The EMBO Journal vol.11 no.11 pp.4145–4152, 1992

Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription

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Communicated by G.Thireos

When yeast cells are grown under conditions of amino acid limitation, transcription of amino acid biosynthetic genes is increased through the action of the GCN4 transcriptional regulator. *gcn5* mutant strains exhibit poor growth under such conditions. We have established that GCN4 requires the function of *GCN5* in order to promote normal levels of transcriptional activation. In addition, we have shown that GCN5 is also required for the activity of the HAP2–HAP3–HAP4 transcriptional activation complex, which mediates the transcription of genes involved in respiratory functions. Thus, GCN5 is a new member of the recently revealed general class of transcriptional regulators that collaborate with certain specific DNA binding activators to promote high levels of transcription. We have cloned and sequenced the *GCN5* gene. The deduced GCN5 protein contains a region conserved in other yeast, *Drosophila* and human proteins, all members of this new class of transcriptional activators.

Key words: GCN4/HAP2–HAP3–HAP4

Introduction

The yeast protein GCN4 is one of the best characterized transcription activation factors in eukaryotes. This protein contains the leucine zipper motif, binds to DNA as a homodimer and its transcriptional activation capacity is mediated by a short highly acidic region (Hope and Struhl, 1986, 1987; Hope *et al.*, 1988). GCN4 activates the transcription of a large number of amino acid biosynthetic genes when yeast cells are starved for amino acids (Hinnebush, 1990). GCN4 is regulated quantitatively by the conditions that require its activity. The *GCN4* mRNA is constitutively expressed at relatively high levels, but this message is efficiently translated only when amino acid limitation conditions are imposed (Hinnebush, 1984; Thireos *et al.*, 1984). Under such conditions a protein kinase, encoded by *GCN2*, is activated and phosphorylates the α-subunit of the translation initiation factor eIF2 (Roussou *et al.*, 1988; Dever *et al.*, 1992). This modification results in the reduction of the rates of formation of 40S met-tRNA complexes, a condition that alleviates the negative translational effects of the small upstream ORFs located in the 5′ untranslated region of the *GCN4* mRNA (Tzamarias *et al.*, 1989; Abastado *et al.*, 1991).

Proper translational activation of the *GCN4* mRNA is necessary in order for the GCN4 protein to mediate transcriptional regulation at its full extent (Hinnebush, 1990). However, the magnitude of the transcriptional activation on the regulated genes differs, being determined by the nature of the GCN4 binding site (Hill *et al.*, 1986). The optimum binding site for GCN4 is the perfect palindromic sequence TGAC/GTCA (Hill *et al.*, 1986; Oliphant *et al.*, 1989). The left hand side of this sequence contributes more to the overall affinity of the GCN4 binding and the right hand side can tolerate more alterations (Sellers *et al.*, 1990). Such alterations reduce the binding affinity of the protein to the DNA target accounting for the observed differences in the magnitude of transcriptional activation that GCN4 promotes (Arndt and Fink, 1986; Sellers *et al.*, 1990).

In this study we have demonstrated that just the amount of GCN4 is not sufficient to determine the transcriptional levels of GCN4 regulated genes. By analysing the effects of a mutation we have found that the function of a novel protein encoded by *GCN5* is necessary for the ability of GCN4 to promote maximal levels of transcription. In addition, we have found that this protein is also required for maximal transcriptional activation of genes involved in respiratory functions mediated by the HAP2–HAP3–HAP4 complex (Forsburg and Guarente, 1989). The function of GCN5 is thus required for the activity of at least two sequence specific transcriptional regulators. GCN5 shares a region of similarity with other yeast and *Drosophila* proteins which also collaborate with multiple transcriptional factors.

Results

Growth phenotypes of the gcn5-1 mutant strain

The *gcn5-1* mutation was isolated in a genetic screen designed to isolate mutant strains unable to grow in media imposing amino acid limitation (Penn *et al.*, 1983). A strain carrying this recessive mutation was sensitive for growth in media containing 3-aminotriazole (AT, histidine starvation), methyl-tryptophane (tryptophane starvation), a high concentration of leucine (limitation for valine and isoleucine) and *o*-methyl-threonine (limitation for threonine). As shown in Figure 1, the *gcn5-1* strain exhibited sensitivity for growth under such conditions compared with the growth of an isogenic wild type strain. However, this sensitivity was less severe than that of a *gcn2-11* strain (Figure 1) or *gcn1*, *gcn3* and *gcn4* strains (not shown). We have concluded that the *gcn5-1* mutation confers a distinctly weaker phenotype than other *gcn* mutations.

Translational activation of the GCN4 mRNA is normal in the gcn5-1 strain

The inability of *gcn1*, *gcn2* and *gcn3* strains to grow under amino acid limitation conditions results from an impairment of the mechanism that activates translation of the *GCN4* mRNA (Hinnebush, 1985). However, these strains can grow under these conditions when transformed with a translation-

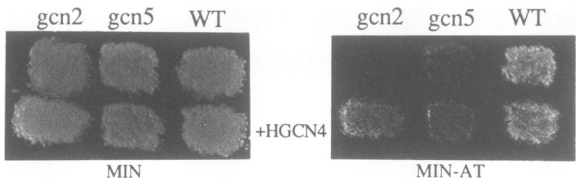


Fig. 1. Growth phenotype of the *gcn5-1* mutant strain. (Top row) Growth of the indicated yeast strains (*gcn2-11*, *gcn5-1* and wild type, WT) on minimal solid media (MIN) and on minimal media containing 10 mM 3-aminotriazole (MIN-AT). (Bottom row) Growth under the same conditions of the same strains transformed with the *ΔORFGCN4* gene derivative which expresses high levels of GCN4 (+HGCN4). Growth was allowed for 3 days at 30°C.

