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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 α-aminoadipate 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 α-aminoadipate step. The *lys14* strains, like *lys9* mutants, accumulated α-aminoadipate-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 α-aminoadipate (AA) pathways characteristic 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), *Saccharomycopsis 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 complementation 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 α-aminoadipate-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 multiple genes are needed for the synthesis of this enzyme. Results from the complementation, recombination, and biochemical studies demonstrated that mutants previously classified as *lys9* and *lys13* (3, 9) belong to the same complementation group and that *lys9* and *lys14*, representing two distinct loci, are required for the biosynthesis of saccharopine 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 μg/ml, respectively. Cells grown in liquid medium were shaken moderately. 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 spectrophotometrically at 550 nm. Complementation and genetic analyses, including tetrad analysis for the monogenic segregation 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 examined 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 μg 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). Saccharopine 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 saccharopine, 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*-dimethylaminobenzaldehyde adduct was determined as described for the AA reductase assay (19, 24). Saccharopine dehydroge-

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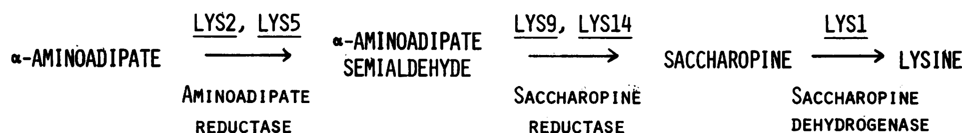

FIG. 1. Lysine mutants of *S. cerevisiae* blocked at the steps between AA and lysine.

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 13*, and *lys1*) 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 α, *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 AU363 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

TABLE 1. Analysis of tetrads from crosses between prototrophic strains and selected lysine auxotrophs

Cross no.	Strains used	No. of tetrads examined	No. of tetrads exhibiting segregation for *LYS*:*lys*[a]	
			2:2	3:1 or 1:3
8221	STX4-4A α *lys1* / X2180-1A **a** *LYS*	18	18	0
8211	P49 α *lys2* / X2180-1A **a** *LYS*	15	15	0
8305	8209-3A α *lys5* / X2180-1A **a** *LYS*	21	21	0
8125	8102-2B **a** *lys9* / X2180-1B α *LYS*	21	19	2
8206	8201-7A **a** *lys14* / X2180-1B α *LYS*	12	12	0
8110	7305d α *lys14* / X2180-1A **a** *LYS*	13	13	0
8104	AU363 **a** *lys14* / X2180-1B α *LYS*	20	15	5

[a] Asci from respective crosses were dissected, and tetrads (four segregants from each ascus) were tested for growth on mm and lysine-supplemented agar medium.

TABLE 2. Tetrad distributions from crosses between *lys14* strains and other lysine auxotrophs[a]

Cross no.	Strains used	No. of tetrads examined	No. of tetrads showing:[a]		
			Parental ditype	NPD Nonparental ditype	Tetratype
8121	STX4-4A α *lys1* / 8110-2C **a** *lys14*	38	8	9	21
8321	8221-13B α *lys1* / 8201-7A **a** *lys14*	31	9	10	12
8124	P49 α *lys2* / 8201-7A **a** *lys14*	27	2	6	19
8320	8209-3A α *lys5* / 8201-7A **a** *lys14*	43	4	14	25
8323	8103-21C α *lys9* / 8201-7A **a** *lys14*	40	7	11	22
8207	8108-8D α *lys13* / 8201-7A **a** *lys14*	36	4	6	26
8308	8104-1C α *lys14* / 8201-7A **a** *lys14*	35	35	0	0
8205	7305d α *lys14* / 8201-7A **a** *lys14*	40	39	0	1
8127	8102-2B **a** *lys9* / 72 α *lys13*	31	29	0	2

[a] Asci from respective crosses were dissected, and tetrads (four segregants from each ascus) were tested for growth on mm and lysine-supplemented agar medium.

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 *lys1* 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 (4, 5, 24). The *lys9* and *lys13* mutations are 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 nonidentity 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.

TABLE 3. Enzyme activities and the accumulation of ASA by *lys14* and other lysine auxotrophs

Strains examined	ASA[a]	Sp act		
		AA reductase[b]	Saccharopine reductase[c]	Saccharopine dehydrogenase[d]
8221-13B *lys1*	0.20	3.66	ND[e]	0.0
P49 *lys2*	0.0	0.03	ND	0.77
8209-3A *lys5*	0.0	0.0	ND	1.09
8103-21C *lys9*	2.15	7.40	0.0	10.2
8108-8D *lys13*	0.81	8.80	0.0	9.92
8201-7A *lys14*	0.54	4.11	0.50	3.50
AU363 *lys14*	0.13	3.19	0.23	3.22
7305d *lys14*	0.56	3.87	0.51	3.23
X2180-1B *LYS*[f]	0.0	4.40	3.97	5.30

[a] The accumulation of semialdehyde was examined by adding *p*-dimethylaminobenzaldehyde to the culture supernatant, boiling the mixture for 20 min, and reading the absorbance at 460 nm. The values are absorbance units at 460 nm.

[b] Specific activity expressed as the absorbance at 460 nm/h per mg of protein.

[c] Specific activity expressed as the absorbance at 460 nm/2 h per mg of protein.

