THE INDUCTION OF MITOTIC RECOMBINATION BY MITOMYCIN C IN USTILAGO AND SACCHAROMYCES[1]

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VEGETATIVE heterozygous diploid strains of *Ustilago maydis* undergo spontaneous mitotic crossing over at low frequency, but this frequency can be increased up to 100-fold if the cells are treated with ultraviolet light (UV) (HOLLIDAY 1961b, 1962a, b, 1964). There is no indication that the induced crossing over differs in mechanism from that which has been described for spontaneous mitotic crossing over in *Aspergillus nidulans* (PONTECORVO and KÄFER 1958); but unlike Aspergillus, Ustilago does not produce haploid somatic segregants, either spontaneously or after UV treatment. Vegetative Saccharomyces diploids do not haploidise either; their somatic segregation occurs as a result of crossing over and is stimulated by UV, but the recombination process involved appears to be distinct from normal mitotic crossing over (WILKIE and LEWIS 1963).

On the basis of the results with Ustilago, it has been suggested that UV may promote recombination by bringing about homologous pairing of mitotic chromosomes; and that this might be the consequence of the specific inhibition of DNA synthesis by UV producing a state of unbalanced growth akin to the condition of the meiotic cell (HOLLIDAY 1961b).

If UV acts in the way suggested, then other inhibitors of DNA synthesis should also stimulate mitotic crossing over. The antibiotic mitomycin C (MC) has been shown to interfere specifically with DNA synthesis in bacteria (SHIBA, TERAWAKI, TAGUCHI and KAWAMATA 1959), in Saccharomyces (WILLIAMSON and SCOPES 1962) and in animal cells (BEN-PORAT, REISSIG and KAPLAN 1961). In the present paper it is shown that MC stimulates mitotic crossing over in Ustilago with an efficiency comparable to that of UV, and furthermore, that it does not appear to have mutagenic activity under the same conditions of treatment. A similar effect on mitotic recombination in Saccharomyces is also reported. In another study the effect of 5-fluorodeoxyuridine (FUDR), another inhibitor of DNA synthesis, is examined: it too stimulates mitotic crossing over in Ustilago and appears to be nonmutagenic (EASTON and HOLLIDAY 1964).

EXPERIMENTAL

Ustilago maydis

Most of the genetic markers, techniques and media employed in these experiments have been

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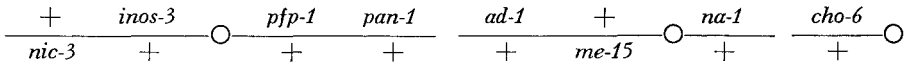
described in earlier publications (HOLLIDAY 1961a, b, 1962b). All the recombination experiments were carried out with Diploid H which has the genotype:



This diploid is prototrophic and sensitive to p-fluorophenylalanine (PFP). The recessive mutation to resistance to this amino acid analogue, *pfp-1*, arose spontaneously in a haploid strain streaked on complete medium supplemented with 0.01 percent PFP. Haploids carrying *na-1*, or diploids homozygous for this marker, are unable to use nitrate as sole source of nitrogen, since this mutation has resulted in the loss of activity of the enzyme nitrate reductase (HOLLIDAY 1961c). The other markers indicate recessive requirements for nicotinic acid, inositol, pantothenic acid, adenine, methionine and choline. The evidence for the location of several of the markers has been previously discussed. The reasons for assigning chromosome arms to a common centromere is indirect, since no meiotic linkage has been detected between markers astride the centromere. The evidence, which is not conclusive, comes from a study of aneuploids and will not be discussed here. Data presented in this paper and elsewhere (HOLLIDAY 1964) indicate that *cho-6* is situated on a fifth chromosome arm. If this is so, then clearly the genetic data are not reconcilable with cytological observations that the haploid chromosome number is two (KHARBUSH 1928; and see HOLLIDAY 1961b).

