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Repression of the Genes for Lysine Biosynthesis in *Saccharomyces cerevisiae* Is Caused by Limitation of Lys14-Dependent Transcriptional Activation

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The product of the *LYS14* gene of *Saccharomyces cerevisiae* activates the transcription of at least four genes involved in lysine biosynthesis. Physiological and genetic studies indicate that this activation is dependent on the inducer α-aminoadipate semialdehyde, an intermediate of the pathway. The gene *LYS14* was sequenced and, from its nucleotide sequence, predicted to encode a 790-amino-acid protein carrying a cysteine-rich DNA-binding motif of the $Zn(II)_2Cys_6$ type in its N-terminal portion. Deletion of this N-terminal portion including the cysteine-rich domain resulted in the loss of *LYS14* function. To test the function of Lys14 as a transcriptional activator, this protein without its DNA-binding motif was fused to the DNA-binding domain of the *Escherichia coli* LexA protein. The resulting LexA-Lys14 hybrid protein was capable of activating transcription from a promoter containing a *lexA* operator, thus confirming the transcriptional activation function of Lys14. Furthermore, evidence that this function, which is dependent on the presence of α-aminoadipate semialdehyde, is antagonized by lysine was obtained. Such findings suggest that activation by α-aminoadipate semialdehyde and the apparent repression by lysine are related mechanisms. Lysine possibly acts by limiting the supply of the coinducer, α-aminoadipate semialdehyde.

The yeast *Saccharomyces cerevisiae* synthesizes lysine through the α-aminoadipate pathway (Fig. 1), which is characteristic of the higher fungi (6). Three types of regulatory effects have been found to influence gene expression in this pathway. The general control of amino acid biosynthesis leads to increased levels of at least four enzymes of the pathway in response to starvation for any one of a number of amino acids (32, 38, 39). The synthesis of six of the eight enzymes of the pathway is reduced when an excess of lysine is present in the growth medium; this apparent repression is impaired by the *lys80* mutations previously proposed to affect a negative regulator of *LYS* gene expression (32). Finally, several enzymes of the pathway were found to respond to an induction mechanism involving the product of gene *LYS14* and an intermediate of the pathway, α-aminoadipate semialdehyde, which acts as an inducer (31).

The existence of the induction mechanism was originally suggested by the behavior of *lys14* mutants. Such mutants are leaky lysine auxotrophs displaying a low but significant residual activity of saccharopine dehydrogenase (glutamate forming); in contrast, *lys9* mutants are totally devoid of this activity (8, 31). In addition, *lys14* mutants were found to be suppressed by the amplification of the *LYS9* gene, suggesting that the product of *LYS14* was required for the expression of *LYS9* (31). The *lys9* mutants lack saccharopine dehydrogenase (glutamate forming) and require lysine for growth, yet even when grown in the presence of lysine in excess, they exhibit derepressed levels of homocitrate synthase, α-aminoadipate reductase and the lysine-forming saccharopine dehydrogenase (31). Such a derepression does not occur in a *lys2 lys9* mutant, which is devoid of the activity of α-aminoadipate reductase in addition to that of saccharopine dehydrogenase (glutamate forming). Since such a double mutant fails to accumulate α-aminoadipate semialdehyde intracellularly like a *lys9* mutant, this observation was taken as evidence that this intermediate, or a closely related derivative, is required in addition to the Lys14 protein for the induction of the *LYS* genes (31).

LYS1, encoding saccharopine dehydrogenase (lysine forming), *LYS9*, and *LYS14* have been cloned, and their mRNAs have been assayed by Northern (RNA) hybridization in different strains and under various growth conditions. The levels of *LYS1* and *LYS9* mRNAs showed variations that were parallel to those of the cognate enzymes, supporting the view that both the apparent repression by lysine and the *LYS14*-dependent induction by α-aminoadipate semialdehyde operate transcriptionally. The level of *LYS14* mRNA was low and did not vary under the conditions investigated (31).

In this study, we have achieved a more extensive analysis of the *LYS14* gene product, with the purpose of further identifying its function in the control of the expression of the *LYS* genes. Our results confirm the transcriptional activation function of Lys14. Furthermore, we obtained evidence that this activation function, which is dependent on the presence of α-aminoadipate semialdehyde, is antagonized by lysine. Such findings suggest that activation by α-aminoadipate semialdehyde and the apparent repression by lysine are related mechanisms. Lysine could act by limiting the supply of the coinducer, α-aminoadipate semialdehyde, or by somehow preventing the activation function of Lys14.

