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Genetic Manipulation of *Saccharomyces cerevisiae* by Use of the *LYS2* Gene

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The structural gene for α-aminoadipate reductase (*LYS2*) was isolated from a *Saccharomyces cerevisiae* genomic DNA library by complementation of a *lys2* mutant. Both genetic and biochemical criteria confirmed that the DNA obtained corresponds to the *LYS2* locus on chromosome II. Subcloning and deletion analysis showed that a functional *LYS2* gene is contained within a 4.6-kilobase (kb) *Eco*RI-*Hin*dIII fragment of the original insert, and the slightly larger *Eco*RI-*Cla*I segment (4.8 kb) was used to construct a series of cloning vehicles, including integrating, episomal, replicative, and centromeric vectors. The cloned DNA was also used to generate a genomic deletion that lacks all *LYS2* coding sequences on chromosome II. The level of the *LYS2* transcript (4.2 kb) was 10-fold higher in cells grown on minimal medium than in cells grown on complete medium and was not repressed by the presence of lysine alone. Gene disruption, gene replacement, and promoter analysis of the major α-factor structural gene (*MFα1*) were performed to illustrate the utility of the *LYS2* gene for the genetic manipulation of yeasts. Because all fungi synthesize lysine via the α-aminoadipate pathway, the techniques developed here for using the *S. cerevisiae LYS2* gene should be directly applicable to other fungal systems.

One useful tool for the isolation and analysis of genes from *Saccharomyces cerevisiae* is direct DNA transformation of yeast mutants with plasmid vectors that contain a selectable (functional) gene and that allow segments of the yeast genome to be inserted. This kind of cloning strategy usually requires that the recipient strain carry a recessive mutation (nonfunctional chromosomal copy) at the locus corresponding to the selectable marker. The widely used vector systems involve cloned genes for biosynthetic enzymes (for example, *LEU2* [2, 8], *HIS3* [42], *TRP1* [44], and *URA3* [18]). Unless lesions in one or more of these loci are already fortuitously in the cells to be transformed, such mutations must be introduced by genetic crosses, because no positive selections for mutations in these genes are available, except recently for *ura3* (5). Due to Mendelian segregation, however, it may be very difficult to maintain the genetic background desired in the resulting spores. One approach to avoid this problem is to use a dominant selectable marker on the plasmid (for example, antibiotic resistance [20]). Another solution to this problem is to use as the selectable marker a gene for which it is possible to select directly and conveniently for mutations to the loss of function in any yeast cell. In this way, any yeast strain could be made a potential recipient for transformation.

One marker that has these last features is the *LYS2* gene of *S. cerevisiae*. *LYS2* is the structural gene for α-aminoadipate (α-AA) reductase (40), which catalyzes an essential step in the pathway for lysine biosynthesis in *S. cerevisiae* and other fungi (3). For this reason, *lys2* mutants are lysine auxotrophs. It has been found by Chattoo et al. (9) that wild-type cells plated on a medium containing α-AA as the sole nitrogen source are unable to grow. In contrast, *lys2* mutants can grow under these conditions if lysine is also provided (see references 1 and 50 for an explanation of the metabolic basis of these effects). Most importantly, *lys2* mutants appear spontaneously at a reasonable frequency (10^{-6} to 10^{-5}) when wild-type cells are plated on such a medium. Hence, a positive selection exists for constructing *lys2* strains that does not require either chemical mutagenesis or genetic crosses. Furthermore, because only cells proficient for lysine biosynthesis can grow on medium lacking lysine, a positive selection also is available for detecting those *lys2* cells that have recovered a functional *LYS2* gene. A vector-recipient transformation system based on the genetic properties of the *LYS2* gene requires that the DNA for this locus be available for use as the selectable marker on a plasmid. This paper describes the identification and characterization of the *LYS2* gene and the construction of a variety of yeast vectors containing it. In addition, we demonstrate the utility of these tools for a variety of genetic manipulations of yeasts.

(Certain aspects of these studies have been presented in preliminary form elsewhere [West Coast Bacterial Physiologists Meeting, Pacific Grove, Calif., 1982; Abstr. Meet. Mol. Biol. Yeast, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1983, p. 400; Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, H208, p. 142]. While this work was in progress, two independent reports of the isolation of the *LYS2* gene appeared [13, 39].)

MATERIALS AND METHODS

Organisms, growth conditions, and DNA-mediated transformation. The compositions of minimal medium (SD), synthetic complete medium (SC), drop-out media (SC − Lys, SC − Ura, etc.), and rich medium (YPD) are presented elsewhere (37). Selective medium for the isolation of *lys2* mutants was prepared as described by Chattoo et al. (9) and,

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source or reference
381G	*MATa his4-580*(Am) *trp1*(Am) *ade2*(Oc) *lys2*(Oc) *tyr1*(Oc) *SUP4*-3(Ts, Am)	L. Hartwell
SX50-1C	*MATα his3Δ leu2-3,112 ura3-52 trp1*	J. Hicks
DA4B[a]	*MATa ade2-1 his4-580 lys2*(Oc) *SUP4-3 trp1 ura3-52*	This work
AB35-13D	*MATa ade2 his4 trp1 leu2-3,112 ura3-52*	A. Brake
DA100[a]	*lys2* mutant derived from AB35-13D	This work
DA200[a]	*lys2* mutant derived from SX50-1C	This work
320	*MATa rme1 ade2 leu1 ura3 can1-11 cyh2-21*	Y. Kassir
DA320[a]	*lys2* mutant derived from 320	This work
DBY746	*MATα his3Δ-1 leu2-3,112 trp1-289 ura3-52*	D. Botstein
DA746[a]	*lys2* mutant derived from DBY746	This work
DBY747	*MATa his3Δ-1 leu2-3,112 trp1-289 ura3-52*	D. Botstein
DA747[a]	*lys2* mutant derived from DBY747	This work
A364A[a]	*MATa ade1 ade2 gal1 his7 tyr1 ura1 lys2*	L. Hartwell
AB35-14A	*MATα leu2-3,112 ura3-52*	A. Brake
K382-19D[b]	VIII V V VII XV II V II *MATα spo11 ura3 can1 cyh2 ade2 his7 hom3 tyr1*	R. Esposito
K381-9D	VIII V V VII XV XII V II XV IV *MATα spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	R. Esposito
K393-35C	VIII V VI VII IX XVII XIV *MATα spo11 ura3 his2 leu1 lys1 met14 pet8*	R. Esposito
K396-22B	VIII V I V III XII X VII *MATα spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	R. Esposito
YNN217[a]	*MATa his3Δ-200 lys2-801*(Am) *ura3-52 ade2-101*(Oc)	C. Mann
STX20-1C	*MATa lys2 gal1*	YGSC[c]
P49	*MATα lys2 gal2*	YGSC
AB9	*MATα lys5 gal5*	YGSC
X4004-3A	*MATa lys5 trp1 met2 ura3*	YGSC
689	*MATa leu2-3,112 ura3-50 can1*	G. Zakian
S288C	*MATα mal gal2 CUP1 SUC2*	YGSC
X2180-1B	*MATα mal gal2 CUP1 SUC2*	YGSC
SEY2102	*MATα leu2-3,112 ura3-52 his4 suc2-Δ9*	15
DA2102	*lys2* mutant derived from SEY2102	This work
DA2102-375-R	*MATα leu2-3,112 ura3-52 his4 suc2-Δ9 lys2 mfα1::LYS2 (SYN)*	1
DA2102-375	*MATα leu2-3,112 ura3-52 his4 suc2-Δ9 lys2 mfα1::LYS2 (ANTI)*	1
DA2100	*MATα leu2-3,112 ura3-52 his4 suc2-Δ9 lys2-Δ1::URA3*	This work
DA2189-1C	*MATa leu2-3,112 ura3 his4 lys2-Δ1::URA3*	This work
DA2137-2A	*MATα leu2-3,112 ura3 his4 lys2-Δ1::URA3 mfα1::LYS2 (SYN)*	This work
DA2137-4C	*MATa leu2-3,112 ura3 his4 lys2-Δ1::URA3 mfα1::LYS2 (SYN)*	This work
DA2137-7A	*MATa leu2-3,112 ura3 lys2-Δ1::URA3*	This work
DA72	*MATa/MATα leu2-3,112/leu2-3,112 ura3/ura3 his4/HIS4 lys2-Δ1::URA3/lys2-Δ1::URA3 MFα1/mfα1::LYS2 (SYN)*	This work
DA8902-1C	*MATa leu2-3,112 ura3 his4 lys2-Δ1::URA3 mfα1::LYS2 (ANTI)*	This work
DA8902-5D	*MATα leu2-3,112 ura3 his4 lys2-Δ1::URA3 mfα1::LYS2 (ANTI)*	This work
DA57	*MATa/MATα leu2-3,112/leu2-3,112 ura3/ura3 his4/HIS4 lys2-Δ1::URA3/lys2-Δ1::URA3 MFα1/mfα1::LYS2 (ANTI)*	This work

[a] Strain used to test transformation efficiency of pDA6200 (by production of Lys$^+$ transformants).
[b] Roman numerals above the marker refer to the yeast chromosome on which that marker is known to reside (27).
[c] YGSC, Yeast Genetic Stock Center, Donner Laboratory, University of California, Berkeley, Calif.

when necessary, was supplemented with the appropriate nutrients. All yeast cultures were grown at 30°C unless otherwise specified.

