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Studies on mitotic gene conversion in *Ustilago*

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1. INTRODUCTION

Previous studies on mitotic recombination in *Ustilago maydis* have been concerned with the analysis of reciprocal mitotic crossing-over between widely spaced markers (Holliday, 1961*b*, 1965*a* and references therein). In this paper the studies are extended to include analysis of recombination events within a marked gene, with the specific aim of developing a system for testing hybrid DNA models of gene conversion and crossing-over (Holliday, 1964; Whitehouse & Hastings, 1965). Since it might be necessary to relate intragenic recombination to the nature of the recombinant gene product, it was considered desirable to use mutants of a gene which coded for a known enzyme. To this end, mutants were obtained which are unable to use nitrate as sole source of nitrogen; as will be explained, one group of these mutants is almost certainly defective in the structural gene for nitrate reductase, an enzyme for which standard assay and purification procedures are available. These mutants are easy to obtain in large numbers and, unlike many auxotrophs, they have no effect on fertility when crossed.

Recombination has been studied in heteroallelic diploids containing non-complementing or weakly complementing pairs of mutants. Such diploids behave in much the same way as do those of yeast, which were first extensively investigated by Roman (1956, 1958) and Roman & Jacob (1958). They generate wild-type recombinants spontaneously, and with high frequency after low doses of ultraviolet (u.v.) light. The recombination is due to gene conversion (i.e. is non-reciprocal) at one or other of the mutant sites, and it is not strongly correlated with outside marker exchange. In addition, it has been shown that conversion can be most easily induced at a particular stage of the division cycle, as is also the case for mitotic crossing-over (Holliday, 1965*a*), and that a fine structure map of mutants can be constructed on the basis of u.v.-induced conversion frequencies.

2. MATERIALS AND METHODS

(i) *Media*

The complete and minimal media have been previously described (Holliday, 1961*a*, as modified in 1961*b*). The source of nitrogen in standard minimal medium is

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0·15% ammonium nitrate; in nitrate minimal medium this is replaced by 0·3% potassium nitrate, and in ammonium minimal medium by 0·3% ammonium sulphate.

(ii) *Isolation of mutants*

The inositol starvation technique was used to select mutants unable to assimilate nitrate, but the original procedure (Holliday, 1962) had to be modified. It was necessary to carry out the experiments in liquid media, since agar contains sufficient reduced nitrogen to preclude the survival of nitrate mutants on nitrate minimal lacking inositol, and it was also necessary to starve the cells of nitrogen before depriving them of inositol. Cells of the inositol mutant, *inos-3*, were grown in liquid ammonium minimal medium containing 10 μg. inositol/ml., log phase cells were washed thoroughly and then suspended in the same medium lacking ammonium sulphate for 24 hours. After washing they were suspended in water and treated with a dose of u.v. light which killed 95% of the cells. The irradiated cells were added to nitrate minimal medium and starved in a shake culture for 3 days. The surviving cells (0·05% of those not killed by u.v.) were spread on plates of minimal medium supplemented with inositol and replicated to nitrate minimal medium supplemented with inositol. About 2% of the survivors are mutants. In addition, nitrate mutants can be isolated by the non-selective replica plating technique. In the original experiments (Holliday, 1961*a*) the minimal medium used did not allow the detection of these mutants, but subsequent experiments have shown that about one-quarter of all auxotrophs are nitrate non-utilizers.

(iii) *Crosses*

Haploids of opposite mating type (i.e. $a_1b_1 \times a_2b_2$ or $a_1b_2 \times a_2b_1$) are inoculated into young maize seedlings by either of the methods previously described (Holliday, 1961*a*, *b*), or into larger potted plants in growth room or greenhouse. Mature brandspores are treated with 1·5% copper sulphate overnight, washed, and plated on complete medium. Sometimes 10 μg. per ml. achromycin (tetracycline hydrochloride) was added to the medium to insure against bacterial contamination. After 30–40 hours' incubation the sporidia from the brandspore colonies were washed off the plate(s) and spread on fresh plates of complete medium at a density of about 100 cells per plate. The genotypes of the colonies were identified by replica plating to test media. This method of meiotic analysis, after early difficulties (Holliday, 1961*a*), has been routinely used for linkage studies and for obtaining recombinant genotypes. Provided certain auxotrophic markers are avoided, a 1 : 1 segregation is normally observed for each marker in a cross. It should be mentioned that *pan-1* is routinely used in crosses since it is tightly linked to the *a* mating-type locus. Thus in determining mating types of the progeny of a cross only tests involving the *b* mating-type have to be made. *pdx-2* is also closely linked to *a* and this marker has sometimes been used in place of *pan-1*.

