The EMBO Journal vol.13 no.20 pp.4807–4815, 1994

Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors

Gregory A.Marcus, Neal Silverman, Shelley L.Berger[1], Junjiro Horiuchi and Leonard Guarente

Department of Biology, MIT, Cambridge, MA 02139 and
[1]Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA

Communicated by H.Ploegh

A selection for yeast mutants resistant to GAL4–VP16-induced toxicity previously identified two genes, *ADA2* and *ADA3*, which may function as adaptors for some transcriptional activation domains and thereby facilitate activation. Here we identify two new genes by the same selection, one of which is identical to *GCN5*. We show that *gcn5* mutants share properties with *ada* mutants, including slow growth, temperature sensitivity and reduced activation by the VP16 and GCN4 activation domains. Double mutant studies suggest that ADA2 and GCN5 function together in a complex or pathway. Moreover, we demonstrate that GCN5 binds to ADA2 both by the two-hybrid assay *in vivo* and by co-immunoprecipitation *in vitro*. This suggests that ADA2 and GCN5 are part of a heteromeric complex that mediates transcriptional activation. Finally, we demonstrate the functional importance of the bromodomain of GCN5, a sequence found in other global transcription factors such as the SWI/SNF complex and the TATA binding protein-associated factors. This domain is not required for the interaction between GCN5 and ADA2 and thus may mediate a more general activity of transcription factors.

Key words: ADA2/co-activator/*GCN5*/genetics/transcription

Introduction

Transcriptional activation in eukaryotes involves functional interaction between transcriptional activators bound at enhancers or UASs and the general transcription factors bound at the TATA box. Activators are modular, containing DNA binding domains and activation domains (Hope and Struhl, 1986). One class of activation domains is enriched in amino acids with acidic side chains and can function in a wide variety of eukaryotes, ranging from yeast to mammals (Sadowski *et al.*, 1988). Acidic activators function when bound at sites very distant from the TATA box. Models for activation include direct protein–protein contact between activation domains and general factors (Lin and Green, 1991; Lin *et al.*, 1991) (looping out intervening DNA) and disruption of chromatin, which results in an alleviation of repression (Han and Grunstein, 1989; Workman and Kingston, 1992; Croston and Kadonaga, 1993).

Whatever their mechanism of action, activators require novel protein factors to potentiate their full activity. One class of these factors, termed co-activators, are tightly associated with the TATA binding protein (TBP) and comprise a TFIID complex (Dynlacht *et al.*, 1991). These TBP-associated proteins (TAFs) evidently serve as sites in the general machinery to which activators can bind (Goodrich *et al.*, 1993; Hoey *et al.*, 1993). Another class are products of yeast genes SWI1-3 and SNF5-6, which comprise a single complex (Peterson *et al.*, 1994; Cairns *et al.*, 1994). These proteins may function through chromatin because suppressors that bypass the requirement for them lie in histone (Hirschhorn *et al.*, 1992) and non-histone chromatin proteins (Winston and Carlson, 1992). In addition, the SWI/SNF complex promotes the binding of GAL4 derivatives to nucleosomal DNA in an ATP-dependent manner (Côté *et al.*, 1994).

A third class of cofactors required for activation includes products of the yeast *ADA2* and *ADA3* genes. Mutations in these genes were selected since they confer upon cells resistance to the toxic chimeric activator GAL4–VP16, containing the DNA binding domain of GAL4 and the acidic activation domain of VP16 (Berger *et al.*, 1992). The toxicity of the chimera correlates with its unusual potency as an activator, because mutations in VP16 which reduce activation also reduce toxicity (Berger *et al.*, 1992). Mutations in *ADA2* and *ADA3* allow cells to tolerate the chimera and also reduce their ability to respond to certain transcriptional activators, including VP16 and GCN4 (Berger *et al.*, 1992; Piña *et al.*, 1993).

We have argued that ADA2 and ADA3 could be adaptors that bridge interactions between activation domains and general factors at promoters. This conclusion comes from two observations. First, the VP16 activation domain can be made to bind and sequester a factor(s) needed for transcriptional activation but not for basal transcription *in vitro*, demonstrating that adaptors exist (Berger *et al.*, 1990). Second, mutations in *ADA2* or *ADA3* reduce activation by some, but not all, acidic activation domains *in vivo* and *in vitro* (Berger *et al.*, 1992; Piña *et al.*, 1993). This specificity argues for a functional interaction between the ADAs and specific activation domains.

Another yeast gene product that has been implicated in transcription is GCN5. Mutations in *GCN* genes cannot derepress *HIS3* and other genes that respond to the general amino acid control system (Hinnebusch and Fink, 1983; Penn *et al.*, 1983). This failure to derepress results from a defect in the synthesis, stability or activity of the activator, GCN4. Whereas mutations in *GCN1-3* exert their effects by lowering translation of *GCN4* mRNA (Hinnebusch, 1985), mutations in *GCN5* do not affect the level of GCN4 protein, but rather reduce its ability to activate transcription (Georgakopoulos and Thireos, 1992). Thus, it has been proposed that GCN5 could be

a co-activator that augments the activity of GCN4 (Georgakopoulos and Thireos, 1992).

The *GCN5* sequence has a domain at the C-terminus, the bromodomain, that is highly conserved in other proteins involved in transcription, including brahma from *Drosophila* (Tamkun *et al.*, 1992), yeast *SWI2* (*SNF2*) (Laurent *et al.*, 1991), yeast *SPT7* (Haynes *et al.*, 1992), the EIA-associated protein p300 (Eckner *et al.*, 1994) and mammalian TAF250 (*CCG1*) (Ruppert *et al.*, 1993). The conservation is very high, as illustrated by the 50% identity between *GCN5* and *CCG1* across the 70 amino acid bromodomain. The presence of the bromodomain in this apparently diverse set of transcription factors suggests that it is an important functional domain. However, attempts to show functionality of the bromodomain in these proteins have not yet succeeded (Laurent *et al.*, 1993; Elfring *et al.*, 1994).

Previously, we isolated 10 alleles of *ADA1*, but only two alleles of *ADA2* and one allele of *ADA3*. Here we demonstrate use of the same selection on a much larger scale to identify more genes. In addition to isolating more alleles of *ADA1*, *ADA2* and *ADA3*, we identify two new genes with similar properties. We show that one of these genes is *GCN5* and demonstrate a physical interaction between GCN5 and ADA2 *in vivo* and *in vitro*. This provides the first direct indication that GAL4–VP16 resistant mutants might define a set of proteins that comprise a single multi-protein complex involved in transcriptional activation. Finally, we show that the bromodomain is important in the function of GCN5.

Results

Selection of GAL4–VP16-resistant mutants

The yeast strain BP1, which was used in the selections that yielded *ada2* and *ada3* mutants (Berger *et al.*, 1992), was mutagenized and transformed with a high copy plasmid expressing GAL4–VP16 from the constitutive ADH1 promoter. Three hundred colonies showing resistance to GAL4–VP16 were analyzed as summarized in Table I. In order to identify recessive chromosomal mutations, the candidates were mated to a wild-type strain. Fifty of the resulting diploid strains displayed sensitivity to GAL4–VP16, indicating that the mutation conferring resistance in the haploid was recessive. In the remaining 250 candidates the plasmid was removed and the resulting strains were mated to an *ada2* mutant bearing GAL4–VP16. All 250 diploids were sensitive to GAL4–VP16, indicating that resistance of the haploid mutants was due to a mutation on the original GAL4–VP16 expression plasmid. Thus, in none of the 300 strains was resistance due to a dominant chromosomal mutation.

