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Genetic characterization of *rec*-1, a mutant of *Ustilago maydis* defective in repair and recombination

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SUMMARY

Detailed physiological and genetic studies of haploid and diploid strains have revealed a complex phenotype for the *rec*-1 mutation in *Ustilago maydis*. The mutant is defective in the repair of damage by UV light, ionizing radiation and nitrosoguanidine. Four alleles are all recessive and have the same sensitivity to UV, suggesting the loss of a single cellular function. A significant fraction of non-viable cells is formed during growth, and in diploid strains considerable variation in colony size and morphology is seen. The spontaneous frequency of mutation is greater than in wild-type cells, but there is little, if any, enhancement by irradiation.

rec-1 also has pleiotropic effects on genetic recombination. The spontaneous level of mitotic allelic or non-allelic recombination is abnormally high, but the relative increase after irradiation is much lower than in control diploids. Allelic recombination is strongly associated with the expression of a hetozygous recessive distal marker, and it is shown that this is often due to hemizygosity rather than to homozygosity of this marker. The results indicate that allelic recombination is due to crossing over rather than gene conversion, but that the cross over is often associated with a chromatid break. *rec*-1 interacts with other radiation sensitive mutants, such as *rec*-2. Diploids homozygous for both are totally deficient in allelic recombination. In crosses between *rec*-1 strains meiosis is defective, with a low viability of meiotic products and frequent production of aneuploids or diploids among the survivors. The overall phenotype of *rec*-1 strains can best be explained in terms of the loss of a regulatory function, which leads to uncontrolled recombination during mitosis and meiosis, and the loss of a recombination repair pathway which is normally induced by agents which damage DNA.

1. INTRODUCTION

Interest in the relationship between recombination and repair arose on the one hand from the discovery that recombination defective mutants in *Escherichia coli* were sensitive to radiation (for reviews, see Howard-Flanders, 1968; Witkin, 1969), and on the other from the theory that gene conversion in fungi could be due to the repair of mismatched bases in heteroduplex DNA (Holliday, 1962*a*, 1964*a*; Whitehouse, 1963). The first radiation-sensitive mutants in any eukaryote were

obtained using the yeast-like smut fungus *Ustilago maydis* (Holliday, 1965*a*). When the effect of three of these on recombination was examined, it was found that two of them, *rec*-1 (previously designated *uvs*-1) and *rec*-2 (previously designated *uvs*-2), had pronounced effects on mitotic recombination or meiosis (Holliday, 1967). In particular, both blocked radiation-induced allelic recombination (gene conversion) in hetoallelic diploids, a process which is believed to be due to recombination mediated repair. Double mutant strains were more radiation sensitive than either single mutant and appeared to be totally deficient in genetic recombination. Other radiation sensitive mutants, such as *uvs*-3 (Holliday, 1967) or pyrimidine auxotrophs (Moore, 1975*a*, *b*), are proficient in recombination.

Further studies on radiation-sensitive, as well as temperature-sensitive (Unrau, & Holliday, 1970) and DNase-deficient mutants (Badman, 1972), have been carried out in association with biochemical investigations of enzymes or proteins which may be involved in replication, repair or recombination in *U. maydis* (Holloman, 1973; Holloman & Holliday, 1973; Banks, 1974; Holliday *et al.* 1974; Banks & Spanos, 1975; Banks & Yarranton, 1975; Banks *et al.* 1975; Holloman, 1975; Jeggo & Banks, 1975). The aim is to identify the specific biochemical defect in mutants which are altered in one or more of these genetic processes. As well as this, it is essential to characterize by physiological and genetic means the full phenotype of each mutant. In this study it will be shown that the *rec*-1 mutation has pleiotropic effects on the phenotype. Since repair, recombination, mutation, viability, growth and meiosis are all affected, it can be concluded that a single mutation can completely alter the normal genetic system of *U. maydis*. In studies of allelic recombination it will be demonstrated that the process of gene conversion is usually replaced by an aberrant form of crossing over.

2. STRAINS

(i) *Nomenclature*

In view of its several effects on recombination *uvs*-1 has been renamed *rec* 1–1. Similarly, *uvs*-2 has been renamed *rec* 2–1. *uvs*-3 becomes *uvs* 3–1. These three mutants were induced by UV light. Three alleles of *rec* 1–1 are known: *rec* 1–2 was induced by UV, and *rec* 1–3 and *rec* 1–4 by nitrosoguanidine (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) mutagenesis. Other mutants of *U. maydis* have also been given locus and allele numbers. A full list can be found in Holliday (1974).

(ii) *Haploids*

By crosses to multiply marked strains, a variety of *rec* 1–1 and *rec* 1–2 haploids have been recovered from the products of meiosis, and some of these were used in the synthesis of diploid strains. Unless otherwise stated, experimental studies of *rec* 1–1 haploids were carried out either in the original isolate in a wild-type a_2b_1 strain or with a double auxotroph *rec* 1–1 *nar* 1–1 *pan* 1–1 a_1b_2. Strains carrying two or three radiation-sensitive mutants were identified in tetrads and their genotype confirmed by complementation tests.

(iii) *Diploids*

rec 1–1 homozygous diploids were obtained either by direct selection from haploid parents or by first constructing diploids heterozygous for *rec* 1–1 and then obtaining the homozygous derivative by mitotic crossing over. The full genotypes of the diploids used in these experiments are given in Table 1.

Table 1. *Diploids used in recombination experiments*

Strain no.	Genotype
50r	$\frac{rec\ 1\text{–}1}{+}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}6}\ \bigcirc\frac{+\quad +}{me\ 1\text{–}2\ ad\ 1\text{–}1}\ \frac{pan\ 1\text{–}1\ a_1}{+\quad a_2}\ \frac{b_2}{b_1}$
50s*	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}6}\ \bigcirc\frac{+\quad +}{me\ 1\text{–}2\ ad\ 1\text{–}1}\ \frac{pan\ 1\text{–}1\ a_1}{+\quad a_2}\ \frac{b_2}{b_1}$
MT8	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \frac{rec\ 2\text{–}1}{rec\ 2\text{–}1}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}6}\ \bigcirc\frac{+\quad +}{me\ 1\text{–}2\ ad\ 1\text{–}1}\ \frac{pan\ 1\text{–}1\ a_1}{+\quad a_2}\ \frac{b_2}{b_1}$
M35s*	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \bigcirc\frac{+\quad ad\ 1\text{–}1\ leu\ 1\text{–}1}{me\ 1\text{–}2\quad +\quad\ +}\ \bigcirc\frac{inos\ 1\text{–}3\quad +}{inos\ 1\text{–}3\ nic\ 1\text{–}1}\ \frac{+}{nar\ 1\text{–}1}\ \frac{pan\ 1\text{–}1\ a_1}{+\quad a_2}$
M58	$\frac{rec\ 1\text{–}1}{+}\ \bigcirc\frac{+\quad ad\ 1\text{–}1}{me\ 1\text{–}2\quad +}\ \bigcirc\frac{inos\ 1\text{–}5\ nic\ 1\text{–}1}{inos\ 1\text{–}4\quad +}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}$
M58s*	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \bigcirc\frac{+\quad ad\ 1\text{–}1}{me\ 1\text{–}2\quad +}\ \bigcirc\frac{inos\ 1\text{–}5\ nic\ 1\text{–}1}{inos\ 1\text{–}4\quad +}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}$
M60	$\frac{rec\ 1\text{–}1}{+}\ \bigcirc\frac{+\quad +}{me\ 1\text{–}2\ ad\ 1\text{–}1}\ \bigcirc\frac{inos\ 1\text{–}5\quad +}{inos\ 1\text{–}4\ nic\ 1\text{–}1}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}$
M60s*	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \bigcirc\frac{+\quad +}{me\ 1\text{–}2\ ad\ 1\text{–}1}\ \bigcirc\frac{inos\ 1\text{–}5\quad +}{inos\ 1\text{–}4\ nic\ 1\text{–}1}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}$
M53	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}1}\ \bigcirc\frac{me\ 1\text{–}2\ ad\ 1\text{–}1}{+\quad +}\ \bigcirc\frac{+\quad nic\ 1\text{–}1}{inos\ 1\text{–}3\quad +}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}1}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}$
M133s	$\frac{rec\ 1\text{–}1}{rec\ 1\text{–}2}\ \frac{ad\ 1\text{–}1}{+}\ \bigcirc\frac{inos\ 1\text{–}5\ nic\ 1\text{–}2}{inos\ 1\text{–}4\ nic\ 1\text{–}1}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}6}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}\ \frac{b_2}{b_1}$
M133†	$\frac{rec\ 1\text{–}1/2}{+}\ \frac{ad\ 1\text{–}1}{+}\ \bigcirc\frac{inos\ 1\text{–}5\ nic\ 1\text{–}2}{inos\ 1\text{–}4\ nic\ 1\text{–}1}\ \frac{nar\ 1\text{–}1}{nar\ 1\text{–}6}\ \frac{+\quad a_2}{pan\ 1\text{–}1\ a_1}\ \frac{b_2}{b_1}$

Linkages. In standard *rec*$^+$ crosses recombination frequencies vary. The range for *ad*-1 and *me*-1 is 15–30 %; for *nic*-1 and *inos*-1, 20–35 %; for *pan*-1 and *a*, 2–10 %. No other pairs of markers show linkage.

* Derived by mitotic crossing over from diploids (50r, M35, M58, M60) heterozygous for *rec*–1.

† Derived from M133s by selecting a radiation-resistant allelic recombinant. It is not known if the diploid is heterozygous for *rec* 1–1 or *rec* 1–2.

3. METHODS

(i) *Media*

Cultures were routinely grown on solid or in liquid complete medium (CM). Nitrate minimal medium (NM) with or without appropriate supplements were used for selecting or classifying mitotic or meiotic recombinants and segregants. The composition of these media is given in Holliday (1974).

(ii) *Growth conditions*

Stationary phase cultures (2×10^7–10^8 cells/ml, depending on the strain) were prepared by inoculating flasks of liquid CM and incubating at 32 °C for 24–40 h in a New Brunswick G25 gyrotary shaker. Log phase cultures were obtained by overnight growth, followed by dilution in CM to about 10^6 cells/ml and incubation for at least 3 h before use. In quantitative experiments log phase cells were found to give more reproducible results than stationary phase cells, which change their physiological state with age. For this reason log phase cultures were used in all experiments, unless otherwise stated.

(iii) *Treatment with radiation or nitrosoguanidine*

Cells to be irradiated by UV light were washed once with sterile water and then suspended to a concentration of not more than 10^6/ml unless otherwise stated. In some experiments cells were irradiated after spreading on plates. The source of UV was a Hanovia germicidal lamp, and the output was calibrated with a Latarjet dosimeter. Apart from liquid holding experiments, the cells were immediately spread on plates and incubated in the dark. Photoreactivation was carried out with two 40 W daylight fluorescent tubes at a distance of 6 cm. γ-Irradiation was carried out with a ^{60}Co source (Gammabeam 650, Atomic Energy of Canada Ltd.). Cells were irradiated in a stirred and aerated suspension. Nitrosoguanidine was dissolved in alcohol and diluted into an aqueous cell suspension at 10 or 100 μg/ml. Treatment in a 32 °C water bath was ended by a 100-fold dilution and immediate plating.

(iv) *Survival*

Survival was determined either by spreading appropriate dilutions of treated cells on CM and counting colonies after 3–4 days incubation, or by spreading about 10^5 cells/plate of CM and scoring viability under a low power microscope after 16–24 h. Non-viable cells are clearly distinguishable from mini-colonies. This method is also used to determine the spontaneous frequency of non-viable cells during growth in CM.

(v) *Cell counts and growth rates*

Cells were routinely counted with a Coulter counter model A, which is accurate to within 2–3 %.

(vi) *Spontaneous reversion rates*

These were determined by a fluctuation test with 10–20 parallel cultures. Tubes containing 5 ml liquid CM were inoculated with less than 10^3 cells and incubated for 2 days to stationary phase. Each culture then contained the same number of cells. Cells were washed and if necessary concentrated by centrifugation prior to plating appropriate aliquots from each culture on selective medium. The frequencies of revertants per culture were arranged in ascending order and the median value was taken as the best estimate (Lea & Coulson, 1949).

(vii) *Genetic analysis*

Methods for crossing haploids, isolating the random products of meiosis or the components of tetrads have been described elsewhere (see Holliday, 1974). Vegetative diploids were originally isolated by the method described by Holliday (1961*b*), but this was superseded by the use of mating medium (Day & Anagnostakis, 1971; Holliday, 1974). The same applies to the classification of mating types. Mitotic crossing over was detected by replica plating colonies grown on CM to NM. Non-growing colonies were picked and classified with appropriate supplemented media. Allelic recombination frequencies after irradiation were determined by plating samples of cells on the appropriate selective media.

