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Regulation of *HIS4-lacZ* Fusions in *Saccharomyces cerevisiae*

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The beginning of the *Saccharomyces cerevisiae HIS4* gene has been fused to the structural gene for *Escherichia coli* β-galactosidase. This construction, which contains *HIS4* DNA from −732 to +30 relative to the translation initiation codon, has been integrated into the yeast genome at two chromosomal locations, *HIS4* and *URA3*. At both locations, this 762-base-pair stretch of DNA is sufficient for initiating expression of β-galactosidase activity in *S. cerevisiae* and confers upon this activity the regulatory response normally found for *HIS4*.

The *HIS4* gene of *Saccharomyces cerevisiae* encodes a single polypeptide which contains the enzymatic activities for steps 3, 2, and 10 in the pathway of histidine biosynthesis (5, 12). The *HIS4* gene, like many genes of amino acid biosynthesis, is under a complex general control. Starvation for histidine or for any one of a number of amino acids other than histidine leads to elevated levels of the *HIS4* protein. A number of genes specifying enzymes of the arginine, lysine, isoleucine-valine, and tryptophan pathways are also under this control (21, 27).

There are two classes of mutations which affect the general control response. One class has increased sensitivity to amino acid analogs (*aas*), and the other has increased resistance to amino acid analogs (*tra*). Strains carrying mutations of the *aas* class are unable to elevate the levels of amino acid biosynthetic enzymes; strains carrying mutations of the *tra* class produce these enzymes constitutively at a high level. These mutations affect the regulation of the same genes which respond to amino acid starvation. Recent studies have shown that the general control is exerted at the level of transcription (28; S. Silverman and G. R. Fink, manuscript in preparation; F. Messenguy, S. Silverman, M. Crabeel, C. Ilgen, and G. R. Fink, Abstr. Int. Conf. Yeast Genet. Mol. Biol. 10th, Louvin-la-Neuve, Belgium, p. 92, 1980). The steady-state levels of mRNA for *HIS4*, *ARG3*, and *TRP5* are increased three- to fivefold by amino acid starvation.

We have used a gene fusion between the *HIS4* gene of *S. cerevisiae* and the *lacZ* gene of *Escherichia coli* to locate the sequences required for response to the general control. Previous studies (3, 13, 23) have established the methods and utility of these fusions. In our work, a 762-base-pair fragment from the 5′ end of the *HIS4* gene was fused in frame to a large 3′-end fragment of *lacZ*. The hybrid contains 30 nucleotides of the *HIS4* coding sequence (including the ATG) preceded by 732 nucleotides that are normally 5′ to the start of translation of *HIS4*. This hybrid gene expresses an active β-galatosidase in *S. cerevisiae* both on an autonomous plasmid and in a single copy on a chromosome. The β-galactosidase activity produced from this fusion is regulated by amino acid starvation in a fashion similar to that of the normal *HIS4* region. The *tra* and *aas* mutations which affect normal *HIS4* expression also affect the expression of β-galactosidase in the *HIS4-lacZ* fusion. These studies show that the information required for transcription initiation and for the general control response is located in a 762-base-pair fragment 5′ to the first ATG of *HIS4*.

MATERIALS AND METHODS

Genetic analysis. Procedures for tetrad and random spore analysis were performed as described in the Cold Spring Harbor yeast manual (24).

Growth of cells. To detect the presence of β-galactosidase activity in cells grown on petri plates, a colorimetric indicator was used; solid medium containing 5-bromo, 4-chloro, 3-indolyl-β-D-galactoside (X-gal, Bachem) was prepared as described previously (23). To grow *S. cerevisiae* in conditions which repress the general control, synthetic dextrose (SD) medium (24) plus all nutritional requirements was used. To elicit derepression of the enzymes for amino acid biosynthesis, two growth conditions were used. *S. cerevisiae* strains which contain a leaky arginine auxotrophy (*arg11*; 9) were grown in SD medium plus nutritional requirements but in the presence of a very low concentration of arginine (7.5 μM). For the second method, wild-type *S. cerevisiae* cells were grown in SD medium to 2.5×10^6 to 5×10^6 cells per ml and then 3-amino-1,2,4-triazole was added to 10 mM. In all cases,