The recessive mutants were characterized further by mating to *ada1*, *ada2* or *ada3* tester strains. Candidates that failed to complement an *ada* mutation would give rise to diploids that were resistant to GAL4–VP16. Slow growth of the diploid would provide a further indication of a failure to complement. By these tests, we identified five new alleles of *ADA1*, eight new alleles of *ADA2* and 12 new alleles of *ADA3*. Among the remaining mutants, complementation tests indicated two new groups termed *ADA4* (three mutants) and *ADA5* (one mutant). Complementation tests in other mutants were incomplete and further analysis is needed to group them.

Table I. Selection for mutants resistant to GAL4–VP16 results in additional alleles of *ADA1*, *ADA2* and *ADA3*, as well as alleles of two new genes

Category	Number obtained
Primary transformants	300 000
Plasmid mutants	250
ADA1 alleles	5
ADA2 alleles	8
ADA3 alleles	12
ADA4 alleles	3
ADA5 alleles	1

BP1 was mutagenized and transformed with pGAL4-VP16 URA as described in Materials and methods. Approximately 300 large colonies showing resistance to the toxic plasmid were picked. The majority of these appeared to be linked to the plasmid expressing GAL4–VP16, as described in Materials and methods. Other strains were characterized as *ada1*, *ada2* or *ada3* alleles by mating to a mutant tester strain and scoring the growth of the diploid on minimal medium as well as its resistance to GAL4–VP16 overexpression. Representative strains were transformed with the appropriate clone for confirmation. From tetrads, we obtained some of these resistant mutations in strains of the opposite mating type. Crossing among mutants was used to identify the *ADA4* and *ADA5* complementation groups.

Cloning of ADA4 and its identification as GCN5

We chose to focus on *ADA4*, in part because mutants displayed extremely slow growth on minimal media, a phenotype also seen in *ada2* and *ada3* mutants. Tetrad analysis indicated that slow growth and resistance to GAL4–VP16 co-segregated as a single mutation (not shown). ADA4 was cloned on a 12 kb fragment from a yeast genomic library by restoration of normal growth to an *ada4* mutant strain. This clone also restored sensitivity to GAL4–VP16. The complementing fragment was subcloned to a 2.2 kb fragment as described in Materials and methods. The sequence at one end of the subclone corresponded to a portion of the *PUP2* gene, which is adjacent to *GCN5* (Georgatsou *et al.*, 1992). Therefore, we determined whether the gene complementing the *ada4* mutation was indeed *GCN5*. Restriction analysis revealed that the entire *GCN5* coding sequence lay within this 2.2 kb fragment. Furthermore, a 1.8 kb *Xho*I–*Pst*I fragment containing the *GCN5* sequence (Georgakopoulos and Thireos, 1992) complemented the *ada4* mutant. Lastly, the specific *GCN5* coding sequence amplified by PCR and placed under control of the *ADH1* promoter also complemented the mutant.

To confirm that the *ada4* mutation was in *GCN5*, the 1.8 kb *Xho*I–*Pst*I fragment was cloned into an integrating vector bearing the *URA3* marker and targeted to the *GCN5* locus. The strain containing the integrant was mated to the *ada4-1* mutant and the diploid sporulated. In all of six tetrads, two segregants grew well and were Ura^+ and two grew slowly and were Ura^-, thus showing linkage between *GCN5* and *ADA4* (hereafter designated *GCN5*).

gcn5 mutants exhibit reduced activation by some activation domains in vivo

The *GCN5* gene was deleted as described in Materials and methods. The resulting strain shared several pheno-

Table II. Transactivation by GAL4−VP16 and lexA activation domain fusions in a *gcn5* mutant and *ada2 gcn5* double mutant

	WT	Δ*gcn5*	Δ*gcn5* Δ*ada2*
GAL4−VP16 WT	17 872	814	ND
GAL4−VP16 FA	6406	144	ND
lexA−GAL4	4049	1823	1433
lexA−GCN4	1785	404	300
lexA−HAP4	4133	2508	2303

An ARS-CEN plasmid expressing GAL4−VP16 or GAL4−VP16FA was transformed into a wild-type and a *gcn5*Δ strain. The strains were also transformed with pLGSD5, a reporter plasmid with *lacZ* under the control of the *GAL4* promoter. The lexA activation domain fusions, on an ARS-CEN plasmid, were transformed into those strains, as well as into an isogenic *ada2 gcn5* double deletion strain, along with Yep21-Sc3423 (Hope and Struhl, 1986), which contains the *lacZ* gene under the control of a *lexA* operator site. The specific activity of β-galactosidase averaged from at least three independent experiments (SD < 20%) is presented. pLGSD5 gives a background of 4–5 units and Yep21-Sc3423 plus lexA202 alone gives 10–20 units of activity (not shown). Levels of GAL4−VP16 FA were determined in wild-type and *gcn5-1* strains by gel shift of a GAL4 site and were similar (data not shown). Likewise, levels of each lexA fusion protein were compared in extracts from wild-type and *gcn5-1* cells by Western analysis using anti-lexA antibody and were comparable (data not shown).

types with *ada2* and *ada3* deletion mutants, including resistance to GAL4−VP16, slow growth on minimal medium and temperature sensitivity (not shown) on minimal or rich media.

Transactivation by GAL4−VP16 was tested in the *gcn5* deletion mutant by introducing a low copy plasmid expressing GAL4−VP16 or GAL4−VP16FA (with a Phe442→Ala mutation) (Cress and Triezenberg, 1991). As shown in Table II, the ability of GAL4−VP16 to activate a reporter bearing *lacZ* under the control of GAL1-10 UAS was reduced by >20-fold in the *gcn5* mutant and the activity of GAL4−VP16FA was reduced by >40-fold. The *gcn5-1* mutant strain showed a similar defect in the ability of GAL4−VP16 to activate transcription (not shown). The levels of GAL4−VP16FA protein in the wild-type and mutant strains were determined by gel shift analysis and were similar (not shown).

We next tested the acidic activation domains of GCN4, GAL4 and HAP4, which were each fused to the lexA1–202 moiety and assayed using a *lacZ* reporter under the control of a single lexA site (Table II). The activity of the GCN4 domain was reduced ~4.5-fold in the *gcn5* deletion, whereas the activities of the GAL4 and HAP4 domains were only affected ~2-fold. These activation domains had similar activities in the *gcn5-1* mutant (not shown). The levels of the lexA fusion proteins were comparable in the wild-type and *gcn5-1* mutant as judged by Western blot analysis using anti-lexA antibody (not shown). This pattern of activation domain defects in the *gcn5* strain recapitulated effects observed in *ada2* and *ada3* mutant strains (Piña *et al.*, 1993).

ada2 gcn5 and ada3 gcn5 double mutants

Since *gcn5* null mutations displayed very similar properties to null mutations in *ADA2* and *ADA3*, we constructed double mutants between *GCN5* and the *ada* mutants. If the genes operated in the same pathway or as a complex, the double deletion strain should not have a more severe phenotype than either of the single mutants. *gcn5 ada2* and *gcn5 ada3* double deletion mutants were generated in the BWG1-7A background as described in Materials and methods. The slow growth phenotype of these strains could be restored to wild-type only if they were transformed with both a plasmid bearing *GCN5* and a plasmid bearing the appropriate *ADA* gene. Importantly, these double mutants behaved similarly to *ada2 ada3* double mutants (Piña *et al.*, 1993), in that they grew no more slowly than the single mutants did (data not shown). Furthermore, the level of transactivation by lexA-GCN4, lexA-HAP4 and lexA-GAL4 in an *ada2 gcn5* double mutant is similar to that in a single deletion mutant in *gcn5* (Table II) or *ada2* (not shown) This is strong genetic evidence that ADA2, ADA3 and GCN5 function in the same pathway or as a complex *in vivo*.

