A study of the lys-3 locus in Neurospora crassa

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Abstract
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Ahmad, M. A study of the lys-3 locus in Neurospora crassa.

Conidia of the strain Em5297a of Neurospora crassa were exposed to ultraviolet rays and 59 lysine mutants were obtained using the media and methods of Ahmad and Catcheside (1960 Heredity 15: 55). Of these 59 mutants, 8 were found to belong to the same locus as lys-3. None of them showed any complementation amongst themselves in heterocaryon tests, although they were heterocaryon positive with other mutants against which they were tested. The locus lys-3 therefore appears to be a simple one.

For determining the location of these mutants, one of them, A212, was crossed with nicatinic-l (3416). A count of 1,046 spores from this cross showed 1 wild type and 1,045 mutant spores. This gave a linkage value of A212 to nic-l as 0.19 centimorgans. Next the order of lys-3 and nic-l was determined because Barratt, et al. (Barratt, Newmeyer, Perkins and Garnjobst 1954 Adv. Genet. 6:1) had shown the two loci superimposed on one another in their map of linkage group 1.

A212, as a representative of the lys-3 locus, was crossed with albino-2 (15300) and a double mutant A212, al-l2 was obtained. This double mutant was then crossed to nic-l and the spores from this cross were plated on Y. M. Out of 17,425 spores studied, 17,396 were nutritional mutants, 27 were wild type, and 2 were albino. Since the majority of the recombinants proved to be wild type, the order of loci on linkage group 1 is al-l2, lys-3, nic-l. The distance between lys-3 and nic-l comes to 29 x 2 x 100 or 0.33 nearly.

lys-3 is thus proximal to nic-l in relation to al-l2 and the distance between lys-3 and nic-l is about 0.33 centimorgans.

Further, the distance of A212 from albino-2 was found to be roughly 11.5 centimorgans. On classification of 122 spores from a cross A212 x al-l2, 50 single spore cultures proved to be albino, 55 lysine, and 7 wild type.

\[ \text{al-l2} \quad \text{lys-3} \quad \text{nic-l} \]

Fig. 1. Relative positions of al-l2, lys-3 and nic-l on linkage group 1.

These findings support the observation of St. Lawrence (1956 Proc. Natl. Acad. Sci. U. S. 43: 189) with regard to the relative positions of lys-3 and nic-l. In her studies, St. Lawrence utilized osmotic as a marker, which lies to the right of lys-3 and nic-l, whereas in the present studies albino-2, lying to the left of lys-3 and nic-l, has been used.

Baer, D. and P. St. Lawrence. Autoradiographic determination of the location of radioactivity in asci grown on some tritiated pyrimidines.

The intent of this project was to determine the period(s) of DNA synthesis in relation to the chromosomal events during ascus formation. Preliminary experiments were designed to test whether labeling in the nuclei of cultures maintained on various tritiated pyrimidines throughout the sexual cycle could be detected by autoradiography. The results demonstrate that, under these conditions, commercially available tritiated thymine, thymidine and deoxyuridine do not yield DNA with sufficient radioactivity to be useful for studies of DNA synthesis by autoradiography. The biochemical studies of Fink and Fink (1962 J. Biol. Chem. 237: 2289 and 2889) suggest an explanation of these observations.

Cultures of wild type ST74A were grown for 5 days at 25°C on synthetic crossing medium (Westergaard and Mitchell 1947 Am. J. Bot. 34:573) containing approximately: 0.05 mc/ml of either H3-thymine (S.A. 6.6 c/mM, New England Nuclear) or thymidine-methyl-H3 (S.A. 6.7 c/mM, New England Nuclear); or 0.03 mc/ml H3-deoxyuridine (S.A. 1.3 c/mM, Schwarz). The position of the label on the H3-thymine was unspecified but presumably some of the ring carbons carried tritium. Position of the label in deoxyuridine was unspecified but presumably, C5 and C6 were tritiated. Portions of the mycelia were fixed after 11 and 49 hours of growth. Fertilization with wild type conidia was followed by fixation of asci and mycelium six days later. Standard radioautographic procedures were used, including treatment of some fixed material with DNAase or RNAase prior to covering with film.