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Amino Acid and Adenine Cross-pathway Regulation Act through the Same 5′-TGACTC-3′ Motif in the Yeast *HIS7* Promoter*

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The *HIS7* gene of *Saccharomyces cerevisiae* encodes a bifunctional glutamine amidotransferase:cyclase catalyzing two reactions that lead to the formation of biosynthetic intermediates of the amino acid histidine and the purine adenine. The *HIS7* gene is activated by GCN4p under environmental conditions of amino acid starvation through two synergistic upstream sites GCRE1 and GCRE2. The BAS1p-BAS2p complex activates the *HIS7* gene in response to adenine limitation. For this activation the proximal GCN4p-binding site GCRE2 is required. GCN4p and BAS1p bind to GCRE2 *in vitro*. Under conditions of simultaneous amino acid starvation and adenine limitation the effects of GCN4p and BAS1/2p are additive and both factors are necessary for maximal *HIS7* transcription. These results suggest that GCN4p and BAS1/2p are able to act simultaneously through the same DNA sequence *in vivo* and use this site independently from each other in a non-exclusive manner.

Promoters of yeast genes contain two types of transcriptional control elements. TATA and initiator elements, located near the transcriptional start site, comprise the binding sites for general transcription factors and ultimately the RNA polymerase holoenzyme complex. Elements located upstream of the TATA element are recognized by gene-specific transcription factors. The simplest regulated promoter would contain a single upstream element bound by a single protein. The situation in natural promoters is more complex. Naturally occurring promoters often contain multiple binding sites for several proteins which act synergistically. In addition, the same target sequences in a promoter can be recognized by different proteins in a mutually exclusive manner (1). Here we report evidence for a novel, non-exclusive activation of *HIS7* transcription by the transcriptional activators GCN4p and the BAS1/2p complex through the same target sequence.

Co-regulation of genes involved in different metabolic pathways in yeast is usually achieved by the same transcription factor that binds to a common target sequence in the different target promoters. An example of such a cross-pathway regulation is the general control of amino acid biosynthesis in yeast (2). The transcriptional activator GCN4p mediates the coordinate derepression of genes coding for amino acid biosynthetic enzymes, aminoacyl-tRNA synthetases (2), and purine biosynthetic genes (3–5) upon the environmental signal of amino acid starvation.

De novo biosynthesis of the amino acid histidine and purines are metabolically interconnected because both pathways share common substrates and intermediates (4). This connection on the metabolic level seems to be reflected by the cross-pathway regulation on transcriptional level of certain *HIS* and *ADE* genes by the transcription factors GCN4p and BAS1/BAS2p. The BAS1-BAS2p complex mediates thereby the derepression of regulated genes upon adenine limitation.

HIS4 is an example of a yeast gene which is subject to both cross-pathway regulatory systems. Transcription of the gene is elevated under conditions of amino acid starvation mediated by the binding of GCN4p to a high affinity site in the *HIS4* promoter (6). In addition, the gene is activated independently of GCN4p by the joint action of BAS1p and BAS2p (7) binding adjacent to each other to a site upstream of the GCN4p-binding site (8). BAS1/2p-mediated activation of the *HIS4* gene is significantly repressed in the presence of adenine (8). Therefore BAS1/2p seem to be involved not only in adenine repression but also in basal expression of the *HIS4* gene (4).

BAS2p (=PHO2p=GRF10p) represents a global transcriptional activator. In combination with different proteins BAS2p has an effect on various regulatory networks. Together with PHO4p, BAS2p is involved in phosphate regulation (9). Thus BAS2p confers phosphate regulation to *HIS4* independently of BAS1p (7, 8). In addition, BAS2p is involved in the regulation of the *TRP4* gene involved in tryptophan biosynthesis (10). In cooperation with SWI5p, BAS2p is involved in the regulation of the *HO* gene (11). Finally, together with BAS1p, BAS2p is involved in the adenine regulation of either purine (5) or amino acid biosynthetic genes.

The *HIS7* gene product is at the crossing point of the histidine and purine biosynthetic pathways. It codes for a bifunctional glutamine amidotransferase:cyclase catalyzing the fifth and sixth step in the *de novo* histidine biosynthesis (12). This enzyme produces an intermediate of histidine biosynthesis and in addition, 5-aminoimidazole-4-carboxamide ribotide (AICAR), which is an intermediate of purine biosynthesis (Fig. 1). Here we have analyzed whether the metabolic role of the *HIS7* gene product at the crossing point of two biosynthetic pathways is also reflected in the transcriptional regulation of the *HIS7* gene. We show that GCN4p activates *HIS7* transcription under conditions of amino acid starvation, BAS1/2p under adenine limitation, and both factors act independently through the same promoter site (Fig. 2).

MATERIALS AND METHODS

Strains and Media—All yeast strains used were derivatives of the *Saccharomyces cerevisiae* laboratory strain S288C (*MAT*a *gal2 SUC2*

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mal CUP1). The three yeast strains RH1371 (*aro3–2* Δ*ura3 gcd2–1*), RH1372 (*aro3–2* Δ*ura3*), and RH1381 (*aro3–2 ura3–52 gcn4–101*) harboring high, low, and zero GCN4p levels, respectively, were described previously (12). The yeast strain RH2151 (*gcn4–1 bas1–2 bas2–2 ura3–52*) was described in Tice-Baldwin *et al.* (8). Yeast cells were made competent for transformation by treatment with lithium acetate (13). *Escherichia coli* strain MC1061 (14) was used for plasmid propagation. Cultivation of yeast was performed at 30 °C in either YEPD complete medium (15) or MV minimal medium (16) with recommended amounts of supplements (15). *E. coli* was grown at 37 °C in LB complete medium (17) containing 50 mg/liter of ampicillin when selecting for transformants.