Table III. lexA−ADA2 and lexA−ADA3 activate transcription in a GCN5-dependent manner

	WT	Δ*gcn5*
lexA−ADA2	179	63
lexA−ADA3	173	42

The wild-type and *gcn5* deletion strains BP1 and GMy25 were transformed with plexA-ADA2 or plexA-ADA3 and the *lacZ* reporter Yep21-Sc3423 (Hope and Struhl, 1986). Levels of β-galactosidase were measured as in Table II.

lexA−ADA2 and lexA−ADA3 activate transcription in a GCN5-dependent manner

ADA2 and ADA3 were tested for their ability to activate transcription when fused to the lexA1–202 moiety. These fusions both complement a mutation of the cognate *ADA* gene. Table III indicates that these fusions were transcriptionally active and that their activities were greatly reduced in a *gcn5* mutant strain. Further, the activity of lexA−ADA2 was reduced in an *ada3* mutant and the lexA−ADA3 activity was reduced in an *ada2* mutant (unpublished data). These findings provide further evidence for a functional interdependence between *GCN5* and the *ADA* genes, but they must be interpreted with caution (see Discussion).

GCN5 binds to ADA2 in vivo and in vitro

The above observations are consistent with the possibility that GCN5 binds to ADA2. To test whether ADA2 and GCN5 do indeed interact, we carried out two-hybrid studies (Fields and Song, 1989) between lexA−GCN5 and ADA2 fused to a portion of the VP16 activation domain (residues 452–490, see Materials and methods). Both the GCN5 and ADA2 fusion proteins retain the ability to complement the respective mutations *in vivo* and thus retain function. As shown in Figure 1, the activity of lexA−GCN5 is stimulated about 50-fold by ADA2−VP16 as compared with overexpression of ADA2 alone. The lexA DNA binding domain (1–202) alone was not affected at all by ADA2−VP16. This finding suggests that GCN5 and ADA2 interact *in vivo*.

The two-hybrid experiment does not distinguish direct binding of GCN5 to ADA2 from an interaction that may be mediated by other proteins. In order to determine whether GCN5 and ADA2 interact with each other directly,

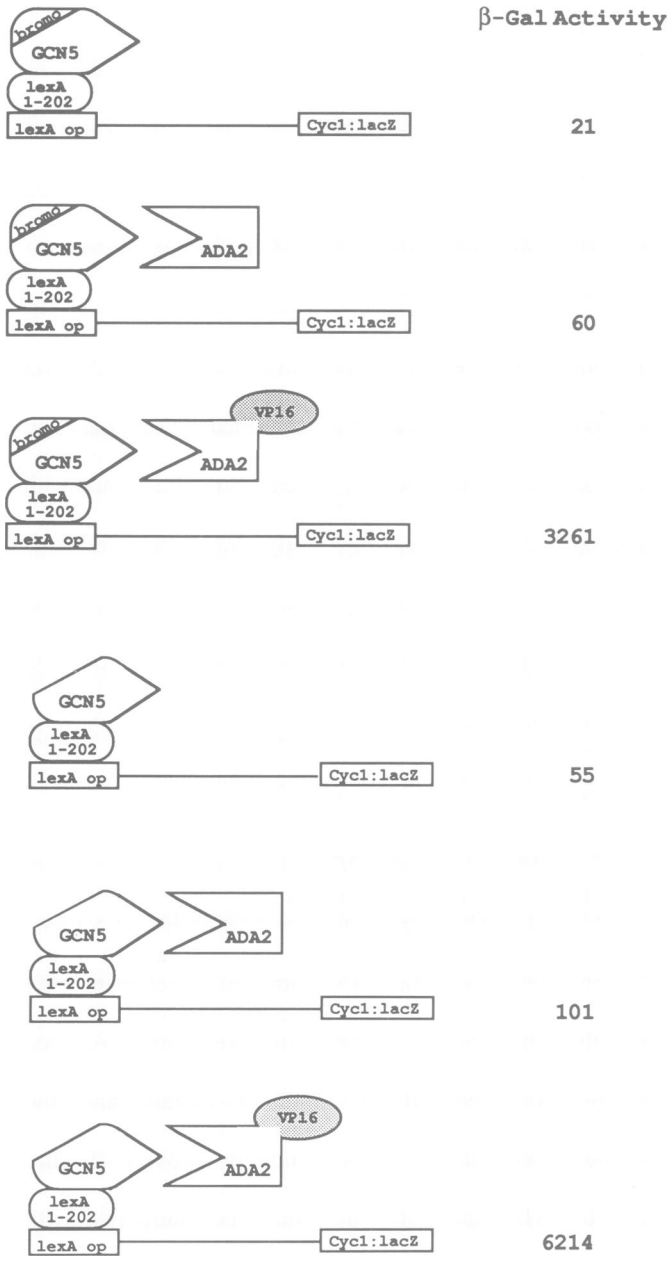

Fig. 1. GCN5 interacts with ADA2 as shown by two-hybrid analysis. BWG1-7a was transformed with a plasmid containing the lexA DNA binding and dimerization domains fused to *GCN5* or *GCN5Δ*. A second plasmid expressed either ADA2, ADA2−VP16 or neither protein. The strain also contained the *lacZ* gene under the control of a single lexA operator in plasmid pRbHis (a gift of J.Fikes). Specific activity of β-galactosidase is shown, which represents the mean of at least three independent experiments with an error of <20%. In addition, the control of lexA1–202 alone gave 25 units of activity and varied by less than 2 units when ADA2 or ADA2−VP16 were co-expressed (data not shown).

we translated both proteins in a reticulocyte lysate programed with mRNA from the *ADA2* and *GCN5* genes. As a control we co-translated each gene with luciferase. Precipitation was carried out with antibody to ADA2 (see Materials and methods). Figure 2 shows that GCN5 was clearly co-precipitated with ADA2. In the absence of ADA2, the antibody did not precipitate any GCN5. Further, luciferase was not co-precipitated when translated with ADA2. These results suggest that there is a direct physical interaction between GCN5 and ADA2.

The GCN5 bromodomain is functional

In order to test whether the bromodomain is important in the function of GCN5, we generated a version of GCN5 by PCR that deleted the bromodomain (see Materials and methods). The N-terminal primer was designed to fuse the influenza hemagglutinin (HA) epitope at the N-terminus of the gene. As shown in Figure 3, the HA epitope tag itself had no effect on the ability of GCN5 to complement a mutant. However, GCN5 missing its bromodomain (*GCN5Δ*) only weakly complemented a *gcn5*-deleted strain for growth on minimal plates. We suspected the growth defect in a *GCN5Δ* strain was due to a defect in transcription. Therefore, we assayed lexA−GCN4, lexA−HAP4 and lexA−GAL4 for their ability to transactivate in a *gcn5* deletion mutant complemented with either full-length *GCN5* or *GCN5Δ*. The ADA-dependent activation domain of GCN4 showed a partial reduction in its ability to activate transcription in the absence of the bromodomain, whereas the largely ADA-independent GAL4 and HAP4 activation domains did not (Figure 3B). Finally, restoration of toxicity by GAL4-VP16 was only partial in the strain with the *GCN5Δ* construct compared to *GCN5* (Figure 4). Thus, in three functional assays the bromodomain was important for GCN5 function. To demonstrate that deletion of the bromodomain did not result in degradation of GCN5, we carried out Western blot analysis using antibody to the HA epitope (Figure 5). The levels of GCN5 and GCN5Δ proteins were similar in cell extracts.

The bromodomain could be important in aiding the GCN5−ADA2 interaction, or in facilitating the activity of the assembled ADA complex. To determine whether the bromodomain was important for the ADA2−GCN5 interaction, we carried out *in vivo* and *in vitro* assays for this interaction with GCN5Δ. GCN5Δ was at least as active as full-length GCN5 in the two-hybrid assay (Figure 1). Further, GCN5Δ was co-precipitated with ADA2 in a manner similar to GCN5 (Figure 2, lanes 1 and 2). Thus, we conclude that the bromodomain is not an important determinant of the GCN5−ADA2 interaction.

