investigator-initiated research project grant. By providing generous support and maximum flexibility, the Howard Hughes Medical Institute has assisted in the funding of large units with specific program orientations in more than 20 leading institutions. But the issue of the role of big science in biological research has never been seriously addressed as public policy. The topic is scheduled to receive that attention in an upcoming study to be conducted by the Institute of Medicine of the National Academy of Sciences.

Reflections

Nobelist Christian de Duve has written (*5*), "Although it is always difficult to judge one's own time in historical perspective, one cannot help the feeling that the second half of this century will be remembered for one of the great breakthroughs of human knowledge—perhaps the greatest to date, as it concerns the basic mechanisms of life." In this centennial year, scientist and author Lewis Thomas has said (*6*), "I think the general public is aware of the fact that we are in the early stages of a genuine revolution in biological science. We're beginning to understand at a deep level how living cells and tissues really work. The effects that this revolution is now having and will have in the years ahead on medicine itself are simply incalculable. All of this had its beginnings in the NIH, starting around 40 years ago. All by itself this magnificent institution stands as the most brilliant social invention of the 20th century anywhere."

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5. C. de Duve, *A Guided Tour of the Living Cell* (Rockefeller Univ. Press/Freeman, New York, 1984), vol. 1, p. 17.
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Research Articles

Multiple Global Regulators Control *HIS4* Transcription in Yeast

KIM T. ARNDT, CORA STYLES, GERALD R. FINK

Gene expression is dependent on the interaction of DNA binding factors with distinct promoter control elements to activate RNA synthesis. The expression of the *HIS4* gene in yeast is under two different control systems. One of these, general amino acid control, involves a DNA binding protein, GCN4, that stimulates transcription in response to amino acid starvation by binding to 5′-TGACTC-3′ sequences in the *HIS4* promoter region. A second system, the basal level control, stimulates *HIS4* transcription in the absence of amino acid starvation. The basal level transcription of the *HIS4* gene is under the control of two genes, *BAS1* and *BAS2*, which are also required for the control of purine biosynthesis. In addition, *BAS2* is required for the utilization of organic phosphates in the growth medium. Genetic mapping and DNA sequence analysis show that *BAS2* is *PHO2*, a gene previously identified as a regulator of phosphate metabolism. Direct biochemical analysis shows that the *BAS2* gene encodes a protein that binds to both the *HIS4* and *PHO5* promoters. The involvement of a single DNA binding protein in the regulation of histidine, adenine, and phosphate metabolism suggests that yeast may use a few key DNA binding proteins to coordinate the regulation of diverse metabolic pathways.

THE SEQUENCE OF ENZYME-CATALYZED REACTIONS RESULTing in the biosynthesis of amino acids is virtually identical in the yeast *Saccharomyces cerevisiae* and bacteria, but the regulation of genes that encode these enzymes is very different. In bacteria, starvation for a single amino acid leads to increased transcription of only those genes in the cognate pathway. For example, enteric bacteria respond to starvation for histidine by increased expression (derepression) of all ten enzymes in the pathway for histidine biosynthesis (*1*) but do not derepress the genes for other amino acid biosynthetic enzymes. In contrast, yeast and many other fungi respond to starvation for a single amino acid by turning on the transcription of many unrelated amino acid biosynthetic pathways (*2*). For example, starvation for histidine leads not only to derepression of the enzymes for histidine biosynthesis but also the biosynthetic enzymes for arginine, isoleucine, leucine, tryptophan, and lysine (*3*). This cross-pathway regulation, known as general amino acid control, has been shown to act at the level of transcription (*4*).

A second difference is that bacteria completely stop transcription of the genes for their amino acid biosynthetic enzymes when the amino acids are present in the growth medium. Under similar conditions of surfeit, addition of amino acids to the growth medium or the presence of large internal pools of the amino acids, yeast cells maintain high levels of amino acid biosynthetic gene expression. We call the high level of transcription in the presence of amino acid excess the basal level control.

In this article, we identify cis- and trans-acting elements that mediate the control of the basal transcription levels of the *HIS4* gene and show that these elements are distinct from those that regulate the general control starvation response. Although we anticipated that the basal level control might be specific to the histidine genes,

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we found that the trans-acting proteins mediating the basal level control, like those mediating the general control, are global regulators.

Basal control is distinct from general control. The general amino acid control response is mediated by a trans-acting protein, GCN4, and a cis-acting DNA sequence 5′-TGACTC-3′ found in multiple copies in the 5′ noncoding regions of genes that respond to amino acid starvation (*5*). GCN4 is a protein transcription factor that binds at TGACTC repeat sequences to stimulate transcription under conditions of amino acid starvation (*6*). Strains containing a deletion of the *GCN4* gene are unable to elevate the transcription of amino acid biosynthetic genes when grown on amino acid starvation medium (*7*). Nevertheless, *gcn4* deletion strains grow well on minimal medium (nonstarvation) because the expression of amino acid biosynthetic genes [as measured both by enzyme assay and messenger RNA (mRNA) levels] is not much reduced from that found in strains carrying a wild-type *GCN4* gene (*8*). The fact that amino acid biosynthetic genes are transcribed efficiently in the absence of GCN4 suggests that additional trans-acting factors are required to maintain the basal levels of transcription.

The trans-acting factors that mediate the basal control recognize cis-acting elements other than the TGACTC sequences as shown by studies with a *CYC1-lacZ* fusion deleted for its upstream activation sequences (UAS). In place of the *CYC1* UAS, we inserted either two copies of an 18-bp synthetic oligonucleotide containing the TGACTC sequence or an intact 169-bp *HIS4* promoter fragment (Fig. 1). Both of these constructions achieved equal levels of GCN4-dependent transcription when the strains were starved for an amino acid. However, without starvation the basal level of transcription resulting from the two TGACTC elements was only 10 percent of that achieved by the intact *HIS4* promoter fragment. These studies show that cis-acting sequences present in the intact *HIS4* promoter fragment, and missing from the TGACTC elements, are required for the basal level of transcription.

