF-2α phosphorylation. Shown are Western blots in which the levels of phosphorylated and nonphosphorylated eIF-2α were analyzed. Lysates were prepared from cells of the SP1 strain at the indicated time points prior to and after addition of glucose to glucose-starved cells. Cells from another culture that was exposed to 3-AT treatment were used as a positive control (compare YPD to 3-AT).

DISCUSSION

In this study we describe a novel glucose response in yeast at the level of gene expression. We show that addition of glucose to glucose-starved cells caused a transient increase in the level of HIS4 mRNA, a target gene of Gcn4 transcription factor (Fig. 1). We verified that glucose-dependent HIS4 induction is indeed mediated through Gcn4 (Fig. 2C). This is an unexpected finding because Gcn4 is usually activated not by growth stimuli but rather by stress conditions. Yet, the findings described here are reminiscent of the situation in mammalian cells where AP-1 factors are activated by either stress or growth stimuli (9, 12, 15, 18). Thus, our results show that activation of AP-1 factors by these contradictory signals is conserved from yeast to mammals. This conservation points at the importance of AP-1 activation by these signals but does not explain the paradox of induction of an identical response by mitogenic signals on one hand and by stress signals on the other hand.

Induction of Gcn4 in response to amino acid starvation is explained readily because it leads to activation of de novo biosynthetic pathways of amino acids. It could be that the same biosynthetic pathways need to be activated when glucose becomes available after starvation to ensure availability of amino acids and nucleotides for reinitiation of cell cycle. These biosynthetic pathways seem to be suppressed under glucose starvation as is manifested by the undetectable levels of HIS4 mRNA under these conditions (Figs. 1 and 2). The question remains however, why is Gcn4 induced in medium so rich in...
Gcn2 Mediates UV- and Glucose-dependent Gcn4 activation

amino acids? It could be that amino acid permeases and sensors are shut off under glucose starvation (to ensure maintenance of amino acids?) It could be that amino acid permeases and sensors in the medium, leads to induction of biosynthetic pathways as is manifested by the rise in GCN4 target genes (Figs. 1 and 5), but simultaneously it may reactivates the amino acid permeases. Consequently, the high concentrations of nutrients in YPD medium immediately suppress the cascades again. This model explains the transient mode of the response. Such a pattern of transient response to glucose has not been described previously at the transcriptional level but only at the enzymatic level showing activation of Ras and adenyl cyclase (26, 29, 33). As we showed here the two responses are connected (Fig. 2; see below). In fact, the case shown here for HIS4 is the first example for a gene whose expression reflects the cAMP response.

The effect of glucose addition on Gcn4 activity points at a previously unidentified link between glucose signaling and amino acids and purine biosynthesis. Clearly, to resume growth after starvation cells must produce ATP but concomitantly have to synthesize nucleotides and amino acids. It seems that the glucose-induced cAMP burst orchestrates coinduction of glucose metabolism for the production of energy (26, 30) and amino acids/purine metabolism as is shown in this work. Most interestingly however, the transient rise in cAMP is essential but not sufficient for Gcn4 activation. Only metabolized sugars such as glucose or fructose activate Gcn4 (Fig. 1 A and B), whereas sugars that are transported to the cell but not metabolized, like xylose and 2-deoxyglucose, do not activate Gcn4 (Fig. 1B). These non-metabolizable sugars were shown to cause a transient or permanent rise in cAMP (29). Similarly, deletion of the RAS2 gene has no effect on the transient rise in cAMP (54) but dramatically suppresses Gcn4 activation (Fig. 2). Thus sugar metabolism and intact Ras2, which are not essential for the cAMP response, are essential for Gcn4 activation.

