ULTRAVIOLET-INDUCED GENETIC RECOMBINATION IN A PARTIALLY DIPLOID STRAIN OF *ESCHERICHIA COLI*[1]

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UNDERSTANDING the mechanism of genetic recombination is of prime concern to biologists. Breakage-reunion-type recombination events account for most, if not all, recombinant structures in bacteriophages λ (MESELSON and WEIGLE 1961; KELLENBERGER, ZICHICHI and WEIGLE 1961; MESELSON 1964) and T4 (TOMIZAWA and ANRAKU 1964, 1965; ANRAKU and TOMIZAWA 1965a, b; TOMIZAWA, ANRAKU and IWAMA 1966). These studies have also revealed the nature of some of the steps intermediate to recombinant formation.

The genetic systems available in *Escherichia coli* K-12 are unusual in that they offer opportunities to study both reciprocal and nonreciprocal recombination events. Genetic (TOMIZAWA 1960) and isotopic labeling (OPPENHEIM and RILEY 1966) experiments have revealed some aspects of the recombination process following conjugal transfer of donor genetic material to recipient strains. However, problems concerned with replication of the donor chromosome during conjugation and vegetative chromosome replication in the recipient (CURTISS 1966) make complete analysis of recombination events following conjugation difficult. Therefore, it was decided to investigate recombination events in a partially diploid strain of *E. coli* K-12 (CURTISS 1962, 1964) in order to eliminate problems concerned with gene transfer. LEDERBERG, LEDERBERG, ZINDER and LIVELY (1951) and BECKHORN (see DEMEREC, WITKIN, BECKHORN, VISCONTI, FLINT, CAHN, COON, DOLLINGER, POWELL and SCHWARTZ 1951) showed that small doses of UV (ultraviolet light, 2537 Å) would induce haploidization in partially diploid strains of *E. coli* K-12, but they did not study the kinetics of the process, since all surviving colonies still contained partially diploid cells. UV is known to stimulate genetic recombination in phage (JACOB and WOLLMAN 1955; HERSHEY 1958; LEVINE and CURTISS 1961), in *E. coli* K-12 (JACOB and WOLLMAN 1961), in yeast (ROMAN and JACOB 1958), and in the phage P22-*Salmonella typhimurium* transduction system (GAREN and ZINDER 1955). Thus, it was felt that the use of UV to stimulate genetic recombination in a partially diploid strain of *E. coli* K-12 (CURTISS 1964) would provide a closed system, not involving gene transfer, with which meaningful results could be obtained. This communication reports findings on UV-induced haploidization, on recombination leading to homozygosity

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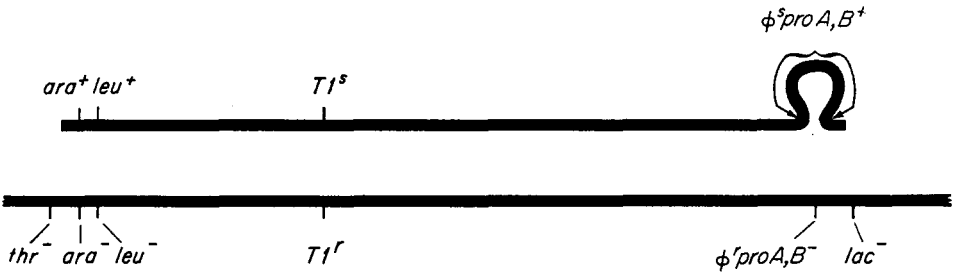


FIGURE 1.—Genetic structure of the F$^-$ partially diploid strain χ 137Ex2. The following abbreviations for loci and allelic states are used: *thr*—threonine; *ara*—arabinose; *leu*—leucine; *pro*—proline; *lac*—lactose; *T1*—bacteriophage T1; ϕ—bacteriophages T3, T7, λ, and P1*kc*; +—ability to synthesize or utilize; —inability to synthesize or utilize; s—sensitive; and r—resistant. The *T1^r* mutation confers resistance to bacteriophages T1 and T5. The ϕ^r*proA-B$^-$* mutation is a deletion about 2.5 minutes of transfer time long which results in resistance to bacteriophages T3, T7, λ and P1*kc* and the abscence of the information to code for the first two enzymes in proline biosynthesis (CURTISS 1965; CURTISS and CHARAMELLA 1968). The *proB* locus is about 0.1 minutes of transfer time from the right end of the exogenote and the *proA* locus is about 2 to 2.5 minutes of transfer time to the left of the *proB* locus. Note that the mutations *proA$^-$*, *proB$^-$*, and ϕ^r*proA-B$^-$* have previously been referred to as *pro$^-_1$*, *pro$^-_2$*, and ϕ^r*pro$^-_{1,2}$*, respectively (CURTISS 1965).

The exogenote is 10 minutes long as adjudged by interrupted mating experiments (CURTISS 1964) and must form a loop to pair on either side of the ϕ^r*proA-B$^-$* deletion mutation in the endogenote. The exogenote does not complement the *thr* or *lac* loci.

for one or more markers with retention of the partially diploid state, and on reciprocal exchange with retention of the partially diploid state. Preliminary accounts of these studies have been made (CURTISS 1962, 1963).

MATERIALS AND METHODS

Description of the partially diploid strain: The partially diploid strain was isolated as a proline-independent recombinant in a mating between the F$^-$ strain χ 85 and Hfr CAVALLI (CURTISS 1962, 1964). The recombinant was F$^-$ and was diploid for the *ara* to *proB* segment of the *E. coli* K-12 genome. This partially diploid strain was infected with the fertility factor F, and by a series of steps the partial chromosome (exogenote) was transferred to the F$^-$ strain χ 137. One of the F$^-$ partially diploid recombinants obtained was designated χ 137Ex2 (CURTISS 1964) and was used for all experiments in this report.

Figure 1 shows a detailed diagram of the genetic structure of χ 137Ex2. The partial chromosome will be referred to as the exogenote, and the region complemented by the exogenote as the endogenote (MORSE, LEDERBERG and LEDERBERG 1956; CURTISS 1964). Genetic markers on the exogenote will be referred to as exogenote markers; markers on the endogenote as endogenote markers; and markers on the remainder of the chromosomes as chromosomal markers. This partially diploid strain spontaneously undergoes haploidization at a rate of 0.4%/bacterium/generation and recombination leading to homozygosity for *T1^s* or *T1^r* with retention of the partially diploid state at a rate of 0.04% /bacterium/generation. (Rate determinations were done in broth or in fully supplemented minimal medium so that all segregants could grow. The results were the same under either condition.) The relative stability of χ 137Ex2 is due to the ϕ^r*proA-B$^-$* endogenote marker. This mutation is a deletion of 2 to 2.5 percent of the bacterial chromosome (CURTISS 1965), which prevents effective pairing between the right ends of the exogenote and endogenote (CURTISS 1964). When a *proA$^-$* or *proB$^-$* point mutation is present in the endogenote, the spontaneous haploidization rate increases to 5 to 10%/bacterium/generation (CURTISS 1964).

Use of F+ partially diploid strains revealed that the exogenote is transferred linearly by conjugation, with either the *ara*+ *leu*+ end or the ϕ^s*proA-B*+ end entering the recipient first and with the entire exogenote requiring approximately 10 minutes for transfer (CURTISS 1964). Joint transfer of exogenote and chromosomal and/or endogenote markers has not been observed (CURTISS, 1964; unpublished). Therefore, it has been concluded that the exogenote is neither in any way attached by covalent bonds to nor integrated into any part of the bacterial genome. Since the *proB* locus is within 0.1 minutes of transfer time from the right end of the exogenote and the *ara* locus is within 0.2 minutes of transfer time from the left end of the exogenote, an attempt was made to cotransduce *ara*+ and *proB*+ with phage P1*kc* to see if the exogenote existed as a circular structure. No *ara*+ *proB*+ cotransduction was observed. Based on these results from conjugation and transduction experiments and on some of the results presented in this communication, it has been inferred that the exogenote is a linear structure and not circular.