(iv) *Synthesis of heteroallelic diploids*

Crosses between allelic nitrate mutants have always been fully fertile. To synthesize a heteroallelic diploid, one parent is *nar inos-3* and the other *nar pan-1* of opposite mating-type, where the two *nar* mutants are different; for homoallelic diploids the *nar* mutants are the same. Pieces of infected gall tissue are placed on plates of ammonium minimal medium; the diploid cells grow out in about 5 days. In addition to diploids with the *nar* phenotype it has also been possible to synthesize diploids heteroallelic for any mutants which are pathogenic when inoculated together.

(v) *UV irradiation of diploids*

The source of UV light was a low-pressure mercury lamp emitting 1300 ergs/cm.^{2}/sec. at the target distance of 16 cm. Cells were grown in liquid complete medium overnight to stationary phase; they were then diluted into fresh complete medium and at least two divisions allowed to occur (approximately 6 hours' incubation). The cells were then washed, counted with a Coulter electronic particle counter, and appropriate numbers spread on plates of nitrate minimal medium. The cells were irradiated on the plate, and *nar*$^+$ colonies counted after 6 days. The viability of the cells after UV treatment is determined in two ways. Nitrate minimal solid medium contains sufficient reduced nitrogen to allow the formation of small countable colonies, when the cells are plated at low density. The cell survival can therefore be measured by plate counts on the same medium which selects the *nar*$^+$ recombinants. Alternatively, the viability can be determined by microscopic examination of small samples of cells on the same medium after 20–24 hours' incubation. Viable cells form micro-colonies which are clearly distinguishable from non-growing cells. This method has been found to be more accurate, particularly for measuring survival after very low doses of UV. Nitrate minimal medium is unsuitable for measuring survival after high doses of UV, since then the viability is greater when cells are crowded than when they are dispersed on the agar. The reason for this is unknown.

3. EXPERIMENTAL RESULTS

(i) *Phenotypes of the mutants*

Mutants unable to use nitrate as sole source of nitrogen fall into three categories on the basis of their response to other nitrogen sources. Of thirty-three mutants isolated by the inositol starvation technique, twelve were unable to use nitrite as a nitrogen source. When incubated on nitrate minimal medium nitrite rapidly accumulates in the medium: they therefore have nitrate reductase activity whilst lacking nitrite reductase (or possibly an enzyme further along the pathway from nitrite to ammonia) and are designated *nir* mutants. Twenty-one mutants were able to grow on nitrite as sole source of nitrogen: they therefore lack nitrate reductase activity and are designated *nar* (*nar-1* was previously referred to as *na-1*; Holliday, 1965*a*). Of these twenty-one, nine will grow vigorously on hypoxanthine

as sole nitrogen source whilst the others will not do so. It has been shown that mutants of *Aspergillus* which are able to grow on nitrite but not nitrate, belong to at least six loci and that mutants at only one locus will use hypoxanthine as sole source of nitrogen (Pateman, Cove, Rever & Roberts, 1964). This locus is believed to be the structural gene for nitrate reductase, whereas the others are thought to lack an essential co-factor involved in the reduction of both nitrate and hypoxanthine. Further evidence that this is so comes from a study of one of the *Ustilago* mutants, *nar-10*, which will utilize hypoxanthine. This mutant is temperature sensitive: it will not grow on nitrate minimal medium at 30°C., whereas it will grow slowly on the same medium at 20°C. It has been shown by Miss C. Lewis (unpublished) that nitrate reductase activity can be demonstrated in crude extracts of cells grown at the low temperature, but that this activity rapidly disappears at 30°C. Enzyme from wild-type cells is much more stable at the higher temperature. The result indicates that *nar-10* produces a thermolabile protein, and that the mutation is in the structural gene for the enzyme.

(ii) *Complementation in heteroallelic diploids*

Five of the mutants presumed to be defective in the structural gene for nitrate reductase were selected for genetic studies. These were *nar-6*, *-10*, *-11*, *-12*, *-13*. All ten possible heteroallelic diploids were synthesized, together with diploids homoallelic for *nar-6*, *-11*, and *-13*. The four heteroallelic diploids containing *nar-13* had an unaltered mutant phenotype, whereas all the other diploids showed at least some growth on nitrate minimal medium. This varied from barely detectable growth on agar plates (*nar-12/6* and *nar-12/11*) to slow growth (e.g. *nar-10/6*, which has a doubling time in liquid nitrate minimal of 660 min., compared with 150 min. for wild-type). The interallelic complementation relationships are shown in the lower half of Fig. 3. In no case was the complementation strong enough to prevent or obscure the growth of nar^+ colonies on nitrate minimal plates, but this was not so for some diploids containing mutants which were not used in recombination studies. For instance, *nar*-6/9 has a doubling time of 300 min. and *nar-6/18* and *nar-6/19* have the wild phenotype. On this test alone the allelism of *nar-18* and *19-* with the other mutants is therefore not established. Complementation tests involving the other *nar* mutants which do not respond to hypoxanthine have not been carried out; but it is known that *nar-1* and *-2* are alleles and unlinked to *nar-6*.