Discussion

We describe an exhaustive application of the selection for mutations resulting in resistance to GAL4−VP16. We uncovered more alleles of three genes previously identified, *ADA1*, *ADA2* and *ADA3*, and also describe mutations in two additional genes that arose from the selection, *ADA5* and *GCN5*. We argued previously that *ADA1* might be mechanistically different from *ADA2* and *ADA3* because *ada1* mutants displayed vastly reduced levels of the toxic chimera, while *ada2* and *ada3* mutants did not (Berger *et al.*, 1992). Mutations in either *ADA5* (data not shown) or *GCN5* allow accumulation of GAL4−VP16, suggesting that they are similar to *ADA2* and *ADA3*. The properties of the *gcn5* mutant and the interaction between GCN5 and ADA2 are the subject of this report.

On the basis of five criteria, we conclude that GCN5 and ADA2 interact physically and may comprise a part of a multi-protein complex. First, *gcn5* mutants display a very similar phenotype to *ada2* or *ada3* mutants. In

	PRECIPITATE					INPUT				
ADA2	+	+	+			+	+	+		
GCN5	+			+		+			+	
GCN5Δ		+			+		+			+
Luciferase			+	+	+			+	+	+
Lane	1	2	3	4	5	6	7	8	9	10

Fig. 2. The GCN5 protein co-precipitates with ADA2. ADA2 was co-translated with GCN5, GCN5Δ or luciferase in a reticulocyte lysate incorporating [^{35}S]methionine. GCN5 and GCN5Δ were also co-translated with luciferase as a control. Lanes 6–10 show the products of these translations as the 'input'. + Indicates which proteins were translated. These lysates were precipitated with anti-ADA2 antibody and the pellets were boiled and loaded on a 10% SDS–polyacrylamide gel as described in Materials and methods. Lanes 1–5 show the 'precipitate'.

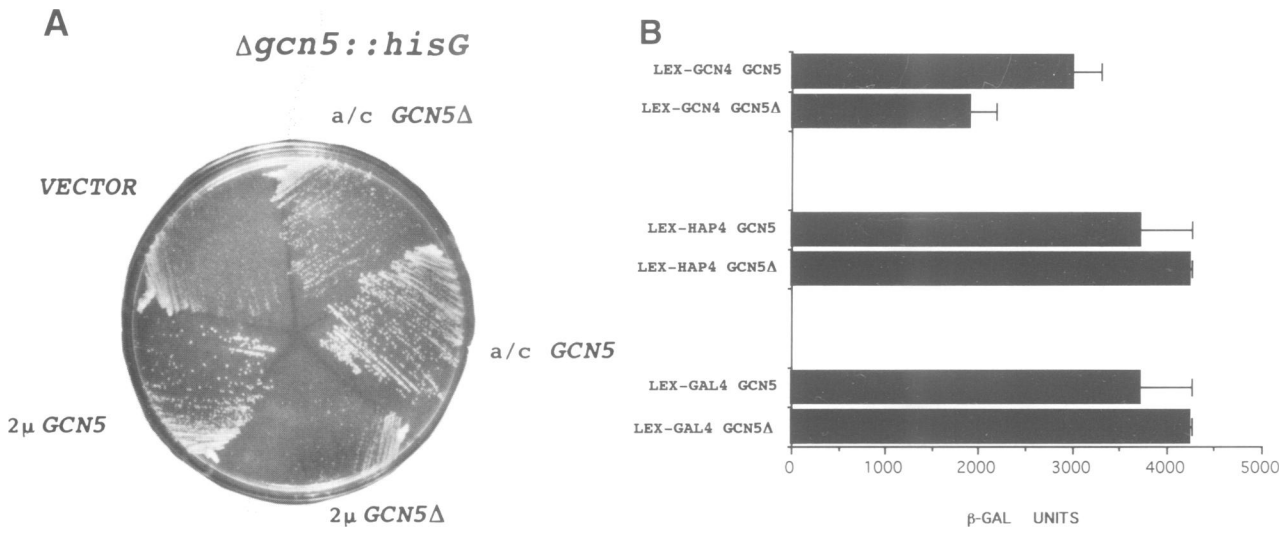

Fig. 3. *GCN5* deleted of the bromodomain (*GCN5Δ*) has reduced ability to complement a *gcn5* deletion. (**A**) GMy25, a *gcn5* deletion strain, was transformed with vector, high copy (2μ) or low copy ARS-CEN (a/c) plasmids expressing HA-GCN5 or HA-GCN5Δ from the ADH promoter. Transformants were restreaked on minimal medium containing glucose. (**B**) GMy23, a *gcn5* deletion strain was transformed with the lexA activation domain fusions, as well as a second plasmid expressing *GCN5* or *GCN5Δ* from the natural *GCN5* promoter. The strain also contained the *lacZ* gene under the control of a single *lexA* operator in plasmid pRbHis. Levels of β-galactosidase were assayed as in Table II. Error bars are shown. As an additional control to show that the mutant strain is indeed defective for transactivation, the lexA fusions were also assayed in the same experiment with a vector that did not express any version of *GCN5*. lexA–GCN4 gave 151 units, lexA–HAP4 gave 1318 units and lexA–GAL4 gave 1029 units.

particular, strains grow slowly on minimal medium, are temperature sensitive on any medium and greatly reduce transactivation by the GCN4 and VP16 activation domains, with smaller effects on the GAL4 and HAP4 activation domains. Second, doubly null mutants, *ada2 gcn5* or *ada3 gcn5*, do not have a more severe phenotype than single mutants. Third, lexA–ADA2 and lexA–ADA3 display transactivation activities that are dependent upon GCN5. [Other interpretations of these data are possible. For example, lexA–ADA2 and lexA–ADA3 may contain cryptic activation domains that are GCN5 dependent, much as the VP16 activation domain is GCN5 dependent. However, given the other evidence for an ADA2–GCN5 interaction and the utility of lexA fusions for studying interactions among HAP2, HAP3 and HAP4 (Olesen and Guarente, 1990), as well as SNF2, SNF5 and SNF6 (Laurent and Carlson, 1992), it is reasonable to argue that the activity of lexA–ADA2 and lexA–ADA3 represents the activity of an ADA complex.] Fourth, ADA2 and GCN5 show a strong interaction *in vivo* by two-hybrid analysis. Fifth, ADA2 and GCN5 co-precipitate. This final experiment suggests that the interaction between the two proteins is direct and requires no other yeast proteins. Furthermore, recent experiments have shown that GCN5 co-fractionates with affinity-purified ADA2 protein from yeast extracts (N.Silverman, unpublished results).

Thus, we envision a complex containing these two proteins and perhaps ADA3 and ADA5. There may be additional factors in this set among those strains that are resistant to GAL4–VP16 that have not yet been characterized. Several other multi-protein complexes have been shown to play a role in eukaryotic transcription. The *SWI1 SWI2/SNF2 SWI3 SNF5* and *SNF6* genes are important for transcription of many yeast genes. They were first classified together genetically (Winston and Carlson, 1992) and have now been shown to comprise a