S. cerevisiae strains used in this study are listed in Table 1. Strain DA4B was derived from a cross between SX50-1C and 381G. Strains DA100, DA200, DA320, DA746, DA747, and DA2102 are *lys2* mutants isolated by plating various strains listed in Table 1 on selective medium (9). The assignment of lysine auxotrophy to *lys2* lesions was confirmed by complementation tests in which the strain to be tested was crossed against strains bearing mutations in either the *lys2* or *lys5* genes (9). All strains obtained yielded prototrophic diploids when crossed against *lys5* mutants (AB9 or X4004-3A) but not against *lys2* mutants (STX20-1C or P49).

Escherichia coli HB101 was transformed with crude or purified DNA preparations by minor modifications (11) of the method of Mandel and Higa (24), selecting for ampicillin resistance on LB plates (26) containing 100 μg of ampicillin per ml. Yeast strains were transformed with 5 to 10 μg of plasmid DNA or a linear DNA fragment essentially by the procedure described by Beggs (2).

Preparation and analysis of plasmids and genomic DNA. DNA was prepared from normal or transformed yeast cells

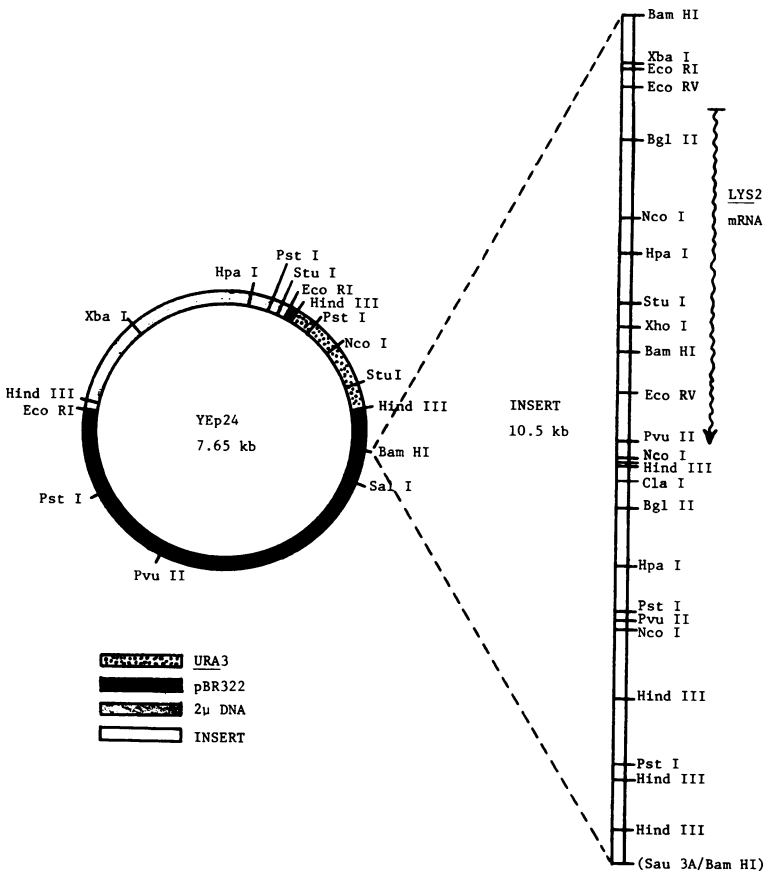

FIG. 1. Restriction endonuclease cleavage site map of pDA6200 containing the *LYS2* gene. This plasmid was identified, purified, and digested with a variety of restriction endonucleases (singly and in various combinations) as described in the text. For determining the sizes of the segments indicated by electrophoresis in agarose gels, the length markers used were fragments of bacteriophage λ DNA generated by digestion with *Hin*dIII. The complete nucleotide sequence of the entire vector, YEp24 (also known as pFL1) (18), is available because all of its elements (pBR322, the small *Eco*RI fragment of the B form of the 2μm circle DNA, and the *URA3* gene) have been completely sequenced (see citations in reference 1).

by minor modifications of the method of Brake et al. (6). Crude plasmid preparations from *E. coli* transformants were obtained by the rapid alkaline extraction method of Birnboim and Doly (4).

Purified plasmids were prepared from *E. coli* transformants grown at 37°C in 1 liter of LB to an optical density of 0.6 to 0.8 at 600 nm and were amplified by treatment with 100 μg of chloramphenicol (Sigma Chemical Co.) per ml for 20 h. Cleared lysates were prepared and extracted with phenol and chloroform-isoamyl alcohol (24:1) as described elsewhere (25). DNA was precipitated once with 2 volumes of ethanol and subjected to CsCl-ethidium bromide buoyant density sedimentation at 42,000 rpm in a Beckman 50Ti rotor for 48 h at 15°C.

Plasmid DNAs were digested with various combinations of restriction endonucleases under the conditions recommended by the suppliers (New England BioLabs, Inc., or Bethesda Research Laboratories, Inc.). The digested DNA was then subjected to electrophoresis, usually at 40 V for 12 to 15 h, in horizontal slab gels at an agarose concentration of 0.8%.

DNA fragments were visualized in agarose gels by staining them with ethidium bromide (1 μg/ml) and were isolated by cutting the appropriate band from the gel. Excised gel slices were placed in dialysis tubing containing 0.2 ml of 1 mM EDTA and 10 mM Tris hydrochloride (pH 7.5) and were subjected to electrophoresis at 100 V for 2 h. The DNA-containing solution within the dialysis bag was removed, extracted with phenol, and precipitated with ethanol. Ligations were carried out as described by others (25) at 16°C with T4 DNA ligase (New England BioLabs).

Analysis of DNA and RNA by blotting and hybridization. DNAs were transferred from agarose gels to nitrocellulose paper sheets as described by Southern (41).

Poly(A)$^+$ RNAs were subjected to electrophoresis at 100 V for 2 h in 1.5% agarose gels containing 2 M formaldehyde (Mallinckrodt, Inc.), 1 mM EDTA (Sigma), and 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [pH 7.8]; Mallinckrodt). Approximately 10 μg of poly(A)$^+$ RNA was run in each lane as determined by A_{260}. The gels were blotted to nitrocellulose paper (Sartorius) as described by Thomas (43).

Strain S288C was grown in four different media (SD, SD+Lys, SC, or YPD) to a density of 100 on a Klett-Summerson colorimeter to prepare the RNAs. Nucleic acids were extracted from broken cells, and poly(A)$^+$ mRNA was prepared by minor modifications of the methods of Tuit et al. (45).

Probes (usually pBR322 carrying the appropriate yeast DNA inserts or appropriate restriction fragments purified by agarose gel electrophoresis) were labeled with [α-^{32}P] deoxynucleoside triphosphates by nick translation (32). Hy-

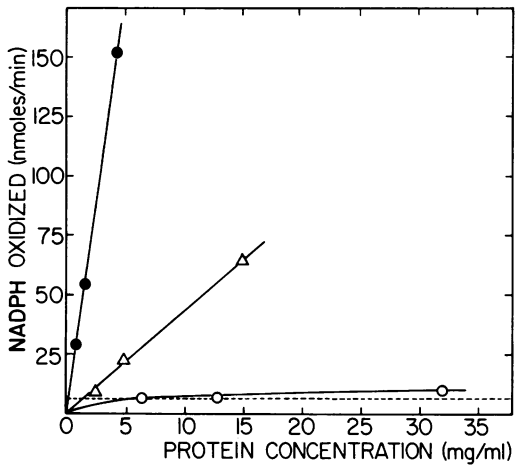

FIG. 2. Overproduction of α-AA reductase activity in yeast cells carrying the *LYS2* gene on a multicopy plasmid. Extracts from the normal yeast strain AB35-14A (△), the *lys2* mutant DA4B (○), and the *lys2* mutant DA4B transformed with pDA6200 (●) were prepared and assayed for α-AA reductase activity as described in Materials and Methods. The spontaneous rate of NADPH oxidation in a reaction mixture lacking added extract (– – –) is also shown.

bridizations were carried out in sealed plastic bags with the use of approximately 4×10^6 cpm of the labeled probe at 65°C for 16 to 20 h in 1% sodium Sarkosyl, 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA [pH 7.0]) and 100 μg of salmon sperm DNA per ml (as carrier). In some experiments, hybridization was performed at 42°C for 15 h in 10% dextran sulfate–45% formamide–1% sodium Sarkosyl–0.1% sodium PP_i–100 μg of salmon sperm DNA per ml. Filters were rinsed twice in 2× SSPE containing 0.1% sodium dodecyl sulfate and were then washed twice in the same solution for 20 minutes at 50°C. Autoradiography was carried out with Kodak X-Omat AR film and intensifying screens at −70°C.

