

THE EFFECT OF ULTRAVIOLET LIGHT ON RECOMBINATION IN YEAST

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IN several species of fungi, diploid cultures that are heterozygous for one or more genes produce somatically, without the intervention of sexual reproduction, diploid segregants which are homozygous for one or more of these genes. PONTECORVO, ROPER, HEMMONS, MACDONALD and BUFTON (1953) and PONTECORVO and KÄFER (1958) have shown that such segregations of homozygous diploids in *Aspergillus nidulans* are the results of either of two mechanisms: *mitotic crossing over*, as first discovered in *Drosophila melanogaster* by STERN (1936), in which crossing over occurs at the four-strand stage as in meiosis but the centromeres divide as in mitosis; or *nondisjunction* of sister chromatids at mitosis without crossing over.

In untreated *A. nidulans* the frequency of mitotic crossing over is less than meiotic crossing over by a factor of about 10^4 (PRITCHARD 1960). The frequency of mitotic nondisjunction is even rarer than the frequency of mitotic crossing over (but the relative frequencies of these two mechanisms depends upon the selective method used [PONTECORVO *et al.* 1953]). Various treatments given to induce somatic segregation have increased the frequency of segregation. FRATELLO, MORPURGO and SERMONTI (1960) found that in *Aspergillus nidulans* increasing doses of ultraviolet light (UV) and nitrogen mustard increased the frequency of mitotic nondisjunction but had no effect on mitotic crossing over. However, HOLLIDAY (1961) working with *Ustilago maydis* obtained a 90-fold increase over the spontaneous rate of mitotic crossing over with UV treatment.

A further important difference in mitotic crossing-over in different organisms has been found when intragenic recombination is analysed. PRITCHARD (1960), working with the *ad-20* gene in *A. nidulans*, found the reciprocal products, the + allele and the double mutant, in the same diploid nucleus in the proportion expected from the mechanism of reciprocal mitotic crossing over. ROMAN and JACOB (1958), on the other hand, working with a diploid heteroallelic for *is-1* in *Saccharomyces*, did not find the reciprocal double mutant and concluded that the recombination was not the result of a reciprocal crossover (see also KAKAR 1963).

Spontaneous mitotic crossing over in *Aspergillus nidulans* and spontaneous and induced mitotic crossing over in *Ustilago maydis* are so rare that in general only segregants for homozygosity in one arm of one chromosome are found in any one nucleus. In *Saccharomyces cerevisiae*, on the other hand, a high frequency of simultaneous homozygosity of unlinked genes on different chromosomes after UV treatment was described by WILKIE and HAWTHORNE (1961). These results

could be explained if crossing over was frequent in all chromosomes in any one nucleus. In the present paper these segregations in yeast are described in detail and are discussed in terms both of the mechanism of mitotic crossing over and of a mechanism of meiotic crossing over followed by restitution after first division of meiosis, as described by JAMES and LEE-WHITING (1955) and rejected by them on the basis of their data. It is also hoped that some of the differences between *Aspergillus*, *Ustilago* and *Saccharomyces* may be explained.

Theory of mechanisms of somatic homozygosis

Mitotic crossing over. This has been fully analyzed by STERN (1936) for *Drosophila melanogaster* and by PONTECORVO *et al.* (1953) for *Aspergillus nidulans*, and is given here only for comparison with the other mechanisms. It is assumed that some degree of somatic pairing occurs. This has been observed in *Drosophila* and implied but not observed in *Aspergillus*. Crossing over occurs at the four-strand stage and the centromeres replicate and disjoin normally as in mitosis. Each crossover leads to homozygosis of the genes distal to the crossover in 50 percent of the derivative nuclei, as shown in Figure 1.

The consequences of mitotic crossing over are: (1) All genes distal to the crossover will be homozygous in 50 percent of the nuclei. (2) If crossing over has occurred between two allelic mutants as illustrated in the figure, wild-type recombinants will be accompanied by one of the original mutants, i.e. a nonreciprocal product in 50 percent of the nuclei and the reciprocal double mutant in the remainder. The wild alleles and the reciprocal double mutant are on the pair of chromosomes which is heterozygous for the distal markers (ROPER and PRITCHARD 1955; PRITCHARD 1960). (3) Selection for homozygosis for a distal marker will select all single and all 3-strand double crossovers between the centromere and the distal marker. (4) Positive correlation will exist between distance from the centromere and frequency of homozygosis of a gene.

