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

II. Organization of locus lysine-1.

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Abstract

A study of 12 new U.V. induced and one previously known lys-1 allele 39933 has shown that the locus lysine-1 occupies a length of about 1.840 centimorgans on the established genetic map. No evidence for the existence of any hot spot was found. In terms of organizational complexity the locus comprises a number of recons and at least two complons.

Introduction

Locus lysine-1 (lys-1) was shown by Grant (1945) to be located in linkage group V (Barratt et al, 1956). Turpin& Broquist (1965) demonstrated that it controls the structure of the enzyme responsible for the conversion of alpha-ketoadipic acid to alpha-aminoadipic acid.

As no studies on the organization of this locus had been carried out before, it was decided to study it with the help of mutants collected by Ahmad et al (1977).

Materials and Methods

Twelve new lysine-1 mutants studied, were derived from Emerson a (5297) by irradiating conidia with ultra violet-light. These mutants were designated as A954, A970, A971, A976, A984, A989, A997, A1013, A1014, A1047, A1052 and A1074 Emerson A (5296) was used for obtaining non-leaky isolates and for improving fertility of markers and double mutants by back crossing.

We are indebted to Dr. W. N. Ogata for the supply of histidine-1 a (K948), isoleucine-valine-1 (16117a), lysine-1A (33933R7a) and lysine-2a (37101) which have been used in genetic studies of locus lys-1.

Table 1. Linkage test results of 12 new lysine-1 mutants with lys-1 allele 33933 and 9 new lys-1 mutants with lys-2 allele 37101.

Designation of the crosses	Spores shed	No. of spores counted			Percentage of recombinants.	Inference
		Germinating	Wild type growing	Total		
A954 x lys-1 A	Yes	80	17	97	35	Linked to lys-1
A954 x lys-2 A	Yes	1060	105	1165	18	Linked to lys-2
A971 x lys-1 A	No	–	–	–	–	Sterility may be due to allelism to lys-1
A971 x lys-2 A	Yes	1830	262	2092	25	Linked to lys-2
A976 x lys-1 A	No	–	–	–	–	Sterility may be due to allelism to lys-1
A976 x lys-2 A	Yes	147	28	445	12	Linked to lys-2
A984 x lys-1 A	Yes	513	18	531	6.8	Linked to lys-1
A984 x lys-2 A	Yes	834	30	864	6.9	Linked to lys-2
A989 x lys-1 A	Yes	102	21	123	34.2	Linked to lys-1
A989 x lys-2 A	Yes	2365	254	2619	19.4	Linked to lys-2

A997 x lys-1 A	No	–	–	–	–	Sterility may be due to allelism to lys-1
A997 x lys-2 A	Yes	423	29	452	12.8	Linked to lys-2
A1013 x lys-1 A	No	–	–	–	–	Sterility may be due to allelism to lys-1
A1013 x lys-2 A	Yes	407	38	445	17	Linked to lys-2
A1047a x lys-1 A	Yes	226	1	227	0.881	May be allelic to lys-1
A1047a x lys-2 A	Yes	1903	233	2136	21.8	Linked to lys-2
A1052a x lys-1 A	Yes	600	30	630	9	Linked to lys-1
A1052a x lys-2 A	Yes	619	27	646	8	Linked to lys-2
A970 x lys-1 (2nd)* 11 A	Yes	1872	70	1942	7.2	Linked to lys-1
A1014 x lys-1 (2nd)* 11 A	Yes	1025	95	1120	17.1	Linked to lys-1
A1074 x lys-1 (2nd)* 11 A	Yes	1055	145	1200	24.2	Linked to lys-1

* (2nd) within brackets denotes second generation isolate.

Media and methods used were the same as reported by Ahmad et al (1964) except that heterocaryon tests were made both in solidified and liquid V.M. in triplicate. Observations were taken up to 21 days. In the case of three leaky mutants, A970, A1047 and A1074, observations were taken from 12 to 52 hours.

Interallelic complementation.