(iii) *Recombination in heteroallelic diploids*

The five mutants used in recombination studies are very stable, with reverse mutation rates of the order of 10^{-8} or lower, and this is true also of the homoallelic diploids. The heteroallelic diploids are much less stable and revert to nar^+, as a result of intragenic recombination, at rates of 3×10^{-7} to 5×10^{-6}. After low doses of UV light the recombination frequency is enormously increased, approximately 100-fold after a dose of light which kills 50% of the cells. The reversion rates of haploids or homoallelic diploids after UV are negligible by comparison.

In order to determine whether the recombination frequencies could be used to map the mutants in a linear order, it was decided to measure the UV-induced recombination frequencies under standard conditions rather than the spontaneous frequencies. In yeast, Manney & Mortimer (1964) found that the rate of induced recombination in heteroallelic diploids increased linearly with X-ray dose; consequently the angle of the linear plot could be used as the index of induced recombination. On this basis the alleles could be mapped in a linear order. In *Ustilago*, the relationship between UV dose and recombination frequency turned out to be surprisingly complex. This relationship has been examined with all ten

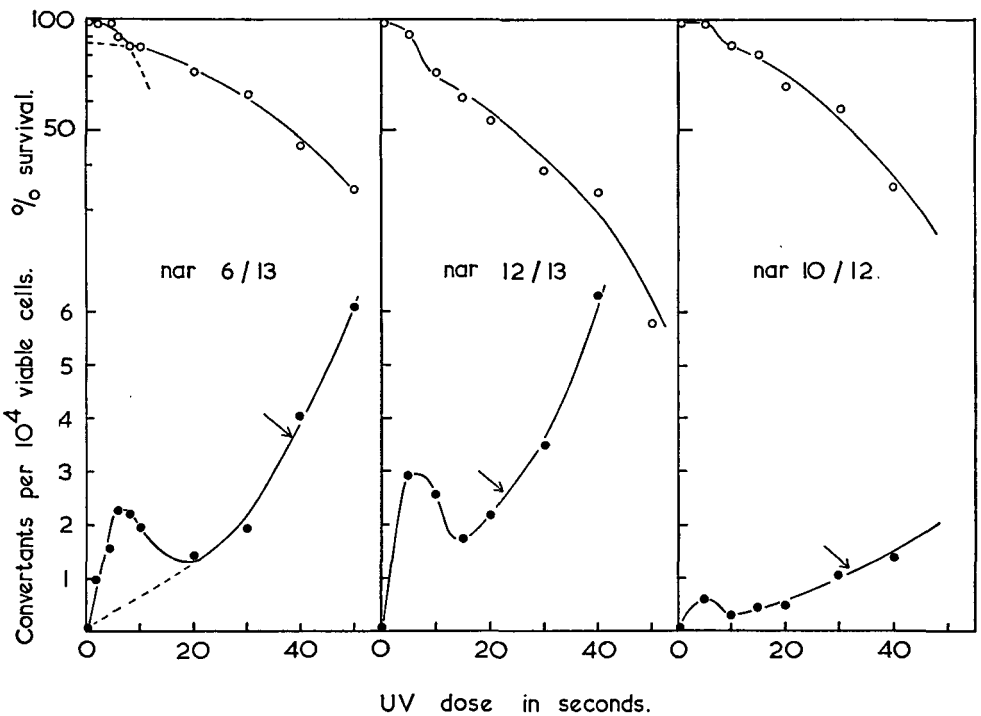

Fig. 1. The relationship between UV dose, survival and induced recombination frequency for three of the ten heteroallelic diploids. The arrows indicate recombination frequency at the 50% level of survival.

diploids and is in each case the same: examples of three typical experiments are shown in Fig. 1, and the actual data for just one of the diploids are given in Table 1. In these and a number of other experiments it has been found that whereas the UV survival curve for non-growing cells consists of an initial shoulder followed by a curve of increasing steepness, the survival of growing cells appears to consist of two superimposed curves: one for a sensitive fraction of cells (10–20%) and the other for a resistant fraction. This is in agreement with studies on synchronized populations of *Ustilago* (Holliday, 1965*a*) where it was found that a particular stage of the division cycle (probably the period of DNA synthesis) was very sensitive to UV. Similarly, the recombination curve consists of two components: a rapid rise followed

