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Induced mitotic crossing-over in relation to genetic replication in synchronously dividing cells of *Ustilago maydis*

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1. INTRODUCTION

Many attempts have been made in a number of organisms to influence the frequency of crossing-over by the experimental manipulation of the environment of cells undergoing meiosis; for instance, by treating such cells with radiation of various types, chelating agents, metabolic inhibitors or different temperatures. This work will not be reviewed here, suffice it to say that such treatments have had rather small or no effects on the frequency of recombination. When an effect has been demonstrated, it has been difficult to interpret in a way that adds very much to the knowledge of crossing-over which has been gained by standard genetic analysis of the products of untreated cells. The situation is entirely different with regard to genetic recombination in mitotic cells. Following the discovery of mitotic crossing-over in fungi (Pontecorvo, 1953; Pontecorvo & Käfer, 1958) it was found that in *Saccharomyces* (James & Lee-Whiting, 1955; Roman & Jacob, 1958; Fogel & Hurst, 1963; Wilkie & Lewis, 1963), *Ustilago* (Holliday, 1961*b*, 1964*a*) and *Aspergillus* (Morpurgo, 1962, 1963), somatic recombination could be greatly increased in frequency by treating diploid cells with radiation, chemical mutagens or metabolic inhibitors. As yet, this work has perhaps yielded less new information concerning the mechanism of genetic recombination than might have been hoped: but if it is possible to change radically the frequency of a process, then, as is the case with studies on mutation, there is the prospect of devising experiments which may obtain such information.

In this study further information about the effect of ultra-violet light on mitotic crossing-over is gained by treating cells at particular stages of the division cycle. This could only be done by using synchronized populations of cells: such populations have been obtained in *Ustilago* by applying a method which has been developed for use with *Saccharomyces* (Williamson & Scopes, 1962). The preliminary experiments to be reported here were carried out with a diploid which has been used routinely for recombination studies. The genetic markers which it carries are not such as to make

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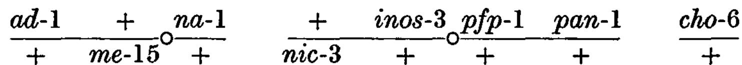
it particularly suitable for detecting the position of mitotic crossing-over; nevertheless, evidence for sequential recombination within chromosome arms was obtained when cells were treated with ultra-violet light at intervals throughout the period of genetic replication within the division cycle. Subsequent experiments using the same markers have confirmed this result. This pattern of recombination is most simply explained if genetic replication within chromosome arms is also sequential.

2. METHODS

Reference should be made to earlier papers for details of the methods which are not described here (Holliday, 1961*a* as modified in 1961*b*).

(i) *Genetic markers and the diploid strain*

The following auxotrophic markers were employed: *nic*-3, *inos*-3, *pan*-1, *cho*-6, *ad*-1 and *me*-15. They indicate requirement for nicotinic acid, inositol, pantothenic acid, choline, adenine and methionine respectively. The marker *na*-1 indicates inability to utilize nitrate as sole source of nitrogen, and *pfp*-1 confers resistance to the amino acid analogue *p*-fluorophenylalanine. This marker makes it possible to select rare mitotic recombinants from a population of diploid cells (Holliday, 1964*a*), although this method of estimating recombination is not used in the experiments reported here. These eight recessive markers are distributed on five chromosome arms in a prototrophic diploid, designated Diploid H, of the following genotype:



By analysis of the random products of meiosis it has been shown in several crosses that *ad*-1 gives about 15% recombination with *me*-15, and *nic*-3 about 35% recombination with *inos*-3. By analysis of mitotic crossing-over it has been shown conclusively that *ad*-1 is distal to *me*-15 and that *nic*-3 is distal to *inos*-3. Some of these data have been previously published, some are unpublished and some are presented in this paper. *pfp*-1 is rather rarely separated from *pan*-1 by mitotic crossing-over. None of the other markers show linkage, but indirect evidence from the study of the behaviour of a small sample of aneuploids indicates that *na*-1 is on the same chromosome as *ad*-1 and *me*-15, and that *pan*-1 and *pfp*-1 are on the same chromosome as *nic*-3 and *inos*-3.

(ii) *Media*

(*a*) *Complete and minimal medium*

0·3% potassium nitrate replaces ammonium nitrate as nitrogen source in the minimal medium, otherwise the media are the same as previously used.

(*b*) *Yeast extract peptone medium* (*YEP*)

1% yeast extract (Difco), 2% bactopeptone and 2% glucose (Roman, 1956).

(*c*) *Starvation medium*

An aqueous solution of 0·01 M KCl, 0·0025 M $CaCl_2$ and 0·0025 M $MgCl_2$ (Williamson & Scopes, 1960).