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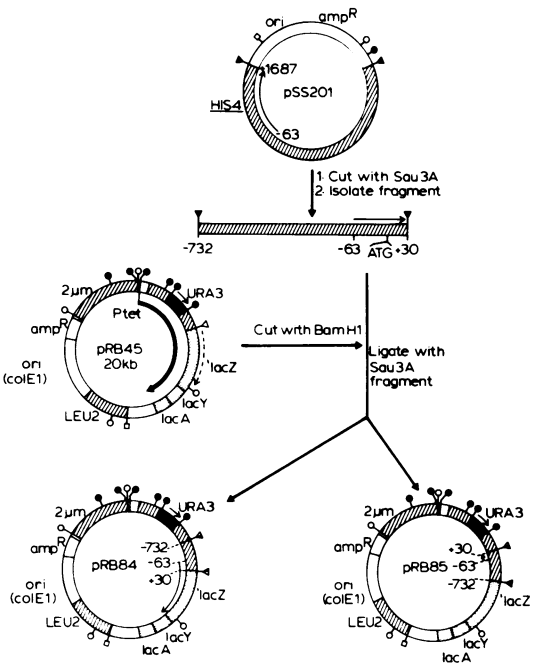

FIG. 1. Construction of autonomously replicating *HIS4-lacZ* fusion plasmids. Procedure for plasmid constructions is discussed in the text. Arrows adjacent to plasmids indicate direction of transcription. Numerals indicated on circles represent the number of nucleotides 5′ (negative) or 3′ (positive) relative to the site of translation initiation. *S. cerevisiae* sequences, except for the *URA3* gene, are hatched. The stippled area is derived from the *E. coli lac* operon. The *LEU2* gene is carried on a *Sal*I-*Xho*I fragment of *S. cerevisiae* chromosomal DNA. Abbreviations: ori, origin of DNA replication of *E. coli*; 2 μm, fragment of *S. cerevisiae* 2-μm DNA; amp^R, ampicillin resistance-encoding gene; tet^R, tetracycline resistance-encoding gene; Ptet, promoter for tet^r. Restriction endonuclease cleavage sites: ⚲, *Eco*RI; ⚳, *Bam*HI; ⚴, *Sal*I; ⚲, *Hin*dIII; ⚳, *Sau*3A; ⚳, *Bam*HI-*Sau*3A hybrid site. Only relevant *Sau*3A sites are indicated.

S. cerevisiae cells were harvested at about 0.5×10^7 to 2×10^7 cells per ml.

DNA preparations. Procedures for transformation of *S. cerevisiae* (15) and preparation of plasmid DNA and subcloning (11, 16, 22) have been described previously.

Plasmid constructions. The manipulations used for plasmid construction are shown in Fig. 1 and 2. pRB84 is the plasmid pRB45 (23) with the *HIS4 Sau*3A fragment extending from −732 to +30 (relative to the initial ATG) inserted in the *Bam*HI site. The orientation of the *Sau*3A fragment is such that a transcript initiated by the *HIS4* promoter should proceed towards the β-galactosidase gene. The coding sequence of *HIS4* is in frame with that of the β-galactosidase gene, and the resultant polypeptide should contain the first 10 amino acids of *HIS4* fused at amino acid 8 of β-galactosidase (8). Plasmid pRB85 has the same *Sau*3A fragment inserted at the same *Bam*HI site, in the opposite orientation from pRB84. pSS202, pSS203, and pSS204 are subclones of the respective parent plasmids. They were constructed in the following way (Fig. 2). pRB84, pRB85, and pRB45 were cleaved with *Hin*dIII and *Sal*I. The 7.8-kilobase-pair fragment containing the *HIS4*-β-galactosidase gene fusion (from pRB84 or pRB85) or the corresponding fragment from pRB45 were ligated into *YIP5* (2), which had been cleaved with the same restriction enzymes.

Plasmids thus constructed are capable of replication in *E. coli* conferring ampicillin resistance. YIP5 also contains the *URA3* gene of *S. cerevisiae*, which is capable of complementing an *S. cerevisiae ura3* mutation or an *E. coli pyrF* mutation. When transformed into *S. cerevisiae*, these plasmids can integrate into a chromosome by homologous recombination.