With this diploid, mitotic crossing over can be detected and measured by two methods. Recombinants can be selected by plating cells on a medium containing PFP. Mitotic crossing over between the centromere and *pfp-1* will produce homozygosity for the recessive marker in half the cases, and hence the ability of the recombinant cell to grow on the selective medium. This method for selecting recombinants is similar to those which have been used extensively in Aspergillus (PONTECORVO and KÄFER 1958), and MORPURGO (1962, 1963) has indeed used resistance to the same analogue in experimental studies on induced mitotic crossing over in this fungus. A number of different media in general use in the laboratory were supplemented with varying amounts of PFP and tested for their ability to select recombinants. It was found that a yeast synthetic complete medium (ROMAN 1956) containing 0.01 percent PFP allowed good growth of recombinant colonies, whilst eliminating most of the background growth of the large numbers of nonrecombinant cells. The great majority of the recombinants had become homozygous for *pan-1*, thus locating *pfp-1* between *pan-1* and the centromere.

Mitotic crossing over can also be measured by the nonselective method in which cells are plated on complete medium and the subsequent colonies replicated to nitrate minimal medium. This medium has the usual source of nitrogen, ammonium nitrate, replaced by 0.3 percent potassium nitrate. Colonies which are homozygous for an auxotrophic marker or *na-1* do not grow on the replica and can be readily detected. With this method, the reciprocal products of the exchange can often be detected as mosaic colonies: according to the position of the exchange such colonies may be half-wild and half-mutant in phenotype, and therefore produce a semicircular replica, or consist of two mutant phenotypes. The latter are identified if the recombinant colony on complete medium is phenotypically mosaic, or if the nongrowing cells picked from the minimal plate are found to be a mixture of two phenotypes. In some cases the nongrowing replica is recognisably mosaic; for instance, *nic* homozygotes divide once or twice on minimal medium, whereas *inos* homozygotes do not. *pan* segregants were not routinely tested for PFP resistance, but the majority of those that were examined, in these and other experiments, had acquired such resistance.

In all the experiments cells growing logarithmically in liquid nitrate minimal medium were transferred to the same medium containing mitomycin C (obtained from the Sigma Chemical Co., St. Louis, Missouri). Before and during treatment the cultures were incubated at 30°C in a New Brunswick shaker, model G-25. As has been found with yeast (WILLIAMSON and SCOPES 1962) concentrations of MC as high as 400 μg per ml of medium are necessary to inhibit cell growth, and this concentration was used in all the experiments reported here. After transfer to the MC medium, about half the cells divided once, there was then usually no further increase in cell number for several hours. The viability of the cells throughout this period of inhibition

varied in different experiments from 60 percent to 100 percent of the initial population. There was also variation in the period of inhibition by MC; normally if the culture was incubated overnight the cells had begun dividing again and would eventually reach the usual stationary phase concentration, but sometimes the period of inhibition was more extended. The exact pattern of inhibition and recovery was not always followed in detail, but whenever a sample of treated cells was assayed for recombinants the viability was determined.

In two experiments the nonselective method was used. In the first the cells were treated for 7½ hours with MC; the viability of the plated cells was 70 percent, but the viable population was almost equal to the initial population. In the second experiment the cells were allowed to pass through the period of inhibition into that of recovery, the final population after 23½ hours treatment was eight times higher than the initial population, but only 60 percent of this population was viable. Along with cell growth during the recovery period there must also be some cell death. The results of the two experiments are shown in Table 1.

A large increase in segregation over the control had occurred and the phenotypes of both the whole colony and the mosaics are what would be expected following mitotic crossing over. In the second experiment the proportion of mosaics was lower than in the first, but with the longer treatment the cells had divided two or three times so that any reciprocal products of crossing over from an early division would be separated out at the time of plating. For comparison, Diploid H was also treated with UV. (The source was a Hanovia low-pressure mercury lamp emitting about 200 ergs/cm²/sec at the target distance of 12 inches). A comparable frequency of crossing over was induced, but the fraction of surviving cells was much lower: in relation to lethality UV is less efficient than

TABLE 1

Assay of recombinants among cells treated with mitomycin C or UV, using the nonselective system

