Pak. J. Bot., 11 (2): 179-184, 1979.

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

III. Studies on the organization of loci lysine-3 and lysine-4.

MAJEED AHMAD

Institute of Biological Sciences, Quaid-e-Azam University, Islamabad, Pakistan

and

A. MOZMADAR, A. BASET, M. FAYAZ, Md. A. RAHMAN, AND B.C.SAHA,

Department of Botany, University of Dacca, Dacca, Bangladesh.

Abstract

Studies on the organization of loci lys-3 and lys-4 with the help of 24 mutants for lys-3 and 19 mutants for lys-4, have shown that interallelic complenentation is exhibited in both cases. Both are, therefore, organizationally complex but the complexity is minimal as both the loci seem to comprise just two subunits or complons.

Genetic fine structure studies on locus lys-3 have revealed that it occupies a considerable length and possesses many recombineable sites.

Introduction

Loci lysine-3 (lys-3) and lysine-4 (lys-4) were discovered by Beadle & Tatum (1945). Lys-3 was reported to be located in linkage group-I, right arm (Barratt *et al*, 1954) and was mapped by Ahmad (1964). It utilises eta-hydroxynorleucine or α-amino-e-hydroxy caproic acid (Good *et al*, 1950). Turpin & Broquist (1965) reported that it controls the enzyme responsible for the conversion of α-aminoadipic acid to α-amino-δ-samialdehyde.

Locus lys-4 was shown to be located in linkage group-I right arm by Perkins (1959) and Perkins *et al* (1962). Good *et al* (1950) found that it will not use eta-hydroxy-norleucine. Saunders & Broquist (1966) established that it controls the structure of saccharopine dehydrogenase, which brings about the formation of lysine from saccharopine.

No studies on the organization of loci lys-3 and lys-4 had been undertaken. As

Ahmad *et al* (1977) had induced a number of mutants for these two loci, it was decided to study their organization with the help of the available mutants.

Materials and Methods

The following strains were used:

New lysine-3 mutants: 23

A904, A921, A931, A932, A936, A938, A943, A948, A949, A952, A964, A966, A993, A1004, A1007, A1011, A1012, A1036, A1038, A1039, A1056, A1061 and A1068.

Previously known lys-3 allele, 4545.

New lysine 4 mutants: 19

A903, A905, A911, A917, A919, A930, A965, A978, A986, A987, A990, A994, A1009, A1015, A1035, A1054, A1066, A1070 and A1077.

Other strains used:

Emerson A (5296), Emerson a (5297).

Media and methods used were the same as reported by Ahmad *et al* (1964, 1967), Ahmad & Islam (1969) and Ahmad, Choudhry & Islam (1969).

Table 1. Distances of 5 new lys-3 mutants from lys-3 alele 4545 as marker.

Cross	Number of asco-spores					Map distance		
	Germinating	Growing	Total viable	lys-3+	Pseudo-wild			
A904 x lys-3 4545A	2622	17	2639	17	–	A904	1.288	4545
A921 x lys-3 4545A	3718	29	3747	28	1	A921	1.495	4545
A931 x lys-3 4545A	2742	29	2771	29	–	A931	2.093	4545
A932 x lys-3 4545A	2041	29	2070	28	1	A932	2.705	4545
A966 x lys-3 4545A	2267	71	2338	67	4	A966	5.731	4545

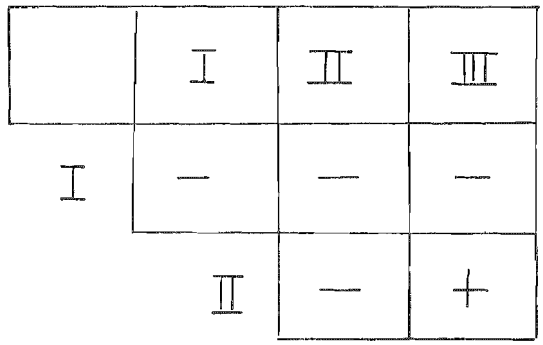

Fig. 1. Complementation matrix of 24 lys-3 mutants. Roman numberals I-III represent the three groups of mutants. Heterocaryon positive +. Heterocaryon negative-.

Results

In order to find whether interallelic complementation was present at locus lys-3, heterocaryon tests of the 23 new lys-3 mutants were made in all possible pairwise combinations. No pair formed a heterocaryon. Thus the heterocaryon tests did not give any evidence of interallelic complementation at locus lys-3.

Next the genetic fine structure of locus lys-3 was studied using the previously known lysine-3 allele 4545 as a marker. Crosses of only five new lyse-3 mutants proved to be fertile with lys-3 allele 4545 successfuly. The data obtained from these two point linkage tests have been tabulated in Table 1. These studies have revealed that the locus occupies a considerable length and comprises a number of recombinable sites. All the 5 new mutants have show their capacity to undergo recombination with the site occupied by the previously known lys-3 allele 4545. The length of the locus comes to atleast 5.731 centimorgans.

