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

VI. Interallelic complementation at locus lysine-5.

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Abstract

Thirty eight lys-5 mutants have been studied for interallelic complementation. They fall into 17 groups which can be arranged on a complementation map comprising 8 complements. While the defects in 16 groups permit a linear complementation map, defect in one group seems to be discontinuous which leads to an overall circular complementation map for the 17 groups considered together. No polarity effect has been met with at this locus. These studies have thus shown that organizationally, locus lys-5 is one of the more complex loci known.

Introduction

Preliminary studies on the interallelic complementation at locus lysine-5 (lys-5) were reported by Ahmad et al (1960). They stated that although 40 new lys-5 mutants fall into four groups, yet the pattern was complex. In order to carefully unravel this complex pattern, detailed studies were carried out on interallelic complementation amongst 38 lys-5 mutants. Thirty seven of these were induced by Ahmad et al (1960) and one previously known mutant for this locus, asco (37402) was obtained by Good (1951). Results of these investigations are reported in this paper.

Materials and Methods

A277, A281, A284, A286, A287, A289, and A290; one previously known lys-5 mutant, asco (37402); Emerson A (EmA) (5296); Emerson a (Ema) (5297); Tryptophan-1 (tryp-1 A 106-2a and A28-1A); Tryptophan-3 (tryp-3) A 78-9a and A18-7a and leucine-1 (leu-1) 33757A. Asco, 37402, was kindly sent by Dr. D.R. Stadler, rest of the mutants were used from author’s own stock.

Media and methods used were the same as described previously (Ahmad et al. 1964). Mutants which showed evidence of leakiness were back-crossed with Em and non-leaky or less leaky isolates of the respective mutant strains were obtained from amongst the progeny. All heterocaryon tests were done in triplicate and controls were maintained in duplicate. The sensitivity of the test was increased through the use of a solid as well as a liquid Vogel’s minimal medium (V.M.) (Vogel, 1956); selected mutant isolates from back-crosses to Em; forced heterocaryon tests and observation of heterocaryon tests for 30 days.

Results

While most pairs gave clear cut results in interallelic complementation tests, in a number of cases it remained doubtful whether the two mutants were able to complement each other or not. Attempts were first made to clarify these cases through repeated heterocaryon tests. Forced heterocaryon studies were next attempted when no decision could be reached through the usual heterocaryon tests. For forced heterocaryon tests lys-5 mutants in question were combined with either Leu-1 (33757) or tryp-1 (A28-1A). Forced heterocaryons were next made using lys-5 + leu-1 or lys-5 + tryp-1 double mutants. These tests gave clear cut results in some cases but in a number of cases either no growth of the forced heterocaryon was initiated or the growth of the forced heterocaryon was too poor and no conidia were formed on the lysine supplemented medium.

It was, therefore, decided to backcross such double mutants to Em and get more vigorous and more profusely conidiating isolates. When such isolates were obtained the growth of the forced heterocaryons was initiated and heterocaryons formed good conidia. Consequently, the forced heterocaryons could be tested on V.M. for their positive or negative nature.

Asco, 37402, was found to be heterocaryon negative to all the 37 newly lys-5 mutants with which it was tested. It was suspected that it possibly carried heterocaryon incompatibility factors (Garnjobst, 1953; Holloway, 1955). This was found to be so, as it failed to form heterocaryon with tryp-1 106-2a, the indole utilising tryp-3 mutant A78-9a, and tryp-3 mutant A 18-7a. It was only after 8 generations of back crossing of asco to Em that an isolate, asco (8th) Ext-2a could be obtained which formed vigorous heterocaryons with all the three testers mentioned above.
Fig. 1. Complementation matrix of 38 lys-5 mutants. Heterocaryon positive, +; heterocaryon negative, −. Roman numerals I-XVII represent the seventeen groups of mutants. Mutants under each are given on the side.

The 38 lys-5 mutants were tested for heterocaryosis in all possible pairwise combinations. In case of ascoc, ascoc (8th) Ext-2a was used for making heterocaryon tests with the remaining 37 mutants. If two mutants did not form heterocaryons but yielded pseudo-wilds (Pittenger, 1954) in recombination tests, they were classed as heterocaryon positive. Combining the data from these two sources a two dimensional interallelic complementation matrix has been presented in Figure 1. The 38 mutants fall into seventeen groups. Group I includes 7 mutants which do not complement any other mutant. The remaining sixteen groups comprise 31 mutants which complement one or more group of mutants. The intensity of complementation is not uniform but the reactions have been recorded only as positive or negative.

A one dimensional complementation map was made from the above data (Figure 2) assuming that mutants having common defects do not complement. The map has 8 subunits or complons (A-H), arranged in a linear manner. Interactions of mutants in groups XI, XII, XIII, XVI can be satisfied by assuming that they were each defective in one complon, while mutants in other groups seemed to be defective in 2 to 8 complons. The defective complons in 11 of these groups were continuous, while in one group, Group IV only a discontinuous defect could satisfy the interactions of this group with the other groups of mutants. The map did not show any polarity.
Fig. 2. Complementation map of the lys-5 locus. Hollow bars A-H at the top indicate eight complons. Roman numerals I-XVII on the right represent the seventeen groups of mutants. Mutants under each group are on the left. Functionally defective regions in each group of mutants have been represented by solid bars.

Discussion

Of the lys-5 mutants tested 81.8% have shown complementation as against 75.5% found in a previous study by Ahmad et al (1960) besides recognising 4 broad groups amongst 40 lys-5 mutants and the complementation map comprised three complons. In the present study the 38 lys-5 mutants have been found to fall into 17 groups and the complementation map comprises 8 complons. These increase in the percentage of mutants which have the capacity to complement, in the number of complementation groups and in the number of complons follow from the increased sensitivity of the tests through the utilisation of repeated heterocaryon tests in both solid and liquid media, the use of forced heterocaryon tests and the inclusion of data from yields of pseudowilds in recombinational studies as evidence of interallelic complementation.

The defects in the four groups of mutants reported by Ahmed et al (1960) could be represented by single complon defects in three groups (B, C and D) and 3 complon continuous defects in the fourth group (Group A). In the present studies, in the case of one groups, group IV, a discontinuous defect had to be assumed (Figure 2). This suggests a circular lys-5 complementation map for locus lys-5 instead of the linear complementation map reported by Ahmad et al (1960).
When the organization of locus lys-5 is compared with the organization of other loci in *Neurospora*, it is seen that it is one of the more complex loci. Thus the mutants studied for locus tryp-1 (Ahmad et al., 1964) fall into 9 groups and the complementation map for the locus comprises 5 complons, the mutants for locus tryp-3 (Ahmad et al., 1969) fall into 13 groups and the complementation map for the locus comprises 6 complons. Mutants for locus adene-4 have been reported by Giles (1959) to fall into 17 groups and the complementation map of the locus comprises 7 complons. Hence the locus lys-5, 38 mutants of which fall into 17 groups and whose complementation map comprises 8 complons seems to be similar in complexity to locus ad-4 but it is apparently more complex than loci tryp-1 and tryp-3 in *N. crassa*.

References


