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The UV Response Involving the Ras Signaling Pathway and AP-1 Transcription Factors Is Conserved between Yeast and Mammals

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Summary

UV irradiation of mammalian cells activates AP-1 through a Ras-dependent pathway, independently of DNA damage. We show that the yeast S. cerevisiae has a remarkably similar UV response involving the AP-1 factor Gcn4, which is distinct from the DNA damage response. Transcriptional activation of *HIS3* and *HIS4* by Gcn4 is triggered by UV irradiation in a Ras-dependent fashion. Moreover, resistance of yeast to UV irradiation is correlated with the level of Ras activity and Gcn4 function. Like mammalian cells in which activated Ras leads to increased c-Jun synthesis and phosphorylation, the effects in yeast involve increased translation of *GCN4* mRNA and a posttranslational event. However, this effect on *GCN4* translation is different from the response to amino acid or purine starvation. Therefore, a UV signaling pathway involving Ras and AP-1 is an ancient and universal mechanism involved in protection against damage to cellular components other than DNA.

Introduction

Exposure of bacterial or yeast cells to ultraviolet (UV) light or other DNA damaging agents results in induction of many genes involved in repair of DNA damage (Little and Mount, 1982; Walker, 1985; Friedberg, 1985; Friedberg et al., 1991). By contrast, none of the UV-inducible genes identified in mammalian cells appear to be involved in DNA repair (Karin and Herrlich, 1989; Holbrook and Fornace, 1991). The mammalian response was named the UV response and is regulated by transcription factors such as AP-1 and NF-κB (Karin and Herrlich, 1989; Holbrook and Fornace, 1991).

Activation of both AP-1 and NF-κB by UV irradiation is mediated by a signaling pathway in which the membrane associated Ha-Ras protein plays a major role. Stimulation of Ras leads to activation of cytoplasmic protein kinases that eventually increase AP-1 activity (Devary et al., 1992; Radler-Pohl et al., 1993) and induce nuclear translocation of NF-κB (Devary et al., 1993). The increase in AP-1 activity is mediated both by induction of c-*jun* and c-*fos* expression and posttranslational modification of c-Jun (and possibly ATF-2) (Devary et al., 1991, 1992; Radler-Pohl et al., 1993). The UV-induced modification of c-Jun is mediated by the Ser/Thr kinase JNK, which is rapidly activated following UV irradiation (Hibi et. al., 1993). The membrane location of Ha-Ras and the rapid kinetics of JNK activation suggest that the signal eliciting this response is not generated by DNA damage. Indeed, both NF-κB and JNK were shown to be fully responsive to UV in enucleated cells (Devary et al., 1993). Together with the absence of any inducible genes involved in DNA repair, these results suggest that the mammalian UV response, mediated by AP-1 and NF-κB, is involved in a protective function other than DNA repair. Therefore, the mammalian UV response is mechanistically and physiologically distinct from the DNA damage responses of bacteria (SOS response) and yeast. The functional significance of the mammalian UV response is not clear.

The Ras signaling pathway is activated in many types of cancers (Barbacid, 1987; Bos, 1989). The ability of UV to activate this pathway may explain why UV is a potent tumor promoter (Romerdahl et al., 1989; Weinstein, 1988). It may also explain why Ras-transformed cells are resistant to UV and other types of radiation (Chang et al., 1987; Kasid et al., 1987; 1989; Sklar, 1988; Shimm et al., 1992).

To determine the universal importance of a UV response homologous to the mammalian UV response, we examined whether it exists in a nonmammalian organism. Such studies are feasible because AP-1 transcription factors are found throughout the eukaryotic kingdom and are highly related in structure and function (Angel and Karin, 1991; Perkins et al., 1988; Zhang et al., 1990; Vogt et al., 1987; Struhl, 1987, 1988). The Fos and Jun oncoproteins and yeast Gcn4 have similar basic–leucine zipper domains (Vogt et al., 1987), bind DNA with the same specificity (Struhl, 1987; Bohmann et al., 1987; Distel et al., 1987; Angel et al., 1988), and are functionally interchangeable between yeast and mammalian cells (Struhl, 1988; Oliviero and Struhl, 1991; Oliviero et al., 1992). Despite these similarities, yeast and mammalian AP-1 factors are believed to be regulated by different physiological signals.

In mammalian cells, a variety of extracellular signals induce *fos* and *jun* expression and change the phosphorylation state of their protein products, thereby increasing both AP-1 binding and transcriptional activities (reviewed by Angel and Karin, 1991; Karin and Smeal, 1992; T. Deng, unpublished data). Although many of the signals that induce AP-1 activity also stimulate cell proliferation, others such as UV inhibit cell proliferation. In yeast, Gcn4 activity is induced by starvation for amino acids or purines (Hinnebusch, 1988; Rolfes and Hinnebusch, 1993). This response is mediated by Gcn2, a protein kinase that phosphorylates translational eukaryotic initiation factor 2α (eIF-2α) (Dever et al., 1992), leading to increased ribosomal scanning through upstream AUG codons (uAUGs) and translation of *GCN4* mRNA. The resulting increase in Gcn4 level activates target genes, such as *HIS3* and *HIS4*,

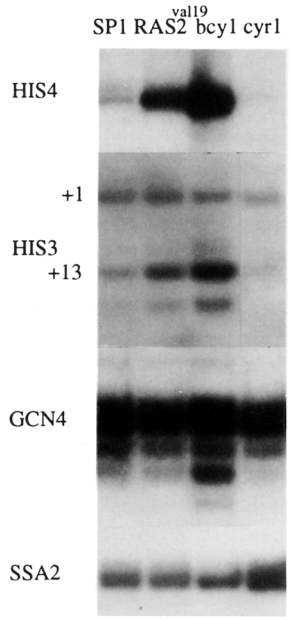


Figure 1. Expression of *HIS3* and *HIS4* in *RAS2^{val19}* and *bcy1* Mutants

Total cellular RNA was isolated from the indicated strains and analyzed by primer extension for *HIS3*, *HIS4*, *GCN4* and *SSA2* expression. *SSA2* is a constitutively expressed gene of the HSP70 gene family and was used as an internal control. The two distinctly initiated *HIS3* transcripts are indicated by +1 and +13. The strain numbers are as follows: *RAS2^{val19}*, TK161R2V; *bcy1*, TTS121; *cyr1*, AM18–5CM1.

that are involved in amino acid biosynthesis (Hinnebusch, 1988; Dever et al., 1992).