We have isolated 17 independent partially diploid strains, and all have exogenotes of the same length as well as similar properties. A complete discussion of the properties possessed by one of these strains has been published (CURTISS 1964). It should be emphasized that χ 137Ex2 and the other partially diploid strains isolated differ in three important ways from F′ partially diploid strains. First, χ 137Ex2 is a non-donor F− strain and the exogenote cannot be cured by acridine orange treatment whereas F′ partially diploid strains possess the fertility factor F and the F′ factor (exogenote) is cured by acridine orange treatment. Second, the exogenote in χ 137Ex2 is believed to be linear, whereas the F′ factor is believed to be circular. Third, the genetic stability of χ 137Ex2 is dependent upon the presence of a long deletion in the endogenote to prevent excessive rates of haploidization with recombination, whereas the genetic stability of F′ partially diploid strains is not appreciably affected by deletions in the endogenote.

Nomenclature: The genetic nomenclature used in this manuscript has been altered to conform to the recommendations of DEMEREC, ADELBERG, CLARK, and HARTMAN (1966), with certain exceptions. These exceptions are predicated on the belief that genotype abbreviations should be comprehensible to all scientists, useful in both written and oral communication, and reflect, if possible, the phenotype associated with mutations at any particular locus. Thus, I have continued to use the superscripts +, —, *r*, and *s* in conjunction with gene symbols, since they clearly indicate the allelic state at a given locus. Abbreviations for genes controlling response to bacterial viruses have been abbreviated by italicizing the phage symbol (i.e., *T1*, *T6*, etc.), since (1) this convention has historical precedent, (2) many of the substitute abbreviations proposed by DEMEREC *et al.* (1966) are cacophonous, and (3) the assignment of cistron designations to mutations affecting response to phages is invalid, and will remain so until appropriate genetic and biochemical studies have been conducted to define the number of cistrons involved. The third exception to the DEMEREC *et al.* (1966) proposal concerns the use of abbreviations with less than (*T1*) and more than (*φproA-B*) three letters. I believe that a gene symbol abbreviation should be chosen primarily for its clarity of meaning, and that the number of letters in the abbreviation should be of secondary importance. Certainly, this will not cause any difficulty for computer cataloguing of bacterial strains and will accommodate future developments in bacterial genetics where assignment of three-letter symbols to loci coding for specific transfer RNA species, etc., would be too restrictive.

Media: ML(minimal liquid) and MA (minimal agar) (CURTISS 1965) were supplemented with L-threonine (40 μg/ml), L-leucine (20 μg/ml), thiamine HCl (2 μg/ml), and with or without L-proline (30 μg/ml). Glucose at 0.5% final concentration was used as the energy source. Penassay broth and agar (Difco) and EMB (eosin-methylene blue) agar (CURTISS 1965) modified to contain 0.5% instead of 0.1% yeast extract and supplemented with either 1.0% L-arabinose or 0.1% glucose were used as complex media. BSG (buffered saline with gelatin, CURTISS 1965), ML, and Penassay broth were used as diluents.

Bacteriophages: The preparation and storage of phage stocks and the methods of testing bacterial cultures for complete and partial resistance by cross-streaking on EMB agar containing 0.1% glucose were previously described (CURTISS 1964, 1965).

Determination of cell genotypes: The genotype of cells contained in colonies of χ137Ex2 formed on MA containing proline, Penassay agar, or EMB arabinose agar was determined by

picking colonies into tubes containing 2 ml of Penassay broth. Prior to incubation, these dilute cultures were streaked on EMB arabinose agar to determine uniformity of colony morphology and fermentation reaction. After incubation at 37°C the cultures were cross-streaked against T1 and T7 on EMB containing 0.1% glucose to distinguish complete or partial resistance or sensitivity of the culture to each phage. Phage sensitivity is dominant to phage resistance (LEDERBERG 1949; CURTISS 1964), and all cells which are heterozygous for any phage-resistance marker will be phage-sensitive. However, haploid segregants in a partially diploid culture seldom incorporate the ϕ^s*proA-B*+ exogenote marker (about 1%, see CURTISS 1964), and therefore are almost always resistant to T3 and T7. Since such haploid segregants are present at a frequency of about 1% in a partially diploid culture, about 1% of the cells will survive to the right of the T7 streak. Thus, partial resistance of the culture in this test is used as the criterion for establishing heterozygosity (diploidy) at the *T1* and *φproA-B* loci. These cultures were also spotted or streaked on MA deficient for either leucine or proline to distinguish ability or inability to synthesize leucine or proline, respectively.

UV irradiation: UV irradiation was accomplished by use of two parallel 15-watt General Electric germicidal lamps (G15T8) with all but the central 10 cm shielded. The lamp height was adjusted to give an incident dose rate of 10 ergs/mm^2/sec as measured with a dose rate meter designed by JAGGER (1961). All irradiations and subsequent experimental procedures were conducted in the presence of yellow light to prevent photoreactivation. Bacteria suspended in either ML or BSG at densities of from 5×10^6 to 2×10^7 per ml were irradiated at room temperature in a variety of flat-bottomed vessels, depending on the volume irradiated. In all cases the depth of the suspension was approximately 1 mm. Since both killing and recombination increased when cells were held in the medium in which they were irradiated, all cultures were diluted either 1:1000 or 1:5000 into fresh medium (usually prewarmed to 37°C) immediately (within 5 sec) after irradiation. In several experiments, irradiated suspensions were sedimented by centrifugation and resuspended with little effect on survival or recombination.

Photoreactivation: Photoreactivation was done by either of two methods. Most experiments employed two parallel 15-watt, black-light General Electric fluorescent lamps (F15T8-BLB) mounted 10.0 cm above the bacterial suspensions contained in open, 3-cm-diameter glass Petri dishes. A 1.0-cm thick glass plate was used to cover the Petri dishes and to remove essentially all wavelength radiations below 3100 Å. The bacteria were suspended in BSG at densities of 1×10^3 to 5×10^3 cells/ml, and the photoreactivating light was administered at room temperature (22 to 23°C). In several experiments, a Hilger quartz prism monochromator delivering 4047 Å light at a band width of approximately 175 Å with an incident dose rate of 3150 ergs/mm^2/sec was used. Photoreactivation was conducted at 37°C, with 1.7 ml of bacterial culture suspended in BSG contained in a quartz cuvette with a 1.0 cm light path.

Phase contrast microscopy: Unirradiated and irradiated bacterial suspensions were observed at 1200× magnification by using medium-bright, high phase contrast microscopy with a Zeiss-Winkler microscope having a warm stage at 37°C. The gelatin technique of MASON and POWELSON (1956) was employed to observe cell division and the nuclear pattern. To obtain more compact nuclei, cultures were grown in ML lacking proline containing 25% gelatin and 2% monovalent cations (total concentration) (KELLENBERGER 1960).

Experimental reproducibility: The experiments cited in this communication were initiated in April 1962 and completed in May 1965. Each type of experiment was done three or more times, with as much as 2 years between repeat experiments and with essentially similar results each time. Frequently the more extensive and elaborate experiments are presented in the figures. Most of the tables contain composite data from several experiments.