Experiment No.	Treatment: Mitomycin (hours)	Treatment: UV (minutes)	Viability (percent)	Colonies examined	Recombinants: Number	Recombinants: percent
1	0	..	100	1521	0	...
1	7.5	..	70	2991	38	1.27
2	23.5	..	60*	1005	31	3.08
3	...	0	100	1912	4	0.21
3	...	6.5	9	2439	56	2.30

Phenotypes of the recombinants

Experiment No.	Whole colonies: *nic*	*inos*	*ad*	*me*	*pan*	*cho*	*na*	*nic*+ *pan*	*ad*+ *inos*	Mosaic colonies: *nic/inos*	*nic/+*	*ad/me*	*pan/+*	*na/+*
1	4	5	3	2	1	2	5	0	1	3	1	5	1	5
2	3	2	6	2	9	2	3	0	0	1	2	1	0	0
3(Control)	0	2	0	1	0	1	0	0	0	0	0	0	0	0
3(UV)	18	11	4	2	1	0	7	1	0	5	2	2	3	0

* 60 percent of the final population was viable, but this population was eight times greater than that at the start of the experiment.

MC in inducing recombination. With UV the marker *nic-3* always shows the highest frequency of segregation (HOLLIDAY 1961b, 1964), but following the MC treatment all the segregants occur with approximately equal frequency. This would be the case if most of the MC induced crossing over was close to the centromere. A similar effect was observed with caffeine (HOLLIDAY 1961b).

A number of experiments have been carried out with the selective assay of recombinants. Cells were plated directly from the MC medium to the selective medium. All the experiments showed a large increase in recombination among MC treated cells, and the most detailed result is shown in Table 2 and Figure 1. In this experiment the number of viable cells increased for nearly 2 hours and then declined to 60 percent of the initial count at 7½ hours. The recombination appeared to increase linearly with time during this period of cell growth and then continued to increase at a slower rate until the end of the experiment. The period of the steep rise in recombination corresponds approximately to the generation time, which suggests that treatment during a particular stage of the division cycle tends to induce recombination: in a 2-hour period all the cells would have come into this stage. Other experiments gave a similar pattern of induced re-

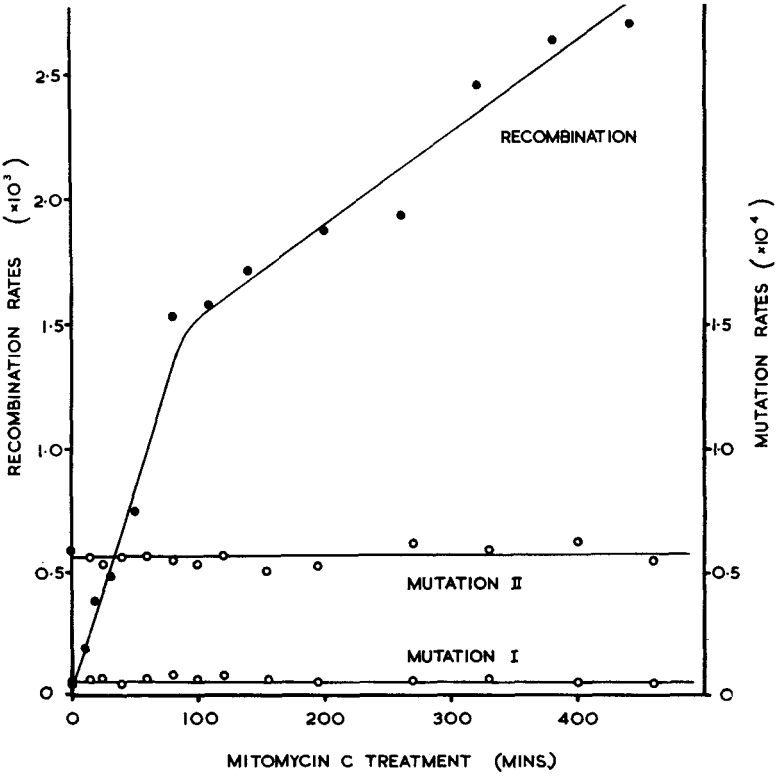


FIGURE 1.—The effect of mitomycin treatment on recombination leading to resistance to p-fluorophenylalanine in Diploid H; and on mutation to resistance to the same analogue in a haploid strain.

