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STUDIES ON THE ORGANIZATION OF GENES CONTROLLING LYSINE BIOSYNTHESIS IN *NEUROSPORA CRASSA*

1: Isolation and characterization of lysine mutants belonging to 4 loci.

MAJEED AHMAD

Institute of Biological Sciences, University of Islamabad. Islamabad.

AND

A. MOZAMDER, A. BASET, MD. FAYAZ, A. BADRUL, MD. A. RAHMAN, AND B. SAHA.

Department of Botany, University of Dacca.

Abstract

The classification through heterocaryon and linkage tests of 189 U.V. induced, lysine requiring mutants of *Neurospora crassa* showed that they occupy four different loci: 21 belong to locus lys-1, 34 to locus lys-3, 21 to locus lys-4 and 113 to locus lys—5. None of the 189 mutants belong to locus lys-2. This uneven distribution of the mutants amongst the five loci controlling lysine biosynthesis is perhaps due to the methodology used for collecting these mutants and possibly also due to the different mutation rates of these loci when subjected to ultraviolet radiation.

Lysine was first isolated from casein hydrolysate by Dreschel in 1889. Its biosynthesis is known to proceed through two distinct routes (Work, 1955, Vogel, 1960, Battacharjee & Tucci, 1969). One pathway involves diaminopimelic acid as an intermediate which occurs in bacteria, lower fungi, algae and higher plants. The other pathway proceeds through alpha-aminoadipic acid and is met with in *Neurospora*, yeast and other fungi.

The sequence of Lysine biosynthesis in *Neurospora* alongwith the enzymes involved, and the genes coding for them is shown in Figure 1. A condensation of acetyl Co A and α-Ketoglutaric acid gives homocitric acid, which is converted through a series of reactions, analogous to those of citric acid cycle, into cis-homoaconitic acid (B), homoisocitric acid (C), Oxaloglutaric (D), α-Ketoadepic acid (E), α-aminoadipic acid (F), α-amino-delta—semialdeyde (G), Saccharopine (H), and finally to lysine (I).

Doermann (1946) demonstrated that lysine biosynthesis in *Neurospora* is controlled by at least 4 different loci: lysine-1 (lys-1), lysine-2 (lys-2), lysine-3 (lys-3) and lysine-4 (lys-4). Lys-1 was shown by Grant (1945) to be located in linkage group V (Barratt et al 1954). Turpin & Broquist (1965) demonstrated that it controls the conversion of α-Ketoadipic acid to α-aminoadipic acid. Lys-2 was shown by Ahmad (1966) to be located in the right arm of linkage group V. Jones & Broquist (1966) have established that it controls the structure of aminoadipic semialdehyde glutamate reductase which is responsible for the formation of saccharopine from alpha-amino-delta-semialdehyde. Lys-3 was mapped by Ahmad (1964) in linkage group I, Turpin & Broquist (1965) illucidated that it controls the conversion of alpha—aminoadipic