ally active derivative of *GCN4* that has had all its upstream ORFs deleted (Hinnebush, 1984; Thireos *et al.*, 1984). By contrast, this *GCN4* derivative did not rescue the *gcn5-1* phenotype (Figure 1). This observation suggested that the *gcn5-1* mutant phenotype was not correlated with the amount of GCN4 and that the *GCN5* gene product did not affect the translational activation of the *GCN4* mRNA. This was confirmed by assaying the ability of the *gcn5-1* strain to support translational activation when grown under starvation conditions. The results presented in Table I show that the levels of β-galactosidase activity produced by various *GCN4–lacZ* fusion genes in the *gcn5-1* background were identical to those produced in an isogenic wild type strain under both repressing and inducing conditions. Three *lacZ* fusion genes were used in this study. The *GCN4(52)–lacZ* fusion gene, routinely used to assay for the translational regulation of the *GCN4* mRNA (Hinnebush, 1984; Thireos *et al.*, 1984), contains the 5′ untranslated region and the coding region of the first 52 amino acids of *GCN4*, whereas the *GCN4(224)–lacZ* gene contains the first 224 GCN4 amino acids. Although these two fusion genes produced approximately the same levels of β-galactosidase activity when expressed in *Escherichia coli* (data not shown), the extended fusion gene, which contains almost all of the GCN4 protein except the last 57 amino acids, resulted in four times less enzymatic activity when expressed in yeast, irrespective of growth conditions and strain background. Both these fusion mRNAs were activated translationally to the same extent in wild type and *gcn5-1* strains (Table I). The third fusion gene used, *ΔORFGCN4–lacZ*, contains the same coding region as the first one, but all four upstream ORFs are deleted. This deletion relieves this message from translational regulation (Tzamarias and Thireos, 1988). The amount of β-galactosidase produced by this fusion gene reflected both the strength of the *GCN4* promoter and the reduction in the translational rates when growth occurred under amino acid limiting conditions (Tzamarias *et al.*, 1989). These results have demonstrated that the expression of *GCN4* was not affected by the *gcn5-1* mutation either at the transcriptional or the translational level.

The gcn5-1 mutation affects the mRNA levels of GCN4 regulated genes

Since the translational activation of the *GCN4* mRNA was normal in the *gcn5-1* strain, we next examined the levels of mRNAs produced in these strains under both repressing and derepressing conditions for genes subject to transcriptional regulation by the GCN4 protein. As compared with a wild type strain, a *gcn5-1* strain accumulated less *HIS3*,

Table I. Translational activation of *GCN4–lacZ* fusion mRNAs in wild type and *gcn5-1* backgrounds

Fusion	β-Galactosidase activity			
	wt		*gcn5-1*	
	Min	Min+AT	Min	Min+AT
GCN4(52)–lacZ	3.2	35	3.4	38
GCN4(224)–lacZ	0.8	9.8	0.9	11
ΔORFGCN4(52)–lacZ	150	95	170	100

β-Galactosidase activity was measured in the wild type (wt) or the *gcn5-1* strain transformed with the indicated fusion genes after growth in minimal media or for 6 h in minimal media containing 10 mM 3-aminotriazole (AT). Numbers in parentheses in each fusion gene indicate the included amino-terminal amino acids of GCN4. Values represent the average of five independent measurements with < 10% deviation.

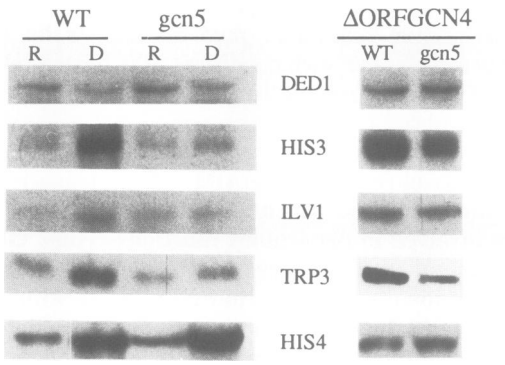
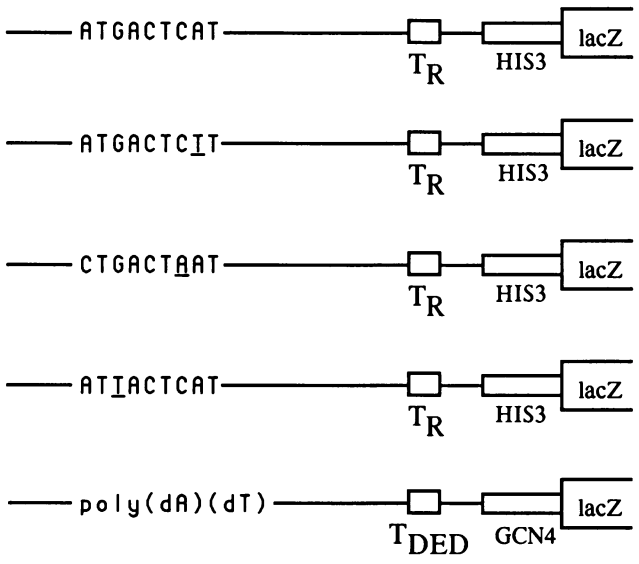


Fig. 2. Effects of the *gcn5-1* mutation on the mRNA levels of amino acid biosynthetic genes. (Left) Total RNAs were extracted from a wild type (WT) and a *gcn5-1* strain (gcn5) grown either in minimal media (repressed) or in minimal media supplemented with 10 mM 3-aminotriazole for 6 h (derepressed). (Right) Total RNAs were extracted from the same strains transformed with the *ΔORFGCN4* gene derivative grown in minimal media. The RNAs were electrophoretically separated and transferred onto nylon membrane. The membrane was sequentially hybridized (following dehybridization) with ^{32}P-labelled DNA probes specific to the *DED1*, *HIS3*, *ILV1*, *TRP3* and *HIS4* genes.