In this paper, we show that, in the yeast Saccharomyces cerevisiae, both UV exposure and Ras activation result in increased transcription mediated by the yeast homolog of AP-1, Gcn4. This response is distinct from the response to DNA damage, mediated by the recently identified protein kinase Dun1 (Zhou and Elledge, 1993). Similar to UV induction of AP-1 target genes in mammalian cells, the activation of Gcn4 target genes in yeast is mediated by increased translation of *GCN4* mRNA and a posttranslational event. The UV induced increase in *GCN4* translation is not mediated by the previously described pathway involving Gcn2 (Dever et al., 1992). Finally, we show that the Ras-dependent, UV induction response has a protective function.

Results

Transcription of Gcn4 Target Genes Induced by Activated Ras

To determine whether in yeast, as in mammals, UV irradiation induces gene expression through the Ras pathway, we searched for genes whose transcription is activated by Ras. Since in mammals Ha-Ras stimulates AP-1 activity (Binetruy et al., 1991), we focused our attention on *HIS3* and *HIS4*, genes that are regulated by the yeast AP-1 factor Gcn4 (Struhl, 1982; Donahue et al., 1983; Hill et al., 1986). Primer extension analysis of RNAs from isogenic wild-type (SP1) and *RAS2^{val19}* strains indicates that the *HIS4* transcript is approximately 5.5-fold more abundant in the activated Ras strain. The +13 transcript of *HIS3* is similarly elevated (Figure 1). *HIS3* has two primary mRNA start sites, +1 and +13, with +13 being the Gcn4 responsive initiation site (Struhl et al., 1985).

The Ras signaling pathway in yeast functions by affecting protein kinase A (PKA) activity (Toda et al., 1985; Broach and Deschenes, 1990). Therefore, we determined *HIS3* and *HIS4* RNA levels in isogenic strains with either high or low PKA activity. In *bcy1*, which lacks the regulatory subunit of PKA and has constitutively active PKA, the levels of *HIS3* (+13) and *HIS4* transcripts are increased (Figure 1, lane 3). The magnitude of this effect is larger than that of activated Ras. By contrast, expression of both *HIS4* and *HIS3* (+13) was reduced in the *cyr1* strain, which lacks adenylyl cyclase and therefore has low PKA activity (Matsumoto et al., 1982). The level of *GCN4* transcription was not affected by any of the mutations. These results indicate that PKA, the effector of the Ras pathway, is an important regulator of Gcn4 target genes.

UV Irradiation Activates Gcn4 Target Genes

Next, we examined whether UV irradiation induces Ras-dependent gene expression as it does in mammalian cells (Devary et al., 1992). Following UV irradiation, *HIS4* is induced about 11- to 13-fold, whereas the *HIS3* (+13) transcript is induced about 2.5- to 3-fold; the basal *HIS3* (+1) transcript remains relatively unchanged (Figure 2). The kinetics of *HIS4* and *HIS3* induction by UV were much more rapid than that of *DIN1/RNR3*, which encodes the regulatory subunit of ribonucleotide reductase, a component of the DNA repair machinery (Yagle and McEntee, 1990; Elledge et al., 1993). *DIN1/RNR3* is induced by various DNA damaging agents (Yagle and McEntee, 1990) through activation of the DNA damage-activated protein kinase Dun1 (Zhou and Elledge, 1993). Maximal *HIS4* induction occurred about 15 min after irradiation, whereas maximal *DIN1/RNR3* induction required 80 min. The level of *GCN4* transcription was unaffected (Figure 2).

UV-Dependent Activation of *HIS4* Requires an Active Ras Pathway

If the AP-1 response to UV irradiation is mediated by the Ras pathway, then yeast strains deficient in key components of this pathway should not show the response. We therefore examined *HIS4* induction in the *cyr1* strain and in strains in which either *RAS1* or *RAS2* has been disrupted. No change in *HIS4* mRNA levels in response to UV irradiation was found in *cyr1* cells (Figure 3A). While the UV activation of *HIS4* is normal in the *ras1* strain, it is dramatically attenuated in the *ras2* strain (Figure 3B). These findings are consistent with the suggestion that Ras2 is the major activator of adenylyl cyclase, while Ras1 may be only a minor activator (Toda et al., 1985). These results indicate that the Ras pathway mediates the UV effect on *HIS4* transcription.

