THE SIZE DISTRIBUTION OF X-RAY INDUCED MULTILOCUS DELETIONS IN THE *ad-3* REGION OF *NEUROSPORA CRASSA*¹

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THE genetic analysis of X-ray induced adenine-3 (*ad-3*) mutations in a genetically marked two-component heterokaryon of *Neurospora* (DE SERRES and OSTERBIND 1962; WEBBER and DE SERRES 1965) has shown that these mutations result from different types of genetic alterations in the *ad-3* region. These alterations produce (a) repairable (*ad-3^R*) mutants which grow on adenine-supplemented medium and (b) irreparable (*ad-3^{IR}*) mutants which do not grow as homokaryons even on complete medium. [Included in the former group are those *ad-3^R* mutations with a separate site of recessive lethal damage elsewhere in the genome (*ad-3^R* + RL mutations)]. The dose-action curves obtained with X-irradiation (WEBBER and DE SERRES 1965) show that the *ad-3^R* mutations increase in direct proportion to dose, and *ad-3^{IR}* mutations increase as the square of the dose. These same data also show that each *ad-3^{IR}* mutation results from two separate single-hit events that occur outside of the *ad-3A* and/or *ad-3B* genes and that these single-hit events are detected about 74 times more frequently than those single-hit events which occur at different sites within the *ad-3A* or *ad-3B* gene and result in *ad-3^R* mutations. Furthermore, experiments where the repair of these single-hit events was studied by varying X-ray dose rate have shown that the events that give rise to *ad-3^{IR}* mutations are qualitatively different from the single-hit events that give rise to *ad-3^R* mutations (DE SERRES, MALLING and WEBBER 1967). More recent genetic analysis of the *ad-3^{IR}* mutations, by means of homology tests (DE SERRES 1967a, b), has shown that these mutations result from multilocus deletion, whereas *ad-3^R* mutations result from point mutations.

In the present paper, the relative proportions of *ad-3^{IR}* mutations of different genotypes actually recovered in our earlier X-ray experiments (WEBBER and DE SERRES 1965) are considered in relation to the relative proportion expected on the basis of the map distances between genetic markers in this region of linkage group I.

MATERIALS AND METHODS

Origin of ad-3 mutations: The technique for quantitative recovery of *ad-3* mutations in a dikaryon (actually a two-component heterokaryon) has been described in detail previously (BROCKMAN and DE SERRES 1963; WEBBER and DE SERRES 1965), as well as the heterokaryon tests used to determine their genotypes and the dikaryon and trikaryon tests used to determine the nature of the genetic damage in the *ad-3* region. Histidine is not added to the medium in

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forward-mutation experiments because it affects the pigmentation of *ad-3* mutant colonies; therefore, the presence of the *hist-2* marker in component I of the dikaryon prevents the growth of colonies in which the *hist-2* locus in component II has been simultaneously deleted. The *ad-3^{IR}* mutations recovered should include only those in the right arm of linkage group I in which the deletion is distal to the *hist-2* locus and affects one or both of the *ad-3* loci.

With the methods described, *ad-3^{IR}* mutations of five different genotypes can be distinguished: (1) *ad-3A*, (2) *ad-3B*, (3) *ad-3A ad-3B*, (4) *ad-3A ad-3B nic-2*, and (5) *ad-3B nic-2*.

Terminology: The following symbols are used: *hist-2*—histidine-requiring; *ad-3A* and *ad-3B*—adenine-requiring; *nic-2*—niacin-requiring.

RESULTS

Relative incidence of five classes of *ad-3^{IR}* mutants: In Table 1 the irreparable mutants recovered at each dose of 250 kv X rays are classified according to their genotype. These data show that, although the total yield of *ad-3^{IR}* mutants increases as the square of the dose, there is *not* a statistically significant heterogeneity in relative genotype frequencies from dose to dose. If the data on the numbers of mutants of genotypes *ad-3A*, *ad-3B* and *ad-3A ad-3B* from each dose (with the data from 5 krad and lower doses pooled), are subjected to a chi-square test for heterogeneity from dose to dose, the value of chi-square is 9.0 with 6 degrees of freedom, so $P > 0.10$. It should therefore be reasonable to use the totals—29 *ad-3A*, 62 *ad-3B*, 92 *ad-3A ad-3B*, 10 *ad-3A ad-3B nic-2*, 4 *ad-3B nic-2*—to estimate the relative frequencies of irreparable mutants of these five classes.