Studies on interallelic complementation were done by testing the 12 mutants in all possible pairwise combinations for heterocaryosis. The tests did not show any clear-cut case of complementation. In some tests A971 showed complementation with A984, A1014 and A1052 while in other tests it did not. A971 showed erratic behavior in controls as well. Thus it some times grew in solidified Vogel's Minimal Medium (V.M.) (Vogel, 1956) but not in liquid V.M. controls, while in other tests it grew in liquid V.M. but not in soldified V.M. Since A971 showed its capability of growing at times in controls it seems that A971 + A984, A971 + A1014 and A971 + A1052 are in all probability heterocaryon negative.

Genetical Studies

Linkage studies of 9 out of 12 lysine group I mutants with the previously known lys-1 allele 33933 and lys-2 alle, 37101, gave varied results. With lys-1, crosses of 4 out of 9 mutants were sterile and the crosses of the remaining 5 mutants (A954, A984, A989, A1047 and A1052(gave linkage values ranging from 0.881 to 35 (Table -1). With lys-2, crosses of all of these 9 mutants were fertile and their linkage values with lys-2 ranged from 6.944 to 25.

Crosses of the remaining three new lysine group-1 mutants, A970, A1014 and A1074 were fertile with lys-1 (2nd) 11A obtained after back crossing lys-1 33933 with Em. The linkage values of these three mutants with 33933 were 7.2, 17.1 and 24.2, respectively. These genetic studies though gave suggestive evidence that 12 new lysine mutants under study may belong to locus lys-1, yet the data left one mostly in doubt whether they belonged to locus lys-1, locus lys-2 or to a new locus.

Three kinds of experiments were performed to clarify the position:

(i) **Heterocaryon tests:**

It was argued that if the 12 new mutants occupied a new locus they will form heterocaryons both with lys-1 allele 33933 and lys-2 allele 37101, (Beadle & Coonradt, 1944). If they were allelic with lys-allele 33933, they will not form heterocaryons with it but will form heterocaryons with lys-2 allele 37101. On the contrary, if they were allelic with lys-2 allele 37101, they will not form heterocaryons with it but will form heterocaryons with lys-1 allele, 33933.

When the 12 new mutants were tested for heterocaryosis with lys-1 allele 33933

and lys-2 allele 37101, they were all heterocaryon negative with lys-1but heterocaryon positive with lys-2. These tests showed that this group of 12 mutants most probably is allelic with lys-1.

(ii) Testing for growth in alpha-aminoadipic acid:

Baratt et al (1954) have mentioned that lys-1 mutants utilise alpha-aminoadipic acid for growth whereas lys-2 mutants do not. To investigate whether the 12 new lysine mutants under investigation belonged to locus lys-1 or lys-2, these 12 mutants alongwith lys-1 mutants 33933 and lys-2 mutant 37101 were tested for growth on V.M. supplemented with alpha-aminoadipic acid. The 12 new lysine mutants and lys-1 mutant 33933 utilized alpha-aminoadipic acid but lys-2 mutant 37101 did not. These tests thus showed that the 12 new mutants may belong to locus lys-1 but not to locus lys-2.

(iii) Linkage studies with the help of two linkage group V markers histidine-1 (K948) (hist-1) and isoleucine-valine-1 (16117) (iv-1).

Whether the 12 new lysine group-1 mutants occupied a new locus or were allelic with lys-1, decisive evidence could only by obtained by mapping them as well as lys-1 with respect to two known linkage group V markers hist-1 and iv-1.

The data obtained from triple point crosses of hist-1 + vi-1 double mutant with lys-1 and six lysine Group-1 mutants have been tabulated in Table 2 and represented in Fig. 2. It is seen that one of the new mutants, A1074, lies proximal to the previously known lys-1 allele 33933 at a distance of about 0.58 map units, while five of them lie distal to it, their distance ranging from 0.166 to 0.974 map units from lys-1 allele 33933. Hence the group-1 lysine mutants do not occupy a new locus. They belong to locus lys-1.