Table 1. *The relationship between UV dose and recombination frequency: the detailed data from an experiment with one of the ten heteroallelic diploids* (nar-10/nar-11). $5{\cdot}3 \times 10^6$ *cells were plated for each treatment.*

UV dose (sec.)	Survival (%)	*nar*+ colonies	Recombination frequency ($\times 10^4$)
0	99	36	0·07
5	88	855	1·83
10	76	636	1·57
15	69	518	1·41
20	55	608	2·07
30	40	686	3·22
40	30	799	5·02
From graph:	50		2·4

by a fall, then a slower rise with dose. The clear implication is that in the sensitive fraction of cells—or part of that fraction (see below)—recombination is induced readily by low doses of UV, but at doses above 5 sec. the rate of observed recombination falls off owing to the preferential killing of the cells by UV This can be confirmed by using a synchronized population and irradiating samples of cells with a low dose of UV at intervals throughout the division cycle. Unfortunately, the synchronizing procedure (Holliday, 1965*a*) depends on growing the initial population in nitrate minimal medium, which cannot be done for the diploids under examination. However, it is possible to synchronize other heteroallelic diploids, although so far less successfully than with prototrophic cells. An experiment with a diploid heteroallelic at the *nic-3* locus is shown in Fig. 2. The dose used in this experiment killed about half the cells during the sensitive period. During this period also the recombination frequency was about six times higher than during the non-sensitive period and about 100 times higher than the spontaneous rate. In another experiment similar peaks in recombination frequency were obtained with an even lower dose of UV, which produced no detectable killing in the sensitive period.

In the experiments with log phase cells, there was some variation in the shape of the survival curves in different experiments, as is shown for instance in Fig. 1. Since stationary phase cells are partially synchronized, it may be that even populations grown for two divisions from stationary phase could have some residual synchrony; this could result in slight differences in the ratio of sensitive to non-sensitive cells in different experiments. It is also probable that the diploids differ slightly in their inherent sensitivity to irradiation. In order to make a fine structure map, it therefore seemed more reasonable to use the recombination frequency after a dose which killed a constant fraction of cells as an index of recombination, rather than that after a constant dose. When this is done for a dose which kills half the cells, the map shown in Fig. 3 is obtained. The recombination frequencies appear to be compatible with a linear order of sites. The main discrepancy appears to be