Strains. *E. coli* and *S. cerevisiae* strains used are listed in Table 1. The plasmid host in all *E. coli* strains was DB6507 (HB101, *hsdR hsdM recA thr leu thi endA pro supE44 pyrF74*::Tn*5*, Kan^r; 6). Plasmid DNA was then prepared (see above) for *S. cerevisiae* transformation. Plasmids pRB45, pRB84, and pRB85 are capable of autonomous replication in *S. cerevisiae* (with the 2-μm DNA replicator). They were used to transform *S. cerevisiae* strain 7881-6A (*MATa his4-912 arg11 leu2-3*) selecting for expression of the *LEU2* gene residing on the plasmids. *S. cerevisiae* strain TD28 (*MATa ura3-52 inos1 can1-100*) was transformed to Ura$^+$ with pSS202 or with pSS204 DNA. Strain DBY629 contains a *URA3-lac* fusion integrated at *URA3* (23).

Enzyme assays. Lysis of *S. cerevisiae* cells and

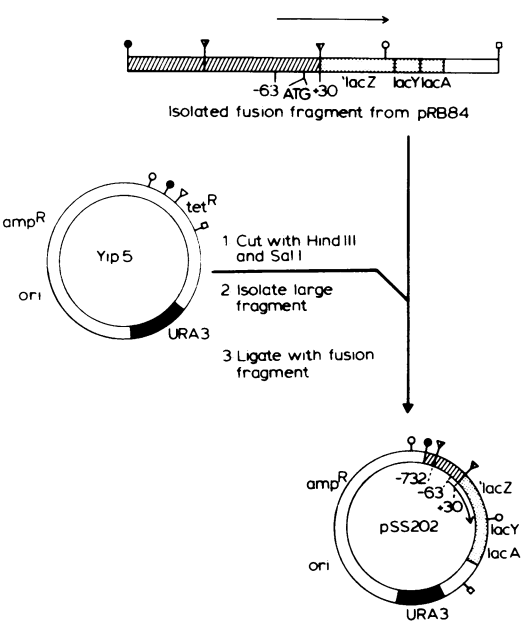

FIG. 2. Construction of integrating *HIS4-lacZ* fusion plasmid. See text for details. Restriction endonuclease cleavage sites are described in the legend to Fig. 1. The *URA3* gene in YIP5 is carried on a *Hin*dIII *S. cerevisiae* DNA fragment inserted into the *Ava*I site of pBR322.

TABLE 1. Strains

S. cerevisiae strain	Genotype	Plasmid used for transformation[a]	Chromosomal location of integrated plasmid
7881-6A	*MATa his4-912 arg11 leu2-3*	pRB45	Autonomous
SS101	*MATa his4-912 arg11 leu2-3*	pRB45	Autonomous
SS102	*MATa his4-912 arg11 leu2-3*	pRB84	Autonomous
SS103	*MATa his4-912 arg11 leu2-3*	pRB85	Autonomous
T9	*MATa ura3-52 inos1* Can^r	pSS202	*URA3*
T14	*MATa ura3-52 inos1* Can^r	pSS202	*HIS4*
DBY629	*MATa ura3-50*	pRB102 (*URA3-lacZ fusion*[b])	*URA3*
293-1D	*MATa tra3-1*	pSS202	*URA3*
293-1B	*MATa* wild type	pSS202	*URA3*
294-8B	*MATa tra3-1*	pSS202	*HIS4*
295-8A	*MATa aas1-1*	pSS202	*URA3*
296-13A	*MATα aas1-1*	pSS202	*HIS4*
GRF88	*MATα his4-38*	None	
7853-5B	*MATα his4-Δ29, arg11*	None	
TD1	*MATα ura3-52, his4-38, trp1*	None	

[a] Plasmids used for transformation of *E. coli* strains B207, B247, B206, B248, and B210 were pRB84, pSS202, pRB45, pSS204, and pSS203, respectively.
[b] M. Rose and D. Botstein, unpublished data.

assays of β-galactosidase activity were performed with a modification of a procedure previously described (23). Ten milliliters of *S. cerevisiae* cells were grown to an absorbancy at 590 nm of approximately 1.0, concentrated by centrifugation, and lysed by blending in a Vortex mixer with 0.4 ml of extraction buffer (0.1 M Tris-hydrochloride [pH 8.0]–20% [vol/vol] glycerol–1 mM dithiothreitol–1 mM phenylmethylsulphonyl fluoride [Sigma Chemical Co.]) and (0.250- to 0.300-mm) glass beads. Assays for β-galactosidase activity were performed as described previously (20), except that activity was normalized to protein concentration with bovine serum albumin as a standard (7). Units were those used in a previous publication (23) (nanomoles of *O*-nitrophenyl galactoside cleaved per minute per milligram of protein).

