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Abstract

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Genetic analyses of recessive lethal mutations induced in a heterokaryon suggest that the majority of X ray-induced ad-3 mutations are the result of deletions involving the ad-3A or ad-3B locus or both (de Serres and Osterbind, Genetics 47: 793, 1962, and unpublished data). The presence of closely linked biochemical markers in the adenine-requiring component of the dikaryon used in these experiments and the use of medium supplemented only with adenine prevented the recovery of genetic alterations extending into the nearby hist-2 locus or nic-2 locus. In theory, ad-3 mutations resulting from large deletions involving loci in adjacent regions should be recoverable by appropriate supplementation of the incubation medium. The ability to recover and maintain *such intergenic alterations in a balanced heterokaryon may be inferred from the experiments of* Atwood and Mukai (Proc. Natl. Acad. Sci., U.S. 39: 1027, 1953). In their tests, those recessive lethal mutations participating in "inclusive complexes" were assumed to represent chromosome deficiencies of indeterminate size. The present experiments show that some recessive lethal mutations result from events that simultaneously inactivate a series of linked loci. These are most simply interpreted as genetic deletions.

The strain numbers and genetic markers in each component of the dikaryon used are given in Table I. With this heterokaryon one should expect to recover as a purple colony in adenine- and niacin-supplemented medium almost any intergenic alteration on the right arm of linkage group I in component B which inactivates at least one of the ad-3 loci.

Table I

Linkage group	*Component A* (74-OR60-29A)	*Component B* (74-OR31-16A)
IR	hist-2 ad-3A ad-3B nic-2	al-2
III	ad-2	-
IV	-	cot
V	inos	-
VI	-	pan-2

In a pilot experiment conidia of this dikaryon were incubated either untreated or after X-ray doses of 10, 15, or 20 Kr. Three spontaneous and 69 X ray-induced purple colonies were recovered. There was a 21-fold increase in the frequency of purple colonies among the total surviving colonies after X-ray treatment. Subsequent tests showed that all of the purple colonies resulted from intra- or intergenic mutation in the ad-3 region in component B. Previously, conidia from a heterokaryon lacking the ad-2 marker produced numerous purple colonies which did not result from mutation in the ad-3 region. Preliminary analysis suggests that such purple colonies may have been caused by incompatibility factor mutations that permit or force extreme nuclear ratios with an excess of component A. Since the ad-2 block precedes the ad-3 block in purine biosynthesis (Mitchell and Houlahan, Fed. Proc. 5: 370, 1946), component A of the present dikaryon cannot accumulate purple pigment.

The present sample of 72 mutants was analyzed (1) in heterokaryon tests, with ad-3A, ad-3B, and hist-2 nic-2 testers to determine the genotype of individual mutations, and (2) in conidial platings, to determine whether the ad-3 mutations are homokaryotic viable (ad-3^{V}) or recessive lethal (ad-3^{RL}). Both the original adenine-requiring dikaryon and a trikaryon carrying a recessive lethal mutation covering the ad-3A ad-3B and nic-2 loci as component C (A ad-3A ad-3B nic-2 al-2; cot; pan-2) were plated. In this way, a distinction can be made between two alternative situations that can lead to an absence of cot colonies in conidial platings of the adenine-requiring dikaryon: ad-3^{RL}, recessive lethal damage in the ad-3 region; or ad-3^{V} + RL, viable ad-3 mutation with recessive lethal mutation elsewhere in the genome. Some ad-3 mutants scored initially as ad-3^{RL} in conidial platings of the dikaryon were shown to be ad-3^{V} + RL by conidial platings of the trikaryon. The results of both the heterokaryon tests and conidial platings of the trikaryons involving all of the ad-3 mutations recovered in this experiment are summarized in Table 2. (Since the sample sizes are small, data from the three different X-ray doses have been pooled.)

Table 2

Treatment	No. of Mutants	Genotype									
		ad-3A		ad-3B		ad-3A ad-3B		ad-3B nic-2		ad-3A ad-3B nic-2	
		V	RL	V	RL	V	RL	V	RL	V	RL
None	3	0	0	0	1	0	0	0	2	0	0
X Irradiation	69	8	4	17	10	0	24	0	3	0	3

These results show that 8 of 72 of the ad-3 mutations cover the nic-2 locus (located 3-5 units distal to the ad-3 region) and that these 8 mutations are recessive lethal. The extent of genetic damage in component B of the adenine-requiring dikaryon can be determined by making a trikaryon by using any mating type A strain marked with a LGI marker, cot, and inos (as component C) and screening for cot colonies by plating conidia from the trikaryon at 37°C on minimal medium. At present, such tests have been made only with a nic-1 marker (located about 25 units distal to the nic-2 locus). These tests have shown that the 6 X ray-induced recessive lethal mutations do not inactivate or delete the nic-1 locus; tests on the 2 spontaneous mutants are incomplete. These data also show that 24 of 24 ad-3A ad-3B double mutants are recessive lethal. The simplest explanation for such mutations is that they result from small deletions covering all, or part, of both the ad-3A and ad-3B loci. Homology tests (de Serres, in preparation) on recessive lethal ad-3A and ad-3B mutants have provided evidence for homologous genetic damage in certain ad-3A+ ad-3B combinations, as might be expected in tests on mutants resulting from a series of overlapping deletions.

The present genetic tests have shown that it is possible to induce, recover, maintain, and analyze recessive lethal mutations resulting from presumptive deletion of particular linked loci in balanced dikaryons of Neurospora. It is noteworthy that none of the ad-3 mutations were associated with (1) gross deletions covering the major portion of the right arm of LGI, or with (2) terminal deletions. These results are entirely consistent with those of Atwood and Mukai (Radiation Res. 1: 125, 1954).

This system is now being used to study the kinetics of survival and mutation-induction, and to analyze the types of induced ad-3 mutants as a function of X-ray dose. ---Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Operated by Union Carbide Corporation for the United States Atomic Energy Commission.