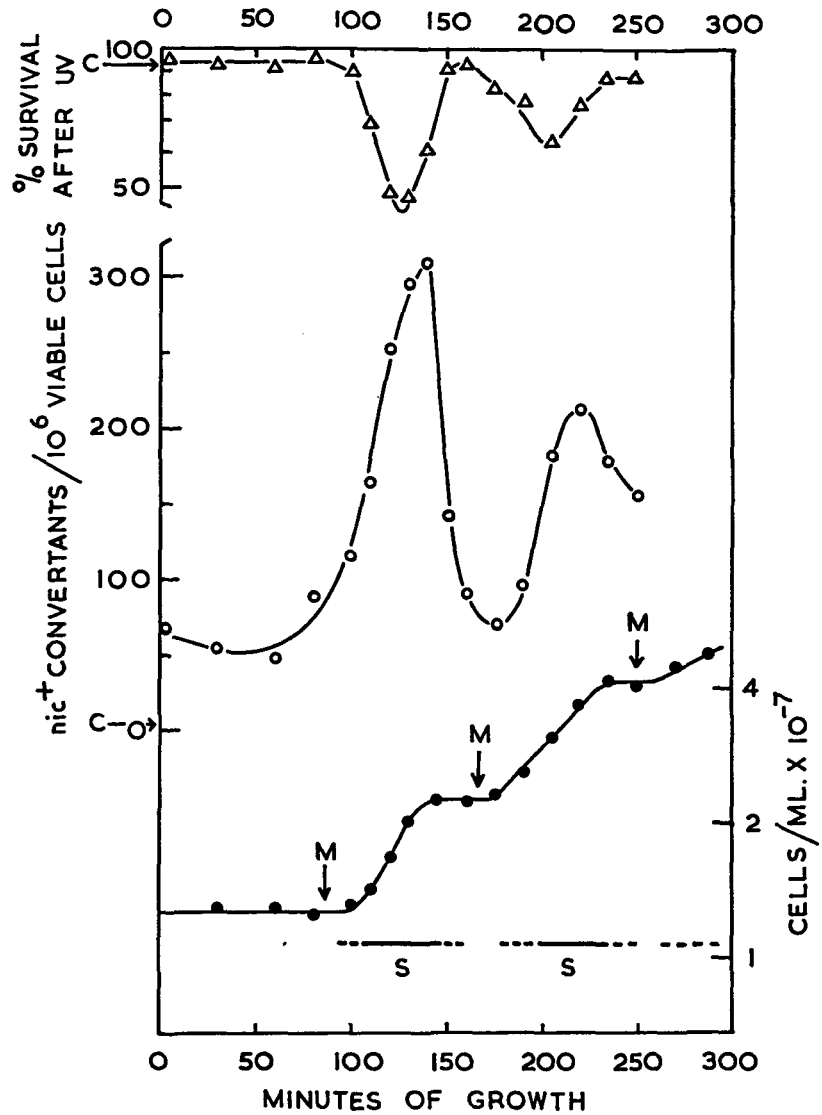

Fig. 2. An experiment with synchronized cells of a diploid heteroallelic for *nic-3* and *nic-17*. Cells were synchronized by a feeding and starving procedure (Holliday, 1965*a*); samples were removed at intervals and given a 10 sec. dose of UV; *nic*+ recombinants were selected on minimal medium, and survival was measured by microscopic examination of cells 20 hours after streaking on supplemented medium. S indicates the period of DNA synthesis: the relationship of this to the time of cell doubling and nuclear division (M) is known from several other experiments with various diploids. C indicates the survival and recombination values for the untreated cells.

that diploids containing *nar-10* give lower values than expected. In Table 2 this is shown by summing the recombination frequencies from the groups of four diploids having a mutant in common. In an additive map these values should be equal.

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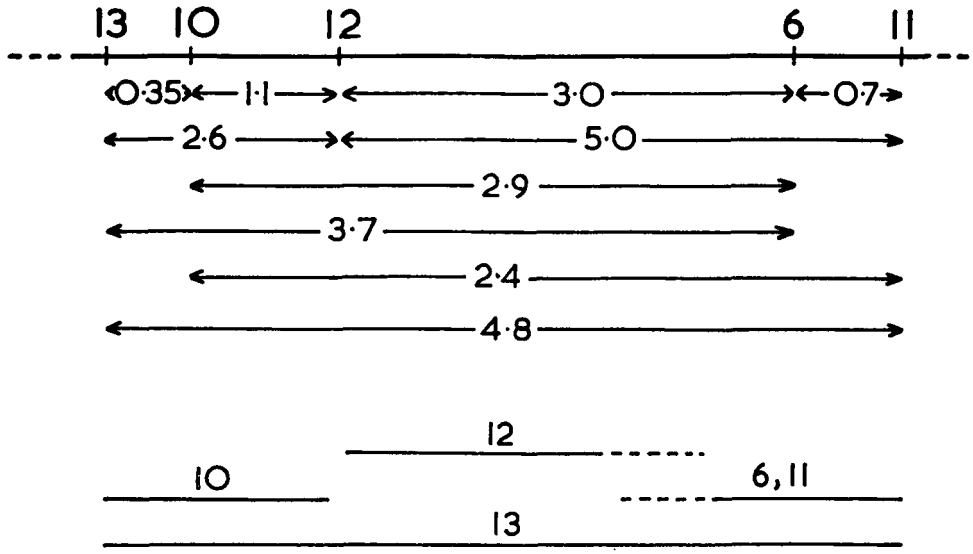

Fig. 3. Fine structure map of the nitrate locus. Values are the frequency of recombination per 10^4 viable cells after a dose of UV which killed 50% of cells. Below are shown the complementation relationships of the mutants. The overlapping broken lines indicate extremely weak complementation.

Table 2. *The sum of the recombination frequencies* ($\times 10^4$) *for the four diploids containing:*

nar-6	*nar-10*	*nar-11*	*nar-12*	*nar-13*
10·3	6·75	12·9	11·7	11·45

(iv) *Half-tetrad analysis*

In order to determine whether the intragenic recombination event is due to gene conversion rather than reciprocal recombination, it is necessary to undertake the laborious task of half-tetrad analysis. In the case of a reciprocal event, the selected *nar*$^+$ allele would be accompanied in the resulting diploid cell by the doubly defective gene in half the cases, whereas with gene conversion, a non-reciprocal event, the doubly defective gene would not be produced. This is shown in Fig. 4.

The steps in the half-tetrad analysis were as follows:

(1) The selected *nar*$^+$ colony was restreaked on nitrate minimal medium and the cells inoculated into maize seedlings. In each case tested the recombinant colony was self-fertile, showing that it was heterozygous for mating type and therefore diploid.

(2) The brandspores were germinated, a small sample of cells from many brandspore colonies was plated on complete medium and the colonies replicated to test media. In each case, segregation for *pan*, *inos* and *nar* occurred.

