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Genetics

Genetic evidence for inducibility of recombination competence in yeast

(UV and x-ray lesions/intragenic recombination)

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ABSTRACT **Recombination between unirradiated chromosomes was induced by UV or x-ray irradiation of haploids followed by a mating with heteroallelic diploids of *Saccharomyces cerevisiae*. The selected event of intragenic recombination did not involve the participation of the irradiated chromosome and apparently was not caused by lesions introduced into the unirradiated chromosomes by some indirect process. The results favor the idea that recombination is repressed in the majority of vegetative cells and that one effect of radiation is the release of some factor(s) necessary for recombination. Consequently, the proportion of competent cells (i.e., cells able to recombine) in the population increases. This competent state seems necessary not only for the recombinational repair of radiation-induced lesions but also, since recombinants are produced in the absence of such lesions, for spontaneous recombination. Photoreactivation of the UV-irradiated haploids led to a decrease in the production of recombinants. Hence, lesions in the DNA appear to be responsible for the induction of the recombinational ability.**

A population of vegetative *Saccharomyces cerevisiae* diploid cells is heterogeneous with respect to genetic recombinational ability, as can be seen from the relatively high coincidence of recombination at unlinked loci (1–4). A possible interpretation of this heterogeneity is that some factor necessary for recombination is repressed in all but a small fraction of the cells. UV and ionizing radiations enhance the production of recombinants in heteroallelic diploids. Holliday (5–7) has suggested that these radiations derepress some recombination processes. One can therefore speculate that there are two effects which lead to the production of recombinants: one enlarges the pool of cells able to recombine, and the other introduces in the chromosomes prerecombinational lesions that promote recombination in the competent cells. A distinction between these effects is not possible when recombination is induced by irradiation of diploid cells or by irradiation of one of the two parental haploids that are mated to produce diploids (8, 9). In both cases, lesions are induced in the chromosomes, and their effects on recombination are inseparable from other hypothetical factors that lead to an increase in the size of the cell pool in which recombination can occur.

In an attempt to separate these two effects, we sought to induce recombination in yeast without inducing lesions in the chromosomes that recombine. Haploids were irradiated with UV light or x-rays and then mated with heteroallelic diploids. The selected events of recombination are limited in the unirradiated chromosomes of the zygotes by the genetic constitution of the strains. An induction of recombinants was indeed observed and can be explained by an increase in the proportion of competent cells—i.e., cells capable of undergoing recombination.

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MATERIALS AND METHODS

Strains. The strains described in the text were derived from the *ade*6 mutants isolated by Jones (10). Diploid homozygotes for the mating type, a/a or α/α, were obtained from a/α strains irradiated with a 5-krad x-ray dose. The α *kar*1 *his*4 *trp*5 *ade*6-21,45 was selected among the meiotic progeny of diploids α *trp*5 *arg*$^-$ *ade*6-21,45/a *kar*1 *his*4 *ade*2. The *kar*1 parental strain of the diploid was isolated by Conde and Fink (11). The presence of the *kar*1 mutation was detected by the poor complementation phenotype and checked by Giemsa staining in zygotes from the cross *kar*1 × *KAR*1.

Media. The complete yeast extract-peptone medium, the minimal medium with glucose or glycerol as carbon source, and the synthetic medium were as described by Mortimer and Hawthorne (12).

Irradiations and Photoreactivation. UV and x-ray sources and the conditions of irradiation were as previously described (13). For each dose, 10 ml of a cell suspension (10^7/ml) in saline (0.9% KCl, wt/vol) was irradiated in an open glass petri dish, 10 cm in diameter. The cells were agitated during UV treatment to keep them in suspension. The UV-irradiated cells were photoreactivated by placing the dish, kept at 30°, between four 20-W F20T12 CW General Electric lamps for 40 min.