MATERIALS AND METHODS

Strains and media. All strains of *S. cerevisiae* were derivatives of the *MAT*α wild-type strain Σ1278b (5): 12T7c (*ura3*), 8903c (*lys14*), 18T8d (*gcn4*), 18T9b (*gcn4 lys14*), 12T2a (*ura3*

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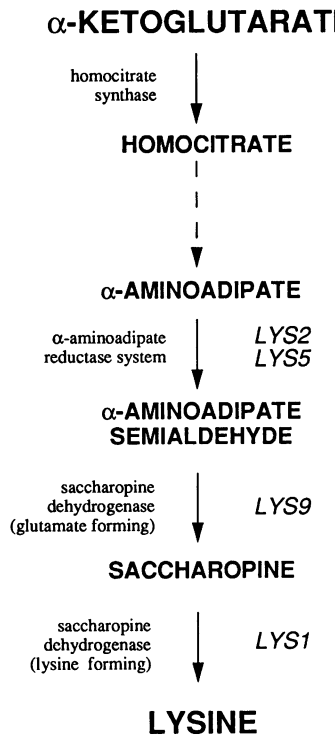

FIG. 1. Lysine biosynthetic pathway of *S. cerevisiae*. Only the intermediates, enzymatic steps, and genes pertinent to the present work are shown.

lys14), 1C2123a (*ura3 leu2*), 02483a (*ura3 leu2 lys14*), 02485d (*ura3 leu2 lys2*), 02485c (*ura3 leu2 lys9*), 02492c (*ura3 leu2 lys2 lys9*), and 20T6b (*ura3 leu2 lys80*). The *Escherichia coli* strains used for plasmid maintenance and for propagation of the single-stranded DNA template were XL1-B {*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*lacZ*ΔM15 Tn*10*(Tet^r)]} from Stratagene and JM103 [*endA1 hsdR supE sbcBC thi-1 strA* Δ(*lac-pro*) (F′ *traD36 proAB lacI*^{q}*lacZ*Δ*M15*)].

All yeast strains were grown on minimal medium (M.am) containing 10 mM $(NH_4)_2SO_4$, 3% glucose, vitamins, and mineral traces as described previously (22). L-Lysine (6 or 66 μg/ml) was added to M.am where indicated below. Where specified, 100 μg of DL-α-aminoadipate per ml, 20 μg of uracil per ml, or 50 μg of leucine per ml was also added to M.am.

Sequence analysis. Restriction fragments were cloned into M13mp18, M13mp19, pBS(+SK), or pBS(+KS). The nucleotide sequences of both strands were determined by the dideoxy-chain termination technique according to the method of Sanger et al. (34). The sequence of single- or double-stranded DNA was determined with the universal primer or synthetic oligonucleotides and α-^{35}S-dATP purchased from Amersham.

Cloning of the *LYS14* gene. Sequencing and Southern blot analysis showed that the pFR3 plasmid described previously (31) does not contain the complete *LYS14* gene. A *Sau*3A-*Sau*3A DNA fragment from the *ARO3* gene had been inserted into the *Bgl*II restriction site of *LYS14* (data not shown), probably during the construction of the library. Therefore, the cloning of the *LYS14* gene was repeated with a new library (a gift from S. Vissers) containing *Sau*3A-*Sau*3A partial fragments of genomic DNA from strain Σ1278b cloned into the *Bam*HI restriction site of pFL38 (7). The DNA of this library was used to transform strain 12T2a (*lys14 ura3*) to lysine prototrophy as described by Ramos et al. (31). The pMEA4 plasmid contains an insert of 6.3 kb bearing the *LYS14* gene. A 3.8-kb *Xho*I-*Xho*I DNA fragment from pMEA4 was inserted into the *Sal*I restriction site from pFL38 to generate the pLAF32 plasmid.

Plasmids. The pLAF1 plasmid is pBS(+SK) in which the 2.75-kb *Bgl*II-*Bam*HI fragment bearing the *LYS14* gene from pFR3 (31) was inserted into the *Bam*HI restriction site. Plasmid pFLAF1 is pFL44 (7) in which the *Eco*RI restriction site was destroyed and from which the *Bgl*II-*Bgl*II *URA3* fragment was removed. Plasmid pQH51 was described by Qiu et al. (30). It contains the sequence encoding the first 87 amino acids of LexA and the promoter of the *ADH1* gene (P_{ADH1}). The *lexA* vector junction allows the construction of in-frame *lexA* gene fusions. The pRB500 plasmid (a gift from R. Brent), which contains the *lexA* gene, and the p1840 plasmid, which bears part of the *GAL1* gene fused to *lacZ* under the control of the *LexA* operator, were described by Brent and Ptashne (9).

Disruption of the *LYS14* gene. The 3.8-kb *Xho*I-*Xho*I fragment of pMEA4 was inserted into the *Xho*I site of pBS(+SK) to obtain pLAF40. The creation of a *Sma*I restriction site just after the ATG of *LYS14* was performed by oligonucleotide-directed in vitro mutagenesis with an Amersham kit to obtain the pME17 plasmid. The 3.8-kb *Xho*I-*Xho*I fragment of pME17 was inserted into the *Xho*I site of pBS(+KS), which has a deletion between the *Eco*RV and the *Sma*I sites of its multiple cloning sites, to obtain pLAF43. The 1.8-kb *Sma*I-*Eco*RV segment from pLAF43 was replaced by the Klenow-blunted 1.1-kb *Bgl*II-*Bgl*II fragment bearing the *URA3* gene, yielding plasmid pLAF44. The haploid *ura3* strain 12T7c was transformed with the *Xho*I-*Xho*I fragment of pLAF44 containing the deleted and disrupted *lys14*::*URA3* gene (Fig. 2).

