Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6746–6750, August 1992
Genetics

Coregulation of purine and histidine biosynthesis by the transcriptional activators BAS1 and BAS2

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Contributed by G. R. Fink, April 20, 1992

ABSTRACT **We have found cross-pathway regulation between purine and histidine biosynthesis in yeast. The transcription factors BAS1 and BAS2/PHO2, which are also regulators of the histidine pathway, participate in the regulation of the purine biosynthetic pathway. Analysis of four genes of the purine pathway (*ADE1*, *ADE2*, *ADE5,7*, and *ADE8*) shows that their expression is repressed by adenine. The maximal basal and induced expression of these purine genes requires the presence of both BAS1 and BAS2. The factor BAS1 has been shown to bind at a site containing the TGACTC hexanucleotide motif in the *ADE2* and *ADE5,7* promoters. This motif is required for both basal and induced activation of the *ADE2* gene by BAS1 and BAS2.**

In *Saccharomyces cerevisiae*, mutations in any one of 10 genes lead to a purine requirement. Eight of these are structural genes encoding enzymes directly required for catalysis of the sequential steps in the *de novo* pathway of purine biosynthesis (see ref. 1 for review). Two loci, *ADE3* and *ASP5*, encode functions required for the synthesis of cosubstrates necessary for reactions in purine biosynthesis.

Mutations in the genes *BAS1* and *BAS2* cause an adenine requirement (2). BAS1 and BAS2 are transcriptional activators identified as basal regulators of *HIS4*. BAS2 (also known as PHO2) activates the expression of the *PHO5* gene together with PHO4 (3). As BAS1 and BAS2 are transcriptional activators, the purine requirement associated with mutations in these genes seemed likely to arise as a consequence of a defect in expression of purine-pathway genes. The possibility of an interconnection between purine and histidine regulation was also suggested by the observation that adenine represses the stimulation of *HIS4* transcription by BAS1 and BAS2 (4). One further connection between adenine and histidine regulation arises from the observation that GCN4, a known transcriptional activator of histidine biosynthetic genes, is required for maximal induction of the *ADE4* gene (5).

In this paper we show that four genes of the *de novo* purine pathway are regulated directly by the transcriptional activators BAS1 and BAS2.

MATERIALS AND METHODS

Yeast Media. SD is 2% glucose/0.5% ammonium sulfate/0.17% yeast nitrogen base. SC is SD supplemented with all amino acids and uracil (6). Adenine was optionally added to SC and SD to a final concentration of 0.3 mM.

Yeast. Strains used were PLY122 (*MAT***a**, *ura3-52*, *lys2Δ201*, *leu2-3,112*), L4224 (*MATα*, *ura3-52 leu2Δ1*, *gcn4-2*, *bas2-2*), L4229 (*MATα*, *ura3-52*, *leu2Δ1*, *gcn4-2*, *bas1-2*), L3079 (*MAT***a**, *ura3-52*, *gcn4-2*), L3080 (*MAT***a**, *ura3-52*, *gcn4-2*, *bas1-2*), and L3081 (*MAT***a**, *ura3-52*, *gcn4-2*, *bas2-2*).

Plasmids. The *lacZ* fusions were constructed as follows in the plasmid vectors described by Myers *et al.* (7). To construct *ADE1–lacZ*, a 1600-base-pair (bp) *Nsi* I–*Xba* I restriction fragment starting 900 bp upstream from the ATG initiation codon of the *ADE1* gene (8) was cloned in YEp356R. For *ADE2–lacZ*, a 680-bp *Hin*dIII–*Eco*RV restriction fragment starting 505 bp upstream from the ATG of the *ADE2* gene (9) was cloned in both multicopy (YEp368R) and integrative (YIp368R) vectors. For *ADE3–lacZ*, a 550-bp *Bam*HI–*Sph* I restriction fragment starting 516 bp upstream from the ATG of the *ADE3* gene (10) was cloned in YEp367. For *ADE5,7–lacZ*, a 900-bp *Bam*HI–*Hin*dIII restriction fragment starting 777 bp upstream from the ATG of the *ADE5,7* gene (11) was cloned in YEp367R. For *ADE8–lacZ*, a 2000-bp *Bam*HI–*Xho* I restriction fragment starting 1700 bp upstream from the ATG of the *ADE8* gene (12) was cloned in YEp367 and YIp367. B1389 is a centromeric plasmid carrying a *HIS4–lacZ* fusion derived from pFN8 (13). *ADE2–lacZ* and *ADE8–lacZ* were integrated in yeast strains L4224 and L4229 after linearization, respectively, at the single *Xba* I and *Nsi* I sites in the promoter sequence.