(3) A haploid of genotype *nar inos* was selected. (This was of mating-type a_2 since *pan* is linked to a_1.) It was inoculated into maize with four tester

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stocks: *nar-x pan* a_1b_1, *nar-x pan* a_1b_2, *nar-y pan* a_1b_1 and *nar-y pan* a_1b_2, where *nar-x* and *-y* are the mutants present in the original heteroallelic diploid. Two inoculations were always fertile and two diploids of *nar* phenotype were isolated from the infected tissue.

(4) Cells from each diploid were tested with a low dose of UV light and plated on nitrate minimal medium to determine whether they revert at high frequency to *nar*$^+$, and thus whether the diploids are homoallelic or heteroallelic.

Thirty-four recombinant colonies from three heteroallelic diploids were analysed in this way; all but five were UV induced. In no case was the doubly defective *nar*

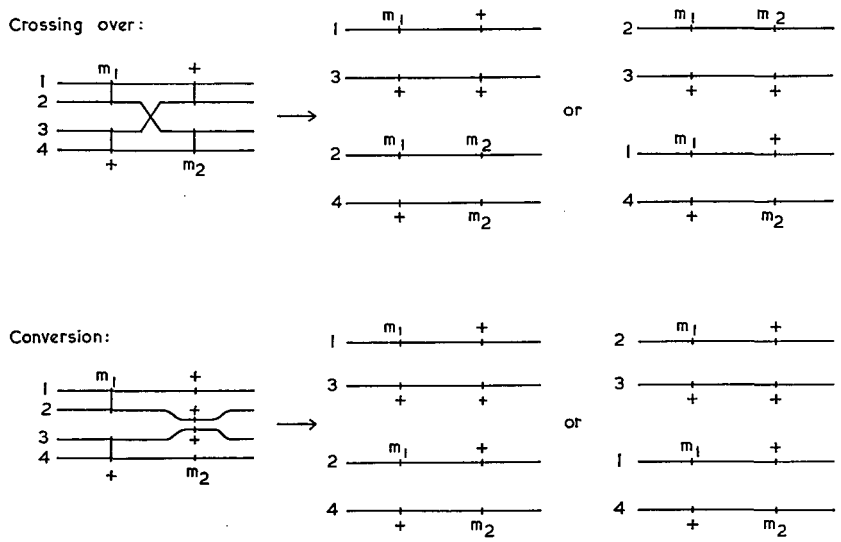

Fig. 4. The genetic consequences of reciprocal exchange and gene conversion in a heteroallelic diploid. At mitosis, sister chromatids move to opposite poles; the daughter cell containing the + + chromosome is selected. If crossing over occurred, it should be in the same daughter cell as the reciprocal product of the exchange, m_1m_2, in half the cases.

gene picked up. The data are given in Table 3; they convincingly demonstrate that intragenic mitotic recombination in *Ustilago* is predominantly, if not entirely, due to gene conversion.

If strict polarity in the conversion event occurred, as is the case in many heteroallelic crosses in *Ascobolus* (Lissouba, Mousseau, Rizet & Rossignol, 1962), then the half-tetrads from any one diploid should always be of the same genotype, i.e. conversion should always occur at the same site. This is clearly not the case in the three diploids which have been examined. There is, however, evidence for relative polarity in the conversion. Taking the linear order of sites as *nar-13*...*nar-6*...*nar-11* (see Fig. 3), then conversion at the site to the left occurred on twenty-four occasions, and at the site to the right on ten occasions.

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Table 3. *Half tetrad analysis. The genotypes of 34 recombinant colonies from three heteroallelic diploids. The dose of UV was 30 sec.*

Heteroallelic diploid	Origin of recombinant	Frequency of recombinants of the genotype: 13 + / + +	+ 6 / + +	+ 11 / + +
nar13- + / + *nar-6*	Spontaneous	1	4	
	UV induced	3	6	
nar-13 + / + *nar-11*	UV induced	3		4
nar-6 + / + *nar-11*	UV induced		3	10

Conversion at site to the left (underlined): 24
Conversion at site to the right: 10
} see Fig. 3 and text.

(v) *The relation of conversion to crossing over*

At present no markers linked to the *nar* locus are known, so no information can be gained concerning the relation between gene conversion at this locus and crossing over. It is, however, possible to synthesize diploids heteroallelic at the inositol locus, which is loosely linked to *nic-3* and proximal to it. The genotypes of the diploids which have been used are given in Table 4. *inos*$^+$ convertants are selected

Table 4. *The incidence of homozygosis for a distal marker amongst gene convertants at the* inos *locus. The convertants were induced by a 60 sec. dose of UV The data are the combined results from three experiments with each diploid.*