The 19 new lys-4 mutants were tested for interallelic complementaton by making

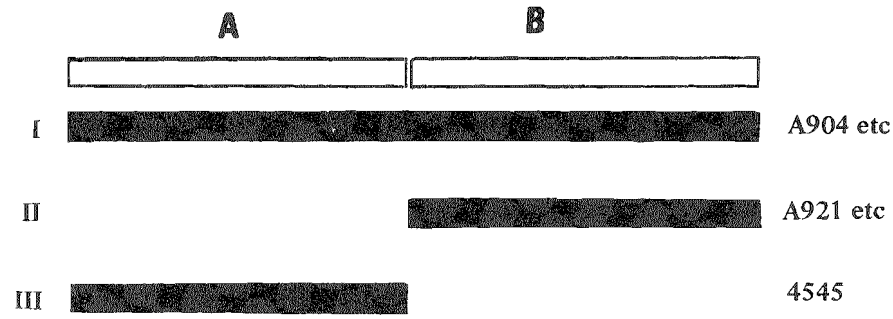

Fig. 2. Complementation map of locus lys-3. Hollow bars A and B at the top indicate two functional subunits. Roman numerals I-III on the left represent the three groups of mutants. One mutant from each group is mentioned on the right. Functionally defective regions in each group of mutants have been represented by solid bars.

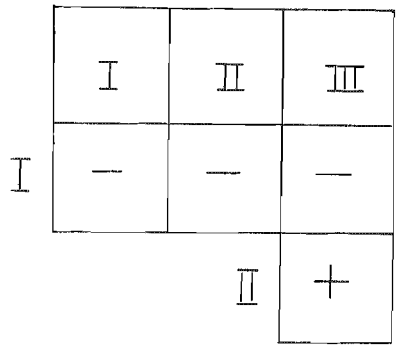

Fig. 3. Complementation matrix of 19 lys-4 mutants. Roman numerals I-III represent the three groups of mutants. Heterocaryon positive +. Heterocaryon negative -.

heterocaryons amongst them in all possible pairwise combination. The data have been presented in the form of a two dimensional matrix in Fig. 3. The mutants fall into three groups.

Group I comprises 17 mutants (A903, A905, A917, A919, A930, A965, A978, A986, A987, A990, A994, A1009, A1015, A1035, A1066, A1070, A1077); none of these 17 mutants complement any other mutant. Groups II and III comprise single mutants A911 and A1054, respectively, and these two mutants complement one another.

A one-dimensional complementation map was made from the above data (Fig. 4) assuming that mutants having common defects do not complement and there by do not form any heterocaryons. This showed that the locus lys-4 comprises at least two functionally distinct subunits (A and B) arranged in a linear manner.

Discussion

Heterocaryon tests between the 23 new lys-3 mutants in all possible pairwise combinations did not reveal any interallelic complementation. When the data on genetic fine structure studies of locus lys-3 tabulated in Table 1 is examined, it is seen that crosses of three new lys-3 mutants, A921, A932 and A966, with lys-3 allele 4545 yielded pseudowild spores in the progeny. Pseudowild spores are formed only if the two mutants alleles carried by the two homologues in such a disomic spore, are able to complement one another (Pittenger, 1954).

This shows that the three new lys-3 mutants (A921, A932 and A966) are able to complement the previously known lys-3 allele 4545. Hence one has to conclude that interallelic complementation is present at lys-3 locus and that the 23 new lys-3 mutants and the mutant strain 4545 can be classified into three groups. Group I, comprising twenty mutants (A904, A931, A936, A938, A943, A948, A949, A952, A964, A993, A1004, A1007, A1011, A1012, A1036, A1038, A1039, A1056, A1061 and A1068 which do not not complement any other lys-3 mutant; Group II, comprising three

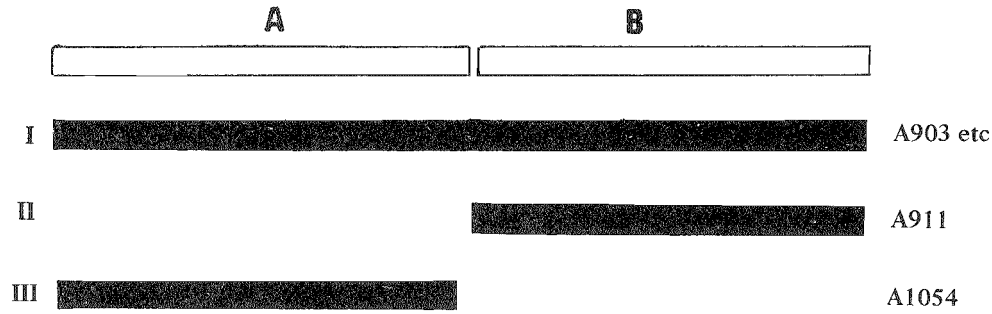

Fig. 4. Complementation map of the locus lys-4. Hollow bars A and B at the top indicate two functional subunits.

II. Roman numerals I-III on the left represent the three groups of mutants. One mutant from each group is mentioned on the right.