The cis-acting elements for basal control. The cis-acting elements required for the control of the basal level were studied by examining the expression of many linker insertion mutations (*9*) in the *HIS4* promoter region (Fig. 1). Analysis of β-galactosidase of a *HIS4-lacZ* fusion controlled by these mutant promoters was carried out in a strain containing a deletion of the *GCN4* gene so that the GCN4-independent cis-acting transcription elements could be identified. The linker insertions fall into three groups with respect to basal level transcription: those that have no substantial effect, those that increase the basal levels, and those that eliminate the basal level. Deletions that nearly eliminate the basal level of transcription are clustered in one region (Fig. 1). Further evidence for the importance of this region in the activation of basal level transcription is provided by insertion of various *HIS4* promoter fragments into the upstream region of the heterologous *CYC1* gene (*10*) lacking its own upstream activation site. In *gcn4* strains containing 2μ plasmids with these constructions, either the intact 89-bp or the intact 169-bp fragment (Fig. 1, top) can activate the basal level of *CYC1* transcription, whereas neither the left or right half of either fragment (divided at the vertical arrow) is capable of any basal level transcription. These studies indicate that the cis-acting region required for the basal level transcription, which we call the basal element, extends from about −245 to −210 relative to the *HIS4* translation start (Fig. 1).

***BAS1* and *BAS2*, trans-acting genes for basal control.** Once the region of the *HIS4* promoter required for the basal level of expression had been identified, we used a segment of DNA containing that region to search for trans-acting factors that regulate the basal level of *HIS4* transcription. The strategy was to find mutations in genes required to promote *HIS4* transcription in the absence of the GCN4 protein. We constructed a *gcn4* strain that contained a *CYC1-lacZ* fusion whose transcription was regulated by the 89-bp *HIS4* promoter fragment (Fig. 1). The levels of β-galactosidase (about 900 units) must result from transcription that is independent of the GCN4-TGACTC system. Colonies with this level of β-galactosidase cleave sufficient amounts of X-gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside) to turn blue on an X-gal plate (*11*) in 2 days. We mutagenized this strain with EMS (ethyl methanesulfonate) and isolated about 400 independent mutants that were either white or light blue (*12*). Only 80 of these candidates had

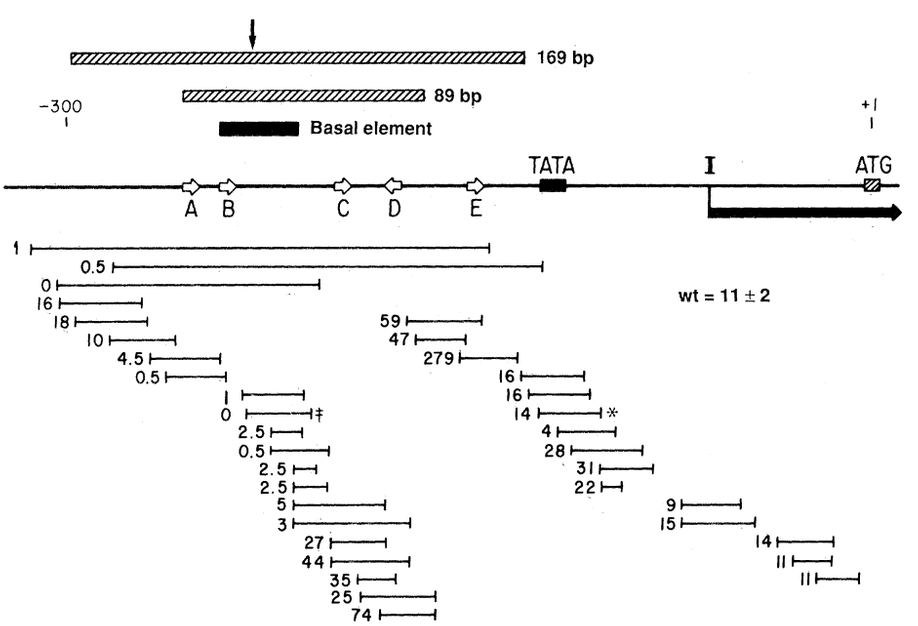

Fig. 1. The cis-acting sequences for the GCN4-independent basal transcription of *HIS4*. The black line in the center is the *HIS4* promoter region showing the position of the five GCN4 binding sites (TGACTC repeats indicated by open arrows labeled A through E), the position (−123) of the TATA sequence through which GCN4 acts (*9*), the position (−63) where most of the *HIS4* mRNA synthesis starts (I site), and the ATG translation start. All positions are indicated relative to the A of the ATG translation start. The *HIS4* promoter fragments are shown by hatched bars. The lines beneath the promoter indicate the extent of each deletion mutation. These *HIS4* promoter mutations (fused to the *E. coli lacZ* gene at *HIS4* amino acid 11 on a yeast centromere plasmid) were transformed into a strain with a deletion of the *GCN4* gene and grown on minimal complete medium (containing all the amino acids, but lacking uracil). Two independent transformants were assayed in duplicate and the values, representing an average of the β-galactosidase activities, are listed to the left of each deletion. The intact *HIS4* promoter [pFN8 (*9*)] gives 11 units under these conditions. The black bar above the line indicates the cis-acting basal control region (approximately −245 to −210). A group of promoter deletions, which are clustered 3′ to the basal element, increases the basal level. The increase could result from either alteration in the spacing of the basal element relative to the TATA sequences or removal of sequences that normally function to repress the basal level. The remaining deletions do not greatly alter the GCN4-independent basal levels (*35*). The ‡ indicates a deletion used in Fig. 5 and the * denotes the TATA deletion referred to in (*35*).