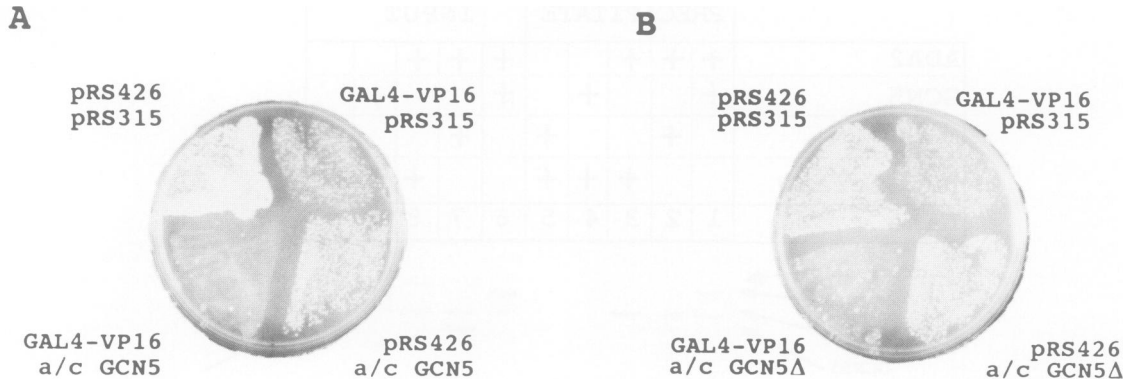

Fig. 4. *GCN5Δ* only partially restores sensitivity to GAL4−VP16 toxicity to a *gcn5* deletion strain. (**A**) GMy25 was doubly transformed with all pairwise combinations of a high copy plasmid expressing GAL4-VP16 (or the matched *URA3* vector control, pRS426) and a low copy ARS-CEN (a/c) plasmid expressing *GCN5* (or the matched *LEU2* control, pRS315). The transformants were plated on drop-out medium on a single plate. The plasmids are listed next to the quadrant in which they were plated. (**B**) The transformants here are identical to those in part (A), except that a plasmid expressing GCN5Δ was used instead of full-length *GCN5*. The severe growth defect of *gcn5* strains observed on minimal medium (Figure 3A) is not observed on the supplemented drop-out medium after 3 days. The few large colonies observed in the *GCN5*/VP16 quadrant result from mutations, presumably in the GAL4-VP16 expression plasmid. Note that in the *GCN5Δ*/GAL4-VP16 quadrant all transformants grow slightly larger than the transformants in the *GCN5*/VP16 quadrant and the frequency of large colonies is also greater.

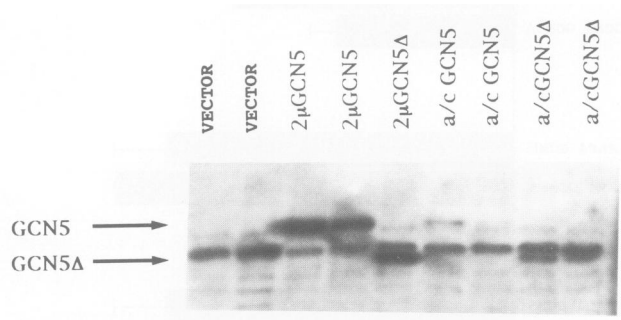

Fig. 5. Western analysis shows similar levels of GCN5 and GCN5Δ protein in a *gcn5* deletion strain. Western analysis using 12CA5 antibody to the HA epitope (Kolodziej and Young, 1991) was performed on whole cell extracts of the transformants of GMy25 described in Figure 3A. The bands corresponding to GCN5 and GCN5Δ proteins are indicated. A background protein, found in all extracts, runs directly above the GCN5Δ band.

complex (Cairns *et al.*, 1994; Peterson *et al.*, 1994). These factors are evidently important for activity of the glucocorticoid receptor in yeast (Yoshinaga *et al.*, 1992) and they promote the binding of GAL4 derivatives to nucleosomal DNA *in vitro* (Côté *et al.*, 1994). Similarly, the *SRB* genes interact genetically with the C-terminal domain of the largest subunit of RNA polymerase II (Thompson *et al.*, 1993). The products of these genes form a complex that co-fractionates with RNA polymerase II and comprise an RNA pol II holoenzyme that also includes TFIIB, the 73K subunit of TFIIH and TFIIF (Koleske and Young, 1994). A third complex may involve products of some *SPT* genes, identified as suppressors of TY1 insertions in yeast promoters (Winston *et al.*, 1984). Based on the similarity of *SPT3*, *7*, *8* and *15* mutants, it is possible that the products of these genes comprise a complex (Winston, 1992). In fact, SPT3 and TBP, the TATA binding protein, which is the SPT15 product, have been shown to interact (Eisenmann *et al.*, 1992). In *Drosophila* and mammalian cells, TBP is a part of a multi-protein complex, TFIID, which also contains TAFs (Dynlacht *et al.*, 1991).

What is the role of the ADA2−GCN5 complex? We have suggested that ADA2 and ADA3 might be transcriptional adaptors which help bridge the interaction between activators and the basal factors. Consistent with this hypothesis, expression of an epitope-tagged version of ADA2 in yeast allows co-precipitation of the tagged ADA2 protein and GAL4−VP16 in yeast extracts (Silverman *et al.*, 1994; R.Candau, N.Bordei, D.Darpino, L.Wang and S.B., unpublished data). We surmise that the ADA−GCN5 complex also contains domains that interact with one or more of the basal factors.

One domain that is a candidate for such interactions is the bromodomain, found at the C-terminus of GCN5 and also in the mammalian TAF complex, the SNF complex, the E1A-associated p300 (Eckner *et al.*, 1994) and in several factors in *Drosophila*, such as brahma (Kennison, 1993). In several cases, deletion of the bromodomain was shown to be inconsequential (Laurent *et al.*, 1993; Elfring *et al.*, 1994). Here we show that deletion of the bromodomain does not lower the steady-state levels of GCN5, but does reduce the ability of the protein to complement a *gcn5* deletion strain and to support the activity of the GCN4 activation domain. In addition, the truncated protein only partially restores toxicity by GAL4−VP16 compared with the full-length GCN5. We have previously proposed that toxicity was due to trapping of basal factors by the potent VP16 activation domain at chromosomal sites (Berger *et al.*, 1992). The bromodomain may be important in this process by helping the ADA complex bind to activation domains, to basal factors, or to DNA.

Although it is also possible that the bromodomain helps interactions within the ADA complex, we do not favor this possibility for two reasons. First, the bromo-deleted GCN5 interacts with ADA2 in the two-hybrid and co-precipitation assays as well as the full-length GCN5 does. Second, the fact that the domain is present in proteins found in other transcription complexes suggests that its function is more general. We infer that the function of the bromodomain is partially redundant in the ADA complex, because the truncated protein still has a partial ability to function. The function of the bromodomain may be redundant in other complexes in which it could be deleted without impairing activity.

In summary, we show that our genetic selection has

converged on at least two proteins, ADA2 and GCN5, that function together by virtue of comprising a heteromeric complex. The importance of such complexes in transcription is just now coming to light. The precise molecular function of this complex, and the activity of the bromodomain in particular, should bring further understanding to the process of eukaryotic transcriptional activation.

Materials and methods

Selection of GAL4–VP16 resistant mutants

pGAL4VP16 URA was generated by ligating a 2.8 kb *Bam*HI fragment from pSB201 (Berger *et al.*, 1992) containing the ADH promoter/terminator cassette with *GAL4–VP16* into the *Bam*HI site of pRS426 (Sikorski and Hieter, 1989).

The strain BP1 (*MATa ade1-100 ura3-52 leu2-3,2-112 his4-519*) was mutagenized with EMS (Guthrie and Fink, 1991), grown for 5 h in YPD, transformed with the 2μ plasmid pGAL4-VP16 URA and plated on the rich medium SD + 0.1% casamino acids, 0.006% adenine, 2% glucose. Three hundred thousand primary transformants were screened, the majority of which were tiny, pinpoint colonies. Three hundred larger colonies were picked and restreaked. Candidate strains with the toxic plasmid were mated to PSY316 (*MATα ade2-101 ura3-52 leu2-3,2-112 his3-Δ200 lys2*), a wild-type tester strain, and diploids that retained the plasmid with GAL4-VP16 were selected. Diploid strains that regained sensitivity to the toxic plasmid were obtained when the original haploid strain contained a recessive mutation that gave resistance to GAL4–VP16. The other strains were presumed to have a dominant chromosomal mutation or a mutation in the GAL4–VP16 expression plasmid. These strains were cured of the plasmid by growth on 5-fluoro-orotic acid (FOA) and mated to strain NSy5B (*MATα, ade2-101, ura3-52, leu2-3,2-112 ada2-2, his⁻*) containing pGAL4-VP16 URA. None of the resulting diploids were clearly resistant to the toxic plasmid, implying that all 250 of these strains had mutations linked to the plasmid. The strains with recessive mutations were mated to *ada1⁻*, *ada2⁻* or *ada3⁻* tester strains to identify additional alleles of these genes by complementation of the slow growth and toxicity resistance phenotypes. *ADA4* and *ADA5* complementation groups were identified among the remaining resistant strains using a segregant that was obtained during tetrad disection. Additional strains resistant to GAL4–VP16 were isolated that do not conform to these complementation groups. In most cases this is because they lack secondary phenotypes or appeared to have multiple mutations responsible for the slow growth phenotype. We also isolated one sterile strain that conferred resistance to GAL4–VP16. However, no GAL4–VP16 protein was detected (not shown).