Designation of genetic regions: The region between the *hist-2* gene and the *ad-3A* gene is designated region I and is expected to contain one of any pair of breaks which results in mutations of genotype *ad-3A*, *ad-3A ad-3B*, or *ad-3A ad-3B nic-2*. The region between the *ad-3A* gene and the *ad-3B* gene is designated region II and is expected to contain one of any pair of breaks which results in mutations of genotype *ad-3A*, *ad-3B*, or *ad-3B nic-2*. The region between *ad-3B* and *nic-2* is designated region III and is expected to contain one of any pair of

TABLE 1
Frequency and number of *ad-3^R* and *ad-3^{IR}* mutations of each genotype obtained after varying X-ray exposures*

Exposure (krad)	Frequency of <i>ad-3</i> mutations (per 10 ⁸ survivors)		Number of <i>ad-3^{IR}</i> mutations of each genotype					Total
	<i>ad-3^R</i>	<i>ad-3^{IR}</i>	<i>ad-3A</i>	<i>ad-3B</i>	<i>ad-3A ad-3B</i>	<i>ad-3A ad-3B nic-2</i>	<i>ad-3B nic-2</i>	
0	0.3	0	0	0	0	0	0	0
1	4.2	0.1	0	1	2	0	0	3
2	10.9	0.5	0	2	2	0	0	4
5	33.3	4.0	2	10	6	2	0	20
10	65.3	30.4	16	19	31	5	2	73
20	148.4	100.5	3	14	24	3	0	44
40	213.9	246.8	8	16	27	0	2	53
			29	62	92	10	4	197

* Modified from WEBBER and DE SERRES (1965) on the basis of more detailed homology tests (DE SERRES 1967a, b).

breaks which results in mutations of genotype *ad-3B* or *ad-3A ad-3B*. The region between *nic-2* and the end of the right arm of linkage group I is designated region IV and is expected to contain one of any pair of breaks which results in mutations of genotype *ad-3B nic-2* and *ad-3A ad-3B nic-2*.

Relative radiosensitivities of regions I, II, III, and IV: The numbers of mutants of different genotypes in the total sample of *ad-3^{IR}* mutations are given in Table 1. These numbers have been modified as a result of more detailed genetic analysis by means of homology tests (DE SERRES 1967a, b) and are different from those published previously for these X-ray experiments (Table 2 in WEBBER and DE SERRES 1965). These revised numbers have been used for computing the relative break frequencies or radiosensitivities of regions I, III, and IV as a function of II (the smallest region). In making these computations, we have assumed that the single-hit events resulting in chromosome breakage are distributed at random along the length of the chromosome and that the probabilities of breaks in different regions are independent.

For region I:

$$\frac{\text{probability of break in region I}}{\text{probability of break in region II}} =$$

$$\frac{\text{probability of break in region I, given a break in region III}}{\text{probability of break in region II, given a break in region III}} =$$

$$\frac{\text{number of } ad-3A \text{ } ad-3B \text{ mutants}}{\text{number of } ad-3B \text{ mutants}} = \frac{92}{62} = 1.5$$

For region III the analogous fraction is:

$$\frac{\text{number of } ad-3A \text{ } ad-3B \text{ mutants}}{\text{number of } ad-3A \text{ mutants}} = \frac{92}{29} = 3.2$$

For region IV, the fraction is:

$$\frac{\text{number of } ad-3A \text{ } ad-3B \text{ } nic-2 \text{ mutants}}{\text{number of } ad-3A \text{ mutants}} = \frac{10}{29} = 0.3$$

Therefore, the ratio of sensitivities of region I:region II:region III:region IV is 1.5:1.0:3.2:0.3.

Genetic length of regions I, II, III, and IV: DE SERRES (1967a) has found a map distance of 4.6 for region I, about 0.25 for region II, and 4.3 for region III; region IV is known to exceed 50 map units (PERKINS 1959). The ratio of the map distances for region I:region II:region III:region IV is 18.4:1.0:17.2: > 200. In Figure 1, these ratios are contrasted with the ratios for relative radiosensitivities of these regions. Although there is some variation in map distance for the same region from different crosses (BARRATT, NEWMAYER, PERKINS and GARNJOBST 1954), these variations are not large enough to account for the difference between the ratios obtained from these two types of data.

FIGURE 1.—Comparison of the relative genetic map distances between closely linked non-allelic genes on linkage group I with the relative radiosensitivity to those X-ray induced single-hit chromosome breaks which cooperate in pairs to produce irreparable recessive lethal mutations in the *ad-3* region.

DISCUSSION

The difference between the radiosensitivity ratio and the map distance ratio, if taken at face value, might be interpreted as indicating that the relative sensitivities of these four regions to X-ray induced breaks are different from their sensitivities to "breaks" involved in meiotic recombination.

Actually, it seems more likely that the difference in these two ratios reflects a bias in the type of irreparable mutations which are produced or the types which can be recovered.