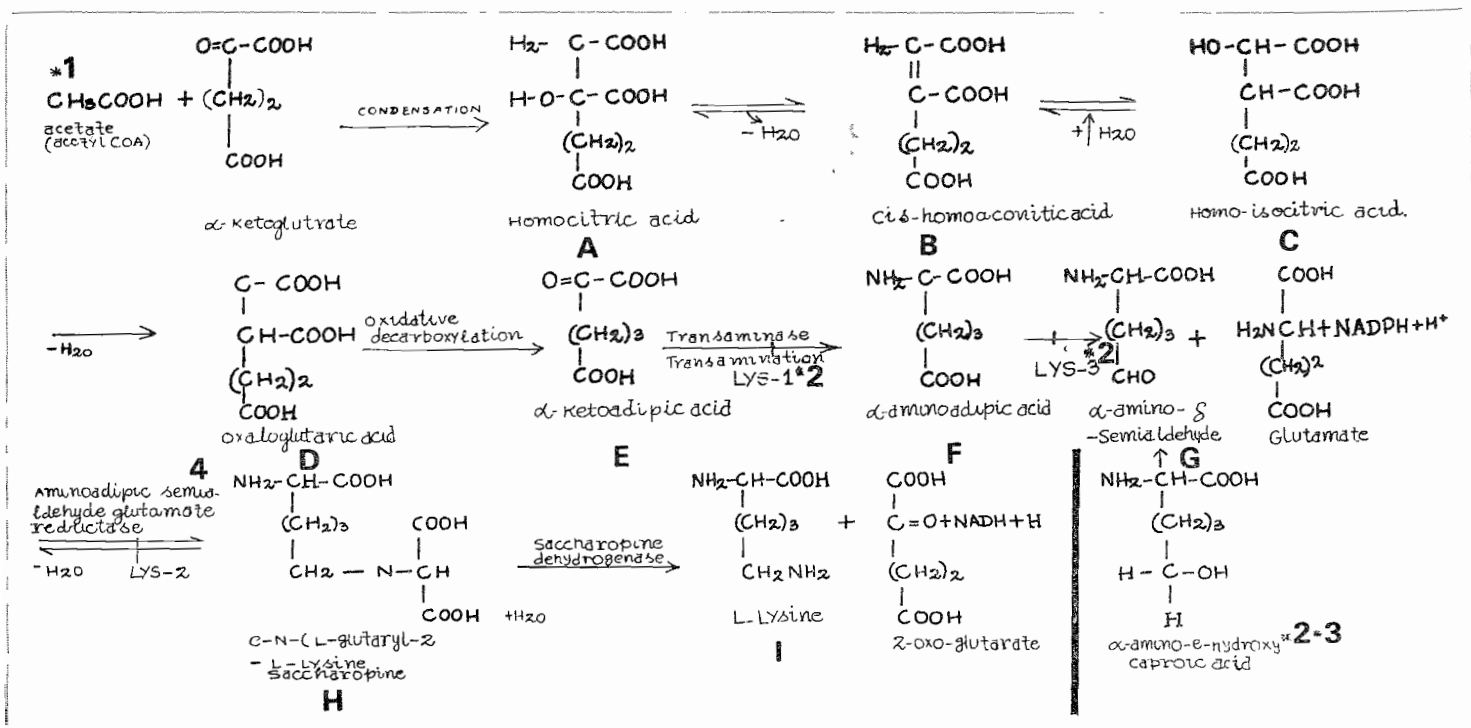

Fig. 1. Lysine biosynthesis in *Neurospora crassa* (reconstructed) after 1* : Bhattacharjee and Tucci 1969, 3* : Yura and Vogel 1959, 2* : Turpin and Broquist 1965, 4* : Jones and Broquist 1966, 5* : Saunders and Broquist 1966.

acid to alpha-amino-delta-semialdehyde. Lys-4 was shown to be located in linkage group I, right arm by Perkins (1959) and Perkins et al. (1962). Saunders & Broquist 1966) established that it controls the structure of saccharopine dehydrogenase which is responsible for the formation of lysine from saccharopine.

As lysine is an essential aminoacid, it was decided to induce further lysine mutants in *Neurospora crassa* and examine whether any additional loci (other than the four already reported) could be uncovered for the synthesis of lysine. It was sought to study the organization and genetic fine structure of the loci controlling lysine biosynthesis in *Neurospora crassa*.

Materials and Methods

One hundred and eighty nine U.V. induced lysine mutants were obtained in two separate experiments from the strain Emerson a (5297), following the techniques of Ahmad & Catcheside (1960). They were grouped by hetercaryon tests.

The following representatives of the seven linkage groups were used as markers:

Linkage group I : Lysine-3, 4545; lysine-4, 1569, nicotinic-1, 3416; arginine-1, 46004.

Linkage group II : arginine-5, 27947; aromatic, Y 7655.

Linkage group III : tryptophan-1, 10575; leucine-1, 33757.

Linkage group IV : tryptophan-4, Y 2198.

Linkage group V : Methionine-3, 36104; lysinel-1, 33933 and lys-2, 537.

Linkage group VI : Tryptophan-2, 75001; Asco, 37402.

Linkage group VII : Nicotinic-tryptophan, 65001.

Media and methods used were the same as reported by Ahmad et al (1964), Ahmad et al 1967 and Ahmad & Islam (1969). Linkage and allelism of different groups of mutants were determined by counting wild-type and mutant ascospores and estimating percentage of recombinants or linkage value by the following formula:

$$\frac{\text{Wild-type spores} \times 2 \times 100}{\text{Total}}$$

Results

Grouping of 189, U.V. induced mutants was first done by heterocaryon tests. Linkage tests were then done to identify the locus to which each group of mutants belonged. Fifty nine mutants collected in the first experiment fell into 4 groups by heterocaryon tests (Table 1) while 130 mutants collected in the second experiment fell into 7 groups. Linkage and allelism tests (Table 2 and 3) showed that mutants in groups I, II, III and IV belonged to loci lys-1, lys-3, lys-4, and lys-5, respectively. Mutants falling under groups V, VI and VII by heterocaryon tests were also found to belong to locus lys-5. Of the 189 mutants, 21 belong to locus lys-1, none to lys-2, 34 to lys-3, 21 to lys-4 and 113 to lys-5.

TABLE 1. Showing grouping of 189 new lysine mutants with the help of heterocaryon tests and the distribution of these mutants amongst the 5 lysine loci.