β-Galactosidase Assays. Strains containing *lacZ* fusions were grown overnight in SC medium lacking leucine and/or uracil and then diluted in the desired medium and grown for 6 hr at 30°C. All the assays were performed on exponentially growing cells. Cells were suspended in 1 ml of Z buffer (60 mM Na_2HPO_4/40 mM NaH_2PO_4/10 mM KCl/1 mM $MgSO_4$) and, after the suspension was vigorously shaken with 0.1 ml of chloroform, 0.2 ml of a 4-mg/ml solution of *o*-nitrophenyl β-D-galactopyranoside was added. After incubation at 28°C until the color developed, the reaction was stopped by addition of 0.5 ml of 1 M Na_2CO_3. After a 10-sec centrifugation in an Eppendorf microcentrifuge, units of β-galactosidase activity were calculated by the formula (1000 × reaction mix OD_{420})/(culture OD_{600} × vol of culture × min of assay). Each value is the average of two to six assays on independent transformants. Variation between assays was <20%.

***In Vitro* DNA-Binding Assays.** BAS1 and BAS2 protein extract preparation, gel retardation, and DNase I protection experiments were performed as described (4). For the DNase I protection experiment (see Fig. 1), the amount of BAS1 added in each reaction is given in arbitrary units, where 1 unit corresponds to 1 μl of heparin-agarose-purified BAS1. In each reaction mix, a sample of control extract (prepared from *Escherichia coli* without a BAS1 plasmid) was added to the BAS1 extract to make a final volume of 50 μl. The complementary oligonucleotides used for gel retardation in Fig. 2 are the following: ADE8 promoter, coding strand, 5′-GGATTC-GAATGAGTGACTCGTTGCTAGTGACTCTGACCT-3′; *ADE8* promoter, noncoding strand, 5′-AGGTCAGAGT-CACTAGCAACGAGTCACTCATTCGAATCC-3′. Oligonucleotides used as probes in Fig. 5 are the following: probe A, 5′-GCTGACAAATGAATTCTTGTTGCATGGC-3′

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Abbreviation: PRPP, phosphoribosyl pyrophosphate.

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Table 1. Effect of adenine (Ade) concentration on expression of various *ADE–lacZ* fusions carried on multicopy plasmids in the wild-type yeast strain PLY122

Construct	β-Galactosidase units*		Repression factor
	No Ade	0.3 mM Ade	
ADE1–lacZ	286	16	17.9
ADE2–lacZ	195	57	3.4
ADE3–lacZ	371	300	1.2
ADE5,7–lacZ	114	31	3.7
ADE8–lacZ	105	32	3.3

*Calculated as described in *Materials and Methods*.

(coding strand) and 5′-GCCATGCAACAAGAATTCATTTGTCAGC-3′ (noncoding strand); probe B, 5′-GCTGACAAATGACTCTTGTTGCATGGC-3′ (coding strand) and 5′-GCCATGCAACAAGAGTCATTTGTCAGC-3′ (noncoding strand); probe C, 5′-CTAAGTGATTGAATTCTTGCTGACC-3′ (coding strand) and 5′-GGTCAGCAAGAATTCAATCACTTAG-3′ (noncoding strand); probe D, 5′-CTAAGTGATTGACTCTTGCTGACC-3′ (coding strand) and 5′-GGTCAGCAAGAGTCAATCACTTAG-3′ (noncoding strand).

