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A method for obtaining double mutants within single genes or gene clusters

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Abstract
Method for double mutants within genes or gene clusters

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Double mutants within the hist-3 region hove been obtained by a technique utilizing heterocaryons similar to that described by de Serres and Osterbind (1962 Genetics 47:793). This procedure makes possible the recovery of double mutants within single genes (cistrons) or within operon-type systems. This technique should be of general applicability for genetic mapping studies at many loci in Neurospora, as well as in other organisms which form heterocaryons producing multinucleate conidio and in other types such as yeast or Aspergillus which produce diploid heterozygous single cells or conidio.

Basically, the procedure in Neurospora involves forming a heterocaryon between two complementing mutants within the same cistron or operon with each of the strains carrying a different, unrelated biochemical mutation. Conidia from such a heterocaryon ore then treated with on appropriate mutagen, subjected to the filtmtion concentration procedure on minimal medium and then plated on minimal medium containing only the growth supplement normally required by the single original complementing mutants. Under these conditions, selection will occur for heterocaryotic conidia containing induced double mutants (in either of the two parental nuclei) which now cannot complement with the original single parental type nucleus.

In the studies at the hist-3 region, two different heterocaryons were used (both mating type A). The first heterocaryon combined a hist-3A mutant (M127) carrying on adenine forcing mutant ad-6 and a hist-3D mutant (M234) carrying a niacin forcing mutant nic-2 (43002). The second heterocaryon involved the same hist-3A parent with the ad-6 mutant and a hist-3B mutant (M1352) with the same nit-2 strain. The double mutants were detected by their inability to grow on minimal medium and were extracted from the heterocaryons either by conidiol plating or by outcrossing. The second site mutants in the resulting homocaryotic double mutants were then characterized by their complementation pattern with the tester strains hist-3A (M127), hist-3B (M1352) and hist-3D (M234), by their mopping pattern with the other hist-3 mutants, ond by enzymatic assays for the three reactions in histidine biosynthesis controlled by the hist-3 region. By using this procedure, a large number of presumptive double hist-3 mutants were obtained. Many of the double mutants involved lethal mutants which could not be extracted from the heterocaryons either by plating or by outcrossing. Fifteen double mutants were completely characterized. Eight double mutants were recovered in the hist-3B strain. Five of these second site mutants were noncomplementing, two were hist-3A mutants, and one was a hist-3D mutant. Seven double mutants were recovered in the hist-3D strain. Six of the second site mutants were noncomplementing, and one was a hist-3A mutant. (Supported by AEC contract AT (30-1)-3098.) - - - Deportment of Biology, Yale University, New Haven, Connecticut 06520.