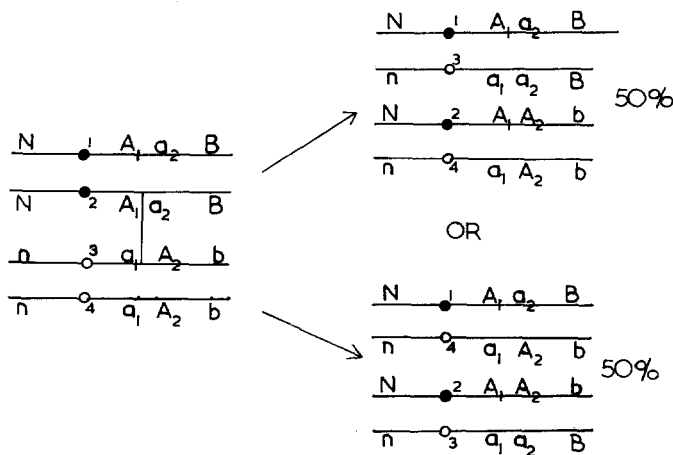


FIGURE 1.—Results of mitotic crossing over.

Nondisjunction of centromeres at mitosis. This has been found in *Aspergillus* by PONTECORVO *et al.* It results in complete homozygosis of all the genes on a chromosome, as shown in Figure 2. The consequences of nondisjunction of centromeres at mitosis are: (1) Homozygosis of all the genes on both arms of the chromosome; (2) no crossing over; and (3) failure to obtain wild-type alleles from two mutant alleles.

Meiotic crossing over and restitution. It is assumed that pairing of chromosomes occurs as in a normal meiosis, crossing over is at the meiotic rate and at the four-strand stage. As in normal meiosis, the centromeres segregate and do not divide at the first division, but the chromosomes do not separate into four separate nuclei at the second division. This may be due to nondisjunction or to restitution of the pairs of haploid telophase nuclei into two diploid nuclei. This is shown in Figure 3.

The consequences of meiotic crossing over and restitution are: (1) All genes proximal to the crossover will be homozygous. (2) The reciprocal products of interallelic crossing over will be present in the same nucleus only in certain cases involving a 3-strand double. The wild-type allele and one of the mutant alleles will be on two chromosomes which are heterozygous for distal markers. (3) There will be negative correlation between the distance from the centromere and the frequency of homozygosis of a gene.

Crossing over at the two-strand stage, meiotic action of centromeres and restitution. It is assumed that pairing of chromosomes occurs as in normal meiosis,

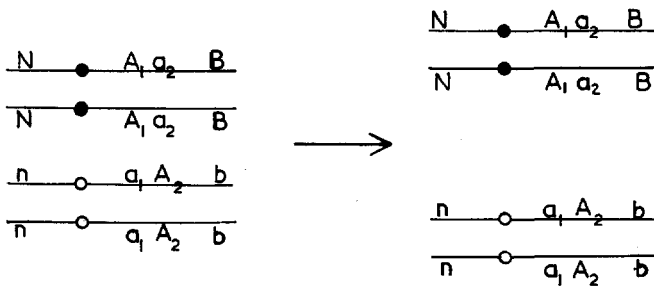


FIGURE 2.—Results of nondisjunction at mitosis.

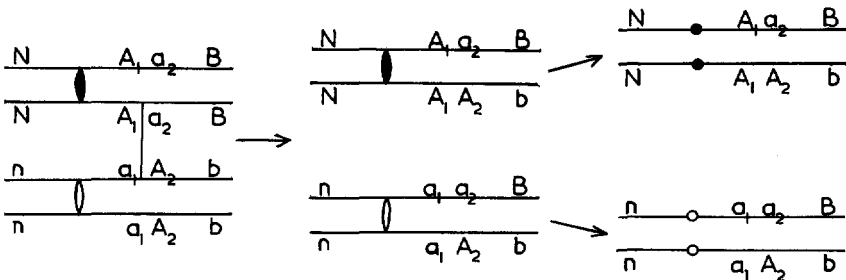


FIGURE 3.—Results of crossing over with meiotic segregation of centromeres and restitution.

but that crossing over occurs as the result of UV treatment at the two-strand stage. Restitution or nondisjunction again occurs at the second division (Figure 4).

The consequences of crossing over at the two-strand stage with meiotic action of centromeres and restitution are: (1) All genes will be homozygous in both nuclei. (2) A higher than normal frequency of crossing over will occur. (3) No correlation will exist between the distance from the centromere and the frequency of homozygosis of a gene.

MATERIALS AND METHODS

Diploid strains of *Saccharomyces cerevisiae* were synthesized having the following gene combinations:

No. 1327	$\frac{hi_6}{+}$	$\frac{tr_1}{+}$	$\frac{ur_3}{+}$	$\frac{me_2}{+}$	$\frac{ad_8}{+}$	$\frac{le_1}{+}$	$\frac{ar_3}{+}$	$\frac{ly_7}{+}$
No. 1380	$\frac{hi_8}{+}$	$\frac{ad_2}{+}$	$\frac{tr_5 + ad_6}{+ le_1 +}$	$\frac{ly_2}{+}$	$\frac{ty_1}{+}$	$\frac{ur_3}{+}$	$\frac{me_2}{+}$	
Diploid I	$\frac{ur_3}{+}$	$\frac{ty_1}{+}$	$\frac{me_2}{+}$	$\frac{ar_4}{+}$	$\frac{ly_7}{+}$	$\frac{hi_8}{+}$	$\frac{ad_2}{+}$	$\frac{tr_5 + ad_6}{+ le_1 +}$
Diploid II	$\frac{hi_6}{+}$	$\frac{ly_1}{+}$	$\frac{ur_3}{+}$	$\frac{thr_3}{+}$				