Plasmids—Plasmid pME694 containing a (wild-type) 1.9-kb[1] *Sph*I-*Bam*HI *HIS7* fragment was described previously (12). Plasmid pME696 carrying an integrative *HIS7-lacZ* fusion is a derivative of pME694 (12). Plasmid AB243 containing a 12-kb *BAS1* fragment was described earlier (7). To construct plasmid pME1399 the 2.8-kb *Sal*I/*Eco*RI fragment of pME1141 was ligated into the appropriate sites of the plasmid pRS316 (18). The 510-bp *Sal*I/*Bst*EII fragment of the resulting plasmid was replaced by the *Sal*I/*Bst*EII fragment of plasmid p238 carrying a *GCN4* allele mutated in all four upstream open reading frames (19). Plasmids pCB159 and pAB291 overexpressing BAS1p and BAS2p, respectively, were previously described in Tice-Baldwin *et al.* (8). The plasmid pME1405 was constructed by cloning the 2.8-kb *Bam*HI/*Xho*I fragment of pME1406 containing the *BAS2-VP16* fusion (20) into the plasmid pRS316 (18).

DNA Techniques—Enzymatic manipulation and cloning of DNA were performed as described by Sambrook *et al.* (17). Nucleotide sequences were determined using the method of Tabor and Richardson (21). Polymerase chain reactions (PCRs) were performed as described previously (12) using Vent DNA polymerase (New England Biolabs, Beverly, MA).

Site-directed Mutagenesis of the HIS7 Promoter—Site-directed mutations in the *HIS7* promoter were introduced using the PCR technique (22). Oligonucleotides carrying specific mutations (mutated nucleotides in lower case) in the *HIS7* promoter sequence were: MKG (5′-GCAAAAAAAAATTtAGTaATATCGTAGC-3′) for mutating the GCN4p-binding site GCRE1 to gcre1 and CHE3 (5′-TCGATGTCTtAgTCTTTTCTCA-3′) for mutating GCRE2 to gcre2. These oligonucleotides were used as primers in a first PCR reaction together with MUTH7 (5′-CGCCATTACCGGTCATG-3′) or CHE5 (5′-TTGAAAGTGGTAAC CTACAGTCACTAACCAATGCAATTG-3′), respectively, as second primers and pME694-DNA as template. The resulting first PCR product was subsequently used as primer in a second PCR reaction with MUTH7 or CHE5, respectively, as second primer and the same template DNA. The final PCR product was cut with *Sty*I and *Eco*RV and exchanged against the corresponding wild-type *HIS7* fragment on pME694 resulting in plasmids pME695 (gcre1) and pME673 (gcre2). For the construction of pME674, which is mutated in both GCREs, an analogous procedure with MKG (gcre1) as primer and DNA of pME673 (gcre2) as template was used. All mutations were verified by sequence analysis. The respective integrative *HIS7-lacZ* fusion plasmids pME698 (gcre1), pME699 (gcre2), and pME700 (gcre1/2) were constructed on the basis of the pME694 derivatives as described previously for pME696 (12).

Construction of HIS7-lacZ Reporter Strains—*HIS7*-lacZ fusion constructs pME698 (gcre1), pME699 (gcre2), and pME700 (gcre1/2) were integrated at the original *HIS7* locus of yeast strains RH1371 (*gcd*), RH1372 (wt), and RH1381 (*gcn4*), yielding strains RH1617 (*gcd2*, gcre1), RH1618 (wt, gcre1), RH1619 (*gcn4*, gcre1), RH1620 (*gcd2*, gcre2), RH1621 (wt, gcre2), RH1622 (*gcn4*, gcre2), RH1623 (*gcd2*, gcre1/2), RH1624 (wt, gcre1/2), and RH1625 (*gcn4*, gcre1/2). The procedure was described previously for the wild-type *HIS7-lacZ* construct pME696 resulting in strains RH1614, RH1615, and 1616, respectively (12). Strains harboring constitutively high amounts of GCN4p independently of the *GCD2* locus were obtained by transformation of the strains RH1616, RH1619, RH1622, and RH1625 with the plasmid pME1399.

Construction of Strains Carrying HIS7 Promoter Mutations—The pME694 derivatives pME695 (gcre1), pME673 (gcre2), and pME674 (gcre1/2) carrying mutated alleles of the *HIS7* promoter were used to introduce the respective mutations into strain RH1381 (*gcn4*) by gene replacement. The plasmids were cut with *Sph*I and *Bam*HI and transformed into the same *his7::URA3* disruption strains as used for the integration of the *HIS7-lacZ* fusion constructs (12). Aro$^+$ transformants were examined for their His$^+$ and Ura$^-$ phenotypes and by Southern blot analysis. This procedure yielded strains RH1545 (*gcn4*, gcre1), RH1565 (*gcn4*, gcre2), and RH1566 (*gcn4*, gcre1/2). Strains harboring constitutively high amounts of GCN4p were obtained by transformation of the strains RH1381, RH1545, RH1565, and RH1566 with the plasmid pME1399.

