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 pattern of expression reflects directly the pattern of the somatic genes (36, 37). Expression of these genes is elevated under glucose starvation and rapidly suppressed upon addition of glucose (32, 34, 35). Suppression of both 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 scavange 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 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 cascade 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 scavange 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 a unique 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% NH₄(SO₄)₂, 2% glucose, and the required amino acids or nitrogenous bases. For glucose-response experiments cultures were grown to logarithmic phase (A₅₀=0.5) on YPD medium, collected, and resuspended in the same volume of YPD medium containing 0.01% glucose.

Table I Yeast strains used in this study

| Strain | Relevant genotype | Source or reference |
|--------|------------------|---------------------|
| SP1   | MATa his3 leu2 ura3 trp1 ade8 can1 | M. Wigler (24) |
| SP1gc4Δ | Isogenic to SP1 but gc4::URA3 | (24) |
| SP1ras2Δ | Isogenic to SP1 but ras2::LEU2 | (24) |
| TK1612ΔV | Isogenic to SP1 but RAS2::LEU2 | M. Wigler (24) |
| SP1gen2Δ | Isogenic to SP1 but gc2::LEU2 | This study |
| SP1gpa2Δ | Isogenic to SP1 but gps2::TRP | This study |
| GCN4Δ | Isogenic to GCN4::orf::URA3 | (24) |
| W303 | MATa can1-100 ade2-1 his3-11,15 leu2-3 trp1-1 ura3-1 | Yeast genetic Stock Center (Berkeley, CA) |
| H4 | MATa trp1-3 115 ura5-52 | A. Hinnebusch (44) |
| Σ5227LH | MATa his3 leu2 ura3 trp1 | R. Wek (49) |
| EG328–1A | MATa leu2 ura3-52 trp1 | |
addition. of the three patterns of gene expression observed in response to glucose (glucose, fructose, xylose, or 2-deoxyglucose). divided to four and each fraction was treated with a different sugar from cells treated as in that are not metabolized. Primer extension analysis of mRNA prepared and reveals a novel pattern of gene expression in response to unexpected and intriguing (see below). including amino acids are not limited, induction of starvation 0.01% glucose was used). As all other nutrients dmium, and only glucose concentrations were manipulated (for respon sponds to growth stimulation and not to stress signals. This result also shows that glucose transport is not sufficient for induction of transcription of stress genes and G1 cyclins, (ii) slow induction of structural and proliferative genes, and (iii) rapid and transient increase in Gcn4 target genes.

The possibility remains, however, that a rapid increase in external glucose concentration could be sensed in fact as a stress signal, such as osmotic shock, rather than as a growth signal. To verify this point we added to glucose-starved cells several sugars: glucose and fructose that are easily metabolized and support growth and xylose and 2-deoxyglucose that enter the cell but cannot be used metabolically (29). Thus, HSP4, known to be induced by stresses such as amino acid limitation and UV radiation, is induced here by a growth signal.

The transient mode of HSP4 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 HSP26 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.
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 HIS4 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 mitogenic 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 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. In this experiment the cells were specifically defective in only 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-
Gcn2 Mediates UV- and Glucose-dependent Gcn4 activation

Fig. 4. Induction of HIS4 mRNA by glucose response is not mediated through increase in Gcn4 translation nor through increase in Gcn4 levels. A, β-galactosidase activity of the GCN4-LacZ reporter gene integrated in the genome of the wild type SP1 strain. Samples for β-galactosidase assay were collected from logaritmically growing cells (Logarithmic Phase), from cells under glucose starvation (Starvation), and from same cells after glucose was added. B, GCN4 translation increases dramatically in response to amino acid starvation. Same cells used in A were grown on rich YPD medium to logarithmic phase, collected, and divided to three cultures. One culture was resuspended in YPD, one in YNB, and one in YNB without histidine supplemented with 20 mM 3-AT. Samples for β-galactosidase assay were removed at the indicated time points. C, Western blot analysis of Gcn4 proteins. Yeast lysates were prepared from logaritmically growing cells (of the wild type strain SP1), from glucose-starved cells, and at the indicated time points after glucose was added. As positive controls, lysates were prepared from cells of the same strain treated with 3-AT (eighth lane from the left), from cells of the GCN4 strain (24) in which GCN4 is constitutively translated, and from cells of the RAS2val19 strain in which GCN4 translation is partially elevated (24). Lysate prepared from gcn4Δ cells was used as a negative control (tenth lane from the left). Lysates were separated on 10% SDS-polyacrylamide gel electrophoresis and blotted to nitrocellulose paper, which was reacted with polyclonal anti-Gcn4 antibodies as described under “Experimental Procedures.”

