ESTIMATION OF THE RELATIVE FREQUENCIES OF X-RAY-INDUCED VIABLE AND RECESSIVE LETHAL MUTATIONS IN THE ad-3 REGION OF NEUROSPORA CRASSA

F. J. DE SERRES AND ROSALIE S. OSTERBIND[1]

Biology Division, Oak Ridge National Laboratory,[2] Oak Ridge, Tennessee

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A DIRECT method (DE SERRES and KØLMARK 1958) has been developed to estimate forward-mutation rates in the *ad-3* region in Neurospora and to obtain essentially unselected samples of mutants with either complete or partial biochemical requirements after various mutagenic treatments. However, since Neurospora is a haploid organism, we can expect such samples of mutants (from treatment of wild-type strains) to include only those mutations that are viable as homokaryons on adenine-supplemented minimal medium.

The specific locus method in mice (RUSSELL and RUSSELL 1959) or Drosophila (WARD and ALEXANDER 1957) has shown that a substantial fraction of X-ray-induced mutations are homozygous lethal. Furthermore, a detailed analysis (RUSSELL and RUSSELL 1960) of mutations in the dilute-short-ear (*d se*) region in mice has shown that the percentage of visible mutations that fail to survive in a homozygous condition at a given locus can vary from about 90 percent (*d*) to 20 percent (*se*). Since any apparent differences in forward-mutation rates at different loci in the Neurospora system could in reality be the result of differences in the relative frequencies of homokaryotic viable and homokaryotic lethal mutation, we have tried to develop a system to recover mutations of both types. ATWOOD (1955) has shown that many different homokaryotic lethal mutations can be recovered and maintained in balanced heterokaryons between strains with the appropriate genetic markers. We have used a variation of his technique to try to recover any such class of lethal mutations in the *ad-3* region that might occur either spontaneously or after mutagenic treatment. We have classified as homokaryotic, or recessive, lethals those *ad-3* mutations that will not grow on minimal medium supplemented with adenine.

Tests of such a system, which has given the first indication of the relative incidence of X-ray-induced viable and recessive lethal mutation in the *ad-3* region, are presented in this report.

METHODS

The test system makes use of a heterokaryon between an *ad-3A ad-3B* double mutant [from an *ad-3A* × *ad-3B* cross (GILES, DE SERRES and BARBOUR 1957)] and wild type. This double strain will not complement any known *ad-3A* or

[1] Student Trainee, Summer 1961. Present Address: Southwestern at Memphis, Tennessee.

[2] Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

ad-3B mutation. Any *ad-3* mutation induced in the wild-type component of this heterokaryon, therefore, can be recovered (since such a heterokaryon accumulates the reddish-purple pigment characteristic of *ad-3* mutants with our direct method) and maintained as a heterokaryon on minimal medium supplemented with adenine. The strain numbers and the location and genotype of genetic markers in each component of the heterokaryon used in the present experiments is as follows:

	LGI						LGIV	LGV	LGVI
74-OR24-1A	A	*hist-2*	*ad-3A*	*ad-3B*	*nic-2*	+ ;	+ ;	*inos;*	+
74-OR31-16A	A	+	+	+	+	*al-2;*	*cot;*	+ ;	*pan-2*

Aliquots of untreated or X-irradiated conidia of this heterokaryon, from a culture grown on minimal medium, were incubated as previously described (DE SERRES and KØLMARK 1958) except that the medium was supplemented with 12.5 μg/ml adenine and 250 μg/ml arginine (to enhance pigmentation) and 1.0 percent sorbose and 0.1 percent sucrose (to induce colonial growth). This medium was autoclaved for the period of time required for maximum viability (DE SERRES, KØLMARK and BROCKMAN 1962). With incubation in the *dark* at 30°C for seven days, the adenine-requiring heterokaryons resulting from mutation in the *ad-3* region in the *al-2*, *cot*, *pan-2* component were bright reddish-purple, whereas the background colonies were white. These purple colonies were transferred to minimal medium supplemented with adenine and then plated on the same medium to recover single-colony isolates from each for subsequent heterokaryon tests. Conidia from these adenine-requiring, single-colony isolates were then used in heterokaryon tests to determine the genotype of the mutations in the *ad-3* region. Such tests were made with *hist-2 nic-2* (as a control), *ad-3A* and *ad-3B* tester strains by mixing suspensions of conidia in liquid minimal medium in test tubes. The type of response of the trikaryons formed by the adenine-requiring heterokaryons and these testers on minimal medium indicates whether an *ad-3A*, *ad-3B* or *ad-3A ad-3B* double mutant has been induced in the *al-2*, *cot*, *pan-2* component.