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(iii) *The induction of synchronous division*

The synchronizing procedure is based directly on the method developed for use with yeast by Williamson & Scopes (1962). All cultures were incubated at 30°C. in flasks or tubes of liquid medium in a New Brunswick incubator shaker, Model G 25. Cultures of *Ustilago* which are grown to stationary phase in nitrate minimal medium consist of cells of fairly uniform appearance and size. Only a small proportion have buds, and these are clearly fully-formed cells attached only by cell-wall material to the parent cell. This population is washed once and then incubated in YEP medium for 40 min. Following this the cells are washed three times in water or starvation medium and then resuspended in starvation medium. After several hours' incubation the cells are given another 40 min. feed in YEP medium followed by another period of starvation. With several cycles of such treatment, during which no buds are produced and the cells remain viable, the cells are brought to a uniform physiological state, such that, when they are finally allowed to divide, they do so in synchrony.

In these particular experiments the periods of starvation were, for convenience, not kept constant. The first feed was given in the evening and the cells starved overnight; the second feed was given in the morning and the third in the evening after 7 hours' starvation throughout the day. This procedure was followed on the third and fourth day, and the synchrony experiment was performed on the fifth day in complete medium after seven cycles of feeding.

(iv) *Cell counts, cell and nuclear division*

Cell numbers were counted in all the experiments with a Model A Coulter electronic particle counter. The proportion of cells which were binucleate, and the timing of the first mitotic division during the growth of the synchronized culture was determined by phase contrast observation with a Zeiss Standard Universal microscope. The proportion of cells which were initially inviable and did not produce buds was determined both by microscopic examination and by viable counts.

(v) *Estimation of deoxyribonucleic acid (DNA)*

In the second experiment a large number of cells (*ca.* 3×10^9) were put through the synchronizing procedure and grown in the final culture so that adequate samples of cells would be available for DNA determinations. These were kindly carried out by Mrs R. Esposito, using a standard method described elsewhere (Esposito & Holliday, 1964). At each time-interval duplicate measurements were made, each being based on a sample of at least 10^8 cells.

(vi) *Ultra-violet treatment and the detection of recombinants*

Samples of cells were removed at intervals from the synchronously dividing culture, diluted and suspended at low concentration in 10 ml. water in a petri dish. This was agitated during a treatment of 120 sec. with ultra-violet light from two Hanovia germicidal lamps emitting about 200 ergs/cm.^{2}/sec. at the target distance

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of 12 in. Appropriate numbers of surviving cells were spread on plates of complete medium. The cell survival was determined by colony counts, and plates with up to 300 colonies were replicated to nitrate minimal medium. Recombinants were detected either as whole colonies which failed to grow on this medium, or as mosaic colonies where only a proportion (usually half the colony) of the cells failed to grow. Some of the whole colony recombinants were mosaic consisting of a mixture of *ad* and *me* auxotrophic cells, or a mixture of *nic* and *inos* auxotrophs. Such mosaics were either recognized by the appearance of the colony on complete medium, or of the cells on the replica; otherwise they were detected as mixed phenotypes during the identification of the nutritional requirements of the recombinants. This identification was carried out by subculturing the segregants to plates of complete medium, and then replicating these to appropriate media. Although occasional mosaic colonies consisting of prototrophic and auxotrophic cells may have escaped detection, it is considered unlikely that any wholly auxotrophic colonies would not be identified.

3. EXPERIMENTAL RESULTS

(i) *Synchronous growth and division*

In this and the subsequent three sections reference should be made to Fig. 1. When the synchronized cells are allowed to grow in complete medium buds begin to appear after 60 min. incubation: the buds are usually produced at a characteristic angle to the long axis of the parent cell. At about 85 min. the nucleus usually passes into the bud and divides there at a mean time of about 95–100 min.: one nucleus passes back into the parent cell. The first cells begin to separate at 120 min. and the last at 150 min. The normal generation time in complete medium is just under 2 hours. In both experiments about 10% of the cells were binucleate, they produced a bud at each end and were not out of synchrony with the rest of the population. About 10% of the cells were inviable, thus after the first division the number of cells doubles almost exactly. Although every attempt was made to make the populations in the two experiments as similar as possible, it was necessary in the second experiment to grow the cells at a higher concentration and in a much larger container which may have been less well aerated. The first division was identical in both experiments, but the second division was somewhat slower in the second experiment. In neither experiment was the synchrony as pronounced at the second division as at the first. This appears to be due to the daughter and parent cells dividing at different rates, thus after one division the population consists of two types of cell that divide out of step with each other. However for the type of genetic experiment which was performed, one well-synchronized division is as much as can be handled.