Preparation of cell extracts and enzyme assay. Yeast cultures were grown overnight in 1 liter of SD (which lacks uracil, providing selection for plasmid maintenance) containing the appropriate supplements, were harvested by centrifugation at 5,000 rpm in a Sorvall GSA rotor at 4°C for 5 min, and were washed by suspension and centrifugation in 10 ml of 100 mM triethanolamine (pH 7.2; J. T. Baker Chemical Co.). The cell pellet was suspended in a volume of buffer (100 mM triethanolamine containing 1 mM phenylmethylsulfonyl fluoride [Sigma], 0.5 μg of pepstatin A [Sigma] per ml) equal to the wet weight of cells. Cells were then broken by vortexing them with glass beads as described by Julius et al. (22). The lysate was then centrifuged at 10,000 rpm in a Sorvall SS-34 rotor for 25 min at 4°C to remove unbroken cells and cell debris. The supernatant fluid was then dialyzed against 100 mM triethanolamine (pH 7.2)–1 mM dithiothreitol (Sigma) for 15 h at 4°C before the assay.

The activity of α-AA reductase was determined by measuring the α-AA-dependent oxidation of NADPH to NADP, with the following modifications of the procedure of Sinha and Bhattacharjee (40). All reaction mixtures in a total volume of 1 ml contained 2.5 mM DL-α-AA (Aldrich Chemical Co., Inc.), 3 mM ATP (P-L Biochemicals, Inc.), 2.5 mM NADPH (P-L Biochemicals), 6 mM $MgCl_2$, 50 mM triethanolamine (pH 7.2), and various amounts of dialyzed cell-free extract. The reaction mix was incubated at 30°C. At different times portions were removed, diluted in a 1% sodium dodecyl sulfate-0.1 N NaOH solution, boiled for 2 min, and centrifuged in an Eppendorf microfuge for 10 min to remove insoluble material. The amount of NADPH remaining in each supernatant solution was determined by measuring its A_{340} in a Zeiss spectrophotometer, assuming an extinction coefficient of 6.22×10^3/M per cm. Under the conditions imposed, the assay was linear with respect to both time and the amount of protein added.

RESULTS

Isolation and characterization of the *LYS2* gene. The *LYS2* gene was isolated by complementation as described in detail elsewhere (1). One plasmid, pDA6200, isolated by the procedure used contained a 10.5-kilobase (kb) insert (Fig. 1) and was chosen for subsequent investigation. A variety of genetic and biochemical criteria demonstrated that the genomic insert in pDA6200 carries the *LYS2* gene. This evidence is summarized here. First, pDA6200 transformed eight independently derived *lys2* strains (Table 1) to lysine prototrophy at high efficiency (5 to 300 transformants per μg of DNA per 10^6 spheroplasts, depending on the recipient). Second, the insert carried by pDA6200 was shown (1) to correspond to the *LYS2* locus on chromosome II by (i) "target integration" (28) after cleavage of the plasmid at a unique site (*Xho*I) in the insert, (ii) chromosome loss mapping (16, 17) of the integrant so generated with the well-marked *spo11* tester strains (23), and most conclusively (iii) an allelism test performed by backcrossing the integrant, DA4B::pDA6200 (*ura3 lys2::LYS2 URA3*), to strain K382-19D (*ura3 LYS2*). In all 30 tetrads examined, every viable spore was Lys^+. This lack of separation of the *lys2* and *LYS2* markers in meiosis proved that the DNA segment carried by pDA6200 had integrated at the *lys2* locus and therefore was indeed derived from the genomic region that includes the *LYS2* gene. Third, a spectrophotometric assay for the *LYS2* gene product by the α-AA-dependent oxidation of NADPH showed that the presence of pDA6200 not only restored α-AA reductase activity to extracts from a transformed *lys2* mutant but

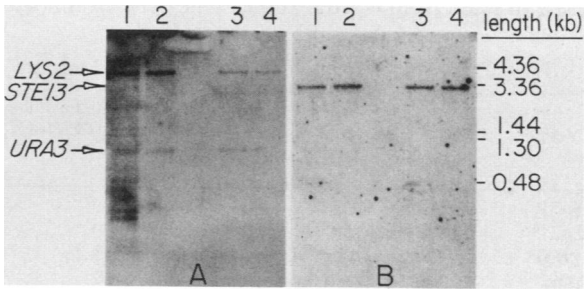

FIG. 3. Expression of *LYS2* transcript under general amino acid control. Poly(A)$^+$ RNA was prepared, as described in Materials and Methods, from a prototrophic haploid strain (S288C) grown in SD (lane 1), in SD+Lys (lane 2), in SC (lane 3), and in YPD (lane 4). The preparations were denatured and separated by electrophoresis in an agarose gel. After transfer to a nitrocellulose filter, they were hybridized to either (A) a mixture of about 4×10^6 cpm each of pBR322-*LYS2* (carrying the large *Hin*dIII fragment of pDA6200 that contains the entire *LYS2* gene; Fig. 1) and YIp5 (42) (carrying the *URA3* gene) or (B) about 4×10^6 cpm of a gel-purified *Nco*I fragment (1.7 kb) derived from the coding region of the *STE13* gene carried on pBR322-*STE13* (D. A. Barnes, Ph.D. Thesis, University of California, Berkeley, 1985). Molecular weight markers for determining the sizes of the transcripts were a mixture of denatured DNA fragments of separate digests (*Eco*RI, *Rsa*I, and *Taq*I) of pBR322.

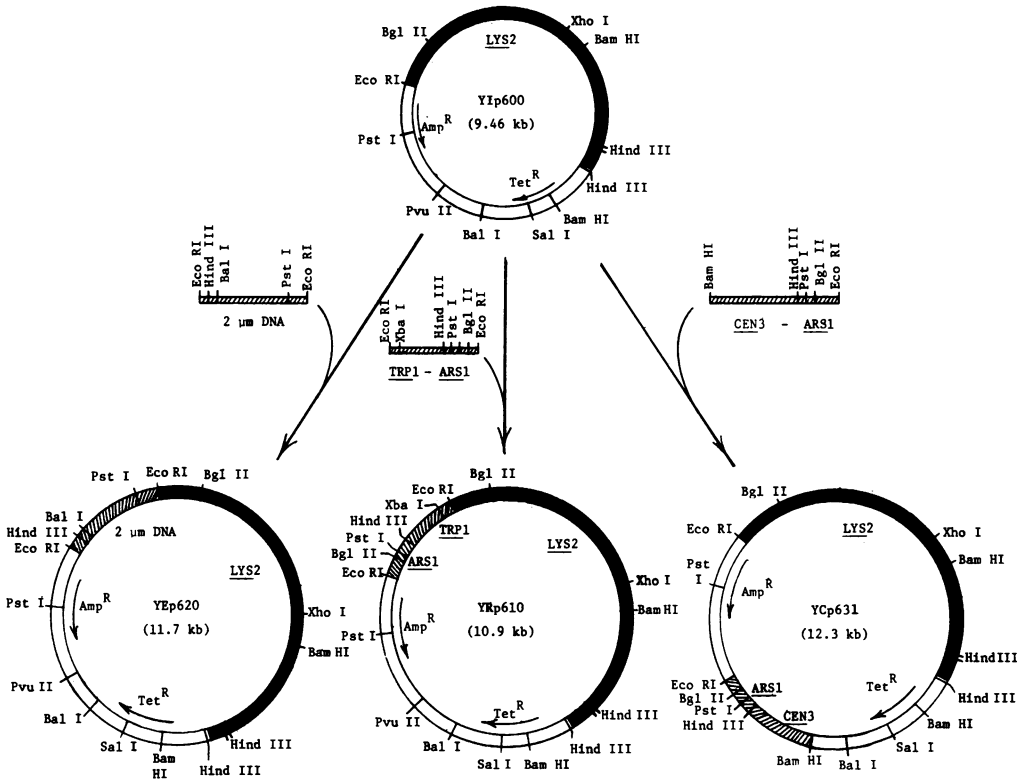

FIG. 4. Yeast cloning vehicles containing the *LYS2* gene. The plasmids indicated were constructed from the components shown as described in detail in the text.

resulted in a seven- to ninefold higher specific activity compared with that of extracts from a wild-type (*LYS2*$^+$) cell (Fig. 2).