Genes determining requirements for adenine, arginine, histidine, leucine, lysine, methionine, tryptophan, tyrosine, and uracil are symbolized by initial letters of the substance required. *ad*₂ is phenotypically distinguishable from other *ad* markers. All of these markers are recessive, consequently each of the diploids was prototrophic. Stocks of the Department of Genetics, University of Washington, Seattle, were used, and strains 1327 and 1380 were synthesized by DR. D. C. HAWTHORNE of that department.

Cultures were grown to the stationary phase in a liquid yeast-extract peptone (YEP) medium, washed and irradiated in suspension in 10 ml. of water in a petri dish at a density of 10⁶ per ml. Cells were then plated out on a medium supplemented with all the above growth factors. The resulting colonies were replated with a velvet pad on to minimal medium, and auxotrophic colonies were

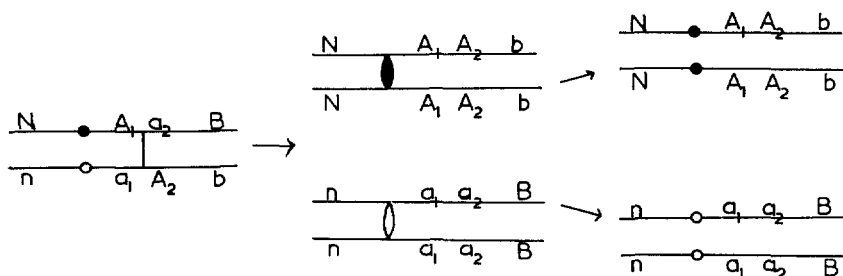


FIGURE 4.—Result of crossing over at the two-strand stage with meiotic segregation of centromeres and restitution.

isolated and their requirements determined. It was initially assumed that a requirement for any one of the growth factors was due to the homozygous condition of the appropriate gene, and in a number of cases this was proved by ascus analysis in the usual way. Ultraviolet radiation was administered in doses giving about 4 percent survival. In experiments with strains 1327 and 1380, the UV source was a Hanovia Vycor lamp (95 percent output at 2537Å), while a Phillips T.U.V. 6-Watt lamp delivering 860 ergs/cm²/sec (95 percent at wavelength 2537Å) was used to induce recombination in Diploids I and II.

RESULTS

Effect of ultraviolet light. A comparison of the type and frequency of diploid homozygous recessive auxotrophs which segregate spontaneously and after UV treatment was made with the stock Diploid I. The results for unlinked genes are given in Table 1.

To test the nature of the homozygotes, a sample of 39 induced recombinants was tested. All were found to sporulate and 4-spored asci, although infrequent, were dissected for tetrad analysis. Thirty-eight gave a 4:0 segregation as expected. Since recombination between linked markers on either side of the centromere was fairly frequent, it was concluded that in general homozygosis was not the result of nondisjunction of mitotic chromosomes, and some mechanism of crossing over was involved. In one case of tetrad analysis, a lethal appeared to be segregating, giving a 2:0 segregation. A hemizygous condition, however, could not be ruled out.

The ultraviolet light has produced a 12-fold increase in the number of cells having one or more genes in the homozygous recessive phase. The distribution pattern in terms of single and multiple recessives does not differ significantly between the spontaneous and induced series, as is shown by comparing the expected and observed figures for the spontaneous segregants.

Frequency of homozygous gene segregants. A more detailed presentation of the results for linked and unlinked genes is given in Table 2. Out of a total of 1720 cells, 151 were auxotrophic for one or more requirements. The frequencies of segregation of individual genes have been calculated in two ways, using the number of cells and using the number of auxotrophs. The expected frequencies for

TABLE 1

The number of diploid segregants from Diploid I having one or more unlinked genes in the homozygous recessive phase

	Number tested		Number of segregants homozygous at the indicated number of loci						Totals
			1	2	3	4	5	6	
Spontaneous	2400	Found	10	2	1	0	0	0	13 (0.5%)
		Expected*	8.5	3.0	0.9	0.3	0.2	.	..
Ultraviolet	1720		77	27	9	4	2	0	119 (6.9%)

* Calculated from the data obtained with ultraviolet treatment.

two or more unlinked genes segregating in the same cell have been calculated from the products of the individual gene frequencies (Table 2).

The number of cells with pairs of genes segregating as homozygotes simultaneously has been summed for the 40 pairs of the ten genes, which represent all the unlinked combinations except *ad*₂ *ad*₆. Expected frequencies for each of the 40 pairs have been calculated both on total colonies and on the number of auxotrophs (Table 3).