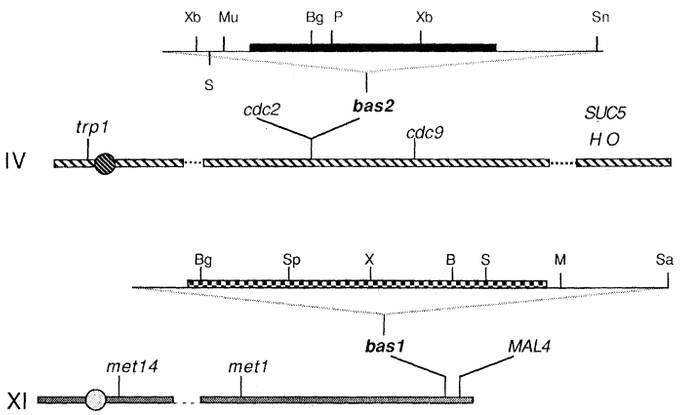

Fig. 2. Chromosomal locations and restriction maps of *BAS1* and *BAS2*. The *BAS1-MAL4* map distance is 2.1 cM (three tetratypes and no nonparental ditypes in 70 tetrads), and the *BAS2-CDC2* map distance is less than 1 cM (all 187 tetrads were parental ditype). The orientation of these markers with respect to the centromere is not known. The position of the *BAS1* gene on the cloned fragment was determined by making small internal deletions in a 6.5-kb *BAS1* subclone in YCp50 and assaying for *BAS1* function after transformation of a *gcn4,bas1-1* strain. The *BAS2* gene was localized by a similar approach and positioned exactly by DNA sequence analysis. The growth phenotypes of the *bas1-2* and *bas2-2* deletion alleles were identical to those of another internal deletion of the *BAS1* gene and a *TRP1* disruption of the *BAS2* gene, respectively. The restriction enzyme sites are: Xb is Xba I, S is Spe I, Mu is Mlu I, Bg is Bgl II, P is Pvu I, Sn is Sna BI, Sp is Sph I, X is Xho I, B is Bam HI, M is Mst II, and Sa is Sac I.

lower levels of β-galactosidase than wild type by direct assay. Of these mutants, two strains had become histidine-requiring (His^-) in addition to having low β-galactosidase levels. These two mutants were pursued further because it seemed likely that a *gcn4* strain would become His^- if it could not produce the putative trans-acting factors needed for the basal level expression of the *HIS4* gene.

The two mutant strains were subjected to extensive genetic tests designed to characterize the nature of the defects leading to low β-galactosidase levels and the His^- phenotype. Transformation of each of these strains with a centromere plasmid containing the *GCN4* gene showed that these mutations conferred a histidine requirement only in a *gcn4* strain. This result, confirmed by crosses to *GCN4* strains, indicated that all further genetic work would have to be done in a *gcn4* background since the GCN4-TGACTC system could provide sufficient transcription of *HIS4* to confer a His^+ phenotype to our mutant strains.

Crosses to *gcn4* strains showed that each mutation segregated as a single gene (2 His^+:2 His^- in at least 50 tetrads) and also demonstrated that the His^- phenotype and low β-galactosidase were completely linked. Our new His^- strains did not carry a mutation in any of the seven known histidine biosynthetic enzymes (*2*). Crosses of the two mutants by *his1* through *his7* (each in a *gcn4* background) showed independent segregation of the known biosynthetic His^- mutation with our new mutations. The new His^- mutant strains complement to give His^+ diploids. Tetrads resulting from these diploids contained His^+ and double mutant recombinants at frequencies expected for unlinked genes. On the basis of these genetic tests, we conclude that these mutations define two genes, *BAS1* and *BAS2*, whose products are required for the basal level of *HIS4* transcription.

Cloning of *BAS1* and *BAS2*. We isolated *BAS1* and *BAS2* from a YCp50 yeast library (*13*) by transforming either a *gcn4,bas1-1* or a *gcn4,bas2-1* strain to His^+ (*14*). Plasmids capable of conferring a His^+ phenotype were recloned in *Escherichia coli*, characterized by digestion with restriction enzymes, and transformed back into yeast for genetic analysis. For each of the basal control genes, the plasmids fell into two classes on the basis of restriction patterns. We describe the analysis of *BAS2* below; the analysis of *BAS1* followed identical protocols and led to similar conclusions. One class of plasmids, expected and readily identified by its characteristic restriction pattern, contained the *GCN4* gene. Analysis of the other class showed that we had cloned the *BAS2* gene. By making small deletions in the yeast insert of the smallest *BAS2* subclone that both suppressed the His^- phenotype and restored normal *HIS4-lacZ* expression, we precisely identified the segment that contained the *BAS2* gene. This segment was subcloned into a *URA3* integrating vector (YIp5), and the resulting plasmid was cut within *BAS2* DNA sequences to direct integration to the *BAS2* locus upon transformation of a *ura3-52,bas2-1* strain. The Ura^+ transformants were all $Bas2^+$ and were shown by tetrad analysis to have integrated at the *BAS2* locus (*15*).

Using the *BAS1* and *BAS2* clones, we probed Southern blots of yeast chromosomes that had been separated by pulse field electrophoresis (*16*). The *BAS1* clone hybridized to chromosome XI and the *BAS2* clone hybridized to chromosome IV. Tetrad analysis then gave the map positions shown in Fig. 2. Internal deletions were constructed in vitro in both *BAS1* (from Xho I to Spe I, mutant *bas1-2*) and *BAS2* (from Mlu I to Bgl II, mutant *bas2-2*) and then transformed into yeast with a *URA3* integrating vector. The duplications resulting from these transformations were resolved by selection on 5-fluoroorotic acid (*17*) and the Bas^-, Ura^- segregants were shown to have the deletion allele by Southern hybridization, tetrad analysis, and growth requirements. These deletions were made in both a *GCN4* and a *gcn4* (deletion) strain. Some of the growth defects displayed by the original *bas1-1* and *bas2-1* strains are more pronounced in strains carrying the deletion alleles.

Both *BAS1* and *BAS2* are required for *HIS4* basal control. The histidine growth defects of *bas1* and *bas2* strains are reflected in *HIS4* expression, as monitored by *HIS4-lacZ* fusions (Table 1). The *HIS4* expression (with a low copy number *HIS4-lacZ* vector) is decreased relative to wild type (50 units) in strains that are *gcn4,BAS1,BAS2* (to 11 units), *GCN4,bas1-2,BAS2* (to 6 units), or *GCN4,BAS1,bas2-2* (to 13 units). This level of *HIS4* transcription is sufficient to give a His^+ phenotype. However, in a *gcn4* background, strains containing either *bas1-2* or *bas2-2* have only about 1 unit of *HIS4* expression and, as a consequence, have a His^- phenotype. Analysis of a *HIS4-lacZ* fusion on a high copy number 2μ plasmid gives similar results and also allows greater sensitivity in the measurement of transcription. Northern analysis shows that *bas1-1,gcn4* and *bas2-1,gcn4* strains have a marked decrease in steady-state *HIS4* mRNA levels (Fig. 3). The direct assessment of *HIS4* mRNA confirms the levels of *HIS4* transcription as measured by β-galactosidase fusions (*18*).