GCN4 Is Required for the Response to UV and Ras

At least two yeast proteins bind to AP-1 sites: Gcn4 (Hope and Struhl, 1985; Arndt and Fink, 1986) and yAP-1 (Moye-Rowley et al., 1989). Of these, only Gcn4 activates *HIS3*

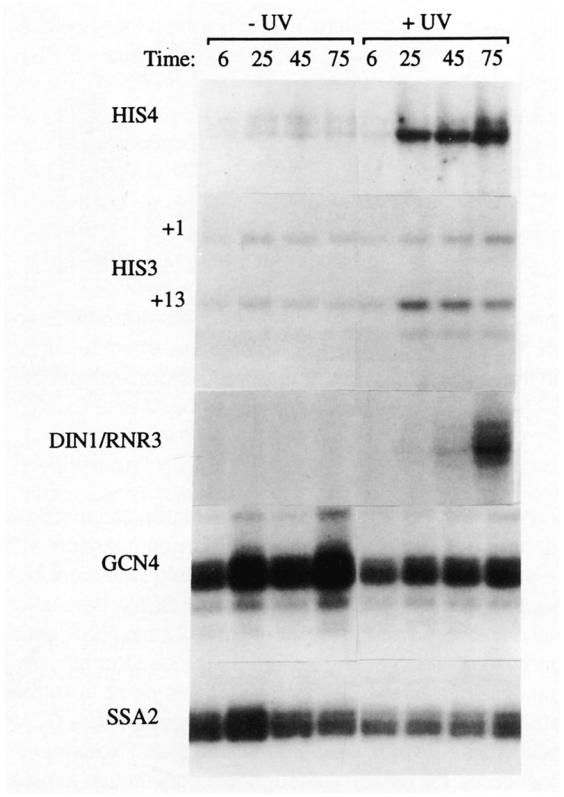


Figure 2. Expression of *HIS3* and *HIS4* Is Induced by UV Irradiation

Total cellular RNAs prepared from SP1 cells at various times (minutes) after exposure to UV irradiation (40 J/m^2) were analyzed by primer extension for *HIS4*, *HIS3*, *D1N1*, *GCN4*, and *SSA2* expression.

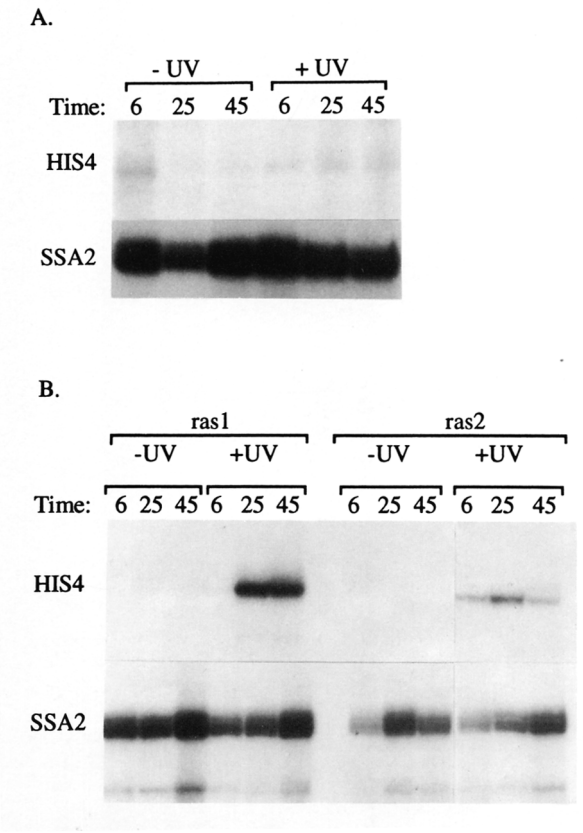


Figure 3. The *cyr1* and *ras2* Mutants Do Not Activate *HIS4* in Response to Irradiation

RNAs prepared from (A) *cyr1* and (B) *ras1* and *ras2* strains at various times after exposure to UV irradiation (40 J/m^2) were analyzed by primer extension for *HIS4* and *SSA2* expression.

and *HIS4* (Hinnebusch, 1988). This suggested that the effects of UV and Ras on *HIS3* and *HIS4* transcription might be mediated by Gcn4. Indeed, unlike a *GCN4* wild-type strain, a *gcn4* strain does not show increased *HIS4* transcription in response to UV irradiation (Figure 4A). Induction of *DINI/RNR3*, however, was unaffected. Thus, unlike the induction of DNA repair genes, the induction of AP-1 target genes by UV irradiation is mediated by Gcn4.

Deletion of *GCN4* also abolishes the induction of *HIS3* (+13) and *HIS4* by Ras2^{val19} (Figure 4B). In addition, deletion of *GCN4* abolishes the induction of the *HIS3* (+13) transcript normally seen in *bcy1* cells (Figure 4C). Therefore, the effect of Ras activation on AP-1 dependent transcription is mediated by Gcn4.

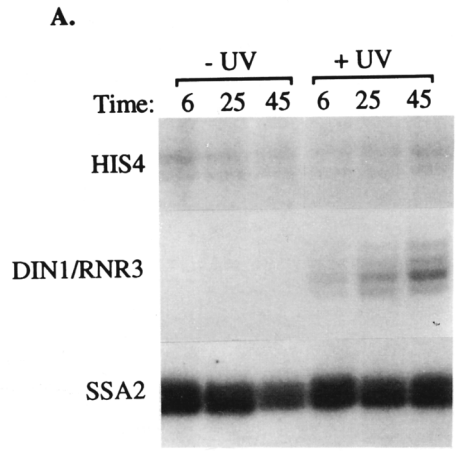
Activation of the Ras Pathway Increases *GCN4* Translation

Activation of Gcn4 in response to amino acid or purine starvation is regulated at the translational level by four uAUGs followed by short open reading frames (uORFs) in the 5′ untranslated region of *GCN4* RNA (Thireos et al., 1984; Hinnebusch, 1984; Mueller and Hinnebusch, 1986; Rolfes and Hinnebusch, 1993). To determine whether activation by the Ras pathway occurred through a similar mechanism, we transformed wild-type, *bcy1*, and *RAS2*^{val19} cells with a *GCN4–LacZ* translational fusion gene (Hinnebusch, 1985). Expression of this fusion gene was about 3-fold higher in *RAS2*^{val19} and *bcy1* as compared with the isogenic wild-type strains (Figure 5A). We also tested a *GCN4–LacZ* fusion that was mutated in the uORFs, and hence lacked the ability to exhibit normal translational control (Mueller and Hinnebusch, 1986). In this case, no major differences in β-galactosidase levels were observed among wild-type, *bcy1*, and *RAS2*^{val19} strains (Figure 5B). Therefore, increased Gcn4 activity in response to Ras activation is likely to involve increased translation of *GCN4* mRNA.