EXPERIMENT 1				EXPERIMENT 2					
Locus	Group by hetero-caryosis	No. of mutants	Designation of mutants	Locus	Group by hetero-caryosis	No. of mutants	Designation of mutants	Total	Total for the two experiments
lys-1	I	9	A210,216,219,228,231,233, 236,270,291.	lys-1	I	12	A954,970,971,976,984,989, 997, 1013,1014,1047,1052,1074.		21
lys-2		0		lys-2		0			0
lys-3	II	8	A204,205,212,215,217,232, 244,258.	lys-3	II	26	A904,910,921,931,932,936,938, 943,948,949,952,964,966,993, 1004,1007,1011,1012,1018, 1033,1036,1038,1039,1056, 1061,1068.		34
lys-4	III	2	A235,239.	lys-4	III	19	A903,905,911,917,919,930,965, 978,986,987,990,994,1009, 1015, 1035,1054,1066,1070, and 1077.		21
lys-5	IV	40	A201,202,203,207,209,213, 218,223,224,226,227,230,240, 242,243,245,247,248,249,252, 254,256,257,259,260,261,262, 263,264,268,273,275,277,281, 284,286,287,289,290,301.	lys-5	IV	56	A901,909,914,915,918,920,923, 924,925,926,927,928,929,933, 934,935,939,941,945,946,947, 950,958,959,967,968,972,975, 979,982,983,985,988,995,996, 998,999,1003,1005,1032,1037, 1042,1043,1044,1046,1048, 1049,1050,1051,1053,1055, 1057,1059,1063,1064,1069.		
		59		lys-5	V	14	A916,937,942,951,957,960, 962,969,973,1008,1071,1073, 1075, 1079.		
					VI	2	A906,907.		
					VII	1	A1010.	73	113
						130			189

TABLE 2. Showing linkage relationship of 4 groups of new lysine mutants with the help of markers belonging to linkage groups I, V and VI.

Group	Representative	Linkage group	Marker used in cross	Spore count from the cross			Linkage value	Inference
				Germinating	Wild	Total		
I	A 233	V	me-3	1021	224	1245	35.98	Linked, may be lys-1.
II	A 212	I	nic-1	1045	1	1046	0.19	Linked, may be lys-3.
III	A 235	I	nic-1	1451	98	1459	12.6	Linked to nic-1 but away from it, may be lys-4.
	A 235	I	arg-1	1418	56	1474	7.598	Linked to arg-1, may be lys-4.
IV	A 202	VI	tryp-2	767	198	965	41	Linked.
	A 203	VI	tryp-2	484	118	602	39.2	Linked.
	A 201	VI	tryp-2	543	138	681	40.5	Linked.
	A 223	VI	tryp-2	1654	421	2075	40.6	Linked.

TABLE 3. Showing linkage and allelism of representatives of the 7 groups of new lysine mutants. Crosses of these representatives of groups V, VI and VII with lys-1, lys-2, lys-3 and lys-4, were fertile and showed no linkage or allelism to them.

Group	Locus	Representatives	Linkage	Marker used	Whether spores shed	Spore count from the cross			Percentage of wild recombinant spores	Linkage
						Germinating	Wild	Total		
I	lys-1	A 989a	V	lys-1,33933A	yes	102	21	123	17.1	Linked to lys-1.
		A 989a	V	lys-2,537A	yes	2365	254	2619	9.7	Linked to lys-2
		A 971a	V	lys-1.3393A	No	—	—	—	—	May be allelic to (ys-1.
		A 971a	V	lys-2,537A	Yes	1734	257	1991	12.9	Linked to lys-2.
		A 954a	V	lys-1 33933A	Yes	80	17	97	17.5	Linked to lys-1.
		A 954a	V	lys-2,537A	yes	357	36	393	9.2	Linked to lys-2.
		A 997a	V	lys-1,33933A	No	—	—	—	—	May be allelic to (ys-1.
		A 997a	V	lys-2,537A	yes	423	29	452	6.4	Linked to lys-2.
		A 1047	V	lys-1,33933A	yes	226	1	227	.44	Allelic to lys-1.
		A 1047	V	lys-2,537A	yes	1903	233	2136	10.9	Linked to lys-2.
II	lys-3	A 938a	I	lys-3,4545A	yes	36	0	36	0	Allelic to lys-3.
		A 938a	I	lys-4,15069A	yes	103	27	13.0	20.8	Linked to lys-4.
		A 1033a	I	lys-3.4545A	yes	218	0	218	0	Allelic to lys-3.
		A 1033a	I	lys-4,15069A	yes	216	54	270	200	Linked to lys-4.
		A 212	I	lys-3,4545A	yes	258	2	260	1.6	May be allelic to lys-3.
III	lys-4	A 990a	I	lys-3,4545A	yes	99	27	126	21.4	Linked to lys-3.
		A 990a	I	lys-4,15069A	No	—	—	—	—	May be allelic to lys-4.
		A 919a	I	lys-3.4545A	yes	103	26	129	20.2	Linked to lys-3.
		A 919a	I	lys-4 15069A	yes	682	1	683	0.146	Allelic to lys-4.
		A 930a	I	lys-3,4545A	yes	160	43	203	21.1	Linked to lys-3.
		A 930a	I	lys-4.15069A	yes	970	2	972	0.206	Allelic to lys-4.
IV	lys-5	A 223	VI	Asco. 37402A	yes	31,583	11	31,594	0.07	May be allelic to Asc o-lys-5.
		A 923a	VI	"	yes	378	7	385	1.8	—do—
		A 934a	VI	"	No	—	—	—	—	—do—
V	lys-5	A 937a	VI	Asco, 37402A	No	—	—	—	—	May be alleli to Asco. lys-5.
		A 960a	VI	"	No	—	—	—	—	—do—
		A 962a	VI	"	No	—	—	—	—	—do—
VI	lys-5	A 906a	VI	Asco, 37402A	No		-	—	—	May be allelic to Asco lys-5.
VII	lys-5	A 1010a	VI	Asco, 37402A	No	—	-	—	—	May be allelic to As.o lys-5.