mRNA analysis. *S. cerevisiae* cells were grown in supplemented SD medium or YEPD (yeast extract peptone dextrose) medium (see above) and harvested at about 2×10^7 cells per ml. They were resuspended in 1 M Sorbitol–50 mM EDTA (pH 8.0)–0.36 M β-mercaptoethanol plus 200 μg of cycloheximide per ml. Zymolyase (35 μg/ml, Kirin Brewery; 60,000 U/g) was added to convert the cells to spheroplasts. When greater than 90% of the cells were converted to spheroplasts (after 10 to 40 min of incubation at 37°C), the cells were harvested, resuspended in 15 ml of 0.5 M NaCl–0.2 M Tris-hydrochloride (pH 7.4)–0.01 M EDTA–0.2% diethylpyrocarbonate, and lysed by addition of 1.5 ml of 10% sodium dodecyl sulfate. The lysate was frozen at −80°C, thawed, and centrifuged at 10,000 × *g* for 15 min. The supernatant solution was extracted with 1:1 phenol-chloroform until no interphase was present (at least two times) and precipitated twice by the addition of 2.5 volumes of ethanol.

The RNA pellet was subjected to chromatography on oligodeoxythymidylate cellulose (1) (type 3; Collaborative Research), and the fraction retained in high-salt polyadenylated [(poly(A)$^+$] RNA was recovered.

Northern gel analysis of the resultant poly(A)$^+$ mRNA was performed as described previously (25), except for the following modifications. RNA was denatured by heating in a solution containing 50% deionized formamide, 2.2 M formaldehyde, and 10 mM $NaPO_4$ (pH 7.0) at 50°C for 15 min before loading on a 1.0% agarose gel containing 6% formaldehyde and 10 mM $NaPO_4$ (pH 7.0). After transfer and baking, the filter was placed in distilled water which had been heated to 100°C. This was allowed to cool at room temperature for at least 15 min. DNA probes were labeled with ^{32}P by nick translation (17). Filters were exposed to Kodak XAR-5 X-ray film with Du Pont intensifying screens.

RESULTS

Expression of β-galactosidase on autonomous plasmids. The *Sau*3A fragment of *HIS4* was fused in vitro at the *Bam*HI site of plasmid pRB45. Two plasmids that contain *HIS4-lacZ* fusions were studied. Plasmid pRB84 contains the *HIS4-Sau*3A fragment oriented in such a way that transcription originating at the normal *HIS4* promoter should proceed toward the *lacZ* gene, and the normal ATG of *HIS4* is in frame with the *lacZ* gene. Plasmid pRB85 has the same *Sau*3A fragment in the opposite orientation. Each plasmid also has an *E. coli* selectable marker (Amp^r), an *E. coli* origin of replication (from pBR322), *S. cerevisiae* selectable markers (*LEU2* and *URA3*), and an *S. cerevisiae* origin of replication (a segment of the *S. cerevisiae* plasmid 2-μM circle).

S. cerevisiae strain 7881-6A was transformed to Leu^+ with each of these plasmids carrying *HIS4-lacZ* fusions and with the parent plasmid (pRB45), which contains no *HIS4* sequences. The transformants segregated Leu^- cells at a

TABLE 2. Linkage of *HIS4-lacZ* fusion to *HIS4* or *URA3*

Cross[a]	Number of tetrads[b]				
	His$^+$: blue colony[c]				
	PD	NPD	TT		
GRF88 (His4$^-$) × T11 (His4$^+$)	21	0	1		
7853-5B (His4$^-$) × T14 (His4$^+$)	17	0	0		
	Ura$^+$: blue colony[d]				
	PD	NPD	TT		
TD-1 (Ura3$^-$) × T6 (Ura3$^+$)	6	0	0		
TD-1 (Ura3$^-$) × T7 (Ura3$^+$)	8	0	0		
TD-1 (Ura3$^-$) × T12 (Ura3$^+$)	4	0	0		
	Ura$^+$:Ura$^-$ segregation[e]				
	4:0	3:1	2:2	1:3	0:4
7853-5B (Ura3$^+$) × T9 (Ura3$^+$)	17	1	0	0	0