Construction of bas1 and bas2 Disruption Strains—Plasmids pME1167 and pUC19-*pho2::URA3*, carrying the 2.8-kb *Hin*dIII *pho2::URA3* fragment of pCS21 (23), were used to disrupt the *BAS1* and *BAS2* genes, respectively, in the *HIS7-lacZ* reporter strains and strains carrying *HIS7* promoter mutations. Plasmid pME1167, carrying a *bas1::URA3* disruption allele, was constructed based on AB243 (7). A 6-kb *Sac*I *BAS1* fragment from AB243 was recloned on pUC18 (Clontech Laboratories, Palo Alto, CA) yielding pME1165. pME1167 was constructed by replacing a 2.2-kb *Bgl*II/*Spe*I *BAS1* fragment in pME1165 by a flushed chromosomal 1.1-kb *Hin*dIII *URA3* fragment. Strains disrupted for both loci were constructed by curing a single mutant for the *URA3* gene using 5-fluoroorotic acid (24) and subsequent disruption of the second locus in the cured strain. Proper recombination was verified by Southern blot analysis.

β-Galactosidase Assay—β-Galactosidase activities were determined using permeabilized yeast cells and the fluorogenic substrate 4-methylumbelliferyl-β-D-galactoside as described previously (12). Routinely, yeast cells were cultivated in minimal medium without adenine overnight, diluted to an optical density of approximately 0.5 at 546 nm (OD_{546}) in minimal medium without or with 0.3 mM adenine, and cultivated for another 6 h before assaying. 1 unit of β-galactosidase activity is defined as 1 nmol of 4-methylumbelliferone h^{-1} ml^{-1} $OD_{546}{}^{-1}$. The given values are means of at least five independent cultures each three times measured. The standard errors of the means are indicated.

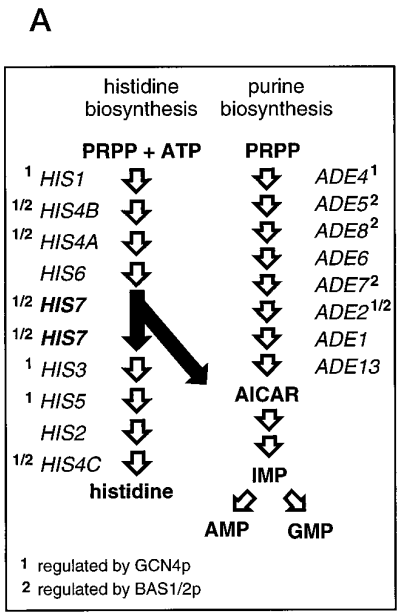
Northern Analysis—Strains were cultivated as for β-galactosidase assays. Total RNA from yeast was isolated as described earlier (25). 10 μg of total RNA of each strain were separated on a formaldehyde agarose gel, electroblotted onto a nylon membrane, and hybridized against ^{32}P-labeled probes prepared from a chromosomal 0.9-kb *Bam*HI-*Xba*I *HIS7* fragment and a 1.1-kb *Hin*dIII *URA3* fragment using the oligolabeling technique described by Feinberg and Vogelstein (26). The endogenous *ura3–52* and Δ*ura3* transcripts were used as internal standards for the amount of RNA.

Gel Retardation Assay—The gel retardation assay using GCN4p produced in *E. coli* was described earlier (10). As DNA probe a ^{32}P-end-labeled 379-bp *Bsa*HI/*Eco*RV fragment of the *HIS7* promoter derived from plasmids pME694 (wild-type), pME695 (gcre1), pME673 (gcre2), and pME674 (gcre1/2) was used. The gel retardation assay using protein extracts from yeast strains overexpressing BAS1p, BAS2p, or both was described previously by Tice-Baldwin *et al.* (8). Different versions of a synthetic ^{32}P-end-labeled 70-bp DNA fragment comprising the *HIS7* promoter between position −186 and −116 relative to the *HIS7* start codon including the GCRE2 were used as DNA probes. The fragments were either wild-type (wt) or carried point mutations in GCRE2 (gcre2).

RESULTS

The HIS7 Gene Is Activated under Conditions of Amino Acid Starvation and Adenine Limitation—The effect of adenine limitation and amino acid starvation on *HIS7* transcription was analyzed. *HIS7* transcription was monitored by determining β-galactosidase activities of strains carrying respective translational *HIS7-LacZ* fusions integrated in single copies at the *HIS7* locus. Under conditions of adenine limitation *HIS7* expression was increased by a factor of two relative to conditions with excess adenine in the growth medium. The effect of the general control system of amino acid biosynthesis was analyzed by comparing *HIS7* transcription in yeast strains harboring constitutively high levels of the transcriptional activator protein GCN4p to strains harboring wild-type levels of GCN4p. Under these conditions we found a 6-fold activation of *HIS7* expression. Under conditions of high expression of the GCN4p and simultaneous adenine limitation we found the highest *HIS7* expression level. Under these conditions *HIS7* expression was activated 8-fold compared to non-starvation conditions (Fig. 1). Thus, the two effects behave in an additive manner. By contrast, *HIS7* transcription was unaffected by the addition of

[1] The abbreviations used are: kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction.