Ras Enhances *GCN4* Translation in a *GCN2*-Independent Manner

Increased translation of *GCN4* under conditions of amino acid or purine starvation is mediated by the Gcn2 protein kinase, which phosphorylates eIF-2α on Ser-51 (Dever et al., 1992; Rolfes and Hinnebusch, 1993). This allows ribosomes to progress from the first uAUG to the correct *GCN4* translation initiation site.

To determine whether the Ras-dependent increase in *GCN4* translation was mediated by the same pathway, we first assayed *HIS3* transcription in isogenic *GCN2* and *gcn2* deletion strains in the presence or absence of *BCY1*. As shown in Figure 6, the PKA-dependent increase in the *HIS3* (+13) transcript is not affected by the absence of



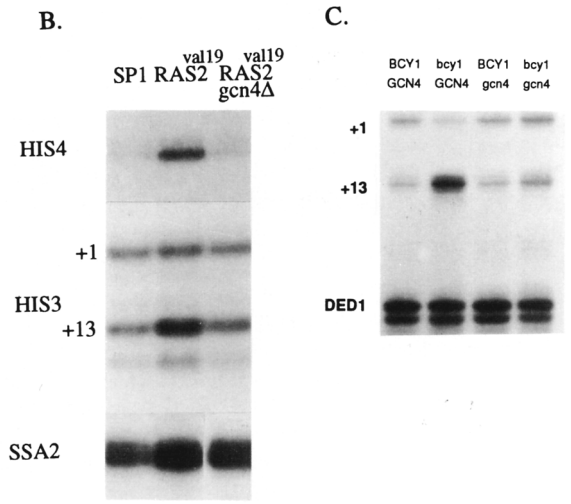


Figure 4. UV- and Ras-Mediated Activation of *HIS3* and *HIS4* Transcription Requires Gcn4

(A) Primer extension analysis of RNAs prepared from *gcn4* deletion strain at various times after exposure to UV irradiation. (B) Primer extension analysis of RNA prepared from isogenic wild-type (SP1), *RAS2*^{val19}, and *RAS2*^{val19} gcn4 strains. (C) S1 nuclease analysis of RNA prepared from an isogenic wild-type (KY2002), *bcy1*, *gcn4*, and *bcy1 gcn4* strains.

Gcn2, while it is abolished in the absence of Gcn4. To evaluate the relationship between Gcn2-dependent and Ras-dependent regulation of *GCN4* further, we analyzed a strain containing a mutated form of eIF-2α in which Ser-51 was substituted by Ala. Although this mutation eliminates induction of *GCN4* translation in response to amino acid limitation (Dever et al., 1992), it had no effect on the PKA-dependent increase in *HIS3*. Therefore, the Ras-dependent and amino acid starvation pathways for induction of *GCN4* are distinct.

Gcn4 Activation by UV Irradiation May Involve a Posttranslational Component

The results described above suggest that increased *GCN4* translation is an important component of Gcn4 induction

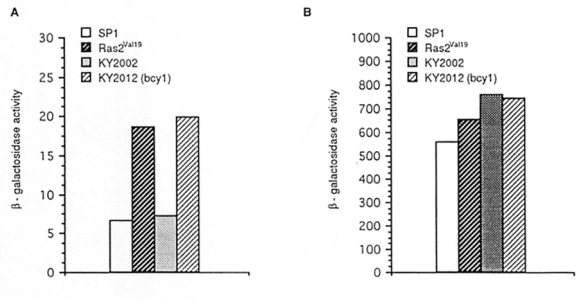


Figure 5. *GCN4* mRNA Is Translated More Efficiently in *RAS2*^{val19} and *bcy1* Strains

β-galactosidase activities (averages of four repeats with two independent clones) of the indicated strains containing integrated *GCN4–LacZ* (A), or *GCN4(-orf)–LacZ* (B) fusion genes.

by UV. However, the induction of *GCN4* translation following UV irradiation is modest (3-fold) in comparison with the effect of amino acid starvation (10-fold induction; Hinnebusch, 1985). Therefore, increased *GCN4* translation may not account for the full extent of *HIS4* and *HIS3* induction by UV, which is larger than their induction following amino acid starvation. To examine this point in further detail, we generated a *GCN4*^{c} strain, containing a *GCN4* allele whose uAUGs were inactivated (see Experimental Procedures for detail). Although the constitutive expression of Gcn4 in this strain led to activation of *HIS4* and *HIS3* (+13) transcription, UV irradiation resulted in residual, but significant, induction of both transcripts (Figure 7). Interestingly, this effect was delayed in comparison with the response seen in the wild-type strain (see Figure 2). These results suggest that the induction of Gcn4 activity by UV irradiation involves both translational and posttranslational components.

The Rapid GCN4 Response to Growth Medium Shift Is Blocked In Low PKA Mutants

Yeast cells that are transferred from rich broth to minimal medium lacking in amino acids undergo a rapid response

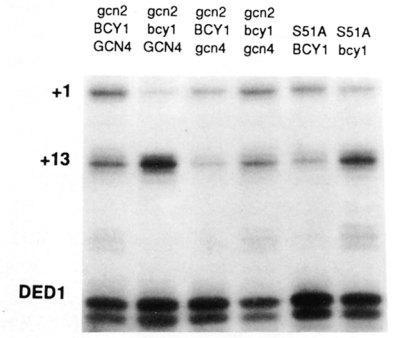


Figure 6. Induction of *HIS3* (+13) in the *bcy1* Strain Is Independent of Gcn2 and eIF-2α Phosphorylation on Ser-51

Total cellular RNAs were isolated from the indicated isogenic strains and analyzed by S1 protection for *HIS3* (indicated as +1 and +13) and *DED1* expression.