Activation of Gcn4 in response to UV radiation (Ref. 24 and Fig. 6) may be required more specifically for induction of nucleotide biosynthesis (needed for DNA repair). However, also in the case of UV radiation it could be that an immediate cellular protective response is the suppression of amino acid transport (as part of the checkpoint/growth arrest system that provides time for repair). Consequently, the cells rely on de novo synthesis until peremeses are activated again. Gcn4 activation in response to UV is indeed not transient and HIS4 mRNA levels remain high for at least 75 min after irradiation (24). Thus, activation of Gcn4 by contradictory stimuli may seem less paradoxical if de novo synthesis of amino acids and purines is required for appropriate cellular response for each of those signals. It is obvious that Gcn4 activation is just one aspect of the complex cellular response to a given stimulus, implying that not Gcn4 activation alone but the particular combination of responses to each stimulus (UV, glucose, amino acid starvation) determines the appropriate overall biological phenotype. Similar to the case described here for Gcn4, the mammalian c-Jun is also activated by either growth stimuli or stress signals. It is tempting to suggest that a similar explanation would resolve the paradox in mammalian cells too. Yet, most c-Jun target genes are currently unknown and it is difficult to predict if c-Jun induces its various responses via the same set of genes. Determination of the subset of c-Jun target genes induced in response to each stimulus is required to test this idea.

As each of the different signals that activate Gcn4 is sensed and transmitted via a specific signal transduction pathway, it seems that Gcn4 is recognized by many cascades. Indeed, in the case of the UV- and glucose-response, it is the Ras/cAMP pathway that activates Gcn4, whereas in response to amino acid and purine starvation this pathway is dispensable for Gcn4 activation. Under these conditions Gcn4 is activated by the Gcn2/eIF2a machinery. The latter pathway also activates GCN4 translation in response to glucose starvation (49). Strikingly, the increased expression of Gcn4 under glucose starvation does not result in transcription of HIS4. In fact, HIS4 mRNA is barely measurable under glucose starvation (Figs. 1, 2, and 5A). This result suggests that an increase in Gcn4 expression is not always correlated with Gcn4 transcriptional activity. There must be regulators that do not affect Gcn4 expression, but rather affect Gcn4 activity. Such a regulator may be for example the multiprotein bridging factor 1 (Mbf1). Mbf1 functions as a mediator between Gcn4 and the basal transcriptional machinery (50). Its expression is essential for Gcn4 transcriptional activity and for cell growth under amino acids starvation. It is not known how Mbf1 is regulated or to which external signal it may respond. Another Gcn4 regulator is the Cpc2 repressor that suppresses Gcn4 under optimal growth conditions (51). It is not known how Cpc2 is inactivated to derepress Gcn4 activity. Analysis of Cpc2 effect on Gcn4 points at a novel mechanism of Gcn4 regulation that is not mediated through GCN4 translation (Figs. 4 and 5). Deletion of Cpc2 in gcn2A cells restores the expression of Gcn4 target genes and the ability to grow under amino acids starvation (51). This result may suggest that Cpc2 is epistatic to Gcn2, but further studies are obviously required to reveal the relationships (if any) between Gcn2, Mbf1, Cpc2, and/or yet unknown regulators of Gcn4. These studies should also reveal which of those Gcn4 regulators is responsive to the Ras/cAMP cascade and mediates Gcn4 activation in response to glucose and UV radiation.

The function of Gcn2 in the glucose response is rather peculiar. Although it is essential for transmitting the signal to Gcn4 (Fig. 6, B and C), translation of GCN4 is not induced (Fig. 4, A and C) and phosphorylation of eIF2a is not changed (Fig. 7). It may be that Gcn2 phosphorylates and modulates a yet unknown substrate, maybe one of Gcn4 coactivators or transcriptional mediators. Alternatively, Gcn2 may function through protein-protein interactions. It was recently suggested that the mammalian homolog of Gcn2, PKR, may recognize more substrates in addition to eIF2a and was shown to physically interact with but not necessarily phosphorylate Stat1 and p53 (Ref. 52 and reviewed in Ref. 53). In any case, Gcn2 functions in the glucose- and UV-response in a novel way that should be explored to fully understand the cellular response to these important signals.

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