Genotype of diploid (linked markers only)	*inos*$^+$ convertants	Convertants which are *nic*$^-$ (No.)	(%)
nic-3 inos-2 / + *inos-3* ○	403	10	2·5
nic-3 inos-3 / + *inos-2* ○	325	7	2·2

on minimal medium supplemented with nicotinic acid, and the colonies replicated to minimal medium. This particular combination of *inos* alleles gives a low frequency of spontaneous and induced conversion, only approximately ten times higher than the combined back mutation rates of the mutants. From Table 4, it can be seen that reversion at the *inos* locus is only weakly correlated with mitotic crossing-over between the centromere and *nic-3*, and that it makes no difference whether

inos-2 or *inos-3* is in coupling with *nic-3*. The weak correlation exists, because in the absence of selection for *inos*$^+$, the same dose of UV light would produce 0·5–1·0% detectable cross-overs proximal to *nic-3*. It can be concluded that the conditions which allow mitotic gene conversion to occur, only infrequently result in reciprocal exchange also. This result was first demonstrated by Roman & Jacob (1958) with yeast.

(vi) *Meiotic conversion*

As would be expected, crosses between different *nar* alleles yield rare *nar*$^+$ haploid progeny. For the mutants *nar-6* and *-13* the frequency of meiotic *nar*$^+$ recombinants is $1{\cdot}2 \times 10^{-3}$, which is comparable to the frequency of mitotic recombinants after high doses of UV light and about 300 times the spontaneous frequency during mitotic division.

In *Ustilago* it should be possible to analyse fairly large numbers of tetrads containing an intragenic recombinant. Brandspores from a cross between two nitrate alleles would be germinated at a concentration of about 200 per plate of complete medium. The colonies would be replicated to nitrate minimal medium; only colonies containing *nar*$^+$ cells (about 0·3%) would produce growing replicas. Each colony would be harvested from the complete plate, dispersed in water and after plating out cells on complete medium, their requirements would be identified in the usual way. If at least two other markers are segregating in the cross, it is possible to tell when all four products of the tetrad have been recovered from the brandspore colony. In these cases the analysis of the tetrads would be completed by making appropriate crosses, or diploids, with the three *nar*$^-$ members of each tetrad in order to determine their full genotype. The same method would also detect the occurrence of post-meiotic segregation. These possibilities have yet to be explored in *Ustilago*.

4. DISCUSSION

The aim of the present study has been to establish a system in *Ustilago* for the experimental investigation of gene conversion along the lines suggested in an earlier publication (Holliday, 1964). The heteroallelic diploids which have been synthesized behave in essentially the same way as do those of *Saccharomyces* (Roman, 1956, 1958; Roman & Jacob, 1958; Fogel & Hurst, 1963; Hurst & Fogel, 1964; Kakar, 1963), *Schizosaccharomyces* (Leupold, 1958) and *Aspergillus* (Roper & Pritchard, 1955; Pritchard, 1955, 1960; Putrament, 1964; Jansen, 1964). Namely, the spontaneous frequency of intragenic recombination is much higher than the back-mutation rates of the haploids or homoallelic diploids, and this frequency is further increased by irradiation of the diploid. The recombination event is due to gene conversion rather than reciprocal exchange, and it is more weakly correlated with outside marker exchange than in meiotic systems, particularly in the case of UV-induced conversion. The first fine structure map based on mitotic recombination used spontaneous conversion frequencies at the *ad-7* locus of *Schizosaccharomyces* (Leupold, 1958). Later Manney & Mortimer (1964) showed that conversion frequencies after X-ray treatments could be successfully used for fine structure

mapping in *Saccharomyces*. It has now been shown that UV-induced conversion frequencies can also be used for this purpose.

In addition to these features of mitotic gene conversion, a further factor has now to be taken into account. This is the demonstration that gene conversion is not equally induced by UV at all stages of the division cycle—there is a fraction of cells which shows a very high rate of UV-induced conversion. It has previously been shown that this is true of UV-induced mitotic crossing over in *Ustilago* (Holliday, 1965*a*) and that the sensitive period corresponds with the period of DNA synthesis. With well-synchronized cells there was a plateau rather than a peak with regard to the combined cross-overs in five marked chromosome arms, which suggests that the period in which crossing over can be maximally induced is quite long. Moreover, the further a marker from the centromere, the earlier during the period of DNA synthesis was crossing-over induced proximal to that marker. This was explained by supposing that DNA synthesis proceeds from the end of the arms to the centromere, that replication in homologues is synchronized, and that a temporary block in replication induced by UV-stimulated crossing-over at, or in the vicinity of, this block. This interpretation is consistent with some recent results in bacterial recombination systems. Gallant & Spotswood (1965) showed that the inhibition of DNA synthesis by thymine starvation in merodiploids of *Escherichia coli* strongly stimulated recombination between the chromosome and the episome, and that the small fraction of cells which was resistant to thymineless death (believed to be the cells not synthesizing DNA at the time of initiating starvation) did not show recombination. In the transformation system in *Bacillus subtilis*, Bodmer (1965) presents strong evidence that incorporation of transforming DNA takes place at the replicating point in competent cells.