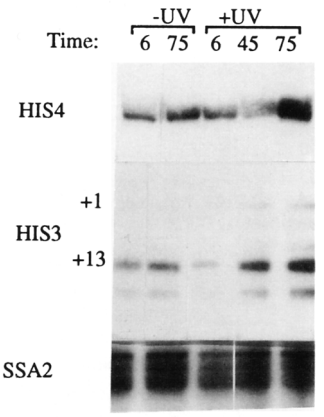


Figure 7. Induction of *HIS4* and *HIS3* (+13) in a *GCN4*^{c} Strain

A *GCN4* construct in which the uAUGs that repress translation were inactivated was integrated into the *URA3* locus of the SP1 strain to generate the *GCN4*^{c} strain (see Experimental Procedures). These cells, which should express Gcn4 constitutively, were exposed to UV as described above, and RNA was isolated at the indicated timepoints and analyzed by primer extension.

in which general protein synthesis is reduced, but *GCN4* translation is transiently increased (Tzamarias et al., 1989). Unlike the long-term starvation response, this rapid Gcn4 induction upon growth medium shift is independent of Gcn2 (Tzamarias et al., 1989). If this response is mediated by Ras via PKA, one would expect that cells with constitutively low PKA activity would not undergo this transient increase in *GCN4* translation. In accord with this hypothesis, a low PKA strain (*tpk1*^{w}) does not show the rapid increase in *HIS3* (+13) RNA levels upon nutritional downshift, whereas the isogenic wild-type strain shows the expected increase (Figure 8A). Thus, the rapid induction of Gcn4 activity upon growth medium shift requires the ability to regulate PKA levels.

To evaluate the relationship between low PKA levels and the long-term amino acid starvation response, we examined *HIS3* induction by 20 mM 3-aminotriazole, a condition of histidine limitation (Hinnebusch, 1988). In this situation, the wild-type strain shows an approximate 8-fold induction of the +13 transcript, while the *tpk1*^{w} strain shows a similar increase (Figure 8B). Therefore, wild-type PKA activity (and presumably the Ras pathway) are not required for the Gcn2-dependent induction of Gcn4 activity.

The Ras Pathway Affects UV Sensitivity

In yeast, the probable role of previously identified UV-responsive genes is to confer protection by enhancing the repair of damaged DNA (Friedberg, 1985; Friedberg et al., 1991). Strains that are mutated in such genes are more sensitive to UV irradiation and other DNA damaging agents (Haynes and Kunz, 1981). Because *HIS3* and *HIS4* encode enzymes involved in histidine biosynthesis, it is unlikely that they are directly involved in DNA repair.

To address whether the Ras-dependent activation of Gcn4 contributes to protection against UV damage, we compared the survival rates of different strains after exposure to UV irradiation (Table 1). The *RAS2*^{val19} and *bcy1* strains, which constitutively activate Gcn4, were found to be approximately 3.5-fold more resistant to UV irradiation than the wild-type strain. In contrast, *gcn4*, *cyr1*, and *ras2* strains, which do not activate Gcn4 target genes, were approximately 5-fold more sensitive than the wild-type strain. However, the *ras1* strain did not differ from the wild-type strain. The increased resistance of *bcy1* and *RAS2*^{val19} cells to UV is quite remarkable, because these strains are much more sensitive than wild type to other types of stress, such as heat shock (Toda et al., 1985, 1987). Altogether, we find a 15.5-fold difference in survival rates between cells containing activated Ras2 and cells lacking this protein. Since a *RAS2*^{val19}gcn4 double mutant is 10-fold more sensitive to UV than the *RAS2*^{val19} strain, the increase in UV resistance in response to activated Ras is mediated, at least in part, through Gcn4.

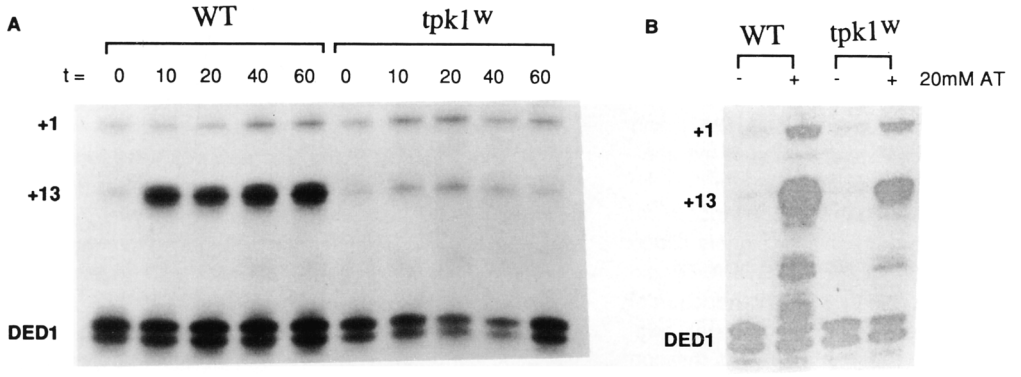


Figure 8. The Rapid, but Not the Long-Term, Response to Amino Acid Limitation Requires the Ras Pathway

(A) Total cellular RNAs isolated from isogenic wild-type (SP1) and low PKA (RS13–58A-1) strains at various times following shift of cultures from YPD to glucose minimal media were analyzed by S1 nuclease protection for *HIS3* (+1 and +13) and *DED1* expression. (B) S1 nuclease analysis of RNAs isolated from the same strains following 15 hr of growth in the presence or absence of 20 mM 3-aminotriazole.