Studies on the fine structure of locus lysine-1.

Isoleucine valine-1, 16117a, was used as a marker in these studies. It was combined with previously known lysine-1 allele 33933. Since the lysine-1 + iv-1 Ext. 7A double mutant was not very fertile, it was back crossed with Em and a highly fertile lys-1 + iv-1. 7A-Ext-1A, was obtained with the help of which three lysine group 1 mutants A954, A989 and A1014 could be mapped. The data have been shown in Table 3 and a fine structure map of locus lysine-1, has been presented in Figure 3.

Discussion

The study of the 12 new lysine group-1 mutants has been interesting. When 9 of them were crossed with lys-1 allele 33933 and lys-2 allele. 37101, crosses of 4 of them were sterile with lys-1 but fertile with lys-2.

Table 2. Spore count of progeny from crosses of lys-1 (33933), lys-2 (37101) and six new lysine Group-1 mutants with the double mutants of two linkage group V markers, iv-1 and hist-1 for determining the locus occupied by lysine Group-1 mutants.

No	Designation of the cross	Spore count of progeny								
		Lysine	Hist-IV	IV	Hist+lys.	IV+lys	Hist	Wild	IV+hist+lys	Total
1.	lys-1a x Hist-1 + IV-1-4A-Ext-2A	197	190	80	66	60	29	31	70	723
2.	lys-2a x Hist-1 + IV-1-4A-Ext-2A	182	127	55	63	38	13	16	40	534
3.	A954 x Hist-1 + IV-1-4A-Ext-2A	192	182	70	80	60	29	18	85	716
4.	A989 x Hist-1 + IV-1-Ext-4A	114	144	42	31	37	19	27	41	455
5.	A1014a x Hist-1 + IV-1-4A-Ext-2A	167	151	63	69	45	22	24	75	616
6.	A1047 x Hist-1 + IV-1-4A-Ext-2A	178	159	66	72	47	27	23	77	649
7.	A1052 x Hist-1 + IV-1-Ext-4A	135	161	72	52	44	22	31	54	571
8.	A1074 x Hist-1 + IV-1-4A-Ext-2A	141	163	69	52	45	20	32	50	572