Experimental Procedure. The cells were precultivated on yeast extract-peptone solid medium during 1 night. They were washed off during the late logarithmic growth phase and suspended in saline, at 10^7 cells per ml for the parental haploids to be irradiated and at 10^8/ml for the parental diploids. After irradiation and in some cases photoreactivation of the haploid, the cells were mixed in a ratio of 1 ml of the diploid to 10 ml of the haploid. The controls were handled in the same way. The cells were then collected on 0.45-μm Millipore filters (HA, 2.5 cm diameter). The filters were placed on yeast extract-peptone medium and incubated at 30° until approximately 10% of zygotes were formed, as seen by microscopic observation. After x-ray treatment the duration of incubation was about 150 min, for all doses. After UV irradiation, an increasing time, as a function of the dose, was necessary: the mating was delayed by approximately 60 min for each additional 50 J/m^2 given to the haploids. Photoreactivation partially suppressed this delay.

The mixed populations of cells and zygotes were then resuspended in saline, washed, and sonicated to dissociate the aggregates. The number of zygotes was counted in a hemocytometer, and the cells usually were resuspended in 1 ml of saline so that the concentration of zygotes was about 1.5×10^7/ml. To detect the recombinants, 0.1 ml of this suspension was plated on an unsupplemented minimal medium. No further formation of zygotes seemed to occur on these plates, as indicated by the correspondence between the dilutions and the number of colonies. To determine the number of viable zygotes, 0.1 ml of

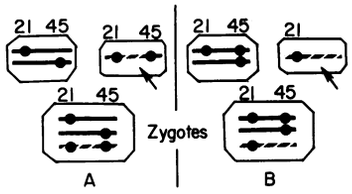

FIG. 1. Schematic representation of the distribution of the *ade*6 mutations in the parental cells and in the zygotes of the crosses α *trp*5 *arg ade*6-21,45 × *a*/*a leu*1/*leu*1 *ade*6-21/*ade*6-45 (*A*) and *a trp*5 *ade*6-21 × α/α *leu*1/*leu*1 *ade*6-45/*ade*6-21,45 (*B*).

a 10^4 dilution was plated on a minimal medium plate supplemented with adenine. All platings were done in triplicate. The glucose plates were incubated 4 days and the glycerol plates 10 days at 30°.

RESULTS

One parent in the cross was a diploid homozygous for mating-type *a*, for a leucine requirement (*leu*1) and heteroallelic for the adenine alleles *ade*6-21 and *ade*6-45—*a*/*a leu*1/*leu*1 *ade*6-21/*ade*6-45. These diploids produce revertants to adenine prototrophy by recombination between the *ade*6 alleles. The other parent was a haploid of mating-type α, tryptophan (*trp*5) and arginine (*arg*) dependent and doubly mutant for *ade*6-21 and *ade*6-45—α *trp*5 *arg ade*6-21,45. After the mating, *Ade*$^+$ cells could be produced by a single event of recombination involving the two chromosomes derived from the diploids (Fig. 1*A*). The participation of the double-mutant chromosome in the production of *Ade*$^+$ cells would imply two independent events between this chromosome and each of the others. The probability of such a double event is expected to be extremely low. The results reported below indeed show that this assumption is correct.

Control experiments have shown that, with the x-ray and UV doses used, the *ade*6-21 and *ade*6-45 mutants do not revert with a frequency that could contribute significantly to the results reported below.

X-ray Irradiation. Fig. 2 (curve a) shows that, when the haploid cells are irradiated and mated with the heteroallelic diploids, the frequency of *Ade*$^+$ recombinants increases among the viable zygotes. The induction generally was not linear as a function of the dose. More-or-less pronounced shoulders in the induction curve were observed. However, for doses between 5 and 20 krads, the induction was linear in all cases and corresponded to 40 to 45 recombinants per 10^6 viable zygotes per 5 krads. The actual numbers of *Ade*$^+$ colonies for a constant number of zygotes plated increased with the doses, which excludes the possibility of a selection of *Ade*$^+$ zygotes derived from preexisting *Ade*$^+$ diploid cells.