Table 1. Survival of Yeast Strains after UV Irradiation

	Percent Survival							
Dose J/m²	Wild Type SP1	*ras1*	*ras2*	*gcn4*	*cyr1*	*RAS2^{val19}*	*bcy1*	*RAS2^{val19}/gcn4*
40	8.5	8.0	4.2	2.9	2.7	18	14	5.5
80	0.04	0.056	0.009	0.0085	0.008	0.14	0.13	0.015

Viability tests were carried out as described in Experimental Procedures. The survival rate of each strain was calculated by dividing the number of colonies that grew after UV irradiation by the number of colonies that grew on the nonirradiated plates. The results shown represent the averages of three different experiments done in triplicates. Standard deviations did not exceed 20%.

Discussion

Evolutionary Conservation of a UV Response Pathway Involving Ras-Dependent Signal Transduction and AP-1 Transcription Factors

Exposure of mammalian cells to UV radiation results in a gene induction response termed the UV response, which is mediated by transcription factors such as NF-κB and AP-1 (Buscher et al., 1988; Devary et al., 1991; 1992; 1993; Radler-Pohl et al., 1993). Since the initial signal triggering the UV response is not provided by damage to DNA (Devary et al., 1993), this UV response is mechanistically different from the prokaryotic SOS response, which is elicited by damage to DNA (Walker, 1985; Friedberg, 1985). In addition, the mammalian UV response differs from the prokaryotic SOS response in the type of genes it affects; none of the mammalian UV-inducible genes identified so far is involved in DNA repair (Karin and Herrlich, 1989; Holbrook and Fornace, 1991). These differences raised questions regarding the generality and physiological relevance of the mammalian UV response. Here, we show that a mechanistically similar response is also present in a simple eukaryote, the yeast S. cerevisiae.

As shown for mammalian cells (Devary et al., 1992, 1993; Radler-Pohl et al., 1993), UV irradiation of yeast cells stimulates the Ras signaling pathway and ultimately leads to increased transcriptional activation by the yeast AP-1 factor Gcn4. The UV-triggered pathway that activates Gcn4 is clearly distinct from the DNA damage response that activates transcription of genes encoding DNA repair enzymes (Friedberg et al., 1991; Elledge et al., 1993). Transcriptional induction of *HIS3* and *HIS4* by UV is considerably faster than that of *DIN1/RNR3*, and it requires the Ras pathway and Gcn4, which are not required for *DIN1/RNR3* induction (Figures 3 and 4). Ras proteins are membrane associated (Barbacid, 1987), and both the Ras pathway and Gcn4 are activated by extracellular signals (e.g., nutrients and metabolites) and not by nuclear signals (Eraso and Gancedo, 1985; Hinnebusch, 1988). It is unlikely that such extracellular signals cause DNA damage. Furthermore, Cdc25, the GDP/GTP exchange factor of Ras, is also required for UV induction of *HIS4* (D. E., H. M., and M. K., unpublished data), suggesting that the UV signal is transmitted to Ras through upstream, membrane-associated components. Induction of *DIN1/RNR3* was recently shown to be mediated by the DNA damage responsive protein kinase Dun1 (Zhou and Elledge, 1993). However, *HIS4* is UV inducible in a *dun1* strain (D. E., H. M. and M. K., unpublished data).

The conservation of the Ras-mediated, AP-1-dependent UV response from yeast to mammals suggests that this pathway is physiologically and universally important. Indeed, Ras-dependent activation of Gcn4 target genes has a role in protecting cells from the effects of UV irradiation (Table 1). There is a 15.5-fold difference in the survival rate after exposure to 80 J/m² of UV between a strain containing activated Ras2 and a strain lacking this protein. The molecular nature of the UV-induced damage and the Gcn4 target genes relevant for the protective response are unknown. However, it is unlikely that UV irradiation merely causes a starvation response (e.g., by degrading amino acids and nucleotides) that leads to activation of Gcn4. Unlike the response to amino acid or purine limitation (Hinnebusch, 1988; Rolfes and Hinnebusch, 1993), UV induction of Gcn4 target genes does not require Gcn2 protein kinase or phosphorylation of Ser-51 of eIF-2α (Figure 6).

The Ras pathway in mammalian cells also has a protective role against UV irradiation (Sklar, 1988; Devary et al., 1992). This UV response involving Ras and AP-1 factors is likely to be relevant for understanding why UV irradiation is a potent tumor promoter. In the mouse skin system, for example, the tumor promoting activity of UV exceeds its tumor initiating activity (Romerdahl et al., 1989). Skin cells as well as unicellular organisms, such as S. cerevisiae, are exposed daily to UV radiation, and an optimal protective response is crucial for both. The evolutionary conservation of the UV response in yeast and mammals suggests an ancient mechanism involved in protection against UV-induced damage of cellular components other than DNA. In addition to DNA damage, exposure to UV is expected to result in damage to biomembranes, various proteins, and most importantly, RNA and ribosomes. A simple way to protect against damage to such components is to replace them with newly synthesized ones. Interestingly, PKA and presumably the Ras pathway were found to be involved in induction of ribosomal protein genes (Klein and Struhl, 1994). Such findings may explain why the UV response in yeast is similar to the response to altered nutritional conditions, while the mammalian UV response is similar to the growth response.

In mammalian cells, the effect of UV and Ras on AP-1 activity is mediated both through increased expression of *jun* and *fos* genes and altered phosphorylation of their protein products (Angel and Karin, 1991; Binetruy et al., 1991; Devary et al., 1991, 1992). As shown here, Ras-dependent activation of Gcn4 is mediated primarily at the translational level. However, there is a residual induction

of both *HIS3* and *HIS4* in a strain containing a *GCN4* allele lacking the uAUGs that repress translation. Since this strain, *GCN4*^{c}, is expected to express Gcn4 constitutively, it appears that in yeast, as in mammals, part of the induction response occurs at a posttranslational level. Further studies are required to determine the nature of this posttranslational event. By contrast, the response to amino acid starvation is regulated only at the translational level (Hinnebusch, 1985).