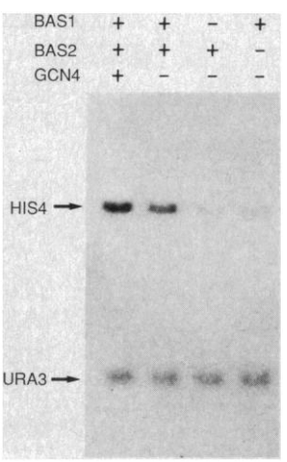

Fig. 3. Northern analysis of *HIS4* mRNA in *bas1* and *bas2* strains. Yeast were grown in minimal complete media to an A_{600} of 1, total RNA was isolated (*9*), and 10 μg of this RNA was placed in each lane. The probes were a ^{32}P-labeled internal *HIS4* fragment (Xho I to Xba I) and the 1.2-kb Hind III fragment of *URA3* (to control for the amount of RNA). The *gnc4* mutation is a deletion allele and the *BAS1* and *BAS2* mutations are *bas1-1* and *bas2-1*, respectively.

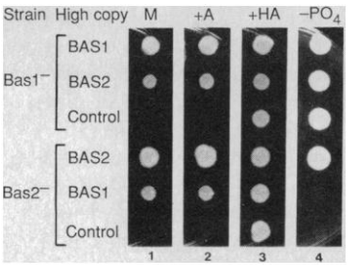

Fig. 4. Growth of high copy *BAS1* and *BAS2* strains. Strains containing a deletion of the *GCN4* gene and a deletion of either the *BAS1* or *BAS2* gene were transformed with a high copy number 2μ plasmid containing either the *BAS1* gene, the *BAS2* gene, or the control *CYC1* gene and spotted on plates (about 5000 cells per spot) containing the indicated nutrients: M is yeast minimal medium with 0.5 m*M* arginine, +A is M plus 0.3 m*M* adenine, and +HA is M plus 0.3 m*M* histidine and 0.3 m*M* adenine (M contains 7 m*M* phosphate). The $-PO_4$ medium is YEPD adjusted to *p*H 5.2 after alkaline $MgSO_4$ precipitation of the inorganic phosphate. The amount of growth after 5 days at 30°C is shown. All strains grow normally on the +HA medium. Growth phenotypes are confirmed by colony sizes when the strains are streaked for single colonies on the same media.

Growth of *bas1-2* and *bas2-2* strains. Studies on the growth of isogenic strains carrying *bas1-2* and *bas2-2* support the dual nature of the regulatory control over *HIS4*. The growth requirements of strains carrying a *bas2-2* mutation in both a *GCN4* and a *gcn4* background were tested by plating cells on different media. The *GCN4,BAS2* strains have the same growth rate as *gcn4,BAS2* strains on all media, including minimal medium lacking histidine and adenine. Strains with both *bas2* and *gcn4* deletions have a His^- phenotype (Fig. 4, bottom row). In addition to the histidine requirement, the *gcn4,bas2* strains have a partial adenine requirement since they do not grow as well as the wild type (*GCN4,BAS2*) when given only histidine but do grow as well as the wild type when supplemented with both histidine and adenine. In a *GCN4* background, *bas2* strains are His^+ (*19*) but still display the partial adenine requirement. The likely explanation is that in *GCN4,bas2* strains, the GCN4-TGACTC system responds to the starvation for histidine and provides sufficient activation of *HIS4* transcription to remedy the histidine requirement, whereas the adenine requirement of *bas2-2* strains is independent of *GCN4*. Growth studies with isogenic strains containing the *bas1-2* mutation indicate that *bas1* strains have amino acid growth requirements very similar to *bas2* strains, except that the adenine requirement is more pronounced.

Unexpectedly, *bas2* strains are unable to grow in the absence of inorganic phosphate. The *bas2* strains grow less well on complex medium (YEPD, which contains low concentrations of inorganic phosphate and high concentrations of organic phosphate) than on defined minimal medium (which contains KH_2PO_4). If the inorganic phosphate is removed from YEPD medium, *bas2* strains fail to grow whether they are *GCN4* or *gcn4* (Fig. 4, column 4). However, *bas2* strains will grow at the same rate as *BAS2* strains if inorganic phosphate is added to a YEPD plate. In contrast, strains containing the *bas1-2* allele, like wild-type strains, grow well on YEPD and can utilize the organic phosphates in YEPD as a sole source of phosphate.

***BAS2* is *PHO2*.** The inability of *bas2* strains to grow on organic phosphate and the proximity of *BAS2* to *PHO2* on the genetic map prompted a comparison between these two regulatory genes. *PHO2* is a trans-acting gene required for the derepression of the inducible acid phosphatases encoded by *PHO5* and *PHO11* under conditions of phosphate starvation (*20*). We obtained a *pho2* strain (*21*) and analyzed its phenotype, which was previously identified as an inability to grow on organic phosphate as the only phosphate source (*22*). Our 3.5-kb *BAS2* subclone that restores *BAS2* function to a *bas2* strain also transforms the *pho2* mutant to Pho^+ [restores the ability of the *pho2* strain to grow on organic phosphate (*23*)]. Construction of the *gcn4,pho2* double mutant revealed that this strain, like our *gcn4,bas2* strains, has both a histidine and a partial adenine requirement. Furthermore, *GCN4,pho2* strains (like our *GCN4,bas2* strains) have a partial adenine requirement, which was not previously reported (*22*). Finally, our DNA sequence of *BAS2* is the same as that obtained by Sengstag and Hinnen for *PHO2* (*24*), which was cloned by complementing the phosphate defect of a *pho2* strain (*25*).

Phosphate regulation of *HIS4*. The finding that *BAS2* is required not only for the regulation of histidine biosynthesis but also for phosphate utilization led us to investigate the effect of alterations in phosphate concentration on *HIS4* expression. Both wild-type (curve A, *GCN4,BAS1,BAS2*) and *gcn4* (curve C, *gcn4,BAS1,BAS2*) strains show increased *HIS4* expression when grown at low phosphate concentrations (normal minimal medium contains 7 m*M* phosphate) (Fig. 5).

One indication that the increase in *HIS4* expression at low phosphate concentrations is specific to *BAS2* is that *HIS4* expression does not vary with phosphate concentration in a strain containing a deletion in the *BAS2* gene (Fig. 5, curve D, *GCN4,BAS1,bas2-2*). Another indication is that a *HIS4* promoter lacking the cis-acting sequences required for *BAS2* function also fails to respond to phosphate starvation (Fig. 5, curve B). Although this deleted promoter (deletion indicated by ‡ in Fig. 1) has all the cis-acting DNA sequence elements for GCN4-TGACTC general control, it is incapable of maintaining the basal level of *HIS4* expression when the phosphate levels are low. These experiments indicate that both *BAS2* and the cis-acting basal control region of *HIS4* are required for the stimulation of *HIS4* transcription in cells grown in low phosphate medium.