In adopting this procedure of calculating the expected frequencies, we assume that the events leading to homozygosis are completely random within all cells when we use the total isolation number as the basis for calculation, and are random within certain cells (approximately 9 percent of the cells of the culture) when we use the number of auxotrophs as the basis.

It is clear that the number found exceeds by tenfold the number expected based on the total isolated. On the other hand, the number found is of the same order as the expected based upon the number of auxotrophs.

This close agreement with expectation based upon the number of auxotrophs leads to the conclusion (1) that only about 9 percent of the cells in the stationary phase in this experiment were competent to respond to the effect of UV on the processes leading to the segregation of homozygotes, and (2) that the events in these cells leading to homozygosis of genes on different chromosomes occur at random and independently.

In a similar treatment of Diploid 1380 with UV the results were the same as for Diploid I. There was however a larger proportion of auxotrophs (111 auxotrophs out of 600 tested) which showed that in this experiment some 20 percent of the cells were competent to respond to UV. This difference may be explained by the fact that the results for 1380 were obtained with the Hanovia lamp, which emits at a greater intensity than the Phillips. Intensity apparently is a factor in the process (WILKIE 1963).

Crossing over and segregation of linked genes: The data on crossing over have

TABLE 2
Frequency of induced homozygosis of the ten individual genes which are heterozygous in Diploid I

Gene	Number homozygous	Frequency/total colonies	Frequency/total auxotrophs
<i>ur</i> ₃	9	0.005	0.060
<i>ty</i> ₁	14	0.008	0.093
<i>me</i> ₂	21	0.012	0.140
<i>ar</i> ₄	12	0.007	0.080
<i>ly</i> ₇	2	0.001	0.013
<i>hi</i> ₈	36	0.021	0.240
<i>ad</i> ₆	21	0.012	0.140
<i>ad</i> ₂	21	0.012	0.140
<i>tr</i> ₅	25	0.015	0.167
<i>le</i> ₁	29	0.017	0.193

Number of auxotrophs: 151. Number of colonies isolated: 1720.

TABLE 3

Distribution of homozygous recessive doubles among auxotrophs of Diploid I

Unlinked gene pair	Number obtained	Number expected	
		On auxotrophs	On isolates
<i>ur ty</i>	0	0.83	0.07
<i>ur me</i>	4	1.26	0.11
<i>ur hi</i>	2	2.16	0.19
<i>ur ad₂</i>	1	1.26	0.11
<i>ur ar</i>	0	0.72	0.06
<i>ur ly</i>	0	0.12	0.01
<i>ur tr</i>	0	1.50	0.13
<i>ur le</i>	0	1.73	0.15
<i>ur ad₆</i>	0	1.26	0.11
<i>ty me</i>	4	1.95	0.17
<i>ty hi</i>	6	3.35	0.29
<i>ty ad₆</i>	3	1.95	0.17
<i>ty ad₂</i>	3	1.95	0.17
<i>ty ar</i>	2	1.11	0.10
<i>ty hy</i>	1	0.18	0.02
<i>ty tr</i>	0	2.33	0.20
<i>ty le</i>	0	2.69	0.23
<i>me ar</i>	3	2.33	0.14
<i>me ly</i>	2	0.27	0.23
<i>me hi</i>	6	5.04	0.44
<i>me ad₂</i>	4	2.94	0.26
<i>me tr</i>	4	3.50	0.31
<i>me le</i>	1	4.05	0.35
<i>me ad₆</i>	3	2.94	0.26
<i>ar ly</i>	0	0.15	0.01
<i>ar hi</i>	3	2.88	0.25
<i>ar ad₂</i>	1	1.68	0.14
<i>ar tr</i>	5	1.98	0.17
<i>ar le</i>	1	2.44	0.19
<i>ar ad₆</i>	3	1.68	0.14
<i>ly hi</i>	1	0.47	0.04
<i>ly ad₂</i>	1	0.27	0.02
<i>ly tr</i>	0	0.32	0.03
<i>ly le</i>	0	0.38	0.03
<i>ly ad₆</i>	1	0.27	0.02
<i>hi tr</i>	8	6.01	0.52
<i>hi le</i>	0	6.94	0.60
<i>hi ad₆</i>	4	5.04	0.44
<i>ad₂ tr</i>	0	3.51	0.30
<i>ad₂ le</i>	2	4.05	0.35
Totals:	69	85.47	7.56

been obtained from strain 1380 and from three experiments with Diploid I, one of which is the same experiment already summarised in Tables 2 and 3 for unlinked genes. All recombinants are from UV-treated cells.

The segregations of homozygotes in chromosome VII are given in Table 4, together with the minimal requirements in crossovers.