RESULTS

1. Colony types after UV irradiation: Irradiated cultures of χ 137Ex2 were initially plated on a variety of media to establish the most ideal conditions for enumerating primary genetic events by colony morphology. EMB arabinose agar

TABLE 1

*Arabinose fermenting colony types arising after UV irradiation of χ137x2**

Colony type	Genotype of cells	Percent at UV dose (in ergs/mm²) of: 0	300	900
(1) 6–8 mm diameter; flattened; medium dark center; white to pinkish fringe.	parental partially diploid	99	64–72	27–42
	partially diploid but homozygous for *T1ˢ* or *T1ʳ*	0	6–8	13–18
(2) 3–4 mm diameter; compact and rounded; pitch black with sharp edge.	haploid *ara*+ ϕ^r*proA-B*⁻	0.8	12–15	20–25
(3) sectored; composed of cells giving colony types 1 and 2.	50 ± 20% partially diploid and 50 ± 20% haploid†	0.2	10–13	25–30

* Log-phase cultures χ137Ex2 were grown in ML lacking proline, irradiated with 300 or 900 ergs/mm² of UV, and immediately plated on EMB arabinose agar. Survival levels for the two doses were approximately 30 and 5%, respectively. Plates were incubated 24 hours at 37°C and 2 days at room temperature.

† About 5% of the sectored colonies contained 95 to 99% *ara*+ ϕ^r*proA-B*⁻ haploid recombinants and 5 to 1% partially diploid cells. These colonies had the compact, rounded morphology with the black coloration but had a bump at the top of the colony which contained the partially diploid cells, and they were scored as being sectored.

was the only medium which allowed this distinction between colonies containing partially diploid cells, haploid cells, and mixtures of both cell types (Table 1). Although most colonies fermented arabinose, arabinose nonfermenting colonies were also observed (0.1 to 0.2% after 300 ergs/mm² and 0.5 to 1.0% after 900 ergs/mm²). Half of these contained partially diploid cells homozygous for the *ara*⁻ endogenote marker and had the diffuse colony morphology. The other half contained only *ara*⁻ haploid cells and had the compact, rounded colony morphology. The sectored colonies (Table 1) were rather heterogeneous and were not too often divided into equal halves. The only criterion used to classify a colony as being sectored was that it contain "nearly" equal numbers of haploid and partially diploid cells, regardless of the geometric distribution. For this reason the enumeration of sectored colony types was subject to considerably greater human error than was involved in the classification of colonies containing only haploid recombinants. With the problem of delayed effects of UV to produce recombinants in cell generations after the one in which UV was administered (to be discussed more fully in later sections), it is probable that the percentage of sectored colony formers due to primary events was overestimated for any given dose of UV.

Since LEDERBERG *et al.* (1951) and BECKHORN (see DEMEREC *et al.* 1951) did not obtain any pure clones of haploid segregants by plating cultures of partially diploid cells immediately after UV exposure, the above results were unexpected. To confirm the existence of pure clones containing only haploid recombinants, two types of experiments were performed. In the first type, a technique developed by LEDERBERG *et al.* (1951) was employed in which a very dilute suspension of partially diploid cells was distributed in a straight line across the top of square or

rectangular plates containing EMB arabinose agar. Several dilutions of the culture were used such that the number of cells distributed would be 1 per cm or less. About 80% of these plates were then irradiated with 300 ergs/mm^2 and incubated at 37°C. Half of the plates receiving no UV and about 75% receiving UV were taken after 2½ and 4 hours of incubation, respectively, and a spreader was used to spread the microcolonies in a line perpendicular to the original line of cells. The plates were then reincubated for another 24 hours.

Data from four independent experiments were analyzed after details of the technique had been worked out. Of 168 lines of descent examined for the UV treatment, fifteen (9%) had colonies containing only haploid recombinants. Of these fifteen, three lines of descent contained four colonies, two contained six colonies, one contained seven colonies, eight contained eight colonies, and one contained fifteen colonies. Twenty-one lines of descent of the 168 (12.5%) contained approximately equal numbers of colonies containing haploid recombinants and colonies containing partially diploid cells. One of these contained seven colonies of haploid recombinants and one sectored colony that included both haploid and partially diploid cells. Based on an examination of the unspread plates receiving UV, it was estimated that there was between 5 and 10% coincidence such that a clone of descent would contain segregants from two microcolonies. There were no clones of descent (out of 102) from the nonirradiated plates which had colonies containing only haploid recombinants. Occasionally, clones of descent were found which contained one or, less frequently, two colonies with haploid recombinants.

In the second experiment agar plugs with colonies containing *ara*$^+$ haploid recombinants were cut out and placed in 1 ml of BSG. These suspensions were agitated with a Vortex Jr. Mixer, 2 ml of 0.75% melted soft agar in BSG was added, and the entire contents poured onto MA plates lacking proline. If the ϕ^s*proA-B*$^+$ exogenote marker was present in any of the cells, then proline-independent colonies should appear after incubation of the plates. Twenty-five colonies were tested in this manner, and no proline-independent colony was observed. Thus, it is concluded (1) that colonies containing only haploid recombinants were in fact formed by plating immediately after UV, and (2) that colony morphology on EMB arabinose agar allowed classification of primary genetic events.

2. Recombinant yield as a function of UV dose: To determine the UV dose response curve for recombinant production, *x* 137Ex2 was grown with aeration at 37°C in ML lacking proline to reduce the frequency of spontaneous haploid segregants. Figure 2 shows the results of a representative experiment in which the numbers of survivors and recombinants are plotted as a function of UV dose. The shape of the survival curve was reproducible in several of the more elaborate repetitions of this experiment. One experiment was analyzed in which the survivors were plated on Penassay agar, and all types *except* sectored colony formers were scored by random picking and testing. Similar results were obtained. There was a 4-fold increase in the number of *ara*$^+$ haploid recombinants between the unirradiated sample and the sample receiving 300 ergs/mm^2 of UV (Figure 2).

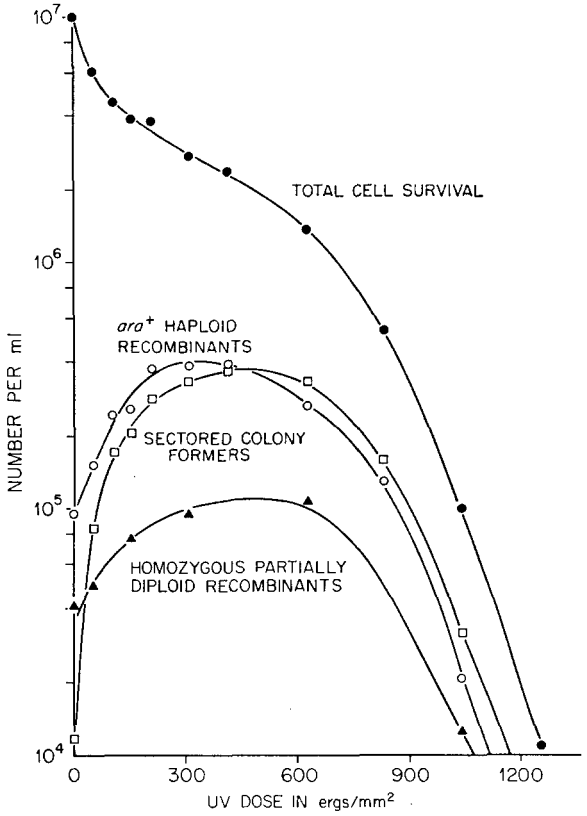


FIGURE 2.—UV survival and recombinant yield in the partially diploid strain χ 137Ex2 as a function of UV dose. The log-phase culture growing in ML lacking proline was irradiated in BSG at a cell concentration of 1.2×10^7 cells/ml, which was normalized to 1.0×10^7 cells/ml for simplicity of presentation. Appropriately diluted samples were plated on EMB arabinose agar and on MA containing proline. The survival on MA was approximately 5% higher for each point than on EMB arabinose agar, the average value being plotted here. The number of *ara*+ haploid recombinants and sectored colony formers was scored by direct observation of colonies forming on EMB-arabinose agar. An average of 1063 colonies was scored for each of the 11 points (855 was the least and 1598 was the most). The frequency of recombinants which had become homozygous for the $T1^s$ or $T1^r$ markers with retention of the partially diploid state was determined by selecting and testing 200 survivors for each UV dose from the MA plates where there is little or no bias due to differences in colony morphology.