***BAS1* is required for *HIS4* but not phosphate regulation.** *GCN4*-independent *HIS4* transcription is maximal when both the *BAS1* and *BAS2* gene products are functional. In a *gcn4* background, *bas1-2,BAS2* and *BAS1,bas2-2* strains have 1 unit of *HIS4-lacZ* expression, whereas *BAS1,BAS2* strains have more than ten times as much *HIS4-lacZ* expression (Table 1, low copy number *HIS4-lacZ*

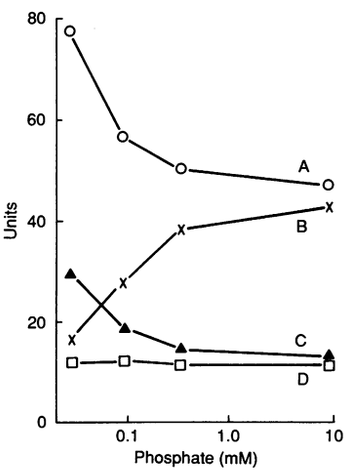

Fig. 5. Phosphate regulation of *HIS4*. The *HIS4-lacZ* activity is shown as a function of phosphate concentration in the growth media. (Curve A) Wild-type yeast (*GCN4,BAS1,BAS2*) containing the normal *HIS4-lacZ* fusion on a low copy number plasmid [pFN8 (*9*)]. (Curve B) Wild-type yeast containing a derivative of pFN8 with a deletion of the cis-acting basal element, marked by ‡ in Fig. 1. (Curve C) Yeast with a deletion in the *GCN4* gene containing the normal *HIS4-lacZ* fusion (pFN8). (Curve D) Yeast with a deletion in the *BAS2* gene containing the normal *HIS4-lacZ* fusion (pFN8). Yeast were grown to mid-exponential phase in a phosphate-free synthetic complete medium to which KH_2PO_4 was added. Potassium concentrations were kept constant by addition of KCl to the media with lower phosphate concentrations, and the *p*H was kept constant at *p*H 4.7 by buffering all media with 25 m*M* sodium citrate. The β-galactosidase activities are the average of two separate experiments (each consisting of duplicate cultures for each point) that showed very similar results. A control *CYC1-lacZ* fusion in wild type, *bas2*, or *gcn4* yeast does not vary by more than 10 percent as the phosphate concentration is changed. When grown at 0.03 m*M* phosphate, wild-type and *gcn4* strains have 20-fold higher acid phosphatase levels than *bas2-2* strains.

Table 1. *BAS1* and *BAS2* regulation of *HIS4*. Each value is the average β-galactosidase activity obtained from two strains of the same genotype containing the indicated *HIS4-lacZ* plasmid. For each strain, two transformants were grown to an absorbancy at 600 nm (A_{600}) of 1 in minimal medium containing 0.5 m*M* arginine, 0.3 m*M* adenine, and 0.3 m*M* histidine. The presence of histidine in the medium prevents GCN4-dependent depression of *HIS4* due to amino acid starvation (*26*). Extracts were made by breaking the cells with glass beads and assayed for β-galactosidase (*34*). Duplicate assays with the same extracts and assays from two strains of identical genotypes varied by <20 percent. The genotype of the strains is indicated by a plus sign for the wild-type allele and a minus sign for the deletion allele. The low copy number plasmid is the yeast centromere plasmid YCp50 containing the *HIS4* gene fused to β-galactosidase at amino acid 11 of the *HIS4* gene [pFN8, (*9*)]. The high copy number plasmid is a 2μ vector containing the 89-bp *HIS4* promoter fragment (Fig. 1) controlling a *CYC1-lacZ* fusion (*10*). As a control, we assayed the *CYC1-lacZ* fusion regulated by *CYC1* promoter sequences and found similar levels of β-galactosidase in *BAS* or *bas* strains.

Strains			*HIS4-lacZ*	
GCN4	BAS1	BAS2	Low copy	High copy
+	+	+	50	3000
−	+	+	11	1000
+	−	+	7	1300
+	+	−	13	1700
+	−	−	6	1300
−	−	+	1	400
−	+	−	1	400
−	−	−	1	270

fusion). These results with low copy number vectors are confirmed by the direct measurement of steady-state *HIS4* mRNA by Northern analysis (Fig. 3).

Although *BAS1* and *BAS2* act synergistically to provide the basal level of *HIS4* transcription, each alone is capable of some degree of *GCN4*-independent basal level transcription. In a *gcn4* background, strains containing both *bas1-2* and *bas2-2* mutations have lower *HIS4* expression than strains carrying only a *bas1-2* or a *bas2-2* mutation (Table 1, high copy number *HIS4-lacZ* fusion). The independent activation of *HIS4* transcription by *BAS1* and *BAS2* is graphically demonstrated by the behavior of *bas1-2* or *bas2-2* strains in which either *BAS1* or *BAS2* is overexpressed (when high copy number vectors are used). The *BAS1* gene in high copy partially suppresses the histidine requirement caused by the *bas2-2* mutation and the *BAS2* gene in high copy partially suppresses the histidine requirement caused by the *bas1-2* mutation (Fig. 4).

BAS1 does not appear to function in phosphate regulation. The *BAS1*, *bas1-2*, or high copy *BAS1* strains grow equally well on media with organic phosphate as the sole source of phosphate. Although *BAS1* on a high copy number vector partially suppresses the histidine requirement of *bas2-2* strains, it fails to suppress the *bas2* phosphate defect (Fig. 4).

BAS1 and *BAS2* act synergistically to provide the high basal level of *HIS4* transcription in the absence of amino acid or phosphate starvation (*26*). By contrast, *BAS2* and *PHO4* act synergistically to stimulate the derepression of *PHO5* transcription, but only under conditions of phosphate starvation. Neither *BAS2* nor *PHO4* appear to stimulate the basal level of *PHO5* transcription in the presence of high phosphate concentrations (*27*).