LexA-Lys14 protein fusions. To construct the LexA-Lys14 protein fusion (extending from residues 278 to 790), we created a *Sna*BI restriction site at positions 826 to 831 in the pLAF1 plasmid to generate the pBLAF1 plasmid. This restriction site was created by oligonucleotide-directed in vitro mutagenesis with an Amersham kit. The 1,555-bp *Sna*BI-*Sna*BI fragment from pBLAF1 was inserted into the *Sma*I restriction site of plasmid pQH51, which allowed the construction of an in-frame *lexA-LYS14* gene fusion, yielding the pBLAF2 plasmid.

The *Bam*HI-*Bam*HI fragment containing P_{ADH1} and the *lexA-LYS14* fusion from pBLAF2 was inserted into the *Bam*HI restriction site of the pAAH5 vector constructed by G. Ammerer (2), yielding pBLAF3.

Deletion of the Lys14 N terminus. The 1,555-bp *Sna*BI-*Sna*BI fragment from pBLAF1, coding for the 513 C-terminal amino acids, was inserted into the *Bam*HI restriction site of the YEp52 plasmid (pBR322 carrying the *LEU2* gene and part of the 2μm plasmid; a gift from F. Lacroute) containing the galactose-inducible *GAL10* promoter, yielding the pLAF16 plasmid. This construction led to the synthesis of Lys14 beginning at methionine 278.

Enzyme assays. Saccharopine dehydrogenase ($NADP^+$, glutamate forming) (EC 1.5.1.10) activity was assayed as described by Jones and Broquist (16) by monitoring the reduction of 0.2 mM $NADP^+$. Saccharopine dehydrogenase (NAD^+, lysine forming) (EC 1.5.1.7) activity was assayed as described by Fujioka and Nakatani (14). The activity of β-galactosidase was assayed by the method described by Miller (25). Argininosuccinate lyase (EC 4.3.2.1) was assayed as described by Delbecq et al. (12). The protein concentration was determined by the Folin method.