Cloning and sequencing of GCN5

GMy47c (BP1 *gcn5-1*) was transformed with a yeast genomic library (Thompson *et al.*, 1993) and colonies which grew well on minimal medium were selected. From these, we isolated a clone, p15-1,2c with a 12 kb insert that restored wild-type growth and sensitivity to GAL4–VP16 to GMy47c, as well as to strains with *gcn5-2* or *gcn5-3* alleles. 15-1,2c was partially digested with *Sau*3A, the DNA was run on a 1.2% agarose gel and a band was cut out with fragments ranging from 1 to 3 kb. The DNA was purified using GeneClean (Bio 101) and ligated into pRS316 cut with *Bam*HI to generate a sub-genomic library. GMy47c was transformed with the sub-genomic library and a 2.2 kb subclone, p5-1,2D, was isolated from a rapidly growing colony that restored wild-type growth and sensitivity to GAL4–VP16 to GMy47c. Restriction analysis later revealed that 5-1,2D is in CT3, the vector of 15-1,2c, and not in pRS316. Thus, the subclone is an internal deletion of almost 10 kb from the insert of 15-1,2C.

The ends of the insert in 5-1,2D were sequenced using the Sequenase kit (USB) using the T3 and −20 primers. The DNA sequences were analyzed using the Blast program (Altschul *et al.*, 1990) and the sequence from the −20 primer matched the yeast sequence for the *PUP2* gene (Georgatsou *et al.*, 1992), which lies adjacent to GCN5

GCN5 plasmids

pRS316-GCN5 was generated by cutting p5-1,2D with *Pst*I, blunting with T4 polymerase and cutting again with *Xho*I to get a 1.8 kb fragment. This was cloned into pRS316 cut with *Xho*I and *Sma*I. This same 1.8 kb fragment was cloned into pRS306 and cut with *Xho*I and *Sma*I to generate pRS306-GCN5.

The PCR-generated fragments were cut with *Not*I and cloned into a high copy vector (DB20L) or a low copy vector (RK15) to generate the following ADH expression plasmids: pDB20L-GCN5 (using primers GCN5N and GCN5C, Table IV), pDB20L-GCN5Δ (using primers GCN5N and GCN5CΔ, Table IV), pDB20LHA-GCN5 (using primers NHAGCN5N and GCN5C, Table IV) and pDB20LHA-GCN5Δ (using primers NHAGCN5N and GCN5CD, Table IV). PCR primers are listed in Table IV. The same fragments were ligated into the *Not*I site of pRK15 (an ARS-CEN ADH expression plasmid based on pRS315, R.Knaus, unpublished data) to generate pRK-GCN5, etc.

pRS315-GCN5 was generated by cloning a 1.8 kb *Xho*I–*Eco*RV fragment containing the *GCN5* gene from pSP72-GCN5 (see below) into the *Xho*I-blunted *Bam*HI site of pRS315 (Sikorski and Hieter, 1989). pRS315-GCN5Δ was generated by removing most of the *GCN5* coding sequence from pRS315GCN5 by cleaving at the unique *Hin*dIII (which cuts 15 bp after the stop codon), filling in the ends with the Klenow fragment of DNA polymerase and then cleaving with *Bam*HI, which cuts 50 bp after the start codon. The remainder of the coding sequence for GCN5Δ was supplied by cutting pRKHA-GCN5Δ with *Not*I to release the GCN5Δ insert, treating with the Klenow fragment of DNA polymerase to blunt the ends and cutting with *Bam*HI.

lexA and VP16 fusion plasmids

plexA-ADA2 was generated by amplifying the *ADA2* gene using primers ADA2LN and ADA2LC (Table IV), cutting with *Not*I and ligating in-frame to the *Not*I site of pADH-lexA202 (a 2μ plasmid). plexA-ADA3 was generated in the same way except primers ADA3N and ADA3CNOT (Table IV) were used to amplify *ADA3*. plexA-GCN5 and plexA-GCN5Δ were generated in the same way except that primers GCN5N and GCN5C or GCN5CΔ (Table IV) were used to amplify *GCN5* and *GCN5Δ* respectively. All three *lexA* fusions were able to complement the slow growth and toxicity phenotypes in the appropriate *ada* mutant strains (data not shown). *lexA-GCN5Δ* was able to complement GMy25 as well as pRKHA-GCN5Δ.

The ADA2-VP16 plasmid was generated in two steps. ADA2 was amplified using primers ADA2PRON and ADA2CNOT (Table IV), cut with *Hin*dIII and cloned into the *Hin*dIII site of pRK25 (a 2μ ADH expression plasmid based on pRS425, R.Knaus, unpublished data) to generate pRK25-ADA2CNOT. Then, the bases encoding residues 452–490 of VP16 were amplified by PCR using primers V452N and VP16C (Table IV), cut with *Not*I and cloned into pRK25-ADA2CNOT cut with *Not*I, which fuses VP16 residues 452–490 in-frame with the C-terminus of ADA2, to generate pRK25-ADA2-VP16.

The *lexA–his* reporter pRBHis (a gift of J.Fikes) was generated by cutting Rb1155 (Brent and Ptashne, 1985) with *Stu*I to excise the *URA3* gene, filling in with the DNA polymerase Klenow fragment and ligating the *HIS4* fragment from pB54 (Donahue *et al.*, 1982).

Deletion plasmids and strains

The *GCN5* deletion plasmid was generated in several steps. First, the *Bam*HI site in pSP72 (Promega) was destroyed by cutting, filling in using the DNA polymerase Klenow fragment and ligation to generate pSP72-Bam. Next, the 1.8 kb *Xho*I–*Pst*I fragment from 5-1,2D, containing *GCN5* and flanking sequences, was cloned into the *Xho*I and *Pst*I sites of pSP72-Bam to generate pSP72-GCN5. The *GCN5* coding sequence was removed by ligating a *Bam*HI linker to a filled in *Hin*dIII site, followed by digestion with *Bam*HI. This served as the backbone to which the 2.4 kb *Bam*HI–*Bgl*II *hisG Ura3* cassette from pNKY51 (Alani *et al.*, 1987) was ligated, to generate pGCN5KO.

The *ADA3* deletion plasmid was generated in several steps also. A 2.9 kb *Xba*I–*Pst*I fragment containing *ADA3* and flanking sequences was cut from the genomic clone pADA3-HHV (Piña *et al.*, 1993) and ligated into the *Xba*I and *Pst*I sites of pSP65 (Promega) to generate pSP65-ADA3. An *Nde*I–*Spe*I fragment encoding the first 588 amino acids of the ADA3 protein was removed from this plasmid. The ends were filled in with DNA polymerase Klenow fragment, ligated with *Bgl*II linkers and cut with *Bgl*II. The 2.4 kb *Bam*HI–*Bgl*II *hisG URA3* cassette (Alani *et al.*, 1987) was ligated into this backbone to generate pADA3KO.