ILV1 and *TRP3* mRNAs when cells were grown under amino acid limitation conditions (Figure 2). By contrast, the presence of the *gcn5-1* mutation apparently did not impair the normal regulation of the *HIS4* mRNA (Figure 2) or the *ARG4* mRNA (not shown), nor did it significantly affect the basal level expression of all tested genes. Since the *gcn5-1* strain was unable to grow properly under starvation conditions, these observations could have been an indirect result of the starvation stress. In order to circumvent this problem we have analysed the mRNA levels in strains transformed with the *ΔORFGCN4* gene derivative, which causes constitutive expression of these genes (Hinnebush, 1985). Both wild type and *gcn5-1* strains transformed with this derivative grow with the same rates in minimal media. Figure 2 (left panel) shows that in such transformed strains the levels of *HIS3*, *TRP3* and, to a lesser extent, *ILV1* mRNAs were reduced in the presence of the *gcn5-1* mutant allele, whereas *HIS4* mRNA was not. Finally, the *DED1* mRNA levels were not affected by the presence of the *gcn5-1* allele (see also below) and were used to control for the amount of RNA in each lane.



UAS	Strain	β-gal activity min	min+AT	+ΔORFGCN4
GCRE	wt	8	15	20
	gcn5-1	6	10.5	14
	gcn5Δ	6.5	10	13
HIS3	wt	3	8	15
	gcn5-1	1.5	3.5	7
	gcn5Δ	1.6	3	6.5
AP1	wt	1	2.8	5.5
	gcn5-1	0.2	0.6	1.2
	gcn5Δ	0.2	0.5	1.5
—	wt	<0.1	<0.1	<0.1
	gcn5-1	<0.1	<0.1	<0.1
	gcn5Δ	<0.1	<0.1	<0.1
DED1	wt	130	95	110
	gcn5-1	132	98	118
	gcn5Δ	138	100	112

Fig. 3. Effects of the *gcn5-1* mutation on the levels of β-galactosidase activity produced from *HIS3–lacZ* fusion genes promoted by GCN4 binding sites of different affinities. Wild type (wt) and *gcn5-1* (gcn5) strains transformed with the indicated *HIS3–lacZ* fusion genes were grown in minimal media (min) or grown in minimal media supplemented with 10 mM 3-aminotriazole for 4 h (min+AT) and the levels of β-galactosidase activity were determined. The same strains co-transformed with the *ΔORFGCN4* gene derivative (+ΔORFGCN4) were grown in minimal media and similarly analysed. GCRE is the optimum GCN4 binding site, whereas the HIS3 and AP1 sites interact with the GCN4 protein with lower affinity. The amount of β-galactosidase that a *GCN4–lacZ* fusion gene produces when expressed from the *DED1* promoter [poly(dA)–(dT)] was also determined for the same strains under the same conditions. TR is the regulated TATA element of *HIS3* and TDED is the *DED1* TATA element. The presented values represent the average of five independent measurements with a < 10% deviation.

GCN4 mediated transcriptional activation requires the GCN5 gene product

The above results have shown that for a subset of genes regulated by the GCN4 protein there was a significant requirement for the GCN5 function for full transcriptional activation. In order to simplify and quantify our analysis, we utilized a minimum reporter gene that contained only the optimum GCN4 binding site to promote the transcription of a *HIS3–lacZ* fusion gene from the regulated TATA element of the *HIS3* gene (Struhl *et al.*, 1985). The activity of this promoter configuration strictly depends on the presence of GCN4. In addition to this reporter gene we have used three derivatives in which the optimum GCN4 binding site (TGACTCA) was substituted for either the natural HIS3 site TGACTCT, which binds GCN4 less efficiently, or an even weaker GCN4 binding site variant TGACTAA (Arndt and Fink, 1986) or a mutation with which GCN4 cannot bind, TTACTCA (Sellers *et al.*, 1990). In addition, we have used another reporter in which the expression of the *ΔORFGCN4–lacZ* gene was driven by the *DED1* promoter (Struhl, 1985). These genes were introduced in wild type, *gcn5-1* and *gcn5Δ* (see below) strains and the levels of β-galactosidase activity produced were used to measure their transcriptional activity in the presence of varying amounts of GCN4. This was accomplished by either growing the cells in repressing conditions (low GCN4), or in derepressing conditions for 4 h (moderate GCN4) or by introducing into these strains the ΔORF derivative of *GCN4* (high GCN4). The results presented in Figure 3 demonstrated that (i) for any amount of GCN4 and for any target there was a requirement for the GCN5 function for full levels of transcriptional activation, (ii) this requirement was greater for weaker GCN4 binding sites and (iii) GCN5 was not required for the activity of the *DED1* promoter. A graphic representation of these results is shown in Figure 4.