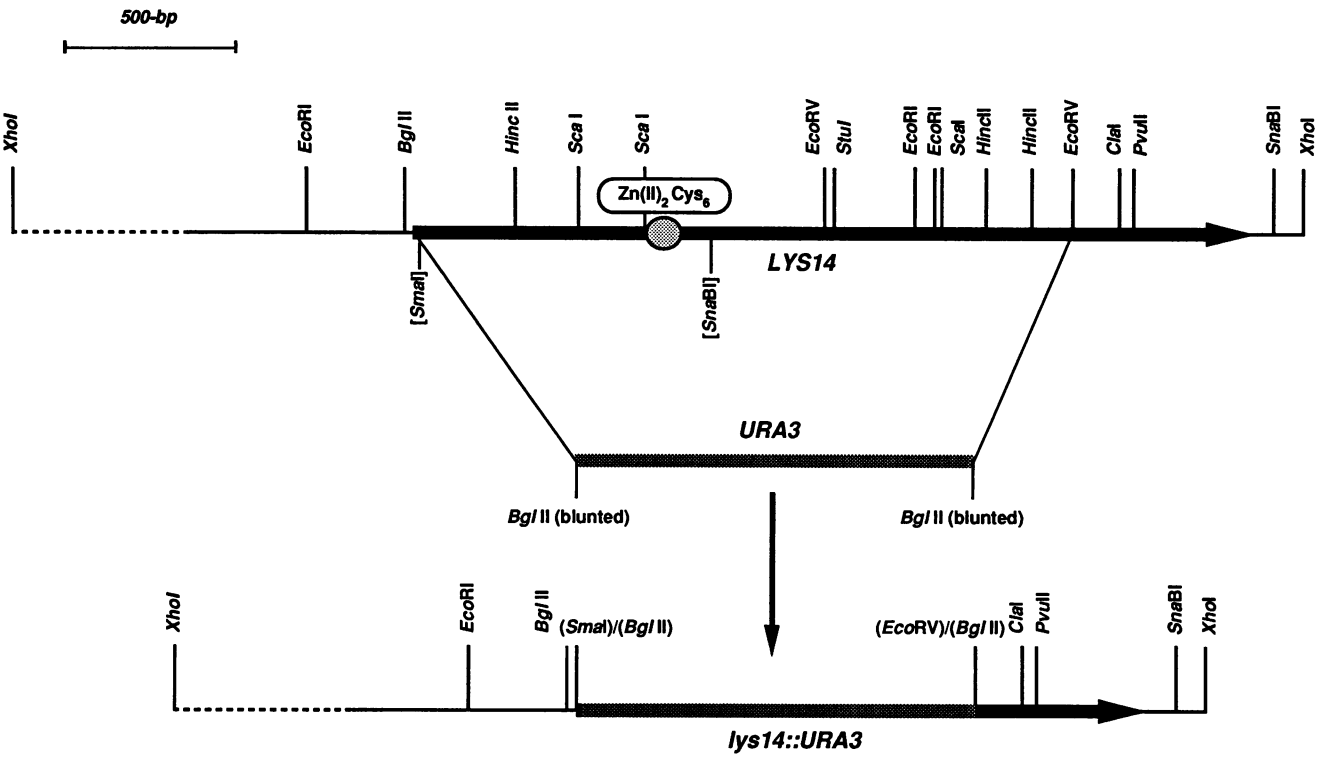

FIG. 2. Restriction map of a 3.8-kb DNA stretch containing the *LYS14* gene, including its disruption by insertion of a segment bearing the *URA3* gene. The large arrow indicates the position of the *LYS14* open reading frame. The dashed line corresponds to the position of the insert, which has not been sequenced. The restriction sites in brackets were created by in vitro mutagenesis. A 1.8-kb *Sma*I-*Eco*RV fragment corresponding to residues 2 to 609 on plasmid pLAF43 was replaced by a *Bgl*II-*Bgl*II fragment containing the *URA3* gene, yielding the disrupted gene *lys14*::*URA3*.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession number X77361.

RESULTS

Sequence of the *LYS14* gene. The pLAF32 plasmid, which contains a 3.8-kb *Xho*I-*Xho*I fragment from pMEA4 (see Materials and Methods), was found to complement a *lys14* mutation, thus indicating that it carries the *LYS14* gene. The sequences of both strands of a 3.1-kb DNA stretch from this *Xho*I-*Xho*I fragment were determined. This stretch contained a single open reading frame of 2,370 nucleotides (Fig. 3), which encoded a predicted protein of 790 amino acids with a calculated molecular weight of 89,424. The protein has a calculated isoelectric point of 6.92. It contains relatively high levels of asparagine (9.2%) and serine (10.6%) but a low level of alanine (3.9%). The codon bias index (35) of the *LYS14* gene is 0.129, indicating a moderately expressed gene.

The predicted amino acid sequence was compared with the sequences in the SwissProt, PIR, and GenPept banks by using the FASTA (28) and BLAST (1) programs. The BLAST computation was performed at the National Center for Biotechnology Information. No important similarity to any known protein was found except for the presence, from residues 158 to 186, of a cysteine-rich motif which is conserved in the amino-terminal region of a number of yeast proteins involved in the regulation of transcription. This domain conforms to the $Zn(II)_2Cys_6$ binuclear cluster proposed in the case of the Gal4 protein (27). It is compared with the cognate domains of several yeast regulatory proteins in Fig. 4.

Disruption of the *LYS14* gene causes a leaky phenotype similar to that of *lys14* mutants obtained in vivo. The fact that *lys14* mutants obtained in vivo are leaky lysine auxotrophs has led to the aforementioned hypothesis that *LYS14* encodes a transcriptional activator of *LYS* gene expression (31). Such a suggestion implies that the same phenotype could be obtained by disrupting *LYS14*. Therefore, *URA3* was inserted between a *Sma*I site created just after the ATG and the second *Eco*RV site at amino acid 609 of the coding sequence of *LYS14*. The resulting fragment (see Materials and Methods and Fig. 2) was used to transform a *ura3* strain. Southern blot analysis was used to confirm that integration had indeed occurred at the *LYS14* locus (data not shown).

A *lys14*::*URA3* disrupted mutant was examined and compared with a previously characterized *lys14* mutant obtained in vivo (31). The disruptant had the same slow-growth phenotype on M.am as the original *lys14* mutant (with a generation time of 240 min, in contrast to 120 min for the wild-type strain). It also exhibited the same low but reproducible level of *LYS9* gene expression: the specific activity of glutamate-forming saccharopine dehydrogenase was about 0.05 U h^{-1} mg of protein^{-1}, in contrast to 1.60 for the wild-type strain. The leaky phenotype and the low but reproducible level of *LYS9* expression of such a disrupted *lys14*::*URA3* mutant are consistent with the idea that the *LYS14* product exerts a positive effect on the expression of *LYS9*.

Transcription activation by a LexA-Lys14 fusion protein. A deletion removing the 277 N-terminal amino acid residues of