***BAS2* binds to both the *HIS4* and the *PHO5* promoters.** The binding of the BAS1 and BAS2 proteins to the *HIS4* promoter was studied by the gel retardation assay (*28*). This method is termed the gel retardation assay because specific protein-DNA complexes migrate more slowly in the native polyacrylamide gel than the free unbound DNA. We examined the pattern of protein-DNA interactions at the *HIS4* promoter by addition of crude extracts of yeast cells to a ^{32}P-labeled *HIS4* promoter fragment and electrophoresis of the mixture on a polyacrylamide gel. The pattern of protein-DNA interactions at the *HIS4* promoter obtained with extracts from wild-type strains is identical to the pattern obtained with extracts from strains containing a deletion of the *GCN4* gene (Fig. 6, left). This pattern is not influenced by the type of medium on which the cells are grown (minimal, rich, or histidine starvation medium). However, GCN4 prepared in vitro or in *Escherichia coli* can easily be detected by this binding assay (*6*). The pattern of protein-DNA interactions for a strain containing a deletion of the *BAS1* gene (Fig. 6, left) and strains containing the *BAS1* gene on a high copy number plasmid is identical to the pattern for wild-type strains. However, failure to detect a BAS1 specific binding activity in yeast extracts does not exclude the possibility that BAS1 is a *HIS4* binding protein since GCN4, in the same assay, also fails to form a detectable protein-DNA complex.

A strain containing a deletion of *BAS2* is lacking a band representing a specific interaction with the *HIS4* promoter (Fig. 6, left). Further evidence for a BAS2-*HIS4* interaction is shown by the increased intensity of that band when the extract is prepared from a strain containing the *BAS2* gene on a high copy number plasmid (Fig. 6, right, lane 4). These data indicate that either BAS2 itself binds to the *HIS4* promoter or that BAS2 regulates another factor that binds.

Analysis of BAS2 protein synthesized in vitro shows that the BAS2 protein itself binds directly to the *HIS4* promoter. The BAS2 protein, free of any yeast factors, was synthesized in vitro by placing the *BAS2* gene under the control of the SP6 promoter (*29*), directing *BAS2* transcription with SP6 polymerase, and translating the *BAS2* RNA with a rabbit reticulocyte lysate. We made both full-length BAS2 protein (559 amino acids) and BAS2 protein that has a deletion of 64 amino acids at the amino terminus (by using an

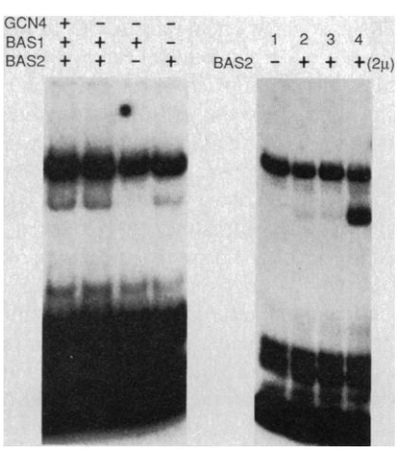

Fig. 6. Protein-DNA interactions at the *HIS4* promoter. (**Left**) Assays were performed with a ^{32}P-labeled 89-bp *HIS4* promoter fragment (Fig. 1) and extracts prepared from cells of the indicated genotype, where all mutations are deletion alleles. (**Right**) Assays were performed with the 89-bp *HIS4* fragment and extracts prepared from *GCN4,BAS1* strains with various *BAS2* genotypes. (Lane 1) *bas2-2*; (lane 2) *BAS2* (integrated) transformant of the *bas2-2* strain used in lane 1; (lane 3) wild-type *BAS2*; (lane 4) *BAS2* on a high copy number 2μ plasmid. Similar experiments with *BAS1* revealed no change in the pattern of protein-DNA interaction with the *HIS4* promoter. Assays were performed as follows. Yeast cultures (100 ml) were grown to an A_{600} of 1, centrifuged, resuspended in 0.4 ml of 0.1*M* tris (*p*H 7.5), 0.2*M* NaCl, 0.01*M* 2-mercaptoethanol, 20 percent glycerol, 5 m*M* EDTA, 1 m*M* phenylmethyl sulfonylfluoride (PMSF) buffer and disrupted by vortexing with glass beads. An equal volume of the same buffer was added, and the extract was centrifuged for 15 minutes at 13,000*g* (4°C). The extracts were diluted to a protein concentration of 1.0 mg/ml as measured by the BioRad protein assay with bovine serum albumin as standard. The assay consisted of 5 μl of yeast extract and 0.75 ng of ^{32}P-labeled 89-bp *HIS4* promoter fragment (Fig. 1) in 25 m*M* tris (*p*H 7.5 at 4°C), 50 m*M* NaCl, 4 percent glycerol, 10 m*M* 2-mercaptoethanol, 1 m*M* EDTA, 0.05 percent Triton X-100, sheared calf thymus DNA at 0.01 μg/μl, and 1 m*M* PMSF buffer (final concentrations) in a total volume of 25 μl. The sample (8 μl) was loaded onto a 4 percent polyacrylamide gel (6.7 m*M* tris, *p*H 7.5 at 4°C, 3.3 m*M* sodium acetate, 1 m*M* EDTA) with electrophoresis at 10 V/cm at 4°C.