***LYS2* transcription under general amino acid control.** To determine if *LYS2* gene expression is controlled at the transcriptional level by the amino acid composition of the medium, as in the expression of many other genes for amino acid biosynthetic enzymes in *S. cerevisiae*, poly(A)$^+$ RNAs were extracted from a prototrophic wild-type strain (S288C) grown in SD, in SD+Lys, in SD containing all 20 amino acids (SC), and in YPD. These RNAs were separated by electrophoresis, transferred to nitrocellulose paper, and examined by hybridization to a nick-translated pBR322 probe containing the entire *LYS2* gene. As a control, the filters were simultaneously hybridized to nick-translated YIp5, which contains the *URA3* gene (42), because *URA3* transcription is known not to be regulated by the presence or absence of amino acids in the medium (31). As an additional control, the blot was washed and rehybridized with a probe containing an internal segment of the *STE13* gene (22), which hybridizes to a 2.9-kb transcript (D. A. Barnes, unpublished data; G. F. Sprague, Jr., personal communication), to confirm that each lane contained essentially equivalent amounts of RNA.

As estimated from densitometer scans of the autoradiogram (Fig. 3), the *LYS2* transcript (4.2 kb) was elevated about 10-fold in cells grown in SD or in SD+Lys compared with its level in cells grown in SC or in YPD, suggesting that transcription of the *LYS2* gene is not regulated by the supply of lysine per se but rather is under repression control by the general amino acid control system (21).

TABLE 2. Transformation efficiencies of *LYS2* vectors

Vector	Selective medium	No. of transformants for following amt of added DNA (μg)		
		0.5	1	5
YEp13[a]	SC − Leu	300	1,000	>3,000
YEp24[a]	SC − Ura	500	>3,000	>3,000
YIp600[a]	SC − Lys	3	15	25
YIp601[a]	SC − Lys	5	18	25
YEp620[a]	SC − Lys	180	~1,000	>3,000
YEp620-R[a]	SC − Lys	220	~1,000	>3,000
YRp610[b]	SC − Lys	200	~1,000	>3,000
YRp610[b]	SC − Trp	330	~1,000	>3,000
YRp610-R[b]	SC − Lys	0	5	12
YRp610-R[b]	SC − Trp	300	~1,000	>3,000
YCp630[c]	SC − Lys	10	25	63
YCp631[c]	SC − Lys	ND[d]	>1,000	ND
YCp631-R[c]	SC − Lys	ND	12	ND

[a] Recipient strain was the same preparation of DA200 spheroplasts.
[b] Recipient strain was a separate preparation of DA200 spheroplasts.
[c] Recipient strain was the same preparation of DA4B spheroplasts.
[d] ND, Not determined.

Construction of vectors containing the *LYS2* gene. Subcloning and deletion analysis of pDA6200 revealed that the 4.6-kb *Eco*RI-*Hin*dIII segment of the insert (Fig. 1) was sufficient to confer a Lys$^+$ phenotype to *lys2* mutants. The slightly larger (4.8 kb) *Eco*RI-*Cla*I fragment was used to construct a variety of yeast cloning vehicles (Fig. 4). Yeast integrative plasmids (YIp600 and YIp601) were prepared by digesting pBR322 to completion with *Eco*RI and *Cla*I and by ligating with the *Eco*RI-*Cla*I fragment that contains the *LYS2* gene. In one of the resulting molecules (YIp600), the *Cla*I site was lost during the ligation; in YIp601, the *Cla*I site was

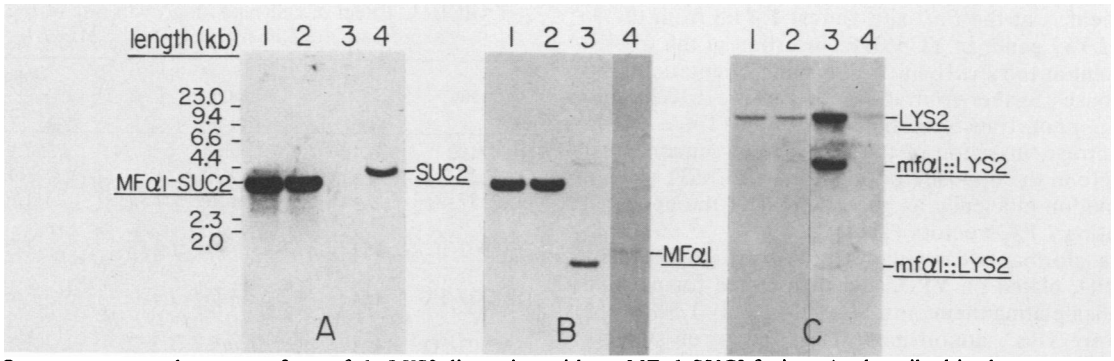

FIG. 5. One-step gene replacement of an *mfα1::LYS2* disruption with an *MFα1-SUC2* fusion. As described in the text, counterselection on α-AA medium was used to select for transformants in which an *MFα1-SUC2* gene fusion displaced a resident *LYS2* gene inserted at the *MFα1* locus (1). The DNA indicated in all panels was isolated, as described in Materials and Methods, from two Suc$^+$ transformants of the parental strain (lanes 1 and 2), the parental strain carrying both a deletion of the *SUC2* locus (*suc2-Δ9*) and the disrupted *MFα1* gene (*mfα1::LYS2*) (lane 3), and a *MFα1 SUC2* wild-type strain (lane 4). After digestion with *Eco*RI, electrophoresis on an agarose gel, and transfer to a nitrocellulose filter, the DNAs were hybridized to a single-stranded *SUC2*-specific probe (0.78-kb *Hin*dIII-*Bam*HI fragment [15] in mp19; M. C. Flessel, unpublished data) (A). The filter was stripped (25) and rehybridized to an *MFα1*-specific probe (pE2) (6) labeled by nick translation (32) (B). Finally, the filter was stripped again and hybridized a third time to a *LYS2*-specific probe (YIp600; see Fig. 4) labeled by nick translation (C). Considerably less DNA was loaded in lane 4 than in lanes 1 to 3. In addition, although approximately 4×10^6 cpm was used in each hybridization mixture, the probes that were used differed significantly in their specific radioactivity. Consequently, different exposure times were used for each sample when necessary to detect complementary DNA fragments. Length standards were *Hin*dIII fragments of phage λ.

preserved. YIp600 was digested with *Eco*RI to completion and ligated to a 2.2-kb *Eco*RI fragment containing the 2μm DNA origin of replication derived from the B form of the 2μm plasmid (7). Two plasmids (YEp620 and YEp620R) were isolated that contained the 2μm DNA segment in either orientation. YIp600 was digested to completion with *Eco*RI and ligated to the 1.5-kb *Eco*RI fragment containing the *TRP1* gene and *ARS1* replicator from YRp7 (44). Two plasmids (YRp610 and YRp610R), containing the *TRP1-ARS1* segment in either orientation, were obtained. YIp600 was digested either completely with *Bal*I or partially with *Pvu*II (which each produce blunt ends) and then ligated to a *Bam*HI-*Eco*RI fragment containing *CEN3* and *ARS1* derived from pYE41(CEN3) (10) that had been filled in with the large fragment of *E. coli* DNA polymerase I (Klenow fragment) to produce flush ends. At the *Bal*I site, only a plasmid (YCp630) containing the *CEN3-ARS1* fragment in the orientation in which *ARS1* is proximal to the 3′ end of the *LYS2* gene was recovered. Plasmids (YCp631 and YCp631R) containing the *CEN3-ARS1* fragment inserted in either orientation at the *Pvu*II site in the pBR322 sequence were obtained.