4. GENETIC REPAIR

(i) *Photoreactivation and liquid holding recovery*

Evidence that *rec*-1 is defective in the repair of pyrimidine dimers comes from photoreactivation experiments. The killing effect of UV is reduced about 5-fold by immediate post-treatment with visible light (Fig. 1). This demonstrates that most of the lethal damage is due to dimers, which are split by the photoreactivating enzyme. Although wild-type cells also show photoreactivation, the effect is relatively smaller than in *rec*-1 and other radiation sensitive mutants such as *uvs*-3 (results not reported). This would be expected if wild-type cells have an extremely efficient dark repair system, and the removal of at least one dark repair pathway by mutation therefore makes the relative contribution of photoreactivation to survival very much greater.

Although wild-type cells show no liquid holding recovery after UV, it was discovered by M. A. Resnick (personal communication) that *rec*-1 strains have very strong liquid holding recovery. This is shown in Fig. 1. Possibly repair pathways which are still intact in *rec*-1 strains can remove most of the dimers if sufficient time elapses prior to active metabolism on nutrient medium.

(ii) *Sensitivity to other mutagens*

It was previously shown that *rec*-1 is sensitive to ionizing radiation (Holliday, 1965*a*). It is also extremely sensitive to the alkylating agent nitrosoguanidine. The other mutant affected in recombination is also very sensitive to these two

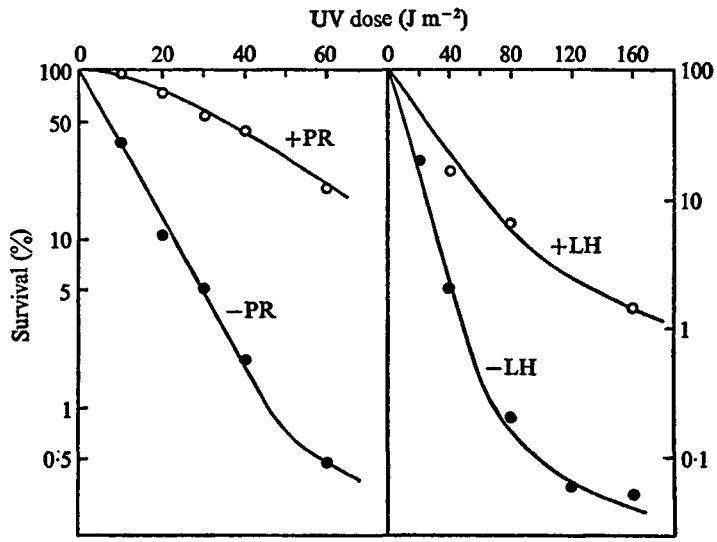

Fig. 1. The effect of photoreactivation (PR) and liquid holding recovery (LH) on survival of *rec*-1 after UV treatment. Visible light treatment was for 2 h immediately after spreading cells on CM. For liquid holding cells were shaken in water at 32 ° for 24 h before plating.

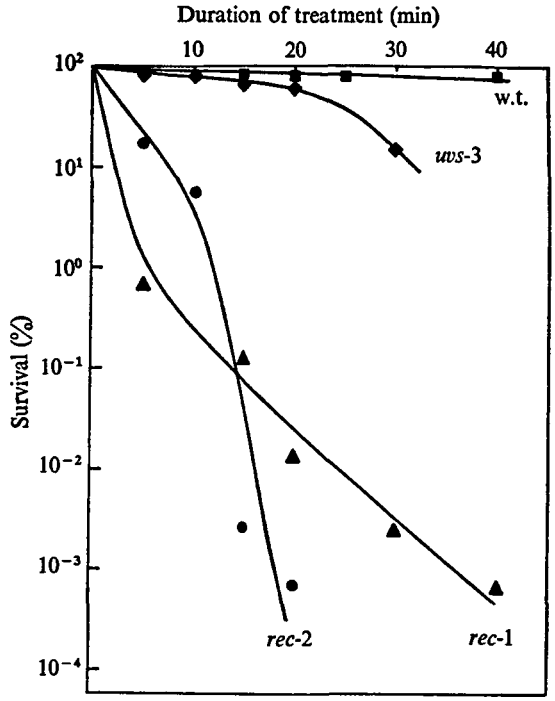

Fig. 2. The survival of *rec*-1, *rec*-2, *uvs*-3 and wild-type cells after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (10 μg/ml at 32 °C.)

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mutagens, but *uvs*-3 is only slightly affected (Fig. 2). The difference in sensitivity between wild-type and *rec*-1 is smaller for UV than for either ionizing radiation or nitrosoguanidine.

(iii) *Interaction with other mutants*

Game & Cox (1973) have attempted to define the number of repair pathways in yeast by examining the radiation sensitivity of double or multiple *rad* mutant strains. For instance, when defects are in the same pathway, the double mutant strain should have the same sensitivity as each single mutant. On the basis of their

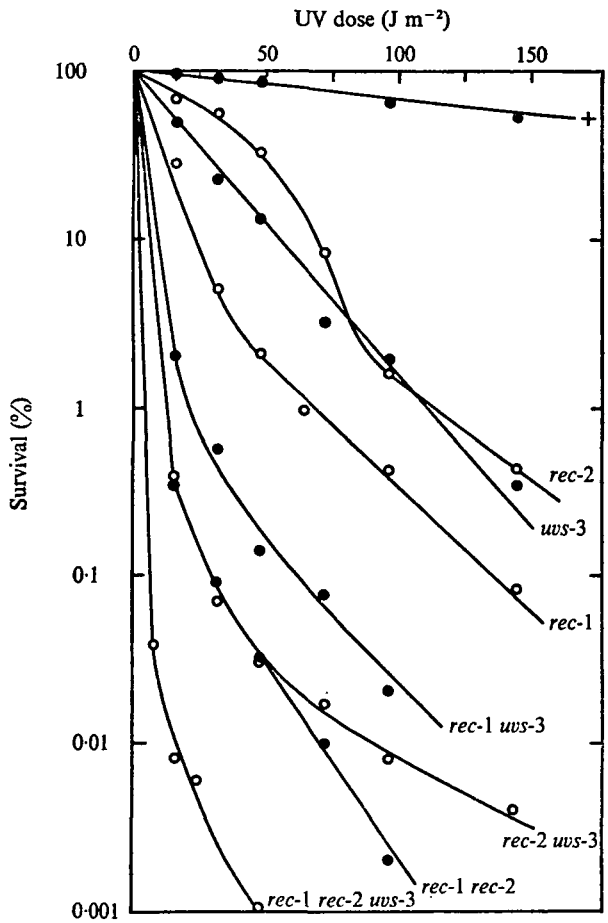

Fig. 3. The survival after UV treatment of *rec*-1, *rec*-2, *uvs*-3, three double mutant strains and the triple mutant strain.

criteria, *rec*-1, *rec*-2 and *uvs*-3 are involved in different repair pathways, since each double mutant is more sensitive to UV than any single, and the triple mutant is more sensitive than any double. These results are shown in Fig. 3. The triple mutant is about 200-fold more sensitive than wild type and 10-fold more sensitive than *rec*-1. In the case of ionizing radiation, these differences are greater.

(iv) *Temperature sensitivity*

It was discovered that *rec*-1 mutants are much more sensitive to UV when grown and plated at 32 or 35 than at 22 or 25 °C. Subsequently it was found that this is true of wild-type strains also. Some of these results are shown in Figs. 4 and 5. Two other radiation sensitive mutants, *rec*-2 and *uvs*-3, also show the same effect. The data are summarized in Table 2.

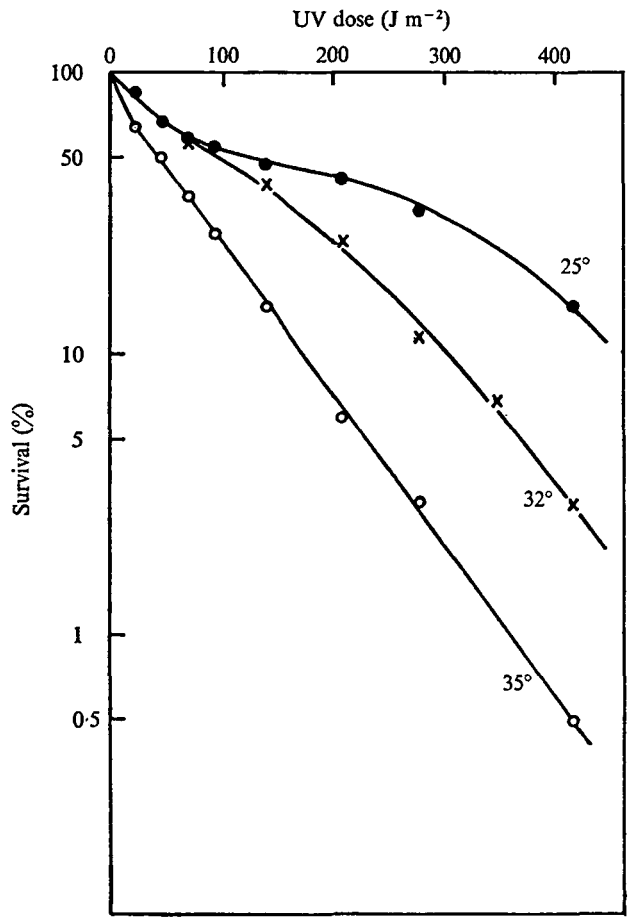

Fig. 4. The effect of temperature on the survival of wild-type cells after UV treatment. Cells were grown on CM at the temperatures indicated both before and after UV.

Since the effect of temperature is much the same for the four strains, it seems possible that there is at least one temperature-sensitive repair pathway that remains intact in all of them. The prediction is that a class of radiation-sensitive mutant should exist which does not show the temperature effect because it is blocked in this pathway. However, it is not ruled out that the effect is due to a complex interrelationship between the rates of repair and of cell killing after irradiation, or an effect on the regulation of repair mechanisms (see Discussion).

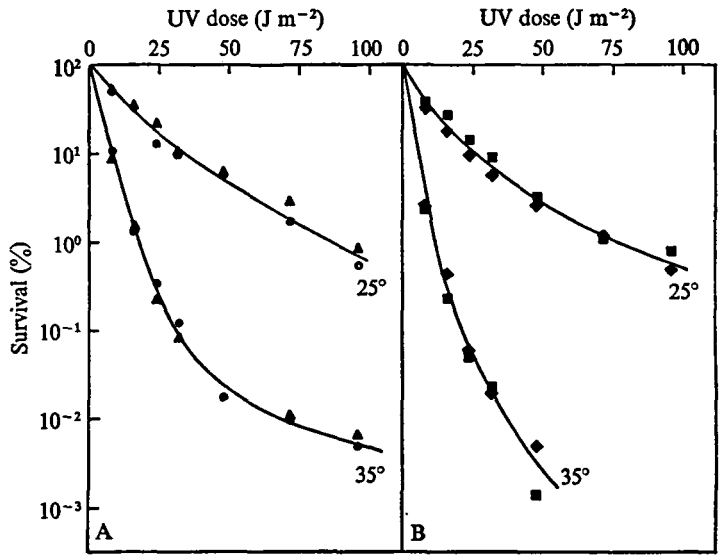

Fig. 5. The survival of four *rec*-1 alleles after UV treatment. In separate experiments cells were incubated throughout at 25 or 35 °C. A: ●—●, *rec* 1–1; ▲—▲, *rec* 1–2; B: ◆—◆, *rec* 1–3; ■—■, *rec* 1–4.

Table 2. *Effect of temperature on the UV-sensitivity of wild-type and mutant strains*

	Dose (J m⁻²) for 50 % survival		Dose (J m⁻²) for 10 % survival		25/35 ° ratios	
Strain	25 °C	35 °C	25 °C	35 °C	50 % survival	10 % survival
Wild type	138	41	470	161	3·4	2·9
rec 1–1	8·4	1·7	40	8·4	4·9	4·8
rec 2–1	45	15	112	42	3·0	2·7
uvs 3–1	40	8·4	175	29	4·8	6·0

Temperature-shift experiments would provide information about the timing of the presumed temperature sensitive process after UV treatment.

(v) *Alleles of* rec *1–1*

The original batch of radiation sensitive mutants contained two with the same phenotype. When these were tested in diploids (e.g. M133s), they were found not to complement. These two mutants, *rec* 1–1 and *rec* 1–2, have identical survival curves (Fig. 5A). The heteroallelic diploid is as sensitive as diploids homozygous for either mutant. Amongst further batches of radiation-sensitive strains induced by nitrosoguanidine mutagenesis, three more mutants were identified which did not complement with *rec* 1–1. The survival of two of these, *rec* 1–3 and *rec* 1–4. has been examined in detail, and they were found to be indistinguishable from the first two alleles (Fig. 5B).

