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Influence of non-lethal doses of radiation on allelic recombination in *Chlamydomonas reinhardi*

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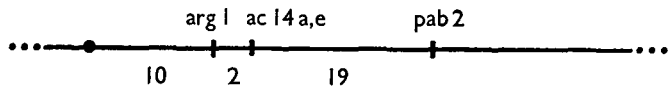
The nature of the relationship between gene and allele recombination and the mechanisms underlying these phenomena are not understood. Some authors (Freese, 1957; Pritchard, 1960) suggest that a single mechanism is sufficient to explain both kinds of data, while others (Winge, 1955; Mitchell, 1957; Lissouba *et al.*, 1962; Stadler & Towe, 1963) postulate two different processes. Gene recombination in *Chlamydomonas reinhardi* responds to non-lethal doses of ionizing radiation in a highly specific way. A response is found only in cells which are passing through one or other of two short stages during



Linkage group I

Fig. 1. Linkage group I of *C. reinhardi* as given by Ebersold *et al.* (1962).

the course of meiosis, the first in preleptotene and the second in late zygotene or pachytene (Lawrence, 1965). The frequency of recombination is decreased at the first and increased at the second stage. The same pattern of response is found with chiasma frequency in *Lilium* and *Tradescantia* (Lawrence, 1961 *a*, *b*). Such a specific response can be used to examine the similarity of the two kinds of recombination. The data reported here concern the response to irradiation of allelic recombination.

Allelic recombination at the *Acetate 14* locus, situated in linkage group I (Ebersold *et al.*, 1962) was examined in synchronously germinating zygospores of *C. reinhardi*. This locus lies within the region used previously to study gene recombination (Fig. 1). The zygospores resulted from the cross *Arginine 1 Acetate 14A* mating type $-/+$ *Acetate 14E* mating type $+$. Since the media were suitably supplemented where necessary, the presence of the *arginine 1* allele was ignored. The cultural and experimental methods used were closely similar to those used previously (Lawrence, 1965), though a thousand times more zygospores per dish were required to estimate the frequency of wild-type recombinants. Different samples of germinating zygospores were irradiated at 30-min. intervals during the period $3\frac{1}{2}$ to 10 hours after the start of germination. A dose of 6 krad of ^{60}Co gamma radiation, delivered at a dose rate of 180 krad/hour, was used and treatments given in the dark at 25°C. The experiment was carried out twice, and the variation between the two sets of data was used to estimate standard errors for tests of significance.

The number of colonies counted is given in Table 1 and the proportion of zygospores giving rise to wild-type progeny (recombinant zygotes) and the zygospore survival are shown in Fig. 2. A marked increase in the frequency of allelic recombination was found

Table 1. *Number of colonies counted on yeast–acetate medium, on which all viable zygospores give rise to a colony, and on arginine-minimal medium, on which only recombinant zygospores produce colonies. A thousand times more zygospores were plated on arginine-minimal medium. Different samples of zygospores were irradiated at the indicated times after the start of germination. Standard errors of the recombination frequency are given in brackets.*

Hours germination	Number of Colonies counted on: Yeast–acetate medium	Number of Colonies counted on: Arginine–minimal medium	Recombinant zygospores $\times 10^{-4}$
3½	1,864	138	0·740 (0·073)
4	1,848	140	0·758 (0·073)
4½	1,855	149	0·803 (0·073)
5	1,833	151	0·824 (0·073)
5½	1,824	160	0·877 (0·073)
6	1,875	156	0·832 (0·073)
6½	1,878	200	1·065 (0·073)
7	1,845	220	1·192 (0·073)
7½	1,871	279	1·491 (0·074)
8	1,871	203	1·085 (0·073)
8½	1,861	231	1·241 (0·073)
9	1,857	274	1·475 (0·074)
9½	1,837	204	1·111 (0·073)
10	1,855	169	0·911 (0·073)
Control	13,267	1,062	0·800 (0·033)

following irradiation in the period 6½ to 9½ hours after the start of germination. A maximum increase of about 1·8 times the control frequency was found at the 7½- and again at the 9-hour stage, an increase which is highly significant ($P < 0{\cdot}001$). Zygospore survival is barely affected by this dose. It is most improbable that this result can be ascribed to mutation, either spontaneous or radiation-induced. No wild-type back mutations were found in the progeny of $3{\cdot}5 \times 10^7$ zygospores homozygous for the *E* allele, and only five such mutants occurred in the progeny of 2×10^8 zygospores surviving an average dose of 25 krad, a dose over four times greater than that used (D. R. Davies, unpublished data). Although the induced mutation rate of the *A* allele has not yet been determined, it seems unlikely that it is more than a thousand times greater than that of the *E* allele, which is the increase in rate necessary to account for the present results.