The first of two possible explanations for this bias is that a greater variety of *ad-3^{IR}* mutations are produced than are actually recovered. The reason for this could be that large deletions, by virtue of their size, behave as dominant lethal mutations or that some vital or indispensable locus or region is located in the right arm of linkage group I distal to the *nic-2* marker. The evidence against this differential recovery explanation is that mutations have been recovered in which many closely linked loci in the right arm of linkage group I have been inactivated (DE SERRES, unpublished). In addition, there is evidence from the behavior of pseudowild types originating from 3:1 segregations in a translocation heterozygote, that large deficiencies do not behave as dominant lethals in a heterozygote (MITCHELL, PITTINGER and MITCHELL 1952; PITTINGER 1954). In the absence of any evidence that interstitial deletions behave as dominant lethals in *Neurospora*, the differential recovery hypothesis seems unlikely.

A second possible explanation for the bias in favor of mutations inactivating only the *ad-3A* or the *ad-3B* locus is concerned with the probability of fusion of randomly distributed chromosome breaks as the distance between them increases. In general, *ad-3^{IR}* mutations would each be expected to arise as a result of two

separate breaks in the right arm of linkage group I that undergo fusion to produce a loss of the intervening chromosome fragment. There is reason to suspect that the probability of fusions resulting in chromosome deletions would decrease sharply as the distance between the two breaks increases (WOLFF and ATWOOD 1954; WOLFF 1959). Thus *ad-3^{IR}* mutations would represent not a random sample of pairs of hits but rather a sample biased in favor of those pairs of hits that occur in close proximity to each other. This line of argument, then, favors the hypothesis that the bias in the recovery of *ad-3^{IR}* mutations inactivating only a single locus is due to a nonrandom production of deficiencies.

Since the map distance between the *nic-2* marker and the end of the right arm of linkage group I is in excess of 50 map units, the probability that one of the two breaks that produce an *ad-3^{IR}* mutation occurs in this region (region IV) should greatly exceed the probability of its occurrence in region I, II, or III. Consequently, we might have expected many more (*ad-3B nic-2*)^{IR} mutations than *ad-3B^{IR}* mutations and many more (*ad-3A ad-3B nic-2*)^{IR} mutations than (*ad-3A ad-3B*)^{IR} mutations. In the experiment (WEBBER and DE SERRES 1965) from which the above data were obtained, only four (*ad-3B nic-2*)^{IR} mutants and ten (*ad-3A ad-3B nic-2*)^{IR} mutants were obtained. Since mutations of these two genotypes should also be produced as one-hit events by terminal deletion in addition to two-hit events, the numbers obtained are an insignificant fraction of those expected. In fact, there is reason to suspect that those *ad-3* mutations resulting from terminal deletion can not be recovered because such events are dominant lethal. More recent data (DE SERRES, MALLING and WEBBER 1967) indicate that the single-hit event that results in the killing of heterokaryotic conidia by nuclear inactivation is a single chromosome break and most probably one that gives rise to terminal deletion. The sample of *ad-3^{IR}* mutations is biased then in favor of the products of those two-hit events that occur in close proximity in the *ad-3* region.

Furthermore, if the relative radiosensitivities of regions I, II, and III were in direct proportion to the length of these regions as measured by recombination, then one would have expected an even greater excess of (*ad-3A ad-3B*)^{IR} mutations that was obtained. Whereas the ratio of *ad-3A^{IR}*:*ad-3B^{IR}*:(*ad-3A ad-3B*)^{IR} mutants obtained was 29:62:92, the numbers expected on the basis of the genetic map distances are more like 66:62:1141! It is well known that recombination distances and actual physical distances along the chromosome may be very different. This may indeed be the basis of the difference between the ratio of *ad-3A^{IR}* to *ad-3B^{IR}* mutants observed and that expected, but it most certainly cannot be the basis for the marked reduction in (*ad-3A ad-3B*)^{IR} mutations.

In summary, it seems likely that breaks which result in *ad-3^{IR}* mutations are distributed at random along the right arm of the linkage group I. However, since the probability that the type of fusion which will lead to chromosome deletion decreases with increasing distance, the samples of *ad-3^{IR}* mutations are biased in favor of two-hit mutations resulting from pairs of breaks close to the *ad-3A* and *ad-3B* loci. It is also of interest that our findings are in agreement with those of DEMEREC and FANO (1941), who found that among the Notch deficiencies the

proportion of short deletions is also much higher than one would have expected on the basis of random breakage and recovery.

SUMMARY

The genotypes of X-ray induced *ad-3* mutations resulting from multilocus deletion (irreparable *ad-3* mutations—*ad-3^{IR}*) in a two-component heterokaryon have been considered in relation to the map distances between genetic markers near the *ad-3* region on linkage group I. The discrepancy between the ratio of the different genotypes expected and that observed is attributed to a decreasing probability for two independent chromosome breaks to fuse and produce a chromosome deletion as the distance between them increases.

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