Two Unlinked Lysine Genes (LYS9 and LYS14) Are Required for
the Synthesis of Saccharopine Reductase in Saccharomyces
cerevisiae

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Three lysine auxotrophs, strains AU363, 7305d, and 8201-7A, were investigated genetically and
biochemically to determine their gene loci, biochemical lesions, and roles in the lysine biosynthesis of
Saccharomyces cerevisiae. These mutants were leaky and blocked after the $\alpha$-aminoacidopate step.
Complementation studies placed these three mutations into a single, new complementation group, lys14.

Tetrad analysis from appropriate crosses provided evidence that the lys14 locus represented a single nuclear
gene and that lys14 mutants were genetically distinct from the other mutants (lys1, lys2, lys5, and lys9)
blocked after the $\alpha$-aminoacidopate step. The lys14 strains, like lys9 mutants, accumulated $\alpha$-aminoacidopate-
semialdehyde and lacked significant amounts of saccharopine reductase activity. On the bases of these
results, it was concluded, therefore, that LYS9 and LYS14, two distinct genes, were required for the
biosynthesis of saccharopine reductase in wild-type S. cerevisiae.

Lysine is synthesized in the facultative yeast Saccharomyces
cerevisiae by the $\alpha$-aminoacidopate (AA) pathways charac-
teristic of higher fungi and blue-green algae (1, 11, 21, 23).
Lysine auxotrophs blocked in the AA pathway have provided
considerable evidence for elucidating the genetic and
biochemical basis of this pathway in Neurospora crassa (1, 22).
Saccharomyces lipolytica (8), Rhodotorula glutinis (12), and S. cerevisiae (1, 2, 9, 10). More than eight lysine
loci for eight enzyme steps have been identified by comple-
mmentation and recombination analysis in S. cerevisiae (1, 3, 9)
and S. lipolytica (8).

Mutants belonging to five different complementation
groups of S. cerevisiae are blocked between the AA step and
lysine (Fig. 1). lys2 and lys5 mutants lack the $\alpha$-aminoacidopate-semialdehyde (ASA) dehydrogenase (EC 1.2.1.31),
commonly known as the AA reductase (2, 5, 10, 19), and lys1
mutants lack the saccharopine dehydrogenase (lysine-forming;
EC 1.5.1.7) activity (2, 10). Saccharopine dehydrogenase
(glutamate-forming; EC 1.5.1.10), commonly known as
saccharopine reductase, catalyzes the conversion of ASA to
saccharopine (1, 6, 10, 20). This enzyme has been partially
purified and shown to exhibit two distinct pH optima for the
forward (pH 7.0) and reverse (pH 9.5) reactions (1, 10). To
date, no evidence has been presented to indicate that mul-
tiple genes are needed for the synthesis of this enzyme.
Results from the complementation, recombination, and bio-
chemical studies demonstrated that mutants previously clas-
sified as lys9 and lys13 (3, 9) belong to the same complementa-
tion group and that lys9 and lys14, representing two
distinct loci, are required for the biosynthesis of saccharo-
pine reductase enzyme.

Lysine auxotrophs were procured from the prototrophic
strain X2180-1A after treatment with ethyl methanesulfonate
and nystatin enrichment (3). Stock cultures were maintained
on a nutrient medium; growth studies and genetic and
biochemical analyses were performed by growing cells in
minimal medium (MM) at 30°C (24). Lysine and AA or other
amino acids were supplemented at 20 and 50 $\mu$g/ml, respec-
tively. Cells grown in liquid medium were shaken moder-
ately. Sporulation medium was as described by McClary et al.
(14). Growth response of lysine auxotrophs to MM, MM
supplemented with AA, or lysine was determined spectrot-
ometrarily at 550 nm. Complementation and genetic
analyses, including tetrad analysis for the monogenic seg-
gregation and recombination studies, were performed according
to published procedures (3, 18). Mutants blocked after the
ASA step accumulate ASA in the culture supernatant when a
mutant is grown in a growth-limiting concentration of lysine.
ASA also accumulated after leaky growth of lys14 mutants in
MM. Segregants plated on minimal agar plates were exam-
ined for lysine requirement within 24 h and were allowed to
grow for an additional 48 h to determine the accumulation of
ASA in the leaky lys14 segregants by suspending cells from
each colony in 0.4 ml of sterile water (17, 19). Prototrophic
strain X2180 and a lys14 mutant served as negative and
positive controls, respectively. Accumulation of ASA was
also determined in liquid cultures.

Prototrophic cells for enzyme assays were grown in liquid
MM, and the mutant cells were grown in MM supplemented
with 20 $\mu$g of lysine per ml. Crude enzyme preparations,
desalting of extracts, and the determination of protein were
performed according to published procedures (7, 19, 24).
The AA reductase activity was assayed by the method
originally described by Sagisaka and Shimura (17) and later
by Larson et al. (13). The reaction mixture and the assay
procedure have been described previously (20, 24). Sacchar-
opine reductase activity was assayed in the reverse direction
according to the method described by Jones and Broquist
(10, 20). The reaction mixture consisted of 2.5 mM sacchar-
opine, 0.25 mM NAD, 400 mM Tris-hydrochloride buffer (pH
9.0), and crude enzyme preparation in a final volume of 1 ml.
The control tube lacked NAD. The mixture was incubated
for 2 h at 30°C, and the formation of ASA and p-dimethyl-
aminobenzaldehyde adduct was determined as described for
the AA reductase assay (19, 24). Saccharopine dehydroge-

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nase (lysine-forming) activity was assayed in the reverse direction (10). The reaction mixture and the assay procedures have been described previously (20, 24).