Cytological examination of the zygospore populations in the manner described previously (Lawrence, 1965) indicates that the movement of cells into successive meiotic stages is normally distributed with respect to time. The two periods of maximum response therefore arise from the presence of two sensitive stages rather than two fractions of the cell population which germinate at different rates and pass through a single sensitive stage. The cytological analysis also showed that 50% of the zygospores had passed through anaphase I 11½ hours after the start of germination. In the previous work on gene recombination the two radiosensitive stages occurred at 5½ and 6½ hours, but 50% of these zygospores had passed through anaphase I by 9 hours. Clearly the two kinds of zygospores, which have different genotypes, germinate at different rates. If allowance is made for the different germination rates it appears likely that radiation influences both kinds of recombination at closely similar, possibly identical, meiotic stages. Work is in progress to verify this conclusion.

On the basis of these results it appears that gene and allele recombination may well be influenced by irradiation at identical meiotic stages, but they do not respond in the same way. Thus, although both types of recombination are increased by irradiation during the second sensitive stage, during the first stage gene recombination is decreased while allele recombination is again increased. With regard to gene recombination, the decrease is found both in a region spanning the centromere of linkage group III (unpublished data) and also in the approximately mid-arm region of linkage group I (Lawrence, 1965). Chiasma frequency in *Lilium* and *Tradescantia* is also depressed at this stage (Lawrence, 1961 *a*, *b*). It is likely, therefore, to be a characteristic response of crossing-over. The possibility that the radiation-induced increase in allele recombination at this stage is characteristic of the particular locus used rather than the process cannot be ruled out.

The present results may be compared with those of Sherman & Roman (1963), who found evidence in yeast for two stages during which allele recombination could occur,

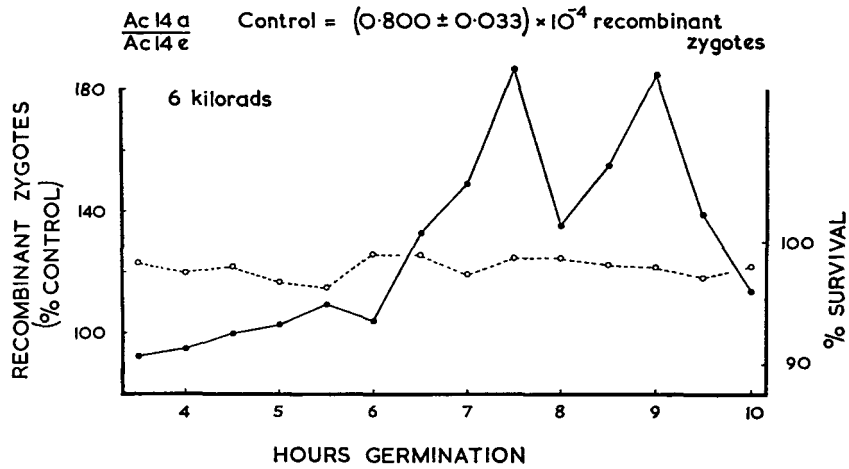


Fig. 2. Frequency of recombinant zygospores, expressed as percent control (solid line) and zygospore survival (dotted line) following irradiation with 6 krad at different stages during germination.

one in late premeiosis, and the other in meiosis. While the *Chlamydomonas* data can be interpreted in this way, an alternative hypothesis is suggested by the comparison of the allele and gene recombination results. Crossing-over appears to occur in at least two sequential steps, the first in the preleptotene and the second in the pachytene stage (Lawrence, 1965, 1961 *a*, *b*). Both steps probably involve the synthesis or turn-over of DNA but not protein (Davies & Lawrence, 1966; Lawrence & Davies, 1966). Allele recombination, at least of the non-reciprocal kind, also implies synthesis of new DNA (Whitehouse, 1963). It is possible, therefore, that the two steps involve processes common to both kinds of recombination. This could explain the correlation, both with respect to position (Case & Giles, 1958) and particular chromatids involved (Whitehouse, 1963), found between reciprocal and non-reciprocal recombination events. Not all the processes at each step, however, are common to gene and allele recombination, as shown by the present data and also by earlier data concerned with the effect of temperature (Mitchell, 1957; Lissouba *et al.*, 1962) and genotype (Catcheside *et al.*, 1964).

The nature of the events at each step is unknown. Possibly the first step involves the formation of structural changes at a number of loci during the course of DNA synthesis.

These are envisaged as potential sites for both kinds of recombination. The second and terminal step, occurring after chromosomes have intimately paired, may then involve the interaction of two such sites, resulting in crossing-over and/or conversion. This interaction requires the occurrence of sites at the same locus on two non-sister chromatids. A mechanism must presumably exist, therefore, which acts during preleptotene or earlier to control the position of crossing-over. Evidence for such a premeiotic determination of the pattern of crossing-over has been found in *Neurospora* (Mitchell, 1960). The processes involved in the second step may well follow the scheme proposed by Whitehouse (1963) in which the formation of hybrid DNA and its mis-repair are proposed, and indeed the finding that allele recombination can occur after the main period of DNA synthesis would support such a model.

SUMMARY

The influence on allelic recombination of non-lethal doses of gamma radiation delivered at different stages during meiosis has been determined and the data compared with previous results concerning gene recombination. Both kinds of recombination are influenced at only two, probably the same two, meiotic stages. During the first sensitive stage, probably in preleptotene, the frequency of allelic recombination is increased, but that of gene recombination decreased. The frequency of both is increased during the second sensitive stage, which is probably in pachytene.

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