The same comparison for the net increase in the number of sectored colony formers gave a 28-fold increase and for homozygous partially diploid recombinants a 3-fold increase. In this particular experiment, there was an unusually high frequency of homozygous partially diploid recombinants in the unirradiated samples (see later experiments). This was due to sampling error and to the fact that these recombinants cannot be selected against during the preirradiation growth period (CURTISS 1964). Usually there was a 10- to 15-fold increase in the absolute number of these recombinants between the no-UV sample and the sample surviving 300 ergs/mm².

The data on net increase in recombinant number described above and presented in Figure 2 indicated that in the partially diploid population the UV did not selectively enrich for pre-existent recombinant types. In accord with this conclusion is the fact that the survival curves for several *ara*$^+$ haploid recombinants and for several partially diploid recombinants having become homozygous at the *T1* locus were essentially indistinguishable from the χ 137Ex2 survival curve shown in Figure 2.

Figure 3 contains the data from the experiment shown in Figure 2 replotted to show percent recombination as a function of UV dose. In this experiment all recombinant types increased linearly up to a dose of 400 to 500 ergs/mm^2; therefore, a dose of 300 ergs/mm^2 was chosen for most other experiments. The 300 ergs/mm^2 dose also gave the largest number of recombinants based on the original partially diploid cell titer (Figure 2).

3. Microscopic observations of irradiated and unirradiated partially diploid cell cultures: The results presented in the preceding two sections led to the prediction of the nuclear pattern in the partially diploid strain. The discovery of pure clones of *ara*$^+$ haploid recombinants and the excess of this class over cells giving rise to sectored colonies (Table 1; Figure 2) indicated that the partially diploid strain χ 137Ex2 had to divide by the formation of uninucleate cells from binucleate cells. To test this prediction, the gelatin technique of MASON and POWELSON (1956) was employed. Out of 515 classifiable unirradiated cells (some 30 to 40% were not classifiable), 346 (67.2%) were uninucleate, 168 (32.6%) were binucleate, and one (0.2%) was tetranucleate. These data were obtained with

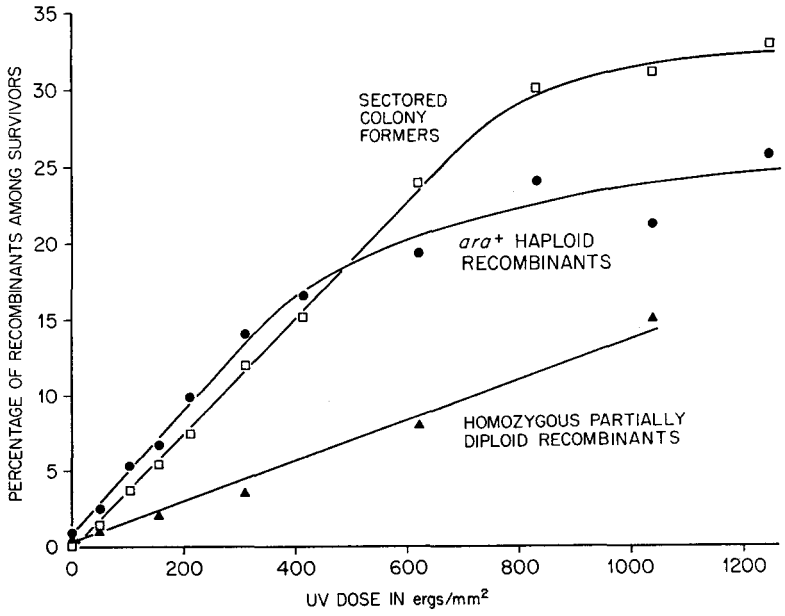


FIGURE 3.—Percent recombination as a function of UV dose in the partially diploid strain χ 137Ex2. See Figure 2 legend for experimental details.

ML-grown cultures; therefore, it cannot be stated whether this pattern of growth and division was due to cultural conditions or to genetic properties of the partially diploid strain.

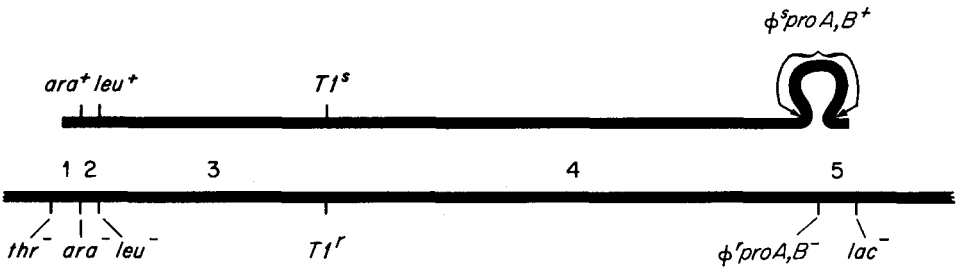
Immediately after 300 ergs/mm^2 of UV, the nuclear pattern of χ 137Ex2 cells was unchanged. These cells were then observed for a 10-hr period. About 60% of the cells had been killed by UV and did not divide or increase in size. Two hours after UV, the frequency of tetranucleate cells had increased to about 1% of the total cell population, and after 3½ hours it increased to 3 to 4%. Many of these tetranucleate cells either divided to yield two binucleate cells, which then divided to yield four uninucleate cells, or divided to give four uninucleate cells more or less simultaneously. These events occurred between 3 and 4 hours after UV. The remaining 36 to 37% of the population underwent normal growth and division of binucleate cells to uninucleate cells. A small proportion of the tetranucleate cells (about 0.5% of the total population) failed to divide and continued to elongate. Individual nuclei were difficult to discern, but from the length of these cells the number of nuclei could be estimated to be between 6 and 12. These elongated cells continued to pinch off uninucleate or binucleate cells from one or both ends for several hours, thus forming microcolonies in the gelatin medium. These were composed of one elongated cell and 20 to 30 uninucleate and binucleate cells. Between 8 and 10 hours after UV, most of these elongated cells divided to give microcolonies containing only uninucleate and binucleate cells. It is guessed that elongated cells like these are responsible for the colonies which are composed of 95 to 99% *ara*$^+$ haploid recombinants and 5 to 1% partially diploid cells (Table 1). The validation of this supposition would require micromanipulation.

The findings on the nuclear pattern of irradiated χ 137Ex2 cells are similar to those obtained by LEDERBERG *et al.* (1951) (who used fixed preparations of UV-irradiated, partially diploid cultures), except that their strain divided by the formation of binucleate cells from tetranucleate cells. χ 137Ex2 was found to divide by the formation of uninucleate cells from binucleate cells as was predicted from the results presented in sections 1 and 2.

4. Spontaneous and UV-induced haploid recombinant types: Table 2 summarizes data collected on the recombinant types obtained after spontaneous and UV-induced haploidization of the partially diploid strain χ 137Ex2. The spontaneous haploid recombinant-type frequencies are similar to those previously published (CURTISS 1964). Among the spontaneous haploid segregants, the exogenote *ara*$^+$, *leu*$^+$, and *T1*^{s} markers were inherited at frequencies of 94.1, 90.8, and 56.6%, respectively. In the UV-induced haploid segregants, the exogenote *ara*$^+$, *leu*$^+$, and *T1*^{s} markers were inherited at frequencies of 94.3, 92.1, and 61.4%, respectively. As shown by the data in Table 2, almost all haploid recombinants integrate the exogenote *ara*$^+$ marker, even though the region for homologous pairing between the *ara* gene and the end of the exogenote is very short (see Figure 1). When the exogenote in χ 137Ex2 is present in an F$^-$ strain with a *proB*$^-$ point mutation instead of ϕ^r*proA-B*$^-$ deletion mutation, about 95% of the frequent haploid segregants inherit the *proB*$^+$ exogenote marker. (The *proB*