It has been reported that mutant alleles of *rad* loci in yeast often have different survival curves (Waters & Parry, 1973). It is clear that at least with regard to the *rec*-1 locus in *U. maydis*, this variation is not seen. This implies that each of the alleles tested completely lacks the product of the *rec*-1 gene.

(vi) *Variability, growth and viability*

It was previously reported that *rec*-1 diploids are extremely variable with regard to colony diameter, shape, colour and surface texture (Holliday, 1967). A large number of *rec*-1 diploids have subsequently been examined, and all have shown this same phenotype. Moreover, whereas M133s (heteroallelic for *rec* 1–1 and *rec* 1–2) is variable, the *rec*$^+$ isogenic derivative is quite uniform. Haploid *rec*-1 strains, on the other hand, are uniform and not distinguishable from wild-type colonies.

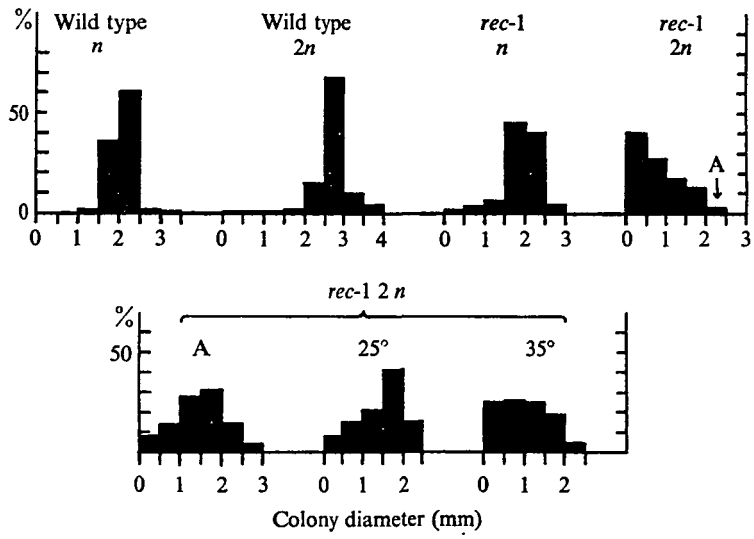

Fig. 6. The variation in colony size of haploid and diploid (50s) *rec*-1 strains, together with wild-type haploid and diploid strains. Top: cells grown at 32 °C for 3 days on CM. Each histogram is based on measurement of over 200 colonies. Bottom: one large 50s colony was dispersed in water and cells respread on CM. In another experiment, cells were grown in liquid medium and spread on CM with incubation throughout at 25 and 35 °C. Each histogram is based on the measurement of over 100 colonies.

The effect of *rec*-1 on colony diameter is documented in Fig. 6. The diploid 50s, homozygous for *rec*-1, has a wide distribution of colony sizes, whereas the haploid *rec* 1–1 is fairly uniform. Replating one large 50s colony shows that the variation persists, although this population of cells is clearly more vigorous than the original sample. In another experiment 50s cells were grown at 25 and 35 °, plated at these temperatures and the colony sizes measured. The variation is much more extreme at the higher temperature (Fig. 6).

The effect of *rec*-1 on growth rate can be directly compared with M133s and its

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radiation resistant derivative. The doubling time of the former in liquid CM at 32 °C is 115 min and the latter 90 min.

All populations of *rec*-1 contain a proportion of non-viable cells (lethal sectoring). This can be easily demonstrated by spreading growing cells on CM, incubating overnight and then scoring mini colonies or non-growing cells. With *rec*-1 haploids the frequency of viable cells is usually 10–15 %; diploid strains have fewer non-viable cells, normally about half the haploid frequency. This feature of the *rec*-1 phenotype is also temperature-sensitive. Representative results are shown in Table 3. The table also demonstrates strong interaction of *rec*-1 and the other two radiation-sensitive mutants. Although *rec*-2 and *uvs*-3 produce very few non-viable cells during growth, in combination with *rec*-1 an extremely high frequency of lethal sectoring is seen (Table 3). *rec* mutants of *E. coli* also produce non-viable cells during growth, particularly *rec*BC strains (Capaldo, Ramsey & Barbour, 1974).

Table 3. *The viability of* rec-1 *and other radiation-sensitive strains during growth in complete medium*

Strains and incubation temperature	Cells examined	Non-viable (%)
Haploids (32 °C)		
Wild type	500	1·4
rec 1–1	595	16·3
rec 2–1	413	1·5
uvs 3–1	204	0·0
rec 1–1 *rec* 2–1	244	41·0
rec 1–1 *rec* 2–1 *uvs* 3–1	259	79·5
Diploid:		
M53 *rec* 1–1		
22 °C	638	3·6
32 °C	332	6·3
35 °C	327	14·7

(vii) *Mutation*

Detailed studies on mutagenesis in *rec-1* strains have not yet been carried out, but there are strong indications that the mutant has the properties of a weak mutator strain. Fluctuation tests show that the reversion of *ad* 1–1 and *pan* 1–1 auxotrophs is significantly increased in a *rec*-1 background (Table 4). There is also evidence that the frequency of forward mutation is elevated. Amongst over 4000 prototrophic colonies tested, four different auxotrophs were found. A similar sample of colonies from untreated wild-type cells would not contain any auxotrophs; even after mutagenesis by UV, the yield of auxotrophs can be as low as 0·1 % (Holliday, 1961*a*).

Preliminary experiments indicate that *rec* strains are refractory to mutagenesis by UV. Treatment of prototrophs does not increase the yield of auxotrophs above the spontaneous level (Table 4). Studies of *ad*-1 reversion have in no case demonstrated an absolute increase in reversion after UV treatment, whereas this could be easily shown with *rec*$^+$ strains. Amongst survivors of UV treatment, there may

be a small increase in *ad*-1 reversion, but again this effect is very much less than in *ad*-1 radiation resistant strains (Table 4).

The results indicate that *rec* is similar to the *rev* mutants in yeast (Lemontt, 1971) and *recA* and *exr* (or *lex*) mutants of *E. coli* (Witkin, 1969) in lacking a cellular function, perhaps error prone repair, which is essential for UV-induced mutagenesis.

Table 4. *Mutation frequencies in* rec-1–1 *and control strains*

A. Reversion of *ad* 1–1 or *pan* 1–1 to prototrophy (fluctuation tests)

Strain	No. of populations	Average population size	Median reversion frequency
ad 1–1	10	$1{\cdot}4 \times 10^{8}$	$3{\cdot}7 \times 10^{-8}$
ad 1–1 *rec* 1–1	10	$5{\cdot}6 \times 10^{7}$	$1{\cdot}4 \times 10^{-7}$
pan 1–1	17	$2{\cdot}4 \times 10^{9}$	$< 3{\cdot}9 \times 10^{-10}$*
pan 1–1 *rec* 1–1	12	$2{\cdot}1 \times 10^{9}$	$3{\cdot}2 \times 10^{-9}$*

B. Reversion of *ad* 1–1 after UV treatment

Strain	UV treatment (J m^{-2})	Survival (%)	Mutation frequency/ survivors
ad 1–1	0	100	$4{\cdot}7 \times 10^{-8}$
	20	100	$9{\cdot}3 \times 10^{-8}$
	100	81	$2{\cdot}9 \times 10^{-7}$
ad 1–1 *rec* 1–1	0	100	$1{\cdot}5 \times 10^{-7}$
	10	76	$1{\cdot}4 \times 10^{-7}$
	20	53	$2{\cdot}1 \times 10^{-7}$

C. Forward mutations to auxotrophy in a *rec* 1–1 prototroph

UV treatment	Colonies scored	Auxotrophs
None	4367	4
35 J m^{-2} ($\sim$ 10% survival)	4322	5

* Unpublished observations of J. E. Pugh.

5. GENETIC RECOMBINATION

(i) *Mitotic crossing over*

A number of studies of mitotic crossing over (MCO) in normal radiation resistant diploids heterozygous for several markers have shown that the spontaneous frequency is about 0·1%, and that this is increased many times after UV irradiation (e.g. Holliday, 1961*b*, 1964*b*, 1965*b*, 1967). Reciprocal products of an exchange are frequently detected as 'twin spot' colonies after UV and, in addition, double exchanges in one chromosome are not uncommon. By contrast, diploids homozygous for *rec* 1–1 have a significantly increased frequency of spontaneous and UV-induced segregation of heterozygous markers (although the relative increase after UV is less than in *rec*$^{+}$ diploids). However, reciprocal products are not observed and double exchanges are very rare (Holliday, 1967). These results have been further documented with a number of diploids homozygous

Table 5. *Frequency of somatic segregation in diploid M53, homozygous for* rec-*1–1**

Incubation temperature (°C)	UV treatment	Colonies scored	Auxotrophs		Phenotypes of segregants						
			No.	%	*nic*	*inos*	*pan*	*ad*	*ad me*	*me*	Others
32	—	716	32	4·7	19	11	0	0	2	0	—
32	33·4 J m^{-2} (3 % survival)	705	91	12·9	37	29	3	8	11	0	3 *nic inos*
22	—	1915	32	1·7	13	15	1	3	0	0	—
35–22†	—	519	22	4·2	8	9	0	0	4	0	—
22–35	—	830	59	7·1	28	28	1	2	0	0	—
32–22	—	995	32	3·2	20	9	1	0	2	0	—
22–32	—	1191	31	2·6	19	9	1	0	0	0	1 *nic pan* 1 *nic*/*nic inos* mosaic
				Totals	144	110	7	13	19	0	

* For full details of genotype, see Table 1.

† The cells were grown at the first temperature, then spread on CM and incubated at the second temperature.

for *rec* 1–1 or heteroallelic for *rec* 1–1 and *rec* 1–2. Representative data are given for diploid M53 (Table 5), which carries the linked markers *nic* 1–1 and *inos* 1–3 in repulsion, as well as the markers *ad* 1–1, *me* 1–1 and *pan* 1–1 which were used in the previous studies.

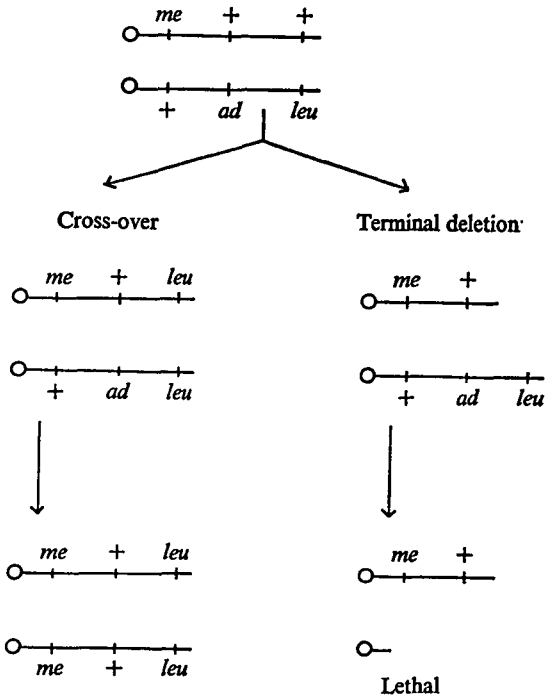

Fig. 7. The effect of mitotic crossing and terminal deletion on the secondary segregation of the proximal marker *me*-1. On the one hand *me*$^-$ segregants can be recovered from *leu*$^-$ segregants, but on the other they cannot.

The frequency of both spontaneous and UV-induced segregation is very high in this strain, and this is largely due to events in the chromosome carrying the *nic* and *inos* markers. Exchanges in this chromosome are about ten times more common than in the other two with markers. Although a proportion of the *nic*$^-$ and *inos*$^-$ segregants appeared as large sectors in prototrophic colonies, in no case was a clear example of a 'twin spot' or reciprocal exchange seen. In diploids which are not *rec* 1–1, such exchanges are easily detected as mosaics, where half the colony is *nic*$^-$ and half *inos*$^-$. The results in Table 5 also confirm the previous observation that double exchanges in one arm are uncommon or absent (Holliday, 1967), since these would give rise to *me*$^-$ segregants. Finally, the effects of elevated incubation temperature on segregation is clearly seen, and this is in line with the studies on the effect of temperature on repair and lethal sectoring. The heat shock which occurs on transfer of cells from 22 to 35 °C has a particularly strong effect on segregation frequency.