The GCN5 protein is required for the HAP2–HAP3–HAP4 mediated transcriptional activation

We had noticed that in addition to the sensitivity of the *gcn5-1* strain for growth under conditions of amino acid limitation, this strain exhibited reduced growth rates when grown in nonfermentable carbon sources such as lactate or ethanol. This suggested that the lack of the wild type GCN5 might also result in lower levels of expression for respiratory genes whose transcription is required to be induced under such growth conditions (Guarente and Mason, 1983). In order to examine this possibility we have used a DNA construct in which the transcription of the *CYC1–lacZ* gene was promoted solely by the UAS2 promoter, a common element involved in the regulation of a number of respiratory genes (Guarente *et al.*, 1984; Trueblood *et al.*, 1988). In addition, we have replaced the GCN4 target of the *HIS3–lacZ* gene in the constructs described above with this element. Both these DNA constructs were introduced in a wild type and a *gcn5-1* strain and the amount of β-galactosidase produced was measured when growth occurred in glucose or lactate containing media. As shown in Table II the presence of *gcn5* mutations impairs the transcriptional activation in both reporter genes under repressing and inducing conditions.

The activity of the UAS2 element depends on the core sequence ATTGGT to which the HAP2–HAP3–HAP4 heterotrimer binds and activates transcription (Forsburg and Guarente, 1988, 1989). This sequence is the reverse CCAAT

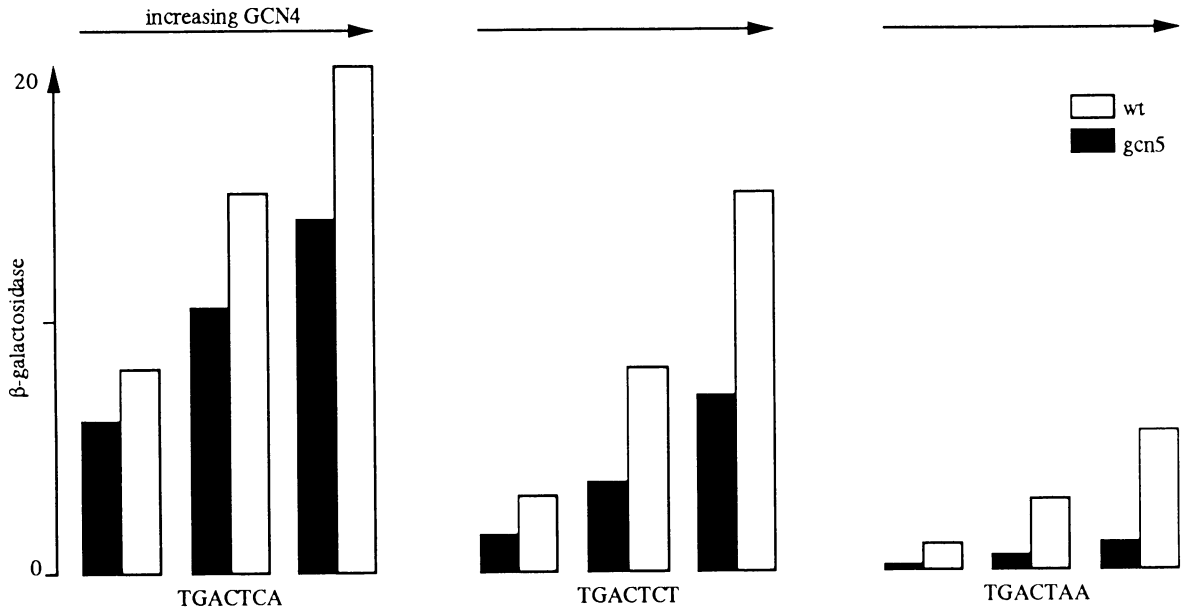


Fig. 4. Graphic representation of the data presented in Figure 3 involving the expression of the *HIS3−lacZ* genes promoted by the three different GCN4 binding sites as a function of strain and amount of GCN4 protein.

sequence that is required for the transcription of a number of mammalian genes. In fact it was shown that the HAP2−HAP3 dimer can interact with one such mammalian element (Chodosh *et al.*, 1988). In order to confirm that GCN5 is required for the HAP2−HAP3−HAP4 mediated transcription, we utilized a *HIS3−lacZ* reporter gene in which the GCN4 element was replaced with a sequence derived from the promoter of the mouse Eβ MHC class II gene (positions −111 to −77; Saito *et al.*, 1983), which contains the sequence TGATTGGCT. As shown in Table II the transcription of this Eβ *HIS3−lacZ* fusion gene was properly induced when growth occurred under strictly aerobic conditions and required the function of *GCN5* for maximal transcriptional levels. We concluded that the *GCN5* gene product is required for the function of the HAP2−HAP3−HAP4 heterotrimer in transcriptional activation.

Table II. Effects of the *gcn5* mutation on the activity of the HAP2−HAP3−HAP4 transcriptional activator complex

Reporter gene/ growth condition	β-Galactosidase activity					
	Glucose			Lactate		
	wt	*gcn5-1*	*gcn5Δ*	wt	*gcn5-1*	*gcn5Δ*
UAS2 CYC1−lacZ	10	4	4.5	122	45	48
UAS2 HIS3−lacZ	2	0.8	0.7	28	12	14
Eβ HIS3−lacZ	0.9	0.4	0.5	11	4	3

β-Galactosidase activity was measured after growth of the wild type (wt), the *gcn5-1* and the *gcn5Δ* strains transformed with the indicated reporter genes in minimal glucose or minimal lactate media. The transcription of Eβ *HIS3−lacZ* fusion gene is promoted by the reverse CCAAT element within the promoter of the mouse Eβ gene. Values represent the average of five independent measurements with <10% deviation.