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 ras2Δ strains. As is shown in Fig. 5, 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 ras2Δ 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 eIF2α (39–41). As we showed previously (24), in the bcy1Δ 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 RAS2val19 strain and in wild type cells. Similar to the case of the bcy1Δ strain, deletion of GCN2 in the RAS2val19 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 RAS2val19 and bcy1Δ strains, it is required for induction of the Ras/Gcn4
Fig. 6. 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, YL5527/LH, 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.

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 factors in the regulation of gene expression.

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...
are shut off under glucose starvation (to ensure maintenance of amino acids? It could be that amino acid permeases and sensors GCN4-LacZ glucose starvation (Figs. 4 and 5), but simultaneously it may reactivitate 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 permisses 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 purine 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/eIF2α 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 gcn2Δ 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 responsible 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 eIF2α 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 eIF2α 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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REFERENCES
1. Walker, G. C. (1985) Annu. Rev. Biochem. 54, 425–457
2. Walker, G. C. (1995) Trends Biochem. Sci. 20, 416–420
3. Prakash, S., Sung, P., and Prakash, L. (1990) Annu. Rev. Genet. 27, 33–70
4. Aboussekhra, A., and Wood, R. D. (1994) Curr. Opin. Genet. Dev. 4, 212–220
5. Wood, R. D. (1996) Annu. Rev. Biochem. 65, 135–167
6. Enoch, T., and Norbury, C. (1995) Trends Biochem. Sci. 20, 426–430
7. Hartwell, L. H., and Weinert, T. A. (1989) Science 246, 629–634
8. Murray, A. W. (1995) Curr. Opin. Genet. Dev. 5, 5–11
9. Devary, Y., Gottlieb, R. A., Smeal, T., and Karin, M. (1992) Cell 71, 1081–1091
10. Herrlich, P., and Rahmsdorf, H. J. (1994) Curr. Opin. Cell Biol. 8, 425–431
11. Iordanov, M., Bender, K., Aide, T., Schmid, W., Sachsenmaier, C., Engel, K., Gaestel, M., Rahmsdorf, H. J., and Herrlich, P. (1997) EMBO J. 16, 1009–1022
12. Radler-Pohl, A., Sachsenmaier, C., Gebel, S., Auer, H. P., Bruder, J. T., Rapp, U., Angel, P., Rahmsdorf, H. J., and Herrlich, P. (1993) EMBO J. 12, 1005–1012
13. Coffer, P. J., Burgering, B. M., Peppelenbosch, M. P., Bos, J. L., and Kruijer, W. (1995) Oncogene 11, 561–569
14. Ip, Y. T., and Davis, R. J. (1998) Curr. Opin. Cell. Biol. 10, 205–219
15. Karin, M. (1995) J. Biol. Chem. 270, 16483–16486
16. Minden, A., and Karin, M. (1997) Biochim. Biophys. Acta 1333, 85–F104
17. Ono, K., and Han, J. (2000) Cell. Signal. 12, 1–13
18. Sachsenmaier, C., Radler-Pohl, A., Zinck, R., Nordheim, A., Herrlich, P., and Rahmsdorf, H. J. (1994) Cell 78, 963–972