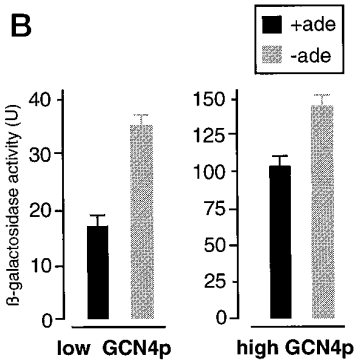


FIG. 1. ***HIS7* transcription responds to the environmental conditions of amino acid starvation and adenine limitation.** *A, de novo* biosynthetic pathways of histidine and purine in yeast. The two biosynthetic pathways share common substrates and intermediates. Both pathways start with phosphoribosylpyrophosphate (*PRPP*). The first step in histidine biosynthesis is the esterification of PRPP with ATP. The reactions catalyzed by the gene product of *HIS7* lead to the by-product 5-aminoimidazole-4-carboxamide ribotide (*AICAR*) which is an intermediate in the purine biosynthetic pathway. The known regulation of the respective genes by the transcription factors GCN4p and BAS1/2p are indicated: *HIS4* (7), *HIS7* (this work), *ADE1*, *ADE2*, *ADE5*, *ADE7* (4, 5), *ADE4* (3). *B, HIS7* gene expression was monitored by measuring the β-galactosidase activities of *S. cerevisiae* strains carrying a translational *HIS7-lacZ* fusion integrated at the genomic *HIS7* locus. Conditions of amino acid starvation were mimicked by using yeast strains harboring constitutively high levels of GCN4p. Strains were cultivated either with 0.3 mM (+*ade*) or without (−*ade*) adenine in the growth medium. The shown values are the results of at least five independent measurements. The standard deviation is indicated and did not exceed 20%. Yeast strains harboring no *E. coli lacZ* gene did not show any detectable β-galactosidase activity (data not shown).

histidine to the growth medium or by a switch from high phosphate to low phosphate growth conditions (data not shown).

Point Mutations within Two GCN4p Recognition Elements (GCREs) Abolish the Activation of the HIS7 Gene by the General Control System of Amino Acid Biosynthesis—The *HIS7* promoter harbors two potential GCN4p recognition elements, which were designated GCRE1 and GCRE2, respectively (12) (Fig. 2). To test the two potential GCREs in the *HIS7* promoter for their significance for the activation of the *HIS7* gene under conditions of amino acid starvation, *HIS7* promoter alleles

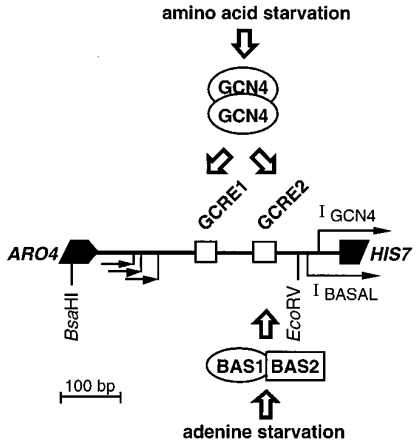


FIG. 2. **Structure of the *HIS7* promoter.** The open reading frames of the *HIS7* gene and the upstream located *ARO4* gene are shown as *solid arrows*. The mapped ends of the *ARO4* transcripts and start sites of basal (I_{BASAL}) and GCN4p-dependent (I_{GCN4}) *HIS7* transcription are indicated (12). The two GCREs in the *HIS7* promoter located at positions −224/−232 and −138/−146, respectively, relative to the *HIS7* start codon are symbolized by *open boxes*. Relevant restriction sites are indicated.

with point mutations (in lower case) in GCRE1 (gcre1: 5′-ATtACTaAA-3′), GCRE2 (gcre2: 5′-CTtAgTCTT-3′), and both GCREs (gcre1/2) were constructed. Both mutated sequences had previously been reported to have a very low affinity to GCN4p (27). *HIS7* transcription was monitored by two approaches: (i) by determining β-galactosidase activities of strains carrying respective translational *HIS7-lacZ* fusions and (ii) in Northern analyses by estimating *HIS7* mRNA levels relative to endogenous Δ*ura3* transcript levels. In both cases the test promoters were introduced in single copy at the genomic *HIS7* locus by gene replacement. The various promoters (wild-type, gcre1, gcre2, and gcre1/2) were also introduced in a *S. cerevisiae* strain with a *gcd* (general control constitutively derepressed) genotype expressing high levels of GCN4 protein. To avoid any interference with the above described adenine effect, all strains were cultivated in media containing 0.3 mM adenine. Under these conditions transcription directed by the wild-type *HIS7* promoter was reduced approximately 4-fold by the *gcre1* mutation and approximately 2-fold by the *gcre2* mutation. An 8-fold reduction was observed when both GCREs were mutated (gcre1/2) (Fig. 3). These results suggest that GCN4p activates *HIS7* transcription synergistically through both GCREs under conditions of amino acid starvation.