(ii) *The period of genetic replication*

It is evident from the results shown in Fig. 1 and from several other experiments with synchronized cultures (Esposito & Holliday, 1964) that the cells at the start of the experiment already have the DNA they require for the first mitotic division, i.e. the nuclei contain the 4C amount of DNA. This contrasts with the situation in

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Saccharomyces, synchronized by a similar procedure, where a doubling of DNA occurs before the first mitosis (Williamson & Scopes, 1960). In *Ustilago*, DNA synthesis starts quite soon after mitosis, at about 100 min., and continues for about

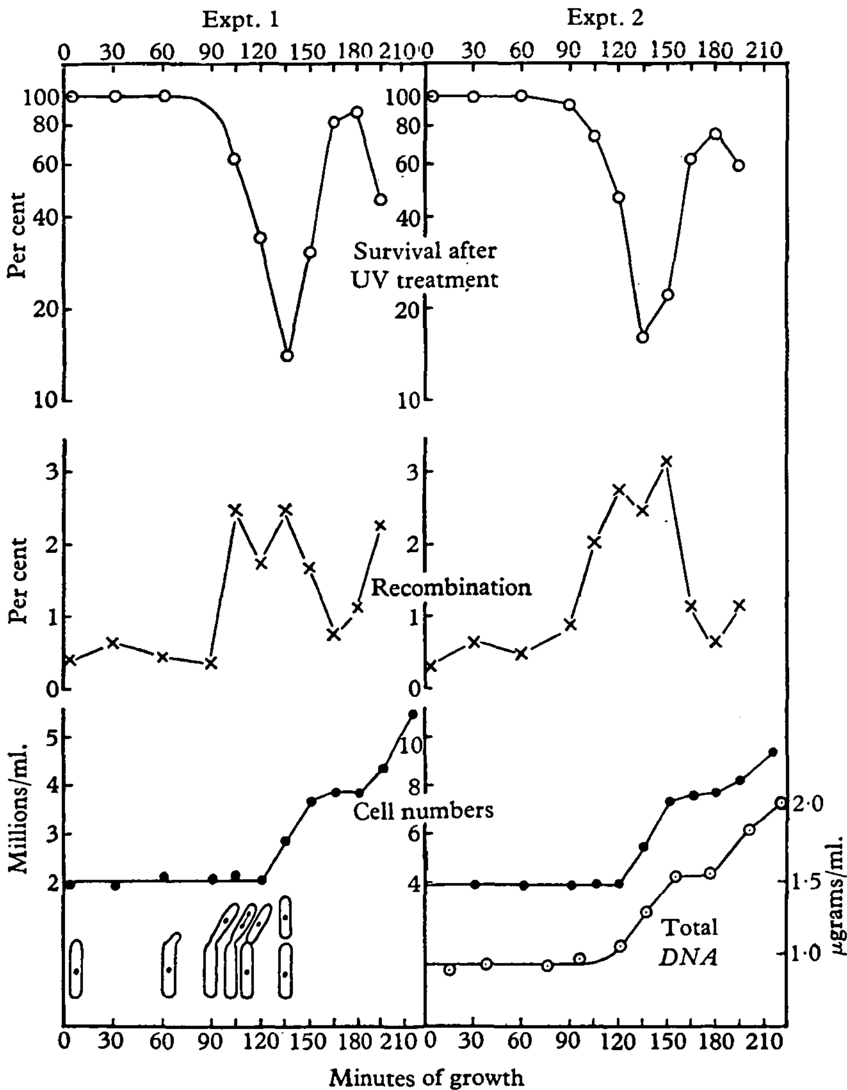

Fig. 1. The overall results from Expts. 1 and 2. From the top: percentage of cells plated which formed colonies after a constant dose of ultra-violet light; frequency of recombinants among the survivors; cell number and total DNA content; approximate mean times for budding, mitosis and cell separation at the first division.

an hour. During this time there is one round of genetic replication if it is assumed that the inviable cells contain the same amount of DNA as the rest but do not synthesize any more. The lag in DNA synthesis that is observed between the two divisions is therefore at the time it would be expected. Since the DNA doubles in less

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time than the whole division cycle and there is a 30-min. scatter in the division time, DNA synthesis must be restricted in these cells to a discrete part of this cycle, as appears to be the case for all organisms which have been examined, other than bacteria.

(iii) *Survival after ultra-violet light treatment*

The cells show a remarkable variation in sensitivity to ultra-violet light throughout the division cycle. With the dose used there was no detectable cell death when cells were treated at 0, 30 or 60 min. At 90 min. the cells begin to be killed, and the sensitivity increases sharply to a maximum at 135 min.; there is then an equally rapid drop in sensitivity from 135 to 165 min. Apart from the two experiments in Fig. 1, this pattern has been observed in several other experiments where ultra-violet survival in a synchronous population has been examined. When the synchrony at the second division is reasonably good, as in Expt. 1, the cells show a second cycle of sensitivity after 180 min. It is evident that the periods when the cells are resistant to ultra-violet correspond fairly well to the periods when they are not undergoing DNA synthesis; and the time at which they are most sensitive is exactly the mid-point in the round of genetic replication after the first mitosis, presumably when all or nearly all the cells are synthesizing DNA. In very detailed studies by Swann (1962) a similar variation in sensitivity to ultra-violet throughout the division cycle has been observed in the fission yeast *Schizosaccharomyces pombe*; and the same observation has been made in *Saccharomyces cerevisiae* (Williamson & Holliday, unpublished data; see below).