Ras-Dependent Induction of *GCN4* Translation Occurs by a Novel Mechanism

Increased translation of *GCN4* mRNA in response to amino acid or purine limitation requires Gcn2 protein kinase, which phosphorylates eIF-2α at Ser-51 (Hinnebusch, 1988; Dever et al., 1992; Rolfes and Hinnebusch, 1993). By contrast, the UV- and Ras-dependent increase in *GCN4* translation does not require Gcn2 or phosphorylation of eIF-2α on Ser-51 (Figure 6). The fact that Ras-dependent induction of *GCN4* translation occurs independently of Gcn2 shows that UV irradiation is not merely activating or mimicking the starvation response. Thus, these results indicate the existence of an alternate pathway for induction of *GCN4* translation.

The Ras- and PKA-dependent increase in *GCN4* translation requires the uAUGs (Figure 5) and, hence, is likely to result from decreased translational reinitiation and increased scanning. In this view, PKA, the target of the Ras pathway, might directly or indirectly cause partial inactivation of a component of the translational initiation machinery such as eIF-2 or eIF-2B, the guanine nucleotide exchange factor. Subunits of the yeast eIF-2B complex are encoded by *GCD1*, *GCD2*, *GCD6*, *GCD7*, and *GCN3*, and mutations in these genes can lead to increased *GCN4* translation that does not depend on Gcn2 (Bushman et al., 1993). Down-regulating the activity of any of these gene products could therefore result in increased Gcn4 levels independent of the Gcn2 kinase.

The fact that the Ras pathway stimulates *GCN4* translation is interesting in light of the effect of Ras activation on translation in mammalian cells (Frederickson et al, 1991; Lazaris-Karatraz et al., 1992). In these cases, Ras and other oncogenes such as Src induce the phosphorylation and activation of translation initiation factor eIF-4E. This phosphorylation and activation are essential for Ras-mediated malignant transformation (Lazaris-Karatraz et al., 1992). Taken together, the results from yeast and mammals show that certain effects of Ras on gene expression and cellular transformation are mediated through control of translation.

Ras as a General Sensor of Different Extracellular Signals in Yeast

In yeast, the Ras pathway has been implicated in the response to signals that stimulate cell growth (reviewed by Broach and Deschenes, 1990). Addition of glucose to yeast cells grown in poor carbon sources results in the rapid activation of Ras and a dramatic increase in intracellular cAMP (Eraso and Gancedo, 1985; Munder and Kuntzel, 1989; Mbonyi et al., 1991; Van Aelst et al., 1991; reviewed by Thevelein, 1991). In addition, activation of the Ras pathway upon carbon-source upshift is required for increased RAP1-dependent transcription of ribosomal protein genes (Klein and Struhl, 1994) and for increased transcription of Gcn4 target genes (unpublished data). In this paper, we show that the Ras pathway also mediates rapid response to environmental stimuli that are not associated with growth. Specifically, the Ras pathway activates Gcn4 in response to UV irradiation and to growth medium shift. Thus, a wide variety of extracellular signals stimulates the Ras pathway.

The ability of yeast Ras to mediate the response to different extracellular signals is similar to that of mammalian Ras, which can be activated by a variety of signals such as growth factors, differentiation factors, cytokines, and UV irradiation (Burgering et al., 1991; Rayter et al., 1992; Wood et al., 1992; Devary et al., 1992). Not all of these signals lead to increased cell proliferation. It remains to be established how each of the different extracellular signals is transmitted to Ras, and how each of the Ras-mediated signals elicits its specific response(s).

Experimental Procedures

Yeast Strains

Yeast strains used are listed in Table 2. Deletions of *BCY1*, *RAS1*, *RAS2*, and *GCN4* were obtained by one step gene replacement and verified by Southern blots. The *BCY1*, *RAS1*, and *GCN4* genes were disrupted by insertion of *URA3* in a deleted copy of each. The *RAS2* gene was disrupted by *LEU2* insertion. Strains KY2009 and KY2004 were made by successive gene replacement of KY2002 using the appropriate *URA3* containing integrating plasmids carrying the *GCN2* or *GCN4* deletions, respectively. KY2010 was generated by successive gene replacement of KY2009 with the *GCN4* deletion molecule. To make the eIF-2α–Ser-51 mutant strains, first pRS306–eIF-2αΔ was introduced by integration, then either the wild-type or a mutant pRS314–eIF-2α plasmid was introduced and trp$^+$ colonies were selected. Then, ura$^-$ colonies were selected on 5-fluoroorotic acid.

Growth Conditions

All strains were grown in YPD, unless otherwise specified. In the growth medium shift experiment, strains were grown to mid-log phase in YPD, washed once in minimal media, resuspended in an equal volume of minimal media, and incubated at 30°C for the indicated period of time. For long-term starvation, induction strains were grown overnight in minimal medium supplemented with 20 mM 3-aminotriazole. Cells of the *cyr1* mutant were grown on YPD supplemented with 1 mM cAMP. β-galactosidase activities were measured by standard methods.

UV Irradiation Procedure

Yeast cultures were exposed to UV radiation as previously described (Madura and Prakash, 1986). In brief, a 100 ml culture was grown to early logarithmic phase (1×10^7 to 2×10^7 cells per milliliter) on YPD. Cells were collected by centrifugation, washed with water, and resuspended in 100 ml of water. The culture was then transferred into two petri dishes (50 ml into each [150 mm diameter] dish). Both halves of the culture were kept under slow stirring, and one of the plates was exposed for 20 s to UV radiation. The irradiation was done with Westinghouse germicidal 254 nm UV lamp at a rate of 2 J/m^2/s. Irradiated and not irradiated cultures were immediately collected by centrifugation, and each was resuspended in 50 ml YPD. Aliquots were removed for RNA preparations at the indicated timepoints.