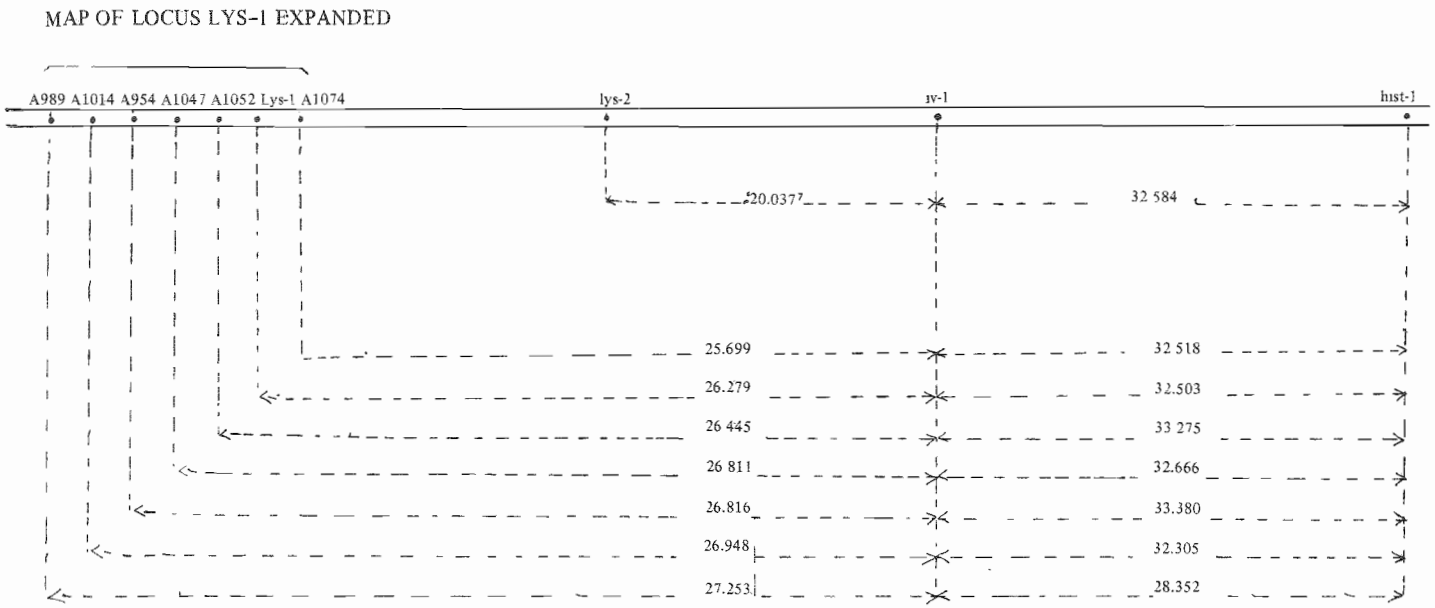

Fig. 2. A map of a part of linkag group V showing allelism of new group-1 lysine mutants with previously known lysine-1 allele 33933, Map based on data from triple point crosses, between IV-1 + hist-1 with lys-1, and lys-1 and 6 new lysine group I mutants, tabulated in table 2.

Table 3. Order and distances of new lys-1 mutants with respect to the previously known lys-1 allele, 33933.

Cross	Number of ascospores						Order and map distance
	Germi-nated	Grow-ing	Total viable	lys-1+ IV-1+	lys-1+ IV-1-	Pseudo-wild	
lys-1 + IV-1-7a-Ext-1A x A954a	3252	26	3278	22	4	0	A954 lys-1 IV-1 1.586
lys-1 + IV-1-7A-Ext-1A x A989a	3015	28	3043	23	5	0	A989 lys-1 IV-1 1.840
lys-1 + IV-1-7A-Ext-1A x 1014a	3000	28	3028	21	5	2	A1014 lys-1 IV-1 1.717

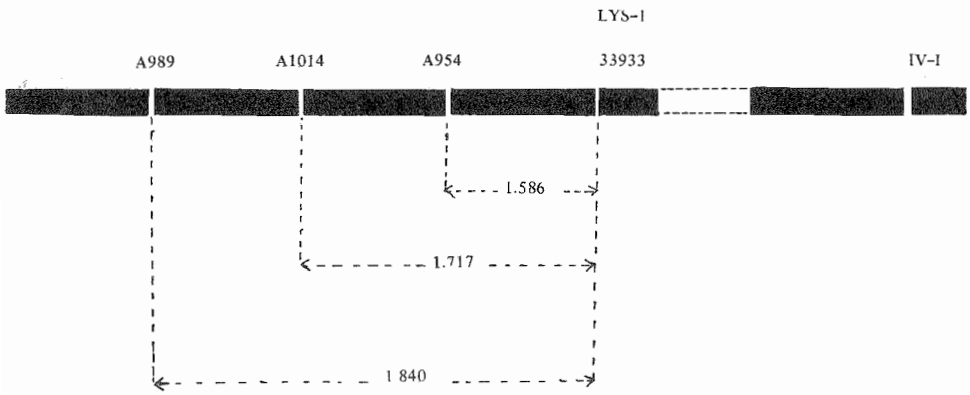

Fig. 3: Gentic fine structure map of locus lys-1.

Since sterility of interallelic crosses is a common experience during gene fine structure studies in Neurospora, the above data were indicative that may be these 9 mutants were allelic with lys-1 and non allelic with lys-2. Secondly it was seen that one of them, A1047 gave a linkage value of 0.881 with lys-1 allele 33933 (Table -1). This linkage value is within the range of two mutants belonging to the same locus as revealed by fine structure studies on several loci e.g. tryp-1 (Ahmad et al,1964), leu-2 (Ahmad et al,1976). It thus appears that these new lysine group 1 mutants belong to locus lys-1. But as the crosses of the remaining 11 of them with lys-1, gave linkage values ranging from about 6.78 to 35, it left one in doubt about their allelism with lys-1.