Oligonucleotide-Directed Mutagenesis. *In vitro* site-directed mutagenesis of the *ADE2* promoter was done with a Bio-Rad Mutagene kit. The oligonucleotides used for the mutagenesis have the following nucleotide sequences: distal, 5′-GCTGACAAATGAATTCTTGTTGCATGGC-3′; proximal, 5′-CTAAGTGATTGAATTCTTGCTGACC-3′.

RESULTS

Genes of the *de Novo* Purine Biosynthesis Pathway Are Repressed by Adenine. Regulation of the purine pathway was analyzed using protein fusions in which the promoter and amino-terminal coding sequence of each of the purine biosynthetic genes examined (*ADE1*, *ADE2*, *ADE5,7*, and *ADE8*) was fused to the *E. coli lacZ* gene. Expression of the fusion protein was assayed in the presence and in the absence of adenine in the growth medium (similar results were obtained with SD and SC media). Strains grown in the absence of adenine express all four purine genes at levels 3–18 times higher than those obtained when the same strains are grown on medium containing adenine (Table 1). The growth rates of strains are the same with or without adenine. The specificity of this regulatory response is shown by the failure of the *ADE3–lacZ* fusion to be repressed when similar concentrations of adenine are added to the culture medium. The ADE3 enzyme, C1-5,6,7,8-tetrahydrofolate synthase (1, 10), is required for purine biosynthesis but is not an enzyme of the *de novo* pathway.

BAS1 and BAS2 Are Required for Regulation of *ADE1*, *ADE2*, *ADE5,7*, and *ADE8*. The effect of mutations in *BAS1* and *BAS2* on expression of purine genes was tested using strains containing either a *bas1* or *bas2* null allele (Table 2) and grown in the presence or absence of adenine. The four gene fusions shown previously to be expressed at higher levels in the absence of adenine, *ADE1*, *ADE2*, *ADE5,7*, and *ADE8*, require both BAS1 and BAS2 for maximal expression whether or not adenine is present in the medium. However, the stimulation of expression by BAS1 and BAS2 is much greater in the absence of adenine. In the absence of either BAS1 or BAS2, these purine gene fusions are still expressed at a low level (Table 2). The expression of *ADE2–lacZ* is the same in the double *bas1 bas2* mutant as it is in either single *bas1* or *bas2* mutant (data not shown), suggesting that stimulation of purine gene expression requires the combined function of both activators.

BAS1 and BAS2 Bind to the Promoter of Purine-Pathway Genes *in Vitro*. Interactions between BAS1, BAS2, and the promoters of some of the purine biosynthetic pathway genes were analyzed by following DNA–protein binding *in vitro*. BAS1 and BAS2, expressed in *E. coli* using the expression vectors described by Tice *et al.* (4), were partially purified on heparin-agarose. *DNase* I protection experiments (Fig. 1) revealed two BAS1 binding sites in the promoter regions of both *ADE2* and *ADE5,7* (Fig. 1). BAS1 also binds to the *ADE8* promoter as shown by a gel retardation assay using a 39-bp oligonucleotide representing sequence from −156 to −194 relative to the ATG (Fig. 2).

Using the same DNase I protection procedures, we were unable to detect BAS2 binding to those segments of the *ADE2* and *ADE5,7* promoters that showed clear evidence for binding by BAS1. Nevertheless, binding of BAS2 to *ADE2* and *ADE5,7* promoter regions was shown by gel retardation experiments (Fig. 3). The specificity of BAS2 binding was shown by competition experiments with unlabeled DNA (data not shown).