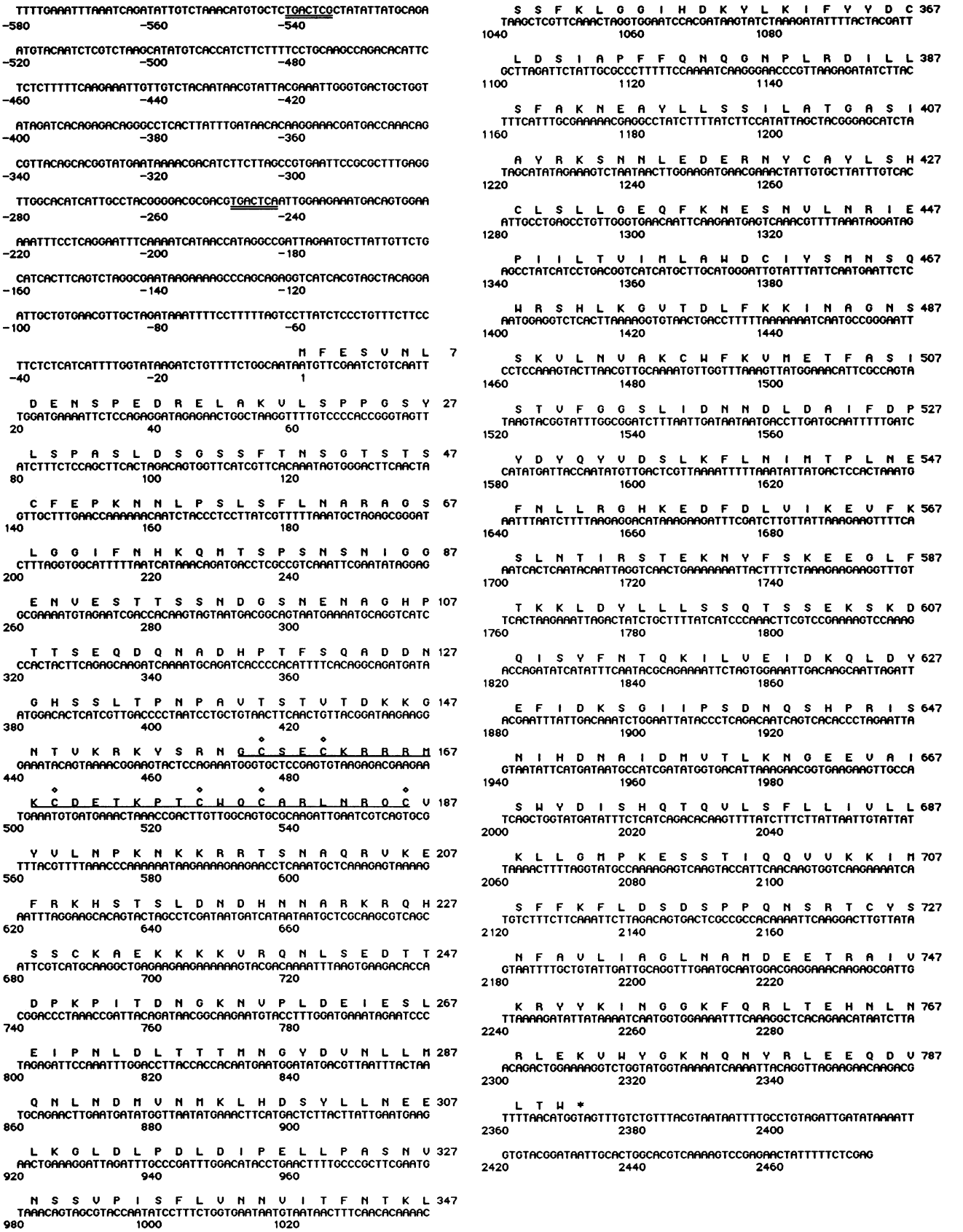

FIG. 3. Nucleotide sequence of the *LYS14* gene and deduced amino acid sequence of the gene product. Nucleotides are numbered under the sequence, and amino acids are numbered on the right. The first ATG of the open reading frame is at the +1 position. Potential Gcn4 protein binding sites are doubly underlined. The amino acid residues forming the $Zn(II)_2Cys_6$ binuclear cluster motif are underlined once. The cysteine residues in this motif are marked with diamonds.

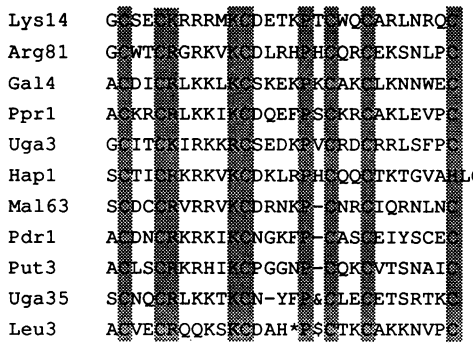

FIG. 4. Sequence similarities between the cysteine-rich motif of Lys14 and sequences of representative yeast regulatory proteins. The sequences of Lys14, Arg81 (24), Gal4 (20), Ppr1 (17), Uga3 (3), Hap1 (29), Mal63 (18), Pdr1 (4), Put3 (21), Uga35 (11), and Leu3 (13, 40) are compared. Amino acids conserved in most of the proteins are shaded. Residues K and R are considered equivalent, as are H and C. Gaps added for maximum match are indicated by hyphens. Insertions made in some of the sequences are indicated as follows: &, insertion of DLGN in Uga35; *, insertion of ERA in Leu3p; $, insertion of EP in Leu3.