The GCN5 protein shares a similar region with other yeast, Drosophila and human proteins involved in transcriptional regulation

We have cloned the *GCN5* gene by complementation using a single copy yeast genomic library. Three independent complementing clones were isolated that all shared the genomic DNA region shown in Figure 5A. Deletion analysis indicated that the complementing function was included within the *Xho*I−*Pst*I DNA fragment. DNA sequence analysis of this fragment identified an ORF 439 amino acids in length (Figure 5B). Close to the 3′ end of this gene we have identified another gene transcribed from the opposite strand that encodes a putative proteasomal subunit (*PUP2*; Georgatsou *et al.*, 1992). Gene replacement with a DNA fragment deleted from the *Bam*HI−*Eco*RI region resulted in viable haploids exhibiting the same phenotypes as the *gcn5-1* strain that were complemented by the *Xho*I−*Pst*I DNA fragment. This deleted strain was not complemented when crossed with the *gcn5-1* strain. Finally, we have cloned the *gcn5-1* allele and have shown that it cannot complement the growth defect of both *gcn5* strains. We concluded that the gene we have cloned was *GCN5*.

By analysing the levels of the mRNAs (Figure 2) results showed that the *gcn5Δ* strain was indistinguishable from the *gcn5-1* strain (data not shown). Moreover, as shown in Figure 3 and Table II, the *gcn5Δ* strain affected the expression of the reporter genes similarly to the *gcn5-1* strain. We concluded that at least for the phenotypes studied, the *gcn5-1* mutation was equivalent to a *gcn5* null mutation.

Comparison of the deduced protein sequence of *GCN5* with the EMBL nucleic acid database revealed that GCN5 shares a region of high similarity with other yeast (SNF2, SPT7 and STH1), *Drosophila* (fsh1 and Brm) and human (FSH1H and CCG1) proteins. Multiple alignment of these similarities is shown in Figure 6. Members of this class of proteins are involved in transcriptional regulation in a manner similar to GCN5 (see Discussion). Outside this region we did not observe any other significant similarities. Overall the GCN5 protein contains a high percentage of charged residues, the first one-third of the protein being acidic whereas the middle part is relatively more basic.

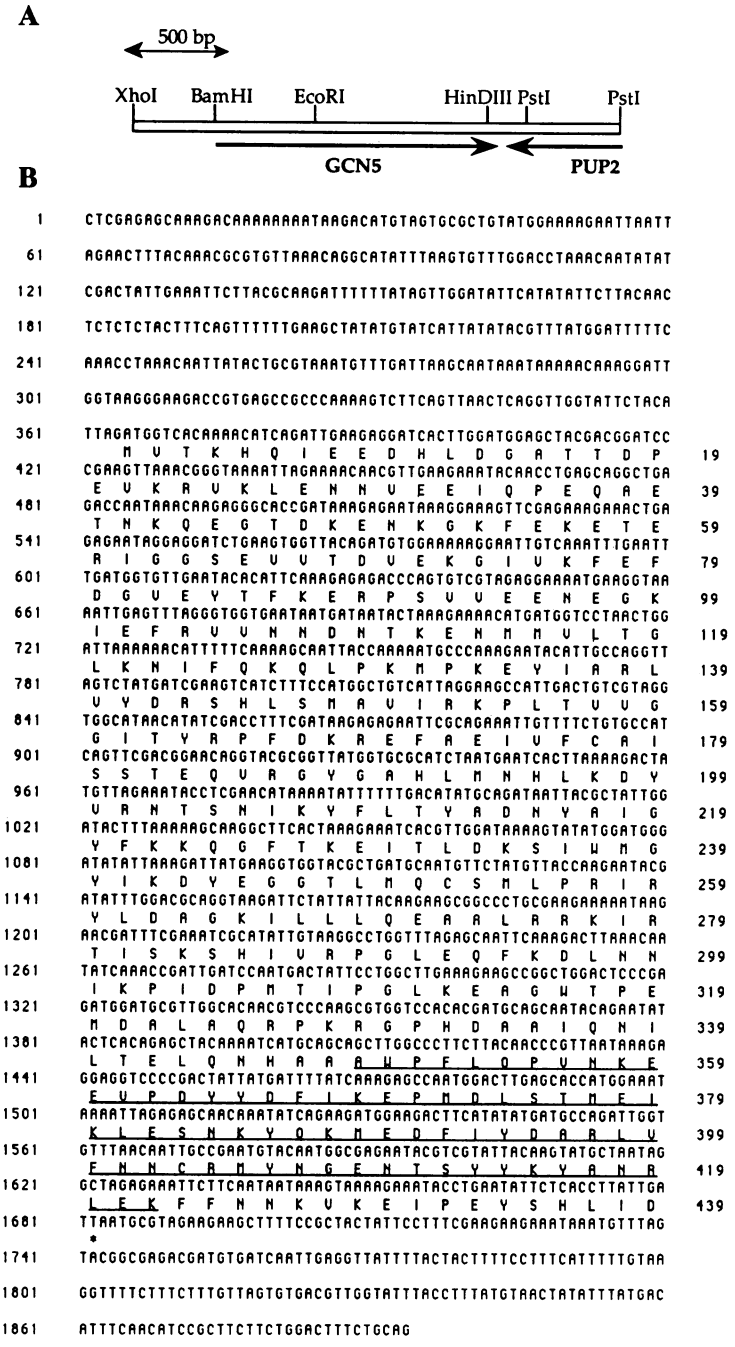


Fig. 5. A. Schematic representation of the genomic DNA fragment that complements the *gcn5-1* mutation. The positions and the direction of transcription of the *GCN5* and *PUP2* genes are indicated by arrows. **B**. Nucleotide sequence of the DNA fragment between the *Xho*I and *Pst*I sites that include *GCN5*. The deduced GCN5 protein sequence is shown. Underlined is the region of the GCN5 protein that is similar to other proteins involved in transcriptional regulation.