TABLE 2

*Recombinant types resulting from spontaneous and UV-induced haploidization**



Genotype of haploids	Recombination in regions	Percent	
		Spontaneous	UV-induced
ara+ leu+ T1^s ϕ^sproA-B+	1 and 5	. . .†	0.2
ara+ leu+ T1^s ϕ^rproA-B−	1 and 4	53.1	60.0
ara+ leu+ T1^r ϕ^rproA-B−	1 and 3	35.8	31.2
ara+ leu− T1^r ϕ^rproA-B−	1 and 2	4.2	2.4
ara− leu+ T1^s ϕ^sproA-B+	2 and 5	. . †.	0.0
ara− leu+ T1^s ϕ^rproA-B−	2 and 4	0.8	0.2
ara− leu+ T1^r ϕ^rproA-B−	2 and 3	1.1	0.5
ara− leu− T1^s ϕ^sproA-B+	3 and 5	. . .†	0.0
ara− leu− T1^s ϕ^rproA-B−	3 and 4	1.7	0.5
ara− leu− T1^r ϕ^sproA-B+	4 and 5	. . .†	0.0
ara+ leu− T1^s ϕ^rproA-B−	1, 2, 3, and 4	1.0	0.5
ara− leu− T1^r ϕ^rproA-B−	None	2.3	4.5
		100.0	100.0

* The spontaneous haploid recombinants were obtained in five experiments in which the χ137Ex2 culture was challenged with T3 and then plated on Penassay agar spread with T3 so that only haploid segregants not inheriting the exogenote *ϕ^sproA-B+* marker would survive (CURTISS 1964). [This method is valid since only 2 out of 217 spontaneous haploid recombinants obtained by random selection inherited the *ϕ^sproA-B+* exogenote allele (CURTISS 1964).] Before testing of genotype, all of these T3-resistant haploid segregants were picked into 2 ml of Penassay broth containing antiserum to T3 at a dilution of 1:300. The UV-induced haploid segregants were obtained in four experiments in which χ137Ex2 had been plated immediately after receiving a 300 erg/mm² dose of UV. About two-thirds of these recombinants were classified after random selection of surviving colonies formed on Penassay agar, and the other one-third from colonies scored as containing haploid recombinants after growth on EMB arabinose agar. There were 525 spontaneous and 615 UV-induced haploid segregants analyzed.

† These classes were excluded by the selection method (see above footnote).

marker is only about 0.1 minutes of transfer time from the right end of the exogenote.) In such unstable partially diploid strains the inheritance of the *ara*$^+$ and *proB*$^+$ exogenote markers is about equal and most frequent, while the inheritance of the *T1^s* exogenote marker is least frequent. Thus, it is apparent that the ends of the exogenote are always more prone to be inherited by haploid segregants, provided that homologous pairing is permitted.

There were only 2.3 and 4.5% of spontaneous and UV-induced haploid segregants, respectively, which did not inherit any exogenote allele (Table 2). It is reasonable to assume that many of these "nonrecombinants" were actually recombinant for unmarked regions wherein recombination could not be detected. Therefore, it is concluded that in the partially diploid strain χ 137Ex2, recom-

bination is obligatory to obtain haploidization either spontaneously or by UV-induction. To explain this it is reasonable to postulate that the exogenote can only be lost by being physically consumed in the process of recombination.

5. *Photoreactivation of UV-induced recombination:* Photoreactivation is the enzymatic removal of pyrimidine dimers from UV-irradiated DNA in the presence of visible light (SETLOW 1966). Photoreactivation experiments, with both low and high dose rates of photoreactivating light, were therefore done to determine whether pyrimidine dimers were involved in stimulating recombination following UV irradiation of χ 137Ex2.

Presented in Figure 4 are data from one of the more extensive of these experi-

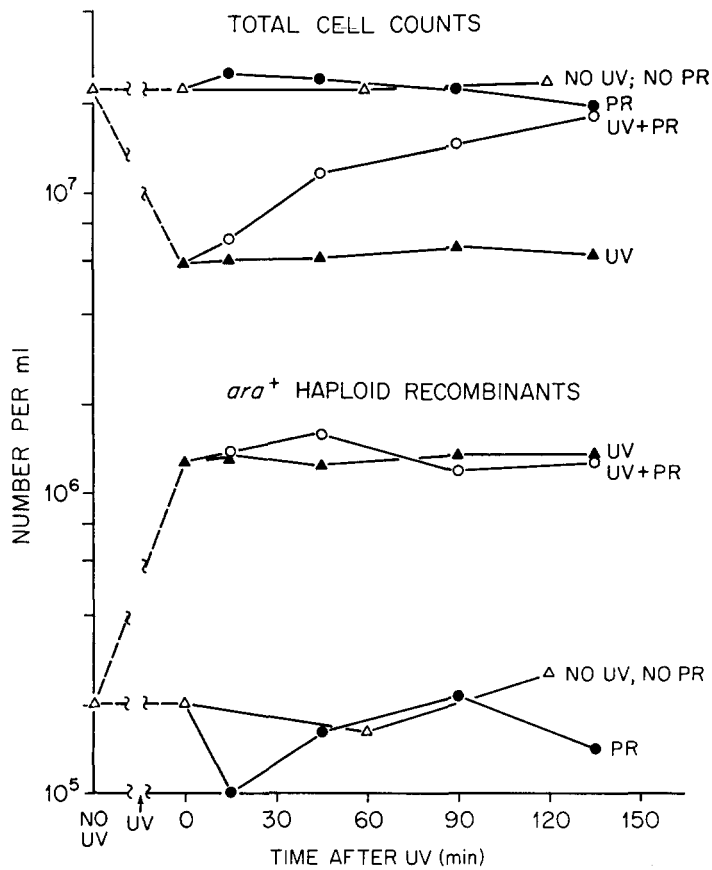


FIGURE 4.—Effect of photoreactivating light (PR) on UV-induced haploidization in the partially diploid strain χ 137Ex2. The log-phase bacteria were grown in ML lacking proline and were washed once and resuspended in BSG prior to administering a UV dose of 618 ergs/mm^2. Immediately after UV the culture was diluted 1:1000 into fresh BSG at room temperature. All cell titers are based on the original concentration of 2.2×10^7/ml. Photoreactivation was accomplished with black-light GE fluorescent bulbs at room temperature as described in MATERIALS AND METHODS. Plating was on EMB arabinose agar, and a mean of 683 colonies was scored for each point (range from 438 to 1923 colonies).

ments in which a low dose rate of photoreactivating light was used. In the culture as a whole there was 26% survival immediately after UV and 81% after 150 minutes of photoreactivation of the UV-irradiated sample. The titer of the unirradiated population remained constant, while photoreactivation alone resulted in 87% survival. There was no discernible liquid holding recovery (JAGGER, WISE, and STAFFORD 1964), since the survival of the irradiated sample was nearly the same after holding in BSG in the dark for 150 minutes (28%) as that occurring immediately after UV. If one considers the slight killing by photoreactivating light alone, it can be concluded that 90% (81%–28% ÷ 87%–28%) of the colony-forming ability lost after UV was photoreactivable.

It was found that there was a 6.2-fold net increase in the number of *ara*$^+$ haploid recombinants immediately following UV (Figure 4) and that their number did not change regardless of whether photoreactivating light was administered. The number of *ara*$^+$ haploid recombinants did not change in the samples given neither UV nor photoreactivating light or in the sample given photoreactivating light alone. The sectored colony formers followed the same behavior as the *ara*$^+$ haploid recombinants in the experiment illustrated in Figure 4 (data not shown). There was no increase or decrease in the number of sectored colony formers initially induced by UV during the 150 minutes of holding in the dark or in photoreactivating light. The mean titer of sectored colony formers for the five determinations after UV and holding in the dark was 8.9×10^5/ml, and that for the four determinations with photoreactivating light was 8.2×10^5/ml.