(iv) *The frequency of induced recombination*

In both experiments a very similar pattern of induced recombination was observed. In Diploid H and similarly marked heterozygous diploids the frequency of spontaneous mitotic crossing-over is of the order of 0·1%. Although there was no ultra-violet killing at 0, 30 and 60 min., there was about a fivefold increase in recombination over this control value. But the bulk of the recombinants were obtained when the cells were treated during the sensitive period: the frequency of recombination was then about twenty-five times the control value. However, the four readings 105–150 min. yielded rather similar recombination frequencies: there was not a peak in recombination corresponding to the peak in sensitivity to ultra-violet, hence the simple quantitative relationship between the lethal effects of ultra-violet and its ability to induce recombination which has previously been observed (see Holliday, 1964*a*) does not apply to the synchronized system. After 150 min. the frequency of recombination drops to below 1% and then rises again. This is shown clearly in Expt. 1, where the rise in recombination occurs at the same time as the fall in ultra-violet resistance after the second mitosis. Thus in this experiment the more rapid second cell division is correlated both with the second period of ultra-violet sensitivity and the second rise in recombination frequency.

The details of the recombination data are given in Tables 1 and 2. Nearly all the *pan* recombinants are also homozygous for *pfp*-1: the scoring of this marker adds

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little information and is omitted from the Tables. Of the 415 segregant colonies which were obtained, 392 appear to have arisen as the result of a single mitotic exchange, 11 as the result of two exchanges in one arm, 8 as a result of two exchanges each in a different arm, and 4 are of uncertain origin—the result of multiple exchange,

Table 1. *Frequency of survival and recombination in cells treated with a constant dose of ultra-violet light at different times during synchronous division*

	Minutes of growth	Survival (%)	Colonies examined	Recombinants (No.)	Recombinants (% and S.E.)
Experiment 1:					
Untreated	0	100	1638	0	— —
U.V. treated	0	100	1987	8	0·40 ± 0·15
	30	100	1553	10	0·64 ± 0·21
	60	100	1631	7	0·43 ± 0·16
	90	90*	1073	4	0·37 ± 0·19
	105	61	1531	38	2·48 ± 0·40
	120	34	917	16	1·74 ± 0·44
	135	14	489	12	2·45 ± 0·71
	150	31	1120	18	1·61 ± 0·38
	165	83	1852	14	0·76 ± 0·21
	180	89	2046	23	1·12 ± 0·23
	195	46	1377	31	2·25 ± 0·40
	Total (U.V. treated)		15,576	181	
Experiment 2:					
U.V. treated	0	100	1304	4	0·31 ± 0·15
	30	100	804	5	0·62 ± 0·27
	60	100	1609	7	0·44 ± 0·16
	90	95	2761	23	0·83 ± 0·17
	105	75	1407	28	1·99 ± 0·38
	120	47	1963	54	2·75 ± 0·37
	135	16	1879	46	2·45 ± 0·36
	150	22	791	25	3·16 ± 0·63
	165	61	955	11	1·15 ± 0·35
	180	77	1108	7	0·63 ± 0·24
	195	60	1768	24	1·36 ± 0·28
	Total		16,349	234	

* Estimated value; a direct viable count was not possible owing to a pipetting error.

aneuploidy or chromosome structural rearrangement. The number of double exchanges in different arms is close to what would be expected on a random basis. About 40% of all the segregants arose as mosaics, mainly of the type where the colony was half auxotrophic and half wild-type, but 40 colonies were *nic/inos* or *ad/me* mosaics. These are derived from cross-overs where the reciprocal products of

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the exchange survived. Most of the other mosaics probably also represent reciprocal cross-over products; but since a significant fraction of the treated cells were binucleate, a cross-over in one nucleus and not in the other could produce a mosaic which was

Table 2. *The total frequency and the simplest origin of the recombinants*

Marked intervals	Recombinants: Phenotype	Recombinants: Frequency	Position of mitotic crossing-over
I, II: o —— + (I) nic-3 (II) / inos-3 (I) + (II)	*nic*	71	I or II
	nic/+	43	II
	inos	22	I
	nic/*inos*	21	I
	inos/+	6	I and II
III, IV: o —— me-15 (III) + (IV) / + (III) ad-1 (IV)	*ad*	32	III or IV
	ad/+	12	IV
	me	21	III
	ad/*me*	19	III
	me/+	5	III and IV
V: o —— + / *na*-1	*na*	47	V
	na/+	31	V
VI: o —— + / *pan*-1	*pan*	15	VI
	pan/+	29	VI
VII: o —— + / *cho*-6	*cho*	15	VII
	cho/+	14	VII
	Two requirements	8	In each of two chromosome arms
	> Two requirements	4	Origin uncertain

$\frac{1}{4}$, $\frac{1}{3}$ or $\frac{1}{2}$ auxotrophic depending on the survival of the four nuclei following division. The *inos*/+ or *me*/+ colonies could in fact have arisen from binucleate cells rather than from the double cross-overs indicated in Table 2.