19. Blattner, C., Kannouche, P., Litfin, M., Bender, K., Rahmsdorf, H. J., Angulo, J. F., and Herrlich, P. (2000) Mol. Cell. Biol. 20, 3616–3625
20. Wisdom, R., Johnson, R. S., and Moore, C. (1999) EMBO J. 18, 188–197
21. Karin, M., Liu, Z., and Zandi, E. (1997) Curr. Opin. Cell. Biol. 9, 240–246
22. Bossy-Wetzel, E., Bakiri, L., and Yaniv, M. (1997) EMBO J. 16, 1695–1709
23. Rodrigues, G. A., and Park, M. (1994) Curr. Opin. Genet. Dev. 4, 15–24
24. Engelberg, D., Klein, C., Martinetto, H., Struhl, K., and Karin, M. (1994) Cell 77, 381–390
25. Struhl, K. (1988) Nature 332, 649–650
26. Thevelein, J. M. (1994) Yeast 10, 1753–1790
27. Broach, J. R., and Deschenes, R. J. (1990) Adv. Cancer Res. 54, 79–139
28. Broach, J. R. (1991) Curr. Opin. Genet. Dev. 1, 370–377
29. Eraso, P., Gancedo, J. M. (1985) FEBS Lett. 191, 51–54
30. Jiang, Y., Davis, C., and Broach, J. R. (1998) EMBO J. 17, 6942–6951
31. Boy-Marcotte, E., Tadi, D., Perrot, M., Boucherie, H., and Jacquet, M. (1996) Microbiology 142, 439–467
32. Mager, W. H., and De Kruijf, A. J. (1995) Microbiol. Rev. 59, 506–531
33. Colombo, S., Ma, P., Cauwenberg, L., Winderickx, J., Crauwels, M., Teunissen, A., Nauwelaers, D., de Winde, J. H., Gorwa, M. F., Colavizza, D., and Thevelein, J. M. (1998) EMBO J. 17, 3326–3341
34. Baroni, M. D., Monti, P., and Alberghina, L. (1994) Nature 371, 339–342
35. Tokiwa, G., Nishida, E., and Fink, G. R. (1984) Mol. Cell. Biol. 4, 1326–1333
36. Klein, C., and Struhl, K. (1994) Mol. Cell. Biol. 14, 1920–1928
37. Neuman-Silberberg, F. S., Bhattacharya, S., and Broach, J. R. (1995) Mol. Cell. Biol. 15, 3187–3196
38. Hinnebusch, A. G. (1988) Microbiol. Rev. 52, 248–273
39. Dever, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F., and Hinnebusch, A. G. (1992) Cell 68, 585–596
40. Hinnebusch, A. G. (1997) J. Biol. Chem. 272, 21661–21664
41. Mueller, P. P., and Hinnebusch, A. G. (1986) Cell 45, 201–207
42. Kaibuchi, K., Miyajima, A., Arai, K., and Matsumoto, K. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8172–8176
43. Engelberg, D., Zandi, E., Parker, C. S., and Karin, M. (1994) Mol. Cell. Biol. 14, 4929–4937
44. Stanhill, A., Schick, N., and Engelberg, D. (1999) Mol. Cell. Biol. 19, 7529–7538
45. Foiani, M., Marini, F., Gamba, D., Lucchini, G., and Plevani, P. (1994) Mol. Cell. Biol. 14, 923–933
46. Hinnebusch, A. G. (1985) Mol. Cell. Biol. 5, 1326–1333
47. Ausubel, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F., and Hinnebusch, A. G. (1992) Cell 68, 585–596
48. Hinnebusch, A. G. (1997) J. Biol. Chem. 272, 21661–21664
49. Mueller, P. P., and Hinnebusch, A. G. (1986) Cell 45, 201–207
50. Kaibuchi, K., Miyajima, A., Arai, K., and Matsumoto, K. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8172–8176
51. Foiani, M., Marini, F., Gamba, D., Lucchini, G., and Plevani, P. (1994) Mol. Cell. Biol. 14, 923–933
52. Williams, B. R. (1999) Oncogene 18, 6112–6120
53. Mbyoni, K., Beullens, M., Detremmerie, K., Geerts, L., and Thevelein, J. M. (1988) Mol. Cell. Biol. 8 3051–3057
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