In view of the extreme morphological variability of *rec*-1 diploids, it was

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previously suggested that the high frequency of somatic segregation could be attributed to chromosome breakage rather than to crossing over. Terminal deletions would allow the expression of recessive auxotrophic markers, and also lead to chromosomal imbalance, variation in growth rate or cell death. Moreover, it would also explain the absence of reciprocal exchanges and double exchanges. A test of this hypothesis was therefore devised using diploid M35s. With the linked markers in the arrangement shown in Fig. 7, two different conclusions are expected from terminal deletion or crossing over. With the former, a *leu*$^-$ segregant could not produce secondary *me*$^-$ segregants, since these would be homozygous for the deletion and therefore lethal. On the other hand, a cross over giving rise to a *leu*$^-$ segregant produces a diploid which could segregate *me*$^-$.

Table 6. *Second-order spontaneous or UV-induced segregation of* me *1–1 and* ad *1–1 from* leu *1–1 segregants from Diploid M35s*

leu$^-$ segregant isolation no.	Origin	Total colonies examined	Second-order segregants		
			ad$^-$	*me*$^-$	*ad*$^-$*me*$^-$
14	Spontaneous	240	7	10	1
17		4587	8	3	
16		435	65*	1	
19		2576	2	2	
29	UV- induced	7838	9	5	
33		3161	3	1	
47		1112	3	8	
49		1004	3	6	
51		4448	0	6	
56		2840	3	1	
65		1896	1	3	
69		550	1	4	
70		3736	10	2	
78		2200	1	2	

* *ad*$^-$ cells appeared early in the growth of isolate 16.

M35s was made homozygous for *inos*-1, because it was originally intended to select *leu*$^-$ derivatives by inositol starvation (Holliday, 1962*b*). In the event, they were obtained by the standard replica plating method. The second order segregation of four spontaneous and ten UV-induced *leu*$^-$ segregants was examined. This series of experiments involved scoring more than 37000 colonies grown on complete medium and then replica plated to minimal medium supplemented with leucine, inositol and pantothenic acid. About half the second-order segregants were spontaneous and half were induced by a dose of UV giving about 10% survival. The results in Table 6 show that all 14 *leu*$^-$ strains segregated *me*$^-$. Moreover in most of the strains the number of *me*$^-$ segregants was comparable to the number of *ad*$^-$ ones. Since we know that double exchanges are rare in *rec*-1 diploids, it is very unlikely that any of the *me*$^-$ segregants could have arisen in diploids that were hemizygous for the terminal region of the chromosome.

These experiments therefore provide strong evidence that the somatic

segregation in *rec*-1 diploids is due to crossing over, rather than terminal deletion. However, as we shall see in a subsequent section, it is very likely that crossing over in these diploids is often associated with breakage of one of the two chromatids involved in the exchange. This might help to account for the rarity of double exchanges. Another possibility is that the 'double exchanges' seen in normal heterozygous diploids are in reality due to gene conversion events, as is often the case in yeast (Nakai & Mortimer, 1969). It will be shown that recombination in *rec*-1 heteroallelic diploids is not due to gene conversion. An absence of conversion might therefore explain the lack of 'double exchanges' in *rec*-1 diploids.

(ii) *Meiotic recombination*

Only two crosses between *rec*-1 strains have been analysed in any detail, but they are sufficient to show that the mutant severely disrupts the normal events of meiosis. Germination of teliospores is initially unaffected and the products of

Table 7. *Survival of products of meiosis in* rec-*1* × rec–*1 crosses*

Observed viability of germinated teliospores: 130/263 = 49·43 %.
Calculated viability of individual members of tetrads ≈ 16 %.

No. of surviving products	Cross M81		Cross M100	
	Observed	Expected	Observed	Expected
1	77	70·3	69	80·3
2	16	20·1	33	23·1
3	0	2·5	5	2·9
4	0	0	0	0
	93		107	

Genotypes of crosses are as follows:
Cross M81: *ad* 1–1 *me* 1–1 *nar* 1–6 *rec* 1–1 × *pan* 1–1 *nar* 1–1 *rec* 1–1
Cross M100: *inos* 1–5 *ad* 1–1 *me* 1–1 *nic* 1–1 *rec* 1–1 × *inos* 1–4 *pan* 1–1 *rec* 1–2

meiosis (basidiospores) are budded off from the promycelium (meiocyte). However, a high proportion of these germinated teliospores give rise to non-viable cells. In the cross M81, which is homozygous for *rec* 1–1, 133 out of 263 germinated spores (50·6 %) did not produce colonies, i.e. none of the products of meiosis survived. If there is a probability P that each haploid product will be non-viable, then $P^4 \approx 0{\cdot}5$ and $P \approx 0{\cdot}84$. If the overall viability of meiotic products is 16 %, then the probabilities of obtaining 1, 2, 3 or 4 products from teliospores are 37·9 %, 10·8 %, 1·4 %, and 0·06 %. In *U. maydis* tetrads are routinely analysed by spreading out small teliospore colonies and recovering the asexual progeny of the immediate products of meiosis (Holliday, 1961*a*). In normal crosses the recovery of triads or tetrads by this technique varies considerably, but is usually in the range expected from the survival of 50–80 % of the meiotic products. Crosses homozygous for *rec*-1 are quite different. The majority of surviving teliospore colonies contain only one phenotype; diads are also seen, but triads are quite rare.

No complete tetrad has been recovered. For cross M81 and M100 the frequencies of each type observed are in good agreement with those expected for about 16% viability of meiotic products, as shown in Table 7.

Table 8. *Effect of* rec-*1 on segregation at meiosis*

Cross M81: *ad* 1–1 *me* 1–1 *nar* 1–6 *rec* 1–1 × *pan* 1–1 *nar* 1–1 *rec* 1–1
(for linkage relationships, see Table 1).

A. Individual teliospore colonies (tetrads)

No. of surviving genotypes	Phenotypes*	Frequency	
One	+ + +	69	77
	+ + p	3	
	a m +	2	
	+ m +	2	
	a + +	1	
Two	+ + + and + + p	9	16
	+ + + and a m +	3	
	+ + + and + m +	3	
	a + + and a m +	1	

B. Auxotrophic random products of meiosis

Phenotype	Frequency	Phenotype	Frequency
(+ + +)†		a + p	2
a m p	9	+ m +	11
+ + p	73	+ m p	4
a m +	33	a + +	14

* a = *ad*⁻, m = *me*⁻, p = *pan*⁻; all progeny are *nar*⁻; of 42 tested, 35 were heteroallelic.
† 75% of the total progeny.

In cross M81, the 77 teliospore colonies with one product and the 16 with two yield 109 progeny, and of these, no less than 84 were *me*⁺ *ad*⁺ *pan*⁺. Since the cross is heteroallelic for *nar* 1–1 and *nar* 1–6, it is possible to test whether these progeny are diploid or aneuploid. Of 42 colonies tested, 35 produced *nar*⁺ recombinants, i.e. they are heteroallelic for the *nar* locus. It is clear, therefore, that a high proportion of the meiotic products must be diploids or aneuploids which are heterozygous for recessive markers. It must be emphasized that although some of the early genetic analyses with auxotrophic markers provided evidence for a high frequency of diploid production during meiosis (see Holliday, 1971), it was later discovered that this was associated with the presence of *leu*-1, a mutant with poor growth and viability. Numerous subsequent studies have shown that the frequency of diploids is very low (0–5%) in *rec*-1 × *rec*⁺ or *rec*⁺ × *rec*⁺ crosses using the same markers employed in M81 or M100.

Amongst random products of meiosis in M81, only 25% were auxotrophic for *ad*, *me* or *pan*, in agreement with the tetrad data. 146 such colonies were classified for their requirements, with the results shown in Table 8. In standard crosses, complementary products of meiosis, for instance the parental classes *ad me* and

pan, are produced in equal numbers, but this is not so in cross M81. The distribution of phenotypes show that recessive markers on individual chromosomes are commonly expressed (131/146), whereas markers on different chromosomes appear infrequently in the progeny (15/146). In other words, the usual random segregation of unlinked markers does not occur. This effect will be seen more clearly in cross M100.

In standard crosses, the recombination between linked markers *ad*-1 and *me*-1 is in the range 15–30 %. In M81, the fact that *ad* and *me* often segregate together indicates that their linkage is preserved, but in view of the distorted ratio of complementary classes, it is not possible to estimate the recombination between these markers. (A previous estimate of 20 % meiotic recombination between *ad*-1 and *me*-1 in *rec*-1 crosses (Holliday, 1967) was based on a similarly abnormal distribution of markers among progeny.)

In cross M100, which is heteroallelic for two *inos* alleles, the additional heterozygous marker *nic* 1–1 is included. Amongst 107 teliospore colonies, 5 triads were obtained. The phenotypes of these are shown in Table 9. They clearly demonstrate that the segregation abnormalities seen in M81 are not due to inviability of certain classes. In these few triads non-Mendelian ratios are seen for all four loci. There is a preponderance of wild-type alleles, giving 3 wild-type:0 mutant ratios in 8 of the possible 20 segregations. In only 3 cases is a mutant allele represented twice in a triad. These results are very reminiscent of those obtained by Moustacchi, Hottinguer-de-Margerie & Fabre (1967) in tetrad analysis of *Saccharomyces* crosses, in which one parent had survived the genetic damage due to ^{32}P decay. Their results were interpreted on the basis of chromosomal abnormalities leading to aneuploidy, and the same conclusion can be drawn here. The diploidy or aneuploidy of many members of these triads was shown by the heteroallelism test (Table 9). All three members of triad 98 were heteroallelic. It can be concluded, as in cross M81, that many of the progeny are heterozygous for recessive markers.

The 107 teliospore colonies yielded in all 150 progeny, the phenotypes of which are given in Table 9. Again, the inequality of the complementary classes is seen and there is an excess of the wild-type allele for each marker. The percentage of progeny which express each of the four markers is given in Table 9. We can see that the frequency of progeny which express two unlinked markers is simply the product of the frequency of each single marker (Table 9). This is not true for the linked markers *ad* and *me*, which segregate together more often than expected on a random basis. In the same way the linked *nic* and *inos* loci tend to segregate together. From tests on a sample of progeny it is apparent that *nic*$^+$ isolates are more frequently heteroallelic for the *inos* locus than *nic*$^-$ ones (Table 9). (A 2-by-2 χ^2 test with Yates's correction gives $\chi_1^2 = 3{\cdot}0$, $P = 0{\cdot}05$–$0{\cdot}1$.) This is expected from linkage, since heteroallelism for *inos*-1 is likely to be associated with heterozygosity for *nic*-1, and *nic*$^-$ haploids or diploids will not often be heteroallelic for *inos*-1.

From all these results we can conclude that meiosis is completely abnormal in *rec* homozygous crosses. Viability of the products is very low and the survivors

Table 9. *Effect of* rec-*1 on segregation at meiosis*

Cross M100: *inos* 1–5 *ad* 1–1 *me* 1–1 *nic* 1–1 *rec* 1–1 × *inos* 1–4 *pan* 1–1 *rec* 1–2 (for linkage relationships, see Table 1).

A. Triads from teliospore colonies

a m + + het		+ + + +
+ m + + het		+ + n +
+ + + + het		+ + + p het
+ + n p		+ + + +*
+ + n +		+ m + +
+ + + + het		a m + +
	a + + + het	
	+ + n +	
	+ + + + het	

All the progeny were *inos*⁻; 'het' indicates that the isolate was heteroallelic for *inos* 1–4/*inos* 1–5

B. Phenotypes from 107 teliospore colonies

a m n +	4	a + n +	0
+ + + p	18	+ m + p	3
a m + +	7	a + + +	3
+ + n p	4	+ m n p	0
a m n p	2	a + n p	0
+ + + +	76	+ m + +	1
a m + p	2	a + + p	3
+ + n +	27	+ m n +	0

Total progeny = 150.