Adenine Limitation Results in HIS7 Activation by BAS1/2p Which Is Mediated through GCRE2—The above set of *HIS7* promoter alleles was also tested in a Δ*gcn4*-strain to reveal any GCN4p-independent function of GCRE1 or GCRE2. Strains cultivated in a growth medium containing 0.3 mM adenine showed all the same basal level of *HIS7* transcription independent of the mutation of the GCREs (Fig. 4*A*). In contrast, under conditions of adenine limitation an approximately 2-fold activation was observed in strains carrying a wild-type (wt) or an in GCRE1 mutated (gcre1) *HIS7* promoter allele. However, no activation of *HIS7* transcription could be observed in strains mutated in GCRE2 (gcre2 and gcre1/2). The levels of GCN4p independent *HIS7* transcription directed by the promoters mutated in GCRE2 in adenine-deficient growth medium were similar to the levels observed with the strain carrying a wild-type *HIS7* promoter allele in adenine containing growth medium (Fig. 4). These results suggest that *HIS7* transcription is activated independently of GCN4p through GCRE2 under conditions of adenine limitation.

To test whether in analogy to the *HIS4* gene (7, 8) the factors

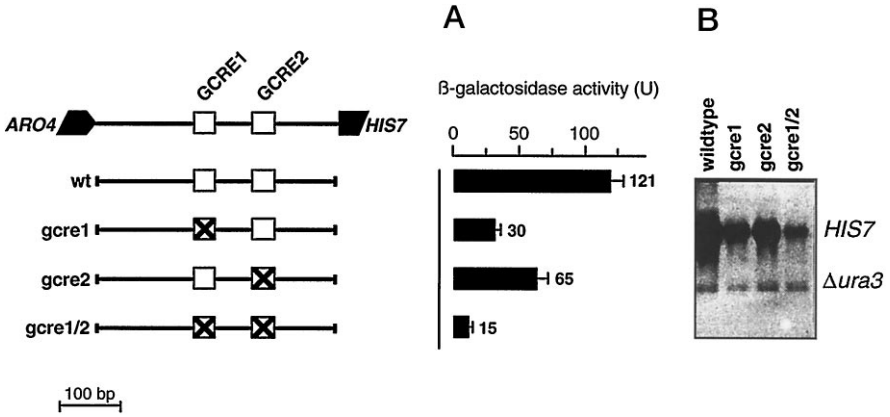


FIG. 3. **Synergistic activation of the *HIS7* gene by GCN4p.** HIS7 transcription directed by wild-type or mutated versions of the *HIS7* promoter was monitored in *S. cerevisiae* strains harboring a constitutively high GCN4p level. *HIS7* promoters were either wild-type or mutated in GCRE1 (gcre1), GCRE2 (gcre2), or both GCREs (gcre1/2). Strains were grown in medium containing 0.3 mM adenine. *A*, β-galactosidase activities of strains carrying respective translational *HIS7-lacZ* fusions integrated at the genomic *HIS7* locus. *B*, Northern analysis of strains carrying respective *HIS7* promoter alleles at the genomic *HIS7* locus. Total RNA of the different strains was hybridized against DNA probes for the *HIS7* and *URA3* transcripts. The endogenous Δ*ura3* transcript level was used as internal control for the amount of RNA. Transcript sizes are: *HIS7* (1.8 kb); Δ*ura3* (0.7 kb).