I. Functionally defective regions in each group of mutants have been represented by solid bars.

mutants (A921, A932 and A966), which complement mutant 4545, and constitutes group III. This information has been presented in a two dimensional matrix in Figure 1. The above data have also been used to draw a complementation map assuming that mutants having common defects do not complement one another (Fig. 2). As seen from the complementation map, locus lys-3 comprises at least two functionally distinct subunits (A and B) arranged in a linear manner.

Genetic fine structure studies on the locus have revealed no hot spots. Five new mutants, mapped by two point linkage tests using previously known lysine-3 allele 4545 as a marker, have been found to occupy separate sites. The order of the 5 new lys-5 mutants with respect to lys-3 allele 4545 is not revealed by the two point linkage tests. Therefore, all that can be said with respect to the length of the locus is that the recombinational data in these studies shows it to be at least 5.731 centimorgans long (Table 1). As far as known to us, this map length is longer than the map length reported for any other locus in any organism. It is, however, obvious that this map length is abnormal; one could not visualize a locus which can be as long as 5.731 centimorgans. It seems that one or more factors are involved which give increased proportion of recombinant progeny. One possible factor can be the involvement of rec genes (Catcheside *et al,* 1964, Jha, 1967). However, Table 1 shows that the interallelic distance of each of the five mutants from the previously known lys-3 allele 4545, is significant. It suggests that the locus lys-3 occupies a considerable length and comprises a number of recombinable sites.

Like locus lys-3, locus lys-4 has given evidence of being complex. The 19 new lys-4 mutants fall into three groups. The largest group is formed by non-complementing mutants both in the case of lys-3 and lys-4 mutants as has been found in many other cases (e.g. pan-2, Case & Giles, 1958; ad-8, Ishikawa, 1962; and tryp-3, Ahmad & Islam, 1969). However in the cases of both lys-3 and lys-4 the complexity in structure seems minimal as the complementation maps for both the loci comprise just two subunits or complons (Fig. 2 & 4).

References

Ahmad, M. 1964. A study of the lys-3 locus in *Neurospora crassa.* Neurospora News Letter, 6: 5.

Ahmad, M., Md. Khalil, N.A. Khan and A. Mozamadar. 1964. Structural and functional complexity at the tryptophan-1 locus in *Neurospora crassa.* Genetics, 49: 925-933.

Ahmad, M., A., Das, M.R. Khan and M.N. Huda, 1967. Improving fertility in crosses of *Neurospora crassa*, lys-5 mutants. Neurospora News Letter, 11: 19.

Ahmad, M. and N. Islam. 1969. Interallelic complementation at the tryptophan-3 locus in *Neurospora crassa.* Heredity, 24: 651-655.

Ahmad, M., Md. K.U. Choudhry and S.M. Islam. 1969. Compementation and recombination between indole utilising tryptophan-3 mutants of *Neurospora crassa.* Heredity, 24: 656-660.

Ahmad, M., A. Mozmadar, A. Basit, Md. Fayaz, A. Badrul, Md. Rahman and B.C. Saha. 1977. Studies on the organization of genes controlling lysine biosynthesis in *Neurospora crassa.* 1. Isolation and characterization of lysine mutants belonging to 4 loci. Pak. J. Bot., 9: 99-106.

Barratt, R.W., D. Newmeyer, D.D. Perkins and L. Garnjobst. 1954. Map construction in *Neurospora crassa.* Adv. Genet., 6: 1-93.

Beadle, G.W. and E.L. Tatum. 1945. Neurospora II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany, 32: 678-686.

Case, M.E. and N.H. Giles. 1958. Recombination mechanisms at the pan-2 locus in *Neurospora crassa.* Cold spr. Harb. Symp., 23: 119-135.

Catcheside, D.G., A.P. Jessup and B.R. Smith, 1964. Genetic control of allelic recombination in *Neurospoa.* Nature, 202: 1942-1943.

Good, N., Heilbronner, R. and Mitchell, H.K. 1950. e-hydroxynoreucine as a substitute for lysine in *Neurospora.* Arch. Biochem., 28: 464-465.

Ishikawa, T. 1962. Genetic studies of ad-8 mutants in *Neurospora crassa* II. Interallelic complementation at the ad-8 locus. Genetics, 47: 1755-1770.

Jha, K.K. 1967. Genetic control of allelic recombination at the histidine-3 locus of *Neurospora crassa.* Genetics, 57: 865-873.

Perkins, D.D. 1959. New markers and multiple point linkage data in *Neurospora* . Genetics, 44: 1185-1226.

Perkins, D.D., M. Glassey and B.A. Bloom. 1962. New data on markers and rearrangements in *Neurospora.* Can J. Genet. Cytol., 4: 187-205.

Pittenger, T.H. 1954. The general incidence of pseudowild types in *Neurospora crassa.* Genetics, 39: 326-342.

Saunders, P.P. and H.P. Broquist. 1966. Saccharopine, an intermediate of the aminoadipic acid pathway of lysine biosynthesis. IV. Saccharopine dehydrogenase. J. Biol. Chem., 241: 3435-3440.

Turpin, J.S. and H.P. Broquist. 1965. Saccharopine an intermediate of the aminoadipic acid pathway of lysine biosynthesis. I. Studies in *Neurospora crassa.* J. Biol. Chem., 240: 2524-2530.