Discussion

The GCN5 protein is required for GCN4 mediated transcriptional activation

Yeast cells with a mutation or a deletion in *GCN5* grow slowly in media imposing amino acid limitation. We have shown that this phenotype results from the failure of such strains to accumulate sufficient amounts of mRNAs for amino acid biosynthetic genes when grown under such conditions. The transcriptional regulation of amino acid biosynthetic genes is mediated by GCN4 and requires the translational derepression of the *GCN4* mRNA (Hinnebush, 1984; Thireos *et al.*, 1984). We have shown that the *gcn5-1* mutation does not affect either the transcription of *GCN4* or the translational activation of *GCN4* mRNA. The GCN5 requirement for normal mRNA levels of the affected genes is evident even when the GCN4 protein is overexpressed. Our simplified analysis using minimum regulated promoter configurations has demonstrated that the function of GCN5 is required for maximal levels of transcriptional activation mediated by the GCN4 homodimer. This study confirmed the strong dependence of the GCN4 mediated transcriptional activation on the nature of the GCN4 binding site (Arndt and Fink, 1986; Hill *et al.*, 1986) and demonstrated that the

Fig. 6. Multiple sequence alignment of the region of GCN5 included between amino acids 349 and 422 with the indicated regions of other proteins. Identical residues between GCN5 and the other proteins are blackened. Stars indicate the identical residues in all proteins and dots indicate conservative substitutions.

requirement of GCN5 for the GCN4 activity is higher for weak DNA binding sites.

These results could be accounted for by an involvement of GCN5 in the post-translational regulation of GCN4. Extensive studies on the GCN4 mediated transcription have shown that the activity of this transcriptional factor is regulated quantitatively and no evidence exists for any post-translational modifications (Hinnebush, 1990). Indirect evidence suggests that GCN4 is unstable (Tzamarias *et al.*, 1989). Consistent with this is our observation of the reduced levels of β-galactosidase activity produced by the *GCN4–lacZ* fusion gene that contains a large portion (224 amino acids) of GCN4. Our results have demonstrated that this intrinsic instability does not depend on the function of *GCN5*. Although this analysis does not rule out the possibility of GCN5 mediated post-translational regulation of GCN4, the requirement of GCN5 for transcriptional regulation by another transcriptional activation complex and its structural similarity to analogous transcriptional regulators in yeast and in other organisms favour the idea of its more direct participation in the transcription process.

In contrast to the minimum reporter gene constructs used in this study and whose expression is solely dependent on GCN4, the effects of the *gcn5* mutation on the mRNA levels of GCN4 regulated genes is more complex. For only a subset of these genes is there clear GCN5 dependence for their transcription and this is only evident for the regulated rather than the basal mRNA levels. Amino acid biosynthetic genes have within their promoter different elements that contribute to basal level expression (Hinnebush, 1990) and our results suggest that expression through these elements does not require the function of GCN5. For the well studied *HIS3* gene promoter, basal level expression depends on a polyd(A)–d(T) stretch and a constitutive TATA element (Struhl *et al.*, 1985). The GCN5 independent basal level expression of this gene is consistent with the similar independence of the *DED1* gene expression, whose promoter has an analogous configuration (Struhl, 1985).

Among the genes whose transcriptional activation is affected by the *gcn5-1* mutation, the *HIS3* and the *ILV1* genes are regulated by GCN4 through the suboptimal binding site TGACTCT (Struhl, 1982; Holmberg and Petersen, 1988). By contrast, *HIS4* and *ARG4*, whose expression is apparently GCN5 independent, are regulated through an optimum GCN4 binding site (Lucchini *et al.*, 1984). In addition, we have observed that overexpression of GCN4 minimizes (HIS3) and almost abolishes (ILV1) the GCN5 dependence on the expression of some genes. Thus, differences in the GCN4 binding affinities in concert with the amount of GCN4 could account for the observed effects of the *gcn5-1* mutation on the mRNA levels of different genes. However, the delineation of the common denominator characterizing the GCN5 dependency of GCN4 regulated promoters may require an exhaustive analysis of more general control regulated genes. Of interest is the strong dependence of *TRP3* expression on GCN5. This gene does not have an apparent TATA element and is regulated through an optimum GCN4 binding site located at position −32 where a TATA element would normally be placed (Aebi *et al.*, 1984; Zalkin *et al.*, 1984). Substitution of the regulated TATA element of the *HIS3* gene by the GCN4 binding site has shown that GCN4 can stimulate transcription in the absence of TFIID binding site (Chen and Struhl, 1989). The sensitivity of the *TRP3* promoter to the absence of GCN5 could have functional implications (see below).

GCN5 is a pleiotropic regulator of transcriptional activation

In addition to the GCN4 regulated promoters, GCN5 is required for maximal levels of transcription mediated by the *CYC1* UAS2 element. This element regulates the transcription of a number of genes involved in oxidative phosphorylation (Guarente *et al.*, 1984; Trueblood *et al.*, 1988). This requirement is consistent with the slow growth phenotype that the *gcn5-1* mutant strain exhibits when grown under strictly aerobic conditions. GCN5 was required for maximal transcription of the *HIS3–lacZ* gene when either the *CYC1* UAS2 element or a mammalian reverse CCAAT element replaced the GCN4 binding site. These results show that the GCN5 requirement was specific to the DNA sequence to which the HAP2–HAP3–HAP4 heterotrimer binds and mediates transcriptional activation (Olesen *et al.*, 1987; Hahn and Guarente, 1988; Forsburg and Guarente, 1989).

gcn5 strains grow normally in media containing either galactose or sucrose as sole carbon sources. This suggests that GCN5 is not required for the function of the GAL4 acidic transcriptional activator (Ptashne, 1988) or the activity of the SNF2–SNF5–SNF6 transcriptional activation complex (Neigeborn and Carlson, 1984). Similarly, constitutive promoters such as those of *DED1* (Struhl, 1985) or *GCN4* do not require the function of GCN5. We conclude that GCN5 is required for normal levels of transcriptional activation in at least two different regulated systems: amino acid biosynthesis and aerobiosis. In this respect GCN5 can be classified as a general transcriptional activator operating in concert with certain DNA binding transcriptional activators.