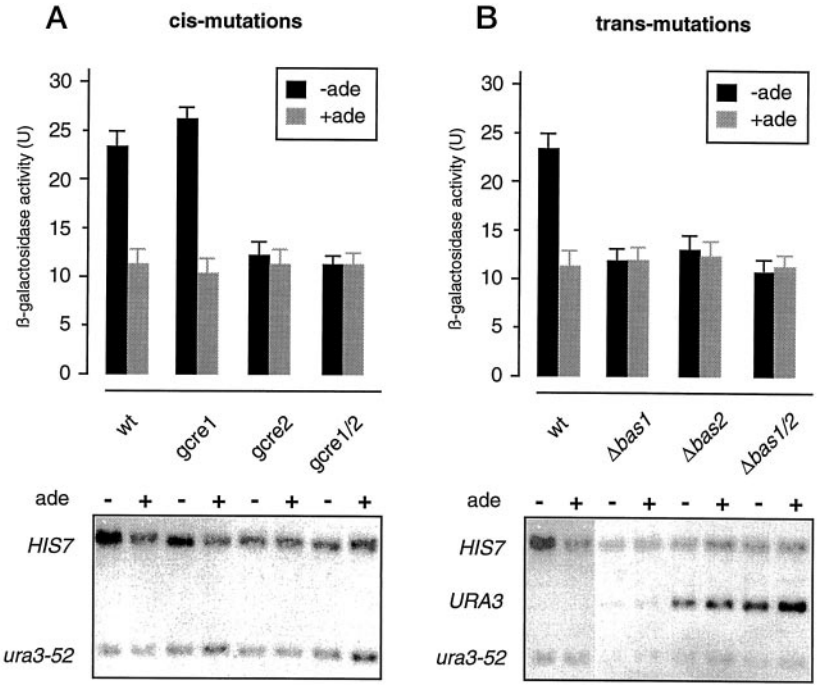


FIG. 4. **The adenine response of *HIS7* transcription is mediated through GCRE2 by the joint action of BAS1p and BAS2p.** *A*, GCN4p-independent *HIS7* transcription directed by the different versions of the *HIS7* promoter (wild-type, gcre1, gcre2, and gcre1/2) was monitored in yeast strain RH1381, harboring no functional GCN4p. Strains were cultivated either without (−*ade*) or with 0.3 mM adenine (+*ade*) in the growth medium. *Upper panel*, β-galactosidase activities of strains RH1616 (wild-type), RH1619 (gcre1), RH1622 (gcre2), and RH1625 (gcre1/2) carrying integrated *HIS7-lacZ* fusions. *Lower panel*, *HIS7* mRNA levels of strains RH1381 (wild-type), RH1545 (gcre1), RH1565 (gcre2), and RH1566 (gcre1/2) carrying different *HIS7* promoter alleles relative to the endogenous *ura3–52* transcript levels. The size of the *ura3–52* transcript is 0.6 kb. *B, HIS7* transcription directed by the wild-type *HIS7* promoter was determined in strains with a genetic *gcn4* (wild-type), *gcn4* Δ*bas1* (Δ*bas1*), *gcn4* Δ*bas2* (Δ*bas2*), and *gcn4* Δ*bas1* Δ*bas2* (Δ*bas1/2*) background as described above. Strains were cultivated either in adenine-deficient medium (−*ade*) or in medium containing 0.3 mM adenine (+*ade*). *Upper panel*, β-galactosidase activities of strain RH1616 (wild-type) and Δ*bas1* or Δ*bas2* derivatives carrying integrated *HIS7-lacZ* fusions. *Lower panel*, relative *HIS7* mRNA levels of strain RH1381 (wild-type) and Δ*bas1* or Δ*bas2* derivatives. The size of the *URA3* transcript is 0.9 kb, the size of the *ura3–52* transcript is 0.5 kb.

BAS1p and BAS2p account for the adenine-dependent activation of the *HIS7* gene, the *BAS1* and/or *BAS2* loci were disrupted in the *HIS7* reporter strains carrying the wild-type *HIS7* promoter and a *gcn4* null allele. GCN4p-independent activation of the *HIS7* gene under conditions of adenine limitation was abolished by a disruption of either *BAS1* (Δ*bas1*) or *BAS2* (Δ*bas2*) (Fig. 4). No additional decrease in *HIS7* transcription was observed in the case of the double mutant (Δ*bas1/2*). These results suggest that *HIS7* transcription is jointly activated by BAS1p and BAS2p under conditions of adenine limitation.

To test whether the BAS1/2p-dependent activation of the *HIS7* gene is mediated through GCRE2, the Δ*bas1* and Δ*bas2* disruptions were introduced into *HIS7-lacZ* reporter strains carrying either wild-type or mutated (gcre1, gcre2, and gcre1/2) alleles of the *HIS7* promoter and a *gcn4* null allele. *HIS7* transcription was monitored under conditions of adenine limitation. Both the Δ*bas1* and Δ*bas2* disruption strains revealed a similar GCN4p-independent *HIS7* transcription level irrespective of the promoter mutation tested (Fig. 5). This level corre-

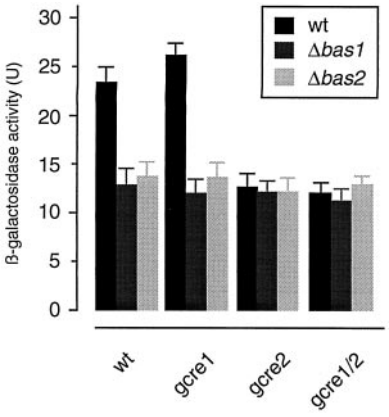


FIG. 5. **BAS1/2p-dependent activation of the *HIS7* gene through GCRE2.** *LacZ* expression directed by wild-type and mutated *HIS7* promoters (wild-type, gcre1, gcre2, and gcre1/2) was monitored in strains with a genetic *gcn4* (wild-type), *gcn4* Δ*bas1* (Δ*bas1*), or *gcn4* Δ*bas2* (Δ*bas2*) background in the absence of adenine in the growth medium.

sponded to the level observed with a promoter mutated in GCRE2 in a BAS1/2p wild-type strain under the same conditions. Taken together, our findings suggest that the *HIS7* gene is activated by the joint action of BAS1p and BAS2p through GCRE2 under conditions of adenine limitation.

BAS1p Binds to GCRE2 in the HIS7 Promoter in Vitro—To test the two GCREs in the *HIS7* promoter for GCN4p binding *in vitro*, *E. coli* produced GCN4p was assayed for binding to the same versions (wt, gcre1, gcre2, and gcre1/2) of a HIS7 promoter fragment as used for the expression studies in a gel retardation experiment (Fig. 6). GCN4p bound *in vitro* to the wild-type promoter fragment and to the promoter fragments containing point mutations either in GCRE1 or GCRE2. No binding was observed with the promoter fragment containing point mutations in both GCREs. These results indicate that GCRE1 and GCRE2 specifically bind to GCN4p *in vitro* and represent the only GCN4p-binding sites in the *HIS7* promoter.

In vitro binding of BAS1p and BAS2p to the *HIS7* promoter was tested accordingly. Protein extracts were prepared from yeast strains (gcn4–2 bas1–2 bas2–2 ura3–52) containing either a high copy number 2-μ *GAL1* promoter expression plasmid with no insert or the same plasmid with the *BAS1* or the *BAS2* open reading frame, respectively, inserted downstream of the *GAL1* promoter (8). Retarded bands were observed when BAS1p containing extracts were incubated with wild-type *HIS7* promoter fragments. No specific retarded bands were obtained either with extracts containing no BAS1p or when in GCRE2 mutated *HIS7* promoter fragments were incubated (Fig. 6). We did not observe any discrete band shifts with the BAS2 protein, suggesting that there is no specific binding of this protein on its own. Therefore we conclude that BAS1p binds *in vitro* to the GCRE2 site of the *HIS7* promoter, whereas BAS2p might primarily be recruited by protein-protein interactions.