* This triad was not tested for heteroallelism

C. The frequency of segregation of individual markers in 150 products of meiosis

Marker	Frequency	%
nic	37	24·6
pan	32	21·4
ad	21	14·0
me	19	12·7

D. Occurrence of two markers in progeny

	Frequency	
Markers	Observed	Expected
ad and *me*	15	1·8
ad and *nic*	6	3·4
ad and *pan*	7	3·0
nic and *pan*	6	5·3
nic and *me*	6	3·1
me and *pan*	7	2·7

E. Tests for heteroallelism at the *inos*-1 locus in a sample of 38 progeny

	Heteroallelic	Haploid, hemizygous or homoallelic
Prototrophs	10	4
All auxotrophs	11	13
nic⁻ auxotrophs	2	8
nic⁺ auxotrophs	9	5

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contain a high frequency of aneuploids or diploids. Since linked markers do not always segregate together, it is probable that crossing over frequently occurs. From these results and those to be reported in a subsequent section it is tempting to conclude that crossing over is abnormal in *rec*-1 × *rec*–1 crosses and leads to chromosome breakage, irregular chromosome segregation and frequent inviable nullisomic progeny. The homozygosis of markers in diploid or aneuploid progeny could be due to crossing over with mitotic segregation of centromeres, or alternatively from a normal first division of meiosis followed by a failure of the second division. The former leads to homozygosis distal to single exchanges, whilst the latter leads to homozygosis of markers proximal to exchanges (see Wilkie & Lewis, 1963). The

Table 10. *Effect of* rec-*1 on allelic recombination at meiosis*

Cross	Meiotic products screened	*nar*+ recombinants	Frequency
M98 *rec* 1–1 *nar* 1–1 × *nar* 1–6	$6{\cdot}0 \times 10^5$	663	$1{\cdot}11 \times 10^{-3}$
M81 *rec* 1–1 *nar* 1–1 + *rec* 1–1 *nar* 1–6	$8{\cdot}66 \times 10^5$	1749	$2{\cdot}07 \times 10^{-3}$
		inos+ recombinants	
M76 *rec* 1–1 *inos* 1–4 × *inos* 1–5	$7{\cdot}75 \times 10^4$	148	$1{\cdot}91 \times 10^{-3}$
M100 *rec* 1–1 *inos* 1–4 × *rec* 1–1 *inos* 1–5	$5{\cdot}2 \times 10^3$*	10	$1{\cdot}9 \times 10^{-3}$

* 3480 teliospore colonies estimated to have 1·5 progeny per colony.

data suggest that the markers which show frequent mitotic segregation, such as *nic*-1 (see Holliday, 1961*b*, 1965*b*), do appear most frequently amongst the progeny. On the other hand, the proximal markers *me* or *inos* sometimes become homozygous without concomitant expression of distal recessive alleles, *ad* or *nic*. It is, in fact, difficult to distinguish between the two possibilities if the frequency of exchanges is quite high. The four markers used probably all have 67 % second division segregation at meiosis in normal crosses (unpublished data). On either hypothesis this would lead to 16·7 % homozygosis in diploid progeny, a value fairly close to those observed (Table 9).

That *rec* 1 need not actually alter the frequency of recombination at meiosis is shown by allelic recombination at the *nar* and *inos* loci. The results with M81 and M100, together with control crosses heterozygous for *rec*, are given in Table 10. The frequency of *nar*+ and *inos*+ progeny amongst the random products of meiosis is similar in both pairs of crosses. The difference between M81 and M98 cannot be regarded as significant, particularly when it is realized that a high proportion of the progeny from M81 contain two copies of the *inos* locus, either one of which could be *inos*+, whereas the progeny from the control cross are almost entirely haploid.

(iii) *Allelic recombination*

Heteroallelic diploid strains of *U. maydis* have essentially the same behaviour as those of yeast (see Roman, 1956; Roman & Jacob, 1958; Hurst & Fogel, 1964). Spontaneous allelic recombination is mainly due to the non-reciprocal process of gene conversion (only weakly associated with outside marker exchange), and its frequency is greatly stimulated by irradiation (Holliday, 1966). It is believed, but has not been rigorously proved, that this induced recombination is due to a repair process. Evidence that this is so in *U. maydis* came from studies of *rec*-1 and *rec*-2 diploids, since these radiation sensitive mutants did not show a strong increase in allelic recombination after UV treatment (Holliday, 1967). Certain radiation sensitive mutants of yeast are also altered in radiation induced recombination (Hunnable & Cox, 1971). These observations made it seem likely that the mutants were defective in the presumed recombination repair process.

(*a*) *Spontaneous mitotic events*

The earlier experiments were carried out only with strains heteroallelic at the *nar*-1 locus. To discover the generality of the effect of *rec*-1 on recombination, diploids heteroallelic for *inos*-1 and *nic*-1 have also been tested (M48s, M58s, M60s). A particularly important strain in the experiments to be presented in this section is the diploid M133s, which is heteroallelic for *nar*-1, *inos*-1, *nic*-1 and *rec*-1. From this a radiation resistant derivative has been selected, following recombination at the *rec*-1 locus, which provides an isogenic control strain.

The frequencies of spontaneous recombination at the three auxotrophic loci in *rec*-1 and control diploids are given in Table 11. It is clear that all three loci are affected in much the same way by *rec*-1. Using smaller numbers of populations, very similar results have been obtained with M133s, and its *rec*-1/+ radiation resistant derivative has the same allelic recombination as diploids 50r, M48 and M58. This conclusively proves that the observed change in spontaneous allelic recombination is indeed due to the *rec*-1 mutation. It should be mentioned that in these and many other experiments there is no evidence that *rec*-1 in the heterozygous condition has any effect on recombination.

Also in Table 11 are given the results with MT8, a diploid which is heteroallelic for *nar*-1 and homozygous for *rec*-1 and *rec*-2. (It has been proved by appropriate tests that the parents of this strain are indeed *nar* 1–1 and *nar* 1–6.) Whereas *rec*-2 diploids appear to have a normal frequency of spontaneous recombination (Holliday, 1967), very few *nar*$^+$ derivatives could be obtained from MT8. Their frequency is two orders of magnitude lower than radiation resistant diploids and close to the expected reversion frequency for *nar* 1–1. Thus an interaction between the *rec*-1 and *rec*-2 mutations produces a *rec*$^-$ phenotype. This shows that it is incorrect to believe that *rec*$^-$ strains cannot be obtained in fungi and that there is no clear evidence for a direct relationship between recombination and repair (see, for instance, Tuveson, 1969).

Table 11. *Effect of* rec-*1 on spontaneous allelic recombination at the* nar-*1,* inos-*1 and* nic-*1 loci*

Diploid	*rec* genotype	Heteroallelic locus	No. of populations	Population size	Median recombination frequency	Increase in *rec*-1 diploid
50r	*rec* 1–1/+	*nar* 1–1/1–6	13	$2{\cdot}3 \times 10^{7}$	$1{\cdot}7 \times 10^{-6}$	$\times 5{\cdot}6$
50s	*rec* 1–1/*rec* 1–1	*nar* 1–1/1–6	10	$1{\cdot}3 \times 10^{7}$	$9{\cdot}5 \times 10^{-6}$	
M48	*rec* 1–1/+	*nic* 1–1/1–2	10	$5{\cdot}7 \times 10^{6}$	$8{\cdot}1 \times 10^{-6}$	$\times 3{\cdot}2$
M48s	*rec* 1–1/*rec* 1–1	*nic* 1–1/1–2	11	$6{\cdot}6 \times 10^{6}$	$2{\cdot}6 \times 10^{-5}$	
M58	*rec* 1–1/+	*inos* 1–4/1–5	20	$7{\cdot}3 \times 10^{7}$	$2{\cdot}9 \times 10^{-7}$	$\times 16{\cdot}9$
M58s	*rec* 1–1/*rec* 1–1	*inos* 1–4/1–5	20	$2{\cdot}3 \times 10^{7}$	$4{\cdot}9 \times 10^{-6}$	
M133s	*rec* 1–1/*rec* 1–2	All 3 above loci	Frequencies, based on smaller numbers of populations, are not significantly different from the above			
M133	*rec* 1–1/+					
MT8	$\frac{\textit{rec}\ 1\text{–}1\ \textit{rec}\ 2\text{–}1}{\textit{rec}\ 1\text{–}1\ \textit{rec}\ 2\text{–}1}$	*nar* 1–1/1–6	12	$4{\cdot}7 \times 10^{7}$	$\sim 4{\cdot}8 \times 10^{8}$	

(*b*) *Recombination after irradiation*

The diploids listed in Table 11 have all been used to show that radiation induced allelic recombination is blocked by *rec*-1 at the three heteroallelic loci. The same results have been obtained with M133 using both UV and γ irradiation. These are

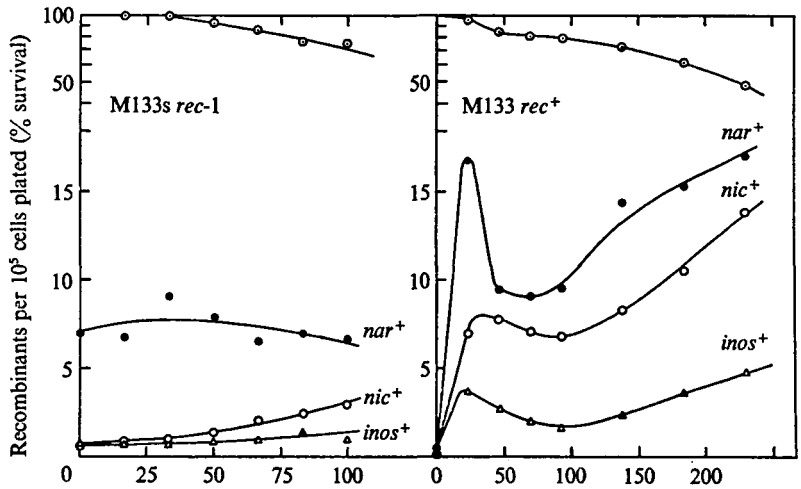

Fig. 8. The effect of UV light on survival and on allelic recombination at three loci in *rec*-1 (M133s) and control (M133) diploids. (For an explanation of the kinetics of induction of recombination in M133, see Holliday (1966). The high background level of *nar*$^+$ recombinants in M133s is due to their continual production on the selective medium (see text). M133s cells were irradiated in suspension at 10^7 per ml. This reduces the actual dose to individual cells 2–3-fold.)

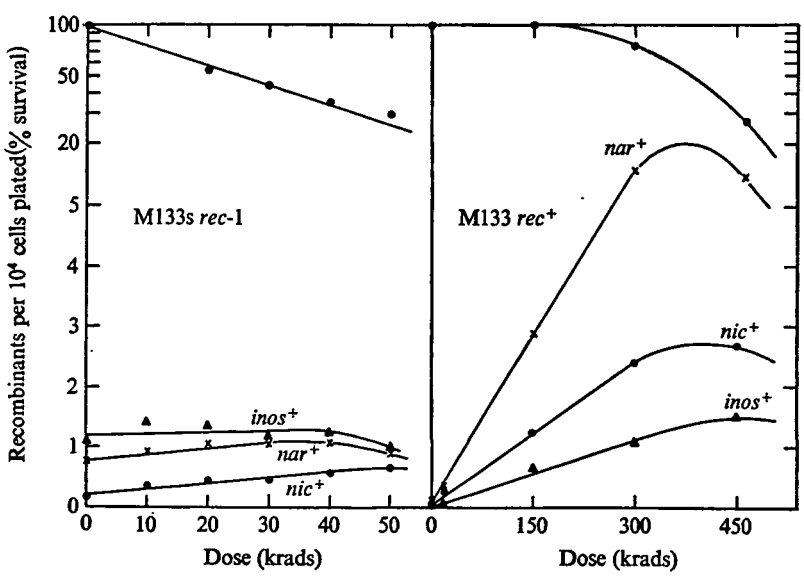

Fig. 9. The effect of γ-rays on survival and allelic recombination at three loci in *rec*-1 (M133s) and control (M133) diploids.

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shown in Figs. 8 and 9, together with those obtained with the isogenic *rec*$^+$ control. In M133 there is no absolute increase in frequency after treatment, but there is a slight rise in recombination at the *nic*-1 and *inos*-1 loci amongst the surviving fraction. That this increase is not seen at the *nar*-1 locus (see also Holliday, 1967) may be due to a difficulty in scoring these recombinants. The reason for this is that new *nar*$^+$ colonies continually arise on the selective medium over a period of many days (see below).

Since *rec* 1 strains show considerable liquid holding recovery after UV, the question arises whether recombination can occur under these conditions. Preliminary experiments indicate that significant numbers of allelic recombinants do appear when the resistance to irradiation is enhanced by liquid holding (Table 12). As might be expected, photoreactivation, which simply removes a proportion of the pyrimidine dimers, increases survival without any significant change in recombination frequency.