TABLE 2

Assay of recombinants among cells treated with mitomycin C, using the selective system

Mitomycin treatment (minutes)	Viable cells examined ($\times 10^{-5}$)	Recombinants	Recombination rates ($\times 10^{4}$)
0	143.0	809	0.57
10	24.0	449	1.87
20	2.60	98	3.77
30	2.95	142	4.81
50	2.90	218	7.52
80	0.80	123	15.4
110	1.50	238	15.9
140	1.45	248	17.1
200	1.40	256	18.2
260	1.40	274	19.6
320	1.35	329	24.4
380	1.35	360	26.7
440	1.33	359	27.0

combination except that the levelling off of recombination frequency after the initial steep rise was more pronounced.

The effect of UV on recombination using the selective system was also examined. Log phase cells were irradiated in water and plated directly to the selective medium, precautions being taken to avoid photoreactivation. After the highest dose of UV the cells were photoreactivated by treating them for 15 minutes with an arbitrary dose of light of wavelength 3000–4000 Å emitted by a fluorescent "black-light" lamp. The results of this experiment are shown in Table 3 and Figure 2. The increase in recombination with UV dose is very nearly linear, but the slight deviation from this relationship is probably significant. There is a more direct proportionality between the frequency of induced recombination and the log of the surviving fraction; and the dose of visible light increased survival and reduced recombination in a way that did not affect this proportionality. A similar result using the nonselective system has previously been obtained (HOLLIDAY 1962a). The scoring of the recombinants in this experiment was much less straightforward than in the experiments with MC: apart from the true recombinant colonies, which had a characteristic brown colour and morphology and a requirement for pantothenic acid, there were also a number of other smaller colonies, of varying morphology and size, on all except the control plates. It is assumed that these arose as a result of induced semi-dominant mutations to PFP resistance at loci other than *pfp-1* (see below). This observation suggested that MC was much less mutagenic than UV under conditions where it induced rather more recombination. This possibility was examined more directly by using a haploid strain. In order to make the conditions under which the mutation was being studied as similar as possible to those in the recombination experiments, it was decided to examine the rates of forward mutation to PFP resistance. Haploid cells were treated with MC as was Diploid H and then plated directly on the

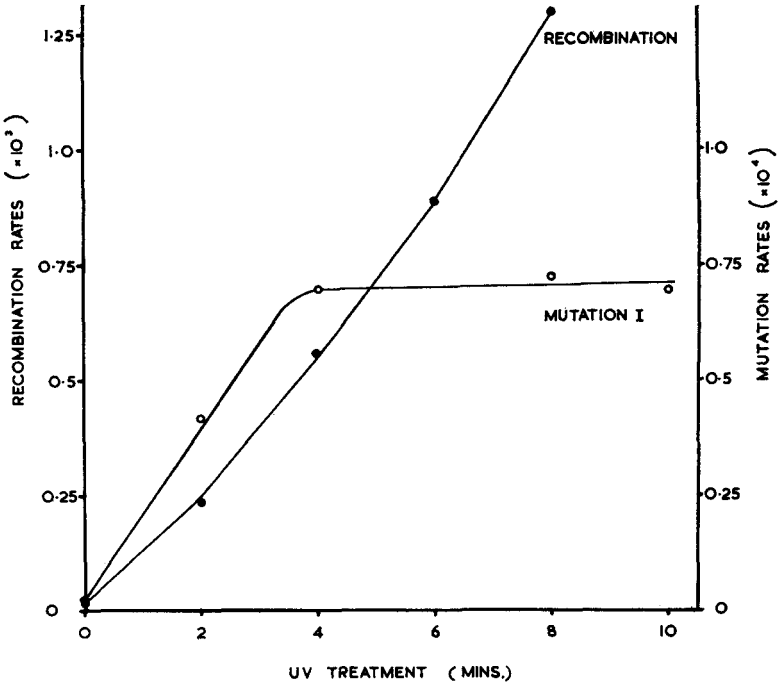


FIGURE 2.—The effect of ultraviolet light treatment on recombination leading to resistance to p-fluorophenylalanine in Diploid H; and on mutation to resistance to the same analogue in a haploid strain.