TABLE 4
Segregation of homozygotes of chromosome VII

		I		II		III		Region	
		tr_5		+		○		ad_6	
		+		le_1		○			
		11		2		31		Map distance	
		Diploid			Mechanism				
Genes		I	1380	Total	Mitotic	Meiotic 4-strand	Meiotic 2-strand		
$\frac{le_1}{le_1}$		36	25	61	1 C.O. in II	No C.O. in II	No C.O.		
$\frac{tr_5}{tr_5}$		28	19	47	1 C.O. in I	1 C.O. in III	1 C.O. in II or III		
$\frac{ad_6}{ad_6}$		22	12	34	1 C.O. in III	1 C.O. in I or II	1 C.O. in I		
$\frac{tr_5 ad_6}{tr_5 ad_6}$		6	0	6	2-strand double in I or II and III	No C.O. in I, II or III	No C.O.		
$\frac{tr_5 le_1}{tr_5 le_1}$		3	5	8	1 C.O. in II, 4-strand double in I	4-strand double in I	1 C.O. in I		
$\frac{le_1 ad_6}{le_1 ad_6}$		3	5	8	4-strand double in II and in III	4-strand double in II or III	1 C.O. in II or III		
$\frac{tr_5 le_1 ad_6}{tr_5 le_1 ad_6}$		2	1	3	1 C.O. in III, 1 C.O. in II, 4-strand double in I	4-strand double in I, 4-strand double in II or III	1 C.O. in I, 1 C.O. in II or III		

The pooled data from Diploids I and 1380 are used. Total number of auxotrophs is 335. The minimum requirement of crossovers (C.O.) is given for three different mechanisms. Where a double crossover is indicated as a 2- or 4-strand double in regions I and II, one crossover is in region I and the other in region II.

It is apparent that many 4-strand double crossovers are required to explain the results both on mitotic crossing over and meiotic 4-strand crossing over. From the data in Table 4 calculations have been made of the frequency of crossovers and of recombination on the three mechanisms. These results can then be compared with the frequencies obtained by DR. HAWTHORNE on the basis of tetrad analysis. The frequency of crossovers, recombination, and the expected numbers of homozygotes resulting from double and multiple crossovers have been calculated as follows:

(1) *Mitotic crossing over.* If p_1, p_2, \dots = the frequencies of recessive homozygotes resulting from a single crossover in chromosome region I, II, . . . , then the frequency of crossovers (exchanges) = $4p_1, 4p_2 \dots$; the frequency of recombina-

tion = $2p_1, 2p_2, \dots$; and the frequency of double crossovers, one in region I and the other in region II = $4p_1 \times 4p_2$. The frequency of recessive homozygotes obtained from such a double crossover = $(4p_1 \times 4p_2)/4$.

If the homozygote is produced only by one of the four types of double crossovers, (2-strand, 3-strand and 4-strand), then the expected frequency of recessive homozygotes is divided again by four. For example the homozygotes $\frac{le_1 ad_6}{le_1 ad_6}$ in Table 4, which require a 4-strand double crossover in regions II and III, are expected in a frequency $(4p_2 \times 4p_3)/(4 \times 4)$.

If the homozygote is the result of a double crossover within one region, e.g. $\frac{le_1 tr_5}{le_1 tr_5}$ which requires, apart from two single crossovers, a double crossover within region I, then the expected frequency of the double crossovers is given by the third member of the Poisson series.

(2) *Meiotic crossing over at the four-strand stage and nondisjunction.* The same conventions are followed as with mitotic crossing over except for the alterations which are necessary due to the different behaviour of the centromeres in mitosis and meiosis. If frequency of recessive homozygotes = p_1, p_2, \dots ; then frequency of crossovers = $2p_1, 2p_2, \dots$ and the frequency of recombination = p_1, p_2, \dots

The calculations of double crossovers and the frequencies of expected homozygotes derived from them follows the mitotic calculations except that $2p_1, 2p_2 \dots$ are used throughout for the frequency of double crossovers, and that this is divided by two instead of four to give the expected frequency of homozygotes. For example the expected frequency of homozygous recessives from a 4-strand double crossover in regions I and II is $(2p_1 \times 2p_2)/(2 \times 4)$. The significance of the use of the factor 4 for mitotic crossing over, and 2 for meiotic crossing over, can be seen from Figures 1-4 in the section on theory.

An inspection of Table 5 shows that the actual frequency of mitotic crossing over is extremely high, in fact it is at a maximum of 50 percent recombination in two of the three regions. Furthermore region II, which is only 2 map units by the tetrad analysis, shows the highest amount of crossing over. The frequency of recombination required for the meiotic mechanism is in good agreement with the values from tetrad analysis. In view of the rarity of mitotic crossing over in other organisms, for example *Aspergillus*, and *Ustilago*, it would appear that these data favour the meiotic view.