Fig. 2 (curve b) also shows the induction curve obtained when a haploid *a trp*5 *ade*6-21 was irradiated and mated with a diploid α/α *leu*1/*leu*1 *ade*6-45/*ade*6-21,45. In this case, the zygotes also contained a triploid nucleus with the same distribution of *ade*6 mutations among the chromosomes, but now the chromosome that contains the x-ray-induced lesions can, by a single recombinational event, give rise to *Ade*$^+$ cells (Fig. 1*B*). The results were 124 *Ade*$^+$ induced per 5 krads and 10^6 viable zygotes. If one assumes that recombination is only due to the lesions in the DNA, one can estimate from this result the frequency of induced *Ade*$^+$ cells expected when the double mutant is irradiated, because two independent recombinational events involving the irradiated chromosome and each of the diploid parental chromosomes are necessary. An estimate of this frequency is the square of the frequency of the single event—

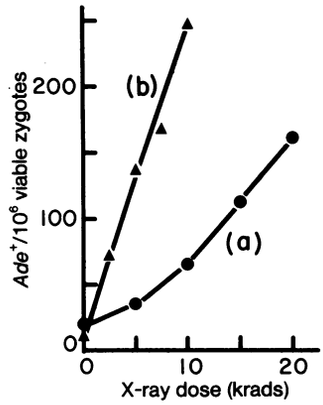

FIG. 2. Frequencies of *Ade*$^+$ recombinants as a function of the x-ray dose given to the haploids for two crosses. *a*. Mated with heteroallelic diploids. *b*. Haploid *a trp*5 *ade*6-21 mated with diploid α/α *leu*1/*leu*1 *ade*6-45/*ade*6-21,45.

that is, $(124/10^6)^2$ or $1.5/10^8$. The value found, $40\text{–}45/10^6$, is 2500 to 3000 times higher, and we can therefore conclude that double events of recombination are not responsible for the induction observed when the double mutant is irradiated. On the contrary, these results support the idea that the production of recombinants is due to an increased proportion of competent zygotes and that recombination occurs by a single event involving the two unirradiated chromosomes.

It could be argued that some repair processes involving interactions between chromosomes could lead to a transfer of a small number of prerecombinational x-ray-induced lesions from the irradiated chromosome to the unirradiated ones, or else that stable free radicals induced in the haploid cells could react with the unirradiated chromosomes. *Ade*$^+$ recombinants could then arise by a single recombinational event originated by DNA lesions in the unirradiated chromosomes. To distinguish between this possibility and the hypothesis of the inducibility of recombinational ability, the following experiment was performed: the haploid cells were irradiated with a constant dose (10 krad) and mated with diploid cells that were given doses of 0, 0.85, or 1.7 krads. At low doses, it is well established that the frequency of induced recombinants is proportional to the dose (14)—i.e., proportional to the number of lesions. If lesions are transferred from the haploid nucleus or are induced by free radicals, the contribution of the irradiated haploids to recombination should therefore be constant, whatever the doses received by the diploids.

According to the hypothesis of the inducibility of recombinational ability, however, the increase in the recombination frequencies, due to the irradiation of the doubly mutant haploid, reflects an increased proportion of competent zygotes. If the diploids are preirradiated, recombinational lesions lead to recombination in the competent cells but not in the noncompetent ones. However, zygotes are formed as well with this last class of diploids. If some of them become competent because of the irradiation of the haploids, the eventual presence of unrepaired potentially recombinational lesions will lead to recombinational events. The number of such lesions will be proportional to the dose given to the diploids, and therefore the contribution of the irradiated haploids to recombination is expected to increase with the dose given to the diploids.