Consensus Binding Site for BAS1 Contains a TGACTC Motif Essential for *in Vivo* Activation. BAS1 binding sites have been identified by *DNase* I protection analysis on *HIS4* (4) as well as *ADE2* and *ADE5,7* promoters (two in each gene, Fig. 1*B*). A sequence comparison of these BAS1 binding sites (Fig. 4*A*) shows that all six contain a common 5′-TGACTC-3′ sequence. A search for this TGACTC motif in other purine

Table 2. Effect of BAS1 and BAS2 on expression of the genes of the purine biosynthetic pathway

Construct*	*BAS1/bas1*		*BAS2/bas2*	
	No Ade	0.3 mM Ade	No Ade	0.3 mM Ade
ADE1–lacZ (M)	33.5 (8)	8.3 (3)	26.8 (10)	3.6 (7)
ADE2–lacZ (I)	5.6 (0.7)	4.3 (0.3)	5.4 (0.7)	1.9 (0.7)
ADE3–lacZ (M)	0.9 (309)	0.8 (219)	0.6 (435)	0.8 (298)
ADE5,7–lacZ (M)	3.9 (27)	1.4 (25)	5.3 (31)	3.9 (24)
ADE8–lacZ (I)	7.5 (0.2)	3.0 (0.1)	5.7 (0.3)	3.5 (0.2)

β-Galactosidase assays were done on cells grown under repression (0.3 mM adenine and derepression (no adenine) conditions. Results are presented as the ratio of β-galactosidase units in the wild-type strain to β-galactosidase units in the isogenic mutant strain (*bas1* or *bas2*). The units of β-galactosidase activity in the *bas1-2* strain (left columns) or *bas2-2* strain (right columns) are indicated in parentheses. The plasmid carrying the *ADE1–lacZ* fusion was introduced by transformation into a set of *BAS1 BAS2*, *bas1 BAS2*, and *BAS1 bas2* isogenic strains (L3079, L3080, and L3081, respectively). All other *ADE–lacZ* fusions were introduced into the yeast strains L4229 (*bas1 BAS2*) and L4224 (*BAS1 bas2*). The L4229 (*ADE–lacZ*) derivatives were then transformed with a centromeric plasmid carrying the *BAS1* gene and a control plasmid, leading to isogenic *BAS1* and *bas1* strains carrying the *lacZ* fusions. Similarly, *BAS2* and *bas2* strains were obtained by transformation of L4224(*ADE–lacZ*) derivatives with a centromeric plasmid carrying the *BAS2* gene and a control plasmid.
*Integrated (I) or multicopy (M) plasmids, as indicated.