GCN5 shares a conserved region with other transcriptional activators

Recent studies have revealed the existence of a number of factors belonging to a general class of transcriptional activators that all share a region of similarity (Tamkum *et al.*, 1992). GCN5 also contains this conserved region. The SNF2/SWI2 protein functions in conjunction with SNF5, SNF6, SWI1/ADR6 and SWI3 proteins to promote high levels of transcription of genes involved in sucrose and alcohol utilization (*SUC2*, *ADH1*, *ADH2*; Neigeborn and Carlson, 1984; Laurent *et al.*, 1991; Peterson and Herskowitz, 1992), in inositol biosynthesis (*INO1*; Stern *et al.*, 1984), in mating type switching (*HO*; Stern *et al.*, 1984), and for GAL4 dependent transcriptional activation (Peterson and Herskowitz, 1992). In addition, SNF2 is required for the transcription of Ty elements (Happel *et al.*, 1991) and *PHO5* (Abrams *et al.*, 1986). It has been suggested that all these proteins form a multimeric complex involved in transcriptional regulation (Peterson and Herskowitz, 1992). Similarly, the SPT7 protein is required for the transcription of *TY* and *SUC2* (Clark-Adams and Winston, 1987; Winston *et al.*, 1987). Finally, the Brm protein of *Drosophila* is required for the transcription of multiple homeotic genes (Tamkum *et al.*, 1992).

This region of similarity is also found in the *Drosophila* fsh1 protein, which is required for the expression of *ubx* (Haynes *et al.*, 1989), its human homologue FSH1H, of unknown function (EMBL accession number M80613), and in the human CCG1 nuclear protein that regulates the cell cycle through an unknown mechanism (Seqiguchi *et al.*, 1991). Interestingly, this region in GCN5 is more similar to these three proteins than to the yeast proteins. Within this family of proteins the STH1, SNF2 and Brm are also similar to DNA helicases (Laurent *et al.*, 1992). *STH1* was in fact isolated by screening a yeast genomic library with an *SNF2* DNA probe and its function is essential for cell viability (Laurent *et al.*, 1992). The highly conserved character of the GCN5 carboxyl-terminal region suggests that this should serve a conserved function, possibly in relationship to transcriptional activation.

Possible functions of GCN5 in transcriptional activation

The results presented in this paper suggest two, but not mutually exclusive, alternative roles for GCN5 in transcriptional activation: (i) GCN5 is required for the transcription activating function of the GCN4 and the HAP2−HAP3−HAP4 transcriptional activators, or (ii) it facilitates their access to or interaction with their DNA binding sites. In addition, the possibility that GCN5 regulates a limited set of protein factors required for the above functions cannot be excluded. According to the first alternative, GCN5 could function as an adaptor that mediates and enhances the interaction of the transcriptional activation domain of the DNA bound activators with the core transcriptional machinery. The TATA binding factor TFIID has been implicated in participating in such interactions (Greenblatt, 1991). Since *TRP3* transcription is independent of the TATA element, our results indicate that the function of GCN5 does not require TFIID binding on this element.

An alternative function for GCN5 could be analogous to that suggested for other global transcriptional regulators such as the SWI−SNF factors or the Brm protein. It has been proposed that such factors alleviate the barrier imposed by the chromatin structure on the access of DNA binding transcriptional regulators to their targets or their communication with the RNA polymerase II complex (Peterson and Herskowitz, 1992; Tamkum *et al.*, 1992). Consistent with this idea is the fact that mutations that alter the chromatin structure, such as in *SIN* genes, relax the dependence of *HO* transcription on the SWI1, SNF2/SWI2 and SWI3 gene products (Kruger and Herskowitz, 1991). Furthermore, deletion of one of the two copies of histones *H2A* and *H2B* genes allows for high levels of transcription of the *SUC2* and *TY* genes in the absence of SNF2 (Happel *et al.*, 1991).

The evidence presented in this paper combined with other recent reports mentioned above, could indicate that groups of different transcriptional activators collaborate with common co-activators. Different co-activators share a region of similarity that is conserved among evolutionarily highly divergent species. It is tempting to speculate that this region is a functional domain that interacts with analogously conserved molecules such as those involved in the chromatin structure or the core transcriptional machinery. The additional different domains of these co-activators would then serve a function required specifically for each group of transcriptional activators. A structure−function analysis of GCN5 and the construction of chimeric co-activators will provide evidence for these speculations.

Materials and methods

Strains and media

All yeast strains used in this study were derivatives of S288C. These were: *ura3-51*, *ura3-52 leu2-1*, *gcn2-11 ura3-52*, *gcn5-1 ura3-52* and *gcn5-1 ura3-52 leu2-1*. The *gcn4Δ ura3-52 leu2-1* and the *gcn4Δ gcn5-1 ura3-52 leu2-1* strains were constructed by two step disruption of *GCN4* as described elsewhere (Hope and Struhl, 1986). Minimal media contained yeast nitrogen base (Difco) and 2% glucose. Histidine limitation was accomplished by supplementing minimal media with 10 mM 3-amino-1,2,4-triazole (AT). For obligatory aerobic growth glucose was substituted by 3% DL-lactate.