In one of the preliminary experiments on photoreactivation (data not shown), the effect of a maximal dose of photoreactivating light on UV-induced recombination with retention of the partially diploid state was studied. In this experiment the absolute number of *ara*$^+$ haploid recombinants, sectored colony formers, and partially diploid recombinants which had become homozygous at the *T1* locus showed a slight but parallel increase following photoreactivation of the UV-irradiated sample. Since enumeration and scoring of recombination events leading to homozygosity at one or more loci with retention of the partially diploid state require random picking of colonies from surviving cells and a minimum of four streaking operations, no further photoreactivation experiments on this recombinant class were conducted. Since photoreactivation had the same effect on all recombinant classes in this experiment, it is felt that the conclusions made for photoreactivation of UV-induced haploidization are generally valid for all types of UV-induced recombination in this partially diploid strain.

If the UV-induced recombination leading to formation of haploid segregants were as photoreactivable as was colony-forming ability, it would be expected that the number of haploid recombinants would have decreased to nearly the level in the no-UV sample. On the contrary, if there were no photoreactivation of haploid recombinant formation, then the number of *ara*$^+$ haploid recombinants would increase in parallel with the colony-forming ability as a function of the dose of photoreactivating light. This latter premise is based on the assumption that the probability of UV-induced recombination would be the same in all cells regardless of whether or not they survived UV. Obviously, neither of these ex-

treme cases is true (Figure 4), and it is concluded that the photoreactivable sector for UV-induced haploidization (in this experiment) is only about one-half of the photoreactivable sector for colony-forming ability.

The observation that colony-forming ability is more photoreactivable than is UV-induced recombination could indicate either (1) that pyrimidine dimers (see SWENSON and SETLOW 1966), which are the photoreactivable lesions (SETLOW 1966) are more important in cell killing than for genetic recombination, or (2) that some recombinants (ca. 50%) are formed or initial events leading to recombination take place immediately (or soon) after UV so that the removal of pyrimidine dimers by the photoreactivating enzyme has no effect. To differentiate between these two possibilities, two types of experiments were done. In the first, the UV-irradiated culture of partially diploid cells was stored in the dark at room temperature for 30 minutes prior to giving the photoreactivating light (Figure 5). In this experiment 19% of the cells survived the UV dose, and 67% of the inactivated colony-forming ability was photoreactivated by the 120-minute treatment. There was a 5-fold increase in the absolute number of *ara*$^+$ haploid recombinants immediately after UV, and 21% of the survivors were haploid recombinants. Holding the irradiated culture in the dark for 30 minutes prior to administering 120 minutes of photoreactivating light resulted in a 2-fold increase in the absolute number of *ara*$^+$ haploid recombinants (Figure 5). This result strongly suggests that most of the initial steps in UV-induced haploidization occurred during the first 30 minutes following UV, and therefore, that the subsequent repair of pyrimidine dimers by the photoreactivating enzyme would be without effect in preventing UV-induced recombinant formation. This result also validates the assumption made above that cells originally killed by UV do contain UV-induced haploid recombinants. Based on the above results, it was reasoned that it might be possible to obtain a decline in the number of *ara*$^+$ haploid recombinants if a maximal dose of photoreactivating light could be given in a short period of time immediately after UV. For this second type of experiment, the Hilger quartz prism monochromator was used, and photoreactivation was at 37°C, the temperature at which most efficient photoreactivation is obtained (see SETLOW 1966). As is shown in Figure 6, the absolute number of *ara*$^+$ haploid recombinants declined significantly when the photoreactivation was carried out under the above described conditions. If UV-induced recombination is equally probable in surviving and nonsurviving cells, an assumption partially supported by the experiment presented in Figure 5, then it can be estimated that about 80% of the potential UV-induced *ara*$^+$ haploid recombinants were photoreactivated (Figure 6). The observed decline in the number of *ara*$^+$ haploid recombinants also proves that pyrimidine-dimer formation is responsible, at least in part, for the increase in recombination caused by UV irradiation.

It is concluded (1) that the initial event(s) leading to UV-induced haploidization occurs in most cells within 30 minutes after UV, (2) that some cells initially killed by UV have also undergone UV-induced haploidization, and (3) that pyrimidine dimers are responsible for some, if not all, of the stimulation of haploidization by UV irradiation. It is felt that these conclusions are also valid

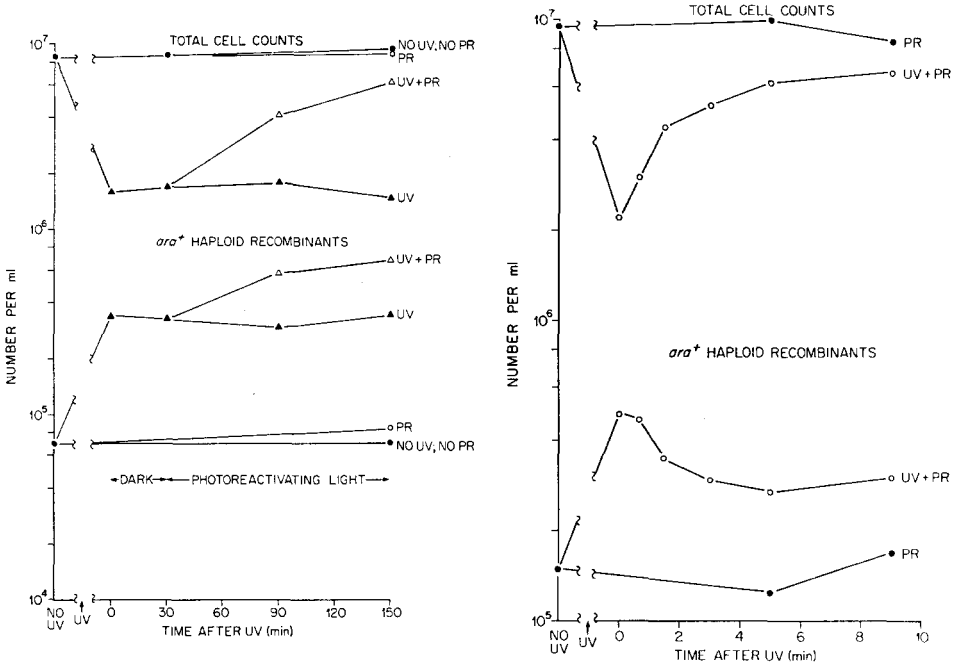


FIGURE 5.—The effect on UV-induced recombination of storage in the dark for 30 minutes prior to administering photoreactivating light. Conditions identical to those described in Figure 4 legend except that the dose of UV was 612 ergs/mm^2. A mean of 427 colonies was scored for each point (range from 315 to 601 colonies). All cell titers are based on the original concentration of .86 $\times$ 10^6 cells/ml.

FIGURE 6.—Photoreactivation of UV-induced haploidization by use of monochromatic (4047Å) light. χ 137Ex2 was grown to log phase in ML lacking proline and was sedimented and resuspended in BSG without washing before receiving a UV dose of 600 ergs/mm^2. (A different UV source similar to that described in MATERIALS AND METHODS was used in this experiment.) The irradiated suspension was diluted 1:5000 into prewarmed (37°C) BSG immediately after UV prior to administering the photoreactivating light. Cell titers are based on the original concentration of 9.5 $\times$ 10^6/ml. A mean of 353 colonies was scored for each point (range of 190 or 535 colonies). Some of the control platings were omitted due to the short experimental duration. Other experimental details are described in MATERIALS AND METHODS.

for UV-induced recombination leading to homozygosity at one or more loci with retention of the partially diploid state, since photoreactivating light had the same effect on these recombinants as on haploid recombinants in one experiment.