the *LYS14* coding sequence was constructed, and the resulting deleted protein was expressed under the control of the galactose-inducible *GAL10* promoter (see Materials and Methods). This construction, leading to the synthesis of a 513-amino-acid truncated Lys14 protein lacking the cysteine-rich motif of the wild-type protein, was unable to complement the *lys14* mutation of strain 02483a (*ura3 leu2 lys14*). To test this truncated protein for function as a transcriptional activator, we applied the method of Brent and Ptashne (9). A fusion between the 87 N-terminal residues of LexA and the 513 C-terminal residues of Lys14 was constructed. The gene coding for this chimeric protein, in which the DNA-binding domain of LexA replaces the cysteine-rich motif of Lys14, was expressed under the control of the *ADHI* promoter in the plasmid pBLAF3 (see Materials and Methods). This plasmid and another plasmid, p1840, bearing part of the *GAL1* gene fused to *lacZ* and under control of the *lexA* operator, were used to cotransform strain 1C2123a (*ura3 leu2*). Activation of the gene *GAL1-lacZ* was estimated by assaying β-galactosidase (Table 1). The doubly transformed cells expressed high levels of β-galactosidase activity compared with cells transformed with p1840 alone or cotransformed with plasmid pRB500, which contains the native *lexA* gene, thus confirming that Lys14 is able to function as a transcriptional activator.

Lysine antagonizes transcription activation by the LexA-Lys14 fusion protein. Previous experiments based on the use of mutants affected at various steps of lysine biosynthesis have suggested that α-aminoadipate semialdehyde is required in addition to Lys14 for activation of *LYS* gene expression (31). Having shown that a LexA-Lys14 fusion is able to activate transcription, we thus considered the role of α-aminoadipate semialdehyde in this activation. The activation capacity of the fusion protein was investigated with various mutant strains and under several growth conditions. As shown in Table 1, LexA-Lys14 stimulated the expression of *GAL1-lacZ* in strain 1C2123a (wild type for *LYS* genes) grown in M.am and in the *lys9* mutant 02458c grown in M.am plus 6 μg of lysine per ml (this low lysine concentration, which achieves limited repression of the *LYS* genes, was used for assessing the activation potential in auxotrophic strains). In contrast, no activation was observed in the *lys2* mutant 02485d or in the *lys2 lys9* double mutant 02492c grown in the presence of the same low lysine concentration. The intermediate α-aminoadipate semialdehyde is known to accumulate intracellularly in *lys9* mutants but not in *lys2* mutants or in *lys2 lys9* mutants, in which the α-aminoadipate reductase step is blocked (31). These observations are therefore consistent with the conclusion that activation by LexA-Lys14 is dependent on the presence of α-aminoadipate semialdehyde.

The effect of lysine on this activation mechanism was also investigated. As shown in Table 1, lysine, when present at a repressing concentration (66 μg/ml) in the growth medium, is markedly antagonistic to the activation function of the chimeric protein. This antagonistic effect, a 50-fold reduction in the wild-type strain 1C2123a, was less marked (4-fold) in strain 20T6b, which carries a *lys80* mutation impairing the repression of *LYS* genes by lysine (32). Two types of evidence support the idea that lysine acts by limiting the supply of α-aminoadipate semialdehyde required for transcription activation by the LexA-Lys14 fusion protein. First, the lysine antagonizing effect was not observed in the *lys9* mutant 02485c. The *lys9* mutants, because of their block in the saccharopine dehydrogenase (glutamate forming) step, have been shown to accumulate substantial amounts of α-aminoadipate semialdehyde even in the presence of lysine in the growth medium (31). Second,

TABLE 1. Influence of various mutations and growth conditions on the transcriptional activation[a] of a *GAL1-lacZ* reporter gene by the chimeric LexA-Lys14 protein

Strain[b] (genotype)	Transcriptional activation capacity of LexA-Lys14 in indicated medium				
	M.am	M.am + lysine (6 μg/ml)	M.am + lysine (66 μg/ml)	M.am + DL-α-aminoadipate (100 μg/ml)	M.am + lysine (66 μg/ml) + DL-α-aminoadipate (100 μg/ml)
1C2123a (*ura3 leu2*)	1,030[c]	810	20	600	960
02485c (*ura3 leu2 lys9*)	NG[d]	1,215	1,545	ND[e]	ND
02485d (*ura3 leu2 lys2*)	NG	10	30	5[f]	10
02492c (*ura3 leu2 lys2 lys9*)	NG	20	35	ND	ND
20T6b (*ura3 leu2 lys80*)	1,400	1,475	330	ND	ND

[a] Expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of protein. Each value is the mean of three independent measurements, which did not differ by more than 20%.

[b] All strains were cotransformed with the reporter plasmid p1840 and the plasmid pBLAF3, which codes for the LexA-Lys14 hybrid protein.

[c] Under the same conditions, cells transformed with the reporter plasmid p1840 alone yielded a value of 4 and cells cotransformed with plasmid pRB500 containing the native *lexA* gene yielded a value of 6.

[d] NG, no growth.

[e] ND, not determined.

[f] Medium was supplemented with 6 μg of lysine per ml.