GCN5 deletion strains were generated by transforming yeast with 10 μg GCN5KO cut with *Xho*I–*Sal*I. Slowly growing Ura$^+$ transformants were tested for resistance to GAL4–VP16 and to see if wild-type growth was restored by DB20L-GCN5. Strains that were resistant to GAL4–VP16 and had wild-type growth restored by the clone were streaked on FOA to select strains that had looped out the *URA3* sequence. In this manner, Ura$^+$ and Ura$^-$ deletion strains GMy22 and GMy23 were generated from BWG1-7a; GMy24 and GMy25 from BP1; and

Table IV. PCR primers

Name	Sequence
GCN5N	CCCGGGAGATCTGCGGCCGCGATGGTCACAAAACATCAG
GCN5C	GAACCCCGGGGCGGCCGCCTAAGATCTTCAATAAGGTGAGAATATTC
GCN5CΔ	GGCCCGGGGCGGCCGCCTAAGATCTTGCTGCATGATTTTGTAGC
GCN5AADC	CCCGGGAGATCTCTAAGAGGCCGCTCAATAAGGTGAGAATATTC
NHAGCN5	CCCGGGGCGGCCGCATGCTTACCCATACGACGTCCCAGACTACGCCATGGTCACAAAACATCAGATTG
ADA2LN	GGGCCGCGGCCGCATGTCAAACAAGTTTCACTGTGAC
ADA2LC	GGGCCGCGGCCGCTTACATCCAATTCTGGCTCTGGAA
ADA2proN	GGGCCCGGAAGCTTCATGAGCAACAAGTTTCACTGTGACGTTTG
ADA2proC	GGGCCCAAGCTTAGTATGGTGATGGTGATGCATCCAATTCTGGCTCTGG
ADA2CNOT	CCCGGGAAGCTTAAGCGGCCGCCATCCAATTCTGGCTCTGG
ADA3N	CCCGGGGCGGCCGCTGGATCCATGCCTAGACATGGAAGAAGAGG
ADA3CNOT	CCCGGGTGCGGCCGCTTAATTTAGTTCCACGTCC
V452N	CCCGGGGCGGCCGCGTCCCCGGGTCCGGGATTTACC
VP16C	CCCGGGATCCGCGGCCGCTACCCACCGTACTCGTCAATTCC

Primers were synthesized at the Biopolymers Laboratory, Howard Hughes Medical Institute, Center for Cancer Research, Department of Biology, MIT. Fifty picomoles of each primer was used for each PCR reaction.

GMy26 and GMy27 from PSY316. 1-7aΔ*ada2*Δ*gcn5* was constructed by transforming GMy23 with ADA2KO (Berger *et al.*, 1992) cut with *Bam*HI and *Xho*I. Transformants were isolated, tested by mating, grown in YPD broth and plated on medium containing FOA to select strains that had excised the *URA3* gene from the *hisG* cassette. The genotype of the strains were confirmed by transformation with the *ADA2* and *GCN5* clones.

GMy28 (BWG1-7aΔ*ada3*Δ*gcn5*) was constructed in a similar manner except that GMy23 was transformed with pADA3KO plasmid cut with *Pvu*II and *Bam*HI. Double mutants were confirmed by mating and by transforming with the *ADA3* and *GCN5* clones.

PSY316-GCN5 was generated by transforming PSY316 with pRS306-GCN5 cut with *Hin*dIII to target the *GCN5* locus. This strain was mated to GMy47c (BP1, *gcn5-1*). The resulting diploid was sporulated and tetrads were dissected.

ADA2 antisera

The *ADA2* coding sequence engineered with a *Bsp*HI site at the ATG, six histidines at the C-terminus and flanking *Hin*dIII sites was generated using PCR and primers ADA2PROC and ADA2PRON (Table IV). This PCR product was cloned into pRK16 (a gift of R.Knaus) as a *Hin*dIII fragment and checked for complementation in yeast. Then, the gene was isolated on a *Bsp*HI–*Hin*dIII fragment and cloned in *Nco*I- and *Hin*dIII-digested pUH24.2ΔCAT. This vector was contructed by modifying the expression vector pDS56/RBSII, *Nco*I (a gift of D.Stüber, identical to pQE-7 from Qiagen) by cutting with *Bsm*I and religating, leaving a unique *Nco*I site. The *ADA2* bacterial expression vector pA26HE produced large amounts of ADA2 protein which was insoluble. Denaturing Ni-bead chromotography (Qiagen) was used to purify this protein.

Purified ADA2 protein (0.5–1.0 mg/ml in saline) was mixed with RIBI adjuvant (RIBI ImmunoChem Research, Inc.) and used to immunize two rabbits according to the standard protocol (Harlow and Lane, 1988). After several boosts, crude sera was assayed for anti-ADA2 antibodies by Western blot analysis. It was demonstrated that one rabbit produced a good titer of anti-ADA2 sera by virtue of its ability to recognize ADA2 protein in *Escherichia coli* extracts from strains with pA26HE, but not in control extracts. ADA2 protein could also be detected in yeast extracts from strains overexpressing ADA2 (data not shown).

In vitro transcription/translation

To generate *GCN5* RNA the transcription plasmid pT7GCN5 was generated by amplifying *GCN5* with the primers GCN5N and GCN5AADC (Table IV), cutting with *Bgl*II and ligating into the *Bam*HI site of T7Plink (Dalton and Treisman, 1992). pT7GCN5Δ was generated in the same way except the PCR fragment was amplified using the GCN5CΔ oligonucleotide (Table IV) instead of the GCN5AADC oligonucleotide. pT7ADA2 was generated by ligating the *Bsp*HII–*Bgl*II fragment from pA2HA (Silverman *et al.*, 1994) into the *Nco*I and *Bam*HI sites of T7Plink.

Transcription reactions were carried out using 2.5 μg T7GCN5 or T7GCN5Δ linearized with *Xho*I in 1× T7 buffer (GIBCO BRL). Trace amounts of rUTP were included in the reaction to measure percent incorporation. RNA pellets were resuspended in H_2O at 0.4 μg/μl. Translations were carried out in 25 μl reactions with 0.6 μg of each RNA following the standard protocol of the Nuclease Treated Lysate (Promega). A methionine-free amino acid mix was used and [^{35}S]-methionine (Amersham) was incorporated in the proteins produced.

Immunoprecipitation

Protein A–Sepharose beads (CL-4b, Sigma) were pre-equilibrated overnight in IP buffer (10% glycerol, 50 mM HEPES–KOH, pH 7.3, 100 mM K-glutamate, 0.5 mM DTT, 6 mM MgOAc, 1 mM EGTA, 0.1% NP40 and 0.5 mg/ml BSA). Bead slurry (20 μl) was spun in a microfuge and the beads were resuspended in 20 μl fresh IP buffer. Reticulosyte lysate (5 μl) containing translated proteins and 1 μl anti-ADA2 sera were added to the beads, mixed and rotated for 3 h at 4°C. The reactions were then spun for 2 min at 7000 r.p.m. and the supernatant was removed. The beads were washed three times with 1 ml IP buffer by inverting and vortexing. Following the last wash, the supernatant was removed and the pellets were resuspended in 20 μl loading dye (Maniatis *et al.*, 1982). Samples were boiled for 3 min, vortexed and boiled again for 3 min prior to loading onto 10% SDS–polyacrylamide gels. The dried gel was exposed overnight on Hyperfilm-ECL (Amersham).

Yeast manipulations, media, Western and β-galactosidase assays

Transformations were by the LiOAc method (Gietz *et al.*, 1992). Tetrad analysis and other yeast manipulations were done using standard techniques (Guthrie and Fink, 1991). β-Galactosidase assays were carried out on yeast extracts made by breaking cells with glass beads (Rose and Botstein, 1983). The activity of β-galactosidase was normalized to total protein. Westerns blots were performed using standard protocols (Harlow and Lane, 1988). Slowly growing *ada* mutants were assayed on SD minimal medium supplemented with amino acids and adenine. Otherwise strains were grown in SD rich drop-out medium containing all amino acids except those needed for plasmid selection.