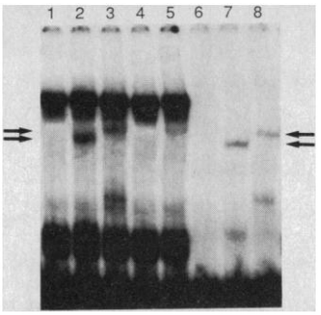

Fig. 7. Binding of in vitro produced BAS2 to the *HIS4* promoter. Gel retardation assays with in vitro produced BAS2 and the 89-bp *HIS4* promoter fragment were performed in the presence (lanes 1 to 5) or in the absence (lanes 6, 7, and 8) of yeast extract. Lane 5 shows the pattern when wild-type yeast extract was used with control reticulocyte lysate (no RNA added prior to translation). The upper arrows show the position of the BAS2-*HIS4* promoter complex. This pattern is similar to the pattern in Fig. 6 but has higher background due to the additional proteins in the reticulocyte lysate. All extracts in lanes 1 through 4 were prepared from a yeast strain with a deletion in the *BAS2* gene and contain in addition: (lane 4) control reticulocyte lysate (no RNA), (lane 3), full-length in vitro synthesized BAS2 (559 amino acids total), (lane 2) in vitro synthesized BAS2 deleted at the NH_2-terminus (amino acids 65 to 559, position of the complex indicated by lower arrows), and (lane 1) in vitro synthesized NH_2-terminal fragment of BAS2 (amino acids 1 to 111). Lanes 6, 7, and 8 are identical to lanes 1, 2, and 3, respectively, except that extraction buffer was used in place of yeast extract. In all lanes, 5 μl of yeast extract or extraction buffer was used plus 4 μl of reticulocyte lysate. The gel retardation assay was performed as in Fig. 6 except that the final NaCl concentration was 100 m*M*. The yeast extracts are added to provide internal standards for the migration of the wild-type BAS2-*HIS4* promoter complex as well as to determine whether any yeast factors are required for the binding. The lower molecular size protein-DNA complex seen in the lanes with full length BAS2 (1 to 559) and NH_2-terminal deleted BAS2 (65 to 559) is due to premature termination of BAS2 during in vitro synthesis. The premature termination fragment of BAS2 can be detected when the BAS2 protein is labeled by [^{35}S]methionine incorporation and displayed on a denaturing polyacrylamide gel in the absence of DNA. The in vitro preparation of BAS2 protein is described in (*36*).

internal, in-frame ATG as the translation start). Full-length BAS2 made in vitro binds to the *HIS4* promoter fragment (Fig. 7, lane 8) to give a complex that migrates at the same position as the *BAS2* protein-DNA complex obtained from crude yeast extracts (indicated by upper arrows). Furthermore, the truncated lower molecular weight BAS2 gives a band at a lower position in the gel than wild-type BAS2 (lower arrows), indicating that BAS2 can bind to the *HIS4* promoter without its amino-terminal 64 amino acids. Addition of in vitro synthesized BAS2 containing just the NH_2-terminal 111–amino acids results in no detectable binding to the *HIS4* promoter (Fig. 7, lane 6).

Our data show that in vitro produced BAS2 binds to the *HIS4* promoter in the presence or absence of yeast crude extracts. By adding in vitro synthesized full-length BAS2 to an extract from a strain containing a deletion of the *BAS2* gene (Fig. 7, lane 3), we were able to reproduce the wild-type pattern of protein-DNA interactions at *HIS4*. Even though efficient BAS2 activation of *HIS4* transcription requires BAS1, the binding of BAS2 to the *HIS4* promoter does not require BAS1 since BAS2 produced in vitro (free of all other yeast factors, including BAS1) binds to the *HIS4* promoter and yeast extracts made from a strain with a deletion of *BAS1* still form the BAS2-*HIS4* promoter complex (Fig. 6, left).

To determine where BAS2 binds within the 89-bp *HIS4* promoter fragment (sequences −260 to −171), we examined BAS2 binding to a set of *HIS4* promoter fragments. BAS2 binds a *HIS4* promoter fragment consisting of sequences from −242 to −131 but does not bind a fragment consisting of sequences from −213 to −131. These results indicate good agreement between the in vitro binding of BAS2 to the *HIS4* promoter and the basal control element defined by the in vivo *HIS4* promoter deletion analysis. BAS2 binding requires sequences between −242 and −213, which when deleted abolishes *HIS4* basal control in vivo.

BAS2 also binds directly to the *PHO5* promoter (Fig. 8). Both full-length BAS2 (lane 3, in each set) and BAS2 missing its amino-terminal 64 amino acids (lane 2, in each case) bind to the *PHO5* promoter fragments B and C with an affinity similar to that of the *HIS4* promoter, whereas BAS2 binding to *PHO5* promoter fragment A is much reduced. Since BAS2 binds to both the *HIS4* and *PHO5* promoters, we searched for a similarity in DNA sequence. *HIS4* (*30*) contains the sequence AAA*TT*AGTTAATT*AATT* (position −233 to −217) and *PHO5* (*27*) contains the sequence AAA*AG*AGTTAATT*GAA*T (Fig. 8, large dot on the *PHO5* promoter). Nucleotide bases that differ between the two DNA sequences are italicized. *PHO11*, the other acid phosphatase gene that is regulated by BAS2, has a similar sequence homology (*27*). The *PHO5* sequence homology is contained on the promoter fragments bound tightly by BAS2. The *HIS4* sequence homology is located within the cis-acting basal control element that contains the sequences necessary (from −242 to −213) for BAS2 binding in vitro. Methylation (*31*) of some of the adenosine residues within this *HIS4* sequence homology region (positions −219, −220, and −223) prevents BAS2 from binding to the *HIS4* promoter.

Multiple global regulators control *HIS4*. The yeast histidine biosynthetic pathway is under multiple transcriptional controls. Amino acid starvation control requires the trans-acting GCN4 protein and basal control requires both BAS1 and BAS2 proteins. The GCN4-TGACTC system is global, controlling more than 25 genes involved in amino acid biosynthesis. The surprise is that the trans-acting proteins of the basal control system also regulate genes in other pathways. We have shown by direct biochemical assays that BAS2 binds to both the *HIS4* and *PHO5* promoters. Genetic and physiological tests suggest that *BAS1* and *BAS2* affect both histidine and adenine pathways and that *BAS2* is a general regulator of phosphate utilization (*32*).

The existence of these global transcription factors and the absence of any specific trans-acting regulatory factors that regulate only

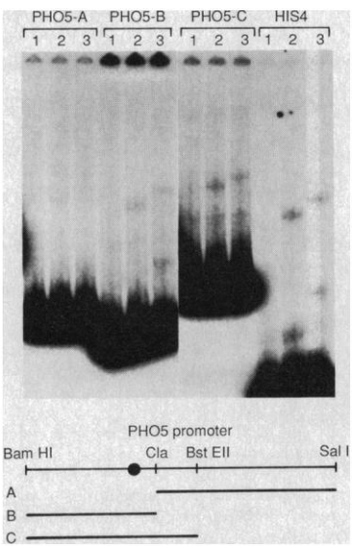

Fig. 8. Binding of in vitro produced BAS2 to the *PHO5* promoter. The assays used in vitro synthesized NH_2-terminal fragment of BAS2 (amino acids 1 to 111, lane 1 in each case), NH_2-terminal deleted BAS2 (amino acids 65 to 559, lane 2), or full-length BAS2 (amino acids 1 to 559, lane 3). Yeast extracts were not used in these assays. The ^{32}P-labeled DNA fragments were either the *PHO5* fragments indicated or the 169-bp *HIS4* promoter fragment (Fig. 1). For the *PHO5* promoter, the Bam H I site is at position −546, the Cla I site is at −274, the Bst EII site is at −173 and the Sal I site is at +82 with respect to the ATG start of translation. The sequence homology with the *HIS4* promoter is indicated by a large dot. Assay conditions are the same as Fig. 7 except that the calf thymus DNA concentration is 0.1 μg/μl.