The fact that linkage values of 9 of these mutants with lys-2 ranged from about 6.944 to 25 showed that they were not allelic with lys-2. However, the high linkage values obtained in the crosses of 11 of them with lys-1 made one cautious in assuming their non-allelism with lys-2.

Three additional studies, which were made, gave conclusive evidence that the 12 new lysine group-1 mutants belong to locus lys-1. The fact that they proved to be heterocaryon negative with lys-1 allele 33933 but positive with lys-2 allele 37101 gave strong evidence of their allelism with lys-1 and non allelism with lys-2 or any other new locus. Their utilization of alpha-aminoadipic acid like lys-1 and failure of lys-2 allele 37101 in utilizing this nutrilite, supported their identity with lys-1. Conclusive evidence that they occupied locus lys-1 came through mapping positions of six of them as well as lys-1 and lys-2 in linkage group V with the help of two markers, iv-1 and hist-1 (Table 2, Fig. 2). One of them, A1074, was located proximal to lys-1 allele 33933 at a distance of about 0.58 centimogran while 5 of them lay distal to it within a map distance of about 0.974 centimogran. The total map length of locus lys-1 through these studies came to about 1.554 map units.

The study of the fine structure of locus lys-1 with the help of the previously known lys-1 allel 33933, and three new lysine group-1 mutants, A954, A989 and A1014,

gave three interesting results. Firstly, all the three mutants could recombine with lys-1 allele 33933 each giving a different proportion of recombinants (Table 3, Fig. 3). This showed that this locus comprises a number of recons. Secondly, the order of the three mutants with respect to lys-1 allele 33933, was exactly the same as was found in the genetic mapping studies using hist-1 and iv-1 in triple point crosses (Table 2, Fig. 2). The map length of locus lys-1 in this study of its fine structure, came to about 1.840 (Table 3, Fig. 3). This is very close to the value of 1.554 which one got for the locus through mapping the positions of lys-1 allele 33933 and 6 of the new lys-1mutants (A954, A989, A1014, A1047, A1052 and A1074). Locus lys-1 thus seems to occupy a length of at least about 1.554 to 1.840 centimograns.

Lastly, among the progeny of the cross of A1014 with lys-1 + iv-1 7A-Ext-1A, two pseudowild spores were recovered. Since pseudowild spores are said to follow from the formation of disomic spores so that the two mutant hetro-alleles carried by the two homologous chromosomes are able to complement one another and form at least a partially functional protein (Pittenger, 1954). The recovery of the two pseudowild spores from the cross under reference thus shows that interallelic complementation is present at the locus lys-1 and that this locus comprises at least two complons (Fig. la, lb).