Lysine auxotrophs blocked between AA and lysine were initially identified by their inability to grow in MM supplemented with AA. Of 336 such mutants, three complemented mutations in all loci (lys2, lys5, lys9 and I3, and Iysl) known to specify products that act after the AA step in the lysine pathway. Mutant 7305d did not grow in MM supplemented with AA and also complemented lys1, lys2, lys5, and lys9 tester strains. Three of the newly procured isolates of this category, including strain AU363, failed to complement with 8201-7A, a segregant from 74622 
a, lys14, trpX X2180-1A hybrid, and 7305d. Thus, the mutations in strains 7305d, AU363, and 8201-7A belong to the same complementation group and are assigned to the lys14 locus. The lys14 strains exhibited good growth in MM supplemented with lysine and a much slower growth with a longer lag phase (leaky) in MM and MM supplemented with AA, suggesting their biochemical block to be between the AA step and lysine (Fig. 1). Of the remaining mutants, 199 failed to complement lys2, 106 failed to complement lys9 and lys13, 17 failed to complement lys5, and 11 failed to complement lys1 tester.

All tetrads examined from hybrids involving the prototrophic strain X2180 and tester strains for lys1, lys2, and lys5 exhibited a 2:2 ratio of segregation for the mating type (results not shown) and lysine requirement, indicative of a single nuclear gene mutation (Table 1). Only two of the tetrads from hybrid 8125, involving a lys9 mutant, exhibited an abnormal ratio of segregation. A second lysine mutation (lys14) is present in the lys9 tester strain X1012-1D (unpublished data). Tetrads from hybrids involving lys14 mutants, 7305d, and 8201-7A also exhibited a 2:2 ratio of segregation for the lysine requirement. Fifteen of the 20 tetrads from the hybrid 8104 involving the strain SA363 exhibited normal segregation (Table 1). A significant number of recombinant tetrads were observed when a lys14 strain was crossed with lys1, lys2, lys5, and lys9 testers (Table 2). The frequency of

![FIG. 1. Lysine mutants of *S. cerevisiae* blocked at the steps between AA and lysine.](http://jb.asm.org/)
parental ditype, nonparental ditype, and tetratype did not indicate any tight linkage between lys14 and other lysine loci examined. The lys14 strains crossed among themselves (hybrids 8205 and 8308) exhibited a total of 74 parental ditype and only one tetratype tetrads. In 54 total tetrads from three different crosses between lys9 and lys13, no significant recombinant tetrads resulted. Results from recombination studies based on tetrad analysis confirmed that all three lys14 mutations belong to the same locus and that the lys14 locus is unlinked to the other lysine loci examined.

The prototrophic strain X2180 and lys2 as well as lys5 mutants did not accumulate any ASA; however, mutants blocked after this step, including lys14 strains, accumulated significant amounts of ASA, with 8103-21C (lys9) showing the maximum accumulation (Table 3). lys2 and lys5 mutants exhibited little or no AA reductase activity, a lys9 and three lys14 strains exhibited normal activity, and lys9 strains exhibited significantly higher levels of this activity compared to the prototrophic strain X2180 (Table 3) (M. K. Winston and J. K. Bhattacharjee, manuscript in preparation). A significant saccharopine dehydrogenase activity was present in all lys14 strains examined, but not in the lys1 mutant. This activity was also significantly higher in lys9 mutants but significantly lower in lys2 and lys5 mutants compared to the prototrophic strain. The saccharopine reductase activity was absent in lys9 strains and significantly reduced in lys14 strains compared to the activity in the prototrophic strain X2180 (Table 3). Results from the complementation and recombination experiments established lys9 and lys14 as two distinct loci. The accumulation data and enzyme activities indicate both to be blocked at the saccharopine reductase enzyme.

All three lys14 strains (7305d, AU363, and 8201-7A) used in this investigation were procured independently. Although mutant strain 7305d was used originally in general regulation studies (16), the gene locus and biochemical block of this mutant were not known until now. In spite of leaky growth in the minimal medium, the lys14 strains are considered mutants because of their growth response to lysine, accumulation of the lysine biosynthetic intermediate, ASA, and a decrease in specific enzyme activity of the lysine pathway. Wild-type strains do not exhibit any of these characteristics. Also, lys14 mutants, like lys2 and lys5 mutants, can use AA as a sole nitrogen source (6, 6, 24). The lys9 and lys13 mutations were concluded to be either the same or very tightly linked. Until evidence is found to the contrary, lys9 and lys13 will be designated as lys9 mutations. Although Hwang et al. (9) reported that lys13 mapped on linkage group II and lys9 mapped on group XIII (the current map shows it on group XIV (15)), the original strain representing the lys13 locus is not available to confirm its noidentity to lys9. Obviously, the lys13 strain which we obtained from the collection of the late M. Ogur is not the same as that used in the original study of Hwang et al. (9). Mutants carrying lys9 are already known to be blocked at saccharopine reductase enzyme (2, 6, 10, 19). The need for LYS9 and LYS14, two unlinked genes, for the synthesis of this enzyme is noteworthy. Since the subunit composition of the pure enzyme is not known, both genes may function as structural genes for a heteropolymeric enzyme or one of the genes may be the structural gene (6, 11) and the other encode a positive regulatory protein required for the synthesis of a homopolymeric enzyme.

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literature cited