(v) *The polarity of induced recombination*

So far as the limited data permit a comparison, it appears that when cells are treated which are not undergoing genetic replication, the spectrum of recombinant phenotypes is quantitatively similar to the overall spectrum. However when

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populations of cells are treated during replication, the spectra of recombinants obtained for the various times of ultra-violet treatment show striking variation. This is shown in Table 3, where each marker is treated independently, and the mosaics are not considered as separate classes since the origin of some of them, as has been described, is somewhat ambiguous. For instance, a *nic*/+ mosaic is scored as a *nic* recombinant and a *nic/inos* recombinant is scored as two recombinants, *nic* and

Table 3. *Summary of the recombination data from Experiments* 1 *and* 2 (*see text*)

Minutes of growth	Expt.	Recombinant phenotypes						
		ad	*me*	*nic*	*inos*	*na*	*cho*	*pan*
0, 30, 60	1st	2	2	13	3	5	1	2
	2nd	1	2	6	2	2	3	3
		3	4	19	5	7	4	5
90	1st	0	0	0	0	3	0	1
	2nd	4	2	10	2	3	2	4
		4	2	10	2	6	2	5
150	1st	6	3	15	5	7	5	3
	2nd	2	3	9	1	8	1	5
		8	6	24	6	15	6	8
120	1st	1	1	5	3	4	1	1
	2nd	5	4	16	3	16	7	7
		6	5	21	6	20	8	8
135	1st	5	0	4	2	0	0	1
	2nd	6	2	12	12	14	3	5
		11	2	16	14	14	3	6
150	1st	4	7	2	1	2	2	2
	2nd	6	6	7	2	4	1	2
		10	13	9	3	6	3	4
165	1st	2	1	3	2	3	1	3
	2nd	4	4	1	0	2	0	0
180	1st	1	0	12	3	2	2	4
	2nd	4	3	0	2	0	0	0
195	1st	7	4	9	6	7	1	1
	2nd	4	1	13	3	3	1	2
	Total	64	45	137	52	85	31	46

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inos. This is considered legitimate since had the *inos* marker been absent from the diploid, the *nic* recombinant would have been detected as a *nic*/+ recombinant and *vice versa*. A cross-over distal to *inos* can produce only a *nic* recombinant, and a cross-over proximal to *inos* has an equal chance of producing a *nic* or an *inos* recombinant; similarly with markers *ad* and *me*. Recombinants which arose as a result of cross-overs in different arms are included in the table. It should be noted that the overall recombination frequency of the proximal markers *inos* and *me* is lower than the overall frequency of the markers distal to them, in agreement with the known order within the arms. Furthermore, the ratio of the difference between the *nic* and *inos* recombination frequencies and the *ad* and *me* recombination frequencies (85:19), is in reasonable agreement with the ratio of the meiotic recombination frequencies for these pairs of markers (35:15).

In Expt. 1 the number of colonies scored after treatments at 90, 120 and 135 min. was not large enough to yield more than a small sample of segregants. At 105 min. the ratio of *ad*, *me* and *nic* recombinants was 6:3:15, this changed to 4:7:2 at 150 min. and then to 1:0:12 at 180 min. and 7:4:10 at 195 min. In Expt. 2 the pattern is even more striking: at 90, 105 and 120 min. the *nic* recombinants (along with *na* recombinants) are very frequent, but at 135 min. the *inos* recombinants are as common. At 150–180 min. the *ad* and *me* recombinants come to exceed in frequency both *nic* and *inos*, but at 195 min. the *nic* recombinants are again the most frequent. As has been pointed out the two experiments are themselves synchronized initially but towards the end of the experiments growth is clearly slower in Expt. 2. This is reflected in the pattern of recombination, the spectrum of recombinants at 180 min. in Expt. 1 is very like that at 195 min. in Expt. 2: and the spectrum at 180 and 165 min. in Expt. 2 is like that at 165 or 150 min. in Expt. 1. It is considered reasonable to add the results from the two experiments up to the 150 min. reading but not thereafter. The actual frequencies of the seven genotypes are given in Fig. 2. Since the values are based on quite small samples of recombinants, they are clearly not accurate measurements of recombination frequency for each marker: the purpose of Fig. 2 is to show the overall pattern of recombination as clearly as possible. It is particularly significant that the two experiments produced the same basic result; and also that the pattern of recombination when ultra-violet treatment was given during the first round of replication, appears to be entering a similar second cycle with the treatments during the initial part of the second round of replication.