Plasmid vectors containing the *LYS2* gene must have a high transformation efficiency to be effective. To test the transformation efficiencies of our constructed vectors, we used different amounts of purified plasmid DNA to transform spheroplasts of the same *lys2* strain (Table 2). For comparison, the commonly used yeast vectors YEp24 (18) and YEp13 (8) were used to transform the same strain to Ura$^+$ and Leu$^+$ prototrophy, respectively. Because YRp610 and YRp610R also carry the *TRP1* gene, it was also possible to test the efficiency with which they transformed the same cells to Trp$^+$ prototrophy.

For every plasmid examined, the number of transformants was proportional to the amount of DNA added. As anticipated for integrative vectors, YIp600 and YIp601 gave transformation frequencies at least 2 orders of magnitude lower than those of the standard episomal plasmids, YEp13 and YEp24. Our episomal *LYS2* vectors, YEp620 and YEp620R, displayed transformation efficiencies nearly identical to those for YEp13 and YEp24. The orientation of the 2μm DNA segment had no effect on the transformation frequency. In contrast, the orientation of the *TRP1-ARS1* segment in the *LYS2* replicating vectors did have a pronounced effect. Both YRp610 and YRp610R gave an excellent transformation efficiency when selection was for Trp$^+$ prototrophy; however, only YRp610 was capable of producing an equivalent number of Lys$^+$ prototrophs. Perhaps an *ARS* sequence too near the *LYS2* promoter in YRp610R interfered with expression of the *LYS2* gene. In a similar way, the position of the *CEN3-ARS1* segment had a marked effect on transformation frequency. YCp630 displayed a very poor transformation efficiency, even though the centro-

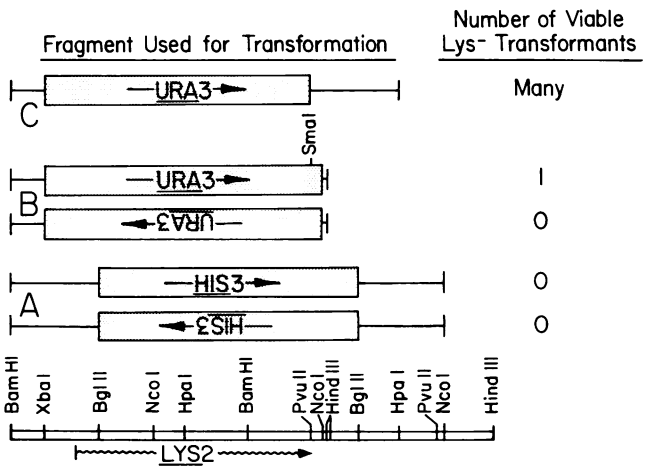

FIG. 6. Construction of a complete genomic deletion of the *LYS2* locus. The indicated regions of the *LYS2* gene were substituted in vitro with the selectable markers given in the orientations shown. The indicated fragments were excised by digestion with the appropriate restriction endonucleases and were used for DNA-mediated transformation of appropriate recipient strains: DBY746 and DBY747 (A), 689 (B), and SEY2102 (C). The marker genes are drawn so as to emphasize the DNA segments of the *LYS2* region that they replace and hence are not necessarily shown to scale.

mere fragment is at the *Bal*I site almost 1.4 kb from the 3′ end of the *LYS2* gene. In YCp631R, insertion of the *CEN3-ARS1* element at the *Pvu*II site in the same orientation (and about 600 bases further from the 3′ end of the *LYS2* gene) also gave a poor transformation efficiency (Table 2). In marked contrast, insertion of the centromere sequences at the *Pvu*II site in the opposite orientation in YCp631 yielded a transformation efficiency as good as that of the episomal and replicating *LYS2* vectors (Table 2).

When transformants carrying YCp631 were grown overnight in YPD, plated on YPD, and then tested for plasmid loss by replica plating them onto SC − Lys, only 1.5% of the colonies were Lys$^-$ auxotrophs. This degree of stability during mitotic growth is very comparable to that observed for other centromere-containing vectors (10).

Use of the *LYS2* gene for gene disruption and replacement. We have pointed out previously (1) that the *LYS2* system offers several distinct advantages for one-step gene disruption (29, 34, 36, 46). (i) Initially, any yeast strain can be made a recipient for transformation by the selection of a *lys2* derivative of the desired strain on α-AA medium. (ii) A functional *LYS2* gene is contained on DNA segments that can be inserted intact into sites for commonly used and readily available restriction endonucleases. (iii), Recombination events that substitute the normal chromosomal allele of a gene with the disrupted copy containing the *LYS2* gene can be readily obtained by selecting for Lys$^+$ transformants. We have previously described (1) a disruption of the major α-factor structural gene, *MFα1*, carried out in just this fashion. (iv) Once the *LYS2* gene has been used to disrupt the genomic copy of a gene of interest, another advantage of the *LYS2* system is that the resultant strain can be used as the recipient for one-step gene replacement by other constructions for which there would otherwise be no direct selection. Altered forms of the gene that have been constructed in vitro (38) can be inserted into the genome in place of the resident copy containing the *LYS2* gene by plating cells transformed with the replacement DNA and by selecting on α-AA medium for Lys$^-$ cells that have lost the *LYS2* sequences.

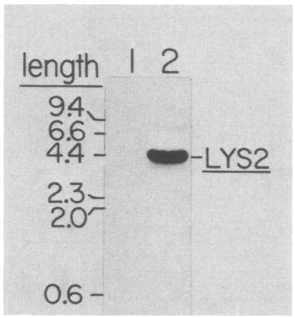

FIG. 7. Deletion of the chromosomal *LYS2* locus and substitution with the *URA3* gene. The *LYS2* gene was removed from its flanking regions and replaced with the *URA3* gene as described in Fig. 6C and in the text. This construction was excised by appropriate restriction endonuclease digestion and was used to transform SEY2102 (Table 1) to Ura$^+$ Lys$^-$. DNA was isolated from one such transformant (DA2100) (lane 1) and from the parental strain (SEY2102) (lane 2), digested with a mixture of *Eco*RI and *Hin*dIII, transferred to a nitrocellulose filter, and hybridized to a *LYS2*-specific probe (YIp601; see text). About 4 × 10^6 cpm was used for the hybridization, and the length standards were *Hin*dIII fragments of phage λ. Visualization of the gel by ethidium bromide staining demonstrated that both lanes of the gel contained equivalent amounts of DNA.

TABLE 3. Effect of cell type on growth rate of *lys2* mutants expressing the *LYS2* gene from the MFα*1* locus

Strain	Genotype	Doubling time (h)[a]	
		−Lys	+Lys
SEY2102	*MATα LYS2 MFα1*	1.9	NT[b]
DA2137-7A	*MATa lys2-Δ1 MFα1*	NT	1.7
DA2137-4C	*MATa lys2-Δ1* mfα*1*::*LYS2 (SYN)*	1.6	NT
DA8902-1C	*MATa lys2-Δ1 mfα1*::*LYS2 (ANTI)*	1.5	NT
DA2137-2A	*MATα lys2-Δ1 mfα1*::*LYS2 (SYN)*	3.6	1.6
DA8902-5D	*MATα lys2-Δ1 mfα1*::*LYS2 (ANTI)*	1.5	NT
DA72	*MATa/MATα lys2-Δ1/lys2-Δ1 MFα/mfα1*::*LYS2 (SYN)*	1.8	NT
DA57	*MATa/MATα lys2-Δ1/lys2-Δ1 MFα1/mfα1*::*LYS2 (ANTI)*	1.5	NT

[a] Cells were grown on SD in the presence or absence of lysine (30 μg/ml), supplemented where necessary with the other nutrients required by the strain (see Table 1).
[b] NT, Not tested.

To test how well this gene replacement ("transplacement") procedure would work, a *MATα lys2 mfα1*::*LYS2* strain (DA2102-375-R) (1) that is α-factor deficient due to disruption of the major α-factor gene was transformed with an *Eco*RI fragment (3.7 kb) containing an *MFα1-SUC2* (prepro-α factor-invertase) translational fusion. This fusion is similar to that constructed previously (15) but retains on both its 5′ and 3′ ends the genomic DNA that normally flanks the *MFα1* gene (M. C. Flessel and J. Thorner, unpublished data). Because the recipient cells carried *LYS2*$^+$, some functional α-AA reductase was probably still present after the transplacement event. Therefore, to permit phenotypic expression before imposing the selection for *lys2*$^-$ transformants, the cells were grown up nonselectively in YPD top agar on YPD plates for 48 h to dilute out any functional α-AA reductase. The top agar was scraped off the plate, macerated in 1 ml of sterile water, and removed by settling out. The cells in the supernatant solution were collected by centrifugation and resuspended in the same volume of sterile water. An aliquot (0.2 ml) of the suspension (about 10^8 cells per ml) was plated on α-AA medium. The colonies appearing on these plates were then replica plated to BCP-sucrose indicator plates (35). In this assay, cells that produce invertase split sucrose, grow on the glucose and fructose so released, and thus generate acids that lower the pH of the medium. The pH drop is monitored by bromcresol purple indicator dye (which turns from royal purple to canary yellow). When 7 to 8 μg of *MFα1-SUC2* DNA was used in a standard transformation mix, about 1% of the α-AA-resistant colonies that arose was Suc$^+$. The Suc$^-$ α-AA-resistant colonies were presumably spontaneously arising *lys2* mutants.