RNA transfer analysis

Total yeast RNA extracted from the indicated strains and growth conditions was electrophoretically separated on a 1.5% agarose gel containing formaldehyde, transferred to nylon membranes and hybridized with appropriate ^{32}P-labelled DNA probes as described elsewhere (Driscoll-Penn *et al.*, 1984). The DNA probes used were: the 1.7 kb *Bam*HI fragment that contains the *HIS3* and the adjacent *DED1* genes (Struhl, 1985), a 2 kb *Eco*RI DNA fragment containing *ILV1* (Kielland-Brand *et al.*, 1984), a 1.3 kb *Bgl*I−*Nco*I DNA fragment containing *TRP3* (Zalkin *et al.*, 1948) and the 1.0 kb *Bgl*II−*Eco*RI DNA fragment internal to *HIS4* (Donahue *et al.*, 1982).

Genes and plasmids

The construction of the *ΔORFGCN4* gene derivative, as well as that of the *GCN4(52)−lacZ* and the *ΔORFGCN4−lacZ* fusions, are described elsewhere (Thireos *et al.*, 1984). All are carried on yeast centromeric vectors with *URA3* or *LEU2* as selectable markers. The *GCN4(224)−lacZ* fusion gene was constructed by inserting into the unique *Bam*HI site of the *GCN4(52)−lacZ* containing plasmid a DNA fragment internal to *GCN4* starting from the *Bam*HI site at Asp52 to the *Sau*3AI site at Asp224 (Thireos *et al.*, 1984).

The *HIS3−lacZ* fusion gene, which is expressed through the optimum GCN4 binding target and the regulated TATA element of the *HIS3* gene, was obtained from K.Struhl. This gene is on a centromeric yeast plasmid carrying *URA3* for selection. The GCN4 binding site in this reporter gene is contained within unique *Bam*HI and *Eco*RI sites and is the synthetic oligonucleotide 5′-GGATCCATGACTCATTTTTTTTTGAATTC-3′. The regulated TATA element is located 18 bp downstream. Three oligo-

nucleotides were synthesized each introducing a mutation at the GCN4 binding site as indicated in Figure 3. These oligonucleotides were inserted between the *Bam*HI−*Eco*RI sites of the above plasmid.

The *DED1GCN4−lacZ* fusion gene was constructed by inserting the *Sca*I−*Sal*I DNA fragment that contains the *ΔORFGCN4−lacZ* fusion gene from the initiation of transcription of GCN4 to the end of the *lacZ* gene (Thireos *et al.*, 1984) into the *Hin*dIII site of YCp88 (URA3 selection), which is located just downstream of the promoter of *DED1* (Hope and Struhl, 1986).

The *UAS2CYC1−lacZ* gene on a YEp24 plasmid was obtained from L.Guarente (clone pLGΔ265, Guarente and Mason, 1983). The *Sma*I−*Xho*I DNA fragment containing the UAS2 element from this clone was inserted as a blunt fragment at the *Bam*HI−*Eco*RI sites of the *HIS3−lacZ* containing plasmid described above, to generate the *UAS2HIS3−lacZ* fusion gene. The E*βHIS3−lacZ* fusion gene was constructed by inserting the *Hin*fI−*Hae*III DNA fragment derived from the promoter of the mouse E*β* gene from positions −111 to −77 relative to the start of transcription (Siato *et al.*, 1983) into the *Bam*HI−*Eco*RI sites of the *HIS3−lacZ* containing plasmid. The sequence of this region is 5′-GACTCCTTTGATGCTGATTGG-CTCCCAGCACTGG-3′.

Molecular cloning of the GCN5 gene

GCN5 was cloned by complementation using a YCp50 based genomic libary (Driscoll-Penn *et al.*, 1984). Three overlapping clones were isolated and following restriction enzyme mapping of the one containing the smaller inserts, the *Xho*I−*Pst*I, as well as the *Bam*HI−*Pst*I genomic fragments (see Figure 5), were inserted at the *Bam*HI site of YCp50. Only the *Xho*I−*Pst*I fragment could complement the *gcn5-1* mutation.

DNA sequence analysis

From the cloned *GCN5* gene the *Xho*I−*Bam*HI, the *Bam*HI−*Hin*dIII and the *Hin*dIII−*Pst*I fragments (Figure 5A) were subcloned into pUC18, and the complete sequence was determined using the dideoxy chain termination method in combination with nested *Exo*III deletions (Guo and Wu, 1983). The sequence and the restriction fragment contiguity were verified by using synthetic oligonucleotides according to the first strand sequence.

Other methods

Yeast transformations were performed using the lithium chloride procedure (Ito *et al.*, 1983). β-Galactosidase assays were performed as described (Tzamarias *et al.*, 1986). Homology searches were done using the TFASTA program. Multiple sequence alignments were done using the CLUSTAL algorithm (Higgins and Sharp, 1989).

Acknowledgements

We thank D.Tzamarias and K.Struhl for providing the plasmid containing the *HIS3−lacZ* reporter gene, L.Guarente for the *UAS2CYC1−lacZ* containing plasmid, J.Papamatheakis for providing the E*β* promoter DNA, E.Maniataki for constructing the E*βHIS3−lacZ* fusion gene, D.Alexandraki for providing the *TRP3* and *ILV1* containing plasmids. M.Averof for helping with the DNA sequence determination and N.Gounalaki for technical assistance. We thank D.Alexandraki and T.Tzamarias for helpful advice and discussions. We also thank L.Kalogeraki and G.Houlaki for photographic and artistic assistance and K.Michelidaki for essential services. This work was supported by funds provided by the Integrated Mediterranean Programmes of the EEC and the Greek Ministry of Industry Research and Technology.

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Received on May 7, 1992; revised on July 17, 1992