6. *Effect of starvation on UV-induced recombination:* In most of the photoreactivation experiments described above, the cells were held in BSG in a nongrowing state for as long as 2.5 hours after UV with no noticeable decline in recombinant frequency. This observation suggested that active growth following UV might not be necessary for the initial step(s) in recombinant formation. Therefore, the effects of starvation before and after UV on the frequency of recombinant induction were studied. A representative experiment is presented in Figure 7. During the period of 6-hour starvation before UV there was a 22% decrease in the percentage of *ara*$^+$ haploid recombinants and an almost equal

increase of 17% in the percentage of sectored colony formers. Independent experiments on a partially diploid strain related to χ137Ex2 have shown that there was about a 20% increase in DNA that was synthesized at some time during the first 2 hours of starvation of a culture handled in the same manner as described for χ 137Ex2 in the Figure 7 legend. As will be proven in the following section, sectored colony formers arise by single haploidization events occurring in binucleate cells and in uninucleate cells with the diploid *ara* to *proB* region of the genome duplicated. Thus, this increase in DNA synthesized during the first 2 hours of starvation could increase the frequency of cells in the population which are either binucleate or uninucleate with the *ara* to *proB* diploid region duplicated. This in turn would give rise to a higher frequency of sectored colony formers and a concomitant decrease in the frequency of *ara*$^+$ haploid recombinants, most of which arise by single recombinational events occurring in uninucleate cells with the diploid *ara* to *proB* region unduplicated.

Starvation for 2 hours after UV caused a decrease in the frequency of *ara*$^+$ haploid recombinants for the cultures having 0 and 2 hours of starvation before UV and a slight increase in the frequency of *ara*$^+$ haploid recombinants for the cultures having 4 and 6 hours of starvation prior to UV (Figure 7). In a repeat of this experiment, starvation for 2 hours after UV resulted in slight decreases in the frequency of *ara*$^+$ haploid recombinants for the cultures having 0 and 6 hours of starvation before UV, and in significant increases in the frequency of *ara*$^+$ haploid recombinants for the cultures having 2 and 4 hours of starvation before UV. Thus, by evaluating all the results of experiments like the one presented in Figure 7, it is concluded that starvation after UV has little or no effect on the frequency of UV-induced *ara*$^+$ haploid recombinants. In all instances, however, starvation after UV caused declines in the frequencies of sectored colony formers regardless of the duration of starvation prior to UV (Figure 7).

The results of these experiments indicate that starvation before or after UV is without effect on UV-induced haploidization. In conjunction with the results obtained in photoreactivation experiments (RESULTS, section 5), it is postulated that pyrimidine dimers induced by UV cause the formation of primary recombinant structures in the absence of all metabolic activity soon after UV and that these primary recombinant structures are stable during prolonged starvation conditions. Presumably, the UV-induced haploidization event would become finalized after biosynthetic activity is resumed.

7. *Growth of partially diploid populations following UV irradiation:* As has already been mentioned, the plating of UV-irradiated, partially diploid cells immediately after UV results in the formation of colonies containing only haploid recombinants and of sectored colonies composed of more or less equal numbers of haploid recombinants and partially diploid cells (RESULTS, sections 1 and 2). Also, it was found that two thirds of the partially diploid cells grown in ML were uninucleate and one third binucleate (RESULTS, section 3). These findings suggest that the pure clones of haploid recombinants arose by UV-induced recombinational events occurring in uninucleate cells having the diploid *ara* to *proB* region of the genome unduplicated. Likewise, single recombinational events occurring in

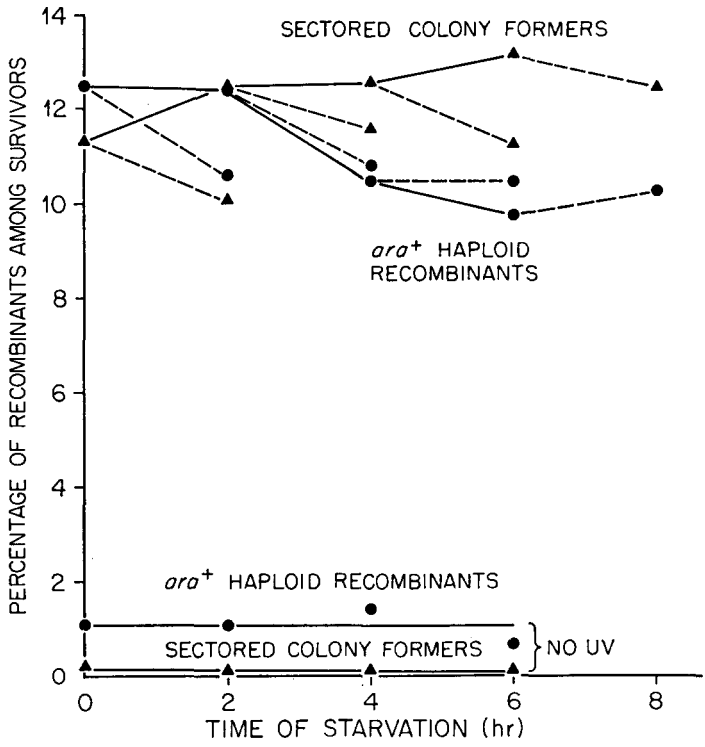


FIGURE 7.—Effect of pre- and post-UV starvation on UV-induced genetic recombination. χ 137Ex2 was grown to log phase in ML lacking proline with aeration at 37°C, after which the culture was sedimented, washed once with BSG, and resuspended at a concentration of 1.8×10^7 cells/ml in prewarmed BSG. This culture was allowed to starve for 0, 2, 4, and 6 hours as a standing nonaerated culture in BSG at 37°C at which times a UV dose of 291 ergs/mm² was administered to 1.0-ml samples. Immediately after UV, the culture was diluted 1:100 in prewarmed BSG at 37°C, a sample plated, and the remainder starved for another 2 hours before plating. The unirradiated cell titers after 2, 4, and 6 hours of starvation were 1.9×10^7/ml, 1.4×10^7/ml, and 1.2×10^7/ml, respectively. Immediately following UV, the survival for the 0, 2, 4, and 6 hours of starvation periods were 39%, 34%, 40%, and 45%, respectively. The cell survival of the cultures receiving 2 hours of additional starvation following UV of the cultures prestarved for 0, 2, 4, and 6 hours were 40%, 31%, 43%, and 39%, respectively. Plating was on EMB-arabinose agar, and a mean of 899 colonies was scored for each sample (range of 579 to 1128 colonies). The solid lines connect data points obtained from platings immediately after UV irradiation of cultures starved for the indicated time before UV. The dashed lines connect data points from platings immediately after UV and from platings after 2 hours of starvation after UV for the same culture given the indicated time of prestarvation.

binucleate cells and in uninucleate cells with the exogenote and the *ara* to *proB* region of the chromosome (endogenote) duplicated would give rise to sectored colonies. It is also suggested that two recombinational events, each occurring either in separate nuclei with the *ara* to *proB* segment unduplicated or in the same nucleus with the diploid *ara* to *proB* region of the genome duplicated, would yield clones containing only haploid recombinants. The experiments described in this

and the following section test these suggested assignments of cell types giving rise to specific recombinant types.

Based on the above reasoning, it would be predicted that by allowing for growth following UV treatment, the sectored colony formers would disappear with a concomitant increase in the number of pure clones of haploid recombinants. Figure 8 presents results obtained from one of the more extensive experiments of this type. In this experiment there was 30.4% total cell survival, a 4-fold increase in the number of *ara*$^+$ haploid recombinants, and a 20-fold increase in the number of sectored colony formers—as determined by plating immediately before and after UV. Following the UV dose, a division delay of nearly 60 minutes occurred before an increase in cell number was observed. After this lag, the concentration of *ara*$^+$ haploid recombinants increased at a faster rate than did the total cell population until about 150 minutes after UV; thereafter, the rates of growth paralleled one another. This more rapid increase in the number of *ara*$^+$ haploid recombinants was balanced by the concomitant decrease in the number of cells giving rise to sectored colonies. Eventually the number of cells giving rise to sectored colonies begins to increase again (Figure 8) because spontaneous haploidization results in about 0.2% of the cells in a partially diploid culture, giving rise to sectored colonies (see Table 1).