RESULTS

In two separate experiments, from aliquots of conidia incubated either untreated or after irradiation with a dose of 30 kr, no spontaneous and a total of 29 X-ray-induced adenine-requiring purple colonies were recovered. Heterokaryon tests on single-colony isolates (recovered from conidial platings) of these purple colonies showed that in this sample there were eight *ad-3A*, 12 *ad-3B* and nine *ad-3A ad-3B* double mutants. These same single-colony isolates of each heterokaryon were then plated on minimal medium supplemented with adenine and pantothenic acid, at 37°C, to determine whether the induced mutations were homokaryotic viable or lethal. We classified as lethal those heterokaryons that did not produce any of the tiny dense colonies made by conidia homokaryotic for *cot* (colonial, temperature-sensitive) in a total of 1000 to 2000 colonies. The data from these tests are given in Table 1. In the present samples (a) 76 percent

TABLE 1

The incidence of viable and recessive lethal mutations among ad-3 *mutants induced in a balanced heterokaryon after X-irradiation (30 kr)*

Genotype	Total number	Homokaryotic viable		Homokaryotic lethal*	
		No.	Percent	No.	Percent
ad-3A	8	3	37.5	5	62.5
ad-3B	12	4	33.3	8	66.7
ad-3A ad-3B	9	0	0	9	100.0
Total	29	7	24.1	22	75.9

* No *cot* colonies in a total of at least 1000–2000 colonies. From a plating of conidia of a single colony isolate on minimal medium supplemented with adenine and pantothenic acid at 37°C.

(22/29) of all of the X-ray-induced mutations in the *ad-3* region are homokaryotic lethal, (b) there is no difference in the percentage of homokaryotic lethal *ad-3A* or *ad-3B* mutations, and (c) mutations that affect both loci simultaneously are always homokaryotic lethal.

DISCUSSION

The data from the present experiments on a heterokaryon of Neurospora are in excellent agreement with those obtained with the specific locus method in mice or Drosophila. Recessive lethal mutations obtained in these three organisms after X-irradiation may be contrasted as follows: Neurospora, 76 percent (22/29); mice (RUSSELL and RUSSELL 1959), 77 percent (71/92) of those induced in spermatogonia; Drosophila (WARD and ALEXANDER 1957), 52 percent (33/64) of those induced in sperm and spermatogonia. Since such a large proportion of the *ad-3* mutants actually induced are homokaryotic lethal, it is evident that our previous estimates (DE SERRES and KØLMARK 1959) of forward-mutation rates are at least four times too low. Furthermore, since these lethals constitute the same percentage of the total *ad-3A* or *ad-3B* mutations, the differences in the forward-mutation rates at these two loci (*ad-3B* > *ad-3A*) must reflect differences in locus size or sensitivity. In this respect, it is noteworthy that present recombination data indicate that the *ad-3A* locus is the smaller of the two (DE SERRES, unpublished data).

It is of considerable interest that nine out of 29 X-ray-induced mutations are *ad-3A ad-3B* double mutants (Table 1) and that all mutations of this type are homokaryotic lethal. These results are identical with those of RUSSELL and RUSSELL (1960) on a similar class of double mutants in mice. They found that the true *d se* mutations derived largely from irradiation of postspermatogonial stages and oocytes were also 100 percent homozygous lethal. If *ad-3A ad-3B* mutations are always lethal and arise by chromosome deletion of a large part or all of the *ad-3* region, then this provides an explanation for the absence of such mutations in much larger samples of X-ray-induced mutations in our earlier work (DE SERRES and KØLMARK 1958) and in many other forward-mutation experiments on wild type (DE SERRES and KØLMARK unpublished observations).

SUMMARY

1. In a sample of 29 X-ray-induced *ad-3* mutations induced in a balanced heterokaryon, 62.5 percent (5/8) of the *ad-3A*, 66.7 percent (8/12) of the *ad-3B* and 100 percent (9/9) of the *ad-3A ad-3B* mutations were homokaryotic lethal.

2. Since 76 percent (22/29) of all X-ray-induced *ad-3* mutations are homokaryotic lethal, forward-mutation rates on wild-type strains using the direct method are at least four times too low.

3. The disparity in the forward-mutation rates obtained with X-ray treatment of wild type cannot be attributed to the relative frequencies of homokaryotic viable and lethal mutation, and must reflect differences in locus size or sensitivity.

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