Two of the Suc$^+$ transformants were subjected to Southern analysis (Fig. 5). DNA was isolated from the two transformants, from the untransformed parent (which was deleted for its normal chromosomal *SUC2* locus), and from X2180-1B (*SUC2*$^+$). These DNAs were digested with *Eco*RI, run on an agarose gel, and blotted to a nitrocellulose filter. The filter was hybridized to a *SUC2* probe. No band was detected in the lane containing DNA from the parental *suc2Δ* strain, and a band of approximately 4.1 kb corresponding to the normal *SUC2* region was observed in DNA from the *SUC2*$^+$ control strain. In the lanes containing DNA from the two Suc$^+$ transformants, the expected 3.7-kb *Eco*RI frag-

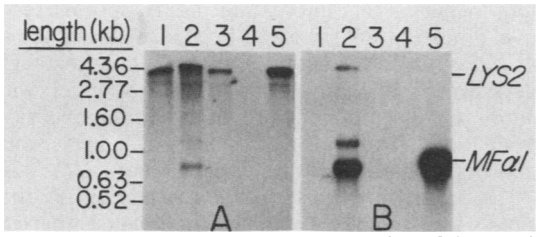

FIG. 8. Effect of yeast cell type on expression of the *LYS2* gene inserted at the *MFα1* locus. Poly(A)$^+$ RNA was prepared, as described in Materials and Methods, from the following strains: *MAT***a** *lys2-Δ1 mfα1::LYS2 (SYN)* (lane 1); *MATα lys2-Δ1 mfα1::LYS2 (SYN)* (lane 2); *MAT***a**/*MATα lys2-Δ1/lys2-Δ1 MFα1/mfα1::LYS2 (SYN)* (lane 3); *MAT***a** *lys2-Δ1 MFα1* (lane 4); and *MATα LYS2 MFα1* (lane 5). After denaturation, electrophoresis in an agarose gel, and transfer to nitrocellulose, the DNAs were hybridized either to a *LYS2*-specific probe (YIp601) labeled by nick translation (A) or to an *MFα1*-specific single-stranded probe labeled by primer extension (generous gift of M. C. Flessel) (B). The length standards were denatured DNA fragments produced from separate digests (*Eco*RI, *Acc*I, and *Eco*RI + *Hin*fI) of pBR322. The filter was also hybridized to both *URA3* and *STE13* probes (not shown), which confirmed that the amount of RNA applied in each lane was approximately equivalent.

ment derived from the *MFα1-SUC2* fusion was observed. To determine if the *SUC2* sequences actually were inserted at the *MFα1* locus, the blot was washed and rehybridized to an *MFα1* probe. A 1.75-kb *Eco*RI fragment corresponding to the normal *MFα1* gene (6) was observed in DNA from the wild-type control. DNA from the parental strain (*mfα1::LYS2*) was hybridized to two fragments because there is an *Eco*RI site within the *LYS2* sequence which splits the flanking homology to the *MFα1* probe into two *Eco*RI fragments (1). In the two Suc$^+$ transformants, a single band was observed with the *MFα1* probe that is identical in mobility to that seen with the *SUC2* probe, because the *SUC2* sequence lacks an *Eco*RI site (15). Finally, with a *LYS2* probe, the Suc$^+$ transformants were found to have lost all detectable *LYS2* sequences at the *MFα1* locus. Thus, it can be concluded with confidence that the *MFα1-SUC2* fusion construction had replaced the disrupted genomic *mfα1::LYS2* copy.

Construction of chromosomal deletion of *LYS2* gene. In certain genetic manipulations of yeasts involving positive selection for either the *lys2* or the *LYS2* genotype (1), it is advantageous to prevent recombination between the genomic *LYS2* sequence and *LYS2* DNA on a plasmid or linear fragment introduced by transformation. To avoid such spurious recombination events, we have deleted the entire transcribed region of the *LYS2* gene and have constructed strains that carry this chromosomal deletion.

When a *Bam*HI fragment containing a functional *HIS3* gene (42) was inserted in either orientation between the two *Bgl*II sites in the *LYS2* region (Fig. 6A), was excised by digestion with restriction enzymes that cleave in the flanking DNA, and was used for transformation, none of the resulting stable His$^+$ transformants was found to be Lys$^-$, even after exhaustive screening. The inability to recover this deletion, which lacks genomic DNA beyond the 3′ end of the *LYS2* gene, suggested that this region of chromosome II contains an essential gene. In an attempt to avoid this problem, the *URA3* gene (1.1-kb *Hin*dIII fragment) was inserted by blunt-end ligation in either orientation between the *Xba*I and *Nco*I sites that flank the *LYS2* region (Fig. 6B), excised appropriately, and used for transformation. Of numerous Ura$^+$ transformants, only one Ura$^+$ Lys$^-$ clone was found after exhaustive screening. Southern analysis indicated that this strain had a deletion of the chromosomal copy of *LYS2* and had the *URA3* gene inserted such that its transcription was in the same direction as *LYS2* transcription (data not shown). However, the cells grew very poorly and had a clumpy phenotype. Diploids arising from a cross of the Ura$^+$ Lys$^-$ transformant to SX50-1C (Table 1) were not clumpy, suggesting that the defect leading to the clumpy trait was recessive. Diploids from this cross were sporulated, and the tetrads were dissected. Ura$^+$ segregated 2:2 in every tetrad, and clumpiness and slow growth always cosegregated with uracil prototrophy. In addition, regardless of their genetic constitution at *MAT*, cells containing this *lys2* deletion-*URA3* insertion produced **a** factor, as determined by the halo bioassay technique described by Julius et al. (22).

To replace an even larger portion of the 3′-flanking region, a 2.2-kb *Pvu*II fragment containing the region immediately 3′ to the *LYS2* gene was inserted into the *Sma*I site at the 3′ side of the *URA3* gene in the previous construction (Fig. 6C) and was used for transformation. Of six Ura$^+$ transformants tested, all were also Lys$^-$, as judged by their inability to grow on SC − Lys. In complete medium, these transformants had a growth rate equivalent to that of the parent strain and appeared to be physiologically normal in all other respects.

To prove conclusively that the genomic copy of *LYS2* had been deleted in the Ura$^+$ Lys$^-$ clones, Southern analysis was used. DNA was isolated from the parent strain (SEY2102) and from one of the Ura$^+$ Lys$^-$ transformants (DA2100), digested with both *Eco*RI and *Hin*dIII, separated on an agarose gel, and blotted to a nitrocellulose filter. The filter was probed with nick-translated YIp601. As anticipated, no *LYS2* sequences were detected in DA2100 (Fig. 7), but the parental strain yielded the expected *LYS2* fragment. This same filter was washed and rehybridized to YIp5 (41) to determine the location of the *URA3* sequences (data not shown). The parental strain produced two bands (2.1 and 3.9 kb, respectively) corresponding to the *ura3-52* allele, which is an insertion of a Ty element into the coding region of the *URA3* locus (33). As expected, DA2100 contained the same two bands and an additional copy of *URA3* of a size (3.3 kb) consistent with substitution of the *LYS2* sequence on chromosome II with the shorter *URA3* gene. Also, as expected for a deletion mutation, we have been unable to recover any spontaneous Lys$^+$ revertants ($\leq 10^{-9}$) from strains carrying this allele (*lys2-Δ1*).

LYS2* transcription at the *MFα1* locus.** It has been shown that expression of the *MFα1* gene is tightly regulated at the transcriptional level depending on the cell type (6, 15). *MFα1* mRNA is made in *MATα* haploids but not in *MATa** haploids or in *MAT***a**/*MATα* diploids. In the gene disruptions we constructed previously (1), the *LYS2* gene with its own promoter intact was inserted into *MFα1* and was expressed in *MATα* cells, regardless of its orientation, because Lys$^+$ transformants were recovered at the same frequency for both insertions. When the insertion mutations were transferred into *MAT***a** and *MAT***a**/*MATα* backgrounds by genetic crosses, the cells also had Lys$^+$ phenotypes and were able to grow on solid medium lacking lysine, just as the *MATα* integrants could (1). Because it is not known what level of *LYS2* gene expression is sufficient to confer a Lys$^+$ phenotype in vivo, however, it was still possible that the level of expression of the *LYS2* gene inserted at *MFα1* was reduced significantly in *MAT***a** and *MAT***a**/*MATα* cells, compared with that in the *MATα* cells.