TABLE 3

Assay of recombinants among cells treated with UV, using the selective system

UV dose (minutes)	"Black light" dose (minutes)	Viability (percent)	Viable cells examined ($\times 10^{-5}$)	Recombinants	Recombination rates ($\times 10^4$)
0	. .	100	690	1059	0.15
2	. .	80	143	350	2.4
4	. .	55	9.8	551	5.6
6	. .	43	7.9	700	8.9
8	. .	23	4.2	551	13.1
8	15	41	4.7	458	9.7

same selective medium. The resistant colonies fell into a number of classes with distinct morphological phenotypes. The frequency of none of these classes increased with MC treatment, and the results for two of them, Class I and Class II, are shown in Table 4 and Figure 1. On the other hand when the haploid was treated with UV there was a large increase in the number of resistant colonies. Class II mutations were too frequent to score, and only Class I mutations are included in Table 5 and Figure 2.

TABLE 4

The absence of an effect of mitomycin C on mutation to p-fluorophenylalanine resistance in a haploid strain

Mitomycin treatment (minutes)	Viable cells examined ($\times 10^{-7}$)	Mutations		Mutation rates	
		Class I	Class II	Class I ($\times 10^{6}$)	Class II ($\times 10^{5}$)
0	9.0	44	528	4.9	5.9
15	6.0	34	344	5.7	5.7
25	6.0	37	321	6.2	5.4
40	6.0	28	342	4.7	5.7
60	6.0	41	339	6.8	5.7
80	6.0	53	329	8.8	5.5
100	6.0	41	317	6.8	5.3
120	6.0	47	351	7.8	5.9
155	3.0	19	152	6.3	5.1
195	3.0	16	158	5.3	5.3
270	4.5	26	281	5.8	6.2
330	3.0	20	177	6.7	5.9
400	3.0	16	190	5.3	6.3
460	3.0	16	166	5.3	5.5

The frequency of induced mutation appeared to increase linearly with increasing dose for the first two readings, but there was then no further increase. This type of mutation response to UV is quite characteristic in fungi (see POMPER and ATWOOD 1955), and the differential response of mutation and recombination to UV treatment suggests that the induction of crossing over is not due to a mutation-like process. It should be pointed out that although the frequency of induced mutation is comparable to that of induced recombination proximal to *pfp-1*, there is reason to believe that these frequent mutations must be recessive or partially so, and must occur at loci different from *pfp-1*: they therefore would not be scored as recombinants in a diploid heterozygous at this locus. First, the colonies scored as recombinants nearly all had a requirement for pantothenic acid, and secondly, their morphological appearance was distinct from that of the more frequent types of mutation induced in the haploid.

TABLE 5

The effect of UV on mutation to p-fluorophenylalanine resistance in a haploid strain

UV dose (minutes)	Viability (percent)	Viable cells examined ($\times 10^{-5}$)	Mutations (Class I)	Mutation rates ($\times 10^{5}$)
0	100	89	17	0.2
2	90	40	167	4.2
4	70	31	217	7.0
8	23	10	73	7.3
10	8	3.4	24	7.0

Saccharomyces cerevisiae

Since MC induces mitotic crossing over but not mutation in Ustilago, it was clearly of interest to know whether MC would also stimulate mitotic gene conversion, or nonreciprocal recombination, in yeast. There is evidence from experiments with UV that this results from a process distinct from that of crossing over (ROMAN and JACOB 1958).