Viability Tests

Viability tests were carried out essentially as previously described (Zhou and Elledge, 1993). In brief, cultures were grown on YPD to 1×10^7 cells per milliliter. A different number of cells was plated on

Table 2. Yeast Strains Used in This Work

Strain	Relevant Genotype	Short Name	Source or Reference
SP1	*MATa, his3, leu2, ura3, trp1, ade8, can*	*SP1*	Toda et al., 1985
SP1Δgcn4	Isogenic to SP1 but *gcn4::URA3*	*gcn4*	This study
SP1Δras1	Isogenic to SP1 but *ras1::URA3*	*ras1*	This study
SP1Δras2	Isogenic to SP1 but *ras2::LEU2*	*ras2*	This study
TK161R2V	Isogenic to SP1 but $RAS2^{val19}$	$RAS2^{val19}$	Toda et al., 1985
TK161R2VΔgcn4	Isogenic to TK161R2V but *gcn4::URA3*	$RAS2^{val19}$, *gcn4*	This study
TTS121	Isogenic to SP1 but *bcy1::URA3*	*bcy1*	Toda et al., 1987
GCN4^c	Isogenic to SP1 but *GCN4(-orf)::URA3*	*GCN4*^{c}	This study
AM18-5CM1	*MATa, ura3, cyr1-1*	*cyr1*	Toda et al., 1985
RS13-58A-1	Isogenic to SP1 but *tpk1*^{w}*, tpk2::HIS3, tpk3::TRP1, bcy1::LEU2*	*tpk1*^{w}	Cameron et al., 1988
KY2002	*MATa, ura3-52, leu2Δ::PET56 trp1-Δ63*	Wild type	Klein and Struhl, 1994
KY2012	Isogenic to KY2002 but *bcy1::URA3*	*bcy1*	Klein and Struhl, 1994
KY2004	Isogenic to KY2002 but *gcn4-Δ1*	*gcn4*	This study
KY2009	Isogenic to KY2002 byt *gcn2-Δ1*	*gcn2*	This study
KY2010	Isogenic to KY2009 but *gcn4Δ1*	*gcn2, gcn4*	This study
KY2024	Isogenic to KY2004 but *bcyl::URA3*	*gcn4, bcy1*	This study

YPD plates to allow accurate counts of colonies (from 500 cells on plates that were not exposed to UV to 1×10^6 cells on plates exposed to 80 J/m^2). Following plating, each dish was exposed to the indicated UV dose and incubated subsequently at 30°C. Colonies were counted 72 hr after treatment.

DNA Manipulations

Integrated *GCN4–LacZ* and *GCN4(-orf)–LacZ* (Figure 5) were prepared by removing the small AvrII–BsaAI fragment from p180 (Hinnebusch, 1985) and p227 (Mueller and Hinnebusch, 1986), respectively, and religating the backbone after fill in reaction using Klenow fragment. This manipulation removed the CEN and the ARS elements of p180 and p227. The resulting plasmids (p180I and p227I) were digested with SmaI and subsequently introduced into yeast by integration at the *URA3* locus. For constitutive expression of *GCN4*, p227I was digested with BamHI to remove the 3 kb *LacZ* fragment. The backbone was then religated to reconstitute the *GCN4* gene. This integrating plasmid was named *GCN4(-orf)I*. It was digested with SmaI and subsequently introduced into SP1 strain where it was integrated at the *URA3* locus. The resulting strain was named *GCN4*^{c}. The plasmid used to disrupt *GCN4* in the SP1 and TK161R2V strains was made by ligating the NruI–SmaI fragment of Yep24, containing the *URA3* gene, to the KpnI site of *GCN4* in pSP64–GCN4 (Hope and Struhl, 1985), using KpnI linkers. The plasmid used to disrupt *GCN4* in KY2002 and its derivatives was made by cutting out a SacI–BamHI fragment from YIp56–3674 encoding most of the *GCN4* structural gene. The *GCN2* deletion plasmid was made by a 2.6 kb deletion of pRS306–GCN2, a *URA3*-containing integrating vector carrying the entire *GCN2* gene. The eIF-2α subunit was cloned from genomic DNA by PCR. An EcoRI to SnaBI fragment was isolated and subcloned into pBluescript. The Ser to Ala mutation at residue 51 was generated by PCR mutagenesis, followed by EcoRV digestion and ligation into pBS–eIF-2α. Both of eIF-2α molecules were subcloned into pRS314, a centromeric vector. The plasmid used to delete the chromosomal copy of the initiation factor was made by SalI–ClaI digestions of pRS306–eIF-2α that contained the entire eIF-2α clone from the EcoRI to the SnaBI site cloned into the yeast integrating vector pRS306.

RNA Preparation and Analysis

Total RNA was prepared from cultures grown to logarithmic phase and was assayed by either primer extension or S1 analysis. For primer extension, 20 μg of RNA was hybridized with 1 ng of ^{32}P-end-labeled specific oligonucleotide in the presence of 133 mm KCl. AMV reverse transcriptase was used for the extension reaction, and the products were separated on 6% acrylamide 7 M urea gel. Oligonucleotides used are the following: *HIS4*, CAAGTGTTCGGCTGTTTTAGCATC; *HIS3*, CGCAATCTGAATCTTGGTTTC; *DIN1/RNR3*, CCGTATGACAAACGGGTGATACG; *GCN4*, ATAATTCGCTAGTGAAACTGATGGGC; *SSA2*, CGAAAACAGTGTTAGCTGGG.

S1 nuclease analysis of *HIS3* and *DED1* was performed on 10 μg of RNA using a 10-fold excess of probe as previously described (Chen et al., 1987).

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