GCN4p and BAS2-VP16p Are Able to Activate HIS7 Transcription Simultaneously through a Single GCRE2 Site—Since we could not find a specific direct interaction of BAS2p with the *HIS7* GCRE2, we were interested how substitution of BAS2p by a more potent activator would affect *HIS7* gene expression. Therefore we substituted BAS1/2p by BAS1p/BAS2-VP16p. In the construct used the weak *BAS2* activation domain was substituted by the strong *VP16* activation domain (20). The *BAS2-VP16* chimera was expressed in the presence of BAS1p in strains harboring no GCN4p or constitutively high levels of GCN4p, respectively. *HIS7* expression was monitored in strains carrying respective translational *HIS7-LacZ* fusions (Fig. 7). The strains carrying a *BAS2-VP16* chimera were compared to strains with no such chimeras cultivated in adenine containing growth medium in order to abolish adenine regulation. In the absence of GCN4p an approximately 3-fold GCRE2-dependent activation of *HIS7* expression due to the *BAS2-VP16* chimera was found. The presence of high levels of GCN4p (gcd2–1 background) lead to an approximately 9-fold activation. This activation was further increased to about 16-fold in the presence of the *BAS2-VP16* fusion construct. The results using different approaches further demonstrate that BAS2p is involved in *HIS7* regulation and support the non-exclusive manner of the activation of *HIS7* transcription by GCN4p and BAS1/2p through GCRE2.

DISCUSSION

The hexanucleotide sequence 5′-TGACTC-3′ was shown to be the binding site for GCN4p (6, 2). In addition, this motif can be recognized by other transcription factors, presumably depending on the sequence context (4, 28). Here, we provide genetic evidence that the same 5′-TGACTC-3′ motif in the *HIS7* promoter is a genetic crossing point of two independent metabolic pathways: amino acid biosynthesis and purine biosynthesis. Accordingly, this motif can be recognized by two different transcriptional activators GCN4p and BAS1/2p in a non-exclusive manner *in vivo*.

In the *HIS7* promoter two GCREs are present. Both GCREs contribute synergistically to the maximal activation of the *HIS7* gene by GCN4p under conditions of amino acid starvation. Besides its role in GCN4p-dependent activation of the *HIS7* gene under amino acid starvation conditions, GCRE2 is the *cis*-acting element of an additional activation of the *HIS7* gene which is repressed by addition of exogenous adenine to the growth medium and requires both the *BAS1* and *BAS2* gene products. This kind of BAS1/2p-dependent adenine repression has also been found for the histidine biosynthetic gene *HIS4* (8) and for the *ADE1*, *ADE2*, *ADE5/7*, and *ADE8* genes involved in *de novo* purine biosynthesis (4, 5). In all these cases, however, BAS1p and BAS2p contribute also to the basal transcription level under adenine repressed conditions. By contrast, *HIS7* transcription requires the Bas1-Bas2p complex only for adenine starvation-dependent transcriptional activation and not for the basal, GCN4p-independent transcription in the presence of adenine.

BAS1/2p-dependent transcriptional activation connects *de novo* histidine and purine biosynthesis on a regulatory level. The *HIS4*, *HIS7*, *ADE4*, and *ADE6* genes have been shown to be repressed by adenine (3, 8, 20, 29). In addition to this cross-pathway regulation, it has been demonstrated that purine biosynthetic genes, including the *ADE4* gene, are also subject to the general control system of amino acid biosynthesis (3–5).

A likely physiological rationale for this 2-fold regulation of the *HIS7* gene is that the reaction which is catalyzed by the *HIS7* gene product results as well in an intermediate of histidine biosynthesis as in an intermediate of adenine biosynthesis (Fig. 1). Both Bas1/2p regulated histidine biosynthetic genes *HIS4* and *HIS7* code for multifunctional enzymes which catalyze steps in the histidine biosynthetic pathway upstream of this metabolic branch point. The *HIS3* gene is not regulated by Bas1/2p and its gene product catalyzes a reaction downstream of this branch point (Fig. 1).

BAS2p, also known as PHO2p or GRF10p (23, 31), has been implicated in diverse transcriptional regulatory mechanisms besides adenine repression. It is also required for phosphate regulation of the *PHO5* gene involved in phosphate metabolism (9, 31), the *TRP4* gene involved in tryptophan biosynthesis (10),