[a] Only relevant phenotypes of strains are indicated.
[b] PD, Parental ditype; NPD, nonparental ditype; TT, tetratype.
[c] Transformants were crossed to a strain containing a *his4* allele. The histidine requirement and the color of the colonies (indication of β-galactosidase activity; see text) were scored on petri plates for the ascospore clones. Linkage of the *HIS4-lacZ* fusion to *HIS4* was demonstrated by finding a predominance of parental ditype tetrads.
[d] Transformants were crossed to a cell containing a *ura3* allele. Uracil requirement and the colorimetric indication of β-galactosidase activity were assayed on petri plates for the resultant spores. Linkage of *URA3* to the *HIS4-lacZ* fusion was demonstrated by finding a predominance of parental ditype tetrads.
[e] Plasmid pSS202 contains the *HIS4-lacZ* fusion and the *URA3*$^+$ gene. Linkage of the fusion to chromosomal *URA3* was assayed by crossing the transformant (originally *ura3-52*) to a Ura3$^+$ cell. The uracil requirement of the resultant spores was assayed on petri plates. Linkage was demonstrated by finding mostly 4:0 tetrads for Ura$^+$:Ura$^-$.

high frequency (approximately 50%), indicating that the plasmids were autonomous. On X-gal medium, transformant SS102 (containing plasmid pRB84) was bright blue, whereas transformants SS103 (containing plasmid pRB85) and SS101 (containing plasmid pRB45) were white. Direct assay of β-galactosidase activity in extracts of cells grown in the absence of leucine (to maintain the plasmid) showed that only SS102 expressed significant levels of this enzyme. The activity present in SS102, 1,480 U, corresponds to about 10% of the activity of *E. coli* when it is fully induced for *lac* operon expression. As compared with SS102, SS103 and SS101 produced 70- and 1,500-fold lower activity, respectively. Thus, substantial enzyme activity is produced only when the *HIS4 Sau*3A fragment is oriented properly for the initiation of transcription and translation to proceed into the *lacZ* gene.

Analysis of integrated *HIS4-lacZ* fusions. We chose to study the regulation of our *HIS4-lacZ* fusions in the integrated rather than the autonomous state. Our preliminary analysis of regulation of enzyme levels in strains containing the fusion on an autonomous plasmid was complicated by the stability and copy number of the plasmid under different growth conditions and in different genetic backgrounds. We constructed integrating vectors by subcloning the appropriate fragments of the *HIS4-lacZ* fusions into YIP5, a plasmid which contains *S. cerevisiae* selectable marker (*URA3*$^+$) but no *S. cerevisiae* origin of replication. Two YIP5 derivatives were studied. Plasmid pSS202 contains the *HIS4-lacZ* fusion from pRB84, and plasmid pSS204 contains the *lacZ* gene fragment from pRB45. The *lacZ* gene fragment in pSS204 has no signals for initiation of transcription or translation in *S. cerevisiae* and served as a control in our experiments. Strain TD28 was transformed to Ura$^+$ with each of these plasmids. On X-gal medium, transformants with pSS202 were blue (5% were white and were not investigated further), whereas all those with pSS204 were white. The pSS202 transformants were picked, purified, and crossed to Ura3$^+$, Ura3$^-$, and His4$^-$ strains of opposite mating type. Since both *URA3* and *HIS4* sequences are present on pSS202, it can integrate by homologous recombination at either *HIS4* or *URA3*. The location of the integrated sequences can be determined by tetrad analysis of such crosses (Table 2). Of six transformants analyzed, two contained the plasmid integrated at *HIS4* and four had the plasmid integrated at *URA3*. Two transformants, one containing plasmid pSS202 integrated at *HIS4* (T14) and one with pSS202 integrated at *URA3* (T9), were subjected to further analysis.

We constructed strains containing pSS202 at both chromosomal locations in different genetic backgrounds to determine whether the β-galactosidase activity produced by the fusion responds to amino acid starvation and to regulatory mutations. Transformants T9 and T14 were crossed to strains containing the *aas1-1* mutation or the *tra3-1* mutation. Ascospores which contained the *HIS4-lacZ* fusion and the desired regulatory mutation or the corresponding wild-type allele were identified by genetic analysis and assayed for β-galactosidase activity. In addition, strain DBY629, which contains an integrated copy of a *URA3-lacZ* fusion, was assayed as a control. The *URA3-lacZ* fusion is a fragment of the *URA3* gene from −223 to +48