(c) *Relationship to crossing over*

In *rec*$^+$ diploids with a heterozygous marker distal to the heteroallelic locus, selection of allelic recombinants does not usually produce homozygosis for the distal locus. In the case of M58 and M60, the frequency of *nic*$^-$ segregants amongst

Table 12. *Effect of liquid holding recovery on allelic recombination in M133s treated with UV light*

	No liquid holding			With liquid holding		
		nar$^+$ recombinants			*nar*$^+$ recombinants	
UV dose* (Jm^{-2})	Survival (%)	No.	Frequency per survivors ($\times 10^4$)	Survival (%)	No.	Frequency per survivors ($\times 10^4$)
0	100	276	1·35	100	107	0·57
100	49	230	2·30	98	177	0·96
150	26	81	1·53	78	279	1·91
200	7·5	21	1·33	72	360	2·67

* Incident dose. Cells were irradiated at 10^7/ml and either immediately plated on selective media and for cell survival or incubated in water for 20 hr at 32 °C prior to plating. Liquid held cells become nitrogen starved and show slower residual growth on the selective medium, hence the lower observed frequency of *nar*$^+$ recombinants from unirradiated cells compared to cells which were not liquid held (see text and Fig. 13).

inos$^+$ recombinants was 12 and 14% respectively (Table 13). This means that about one quarter of recombination events at the *inos* locus are associated with crossing over, since only 50% of such cross-overs are detected. (In a previous experiment with *inos* 1–2 and *inos* 1–3 a much lower frequency of associated crossing overs was observed, but these alleles recombine with very low frequency (Holliday, 1966). The degree of correlation of allelic recombination and outside marker exchange may very well depend on the distance apart of the alleles.) From the results in Table 13, it is not possible to order the *inos* 1–4 and *inos* 1–5 sites

Table 13. *Effect of* rec-*1 on the association of allelic recombination with segregation of a distal marker*

		Populations		Median frequency of *inos*$^+$ recombinants	% *inos*$^+$ *nic*$^-$ segregants		
		No.	Size ($\times 10^{-7}$)		Median*	Mean	Range
M58s	$\frac{rec\ 1-1}{rec\ 1-1} \bigcirc \frac{inos\ 1-5\ nic\ 1-1}{inos\ 1-4\ +}$	20	2·3	$4{\cdot}9 \times 10^{-6}$	65·6 (40)	66·5	47·3–79·5
M60s	$\frac{rec\ 1-1}{rec\ 1-1} \bigcirc \frac{inos\ 1-5\ +}{inos\ 1-4\ nic\ 1-1}$	14	2·2	$1{\cdot}1 \times 10^{-6}$	12·2 (10)	15·0	5·8–24·0
M58	$\frac{rec\ 1-1}{+} \bigcirc \frac{inos\ 1-5\ nic\ 1-1}{inos\ 1-4\ +}$	20	7·3	$2{\cdot}9 \times 10^{-7}$	8·0 (1·5)	12·7	—
M60	$\frac{rec\ 1-1}{+} \bigcirc \frac{inos\ 1-5\ +}{inos\ 1-4\ nic\ 1-1}$	9·2	9·2	$2{\cdot}0 \times 10^{-7}$	12·4 (2)	14·7	—

* Figures in parentheses refer to the median number of *nic*$^-$ segregants observed amongst the *inos*$^+$ recombinants scored.

relative to the centromere and the distal marker, since the 'single exchange' event is not significantly different from the frequency of the 'triple exchange'. This result is reminiscent of much data on allelic recombination and outside marker exchange at meiosis in various fungi.

The situation is entirely different in *rec* diploids, M58s and M60s, which are derived by MCO from M58 and M60. Here selection of *inos*$^+$ recombinants from M58s demonstrates a very high frequency of homozygosis for *nic*-1, whereas M60s has a low frequency (Table 13). This shows that allelic recombination is now strongly associated with crossing over, and it also makes it possible to place the markers in the order: centromere – *inos* 1–5 – *inos* 1–4 – *nic*-1. This experiment provided the first indication that the actual process of allelic recombination in *rec*-1 strains is different from that in wild-type diploids.

If allelic recombination in *rec*-1 diploids is entirely due to a single cross over, and normal mitotic segregation of centromeres occurs, then the maximum homozygosis for the distal locus is 50%. (If both recombinant chromatids segregate to one cell, as will occur in half the cases, the distal marker remains heterozygous.) In fact, the apparent homozygosis for *nic*-1 is 65%. Of the 14 populations examined, 10 produced *inos*$^+$ *nic*$^-$ recombinants, with a frequency significantly higher than 50%. The same result is seen in experiments with M133 (Table 14). Here *inos*$^+$ recombinants are tested for heteroallelism at the distal locus, by a spot test. Amongst 197 spontaneous recombinants, only 48 could still recombine at *nic* and 149 (75·6%) were apparently homoallelic, i.e. homozygous. In the control *rec*$^+$ diploid, the proportion is similar to that seen in M58 and M60. Of 81 *inos*$^+$ recombinants, 70 were still heteroallelic and 11 (14%) were homozygous (Table 14).

(*d*) *Association with chromosome breakage*

The most likely explanation for the anomalous result with M58s and M133 is that a significant proportion of the *inos*$^+$ recombinants are actually hemizygous with a distal marker. The loss of a distal part of the chromosome in question would produce the same *nic* phenotype as a cross over. This is shown in Fig. 10.

Normally to test *inos*$^+$ recombinants for hemizygosity at the *nic* locus one would sporulate each recombinant to see whether a lethal deletion was segregating at meiosis. However, *rec*-1 strains already have very low viability at meiosis, therefore this test is not possible. To circumvent the difficulty, one could select a *rec*$^+$ derivative from each *inos*$^+$ recombinant and then carry out the meiotic test; but this test is also unsatisfactory, as it is quite likely that *rec*-1 diploids accumulate various recessive lethals during their growth, and these would confuse the nterpretation of the results. It was therefore decided to carry out an extensive test of the prediction using mitotic segregation. If an *inos*$^+$ recombinant is hemizygous for the distal marker, then a cross over which becomes homozygous for the recessive *inos* allele will also be homozygous for the deletion and will therefore be non-viable. On the other hand an *inos*$^+$ recombinant which is homozygous for *nic*-1 will yield *inos*$^-$ segregants.

In two series of experiments, 47 spontaneous *inos*$^+$ recombinants of independent

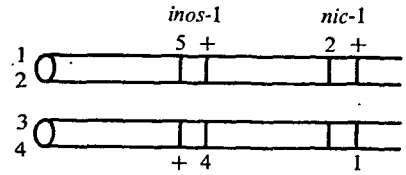
Table 14. *Analysis of* inos$^+$ *allelic recombinants of M133s by their further mitotic recombination or segregation*

M133s genotype: $\frac{rec\ 1\text{–}1}{rec\ 1\text{–}2} \bigcirc \frac{inos\ 1\text{–}5\ nic\ 1\text{–}2\ nar\ 1\text{–}1}{inos\ 1\text{–}4\ nic\ 1\text{–}1\ nar\ 1\text{–}6} \frac{ad\ 1\text{–}1}{+} \frac{+\quad a_2\ b_2}{pan\ 1\text{–}1\ a_1\ b_1}$

Diploid	Expt. no.	No. *inos*$^+$ recombinants tested	Loss of heteroallelism at *nic*-1 No.	*nic*-1 %	*nar*-1 No.	*nar*-1 %	Mitotic segregation of *inos*$^+$ recombinants No. tested	No. segregating *inos*$^-$	No. segregating *pan*$^-$ and/or *ad*$^-$
M133s (*rec*-1)	1	104	76	73·1	2	2·0	22	14	22
	2	93	73	78·5	n.t.		25	13	24
	Total	197	149	75·6			47	27	46
M133 (*rec*$^+$ derivative)	3	81	11	13·6	n.t.				

n.t. = not tested.

origin were examined. In each case several hundred colonies grown on CM were replica plated to minimal medium supplemented with nicotinic acid and ammonium and the phenotypes of the auxotrophic segregants were scored. If *inos*⁻ segregants were not detected in the first experiment, further colonies were



inos⁺ recombinant genotype	Origin of recombinant and frequency (%) C.O. C.O.	+break	Conversion	Conversion +break	Phenotype Segregation for *inos*⁻	Recombination at *nic*-1
3 ++ 2 / 1 5 + 2	50	50	0	0	✓	–
3 ++ 2 / 2 5 4 1	50	0	0	0	✓	✓
2 ++ 2 / 3 or 4 5 + 1	0	0	100	50*	✓	✓
3 ++ 1 / 1 or 2 + 4 2	0	50	0	0	–	–
3 ++ 2 / 2 5	0	0	0	50	–	–
2 ++ 2 / 3 +						
3 ++ 1 / 2 5						

*Chromatids 2+4
3+1

Fig. 10. The expected consequences of crossing over or conversion at the *inos*-1 loci with or without associated breakage of the chromatid participating in the recombination event. (It is assumed events occur at the 4-strand stage and that the segregation of centromeres is random. The scoring of the phenotypes depends on the detection of *inos*⁻ segregants by replica plating, and a qualitative spot test for heteroallelism at the *nic*-1 locus.)

examined until a substantial number (13–30) of *ad*⁻ and *pan*⁻ segregants had been detected. Only then was it concluded that segregation of *inos*⁻ was not possible from that particular *inos*⁺ recombinant. In the first series of experiments (carried out by V.R.), 22 *inos*⁺ recombinants were tested and 14 segregated *inos*⁻. In a

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later series (carried out by M.W.E.), 25 *inos*$^+$ recombinants were tested and 13 segregated *inos*$^-$. These two results are not significantly different from each other. A summary of the overall data is shown in Fig. 11. Although there is considerable scatter of points, it is clear that the *inos*$^+$ recombinants fall into two classes, and we conclude that these are homozygous and hemizygous for the *nic*-1 locus.

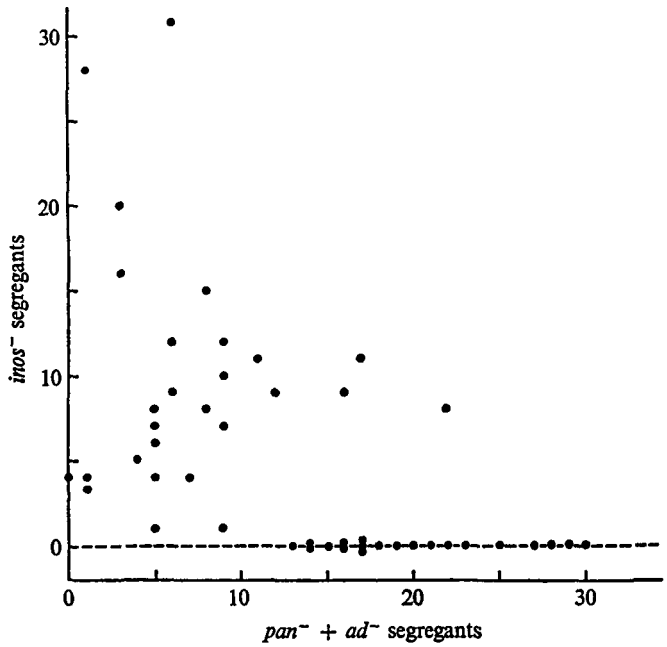

Fig. 11. The numbers of *inos*$^-$, *pan*$^-$ or *ad*$^-$ segregants from 47 spontaneous *inos*$^+$ recombinants from M133s. Twenty-seven isolates segregated *inos*$^-$ and 20 did not.

On the basis of certain assumptions (which are also used in the compilation of Fig. 10), we can now calculate the proportion of allelic recombinants at the *inos*-1 locus which are due to reciprocal (CO) or non-reciprocal (conversion) events and the frequency of associated chromosome breakage (terminal deletion). Since exchange at the 2-strand stage (G1) cannot lead to homozygosis of a distal marker, we assume that recombination occurs at the 4-strand stage, and also that there is random mitotic disjunction of homologous chromatids. In addition, we assume that the selected chromatid, i.e. that with an *inos*$^+$ recombinant allele, is always intact and that any break occurs in the homologous chromatid which was involved in the recombination event.

We observed that 75·6 % of *inos*$^+$ recombinants were no longer heteroallelic for *nic*-1, and amongst 47 tested 20 (42·6 %) were apparently hemizygous and 27 (57·4 %) were homozygous at the distal locus (Table 14). If p is the proportion of cross overs, then $1-p$ is the proportion of conversions; if q is the proportion of events without associated chromatid breakage, then $1-q$ is the proportion with

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breakage. The frequency and origin of the three *inos*$^+$ phenotypes in Fig. 10 in terms of p and q is as follows:

$$\text{heteroallelic } nic^-: \quad 0{\cdot}244 = \frac{pq}{2} + q(1-p) + \tfrac{1}{2}(1-p)\,(1-q) = \tfrac{1}{2}(q-p+1),$$

$$\text{homoallelic } nic^-: \quad 0{\cdot}574 \times 0{\cdot}756 = 0{\cdot}434 = \frac{pq}{2} + \frac{p}{2}(1-q) = \frac{p}{2},$$

$$\text{hemizygous } nic^-: \quad 0{\cdot}426 \times 0{\cdot}756 = 10{\cdot}322 = \frac{p}{2}(1-q) + \tfrac{1}{2}(1-p)\,(1-q) = \tfrac{1}{2}(1-q).$$

From which $p = 0{\cdot}87$ and $q = 0{\cdot}36$, or 87 % crossing over, 13 % conversion and 64 % chromatid breakage.