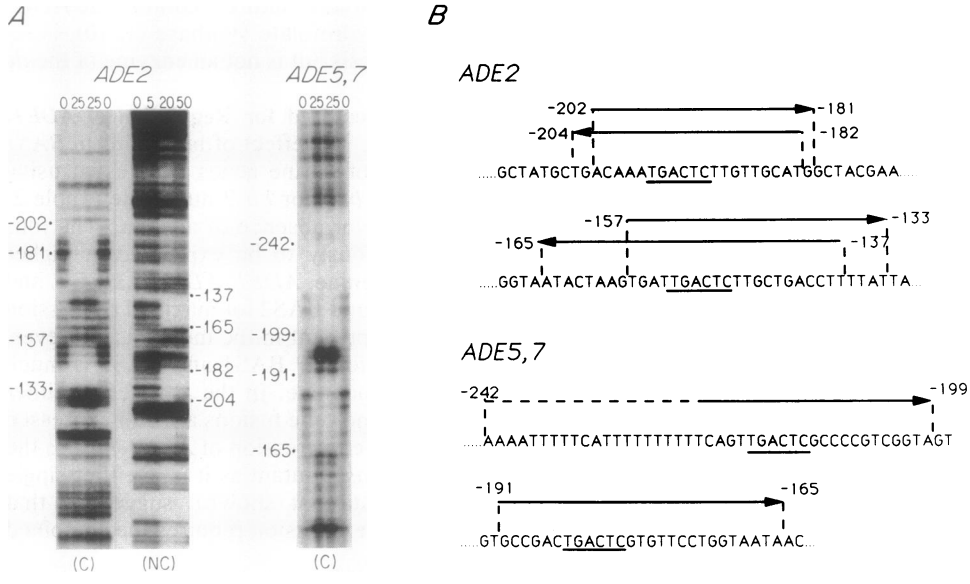

FIG. 1. *ADE2* and *ADE5,7* sequences bound by BAS1. Negative numbers refer to the position relative to the A of the ATG codon (position +1). (*A*) *DNase* I protection analysis of BAS1 binding to *ADE2* and *ADE5,7* promoters. Above each lane, the amount (expressed in arbitrary units) of heparin-agarose-purified BAS1 used in each reaction is indicated. At the bottom, C and NC stand for coding and noncoding strands, respectively, and refer to the ^{32}P-labeled strand in each experiment. (*B*) Nucleotide sequence of the protected regions. Only the coding strand is represented. Solid-line arrows show the regions protected by BAS1. Dashed line shows a large region with no DNase I cleavage site, allowing no accurate identification of the limit of the protected sequence. The TGACTC consensus sequence is underlined.

genes (Fig. 4*B*) uncovered one in *ADE1* (14) and two in *ADE8* (12). Both of these genes require BAS1 for activation (Table 2). The 39-bp region of the *ADE8* promoter where BAS1 binds *in vitro* (see previous section) contains these two TGACTC sequences. A single copy of the TGACTC sequence was also found upstream of the *ADE4* gene, which is known to be repressed by adenine (15).

The effect of the mutant TGACTC motif on regulation was shown by replacing the wild-type *ADE2* TGACTC sequences with TGAATTC mutant sequences. Gel retardation assays using wild-type and mutant oligonucleotides showed that BAS1 did not bind to the mutant binding sites (Fig. 5).

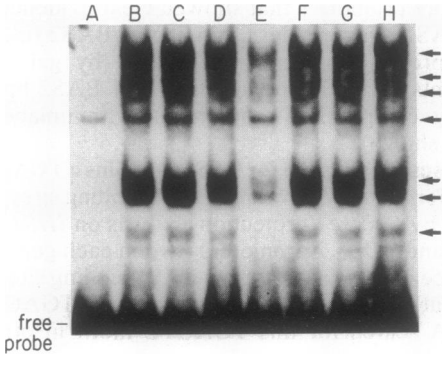

FIG. 2. Binding of BAS1 to the *ADE8* promoter assayed by gel retardation. The probe was a 39-bp double-stranded oligonucleotide spanning the region between nucleotides −156 and −194 in the *ADE8* promoter (*Materials and Methods*). Protein extracts were made from a *bas1-2 bas2-2* yeast strain carrying either a plasmid where BAS1 expression was under control of the GAL1 inducible promoter or a control plasmid (4). The protein concentration of the BAS1 and control extracts was adjusted to 1 mg/ml. Five microliters of control extract was added to the probe in lane A and 5 μl of BAS1 extract was added in each of the other lanes. Unlabeled oligonucleotides, either BAS1 binding competitor (lanes C, D, and E) or random noncompetitor (lanes F, G, and H), were added to the labeled probe in a molar ratio of 1:10, 1:1, or 10:1, from left to right. The competitor oligonucleotide used in lanes C, D, and E was a 24-bp double-stranded oligonucleotide carrying the BAS1 binding site as defined by DNase I protection on the *HIS4* promoter (4). The multiple retardation complexes shown by arrows may correspond to the binding of proteolytic fragments of BAS1 or/and to the binding of more than one BAS1 molecule to the probe. An equally complex pattern was obtained with BAS1 protein purified from *E. coli*. The retardation complex in the control lane A is due to binding of an unidentified protein to the probe.