TABLE 2. Role of the general control of amino acid biosynthesis in repression of the *LYS1* gene by lysine

Strain (genotype)	Medium	Sp act (μmol of product formed/h/mg of protein)[a] of:		
		Glutamate forming saccharopine dehydrogenase (*LYS9*)	Lysine forming saccharopine dehydrogenase (*LYS1*)	Argininosuccinate lyase (*ARG4*)
Σ1278b (wild type)	M.am	1.80	15	1.1
	M.am + lysine (6 μg/ml)	1.80	15	1.1
	M.am + lysine (66 μg/ml)	0.20	2	1.1
8903c (*lys14*)	M.am	0.05	38	4.7
	M.am + lysine (6 μg/ml)	0.05	13	2.7
	M.am + lysine (66 μg/ml)	<0.05	2	1.0
18T8d (*gcn4*)	M.am	1.70	9	0.3
	M.am + lysine (6 μg/ml)	1.30	8	0.3
	M.am + lysine (66 μg/ml)	0.40	3	0.3
18T9b (*gcn4 lys14*)	M.am + lysine (6 μg/ml)	<0.05	2	ND[b]
	M.am + lysine (66 μg/ml)	<0.05	2	ND

[a] Each value is the mean of at least three independent measurements, which did not differ by more than 10%.
[b] ND, not determined.

α-aminoadipate, the direct precursor of α-aminoadipate semialdehyde, was able to reverse this antagonistic effect of lysine on the activation function of LexA-Lys14 (Table 1). The amount of lysine in cells grown under such conditions was 458 μmol/mg of dry cells, in contrast to 314 for cells grown in the presence of lysine (66 μg/ml) alone, thus showing that α-aminoadipate does not interfere with the accumulation of lysine within the cell but rather contributes to building an increased lysine pool.

Taken together, the above results suggest that lysine antagonizes the activation function of Lys14 by interfering with the production of the coinducer, α-aminoadipate semialdehyde, a conclusion which appears at odds with the previous suggestion that repression by lysine and activation by Lys14 are independent mechanisms (31).

Role of the general control of amino acid biosynthesis in repression of *LYS* genes by lysine. The view that repression by lysine functions independently from activation by α-aminoadipate semialdehyde was essentially based on the fact that a significant repression by lysine on the expression of the *LYS1* gene was retained in *lys14* mutants (31). The finding that these two regulations are linked mechanisms led us to reconsider this point and, in particular, to investigate the role of the general control of amino acid biosynthesis in the repression of the *LYS1* gene by lysine. Table 2 compares the effects of lysine on the expression of the genes *LYS9*, *LYS1*, and *ARG4* in a wild-type strain and in mutants impaired in activation by *LYS14* or in the general control of amino acid biosynthesis (e.g., the *gcn4* mutant). Argininosuccinate lyase, the product of the gene *ARG4*, was chosen as a reference enzyme since it obeys the general control of amino acid biosynthesis to the exclusion of any other regulatory mechanism (23).

The *lys14* mutant 8903c, which is impaired in the activation of *LYS* genes, has a low level of *LYS9* expression and behaves like a leaky lysine auxotroph growing slowly on M.am. It exhibits strongly derepressed levels of *LYS1* and *ARG4* expression, suggesting that a derepression of the general control results from starvation for lysine. The addition of lysine (66 μg/ml) to the growth medium does indeed restore a normal growth rate and represses the expression of both *LYS1* and *ARG4* down to the wild-type levels. Repression of *LYS1*, in this case, is apparently linked to the elimination of general control derepression following the supply of excess lysine.

A *gcn4* mutant, which is unable to derepress the general control, nevertheless displays normal expression of *LYS9* but reduced levels of *LYS1* and *ARG4* expression, suggesting that *LYS9* does not respond to the general control.

Finally, a *gcn4 lys14* double mutant, in which both regulations are eliminated, has the same low level of *LYS1* expression whether lysine is present or not. Thus, the *lys14* mutation leads to the absence of repression by lysine of *LYS1* expression. These various results indicate that activation by α-aminoadipate semialdehyde and apparent repression by lysine are related mechanisms in all the cases investigated. The apparent repression of *LYS1* by lysine observed in *lys14* mutants is consequently linked to the derepression of the general control of amino acid biosynthesis during starvation for lysine.

DISCUSSION

On the basis of genetic and physiological studies, it has been proposed that the *LYS14* gene product, in conjunction with α-aminoadipate semialdehyde as an inducer, functions as a transcriptional activator of the *LYS* genes (31). Taken together, the results obtained during this work provide strong support for this assumption. *LYS14* was sequenced and found to encode a protein of 790 amino acid residues in which the presence of separate domains corresponding to DNA-binding and activation functions is suggested. The region extending from residues 1 to 277 was necessary for *LYS14* function but dispensable for transcription activation by a LexA-Lys14 chimeric protein. This region contains, from residues 158 to 186, a presumed DNA-binding domain similar to the $Zn(II)_2Cys_6$ binuclear cluster located near the amino terminus of Gal4 and a number of other fungal transcriptional activators. Further studies will be needed to determine the roles of the amino acid stretches located on both sides of the cysteine-rich motif and, in particular, of the 157 amino acids which separate this motif from the amino terminus of the protein. On the other hand, the region of Lys14 extending from residues 278 to 790, when fused to a portion of LexA comprising its DNA-binding domain, was an effective transcriptional activator of a gene bearing a *lexA* operator site; this suggests that this region of Lys14 contains the domain which interacts with the transcription machinery. A preliminary deletion analysis of this region has failed to identify more limited segments which could be

sufficient for the activation function (12b). Such a failure could result if the activation domain and the effector binding site are located in separate regions of this relatively long portion of the regulatory protein.