[d] Specific activity expressed as the absorbance at 340 nm/10 min per mg of protein.

[e] ND, Not determined.

[f] X2180-1B was used as a control.

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LITERATURE CITED

1. **Bhattacharjee, J. K.** 1983. Lysine biosynthesis in eukaryotes, p. 229–245. *In* K. Herrmann and R. L. Sommerville, (ed.), Amino acid biosynthesis and genetic regulation. Addison-Wesley, New York.
2. **Bhattacharjee, J. L., and A. K. Sinha.** 1972. Relationship among the genes enzymes, and intermediates of the biosynthetic pathway of lysine in *Saccharomyces*. Mol. Gen. Genet. **155**:26–30.
3. **Biswas, G. D., and J. K. Bhattacharjee.** 1974. Induction and complementation of lysine auxotrophs in *Saccharomyces*. Antonie van Leeuwenhoek J. Microbiol. Serol. **40**:221–231.
4. **Chattoo, B. B., F. Sherman, D. A. Azubalis, T. Fjellstedt, D. Mehnert, and M. Ogur.** 1979. Selection of *lys2* mutants of the yeast *S. cerevisiae* by the utilization of α-aminoadipate. Genetics **93**:51–65.
5. **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.
6. **Fjellstedt, T. A., and M. Ogur.** 1970. Effects of suppressor genes on enzymes controlling lysine biosynthesis in *Saccharomyces*. J. Bacteriol. **101**:108–117.
7. **Gornall, A., C. Bardawill, and M. David.** 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751–766.
8. **Heslot, H., C. Gaillardin, J. M. Beckerich, and P. Fournier.** 1979. Control of lysine metabolism in the petroleum yeast *Saccharomycopsis lipolytica*, p. 54–50. *In* O. K. Sebek and A. I. Laskin (ed.) Genetics of industrial microorganisms. American Society for Microbiology, Washington, D.C.
9. **Hwang, Y. L., G. Lindegren, and C. C. Lindegren.** 1966. Genetic study of lysine biosynthesis in yeast. Can. J. Genet. Cytol. **8**:471–480.
10. **Jones, E., and H. P. Broquist.** 1965. Saccharopine, an intermediate of the aminoadipic acid pathway of lysine biosynthesis. J.

Biol. Chem. **240**:2531–2536.

11. **Jones, E. W., and G. R. Fink.** 1982. Regulation of amino acid and nucleotide biosynthesis in yeast, p. 181–299. *In* J. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression, vol. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Kinzel, J., M. Winston, and J. K. Bhattacharjee.** 1983. Role of L-lysine-α-ketoglutarate aminotransferase in catabolism of lysine as a nitrogen source of *Rhodotorula glutinis*. J. Bacteriol. **155**:417–419.
13. **Larson, R. L., W. Sandine, and H. P. Broquist.** 1963. Enzymatic reduction of α-aminoadipic acid: relation to lysine biosynthesis. J. Biol. Chem. **238**:275–282.
14. **McClary, D. O., W. L. Nutly, and G. P. Miller.** 1959. Effect of potassium versus sodium in the sporulation of *Saccharomyces*. J. Bacteriol. **78**:362–368.
15. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *S. cerevisiae*. Microbiol. Rev. **44**:519–571.
16. **Pierard, A., F. Messenguy, A. Feller, and F. Hilger.** 1979. Dual regulation of the synthesis of the arginine pathway carbamoyl-phosphate synthase of *S. cerevisiae* by specific and general controls of amino acid biosynthesis. Mol. Gen. Genet. **174**:163–171.
17. **Sagisaka, S., and K. Shimura.** 1960. Mechanisms of activation and reduction of α-aminoadipic acid by yeast enzyme. Nature (London) **184**:1709–1710.
18. **Sherman, F., and C. W. Lawrence.** 1974. *Saccharomyces*, p. 353–359. *In* R. C. King (ed.), Handbook of genetics. vol. 1, Plenum Publishing Corp., New York.
19. **Sinha, A. K., and J. K. Bhattacharjee.** 1970. Control of a lysine biosynthetic step by two unlinked genes of *Saccharomyces*. Biochem. Biophys. Res. Commun. **39**:1205–1210.
20. **Sinha, A. K., M. Kurtz, and J. K. Bhattacharjee.** 1971. Effect of hydroxylysine on the biosynthesis of lysine in *Saccharomyces*. J. Bacteriol. **108**:715–719.
21. **Strassman, M., and S. Weinhouse.** 1953. Biosynthetic pathways. III. The biosynthesis of lysine in *Torulopsis utilis*. J. Am. Chem. Soc. **75**:1680–1684.
22. **Trupin, J. S., and H. P. Broquist.** 1965. Saccharopine, an intermediate of the aminoadipic acid pathway of lysine biosynthesis. J. Biol. Chem. **240**:2524–2530.
23. **Vogel, H. J.** 1960. Two modes of lysine synthesis among lower fungi: evolutionary significance. Biochem. Biophys. Acta **41**:172–173.
24. **Winston, M. K., and J. K. Bhattacharjee.** 1982. Growth inhibition by α-aminoadipate and reversal of the effect by specific amino acid supplements in *S. cerevisiae*. J. Bacteriol. **152**:874–879.