Table 1 shows the result of one such experiment. After the cross with the unirradiated haploids, the frequency of *Ade*$^+$ among the triploids increased linearly with the dose applied to the diploids. If these frequencies are subtracted from the corresponding ones obtained after the cross with the irradiated

Table 1. Frequencies of Ade^+ recombinants after irradiation of diploids

	Frequency/10^6 viable zygotes		
	at 0 krads	at 0.85 krads	at 1.7 krads
Diploids crossed with unirradiated haploids	30	164	300
Diploids crossed with irradiated haploids (10 krads)	98	297	496
Recombinants due to mating with irradiated haploids	68	133	196

haploids, one finds that the contribution of the haploids is not constant but increases linearly with the doses given to the diploids.

This result strongly argues against the possibility that recombination is due to lesions indirectly introduced in the unirradiated chromosomes and supports the idea that some factor necessary for recombination is brought into the zygotes by the irradiated parental cells (or synthesized in the zygotes), so that a higher proportion of triploids are now competent.

UV Irradiation. With the same strains and methods, it was found that UV irradiation of the double-mutant haploid cells *ade*6-21,45 also induced Ade^+ recombinants in the triploid zygotes. Fig. 3 shows that the induction curve is sigmoidal as a function of the dose and, furthermore, that its shoulder corresponds to the shoulder of the survival curve of the haploid cells. This correlation suggests that the recombination observed is related to the unexcised dimers or to other unrepaired types of lesions because for haploid yeast cells the shoulder of the UV-survival curve is due to the fact that, in this range of doses, the excision mechanism is not saturated (15).

That dimers are at least partially responsible for this induction of recombinants is shown by photoreactivation experiments. Photoreactivation is known to be due to the splitting *in*

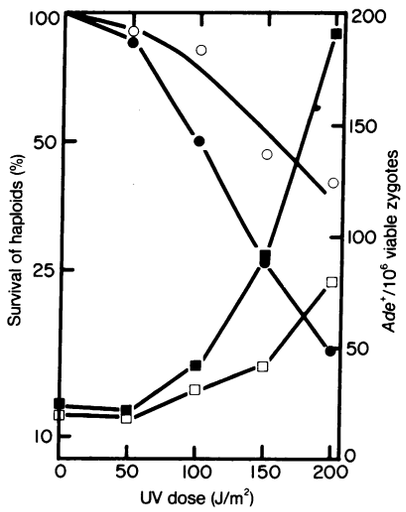

FIG. 3. Survival of haploid double mutant cells (circles) and frequencies of recombinants (squares) as a function of the UV dose given to the haploids. Open symbols refer to photoreactivation of the UV-irradiated haploids.

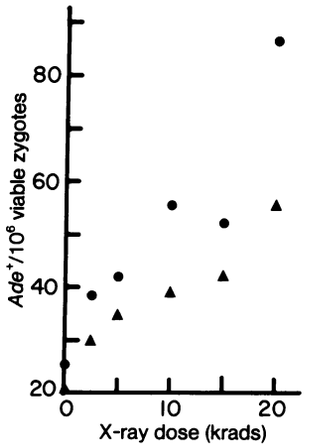

FIG. 4. Frequencies of Ade^+ recombinants as a function of the x-ray dose given to the α *kar*1 *ade*6-21,45 *his*$^-$ *trp*5 haploid (two experiments).

situ of pyrimidine dimers, mediated by the photoreactivating enzyme (16). Illumination of the UV-irradiated haploids with visible light results in an increase in their survival and in a decreased induction of recombination (Fig. 3). This result does not mean that the dimers in their original state are the inducers of the recombinational ability of the cells; it could also be any product of their metabolism or, for instance, the gaps that may be left opposite them during DNA replication.

X-ray Irradiation of a *kar*1 Mutant. One effect of the *kar*1 mutation is to prevent the fusion of nuclei in most of the zygotes formed by the mating of *kar*1 and *KAR*1 cells (11). The clones derived from such zygotes may contain cells with one or the other parental nucleus (dikaryons) and a small proportion of cells with fused nuclei. Thus, a direct interaction between the chromosomes of the two nuclei is for the most part excluded. If, under these circumstances, treatment of the haploid *kar*1 cells enhances recombination in the diploid nucleus of the zygotes, the hypothesis of a derepression of some diffusible factor necessary for recombination is further supported.