TABLE 5

Comparison of recombination frequencies based on different mechanisms

Region	Meiotic tetrad analysis	Meiotic nondisjunction	Mitotic crossing over
I tr_5-le	11%	23.0%	49.2%
II le_1 -centromere	2%	...	52%
III centromere- ad_6	31%	28.4%	33%

A discrimination cannot be made between the three mechanisms on the data presented in Table 6, based upon double and triple crossovers. The data do not differ significantly from any of the expected frequencies.

The recessive homozygotes on chromosome XII, obtained in the diploids I and 1380, are given in Table 7. The minimal requirements in crossovers based upon the three mechanisms are also given. It is clear that with mitotic crossing over the number of the double crossover class is greater than the sum of the two single crossover classes. The mitotic mechanism can therefore be discarded without further calculations of the expected values. With meiotic 4-strand crossing over there is a large class requiring either a 4-strand double in region II or a 2-strand double in regions I and II.

To calculate these frequencies it is necessary to have the frequencies of recombination in regions I and II. The recombination frequency in region II can be calculated from the hi_s and ad_2 homozygote classes: *recombination frequency in II* = $p_1 = [2(61 + 34)] / (304 \times 2) = .31$. To estimate the frequency of recombination in region I we must estimate the proportion of the ++ class that is due to crossing over in region I. The ++ class is comprised of auxotrophs other than hi_s and/or ad_2 . The reciprocal recombinants of all the three homozygous recessive classes will be ++. This is a total of 115, leaving 74 which represent the ++ class resulting from crossovers in region I. To obtain the total crossovers in region I we must add twice the ad_2/ad_2 class: *recombination frequency in I* = $p_2 = [74 + (34 \times 2)] / (304 \times 2) = 2.3$.

The homozygous recessives resulting from 2-strand double crossovers in regions I and II are expected in the frequency $[(.62 \times .46) / (2 \times 4)] \times 304 = 11.2$. The homozygous recessives resulting from a 4-strand double in region II are expected in the frequency $e^{-2}[(2 \times .62^2) / 2] \times 304 = 4.1$. The expected numbers of the

TABLE 6

Comparison of the expected and the observed numbers in classes of homozygotes requiring double or triple crossovers in chromosome VII

Genes	Number observed	Number expected		
		Mitotic	4-strand	2-strand
$\frac{tr_5 ad_6}{tr_5 ad_6}$	6	12.46	...*	..
$\frac{tr_5 le_1}{tr_5 le_1}$	8	2.70	6.38	..
$\frac{le_1 ad_6}{le_1 ad_6}$	8	12.46	9.72	..
$\frac{tr_5 le_1 ad_6}{tr_5 le_1 ad_6}$	3	1.81	0.395	3.4

* Where no figure is given, double or triple crossovers are not required and therefore a calculation cannot be made.

TABLE 7

Number* of recessive homozygotes obtained by recombination in chromosome XII with the minimum requirements of crossovers (C.O.) based on three mechanisms

Genes	Diploid		Total	Mechanisms		
	I	1380		Mitotic	4-strand	2-strand
$\frac{hi_s}{hi_s}$	45	16	61	C.O. in I, C.O. in II	C.O. in II	C.O. in II
$\frac{ad_2}{ad_2}$	30	4	34	C.O. in II	4-strand double in II or 2-strand double in I and III	C.O. in II
$\frac{hi_s ad_2}{hi_s ad_2}$	14	6	20	C.O. in I	No C.O.	No C.O. in II
$\frac{+ +}{? ?}$	104	85	189	No C.O.	No C.O. in II

* Total colonies tested: 3120. Number auxotrophic: 304.

ad_2/ad_2 class on these calculations is 15.3. This differs significantly from the observed number, 34. The mechanism best fitted to the data is two-strand crossing over, the minimal requirements being crossing over in region II with a frequency of 0.3. Data to be considered later, however, exclude two-strand crossing over as the sole mechanism.

The homozygous recessive colonies obtained for chromosome V (given in Table 8) and for chromosome IX (given in Table 9) are not sufficient to discriminate clearly between the three mechanisms. The worst fit, however, is obtained with mitotic crossing over. The double crossover classes ($\frac{hi_6 ly_1}{hi_6 ly_1}$ in chromosome IX and $\frac{ur_s thr_s}{ur_s thr_s}$ in chromosome V) are expected on mitotic crossing over to total 3.1, whereas eight were found. This result is clearly not significant, but the deviation that exists does support the data obtained from the other linkage groups.

The data for chromosomes V and IX are given in Tables 8 and 9. The recombination frequencies obtained from the 4-strand meiotic mechanisms fit the values obtained from tetrad analysis by HAWTHORNE for both chromosomes V and IX (Table 10).