TABLE 3. β-Galactosidase enzyme activity in *S. cerevisiae* strains

S. cerevisiae strain	Fusion chromosomal location	Relevant genotype	Growth in[a]:			Extent of derepression[b]
			YEPD medium	Minimal medium		
				Repressed	Derepressed	
SS101	Autonomous	Wild type	ND	0.95	ND	
SS102	Autonomous	Wild type	ND	1,480	ND	
SS103	Autonomous	Wild type	ND	20	ND	
DBY629	*URA3*	Wild type	ND	83	80	0.96
T9	*URA3*	Wild type	60	330	910	2.8
T14	*HIS4*	Wild type	87	435	1,070	2.5
293-1B	*URA3*	Wild type	24.5	440	1,200	2.7
293-1D	*URA3*	*tra3-1*	2,710	3,030	2,120	0.70
294-8B	*HIS4*	*tra3-1*	3,900	2,540	2,610	1.0
295-8A	*URA3*	*aas1-1*	20.8	697	725	1.0
296-13A	*HIS4*	*aas1-1*	13.7	586	462	0.7

[a] Growth conditions are explained in the text. Units are expressed in nanomoles of *O*-nitrophenyl galactoside cleaved per minute per milligram of protein. ND, Not done.

[b] Ratio of enzyme activity determined for a strain when grown under derepressed conditions versus that for a strain grown under repressed conditions. Each value represents the average of at least two experiments. Values for each determination varied as much as 50%. The extent of derepression varied no more than 20% between different experiments.

nucleotides from the ATG, fused in frame to the large 3′ *lacZ* gene fragment (M. Rose, and D. Botstein, unpublished data). It has the β-galactosidase under uracil regulation and should not respond to the general control. The β-galactosidase activity of these strains grown under various physiological conditions is shown in Table 3.

We compared the extent of derepression of β-galactosidase activity in these strains with that of *HIS4* enzyme levels in a wild-type strain. *HIS4* enzyme activity is about fourfold higher in cells grown under derepressing conditions as compared with that in cells grown under repressing conditions (27). The extent of derepression of β-galactosidase activity in strains T9, T14, and 293-1B, about 2.7-fold (Table 3), compares favorably with this figure. The stability of a hybrid polypeptide not normally found in *S. cerevisiae* may affect the apparent activity. In strain DBY629, which contains the *URA3-lacZ* fusion, the expression of the *URA3* gene does not change in response to the general amino acid control; the level of β-galactosidase activity is essentially the same whether the cells are grown in media which repress or derepress amino acid biosynthetic enzymes.

The effect of regulatory mutations *aas1-1* and *tra3-1* on the *HIS4-lacZ* fusion in plasmid pSS202 was also studied. Cells containing the *aas1-1* mutation have the basal level of amino acid biosynthetic enzymes, and cells with the *tra3-1* mutation have the high (derepressed) level. These levels of activity are independent of the state of amino acid deprivation imposed by the medium. β-Galactosidase activities produced by the integrated plasmids in these two genetic backgrounds act in a way similar to those for amino acid biosynthetic enzymes. The differences between the basal (*aas*) and derepressed (*tra*) levels vary between 2.3- and 5.6-fold (Table 3). These results demonstrate that the *HIS4-lacZ* fusion responds to the general amino acid control by regulating the activity of β-galactosidase in a way similar to *HIS4* or other genes coding for amino acid biosynthetic enzymes. The general control exerted on β-galactosidase activity is the same whether the plasmid containing the fusion is integrated on chromosome III at *HIS4* (T14, 294-8B, 296-13A) or on chromosome V at *URA3* (T9, 293-1B, 293-1D, 295-8A).

Expression of fusion gene in rich media. We also assayed the effect of complex media on β-galactosidase specific activity. It has been shown that the activity of arginosuccinase, an enzyme which is subject to the general control, was decreased by up to fourfold when cells were grown in rich (YEPD) versus supplemented minimal medium (18). This reduction in arginosuccinase on YEPD medium occurred in *aas2* and *aas3* cells as well as in wild-type cells. When the strains listed in Table 3 were grown in minimal medium supplemented with up to 10 times the normal concentrations of all 20 amino acids, there was no significant change in β-galactosidase activity from the basal level (data not shown). However, when cells were grown in YEPD medium, we observed a significant decrease in enzyme activity in wild-type cells or