It might be asked whether the variation in recombination frequencies with the different readings could in fact be fortuitous, since they are based on rather small samples of recombinants. That it is highly probable that the distribution of recombinants for the linked markers is non-random is shown by a χ^2 test on the combined data from Expts. 1 and 2, 105–150 min. The overall recombination data from the experiments are used to calculate the expected frequencies for each of the four readings. The heterogeneity χ^2 (9 d.f.) = 29·3, $P < 0{\cdot}001$. The data from the 165–195 min. readings appear to be equally heterogeneous, but since the data cannot be combined from the different experiments, the samples are not large enough to make

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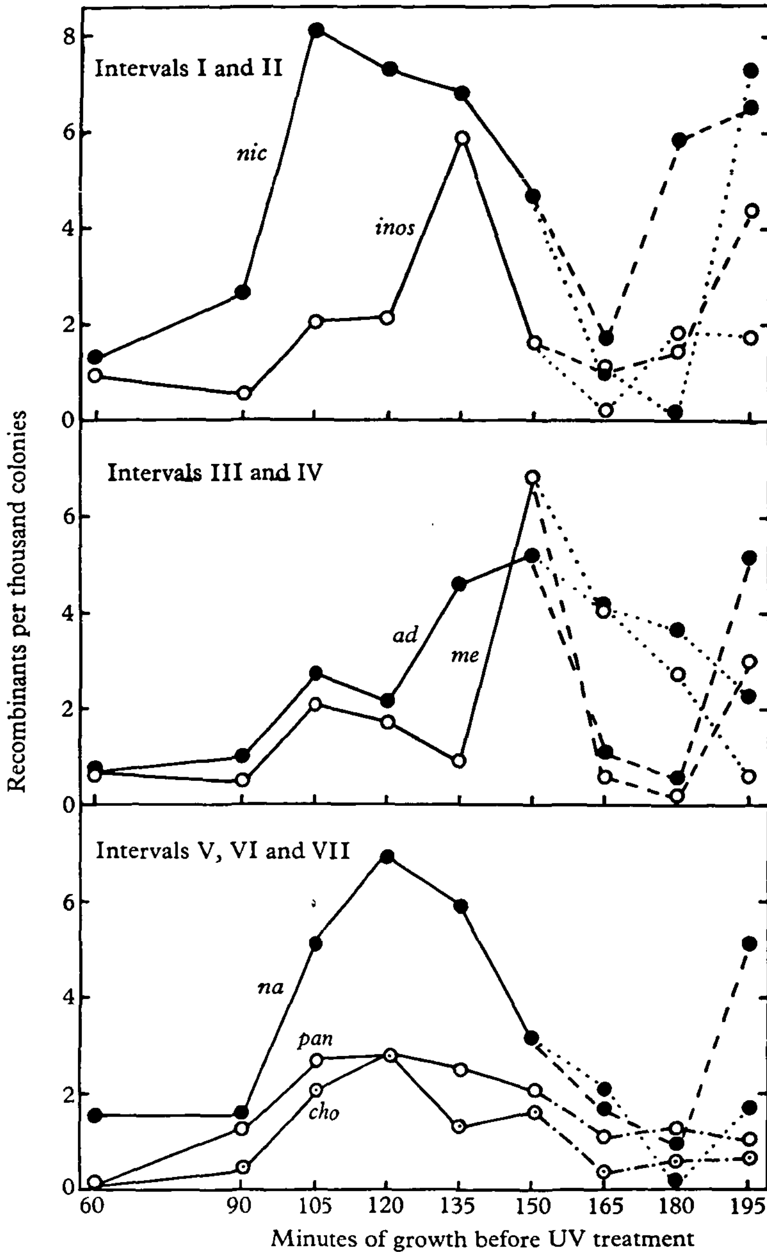

Fig. 2. The frequency of recombination for the seven markers in Diploid H. The data from Expts. 1 and 2 are combined for readings up to 150 min.; for later readings the broken lines indicate the results from Expt. 1 and the dotted lines the results from Expt. 2. For clarity, this distinction is not made for the markers *pan*-1 and *cho*-6.

the same simple test legitimate. There is therefore no reason to believe that the pattern of recombination which emerges from the data is not in fact a genuine one, and it has in fact been confirmed in subsequent experiments with the same genetic markers.