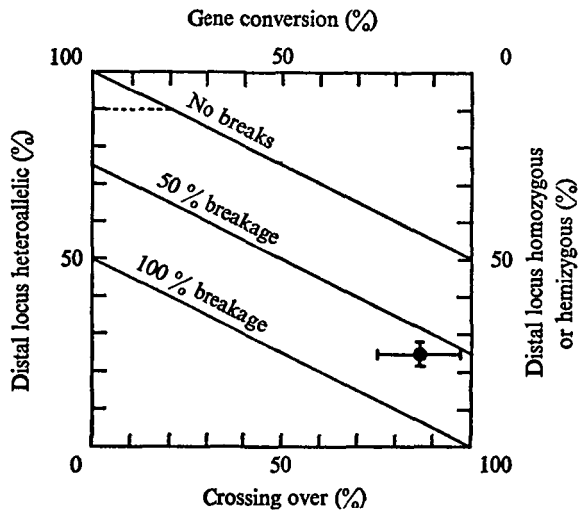

Fig. 12. The quantitative relationship between gene conversion or crossing over at the *inos*-1 locus and the expected frequency of heteroallelism, homozygosis or hemizygosis at the distal *nic*-1 locus. The diagram illustrates the effect of 50 % or 100 % breakage of the chromatid associated with the production of an *inos*$^+$ recombinant (see also Fig. 10). The solid circle indicates the experimental observed value for M133s, and the standard error bars are included. The dotted line indicates the range of possible values for conversion, CO and breakage in control *rec*$^+$ diploids.

The relationship between crossing over, conversion, chromatid breakage and the effect on a distal marker is shown graphically in Fig. 12. The experimentally determined point for allelic recombination at *inos*-1 in M133S is shown with standard error bars. (The 95 % confidence limits are approximately twice the S.E.S.) The estimates for p and $1-q$ would be slightly reduced if a few per cent of the *nic*$^-$ segregants were derived from cross-over events independent of recombination at the *inos*-1 locus (see Table 5). The dashed line in Fig. 12 (top right) indicates possible values for crossing over, conversion and breakage for allelic recombination in control *rec*$^+$ diploids.

From the overall result we can conclude that allelic recombination in *rec*-1 diploids is largely due to crossing over; yet this crossing over is frequently, if not

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always, associated with breakage of a homologous chromatid, presumably the one involved in the initial pairing of homologues. Thus we have clear evidence that the *rec*-1 mutation alters the normal process of genetic recombination.

The possibility that a small proportion of conversion events occurs is suggested by the observation with M60s that about 12 % of *inos*$^+$ recombinants are homozygous or hemizygous for *nic*-1 (Table 13). About half these could be independent segregation events (Table 5), but the rest probably arise by conversion plus associated breakage or crossing over. It is very unlikely that *inos*$^+$ *nic*$^-$ recombinants in a *rec*-1 background could be due to double crossing over (see Holliday (1967) and Table 5).

(iv) *Instability at the* nar-*1 locus*

nar$^-$ strains which lack nitrate reductase are unable to grow on NM containing nitrate as sole source of nitrogen. However, commercial agar contains traces of reduced nitrogen, which allow a considerable number of cell divisions on NM, the

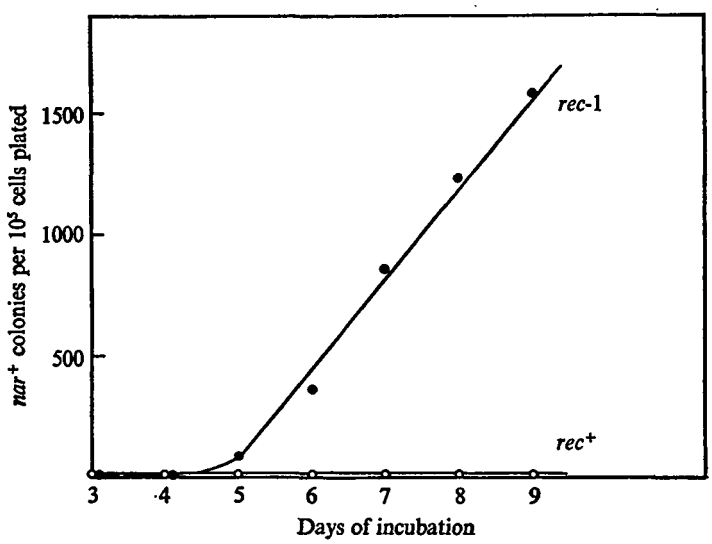

Fig. 13. The accumulation of *nar*$^+$ recombinants with time in a *rec*-1 diploid (50s) heteroallelic for *nar* 1–1 and *nar* 1–6. 50s and 50r cells were spread on NM and the *nar*$^+$ colonies counted on successive days.

degree of leakiness depending on the number of *nar*$^-$ cells plated. Radiation resistant diploids heteroallelic for *nar*-1 which have been treated with UV and plated on NM produce a crop of *nar*$^+$ recombinants, and the colonies are usually counted after 5–6 days. In spite of the background growth, further incubation yields very few more colonies.

Another phenotypic effect of the *rec*-1 mutants is revealed when *nar*-1 heteroallelic diploids are plated on NM. Colonies appear at the same time as would *rec*$^+$ ones, but subsequent incubation continues to yield many further *nar*$^+$ recombinants. They accumulate linearly with time, as is shown in Fig. 13. Thus,

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under conditions of nitrogen starvation, the heteroallelic *nar*-1 locus is unstable and continually throws off recombinants, even though after the first 2–3 days of incubation there could be little residual growth of *nar*$^-$ cells. This effect of *rec*-1 has not been seen in the *nic*-1 or *inos*-1 heteroallelic strains, probably because of the decreasing viability of these auxotrophic cells when deprived of their supplements. The effect of *rec*-1 on recombination at the *nar*-1 locus makes it difficult to measure accurately the actual frequency. The values given in Table 11, Figs. 8 and 9 and in Holliday (1967) are based on counts made after a defined period of incubation, usually 6 days.

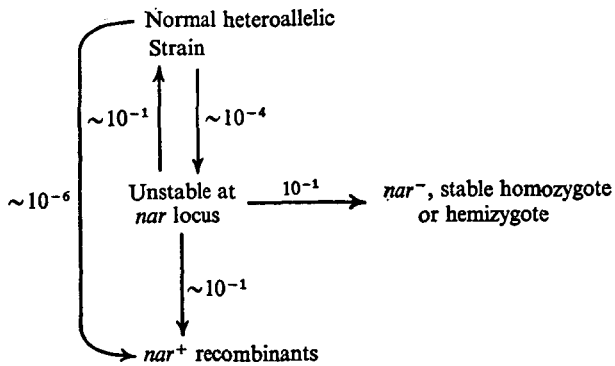

Fig. 14. The changes in the *nar* phenotype of unstable strains, with approximate frequencies.

Colonies replicated from CM to NM usually yield replicas with one or more *nar*$^+$ papillae. Among many thousand such replicas from diploid 50s, a few were seen with numerous *nar*$^+$ papillae. It was hoped that these would be hyper-*rec* strains, with an abnormally high frequency of allelic recombination, but examination of two such isolates showed that they were very unstable strains which segregated several phenotypes with high frequency.

Very small colonies (10–20 cells) were grown on CM and then dispersed to form subclones, the phenctype of which was scored. It was found that the subclones were of four types: (*a*) pure *nar*$^+$ recombinants; (*b*) those with a normal frequency of recombination at the *nar* locus; (*c*) those with a very high frequency of *nar*$^+$ recombinants, and (*d*) those which no longer recombined at the *nar* locus (homoallelic or hemizygous). Types (*a*), (*b*) and (*d*) were quite stable, but (*c*), like the original isolate, continued to produce the four types of subclones. In this subcloning there was no preponderance of the unstable phenotype (*c*); segregation of types (*a*), (*b*) and (*d*) occurred at a frequency of about 10%. The segregation pattern is illustrated in Fig. 14.

In fact, the strains were so unstable that it was very hard to undertake proper quantitative studies or even preserve them in the original state. The results suggest that the unstable strains might arise by unequal crossing over between homologues, which could yield tandem duplications of the *nar* 1 region. If intrachromosomal recombination is very frequent, heteroalleles on one chromatid could

yield stable heteroallelic or homoallelic diploids by loss of one or other of the duplicated regions. Alternatively they could generate *nar*$^+$ recombinants with high frequency.

In extensive searches for similar strains in *rec*$^+$ diploids, none has been detected (J. E. Pugh, unpublished data).

6. BIOCHEMICAL STUDIES

Methods for measuring the excision of pyrimidine dimers from the DNA of fungi have been developed by Unrau, Wheatcroft & Cox (1972). In *U. maydis* it has been demonstrated that dimers begin to disappear from the DNA immediately after the irradiation of wild-type strains (Unrau, 1975). The mutant *uvs*-3, which is specifically sensitive to UV light, does not remove dimers from its DNA until $1\frac{1}{2}$–2 h after irradiation, and this may be mediated by a recombination repair process. The *rec*-1 and *rec*-2 mutants both appear to have normal excision repair, although there are some differences from wild-type in the kinetics of dimer excision. Unrau (1975) favours the hypothesis that dimers are removed sequentially by a normal excision process, followed by a recombination repair pathway. The latter may be an inducible process requiring protein synthesis (Holliday, 1971, 1975; Moore, 1975*b*). The data are consistent with *rec*-1 and *rec*-2 being defective in this pathway.

Recently considerable progress has been made in the isolation and characterization of enzymes or proteins which act on or interact with DNA in *U. maydis*. A DNA polymerase which has been fully purified and characterized (Banks *et al.* 1975; Banks & Yarranton, 1975) is present in normal amount in *rec*-1 strains (Jeggo & Banks, 1975). The same is true of a DNA binding protein (Banks & Spanos, 1975). This protein specifically interacts with *U. maydis* DNA polymerase and has properties very similar to the 'gene 32 protein' which is required for replication and recombination in bacteriophage T4 (Alberts & Frey, 1970). It is therefore very likely that it plays a role in the same processes in *U. maydis*. *rec*-1 strains also contain a normal amount of DNase 1, an enzyme which is implicated in genetic recombination and has recently been shown to recognize mismatched bases in DNA (Holloman & Holliday, 1973; Holloman, 1973; Holliday *et al.* 1974; Ahmad, Holloman & Holliday, 1975). On the other hand, *rec*-1 may contain an increased amount of a DNA binding glycoprotein which has unusual properties (Holloman, 1975). The mutant has about twice the wild-type level (Holliday *et al.* 1974), although this difference may not be significant. Nevertheless, another radiation sensitive mutant, designated γs7, has a 3- to 4-fold increase in this protein and its phenotype is in some ways similar to *rec*-1 (Holloman, 1975; Holliday *et al.* 1974; J. E. Pugh, personal communication). This protein has the unique property of forming an acid-soluble complex with DNA, and it appears to do so by preventing the normal condensation of DNA which occurs at low pH. Since there is evidence that acid treatment can induce condensation of interphase chromatin to give prophase-like structures, it is possible that the protein discovered by Holloman plays a role in the control of chromosome condensation

during the cell cycle. Mutants with an increased level should have more diffuse nuclei, and preliminary observations with γs7 confirm this. In discussions of repair processes in eukaryotes, it is widely assumed that all DNA is accessible to repair enzymes, but this may well not be the case for the chromatin histone–DNA complex itself, let alone for aggregated or condensed chromatin.

7. DISCUSSION

Apart from the mutants of *U. maydis*, radiation-sensitive strains of several fungi have been screened for proficiency in recombination, and some have been found to be altered or defective (Jansen, 1970; Schroeder, 1970; Fortuin, 1971; Hunnable & Cox, 1971; Kowalski & Laskowski, 1975; Resnick, 1975). In addition, direct methods have been used to identify mutants with either reduced or increased recombination frequencies (Rodarte-Ramon, 1972; Rodarte-Ramon & Mortimer, 1972; Simonet, 1973; Fogel & Roth, 1974; Parag & Parag, 1975). Another approach involves the use of variation among wild-type strains in the frequency of recombination. Catcheside and his associates have identified several *rec* mutants in *Neurospora* which have the effect of controlling recombination frequency in different regions of the genome (for a review, see Catcheside, 1974). These mutants appear to have no other phenotypic properties. The characteristics of the *Neurospora rec* mutants and a recognition (*cog*) mutant indicate that the genetic regulation of recombination, particularly its initiation, is extremely complex. The significance of the *Neurospora rec* mutants has been revealed by extremely detailed genetic analysis, but unfortunately comparable studies of artificially induced recombination mutants have not so far been carried out. Usually, the classification of a mutant as recombination defective has depended on one or two standard quantitative tests, such as the frequency of allelic recombination or crossing over in mitosis or meiosis.