TABLE 8

Segregation of homozygotes of chromosome V in Diploid II from 43 auxotrophs obtained from a total isolation of 600

		I	II	Region
		ur_3	thr_3	
		○		
		○		
		+	+	
		7	34	Map distance
Genes	Number observed	Mitotic	Meiotic	
			4-strand	2-strand
ur_3	12	1 C.O. in I	1 C.O. in II	1 C.O. in I or II
ur_3				
thr_3	7	1 C.O. in II	1 C.O. in I	1 C.O. in I or II
thr_3				
$ur_3 thr_3$	4	2-strand double in I and II	No C.O.	No C.O.
$ur_3 thr_3$				

TABLE 9

Segregation of homozygotes of chromosome IX in Diploid II from 43 auxotrophs

		I	II	Region
		hi_6	ly_1	
		○		
		○		
		+	+	
		17	29	Map distance
Genes	Number observed	Mitotic	Meiotic	
			4-strand	2-strand
hi_6	11	1 C.O. in I	1 C.O. in II	1 C.O. in VII or II
hi_6				
ly_1	6	1 C.O. in II	1 C.O. in I	1 C.O. in I or II
ly_1				
$hi_6 ly_1$	4	2-strand double in I and II	No C.O.	No C.O.
$hi_6 ly_1$				

TABLE 10

Comparison of recombination percentages obtained from a meiotic four-strand mechanism with nondisjunction, and from tetrads

		Meiotic 4-strand	Tetrad
Chromosome V	Region I	16%	7%
	Region II	28%	30%
Chromosome IX	Region I	14%	15%
	Region II	25%	30%

Centromere distance and frequency of homozygosis. Because the three mechanisms postulated for the generation of homozygotes produce entirely different frequencies in relation to the distance between the gene and the centromere, data from all four diploids have been pooled and a comparison has been made between the frequency of recombination of markers and the respective map distances from the centromere, as deduced from tetrad analysis (Table 11). These results show no correlation between frequency of homozygosis and map distance from the centromere. As there is no correlation expected on meiotic 2-strand, a positive correlation on mitotic, and a negative correlation with meiotic 4-strand, the data favour the meiotic 2-strand mechanism. To test this conclusion, further platings of irradiated cells of Diploid I were made, and analysis was concentrated on chromosome XII. Homozygous ad_2/ad_2 recombinants (red sectors) were isolated from six different colonies, and three of these were found to be histidine-independent. Ascus analysis of one of these three showed a 4:0 segregation for adenine requirement as expected, and 2:2 segregation for histidine requirement. The heterozygous condition at the *hi* locus thus rules out two-strand crossing over as the recombination mechanism in this particular case.

Effect of sporulating medium: The diploid phase in yeast is stable and is apparently the condition found in nature. The distinction between somatic cells and reproductive cells is not precise since the change from a mitotically dividing cell to a meiotic one can be brought about very quickly by appropriate environmental manipulation. The elaboration of sex organs is not involved, and in this important respect, as in the stability of the diploid, yeast differs from other fungi investigated in connection with somatic recombination. Furthermore, cells on a sporulating (meiotic) medium can revert to the vegetative (mitotic) condition if transferred to YEP medium within about 12 hours of being placed on the former.

The change from heterozygous $ad_2/+$ to homozygous ad_2/ad_2 (red, adenine

TABLE 11

Comparison between frequency of homozygosis and linkage to the centromere

Gene	Distance from centromere in crossover units	Number homozygous	Total tested	Frequency per 10 ⁴
<i>tr</i> ₁	1	12	950	130
<i>le</i> ₁	2	79	3,120	243
<i>ur</i> ₃	5	72	4,670	154
<i>ar</i> ₄	7	20	3,470	58
<i>tr</i> ₅	13	25	3,120	80
<i>hi</i> ₆	17	25	1,150	161
<i>ly</i> ₇	20	8	3,470	22
<i>ly</i> ₁	29	10	600	166
<i>ly</i> ₂	30	11	600	183
<i>ad</i> ₆	31	51	3,120	161
<i>me</i> ₂	33	58	4,070	144
<i>thr</i> ₃	34	16	600	266
<i>hi</i> ₈	35	81	3,120	259
<i>ad</i> ₂	65	54	3,120	173
<i>ty</i> ₁	65	18	1,320	136

requiring) was induced in mitotic (log phase) cells and in meiotic cells (2 hr on sporulating medium) for comparison; results are given in Table 12. The frequency of homozygous recombinants is significantly higher in meiotic than in mitotic cells in the case of spontaneous recombination and recombination induced at low doses. At higher doses, the frequency in mitotic cells approaches that of the meiotic cells where there is a levelling off indicating a saturation effect. These findings lend some support to the hypothesis that somatic recombination takes place mainly in cells that are in an incipient meiotic condition. If the event resulted from an anomaly of mitosis, clearly the highest incidence would be expected in mitotically dividing cells. Recently, SHERMAN and ROMAN (1963) have found an increase in spontaneous intragenic recombination at the isoleucine locus without a corresponding increase in homozygosis at the canavanine locus in cells which had been similarly placed on sporulating medium for a limited period. In their opinion intragenic recombination, which they find to be non-reciprocal, differs from homozygosis and results from a switch in the copying process at replication. In stationary phase cells FOGEL and HURST (1963) found coincidence between the two categories of recombinant, indicating a common mechanism. However, in the case of intragenic recombination at the isoleucine locus, in which ROMAN and JACOB (1958) recovered several nuclei homozygous for the wild-type allele, crossing over at the 2-strand stage is clearly indicated. A first requirement either in a copy-switch or in a crossover mechanism is intimate pairing of homologous strands, and in the former case this would have to take place at the 2-strand stage. Although some of the data presented here are best fitted to a mechanism of crossing over before replication, cytological evidence from other organisms does not favour this interpretation.