Discussion

As a result of irradiation of the conidia of *Neurospora crassa* wild type strain Ema, 189 new lysine mutants were collected. While heterocaryon studies suggested that they fall into 7 groups, linkage and allelism tests revealed that they comprise only 4 groups. This study thus demonstrates that classification of mutants by heterocaryon tests gives only tentative information. The actual number of loci occupied by a set of new mutants can only be determined by recombination tests.

Of the 4 groups identified by genetic studies, the first group comprising 21 mutants belonged to locus lys-1. The second group comprising 34 mutants belonged to locus lys-3. The third group consisting of 21 mutants occupied locus lys-4. The fourth group comprising 113 mutants was found to occupy the locus asco in linkage group VI (Tables 1, 2 and 3).

When group IV lysine mutants appeared to be allelic to asco. Ahmad et al. (1960) tested asco for lysine requirement. It indeed proved to be deficient for lysine. As a matter of fact, asco was discovered as a lysine mutant (37402) by Good (1951) and studied by Stadler (1956). The maturation of ascospores in this mutant is delayed; the spores are white and usually non-viable. Stadler, therefore, classified it as an ascospore lethal and named it 'asco.' But ths designation, i.e. asco, suppressed, the fact that it is deficient in lysine. In the first experiment, 40 out of 59 mutants i.e. about 68% belonged to group IV. When this group of mutants proved to be non-allelic to the four well established loci, controlling lysine biosynthesis in *Neurospora*, Ahmad and co-workers, were surprised as to how such a lysine locus had remained undetected by previous workers. When the linkage studies revealed that it was located in linkage Group VI and was allelic to asco, they were designated the locus as lys-5 (Ahmad et al 1960). this locus was thus finally granted its right place amongst the loci connected with lysine metabolism in *Neurospora*. Later, Perkins et al (1962) reached the same conclusion and designated the locus as lys-5 in the map of linkage group VI.

Two striking facts emerge from analysis of the distribution of 189 mutants amongst the five lysine biosynthesis controlling loci in *Neurospora*. First, no mutant was recovered for locus lys-2 in both the experiments. Secondly mutants for locus lys-5 were about five times as many as mutants for loci lys-1 and lys-4 and about three times as many as for locus lys-3. Hence the distribution of the 189 mutants amongst the five lysine loci is uneven.

Ahmed et al (1976) came across a similar situation of uneven distribution of mutants amongst leucine loci. They conducted a reconstruction experiment by mixing equal proportions of standard alleles for the four leucine loci: leu-1, leu-2, leu-3 and leu-4, and subjected them to the same methodology that they used for isolating lysine mutants. Again, amongst the mutants isolated, recovery of the representatives of the four loci was far from being equal. They, therefore, concluded that the uneven distribution of the leucine mutants induced by them may be ascribed to their technique of collecting mutants. However, they did recover some mutants for each one of the four loci in the reconstruction experiment. Further, frequency of induced mutants for various loci turned out to be different from the frequency of mutants recovered in reconstruction experiment. They, therefore, concluded that part of the uneven distribution of mutants seems to stem from the different mutation rates of the different leucine loci when subjected to ultra-violet radiation. Hence same conclusions have been drawn for lysine mutants as well.

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