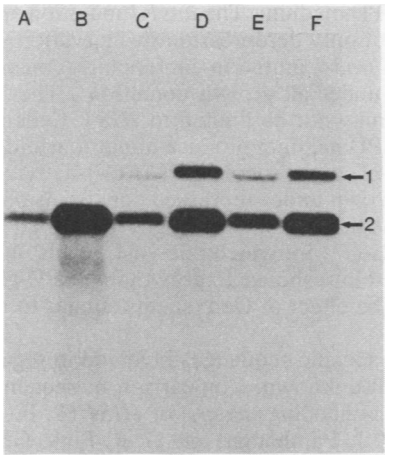

FIG. 3. Analysis of mRNAs produced in *S. cerevisiae* strains. RNA was isolated from three different *S. cerevisiae* strains, each grown under conditions which repress (lanes A, C, and E) and which derepress (lanes B, D, and F) levels of amino acid biosynthetic enzymes. Poly(A)$^+$ RNA was then subjected to Northern gel analysis (see text). The filter was probed first for β-galactosidase-specific sequences by hybridization to radioactively labeled M13 DNA containing the *lacZ* gene. The same filter was then probed with *HIS4*-specific sequences in a similar way. RNA from wild-type *S. cerevisiae* grown under repressing (lane A) and derepressing (lane B) conditions; RNA from transformant T9 (containing the *HIS4-lacZ* fusion integrated at *URA3*) grown under repressing (lane C) and derepressing (lane D) conditions; and RNA from transformant T14 (containing the *HIS4-lacZ* fusion integrated at *HIS4*) grown under repressing (lane E) and derepressing (lane F) conditions are shown.

cells harboring the *aas1-1* mutation, but not in cells harboring the *tra3-1* mutation (Table 3).

mRNA analysis. To determine whether the regulatory response of β-galactosidase activity was a result of a change in the level of mRNA coding for the enzyme, we compared the levels of β-galactosidase-specific mRNA in wild-type cells and cells transformed with pSS202. Poly(A)$^+$ RNA extracted from each strain grown under repressed and derepressed conditions was subjected to electrophoresis on a 1.0% agarose gel and blotted onto a nitrocellulose filter. The filter was then probed with nick-translated ^{32}P-labeled YIP5 plasmid DNA (data not shown). The YIP5 plasmid contains sequences homologous to *URA3*, a gene whose expression does not change with the state of amino acid limitation. Hybridization with this plasmid gave us a measure of the relative amount of poly(A)$^+$ RNA present in each preparation. On the basis of this determination, a filter was prepared with equal amounts of poly(A)$^+$ RNA in each track. This filter was incubated with ^{32}P-labeled M13 mp7 replicative-form DNA (specific activity, approximately 8.6×10^6 Cerenkov cpm/μg of DNA; kindly provided by Forrest Chumley) which contains β-galactosidase-specific sequences (19). Wild-type *S. cerevisiae* contains no mRNA which hybridizes to the M13 mp7 probe (see Fig. 3, lanes A and B). The two original *S. cerevisiae* transformants (T9 and T14) analyzed in Table 3 were found to contain a single mRNA which hybridized to the β-galactosidase-specific probe (the slower-migrating species; Fig. 3, lanes C through F). To show that the general amino acid control in these mRNA preparations was elicited in the expected way, the same filter was probed with *HIS4*-specific ^{32}P-labeled plasmid DNA (pHIS4-PST [14]; specific activity, approximately 2.1×10^7 Cerenkov cpm/μg of DNA; Fig. 3). In each strain tested, the extent of derepression of *HIS4* mRNA was, as determined by comparing band densities on a gel scanner, about fourfold. The mRNA corresponding to β-galactosidase coding sequence was derepressed to about the same extent. Therefore, the level of β-galactosidase-specific mRNA responds to general amino acid regulation in a way similar to that of *HIS4* mRNA (Fig. 3). Consideration of the different specific activities of the DNA probes allow us to infer that the levels of *HIS4* and β-galactosidase-specific mRNAs present in *S. cerevisiae* are roughly the same.

The size of the mRNA corresponding to β-galactosidase coding sequences is about 3.3 kilobase pairs, that expected for a fusion message coding for the *HIS4* amino terminal peptide and the *E. coli* β-galactosidase (Z gene only) fragment. Thus, in these fusions, the entire *lac* operon is not transcribed into a detectable RNA. Either early transcription termination occurs at the end of *lacZ* or rapid processing of a longer message occurs.

DISCUSSION

In *S. cerevisiae*, alterations of 5′ noncoding sequences of several genes seem to affect their regulation (10, 26; Silverman and Fink, manuscript in preparation). We wanted to determine whether the 5′ noncoding sequences in *HIS4* are responsible for its regulation by the general amino acid biosynthetic enzyme control system. If sequences 5′ to the coding region of *HIS4* are necessary and sufficient to elicit this type of control, then fusion of only these sequences to an unrelated gene should place this gene under the general control. Recently, the technique of gene fusions was employed for studying the regulatory sequences of the *S. cerevisiae URA3* (23) and *CYC1* (13) genes.