A heteroallelic diploid strain, J-1-a was used: its full genotype will not be given here, the relevant markers on chromosome VII were:

$$O\frac{try_{5-2} \quad ac_2{}^s}{try_{5-1} \quad ac_2{}^r}$$

This diploid has a requirement for tryptophan and is sensitive to actidione. It can become tryptophan independent following somatic gene conversion, and it can become resistant to actidione if mitotic crossing over, or the related process discussed by WILKIE and LEWIS (1963), produces homozygosity for ac^r. The media used in the experiments to be described are those described by ROMAN (1956). Cells growing in supplemented liquid minimal medium were transferred to the same medium containing 400 μgMC per ml. At intervals cells were removed and plated on (1) complete medium, in order to obtain viable counts; (2) complete medium lacking tryptophan, in order to select convertants; (3) complete medium containing 2 μg actidione per ml, to select crossovers; and (4) after 78 and 180 min, on yeast extract peptone medium (YEP) for varying periods, the cells subsequently being washed off and replated on complete medium plus actidione. It was found that the concentration of viable cells remained constant throughout the experiment, but this does not exclude the possibility that some cell division may have been balanced by an equivalent amount of cell death.

The results of the experiment are shown in Table 6. Since yeast cells in log phase produce small clumps of cells, the cell counts were multiplied by 2.6, the mean number of cells per clump, in order to estimate the total cell population.

TABLE 6

The effect of mitomycin C on somatic conversion and recombination in yeast

Mitomycin treatment (minutes)	try$^+$ convertants		Post-treatment on YEP medium (minutes)	ac^r recombinants	
	Number	Frequency $\times 10^4$		Number	Frequency $\times 10^4$
0	19	0.6	...	115	3.7
20	39	1.2	...	147	4.7
40	84	2.7	...	154	4.9
60	117	3.7	...	130	4.1
90	139	4.4	...	140	4.4
120	147	4.7	...	159	5.0
180	193	6.1	...	241	7.7
240	149	4.8	...	209	6.6
310	148	4.7	...	253	8.0
405	147	4.7	...	196	6.2
580	169	5.4	...	285	9.0
240	...	..	78	326	10.3
240	...	..	180	445	14.1
240	...	..	350	66	21.0
405	...	..	150	59	18.7

The viable counts remained constant throughout the experiment; the mean count, 3.15×10^6, was used to calculate the conversion and recombination frequencies. In the last two readings ten times fewer cells were examined.

The frequency of conversion increased with MC treatment for about 2 hours and then showed no further rise. The increase in conversion over the control is about tenfold, much less than the increase in crossing over in Ustilago, and less also than could be obtained with treatment of the same diploid with UV. However, the initial linear rise for about 2 hours is similar to that obtained with mitotic crossing over. There was only a small increase in the number of actidione resistant colonies when the cells were plated directly on actidione medium, but when they were incubated after treatment on YEP plates before being transferred to the selective medium there was an increased frequency of such colonies. The viable count did not change during the periods of post incubation, so this increase represents a real effect by the MC treatment. Evidently MC treatment followed by actidione treatment suppresses the expression of recombination, incubation on a rich medium allows it. This experiment does not show what length of incubation on YEP after treatment allows the maximum expression of induced recombination, nor was the effect of such incubation on the frequency of conversion examined.

DISCUSSION

A number of mutagens have now been shown to be effective in inducing mitotic crossing over. X rays do so in Drosophila (BECKER 1957; ABBADESSA and BURDICK 1963), X rays, UV and caffeine in Ustilago (HOLLIDAY 1961b and unpublished data), and X rays, UV and alkylating agents in Aspergillus (MORPURGO 1962, 1963; KÄFER and CHEN 1964); and in yeast UV can apparently induce mitotic recombination distinct from normal mitotic crossing over (WILKIE and LEWIS 1963), as well as mitotic gene conversion (JACOB and ROMAN 1958).