An α *kar*1 *ade*6-21,45 *his trp*5 strain was constructed and these cells were mated with an *a*/*a leu*1/*leu*1 *ade*6-21/*ade*6-45 diploid that was cytoplasmically defective (ρ^-). The mating mixture was plated on minimal medium supplemented with leucine. The carbon source was glycerol, so that only those cells that had inherited the ρ^+ respiratory determinant from the haploid parent could grow. The cells selected for on this medium were of three different classes: the dikaryon, the monokaryotic triploid, and the parental diploid. As control platings, for the total number of zygotes initially formed, adenine was added to the medium.

In these experiments, the zygotes required 10 days to form colonies, presumably because glycerol was the carbon source. The plating efficiency of the zygotes was low (around 50%) and the colonies were of different sizes and shapes. For these different reasons, and perhaps others, comparisons with the experiments done with the *KAR*1 haploids are not justified. However, the several experiments that were performed gave the same qualitative results.

Fig. 4 shows the results of two different experiments in which the *kar*1 cells received different x-ray doses before the mating. The points are too scattered to permit determination of the kinetics of induction. However, there is a significant increase as a function of the dose. In these two experiments approximately 15 and 9 recombinants per 10^6 viable zygotes were induced per 5 krads. That most of these recombination events did

Table 2. Percentage of *Leu*$^+$ colonies among *Ade*$^+$ recombinant colonies when irradiated haploid was α *kar*1 *ade*6-21,45 *his*$^-$ *trp*5

Dose to haploid cells, krads	*Leu*$^+$ colonies, % of *Ade*$^+$ colonies	
	Exp. 1	Exp. 2
0	50	66
2.5	49	49
5	51	54
10	53	51
15	55	52
20	49	54

not take place in cells in which nuclei had fused is demonstrated by the following genetic evidence. The adenine prototrophs were replicated on complete medium minus leucine. At all doses (Table 2), approximately 50% of the colonies failed to grow and were thus *leu*$^-$. These arose from cells that were originally dikaryotic but that now carried only the diploid nucleus. If recombination had occurred preferentially in the triploid cells, after nuclear fusion, the proportion of *Leu*$^+$ *Ade*$^+$ colonies would have increased with the dose, and this is clearly not the case.

Among the colonies that appeared to be *Leu*$^+$ *Ade*$^+$ when replica plated, most also contained *leu*$^-$ *Ade*$^+$ cells on closer examination. Fifteen such colonies, derived from the cross in which the *kar*1 haploids were irradiated with 20 krads, were individually resuspended and an aliquot of each suspension was plated on complete medium. The colonies grown on these plates were replica plated on a medium lacking leucine and adenine. Twelve of the 15 colonies contained 3–90% of *leu*$^-$ *Ade*$^+$ cells. Since in control experiments it was found that monocaryotic triploid cells are genetically stable during mitotic divisions, one can conclude that recombination in these cases had occurred before nuclear fusion, probably in the heterokaryotic cells that gave rise to the colonies. Thus, some 90%, and perhaps more, of the induced recombination events arose in cells in which the nuclei did not fuse.

DISCUSSION

These experiments show that intragenic recombination is induced by mating heteroallelic diploids with the corresponding double-mutant haploids irradiated with UV light or x-rays. In the zygotes, recombination occurs between the two unirradiated chromosomes and does not seem due, at least for x-ray irradiation, to lesions indirectly introduced into the unirradiated chromosomes by free radicals or by exchanges between irradiated and unirradiated chromosomes during repair. A qualitatively similar induction is observed with an x-ray-irradiated double mutant containing a *kar*1 mutation (no experiments with UV were done). In this case, nuclear fusion in the zygotes is relatively rare, yet the presence of an irradiated nucleus in the heterokaryon promotes recombination in the unirradiated diploid companion nucleus.