We have found that the fusion of *HIS4* 5′

noncoding sequences to the *E. coli* β-galactosidase gene on an *S. cerevisiae* recombinant plasmid produces β-galactosidase activity when transformed into *S. cerevisiae*. The amount of activity produced is dependent on the orientation of the inserted fragment and on the genetic background of the cell. For any given strain, the extent of derepression of β-galactosidase activity and β-galactosidase-specific mRNA is similar to that found for *HIS4* enzyme or mRNA under the same physiological conditions. This derepression can occur independently of any other *cis*-acting *HIS4* sequences, as is shown by the normal control of *HIS4-lacZ* fusions which are integrated at *URA3*.

We conclude that the fragment from −732 to +30 from the ATG of *HIS4* contains all of the information necessary for transcription initiation as well as for controlling the level of expression of sequences downstream. We believe that this control occurs at the level of transcription initiation, as opposed to mRNA stabilization, because the mRNA produced is an entirely new species in *S. cerevisiae*, except for the short sequence between the normal 5′ end and +30 base pairs into the coding region. This stretch of mRNA from the amino terminus of *HIS4* is not necessary for regulation of *HIS4* mRNA levels, because a mutant lacking sequences from −161 to +92 still regulates the level of the remaining *HIS4* mRNA (Silverman and Fink, manuscript in preparation). If the general control works by selectively stabilizing amino acid biosynthetic enzyme mRNAs, then this β-galactosidase mRNA should not be so stabilized. The simplest explanation for the presence of more β-galactosidase mRNA in derepressed cells is that more of this mRNA is synthesized.

The regulation of *HIS4* is the same whether the *HIS4-lacZ* fusion is integrated at its normal location (at *HIS4* on chromosome III) or at the *URA3* locus on chromosome V. Therefore, not only are all of the necessary regulatory sequences present, but there are no perceptible effects of the chromosomal context.

One of the virtues of the *lac* fusion technique is the ability to measure extremely low levels of β-galactosidase. We had not been able previously to detect any of the three *HIS4* enzymatic activities in cells grown on complete YEPD medium. Our ability to measure low levels of β-galactosidase in the *HIS4-lacZ* fusions has revealed several new aspects of *HIS4* regulation. First, *HIS4* appears to repress at least 5- to 20-fold on YEPD medium as compared with synthetic minimal medium. The overall extent of derepression between cells grown on YEPD medium and cells grown under derepressed conditions is at least 12-fold. Second, the *aas1* mutation does not seem to affect the repression on YEPD medium. The *aas1-1* mutation appears to affect only derepression by starvation. Finally, the *tra3-1* mutation confers high constitutive levels under all growth conditions. The YEPD effect may not be limited to *HIS4*. Cells grown on YEPD medium produce about fourfold lower levels of arginosuccinase (*ARG4*) activity than cells grown under repressed conditions on minimal medium (18). Messenguy described a mutant, Gen^c, in which enzyme levels are not reduced in response to growth in YEPD-glucose (18). The effect of Gen^c seems similar to that of *tra3-1*.

The specific sequences involved in regulation are still unknown. Comparison of sequences in the 5′ noncoding regions of *HIS4* (T. F. Donahue, P. J. Farabaugh, and G. R. Fink, Gene, in press) and *HIS3* (K. Struhl, Ph.D. thesis, Stanford University, Stanford, Calif., 1979) led to the identification of some similarities that could be significant (E. W. Jones and G. R. Fink, *in* J. N. Strathern, E. W. Jones, and J. R. Broach, ed., *Molecular biology of the yeast Saccharomyces*, in press). An interesting structural feature of this region in *HIS4* is the repetition of variants on a "consensus" sequence (Donahue et al., in press). Direct repeats of this sequence have recently been found in the 5′ noncoding region of *HIS1* (A. Hinnebusch, personal communication), and repeats of a different consensus sequence have been found in *TRP5* (28). It has recently been shown that the 5′ flanking region of the human insulin gene contains several nontandemly repeated sequences of about 10 base pairs, one member of which is located in the control region for the gene (4). Structures of this type may be important for defining recognition regions for regulatory molecules (such as the *AAS* and *TRA* gene products) or for determining the structure of chromatin at genes under the general amino acid control under different physiological conditions.

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