DISCUSSION

Somatic recombination (homozygosis) is considered to take place in cells which go through a first meiosis with restitution of nuclei. The evidence for this conclusion can be listed under three headings: (1) pairing occurs between all homologous chromosomes in a cell, as evidenced by simultaneous homozygosis of markers on different chromosomes; (2) a negative correlation exists between the fre-

TABLE 12

Ultraviolet induction of homozygosis at the ad₂ locus in heterozygous mitotic and meiotic cells

Dose (minutes) (T.U.V. lamp)	Mitotic cells*			Meiotic cells†		
	Total colonies scored	Number recombinant	Recombinants per 10 ⁸	Total colonies scored	Number recombinant	Recombinants per 10 ⁸
0	3,920	1	0	2,180	10	5
1	3,533	74	21	4,650	182	40
2	1,629	152	93	2,005	196	98
3	7,355	630	85	6,609	759	115

* Log phase. † Two hours on sporulating medium.

quency of homozygosis and map distance of linked genes; and (3) the highest frequency of homozygosis is seen in cells in an incipient meiotic condition.

In *Aspergillus nidulans* and *Ustilago* simultaneous homozygosis of unlinked markers is not generally seen. However significant departures from a positive correlation between homozygosis and map distance was observed in *Aspergillus* (PONTECORVO and KÄFER 1958). This was most striking in the case of three particular intervals on different chromosomes, each interval being about 20 map units in length and comparatively near the centromere. In these segments relatively high amounts of recombination were obtained. Although these results are probably not explainable on the basis of a meiotic mechanism, it is difficult to fit them into a general hypothesis of mitotic recombination.

Although considerable increase in recombination frequency is possible in yeast and *Ustilago* by UV irradiation, this is not true of *Aspergillus nidulans*, where no increase has been obtained by this means (PRITCHARD, personal communication; FRATELLO *et al.*, 1960). It is tempting to conclude that a basic difference in the mechanism of the event exists between organisms that respond to UV and those that do not, other factors being equal. The fact that other factors are probably not equal makes for caution: for example the diploid spores of *Aspergillus* that are usually subjected to the irradiation contain a pigment which screens them to a certain extent from UV (WILKIE, unpublished).

In the case of yeast, the induction process in log-phase cells is considered to have two components (a lag in the induction gives some evidence of this). The first effect is thought to be the conversion to an incipient meiotic condition, that is to a situation in which homologous chromosomes are predisposed to undergo pairing—a situation already available in cells on sporulating medium. The second effect of UV is believed to be the stabilization of this condition, thus ensuring meiotic pairing and segregation. This of course would correspond to the primary effect of UV in cells placed on sporulating medium for 2 hours, in which the relationship of frequency of recombination and dose appears to be linear (Table 12). Presumably an incipient meiotic condition is unstable in cells on a growth medium, and reversion to a normal mitosis takes place in nearly all cases. These ideas on the effects of UV are difficult to visualize at the molecular level using the data available here. However, action spectra of the induction in different cell types are under investigation, and should give more precise information on the molecules involved in the photochemical reaction. Until more is known of the chemistry and kinetics of chromosome pairing our knowledge of recombination, reciprocal or otherwise, must remain highly speculative.

SUMMARY

Spontaneous and ultraviolet-induced somatic recombination have been studied in four strains of yeast heterozygous for four to ten genes, both linked and unlinked. The frequency and distribution of simultaneous recombinant homozygotes indicate that pairing and crossing over occur between all homologous chromosomes in those cells where the event is taking place.

In the case of linked genes, a negative correlation between the frequency of homozygosis and map distance from the centromere was found. A mechanism of meiotic crossing over and segregation followed by nondisjunction or restitution at the second division is postulated to explain these results. In support of this, it was found that cells grown on sporulating medium produced a higher proportion of homozygous diploids than did cells in the stationary phase or in log phase in a growth medium.

The frequency of homozygosis of individual genes on different chromosomes shows no correlation with centromere linkage, a result which would be expected on the basis of crossing over at the two-strand stage with meiotic segregation and nondisjunction at the second division.

The mechanism of ultraviolet induction is discussed in terms of a change from a mitotic to a meiotic condition in cells which respond to irradiation.

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