These results all are in agreement with the idea that the recombinational ability depends on an inducible mechanism. The irradiation of the haploid cells might result in a derepression or in an increased level of a diffusible product necessary for recombination. When these cells are mated with diploids, a larger proportion of zygotes become able to perform recombination between the unirradiated chromosomes.

That the lesions induced in the DNA are at least partially responsible for the induction of the recombinational ability is supported by the UV experiments (Fig. 3). When pyrimidine dimers in the haploids are partially removed by photoreactivation just after the irradiation and before the mating, the frequency of recombinants is reduced. Furthermore, from the kinetics of the response as a function of the UV dose, it seems that only the dimers that are not removed by the excision repair mechanism are involved in the induction of the recombinational ability. Unrau (17) suggested that such sequential activities occur after UV irradiation of Ustilago cells.

The existence of a UV-inducible repair mechanism has been proposed for different microorganisms (7, 17–22). However, it should be pointed out that recombination does not seem involved in several of these cases.

It is generally accepted that the repair of DNA lesions is a major cause of the occurrence of recombinants (23). In the experiments reported here, the rate of production of *Ade*$^+$ recombinants by x-rays was 3 times higher if the haploid contained only one of the *ade*6 mutations instead of both. This difference can be attributed to the presence of prerecombinational lesions in the chromosome from the haploid which, by necessity, is involved in recombination. Furthermore, if the triploid zygotes were irradiated after the mating, the yield of recombinants was 12 times greater (unpublished results). There is therefore no doubt that, if the DNA contains prerecombinational lesions, the great majority of the recombinational events can be a consequence of their repair. However, the experiment described in Fig. 1, in which the diploids were preirradiated with small x-ray doses before the mating, shows that the lesions are recombinogenic only in a fraction of the population: the competent state of the cells appears to be necessary for x-ray-induced as well as for "spontaneous" recombination, (i.e., recombination not related to the repair of radiation-induced DNA lesions).

Different treatments that do not directly introduce lesions into the DNA, such as with some chemical or physical agents, are recombinogenic. The best known is the induction of meiosis during which the recombination rate is of the order of 10^2 to 10^3 higher than during mitosis. It has been shown that exposure of the cells to sporulation medium for limited times increases recombination although the meiotic division is not induced (24, 25). Hénaut and Luzzati (4) have also reported that the recombination ability of *ade*3 mutants is enhanced by starvation for histidine. In all these cases, the increased production of recombinants could be due to the derepression of factor(s) necessary for recombination. It is possible that these treatments also induce DNA lesions that may in turn be responsible for the induction of recombination ability. One can only speculate about the possible functions of these factors: for instance, proteins necessary for the pairing of the chromosomes, denaturing proteins, or recombinational enzymes could be under the control of inducible regulatory mechanisms.

The frequencies of heteroallelic diploids among the survivors of x-ray-induced reversions increased linearly at moderate doses. However, with higher doses there was a departure from linearity. A rise is often observed and is correlated with the appearance of lethality (unpublished observations; refs. 6 and 26). We suggest, as one possibility based on the present results, that the linear response corresponds to recombination initiated by DNA lesions in the competent cells. At higher doses the accumulation of unrepaired lesions in the noncompetent cells would lead to the induction of a recombinational repair process, resulting in an increase in the pool of competent cells at the expense of the noncompetent ones.

A sequential activity of different repair mechanisms could

therefore occur after x-ray as well as after UV irradiation. It is possible that, when the cells have exhausted the repair capacities of the processes that first occur, their last chance to survive is to induce the factors necessary for performing recombinational repair. Such induced cells could also perform spontaneous recombination. Other experiments (reported elsewhere) show that the recombination ability induced by irradiation is transmitted to the daughter cells for several generations and further support the interpretation given to the present results.

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