The finding that mitomycin C is a strong inducer of mitotic crossing over, and under the same conditions is nonmutagenic, suggests that the action of mutagens in inducing recombination may not be due to their mutagenic activity per se, but rather to an indirect effect on cell metabolism. This conclusion is supported by the complementary observation of MORPURGO (1963) who found that bifunctional alkylating agents induce both mutation and crossing over, whereas monofunctional ones induce only mutation. It is proposed that agents which induce crossing over and not mutation be called *recombinagens*. Apart from MC, FUDR also falls into this category (EASTON and HOLLIDAY 1964). (It should be mentioned that *any* inhibition of cell growth does not promote mitotic crossing over. For instance, toxic concentrations of 2-amino purine or p-fluorophenylalanine do not do so, nor does a general metabolic starvation.)

It is not, of course, ruled out that under different conditions MC might cause mutations in Ustilago. In *Escherichia coli* MC has been reported to have effects very similar to those of UV. At concentrations as low as 0.1 μg per ml MC is bacteriocidal (SHIBA, TERAWAKI, TAGUCHI and KAWAMATA 1959); and it produces mutations, stimulates recombination and induces phage in lysogenic bacteria (IIJIMA and HAGIWARA 1960; YUKI 1962; OTSUJI, SEKIGUCHI, IIJIMA and TAKAGI 1959). Its activity against bacteria is several orders of magnitude greater than against Saccharomyces and Ustilago, where a concentration as high as 400

μg per ml is necessary to inhibit growth and is not markedly fungicidal. In *E. coli* it has been shown that the DNA becomes degraded in the presence of MC (REICH, SHATKIN and TATUM 1961), whereas in Saccharomyces there is a round of DNA replication before inhibition of synthesis occurs (WILLIAMSON and SCOPES 1962).

Assuming that in Ustilago MC has an effect on DNA synthesis comparable to that in Saccharomyces, the absence of associated lethality or mutation and the ability of the cells to recover during treatment, suggests that the mechanism of inhibition is an indirect one. By contrast, the inhibition of DNA synthesis by UV is almost certainly due to the direct production of genetic lesions (SETLOW, SWENSON and CARRIER 1963). The genetic results with MC support the hypothesis that inhibition of DNA synthesis is the important factor in producing the conditions in the cell which favour recombination, rather than the presence of specific lesions in the genetic material as suggested by JACOB and WOLLMAN (1955) and ROMAN and JACOB (1958). Since it appears that genetic replication in the meiotic cell is later than in the mitotic one (see HENDERSON 1962), it is suggested that this inhibition pushes the mitotic cell towards the meiotic condition, thus bringing about pairing and the opportunity for crossing over or gene conversion, although the process does not go so far as to result in the reduction of chromosome number. Other evidence for this view is as follows.

In order for mitotic crossing over to occur two processes must take place: the first is homologous pairing, the second crossing over. The relationship between induced crossing over and the dose of UV is very close to a linear one in growing cells; this suggests strongly that if somatic crossing over involves two sequential processes only one of them is affected by UV. The same relationship appears to hold with short treatments of MC. Therefore either these agents produce somatic pairing and crossing over follows spontaneously, or somatic pairing is already present in some or all of the cells and the process of crossing over is itself induced. The overall evidence is in favour of the first possibility. In Ustilago, double mitotic crossovers within chromosome arms are much more common than double crossovers located in different arms (HOLLIDAY 1961b and unpublished data). Although more information is needed about the frequency of double crossovers with varying dose, it is known that they occur with very low doses. If UV produces fairly localised pairing (and separate evidence for this is available [HOLLIDAY 1964]), double exchanges could occur in these regions. Whereas if the effect of UV was more direct, then double exchanges would be very rare with low doses, and when they did occur, they should be distributed at random. In yeast, FOGEL and HURST (1963) have shown that somatic gene conversion and crossing over—which are believed to occur by distinct mechanisms—tend to be induced concomitantly by UV in a single diploid cell. They conclude that a process common to both mechanisms is stimulated by UV and that this might be chromosome pairing.