HIS4 expression suggests that yeast, like mammalian cells, may use a limited set of DNA binding proteins to regulate genes in many different biochemical pathways. Recent studies have shown that the mammalian transcription factor SP1 binds to many unrelated promoters, including the herpes simplex virus (HSV) thymidine kinase, the metallothionein II_A, and the SV40 early promoters (*33*). Presumably these DNA binding proteins achieve specificity by the multiplicity of interactions with other DNA binding proteins. At *HIS4*, this specificity is achieved by interactions with BAS1, BAS2, and GCN4.

Our studies emphasize the importance of genetics in unraveling complex regulatory pathways. The *gcn4* mutation was crucial in identifying both the GCN4-DNA interactions and the subsequent elucidation of the *BAS1* and *BAS2* basal control system. The binding of GCN4 to DNA could not be detected with yeast extracts. The demonstration of GCN4 binding to TGACTC sequences required GCN4 protein obtained by overproduction in bacteria or by in vitro translation of *GCN4* mRNA. Both of these sources of GCN4 protein required the cloned *GCN4* gene, which could only be obtained by transformation of the *gcn4* mutant. The genetic identification of both *BAS1* and *BAS2* required a *gcn4* strain since neither the *bas1* or *bas2* mutation confers a strong histidine requirement in a *GCN4* strain. The ability of the GCN4-dependent general control system to compensate for defects in the basal control system explains the inability to isolate *bas1* or *bas2* mutations in previous mutant hunts with *GCN4* strains.

The difference in the regulatory responses between bacteria and yeast could be related to differences in cell structure. In yeast, the excess amino acids synthesized during vegetative growth by the basal level of amino acid biosynthetic enzymes are stored in the vacuole, an organelle that is not present in *E. coli*. More than 90 percent of the histidine, lysine, and arginine in a yeast cell are sequestered in this organelle (*2*). The high levels of amino acids stored in the vacuole permit yeast to grow for one or two generations in the absence of any endogenous synthesis. During depletion of the vacuolar pools, *GCN4* expression is elevated, resulting in the general control stimulation of the transcription of the amino acid biosynthetic genes from their already high basal levels. Further information on the role of the vacuole might elucidate the relation between the general and basal control.

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8. Strains containing a *gcn4* mutation have a partial arginine requirement, probably because one of the arginine biosynthetic enzymes has low GCN4-independent levels. For this reason, we add arginine to our minimal media.
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14. The *bas1* and *bas2* mutant alleles obtained from the mutant search are called *bas1-1* and *bas2-1*, respectively, and the deletion alleles made in vitro are called *bas1-2* and *bas2-2*, respectively.
15. When the Ura^+,$Bas2^+$ integrant was crossed to a *ura3-52,bas2-1* strain, no Ura^+,$Bas2^-$ recombinants were observed in 20 tetrads and, when crossed to a *BAS2* strain, no $Bas2^-$ recombinants were observed in 20 tetrads.
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18. We also examined the ability of *BAS1* and *BAS2* to regulate the other amino acid biosynthetic enzymes. Initial observations suggest that *BAS1* and *BAS2* affect *HIS5* basal level regulation. The defect in the regulation of the adenine pathway in *bas1* or *bas2* strains remains to be determined. Northern analysis shows that both *BAS1* and *BAS2* steady-state mRNA levels are unaffected by *bas1-1* or *bas2-1* mutations.
19. *GCN4,bas2* or *GCN4,bas1* strains are His^+ by replica plating, but they show a very slight histidine requirement when grown from single colonies.
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26. The BAS2 activation of *HIS4* is dependent on BAS1 but not GCN4. In addition, the GCN4-dependent general control derepression response at *HIS4* is independent of *BAS1* and *BAS2* since wild-type, *bas1*, or *bas2* strains all give the same GCN4-dependent increase in *HIS4* expression in *gcd1* strains, which constitutively overexpress GCN4 protein.
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35. GCN4 acts entirely through a TATA sequence (at position −123) indicated on the *HIS4* promoter (*9*). Deletion of this TATA site completely eliminates GCN4-dependent derepression. However, the same TATA deletion (indicated by an asterisk in Fig. 1) has no affect on the GCN4-independent basal levels. Three other TATA sequences are located near the GCN4 TATA (at −170, −132, and −102). A small deletion (from −119 to −96, removing part of the GCN4 TATA and the −102 TATA sequence) that lies just 3′ to the GCN4 TATA deletion lowers the basal level reproducibly to <50 percent of normal levels. Perhaps the basal element can use the alternative TATA elements.
36. BAS2 was synthesized in vitro by placing the *BAS2* gene downstream of the SP6 promoter (pGEM-3 vector, Promega Biotec), transcribing the *BAS2* gene with SP6 polymerase (Promega Biotec) to obtain *BAS2* RNA, and then translating the RNA with rabbit reticulocyte lysate (Promega Biotec) under conditions recommended by the manufacturer. Full-length BAS2 was prepared by fusing the SP6 promoter to the *BAS2* gene at position −31 relative to the natural ATG start of translation. The NH_2-terminal fragment of BAS2 (amino acids 1 to 111) was prepared by digestion of this fusion with Bgl II (cuts the *BAS2* gene at nucleotide position +333) prior to in vitro transcription. The NH_2-terminal deleted BAS2 (amino acids 65 to 559) was prepared by fusing the SP6 promoter to the *BAS2* gene at nucleotide position +163 so that the in-frame ATG at position +193 (amino acid position 65) was used as the start of translation.

37. We thank K. Bostian and S. Tarent for the *pho2* strain and M. Christman, E. Elion, R. Last, H. Rudolph, and D. Shevell for comments on the manuscript. Supported by an NIH postdoctoral fellowship (K.T.A.) and NIH grant GM 35010-04 (G.R.F.).

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