In heteroallelic cells of *Ustilago* which are partially synchronized there is a sharp peak in UV-induced conversion, in contrast to the plateau for crossing over. This suggests that the period within which conversion can be stimulated is quite short, and that the peak would be even sharper with better synchrony. As a working hypothesis, it is suggested that conversion might occur at the replicating point by the following mechanism. Inhibition of DNA synthesis by UV at similar points on homologous chromosomes would allow some unravelling of the free end of the newly synthesized strands. In a proportion of cells, either by chance juxtaposition or owing to some unknown 'pairing force', homologues would be close enough to allow the single stranded ends from two chromatids to anneal with opposite partners to produce regions of hybrid DNA and a half chromatid chiasma. The configuration produced would be closely similar to that suggested for the formation of hybrid DNA in meiotic cells (Holliday, 1964). If a heterozygous site is included in the hybrid region, then gene conversion by repair could occur. Since conversion is infrequently associated with outside marker recombination in the mitotic system, it would be necessary to postulate that usually the half chromatids which have exchanged partners would break and rejoin; or alternatively that these half chromatids unravelled and re-annealed with their original partners, which would allow the transmission of genetic information from one chromatid to its homologue via two

conversion events. Occasionally the strands which had not exchanged partners would break and rejoin to give a full chromatid chiasma and reciprocal exchange for outside markers. This would explain why a dose of irradiation which increases the rate of conversion, say, one hundred times, will only increase the rate of mitotic crossing-over approximately ten times.

The results presented in this paper provide further evidence that conversion frequencies are related to distance apart of mutant sites. A difficulty of hybrid DNA models of genetic recombination is that the frequency of conversion by repair would appear to depend on the frequency with which one or other, or both, mutant sites are included in the hybrid region, rather than on their distance apart. The suggestion, and evidence, that mutants interfere with hybrid DNA formation and that this interference relates in some way to distance (Holliday, 1964; and see Lacks, 1966), only goes part of the way towards meeting this problem. Pritchard (personal communication) has pointed out that the difficulty disappears if it is assumed that conversion by repair does not operate simply by the excision and replacement of single bases, but by the excision of one of a pair of mismatched bases in hybrid DNA, followed by the degradation of one strand from the point of excision, very much as is believed to occur in the repair of UV-induced lesions (Setlow & Carrier, 1964, Boyce & Howard-Flanders, 1964). Subsequently, repair synthesis would occur, using the intact strand as template, and double-stranded DNA without mismatched base pairs would be reconstituted. Therefore, if the two heterozygous sites are very close together and are included in a stretch of hybrid DNA, a single repair event covering both sites will *not* produce a recombinant strand, since each of the two strands in the hybrid region carries a mutant base. A wild-type convertant will be formed only if the hybrid DNA ends between the mutants, or, if both are included, a single repair event does not cross both sites. Both these occurrences are, of course, related to the distance apart of the mutants. Moreover, this model can provide an explanation for the phenomenon of map expansion, which occurs in several fine structure maps of fungi (Holliday, 1964). If the region of repair is of fairly constant length, then when two mutants are further apart than this length, there will not be the restriction on the production of wild-type recombinants which applies to two mutants that are close enough for both to be included.

The relationship between repair mechanisms and gene conversion may be clarified by studying the effect of radiation-sensitive mutants on genetic recombination. Such mutants are available in *Ustilago* (Holliday, 1965*b*), their effect on gene conversion at the nitrate locus is under examination, and the results will be published in another paper.

SUMMARY

In order to develop a system for the study of the mechanism of intragenic recombination in *Ustilago*, mutants lacking nitrate reductase activity were isolated, and five alleles were combined in pairs in ten vegetative heteroallelic diploids. The diploids have the mutant phenotype, i.e. inability to utilize nitrate as sole source

of nitrogen, but they will recombine to produce wild-type cells much more frequently than the back-mutation rates of haploids or homoallelic diploids. The spontaneous rate of recombination can be enormously increased by low doses of UV light, particularly if treatment is during the period of DNA synthesis in the mitotic cycle. By means of half-tetrad analysis it has been shown that this process of intragenic recombination, as in other fungi, is due to gene conversion rather than reciprocal exchange. It has also been shown that the frequency of UV-induced conversion under standard conditions gives a rough measure of the distance between two mutant sites, since it was possible to use these frequencies to make a linear fine structure map of the gene. These results are discussed in relation to a hybrid DNA model for gene conversion slightly modified from that previously suggested for meiotic recombination.

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