Constructs were made in which either of the two or both TGACTC sequences were replaced by this mutant sequence. These promoter mutations were tested *in vivo* by replacing the wild-type *ADE2* promoter of an *ADE2–lacZ* construct with mutant versions that contained the TGAATTC sequence and transforming these constructs into yeast (Table 3). Mutation of the distal BAS1 binding site has little effect on *ADE2–lacZ* expression, whereas mutagenesis of the proximal binding site (or both distal and proximal sites) reduces basal expression and abolishes induced expression in the absence of adenine. The effects of these cis-acting mutations further demonstrate the requirement of BAS1 and BAS2 for adenine regulation. Although there are two BAS1 binding sites in the *ADE2* promoter, only one appears to be required for activation in our assay. However, the presence of two sites may be important for some aspect of regulation because at least two other genes, *ADE5,7* and *HIS4* (4), contain multiple BAS1 binding sites.

DISCUSSION

We have shown that the expression of 5 of the 10 enzymes catalyzing synthesis of inosine monophosphate (IMP) from phosphoribosyl pyrophosphate (PRPP) is repressed by ade-

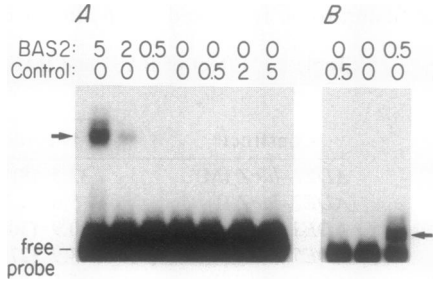

FIG. 3. Binding of BAS2 to *ADE2* and *ADE5,7* promoters assayed by gel retardation. BAS2 was obtained in *E. coli* from an overproducing plasmid and then partially purified on heparin-agarose (4). The control is an extract from the same *E. coli* strain without the BAS2 plasmid. The amounts (μl) of BAS2 and control extracts used in each reaction are shown above the corresponding lane. In each panel the DNA–protein complex is indicated by an arrow. (*A*) Binding of BAS2 to *ADE2* promoter. The probe was a 49-bp double-stranded oligonucleotide spanning the nucleotides −148 to −196 upstream of the A of the ATG start codon. (*B*) Binding of BAS2 to *ADE5,7* promoter. The probe was a 590-bp *Pst* I–*Spe* I restriction fragment containing the whole promoter region.

	5'		3'	
A	TCGAAC	TGACTC	TAATAGTGAC	*HIS4* A
	TAATAG	TGACTC	CGGTAAATTA	*HIS4* B
	GACAAA	TGACTC	TTGTTGCAGG	*ADE2* DISTAL
	AGTGAT	TGACTC	TTGCTGACCT	*ADE2* PROXIMAL
	TTCAGT	TGACTC	GCCCCGTCGG	*ADE5,7* DISTAL
	GCCGAC	TGACTC	GTGTCCTGGT	*ADE5,7* PROXIMAL
B				
	TCAGTC	TGACTC	TTGCGAGAGA	*ADE1*
	ATGTAT	TGACTC	TTCCTGACCG	*ADE4*
	AATGAG	TGACTC	GTTGCTAGTG	*ADE8* DISTAL
	TGCTAG	TGACTC	TGACCTGTTT	*ADE8* PROXIMAL

FIG. 4. Sequence alignment of BAS1 binding sites. (*A*) BAS1 binding sites identified by *DNase* I protection. *HIS4* promoter protection by BAS1 is described in ref. 4; *ADE2* and *ADE5,7* promoter protection are described in this work. (*B*) Potential BAS1 binding sites derived from sequence comparison analysis of *ADE1*, *ADE4*, and *ADE8* promoters (5, 12, 14).

nine. This regulation requires the transcription factors BAS1 and BAS2. However, even in the presence of adenine, BAS1 and BAS2 participate in the expression of the purine biosynthetic genes. BAS1 binds directly to the *ADE2*, *ADE5,7*, and *ADE8* promoters. The hexanucleotide sequence TGACTC is part of the BAS1 binding site. BAS2 binds to both *ADE2* and *ADE5,7* promoter regions as shown by gel retardation analysis, but we could not demonstrate binding of BAS2 to these promoters by *DNase* I protection experiments. Mutations in the TGACTC sequences in the *ADE2* promoter abolish BAS1 binding and affect both basal and induced expression of this gene. We conclude that BAS1 and probably BAS2 participate in the basal and induced expression of all the purine genes we have studied, through a direct interaction at the promoter region.