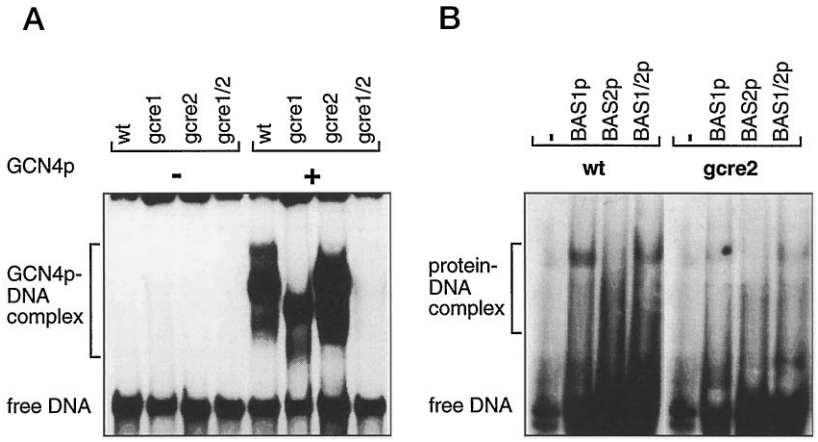


FIG. 6. ***In vitro* binding of GCN4p, BAS1p, and BAS2p to the *HIS7* promoter.** *A, in vitro* binding of GCN4p to the *HIS7* promoter. A gel retardation assay was used to demonstrate *in vitro* binding of GCN4p to the *HIS7* promoter. GCN4p purified from *E. coli* was incubated with different versions of a ^{32}P-end-labeled 379-bp *Bsa*HI/*Eco*RV fragment comprising the whole *HIS7* promoter region including the end of the *ARO4* open reading frame (Fig. 2). The fragments used as DNA probes were either wild-type or carried point mutations in GCRE1 (gcre1), GCRE2 (gcre2), or both GCREs (gcre1/2). *B, in vitro* binding of BAS1/2p to the *HIS7* promoter. A gel retardation assay was used to demonstrate *in vitro* binding of BAS1p and BAS2p to the *HIS7* promoter. Protein crude extracts from yeast strains overexpressing BAS1p or BAS2p or from strains expressing no BAS1/2p were incubated with different versions of a ^{32}P-end-labeled synthetic 70-bp DNA fragment comprising the *HIS7* promoter between position −186 and −116 relative to the *HIS7* start codon including the GCRE2. The fragments used as DNA probes were either wild-type (wt) or carried point mutations in GCRE2 (gcre2).

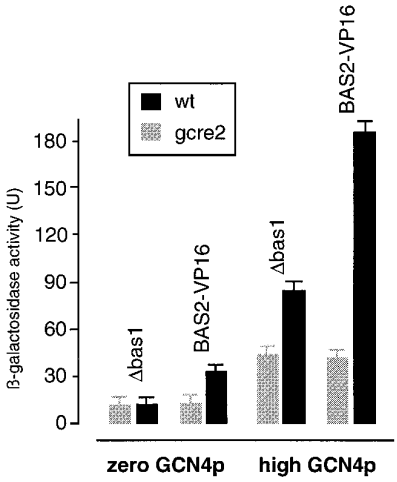


FIG. 7. **Simultaneous activation of the *HIS7* gene by GCN4p and BAS1/2p.** β-Galactosidase activities of strains carrying a wild-type (*wt*) or an in GCRE2 (*gcre2*) mutated *HIS7* promoter allele in either a Δ*bas1* background or in cells expressing the BAS2-VP16p fusion protein was measured. The GCN4p level was either zero or high.

and the *HIS4* gene (independently of BAS1) (7). A phosphate-dependent regulation could not be demonstrated in case of the *HIS7* gene. In addition, BAS2p is required for transcriptional regulation of the *HO* gene (11). As in the case of BAS1/2p-dependent adenine repression, BAS2p usually requires a second DNA-binding protein such as PHO4p for phosphate regulation and SWI5p for *HO* regulation to fulfill its different regulatory functions. Since BAS2p binding to *HIS7* GCRE2 seems to be unspecific, the role of protein-protein interaction and heterodimer formation with BAS1p for BAS2p function remains to be elucidated.

For *HIS4*, *ADE2*, and *ADE5/7* the 5′-TGACTC-3′ motif has been shown to be part of the BAS1p-binding site *in vitro* (8, 4). Similarly to the *HIS7* promoter, a mutation of this motif in one of the two BAS1p-binding sites present in the *ADE2* promoter abolished joint activation by BAS1p and BAS2p, whereas an analogous mutation in the other binding site had no effect (4). Comparison of BAS2p-protected sequences on *HIS4* (8), *TRP4* (10), and *PHO5* (9) promoters did not reveal a clear consensus sequence. In the *HIS4* promoter, however, BAS2p binds to a site adjacent to the BAS1p-binding site. The requirement for an adjacent BAS2p-binding site would explain why not every 5′-TGACTC-3′ motif confers regulation by BAS1p and BAS2p. The fact that BAS2p does not seem to bind to *HIS7* GCRE2 on its own suggests that binding of BAS1p to DNA or protein-protein interaction between BAS1p and BAS2p might be required for BAS2p to fulfill its function.

A major finding of this work is that both GCN4p and BAS1/2p are required for maximal activation of the *HIS7* gene under simultaneous amino acid starvation and adenine limitation conditions. The two transcriptional activators seem to be able to act through the same DNA site in a non-exclusive manner. In addition, it is possible to replace the weak BAS1/2p activator by the strong BAS1p/BAS2p-VP16p activator without interfering with GCN4p activation at the GCRE2 site. It remains to be elucidated whether the observed additive effect is due to a simultaneous interaction of GCN4p and BAS1/2p with the same DNA sequence or whether binding of BAS1p/2p and GCN4 is adjacent to each other but so close together that binding of all factors can be abolished by a single point mutation. Alternatively, the initiation frequency of *HIS7* transcription could also be increased in an additive manner by alternating binding of GCN4p and BAS1/2p to the GCRE2.

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