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4. DISCUSSION

The results which have been obtained show that there is a discrete period in the division cycle when the cells are sensitive to ultra-violet light both with regard to its lethal effect and its ability to induce mitotic crossing-over, and that this period corresponds to the period of DNA synthesis in the cell. Although the cells first become sensitive to ultra-violet light soon after the first mitosis, when the DNA content of the nuclei is halved (from the 4C to the 2C condition), it seems unlikely that this sensitivity can be directly related to the amount of DNA per cell. The rise in sensitivity to the maximum at 135 min. is longer than the scatter in the times of cell division. If the DNA content per nucleus was the important factor, then after mitosis there should be a much sharper rise in sensitivity followed by a slower fall as DNA synthesis returned the nuclei to the 4C condition. A skewed peak of this type was not observed in these or in several other experiments. Furthermore, on genetic grounds it is difficult to see why one diploid cell with a 4C nucleus would be more likely to form a colony after irradiation than two attached cells each containing 2C nuclei at 105 min.; or at 120 min., when the attached cells have already started genetic replication. That the period of ultra-violet sensitivity is the same as that of DNA synthesis is strikingly confirmed by comparable studies in *Saccharomyces* (Williamson & Holliday, unpublished data). Here, synchronized cells undergo a round of DNA synthesis before the first nuclear division. When cells are treated with ultra-violet at intervals throughout the division cycle, the sensitive period coincides with the pre-mitotic period of DNA synthesis and is not related to the amount of DNA per cell or per nucleus.

Considerable information is now available concerning the nature of the chemical changes in DNA following ultra-violet irradiation. It is known that with the type of dose normally used in biological experiments a large number of lesions or hits must occur in the genome and that most of these lesions are thymine dimers which can be removed by repair mechanisms (see Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964). It seems reasonable to suppose that a cell which is not synthesizing DNA would have the opportunity to repair most or all of the damage before the onset of synthesis; whereas if irradiation occurs during replication the damage will block synthesis immediately and the consequent state of unbalanced growth may be lethal before the repair mechanism can operate. Alternatively some of the damage may be stabilized by replication before repair can occur, with the consequent production of dominant lethal mutations. These causes of ultra-violet-induced death are compatible with the fact that in *Ustilago* (contrary to preliminary observations (see Holliday, 1961*b*)), haploid cells are scarcely more sensitive to ultra-violet than are diploids. The first round of DNA synthesis in the synchronized population takes about 60 min., if the scatter in initiation of synthesis is the same as the scatter in division time, namely 30 min., then the cells which begin DNA synthesis earliest will be finishing synthesis at about the time the latest cells begin. Half-way through the period of replication virtually all the cells will be undergoing synthesis whereas before and after this time a proportion will not be doing so. This half-way point is close to the peak of ultra-violet sensitivity.

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It has been suggested that the ability of ultra-violet light to inhibit DNA synthesis, whilst leaving other macromolecular synthesis unaffected, results in a state of unbalanced growth which is related to the condition of the meiotic cell. In this state the chromosomes tend to pair, probably in localized regions of the genome, and consequently there is the opportunity for mitotic crossing-over. The evidence in favour of this hypothesis has been fully discussed elsewhere (Holliday, 1964*a*). The results reported here are compatible with this view and are interpreted as follows.

Considering the scatter in the division times within the synchronized population the ready detection of polarity in the recombination frequencies for the linked markers strongly suggests that a very precise sequential process underlies the observations. It is very difficult to provide any adequate interpretation of the data unless this sequential process is in fact genetic replication. Suppose that genetic replication starts from the ends of all the chromosome arms at about the same time and proceeds towards the centromeres: a dose of ultra-violet light will interrupt DNA synthesis at every replicating point. Suppose further that fairly localized pairing occurs in the vicinity of the point of interruption with the consequent opportunity for crossing-over. Then markers which are distant from the centromere, as judged by the overall frequency of recombination, would show a high frequency of recombination for several successive readings (*nic*-3 and *na*-1). A proximal marker in one of the same chromosome arms (*inos*-3) would show a low frequency of recombination initially, but in the readings late in the period of genetic replication would show a frequency as high as the distal marker. If markers are on a long chromosome arm but nevertheless fairly close to the centromere, they would also show low recombination initially and a peak in the later readings, with the proximal marker having a peak later than the distal (*me*-15 and *ad*-1). Markers which are on short chromosome arms which would finish their replication early could give a low overall recombination frequency but a fairly early recombination peak (*cho*-6 and *pan*-1). Thus on the basis of previous evidence concerning the relationships between ultra-violet irradiation, macromolecular synthesis, pairing and crossing-over, and with the assumptions outlined above, the results fall into a pattern which is entirely consistent with the interpretation which has been suggested. On the basis of this interpretation several other points emerge from the data. (1) The *ad* and *me* recombinants are quite common in late readings when the *nic* and *inos* recombinants are infrequent; the chromosome arm carrying the former markers would therefore be somewhat longer than that carrying the latter markers. (A marker, *leu*-1, distal to *ad*-1 is in fact known (Holliday, 1961*b*).) (2) If marked chromosome arms do indeed belong to the same chromosome then the replication does not cross the centromere. (3) The observation that a marker close to the centromere can recombine for a particular reading, e.g. *me*-15 at 150 min., as frequently as one distant from the centromere in earlier readings, must indicate that the localization of crossing-over is quite precise. If for instance pairing and recombination occurred anywhere from the point of interrupted synthesis to the end of the chromosome arm, i.e. in the chromosome material that was already duplicated at the time of irradi-

