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, aminocyl-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 HIS4 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-aminomimidazole-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 (MATa gal2 SUC2
The three yeast strains RH1371 (aro3-2 Δura3 gcy2-1), RH1381 (aro3-2 Δura3 gcy2-1), and RH1382 (aro3-9 Δura3 gcy2-1) were used. Cultivation of yeast was performed at 30 °C in either YEPD complete medium or YP minimal medium (16) with recommended amounts of supplements (15). E. coli was grown at 37 °C in LB complete medium (17) containing 50 μg/ml of ampicillin when selecting for transformants.

Plasmids—Plasmid pME694 containing a (wild-type) 1.9-kb Spkl-BamHI HIS7 fragment was described previously (12). Plasmid pME696 carrying an integrative HIS7-lacl fusion was a derivative of pME694 (12). Plasmid AB243 containing a 12-kb BAS1 fragment was described earlier (7).

DNA Techniques—Enzymatic manipulation and cloning of DNA were performed as described by Sambrook (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 mutagenesis 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′-GCATATATGATATATCAGTAGTC-3′) for mutating the GCN4p-binding site GCRE1 to gcre1 and che3 (5′-TGATGTGTCTAGTTTTTCATCA-3′) for mutating gcre2 to gcre2. These oligonucleotides were used as primers in a first PCR reaction together with MUTH7 (5′-GGCAATTCAATGCAATTG-3′) or che5 (5′-TTGAGAGTGTAACCTACAGTCACTAACCAATGCAATTG-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 and with PfuTurbo primer extension resulting in a PCR product was used as primer in a Styl and EcoRI digestion reaction in the absence of the corresponding wild-type HIS7 fusion 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-lacl 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-lacl Reporter Strains—HIS7-lacl fusion constructs pME698 (gcre1), pME699 (gcre2), and pME700 (gcre1/2) were integrated at the original HIS7 locus of yeast strains RH1371 (gcy4'), RH1381 (gcy4''), and RH1382 (gcy4''), yielding strains RH1617 (gcy4', gcre1), RH1618 (wt, gcre1), RH1619 (gcy4', gcre2), RH1620 (gcy4'', gcre2), RH1621 (wt, gcre2), RH1622 (gcy4', gcre2), RH1623 (gcy4'', gcre1/ 2), RH1624 (wt, gcre1/2), and RH1625 (gcy4', gcre1/2). The procedure was described previously for the wild-type HIS7-lacl construct pME696 resulting in strains RH1614, RH1615, and 1616, respectively (12). Strains harboring constitutively high amounts of GCN4p independently of the GCDS 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 (gcy4'') by gene replacement. The plasmids were cut with SpAI and BamHI and transformed into the same strain URA3 disruption strains as used for the integration of the HIS7-lacl fusion constructs (12). Aro+ transformants were selected for their His+ phenotype and by Southern blot analysis. This procedure yielded strains RH1545 (gcy4', gcre1), RH1565 (gcy4', gcre2), and RH1566 (gcy4'', 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 HindIII pho2-URA3 fragment of pCS21 (23), were used to disrupt the BAS1 and BAS2 genes, respectively, in the HIS7-lacl 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 SacI BAS1 fragment from AB243 was recloned on pUC18 (Ciontech Laboratories, Palo Alto, CA) yielding plasmid pME1165. Plasmid pME1167 was constructed by replacing a 2.2-kb BglII/SpeI BAS1 fragment in pME1165 by a flushed chromosomal 1.1-kb HindIII 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 activity in 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 (OD546) in minimal medium without or with 0.3 mM adenine, and cultured for another 6 h before the β-galactosidase activity is defined as 1 nmol of 4-methylumbellifereone h⁻¹ ml⁻¹ OD546⁻¹. 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 [32P]-labeled probes prepared from a chromosomal 0.9-kb BamHI-XbaI HIS7 fragment and a 1.1-kb HindIII URA3 fragment using the oligolabeling technique described by Feinberg and Vogelstein (26). The endogenous ura3-52 and ura3-13 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 32P-end-labeled 379-bp BamHI/EcoRV 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 pro-tein extracts from yeast strains overexpressing BAS1p, BAS2p, or both was described previously by Tice-Baldwin et al. (8). Different versions of a synthetic 32P-end-labeled 70-bp DNA fragment comprising the HIS7 promoter between position 186 and 116 relative to the HIS7 start codon including the GCRC2 were used as DNA probes. The fragments were either wild-type (wt) or carried point mutations in GCRC2 (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-lacl 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 translational 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 the 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 1The abbreviations used are: kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction.
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 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 Sura3 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 Δgcd4-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. 4A). 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...
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 gcna null allele. GCN4p-independent activation of the HIS7 gene under conditions of adenine limitation was abolished by a disruption of either BAS1 (Δbas1) or BAS2 (Δbas2) (Fig. 4). No additional decrease in HIS7 transcription was observed in the case of the double mutant (Δbas1/2). 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 GCN4p, the Δbas1 and Δbas2 disruptions were introduced into HIS7-lacZ reporter strains carrying either wild-type or mutated (gcna1, gcna2, and gcna1/2) alleles of the HIS7 promoter and a gcna null allele. HIS7 transcription was monitored under conditions of adenine limitation. Both the Δbas1 and Δbas2 disruption strains revealed a similar GCN4p-independent HIS7 transcription level irrespective of the promoter mutation tested (Fig. 5). This level corre-
Figure 5. BAS1p-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 gcen4 (wild-type), gcen4Δbas1 (Δbas1), or gcen4Δbas2 (Δbas2) 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 and 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 and GCRE2.

In vitro binding of BAS1p and BAS2p to the HIS7 promoter was tested accordingly. Protein extracts were prepared from yeast strains (gcen4Δbas1Δbas2 strain3–5) 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-VP16 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 BAS1p with 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 ablate 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).
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/BAS2p 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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