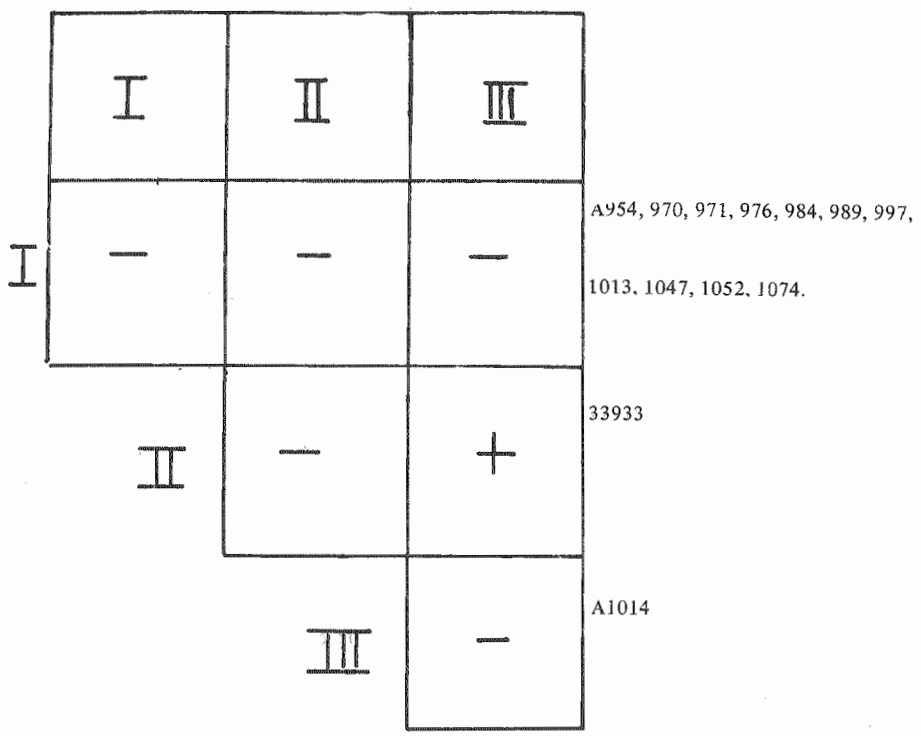

Fig. 1. a: Complementation matrix of 13 lys-1 mutants. Roman numerals indicate complementation groups. Mutants falling under each group are given on the right side of the figure. Heterocaryon positive ,+; heterocaryon negative,·

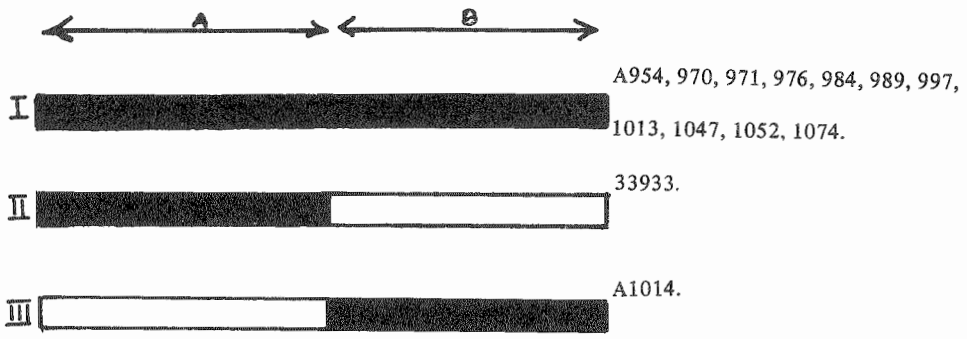

Fig. 1. b: Complementation map of the lys-1 locus. Letters A and B at the top of the figure indicate complementation units or complons. Roman numerals indicate complementation groups. Mutants falling under each group are given on the right side of the figure. Functionally defective regions in each group of mutants have been represented by solid bars.

One of these complons is damaged in the previously known lys-1 mutant 33933 and the other is damaged in the new lys-1 mutant, A1014. This leads to the classification of 13 lys-1 mutants into three groups. Group I includes mutants which do not complement any other mutant. This includes 11 new lys-1 mutants (A954, A970, A971, A976, A984, A989, A997, A1013, A1047, A1052, and A1074) Fig. 1a. Group-II comprises a single mutant 33933 which complements the single Group-III mutant A1014. It is noteworthy that heterocaryon studies of the 12 mutants in all possible pairwise combinations did not reveal any elear out case of interallelic complementation.

During these studies, an abnormally high proportion of prototrophs were recovered in two point crosses between 11 of these mutants and the previously known lys-1 allele 33933 (Table 1) and also in two point crosses of 8 of these mutants with lys-2 allele 37101. This formation of abnormally high proportion of prototrophs was not seen in triple point crosses of 6 of these mutants with iv-1 + hist-1 double mutant and in triple point crosses of three of these mutants with 33933 + iv-1 double mutant (Table -3).

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