The availability of the *lys2-Δ1* mutation on chromosome II made it possible to examine expression of a *LYS2* gene inserted at the *MFα1* locus in all three yeast cell types. Strains carrying both the *mfα1::LYS2* (*SYN*) insertion (1) and the *lys2-Δ1* deletion were constructed by standard genetic crosses. Similar sets of crosses were used to construct strains carrying both the *lys2-Δ1* deletion and the *mfα1::LYS2* (*ANTI*) insertion (1). All of these strains were grown in SD containing the appropriate supplements, and doubling times were determined (Table 3). It is perhaps surprising that only the *MATα* strain, DA2137-2A (wherein the *MFα1* promoter is in the same orientation as, and about 600 bp upstream from, the *LYS2* promoter), grew significantly more slowly than did the others. In fact, this strain was found to be specifically limited for lysine, since addition of only this amino acid to the medium restored a normal growth rate. Similar physiological behavior was observed for other *MATα lys2* mutants into which the *mfα1::LYS2* (*SYN*) allele was transferred by genetic crosses (data not shown).

Thus, contrary to our original expectation, these results seemed to indicate that transcription from the *MFα1* promoter might interfere with, rather than enhance, initiation of transcription from the *LYS2* promoter. To examine this possibility directly, poly(A)$^+$ RNA was isolated from DA2137-2A, DA2137-4C, DA2137-7A, DA72, and SEY2102 grown in SD containing the appropriate supplements. After electrophoresis and blotting of the RNAs, the filter was hybridized to nick-translated YIp601 and then subjected to autoradiography (Fig. 8). As expected, a strain (DA2137-7A) carrying just the *lys2-Δ1* allele produced no detectable *LYS2* transcript, while SEY2102 (*LYS2*$^+$) produced the 4.2-kb *LYS2* transcript derived from the normal *LYS2* locus. The *MAT***a** haploid (DA2137-4C) and *MAT***a**/*MATα* diploid (DA72) produced readily detectable amounts of the normal 4.2-kb *LYS2* transcript. In contrast, in the *MATα* strain (DA2137-2A) the 4.2-kb species was not detectable and three unique transcripts were observed. The largest of these novel transcripts had an apparent length consistent with a molecule resulting from initiation at the *MFα1* promoter and termination at the normal *LYS2* terminator. The two short transcripts (approximately 780 and 1,100 bases) are consistent with their initiation at the *MFα1* promoter and termination within an AT-rich region in the early portion of the *LYS2* coding sequence (14). In support of these suppositions, rehybridization of the filter with an *MFα1* probe confirmed that all three of the unique transcripts contained *MFα1* sequences.

The aberrant nature of the *MFα1-LYS2* readthrough transcripts produced in the *MATα* haploids may interfere with their proper translation and may limit the amount of α-AA reductase made in the cells. Deficiency of the enzyme would account for the "leaky" lysine auxotrophy observed (Table 3).

DISCUSSION

The *LYS2* gene was cloned and characterized. We used a variety of genetic and biochemical criteria to prove that the DNA that was isolated corresponded to the *LYS2* gene, including its ability to integrate by homologous recombination at the *LYS2* locus on chromosome II. The physical map of the DNA we obtained is identical to that reported by Eibel and Philippsen (13) and by S. C. Falco (cited in reference 39).

Even without optimizing its expression, the presence of the *LYS2* gene on a multicopy plasmid resulted in a dramatic overproduction of α-AA reductase activity, which should aid attempts to purify this interesting multifunctional enzyme. The level of *LYS2* transcript was 1 order of magnitude higher in cells grown on SD than in cells provided with a supply of complete amino acids, yet the transcript level was not detectably repressed in cells provided with an excess of the end product of its own pathway, lysine, which was similar to what has been observed for other genes (e.g., *HIS4* [12] and *ARG4* [30, 31]) under transcriptional regulation by the general amino acid control system (21). Because the level of *LYS2* transcript was not measured in cells limited for lysine or any other amino acid (by starvation of a leaky auxotroph or by use of an amino acid analog), it is still possible that *LYS2* transcription can be derepressed to an even greater extent than was observed in SD. Nonetheless, the degree of derepression observed in SD is already sufficient to account for the elevation of enzyme activity (about sevenfold) by histidine starvation observed previously by Wolfner et al. (47).

We used the *LYS2* gene to construct YIp, YEp, YRp, and YCp classes of cloning vehicles for yeast transformation. To our knowledge, at least two similar vectors, an integrative plasmid (YIp333) and an autonomously replicating plasmid (YRp31), were prepared by Eibel and Philippsen (13). However, these plasmids use the significantly larger *Eco*RI-*Pst*I (7 kb) segment of the *LYS2* region (Fig. 1) and, hence, are more cumbersome and less convenient to use than the more compact vectors we constructed.

Aside from serving as the selectable marker on such vectors, the *LYS2* gene is also useful for other types of genetic manipulation of yeasts. We have shown previously that the *LYS2* system offers certain advantages for one-step gene disruption (1). We have demonstrated further in this report that once the *LYS2* gene has been inserted into the yeast genome in place of the normal copy of the gene of interest (in this case, the major α-factor structural gene *MFα1*), one-step gene replacement can be performed conveniently by selecting on α-AA medium for those transformants that have lost the *LYS2* sequences. This procedure should facilitate the introduction of any type of altered gene in a single copy at its normal chromosomal locus. Unlike the *MFα1-SUC2* fusion used here for transplacement, the directed alteration of other genes in other circumstances may not have a readily detectable phenotype. For this reason, the resultant α-AA-resistant colonies may need to be screened by hybridization with an appropriate probe for those colonies in which the target gene has been successfully replaced. This situation should not present a problem, however, because true transplacements are observed at a reasonable frequency (1%) by the technique we have described here. Furthermore, we did not bother to optimize conditions for outgrowth in YPD or determine rigorously how much time was sufficient for phenotypic expression. Judicious attention to this temporal parameter could improve the ratio of true transformants to spontaneously arising *lys2* mutants.

We constructed a deletion-insertion mutation (*lys2-Δ1*) that removes all *LYS2* coding information. Such an allele should be very useful in further genetic applications of the *LYS2* system because recombinational events between the *LYS2* DNA introduced by transformation and the *LYS2* locus on chromosome II will be eliminated. Deletion of the gene had to be very precise because we found that removal of sequences beyond the 3′ end of the transcribed region caused either lethality or slow growth, clumpy morphology, and a *MATα*-specific mating deficiency. This latter constellation of phenotypes is also characteristic of disruptions and deletions in the *SSN6* (*CYC8*) gene, which is known to be

located immediately 3′ to *LYS2*, although a transcription unit (2.5-kb mRNA) of unknown function lies in between (J. Schultz and M. Carlson, personal communication).