Acknowledgements

We would like to thank the Treizman group for pT7Plink, R.Knaus for pRK325, pRK16 and pRK15, D.Stüber for pDS56/RBSII *Nco*I, J.Agapite for construction of plexA-ADA3 and J.Fikes for pRBHis. In addition, thanks are due to Roy Pollock, Rainer Knaus and Nicanor Austriaco for helpful comments on the manuscript, Rachel Kindt for comments on the manuscript and assistance with graphics, members of the Guarente group for fruitful advice and discussions, as well as Myrna Helfenstein for help in preparation of the manuscript. This work was supported by NIH grants GM50207 and ACS NP-755. S.L.B. gratefully acknowledges the support of Smithkline Beecham Pharmacuticals and the Charles King Medical Foundation. J.H. is a Howard Hughes Predoctoral Fellow.

References

Alani,E., Cao,L. and Kleckner,N. (1987) *Genetics*, **116**, 541–545.
Altschul,S.F., Gish,W., Miller,W., Meyers,E.W. and Lipman,D.J. (1990) *J. Mol. Biol.*, **215**, 403–410.

Berger,S.L., Cress,W.D., Cress,A., Triezenberg,S.J. and Guarente,L. (1990) *Cell*, **61**, 1199–1208.
Berger,S.L., Piña,B., Silverman,N., Marcus,G.A., Agapite,J., Regier,J.L., Triezenberg,S.J. and Guarente,L. (1992) *Cell*, **70**, 251–265.
Brent,R. and Ptashne,M. (1985) *Cell*, **43**, 729–736.
Cairns,B.R., Kim,Y.-J., Sayre,M.H., Laurent,B.C. and Kornberg,R.D. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 1950–1954.
Côté,J., Quinn,J., Workman,J.L. and Peterson,C.L. (1994) *Science*, **245**, 53–60.
Cress,W.D. and Triezenberg,S.J. (1991) *Science*, **251**, 87–90.
Croston,G.E. and Kadonaga,J.T. (1993) *Curr. Opin. Cell Biol.*, **5**, 417–423.
Dalton,S. and Treisman,R. (1992) *Cell*, **68**, 597–612.
Donahue,T.F., Farabaugh,P.J. and Fink,G.R. (1982) *Gene*, **18**, 47–59.
Dynlacht,B.D., Hoey,T. and Tjian,R. (1991) *Cell*, **66**, 563–576.
Eckner,R., Ewen,M.E., Newsome,D., Gerdes,M., DeCaprio,J.A., Lawrence,J.B. and Livingston,D.M. (1994) *Genes Dev.*, **8**, 869–884.
Eisenmann,D.M., Arndt,K.M., Ricupero,S.L., Rooney,J.W. and Winston,F. (1992) *Genes Dev.*, **6**, 1319–1331.
Elfring,L.K., Deuring,R., McCallum,C., Peterson,C.L. and Tamkun,J.W. (1994) *Mol. Cell. Biol.*, **14**, 2225–2234.
Fields,S. and Song,O. (1989) *Nature*, **340**, 246.
Georgakopoulos,T. and Thireos,G. (1992) *EMBO J.*, **11**, 4145–4152.
Georgatsou,E., Georgakopoulos,T. and Thireos,G. (1992) *FEBS Lett.*, **299**, 39–43.
Gietz,D., St Jean,A., Woods,R.A. and Schiesti,R.H. (1992) *Nucleic Acids Res.*, **20**, 1425.
Goodrich,J.A., Hoey,T., Thut,C.J., Admon,A. and Tjian,R. (1993) *Cell*, **75**, 519–530.
Guthrie,C. and Fink,G.R. (1991) *Methods Enzymol.*, **194**, 1–933.
Han,M., and Grunstein,M. (1989) *Cell*, **55**, 1137–1145.
Harlow,E. and Lane,D. (1988) *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Haynes,S.R., Dollard,C., Winston,F., Beck,S., Trowsdale,J. and Dawid,I.B. (1992) *Nucleic Acids Res.*, **20**, 2603.
Hinnebusch,A.G. (1985) *Mol. Cell. Biol.*, **5**, 2349–2360.
Hinnebusch,A.G. and Fink,G.R. (1983) *Proc. Natl Acad. Sci. USA*, **80**, 5374–5378.
Hirschhorn,J.N., Brown,S.A., Clark,C.D. and Winston,F. (1992) *Genes Dev.*, **6**, 2288–2298.
Hoey,T., Weinzierl,R.O., Gill,G., Chen,J.L., Dynlacht,B.D. and Tjian,R. (1993) *Cell*, **72**, 247–260.
Hope,I. and Struhl,K. (1986) *Cell*, **46**, 885–894.
Kennison,J.A. (1993) *Trends Genet.*, **9**, 75–79.
Koleske,A.J. and Young,R.A. (1994) *Nature*, **368**, 466–469.
Kolodziej,P.A. and Young,R.A. (1991) In Guthrie,C. and Fink,G.R. (eds), *Guide to Yeast Genetics and Molecular Biology*. Academic Press, San Diego, CA, pp. 508–520.
Laurent,B.C. and Carlson,M. (1992) *Genes Dev.*, **6**, 1707–1715.
Laurent,B.C., Treitel,M.A. and Carlson,M. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 2687–2691.
Laurent,B.C., Treich,I. and Carlson,M. (1993) *Genes Dev.*, **7**, 583–591.
Lin,Y.S. and Green,M.R. (1991) *Cell*, **64**, 971–981.
Lin,Y.S., Maldonado,E., Reinberg,D. and Green,M.R. (1991) *Nature*, **353**, 569–571.
Olesen,J.T. and Guarente,L. (1990) *Genes Dev.*, **4**, 1714–1729.
Penn,M.D., Galgoci,B. and Greer,H. (1983) *Proc. Natl Acad. Sci. USA*, **80**, 2704–2708.
Peterson,C.L., Dingwall,A. and Scott,M.P. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 2905–2908.
Piña,B., Berger,S., Marcus,G.A., Silverman,N., Agapite,J.A. and Guarente,L. (1993) *Mol. Cell. Biol.*, **13**, 5981–5989.
Rose,M. and Botstein,D. (1983) *J. Mol. Biol.*, **170**, 883–904.
Ruppert,S., Wang,E.H. and Tjian,R. (1993) *Nature*, **362**, 175–179.
Sadowski,I., Ma,J., Triezenberg,S. and Ptashne,M. (1988) *Nature*, **335**, 563–564.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning. A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sikorski,R.S. and Hieter,P. (1989) *Genetics*, **122**, 19–27.
Silverman,N., Agapite,J. and Guarente,L. (1994) *Proc. Natl Acad. Sci. USA*, in press.
Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) *Cell*, **68**, 561–572.
Thompson,C.M., Koleske,A.J., Chao,D.M. and Young,R.A. (1993) *Cell*, **73**, 1361–1375.
Winston,F. (1992) In McKnight,S.L. and Yamamoto,K.R. (eds), *Transcriptional Regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1271–1293.
Winston,F. and Carlson,M. (1992) *Trends Genet.*, **8**, 387–391.
Winston,F., Chaleff,D.T., Valent,B. and Fink,G.R. (1984) *Genetics*, **107**, 179–197.
Workman,J.L. and Kingston,R.E. (1992) *Science*, **258**, 1780–1784.
Yoshinaga,S.K., Peterson,C.L., Herskowitz,I. and Yamamoto,K.R. (1992) *Science*, **258**, 1598–1604.

Received on May 20, 1994; revised on July 19, 1994