As in the case of *HIS4* expression (2), both BAS1 and BAS2 are necessary for activation of the purine genes. Therefore, the TGACTC sequence alone may not be sufficient as an upstream activating sequence unless a BAS2 binding site is also present. The requirement for both BAS1 and BAS2 binding sites might explain why not all the genes carrying the TGACTC consensus are regulated by BAS1 and BAS2. Comparison of BAS2-protected sequences on the *HIS4* (4), *TRP4* (16), and *PHO5* (3) promoters does not reveal a clear consensus sequence. Two other genes in the pathway, *ADE4* and *ADE6*, have been shown to be regulated at the transcriptional level by adenine (15, 17). Since the *ADE4* promoter contains the TGACTC motif that we have identified as part of the consensus sequence for BAS1 binding to DNA,

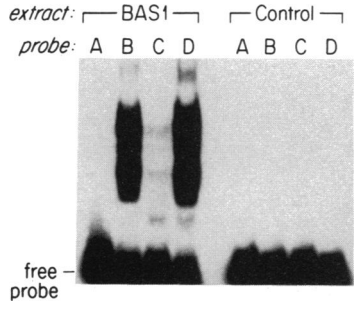

FIG. 5. Binding of BAS1 to wild-type and mutant *ADE2* promoter sequences. The BAS1 extract was the same extract as that used for DNase I protection experiments. Three microliters of BAS1 or control extract was used in each reaction. The probes were mutant (A) and wild-type (B) distal BAS1 binding site and mutant (C) and wild-type (D) proximal binding site. Nucleotide sequences of the probes are given in *Materials and Methods*.

Table 3. β-Galactosidase activity of *ADE2–lacZ* fusions carrying various combinations of wild-type and mutant BAS1 binding sites in the promoter region

BAS1 binding site		No Ade		0.3 mM Ade	
Distal	Proximal	Units	%	Units	%
Wild type	Wild type	195	100	28	100
Mutant	Wild type	156	80	32	114
Wild type	Mutant	28	14	20	71
Mutant	Mutant	14	7	13	46

Activity is given in units and as percent of wild-type activity.

it is likely that *ADE4* will also be regulated by BAS1 and BAS2. The sequence of *ADE6* is unpublished.

The requirement of this TGACTC sequence for the binding of another transcription factor, GCN4, and its presence in many GCN4-regulated genes (18) raised the possibility that GCN4 might participate in the regulation of the adenine pathway. Such an activation of a purine gene by GCN4 has been described for *ADE4* (5). To examine any effect of GCN4 on purine regulation, we tested the five *ADE–lacZ* fusions previously described under conditions of repression and derepression for GCN4. GCN4-dependent derepression of a control *HIS4–lacZ* fusion was 5-fold, whereas under the same conditions, GCN4-dependent derepression of *ADE–lacZ* fusions was 1.5- to 2-fold (data not shown). This slight activation of purine genes by GCN4 was observed only when adenine was added to the growth medium, a situation in which BAS1 and BAS2 do not fully activate transcription. Since it is known that GCN4 can bind *in vitro* to the TGACTC sequences that are part of the BAS1 binding sites in the *HIS4* promoter (18), one can speculate that the activation of the purine genes by GCN4 might reflect a competition between GCN4 and BAS1 for binding to a DNA sequence recognized by both proteins. Similarly, competition has been demonstrated between GCN4 and BAS2 for binding to the *TRP4* promoter *in vitro* (16). Whether this competition plays a physiologically important role *in vivo* remains to be determined. It should be pointed out that the optimal sequence for GCN4 is TGACTCA. This TGACTCA is not found in the BAS1 binding sites, where the nucleotide following the TGACTC motif is either T, C, or G (Fig. 4).