The *lys2-Δ1* mutation we constructed eliminates all detectable *LYS2* mRNA. The use of strains carrying this deletion mutation allowed us to examine the effects of the *MFα1* promoter on the transcription of a *LYS2* gene placed immediately downstream. In the construction used (1), the apparent start of *LYS2* transcription (14) lies about 620 base pairs distal to the putative start of *MFα1* transcription (6; A. Brake, M. C. Flessel, and J. Thorner, unpublished data). In *MAT***a** haploids and *MAT***a**/*MATα* diploids where the *MFα1* gene is normally quiescent, the *LYS2* gene appears to function properly, because a *LYS2* transcript of normal length is produced. However, the level of *LYS2* mRNA made was distinctly lower than that produced from the *LYS2* gene at its normal locus in wild-type cells (Fig. 8). Because the cells were grown in SD (derepressing conditions), this observation suggests that despite the presence of one copy of the consensus sequence for general amino acid control, 5′-A(A/T)GTGACTC-3′ (12; D. Pridmore and P. Philippsen, personal communication), the segment of the *LYS2* promoter region used in the insertion construction may lack other sequences that are necessary for full derepression of the *LYS2* gene. Alternatively, proteins that interact with the *MFα1* promoter may sterically block the action of the factors required for full derepression of the *LYS2* gene. In *MATα* cells, the *MFα1* promoter is active and appears to completely override normal *LYS2* transcription, because only transcripts of unique size were produced. The large *LYS2*-containing transcript is longer than normal *LYS2* mRNA and contains *MFα1* sequences. Its length is consistent with initiation at the *MFα1* promoter, readthrough into the *LYS2* coding sequence, and termination at the *LYS2* terminator. The two short transcripts presumably arise from premature transcription termination. Based on its length, the major small transcript (about 780 nucleotides) appears to initiate at the *MFα1* promoter and terminate about 250 base pairs downstream from the *LYS2* methionine initiator codon in an AT-rich region of the coding sequence (D. Pridmore and P. Philippsen, personal communication). By visual inspection, we have found that this AT-rich region contains a perfect match to the consensus sequence described by Zaret and Sherman (49) that appears to be responsible for efficient transcription termination in yeasts. This AT-rich region of the *LYS2* gene is also the site of preferential integration of Ty elements (14, 39). Remarkably, however, short transcripts were not produced from the *mfα1::LYS2* locus in *MAT***a** and *MAT***a**/*MATα* cells, and we have never observed truncated transcripts produced from the *LYS2* gene at its normal locus on chromosome II, although the gel system we use should resolve RNA molecules of the expected size. Perhaps such a short transcript is made but does not accumulate appreciably because it is rapidly degraded. Alternatively, the fact that transcription termination within *LYS2* occurs but only when tanscription begins about 600 base pairs upstream of the normal transcriptional start suggests either that some novel secondary structure in the RNA is part of the recognition of a termination signal in yeast (like attenuator stem-loop structures in procaryotes [48]) or that normal *LYS2* transcription involves some sort of antitermination mechanism (like bacteriophage λ N protein and its utilization sites [19]).

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LITERATURE CITED

1. **Barnes, D. A., and J. Thorner.** 1985. Use of the *LYS2* gene for gene disruption, gene replacement, and promoter analysis in *Saccharomyces cerevisiae*, p. 197–226. *In* J. W. Bennett and L. L. Lasure (ed.), Gene manipulations in fungi. Academic Press, Inc., Orlando, Fla.
2. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
3. **Bhattacharjee, J. K.** 1983. Lysine biosynthesis in eukaryotes, p. 229–249. *In* K. M. Herrmann and R. L. Somerville (ed.), Amino acid biosynthesis and genetic regulation. Addison-Wesley, Inc., Reading, Mass.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1525.
5. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
6. **Brake, A. J., D. J. Julius, and J. Thorner.** 1983. A functional prepro-α-factor gene in *Saccharomyces* yeasts can contain three, four, or five repeats of the mature pheromone sequence. Mol. Cell. Biol. **3**:1440–1450.
7. **Broach, J. R.** 1981. The yeast 2 μm circle, p. 445–470. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), Molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **3**:121–133.
9. **Chattoo, B. B., F. Sherman, T. A. Fjellstedt, D. Mehnert, and M. Ogur.** 1979. Selection of *lys2* mutants of the yeast *Saccharomyces cerevisae* by the utilization of α-aminoadipate. Genetics **93**:51–65.
10. **Clarke, L., and J. Carbon.** 1980. Isolation of a yeast centromere and construction of functional small circular chromosomes. Nature (London) **287**:504–509.
11. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Donahue, T. F., R. S. Daves, G. Lucchini, and G. R. Fink.** 1983. A short nucleotide sequence required for regulation of *HIS4* by the general control system of yeast. Cell **32**:89–98.
13. **Eibel, H., and P. Philippsen.** 1983. Identification of the cloned *S. cerevisiae LYS2* gene by an integrative transformation approach. Mol. Gen. Genet. **191**:66–73.
14. **Eibel, H., and P. Philippsen.** 1984. Preferential integration of yeast transposable element Ty into a promoter region. Nature (London) **307**:386–388.
15. **Emr, S. D., R. Schekman, M. C. Flessel, and J. Thorner.** 1983. An *MFα1-SUC2* (α-factor-invertase) gene fusion for study of protein localization and gene expression in yeast. Proc. Natl. Acad. Sci. USA **80**:7080–7084.
16. **Falco, S. C., and D. Botstein.** 1983. A rapid chromosome mapping method for cloned fragments of yeast DNA. Genetics **105**:857–872.
17. **Falco, S. C., Y. Li, J. R. Broach, and D. Botstein.** 1982. Genetic properties of chromosomally integrated 2 μm plasmid DNA in yeast. Cell **29**:573–584.
18. **Fasiolo, F., J. Bonnet, and F. Lacroute.** 1981. Cloning of the yeast methionyl-tRNA synthetase gene. J. Biol. Chem. **56**: 2324–2328.
19. **Friedman, D. I., and M. Gottesman.** 1983. Lytic mode of lambda development, p. 21–51. *In* R. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Jimenez, A., and J. Davies.** 1980. Expression of a transposable

antibiotic resistance element in *Saccharomyces cerevisiae*: a potential selection for eukaryotic cloning vectors. Nature (London) **287**:869–871.
21. **Jones, E. W., and G. R. Fink.** 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p. 281–299. *In* J. N. Strathern, E. W. Jones, and J. R. Broach, (ed.), Molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Julius, D., L. Blair, A. Brake, G. Sprague, and J. Thorner.** 1983. Yeast α-factor is processed from a larger precursor polypeptide: the essential role of a membrane-bound dipeptidyl aminopeptidase. Cell **32**:839–852.
23. **Klapholz, S., and R. E. Esposito.** 1982. A new mapping method employing a meiotic Rec⁻ mutant of yeast. Genetics **100**: 387–412.
24. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Mortimer, R. K., and D. Schild.** 1985. Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49**:181–212.
28. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78**:6354–6358.
29. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1983. Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101**:228–245.
30. **Penn, M. D., B. Galgoci, and H. Greer.** 1983. Identification of AAS genes and their regulatory role in general control of amino acid biosynthesis in yeast. Proc. Natl. Acad. Sci. USA **80**: 2704–2708.
31. **Penn, M. D., G. Thireos, and H. Greer.** 1984. Temporal analysis of general control of amino acid biosynthesis in *Saccharomyces cerevisiae*: role of positive regulatory genes in initiation and maintenance of mRNA derepression. Mol. Cell. Biol. **4**:520–528.
32. **Rigby, P., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling of deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
33. **Rose, M. D., and F. Winston.** 1984. Identification of a Ty insertion within the coding sequence of the *S. cerevisiae URA3* gene. Mol. Gen. Genet. **193**:557–560.
34. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
35. **Schaefler, S.** 1967. Inducible system for the utilization of β-glucosides in *Escherichia coli*. I. Active transport and utilization of β-glucosides. J. Bacteriol. **93**:254–263.
36. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
37. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1979. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Shortle, D., D. DiMaio, and D. Nathans.** 1981. Directed mutagenesis. Annu Rev. Genet. **15**:265–294.
39. **Simchen, G., F. Winston, C. A. Styles, and G. R. Fink.** 1984. Ty-mediated gene expression of the *LYS2* and *HIS4* genes of *Saccharomyces cerevisiae* is controlled by the same *SPT* genes. Proc. Natl. Acad. Sci. USA **81**:2431–2434.
40. **Sinha, A. K., and J. K. Bhattacharjee.** 1971. Lysine biosynthesis in *Saccharomyces*. Biochem. J. **125**:743–749.
41. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
42. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
43. **Thomas, P.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
44. **Tsumpfer, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
45. **Tuit, M. F., J. Plesset, K. Moldave, and C. S. McLaughlin.** 1980. Faithful and efficient translation of homologous and heterologous mRNAs in an mRNA-dependent cell-free system from *Saccharomyces cerevisiae*. J. Biol. Chem. **255**:8761–8766.
46. **Winston, F., F. Chumley, and G. R. Fink.** 1983. Eviction and transplacement of mutant genes in yeast. Methods Enzymol. **101**:211–228.
47. **Wolfner, M., D. Yep, F. Messenguy, and G. R. Fink.** 1975. Integration of amino acid biosynthesis into the cell cycle of *Saccharomyces cerevisiae*. J. Mol. Biol. **96**:273–290.
48. **Yanofsky, C., and R. Kolter.** 1982. Attenuation in amino acid biosynthetic operons. Annu Rev. Genet. **16**:113–134.
49. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.
50. **Zaret, K. S., and F. Sherman.** 1985. α-Aminoadipate as a primary nitrogen source for *Saccharomyces cerevisiae*. J. Bacteriol. **162**:579–583.