In the present study, one induced mutant of *U. maydis* and its alleles have been examined in considerable detail, and this has revealed a number of recombination or other abnormalities which would not have been detected on the basis of simple quantitative tests. Indeed, it is clear that the *rec*-1 mutation completely alters the genetic system of *U. maydis*. There is little doubt that effects seen are due to a single *rec*-1 mutation, as many of the comparisons were between wild-type and *rec*-1 diploids which are isogenic. The effects of *rec*-1 on the phenotype of haploid and diploid strains can be summarized as follows:

(1) *rec*-1 strains are sensitive to UV, ionizing radiation and nitrosoguanidine. The survival after treatment is initially exponential, but at high doses survival curves tend to plateau.

(2) The damage by UV is reversed by photoreactivation, and also by liquid holding.

(3) During normal growth, a proportion of non-viable cells is formed. Haploid colonies are fairly uniform, but diploids are extremely variable in size and general morphology.

(4) The spontaneous frequency of mitotic segregation is enhanced, and this is

due mainly to crossing over. This frequency is significantly increased after UV treatment.

(5) The spontaneous frequency of allelic recombination is also enhanced. Unlike wild-type strains, there is very little stimulation of this type of recombination after irradiation.

(6) Allelic recombination appears to be due to crossing over rather than conversion, as it is strongly associated with outside marker exchange. However, it is very likely that only one of the chromatids involved in the exchange survives intact.

(7) Preliminary studies on mutation indicate that the spontaneous frequency is enhanced, but UV light is not mutagenic.

(8) Meiosis is deranged in crosses homozygous for *rec*-1. Most of the products of meiosis are non-viable, and amongst the survivors are a large proportion of diploids or aneuploids.

(9) Those features of the phenotype which have been examined at 35 and 22 or 25 °C (e.g. 1, 3, 4) show that the effect of *rec*-1 is more extreme at the high temperature.

(10) The *rec*-1 mutation interacts with other radiation sensitive mutants, such as *rec*-2, producing a more extreme phenotype with regard to repair or recombination deficiency, or viability during growth.

(11) *rec*-1 probably contains an increased level of a DNA binding glycoprotein which has a strong effect on the physical properties of DNA.

The experiments with M133s and its rec^+ derivative, together with those employing many other diploids, all indicate that the *rec* 1–1 and *rec* 1–2 alleles are recessive. However, P. Unrau and T. L. Olive (personal communication) have recently obtained evidence that *rec*-1 in heterozygous condition can interact with the *pol*-1 mutation when this is also heterozygous. This interaction is seen at the level of temperature sensitivity, and also with regard to UV-induced allelic recombination. In addition, another radiation-sensitive mutant, γs7 (Holliday *et al.* 1974; J. E. Pugh, personal communication), has several phenotypic features like *rec*-1 and contains an even higher level of the DNA binding glycoprotein. This mutant is dominant in almost completely blocking meiosis. Genetic analysis has therefore not been possible, but studies with diploids indicate that γs7 is either allelic to or interacts with *rec*-1. From these observations it cannot be concluded that *rec*-1 and its alleles are invariably recessive; for some features of the phenotype dominance may occur.

The association of crossing over with chromosome breakage and the abnormalities of meiosis suggests that an important component of the recombination process is missing in *rec*-1 strains. Nevertheless, the mutants are not blocked in recombination; indeed the level is enhanced during normal growth of *rec*-1 diploids. It is clear that recombination in eukaryotes is normally a very precise process, which preserves both interacting chromosomes without genetic duplications or deletions. On the other hand in prokaryotes there could frequently be associated chromosome breakage or loss of one of the two interacting genomes. It is possible, therefore,

that *rec*-1 has a 'primitive' recombination system. According to this hypothesis, whatever is lacking in *rec*-1 strains prevents the normal radiation induced enhancement of allelic recombination which is seen in control heteroallelic diploids. Since it is believed that this enhancement is due to a repair process involving recombination, a defect in this would adequately account for the radiation sensitivity of *rec*-1 strains. With regard to the spontaneous damage during growth, this again could be due to a failure of recombination repair, perhaps a process which involves

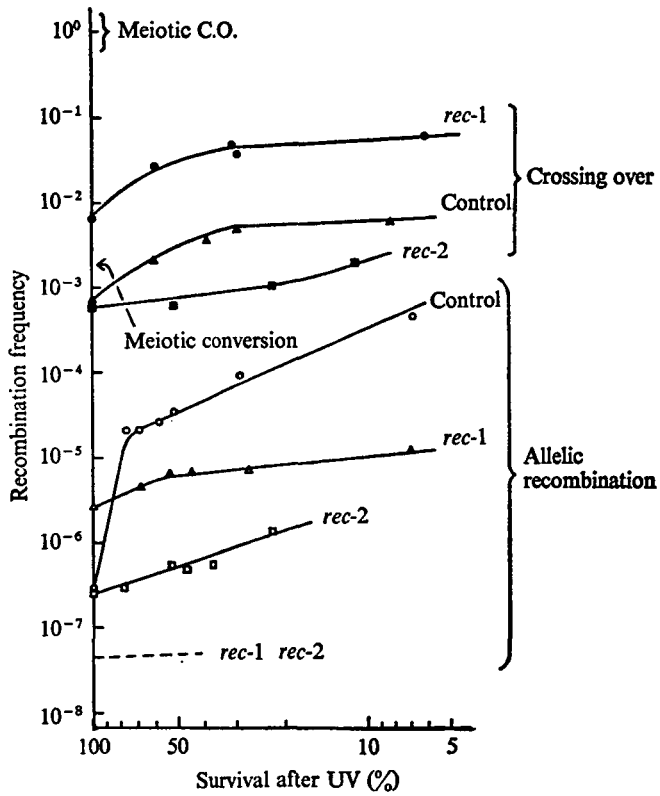

Fig. 15. Summary of spontaneous and UV-induced mitotic recombination frequencies in *rec*-1, *rec*-2 and control diploids. Crossing over refers to the frequency of segregation of *ad*-1, *nic*-1 and *pan*-1 in heterozygous diploids. Allelic recombination refers to the frequency of recombinants from diploids heteroallelic for *inos* 1–4 and *inos* 1–5. In the case of the diploid homozygous for *rec*-1 and *rec*–2 (MT8), the heteroalleles were *nar* 1–1 and *nar* 1–6, and the frequency of *nar*$^+$ colonies is close to that expected for reverse mutation for one or other of these mutants.

sister strand exchange. Finally, if recombination repair is 'error prone', the lack of radiation mutagenesis is accounted for. Nevertheless, whatever mechanism for recombination is favoured, it is not easy to explain the overall phenotype of *rec*-1 in terms of a single missing step, let alone in terms of a lack or insufficiency of a single enzyme which acts on DNA.

For this reason, we favour a completely different interpretation of the *rec*-1 phenotype. This attributes a regulatory function to the *rec*-1 gene product. It has

previously been pointed out that the very different frequencies of recombination at meiosis and mitosis imply that the process is regulated genetically (Holliday, 1968, 1971). Recombination would be induced at meiosis and repressed during the normal mitotic cell cycle. However, since one repair process requires recombination, the damage to DNA of a mitotic cell in some way derepresses one or more of the enzymes required. Evidence for an inducible recombination repair process in *U. maydis* comes from at least three different experimental approaches (Holliday, 1971, 1975; Moore, 1975*b*). It has also been proposed that one repair process in *E. coli* is induced after radiation, although in this case recombination is not involved (Witkin & George, 1973; Radman, 1974, 1975, and personal communication). If radiation is itself the inducer, then one possibility is that the repressor binds more tightly to damaged DNA than it does to the operator region of the structural gene(s) for the recombination enzyme(s). A mutant with an altered repressor which bound weakly to its operator and to damaged DNA would have a high frequency of spontaneous mitotic recombination and have reduced or no induced recombination after irradiation. This is the phenotype of *rec*-1. Its frequency of mitotic recombination is not at the meiotic level, but it is several times higher than that in rec^+ diploids. After radiation there is very little increase in non-reciprocal allelic recombination. Some of these effects of *rec*-1 on recombination are summarized in Fig. 15.

Recently Parag & Parag (1975) have isolated a mutant in *Aspergillus nidulans* which has a very high frequency of mitotic recombination. They also suggest that this is a regulatory mutation which is derepressed for an enzyme required for recombination at meiosis. Irradiation of *E. coli* lysogenic for λ inactivates the repressor and the lytic cycle follows. Sussman & Ben Zeev (1975) have recently obtained evidence that λ repressor binds tightly to DNA which has been damaged by agents which induce the prophage. This suggests a mechanism for induction of λ which is the same as the one we have proposed for the induction of recombination. According to this hypothesis, *rec*–1 would code for a repressor which binds weakly to its operator and not at all to damaged DNA.

Known regulatory mutants of *U. maydis* are partially derepressed for nitrate reductase. These have been asigned four loci, and in one tested instance a double mutant strain was fully derepressed (Lewis & Fincham, 1970). This suggested that the repressor is a complex containing more than one type of subunit. This could also apply to the control of recombination, in which case the various recessive *rec*-1 alleles might all lack one protein subunit of a complex regulatory system. There is already evidence that *rec*-1 interacts with other mutants which affect the genetic system of *U. maydis*, for instance *rec*-2, *pol*-1 and γs7. Further study of these interactions is in progress.

How far can the other features of the *rec*-1 phenotype be explained on this hypothesis? First, the events in pairing and recombination at meiosis must follow a strict temporal sequence and there must be strict genetic control of this process. If the *rec*-1 product plays a role in the control of recombination in meiosis, then crosses homozygous for the mutant could be abnormal, because the normal order

of biochemical events is upset. Secondly, constitutive recombination during normal growth may well upset the cell cycle. In particular, recombination enzymes may undergo 'collisions' with those which are active in transcription and/or replication. This could easily lead to chromosome breaks. For instance, the collision of a replication fork and a recombination intermediate could generate a recombinant and a break, as illustrated in Fig. 16. This would account for lethal sectoring, the

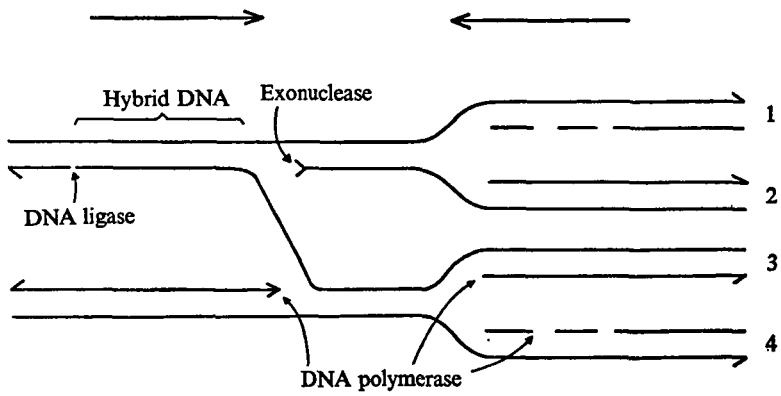

Fig. 16. The failure to coordinate recombination and replication of DNA. One possibility is illustrated, where a recombination event will be in collision with a replication fork. The act of recombination is based on the recent model proposed by Meselson & Radding (1975). The consequence of this collision will be the generation of a recombinant molecule, a broken molecule and two parental molecules. Depending on the pattern and direction of replication, this could lead to a terminal or interstitial deletion, or possibly to loss of a whole chromosome (monosomic). Although the recombination is occurring at the 2 strand stage, since the initial events give rise to a 'half chiasma', the overall effect is the same as crossing over at the 4-strand stage.

morphological variation of diploid strains, with the concomitant production of genetic recombinants and terminal deletions. Thirdly, the sensitivity to radiation would be mainly due to a failure to induce a recombination repair mechanism rather than a defect in this mechanism. If the inducible repair process is error prone, then the absence or low level of mutation after mutagen treatment is accounted for. Fourthly, we need to explain the phenomenon of liquid holding recovery. The constitutive recombination which occurs in *rec*-1 may be capable of repairing damage in DNA, but only at a very slow rate. When cells are held in water, transcription and replication ceases, and there is sufficient time available for partial completion of recombination repair before growth is resumed. Finally, the more extreme phenotype at high temperatures may simply be due to the weaker binding of the repressor to the operator region.

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