BAS1 and BAS2 participate in both the basal and the induced expression of the purine genes. Nevertheless, in the absence of BAS1 or BAS2 the *ADE* genes are still expressed at a low level. This BAS-independent expression probably explains the observation that strains containing either a *bas1* or a *bas2* mutation are not complete auxotrophs and grow slowly in the absence of adenine. The residual expression in the *bas1* strains appears to be repressed by adenine in some genes (*ADE1*, *ADE2*, *ADE8*) (Table 2). As this effect is not found or is much reduced in the *bas2* strains, the repression could be mediated by BAS2 alone, since BAS2 can bind to DNA independently of BAS1.

Activation of the purine genes by BAS1 and BAS2 is more efficient in the absence of adenine. Either the synthesis, the stability, or the function of BAS1 and/or BAS2 could be modified by the presence of adenine in the medium. Expression of *BAS1* (4) and *BAS2* (data not shown) has been shown to be unaffected by the adenine concentration in the growth medium. It is possible that BAS1 and/or BAS2 could be posttranslationally modified or that a third transcription factor could participate in the regulation. We have tested the possibility that purine nucleotides could affect binding of BAS1 and BAS2 to DNA. Several end products (AMP, ADP, ATP, GMP, GDP, GTP) and intermediary compounds (adenylosuccinic acid, IMP, IDP, ITP) of the purine pathway failed to affect either BAS1 or BAS2 binding to DNA when studied by gel retardation (data not shown).

The physiological *raison d'etre* of the coregulation of the histidine and purine pathways by BAS1 and BAS2 is not clear. It is known that BAS1 and BAS2 activate at least two genes of the histidine pathway, *HIS4* and *HIS5* (4), and at least four genes of the purine pathway (this work). Moreover BAS2 binds to the *TRP4* promoter *in vitro* and antagonizes activation of *TRP4* by GCN4 (16). An attractive hypothesis is that BAS1 and BAS2 regulate the pathways in which PRPP is used as a substrate (namely, the histidine, tryptophan, purine, and pyrimidine pathways). It is possible that BAS1 and/or BAS2 sense the purine availability or the size of the purine nucleotide pool and modulate the expression of the PRPP utilization pathways in response to this signal. In *E. coli*, conditions under which the pool of adenine nucleotide is high lead to depletion of the PRPP pool. This effect has been proposed to be mediated through inhibition of PRPP synthetase by ADP (for review, see ref. 19). In yeast, activation of the "PRPP pathways" by BAS1 and BAS2 under conditions of adenine limitation could deplete the PRPP pool. Study of the size of the nucleotide and PRPP pools as well as characterization of the regulation of the PRPP synthetase in yeast will be important further steps in our understanding of the regulation of the complex PRPP utilization pathways. The response to adenine limitation by increased expression of the purine and histidine pathways is reminiscent of the general control of amino acid biosynthesis, in which limitation for one amino acid leads to induction of several biosynthetic pathways (20). Global responses such as purine/histidine and general control must reflect some underlying strategy for coordinating many diverse metabolic systems with the exigencies of yeast growth.

We are grateful to Dr. Henikoff, Dr. Kaback, and Dr. Vogel for providing plasmids. We thank J. Brill, S. Buratowski, S. Kron, and D. Pellman for reviewing the manuscript. B.D.-F. was supported by a Human Frontier Science Program Organization postdoctoral fellowship. This work was supported by a National Institutes of Health grant (GM35010) to G.R.F., who is an American Cancer Society Professor of Genetics.

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