Many attempts have been made to influence the frequency of crossing over at meiosis by treating meiotic or premeiotic cells with various radiations or chemicals. With the exception of the results of EVERSOLE and TATUM (1956), which

could not be repeated by LEVINE and EBERSOLD (1958a), rather slight effects have usually been demonstrated (e.g. WHITTINGHILL 1951; LEVINE and EBERSOLD 1958b; TOWE and STADLER 1964). This is in sharp contrast to the great stimulation of mitotic crossing over by some of these agents. The results appear to be consistent with the idea that these treatments induce pairing and have little or no effect on meiosis, where pairing is complete and crossing over may reach its optimum.

Evidence has been recently obtained for the hypothesis that the insertion of a temperate phage into the bacterial genome and its release to form vegetative phage occur as a result of reciprocal recombination (CAMPBELL 1962, 1963). It is also known that the induction of phage occurs when DNA synthesis is temporarily inhibited in the lysogenic bacterium whether by thymine starvation (MELECHEN and SKAAR 1962), treatment with aminopterin (BEN GURION 1962), MC (OTSUJI *et al.*, 1959) or, of course, UV. Finally, in bacteriophage lambda it has been shown that genetic recombination is greatly increased in frequency under conditions which reduce or prevent genetic replication (J. J. WEIGLE, personal communication). These observations reinforce CAMPBELL's hypothesis and are consistent with the conclusions reached in this paper.

The data presented here and in the related paper dealing with the stimulation of mitotic crossing over by another DNA inhibitor, FUDR (EASTON and HOLLIDAY 1964) can be taken as evidence in support of the precocity theory of meiosis (DARLINGTON 1931). This theory has been out of favour since it was discovered that DNA replication at meiosis does not coincide with chromatid formation (see RHOADES 1961). However, providing that the *effective* replication of the chromosome occurs later than the replication of the DNA, the theory is still compatible with modern observations (HENDERSON 1962). In a mitotic cell an artificially imposed delay in DNA replication will lead to a subsequent delay in chromosome replication, and if the other processes necessary for cell division are proceeding, the prophase will become precocious relative to the timing of chromatid formation. Under these circumstances the precocity theory would predict that genetic pairing and thus recombination would take place. It is still an open question as to whether the observed induced recombination takes place in the genetic material which was already synthesised at the time DNA synthesis is interrupted, or whether it takes place after the recovery of DNA synthesis in the newly formed genetic material. This is experimentally less easy to determine than might be imagined, since normally the act of recombination is detected only by allowing the recovery of synthesis and the growth of the recombinant cell: it is therefore impossible to get information concerning the timing of the recombination event. Experiments are necessary which could detect recombination in the absence of genetic replication. One possibility would be to obtain recessive mutants which conferred resistance to a DNA inhibitor such as MC or FUDR. A diploid heterozygous for such a mutant in the presence of the inhibitor would be unable to synthesise DNA, but if recombination could occur under these conditions then cells homozygous for the mutant could arise and form colonies. Experiments with Ustilago along these lines are now in progress.

I wish to thank DRS. H. L. ROMAN and J. FRIIS for their interest and encouragement during the course of this investigation.

SUMMARY

The suggestion that the stimulation of mitotic crossing over in *Ustilago maydis* by ultraviolet light may be related to its known ability to inhibit genetic replication, has been tested by examining the effect on recombination of another inhibitor of DNA synthesis, the antibiotic mitomycin C. A concentration of mitomycin which was not markedly fungicidal was found to be a very efficient inducer of mitotic crossing over. The same concentration was also shown to induce somatic gene conversion and crossing over in a diploid strain of Saccharomyces. Unlike ultraviolet light, mitomycin C did not appear to induce any mutations in haploid Ustilago under the conditions in which it had a striking effect on recombination in a diploid. Thus the ability to induce crossing over is not necessarily related to mutagenic activity. These results support the hypothesis that an artificially imposed delay in genetic replication in a mitotic cell pushes it towards the meiotic condition and brings about the conditions which favour chromosome pairing and crossing over. The experiments provide no indication of the timing of recombination relative to replication.

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