The LexA-Lys14 fusion protein, which is capable of functioning as a transcriptional activator when bound to a *lexA* operator, proved useful in defining the conditions required for activation. The use of this protein showed that activation occurred only in strains and under conditions expected to promote the accumulation of notable amounts of α-aminoadipate semialdehyde in the cell, thus supporting the view that this intermediate acts as a coinducer in this activation mechanism. A striking result obtained with this protein concerns the fact that activation by the chimeric LexA-Lys14 protein in a wild-type strain was prevented when a high concentration of lysine was present in the growth medium (Table 1). Activation was, however, partially restored in the presence of a *lys80* mutation known to impair lysine repression (32). Such observations appear to be difficult to reconcile with the notion of true lysine repression being exerted solely by a repressor acting at the level of *LYS* promoters, independently of activation by Lys14, as proposed previously (31, 32). The simplest explanation for the interdependence between activation and repression, which is revealed by the present experiments, is that lysine does somehow reduce the flux of metabolites through the pathway and, consequently, limits the production of α-aminoadipate semialdehyde needed for the activation mechanism. Feedback inhibition of homocitrate synthase, observed previously (37), might be responsible for such control of α-aminoadipate semialdehyde production. Nevertheless, the feedback inhibition of homocitrate synthase by lysine is fully retained in *lys80* mutants impaired in apparent lysine repression (32), and therefore the involvement of completely different mechanisms cannot be excluded. The product of the *LYS80* gene could, for example, act in a manner similar to that of Gal80 in the galactose system (15) and, in the presence of lysine, prevent Lys14 from interacting with the transcription machinery. In any case, the role of *LYS80* needs to be reconsidered in light of the results of this study; its cloning and characterization are in progress.

The independence of the apparent repression by lysine and the activation by α-aminoadipate semialdehyde were originally suggested by the observation that a marked repression by lysine of *LYS1* gene expression was still present in *lys14* mutants (31). The study of the effect of the general control of amino acid biosynthesis on the expression of the *LYS1* gene does, however, show that its repression in the leaky auxotrophic *lys14* mutants is mainly due to a lack of induction of the general control system. In this regard, the difference in responses of the *LYS1* and *LYS9* genes to such leaky conditions, as illustrated in Table 2, should be emphasized. Clearly, the *LYS9* gene is solely subject to activation by *LYS14* and does not respond to the general control, in agreement with the absence of TGACTCA sequences, the target of Gcn4, in its 5′ control region (12a). It is actually this lack of response of the *LYS9* gene to the general control which is responsible for the quasi-auxotrophic phenotype of *lys14* mutants. In contrast, a copy of the general control target site has been found in the promoter region of the *LYS1* gene (12a), which is efficiently derepressed under starvation conditions. Also noteworthy is the fact that two copies of this target site are present in the 5′ control region of the *LYS14* gene. It will be of interest to examine the effects of the general control on the expression of this gene as well as the consequence of these effects on the overall regulation of the pathway.

The similarities which exist between the regulation of the lysine pathway and that of the leucine pathway (for a review, see reference 19) need to be stressed. Both pathways are subject to the interplay of the general control of amino acid biosynthesis and a transcriptional activation mechanism which depends on an intermediate of the pathway as the coinducer. Lys14 and α-aminoadipate semialdehyde in lysine biosynthesis appear to play roles that are similar to those of Leu3 and α-isopropylmalate in the leucine pathway. It has also been proposed that in both pathways, the production of the coinducer is modulated by a feedback mechanism exerted by the final product of the pathway and leads to apparent repression by this final product.

More work is needed to assess the precise mechanism of Lys14 activation. The present work, for example, establishes that α-aminoadipate semialdehyde is necessary for the interaction with the transcription apparatus. It does not, however, show whether this effector also modulates the binding of Lys14 to DNA or whether this binding occurs irrespective of the presence of the effector, as shown in the case of Leu3 (10). It will also be of interest to determine whether Lys14, in the absence of the effector α-aminoadipate semialdehyde, acts as a repressor, reducing the levels of the expression of the *LYS* genes below the levels obtained in the absence of activation, as was observed for Leu3 in the absence of α-isopropylmalate (10, 36).

A detailed understanding of the mechanism of activation by Lys14 will also depend on the identification of its targets in the promoter regions of the *LYS* genes. The sequences of the 5′ control regions of three *LYS* genes, *LYS2* (26), *LYS1*, and *LYS9*, are presently available (12a). At least two conserved sequences, with a CCG triplet at each end as found in the target sites of other yeast $Zn(II)_2Cys_6$ proteins (33), have been identified in the promoters of the three genes. Preliminary results indicate that an 81-bp sequence containing these CCG triplets is required for *LYS9* gene activation by Lys14 (5a).

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