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ation, then distal markers would show a higher frequency of recombination than proximal ones in the late treatments. Conversely, if pairing and recombination occurred from the point of interruption to the centromere, i.e. in the region which remained to be duplicated when the cells recovered from ultra-violet inhibition, then the markers near the centromere would recombine as frequently in the early treatments as the late ones. (4) With regard to the low frequency of recombination which is induced when the cells are irradiated before DNA synthesis, this could be due to scattered ultra-violet induced lesions which are not repaired or repairable before synthesis occurs. (5) During a round of genetic replication the initial rate of DNA synthesis should be higher than the final rate, since initially all the chromosomes would be replicating, whereas finally only the longer ones would be doing so. Very frequent and accurate measurements of DNA synthesis during synchronized division might reveal this pattern and therefore provide additional evidence for the suggested explanation of polarized recombination.

It may well be of course that the basic interpretation is the correct one, but that it is a considerable over-simplification. Chromosomes may replicate sequentially but they may not start their replication in synchrony. Less likely perhaps, certain arms or parts of chromosomes may have a reversed direction of synthesis. Further it cannot yet be assumed that the frequency of ultra-violet-induced recombination always gives a valid measure of map distance, although for the linked markers used in these experiments it appears to do so. Only further experiments of the same type with more fully marked chromosomes will establish a convincing overall pattern of replication and recombination. If the interpretation given here turns out to be valid, this will mean that it will be possible to map genes on the chromosome according to where their recombination peak lies in relation to the period of DNA synthesis, a method of mapping in some ways analogous to that used in interrupted mating experiments in *Escherichia coli* (Wollman, Jacob & Hayes, 1956). Further, it should be possible to estimate the distance of any marker not only from the centromere but also from the end of the chromosome arm, a measurement that has so far not been possible by genetic analysis alone.

In other organisms the situation with regard to the pattern of genetic replication is clear-cut only in bacteria. Both by autoradiography and genetic methods it has been shown that the *Escherichia coli* genome replicates sequentially with a single growing point (Cairns, 1963; Nagata, 1963). The same conclusion has been reached in studies with *Bacillus subtilis* (Yoshikawa & Sueoka, 1963*a*,*b*; Wake, 1963). Within the fungi the results of fine structure analysis in *Ascobolus* (Lissouba, Mousseau, Rizet & Rossignol, 1962) have been interpreted to indicate that genetic replication is sequential at least for short regions of the genome. But these results can be explained in a way which does not depend on any particular pattern of replication (Holliday, 1964*b*). An approach to the problem more closely related to that used in this paper has been used in yeast (Gorman, Taruo, Laberge & Halvorson, 1964). It was found that in synchronously dividing cultures, enzymes are synthesized in a stepwise manner, with different enzymes doubling in sequence at different stages of the division cycle. It is difficult to explain this surprising result unless the

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doubling in amount of enzyme follows an ordered duplication of the relevant genes. When the location of the genes is determined by standard methods it should then be possible to draw conclusions about the pattern of genetic replication. In higher organisms the results are confusing: for instance, whereas Pelc and La Cour (1959) and Taylor (1958) found evidence for sequential replication from the ends of the chromosomes arms to the centromere; Taylor (1960), Lima-de-Faria (1961), Peacock (1963) and Stubblefield & Mueller (1962) considered that chromosomes have several points of DNA synthesis. This is certainly the case in the giant salivary gland chromosomes of *Drosophila* (Plaut, 1963). All these results are based on autoradiographic methods, and a difficulty of experiments of this type is to distinguish between true chromosome duplication and the turnover of an unstable DNA fraction, such as has now been demonstrated in higher plants (Sampson, Katoh, Hotta & Stern, 1963). Although the evidence from genetic experiments depends on inference rather than direct observation, these experiments at least have the advantage of being free from this particular difficulty.

SUMMARY

A method is now available for synchronizing the division of populations of cells of the smut fungus *Ustilago maydis*. In two experiments carried out with a diploid strain heterozygous for several biochemical markers, samples of cells were removed at intervals through the synchronized division cycle and treated with a constant dose of ultra-violet light. Cell survival and the frequency of the various recombinants resulting from induced mitotic crossing-over were recorded. In addition the period of DNA synthesis in the unirradiated population has been measured. During this period the cells are very sensitive to ultra-violet light and those that survive contain the highest proportion of induced recombinants. In so far as the markers make it possible to locate the position of cross-overs, cells which are irradiated early in the period of genetic replication show most crossing-over towards the ends of the chromosome arms, whilst cells which are treated late in this period show crossing-over near the centromeres. The data are most easily interpreted by supposing that chromosome replication begins at the ends of the arms and proceeds to the centromere, and that the temporary interruption of this process by ultra-violet light can result in pairing and crossing-over in the vicinity of the points of interruption.

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