Figure 9 presents the data shown in Figure 8 in a different manner, such that the changes in the *ara*$^+$ haploid recombinant and sectored colony former populations are more easily discerned. As the percentage of *ara*$^+$ haploid cells increased,

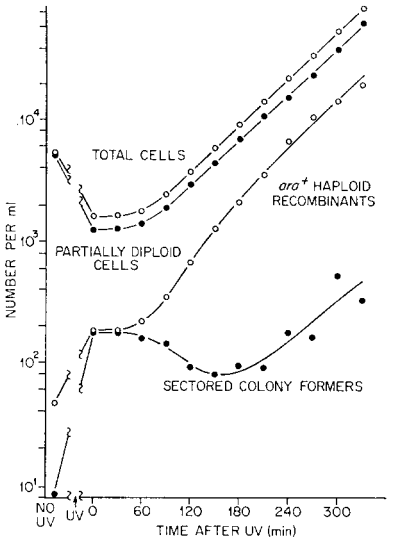


FIGURE 8.—Growth of recombinant and nonrecombinant types following UV irradiation. χ 137Ex2 was grown to log phase at 37°C in ML lacking proline and was irradiated with 300 ergs/mm^2 of UV in this medium at a density of 1.05×10^7/ml and then immediately diluted 1:2000 into prewarmed ML containing proline. All plating was on EMB arabinose agar, and a mean of 943 colonies was scored for each sampling time (range of from 464 to 1795 colonies).

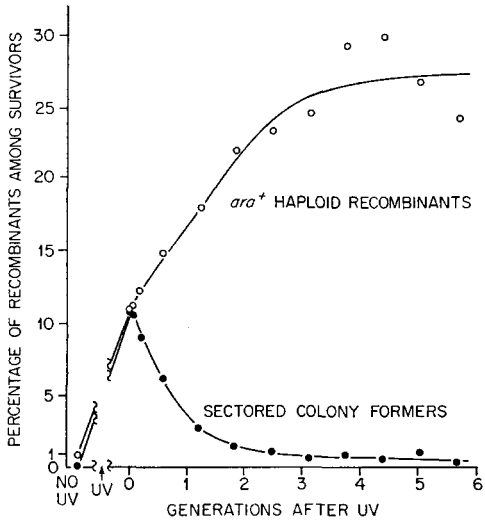


FIGURE 9.—Segregation of sectored colony formers to yield *ara*+ haploid recombinants as a function of generations of growth following UV. Data replotted from Figure 8. Percentages and numbers of generations based on total surviving cell concentrations.

the percentage of cells giving rise to sectored colonies showed a parallel decrease. Theoretically, the *ara*+ haploid population should have leveled off at 22.5%—the sum of the percentages for the *ara*+ haploid recombinants and the sectored colony formers in the zero-time plating immediately after UV. Instead, the *ara*+ haploid population leveled off at about 27%. Presumably, this deviation (also observed in other experiments) from the expected is due to the facts (1) that 5% of the sectored colonies were of the type composed of 95 to 99% *ara*+ haploid recombinants and 5 to 1% partially diploid cells, and (2) that the frequency of cells giving rise to sectored colonies did not decline even after six generations of growth to the value of 0.17% observed in the unirradiated population. While the above two facts are probably related causally, either would account for the observed slight excess in the final frequency of *ara*+ haploid recombinants. The second finding suggests that UV damage inflicted during one generation might cause some recombination during the one or two generations after UV. Such delayed effects of UV would also contribute to an overestimation of the frequency of sectored colonies in platings done immediately after UV.

If the sectored colonies were due to single recombinational events occurring in binucleate cells or in uninucleate cells with the diploid *ara* to *proB* segment of the genome duplicated, then it would be expected that the sectored colony formers should segregate around a mean of one generation of growth after UV to yield *ara*+ haploid recombinants and partially diploid cells. As is shown in Figure 9, this predicted behavior was observed. This behavior was also observed in the pedigree analysis experiments (see RESULTS, section 1).

8. *Recombinant types from binucleate cells:* As previously stated, pure clones of haploid recombinants might arise by two independent UV-induced recombi-

national events occurring in binucleate cells or in uninucleate cells having the diploid *ara* to *proB* region of the genome duplicated. If this were so, then it would be expected that sometimes one of the recombinational events would yield an *ara*$^+$ *T1*^{s} ϕ^r*proA-B*$^-$ recombinant and the other an *ara*$^+$ *T1*^{r} ϕ^r*proA-B*$^-$ recombinant. As shown in Table 3, clones with both types of haploid recombinants were obtained. The frequency of clones containing both *T1*^{s} and *T1*^{r} haploid recombinants is plotted as a function of UV dose in Figure 10. The somewhat exponential increase in these mixed clones for UV doses up to 300 ergs/mm^2 is to be expected, since the occurrence of two independent events should follow a dose-square function. For doses above 300 ergs/mm^2, the frequency of these mixed clones declined steadily; this can be accounted for by the expectation that the proportion among survivors of binucleate cells and uninucleate cells with the diploid *ara* to *proB* segment duplicated that have two viable genomes would likewise decline as the UV dose increased. The occurrence of mixed clones in the unirradiated sample (Table 3; Figure 10) and another cause of mixed clones containing haploid recombinants are discussed in the following section.

For one of the experiments presented in a later section, UV-irradiated partially diploid cultures were allowed to grow for three to four generations in ML containing proline before plating on either MA containing proline or Penassay agar. Out of 402 clones of haploid recombinants analyzed after random selection, not a single one contained both *T1*^{s} and *T1*^{r} haploid recombinants. This provides

TABLE 3

Analysis of colonies containing haploid recombinants for equal numbers of T1^s *and T1*^{r} *haploid recombinants**

UV dose ergs/mm^2	Colonies having haploid recombinants which were:						
	T1^s		*T1*^{r}		*T1*^{s} and *T1*^{r}		
	Number	Percent	Number	Percent	Number	Percent	Total number
0	334	61.3	206	37.8	5	0.92	545
100	144	85.7	21	12.5	3	1.79	168
200	155	84.2	19	10.3	10	5.43	184
300	179	63.5	76	27.0	27	9.57	282
400	85	82.5	9	8.7	9	8.74	103
600	192	86.5	15	6.8	15	6.76	222
800	110	76.4	26	18.1	8	5.55	144
1200	64	87.7	6	8.2	3	4.11	73
	1263	73.4	378	22.0	80	4.6	1721

* Data obtained from ten experiments in which χ137Ex2 was grown to log phase in ML lacking proline prior to UV irradiation with plating immediately thereafter. The given UV doses are approximate averages, since the dose rate of the UV source varied from a low of 9.8 ergs/mm^2/sec to a high of 10.4 ergs/mm^2/sec in the various experiments. Most of the spontaneous haploid recombinants were obtained by selection with T3, and these were included in the Table 2 data. About two thirds of the haploid recombinants obtained after the 300 ergs/mm^2 dose were obtained by random selection and testing of survivors on Penassay agar. The mean cell survival after 300 ergs/mm^2 of UV for the eight experiments in which this dose was given was 28.5%. Most other haploid recombinants were obtained from dose response experiments like that presented in Figure 2 by selecting from EMB arabinose agar those colonies visually scored as containing haploid recombinants. Much of the data for the 100, 200, and 400 through 1200 ergs/mm^2 doses came from the experiment shown in Figure 2, and in this experiment there was a strong bias towards the inheritance of the exogenote *T1*^{s} marker which was not observed in other experiments. After initial testing, all colonies showing a mixed response to T1 were tested further to be sure that the heterozygosity did not persist and was due to the presence of a mixture of *ara*$^+$ *T1*^{s} ϕ^r*proA-B*$^-$ and *ara*$^+$*T1*^{r} ϕ^r*proA-B*$^-$ haploid recombinants. No persisting heterozygosity at the *T1* locus was observed.

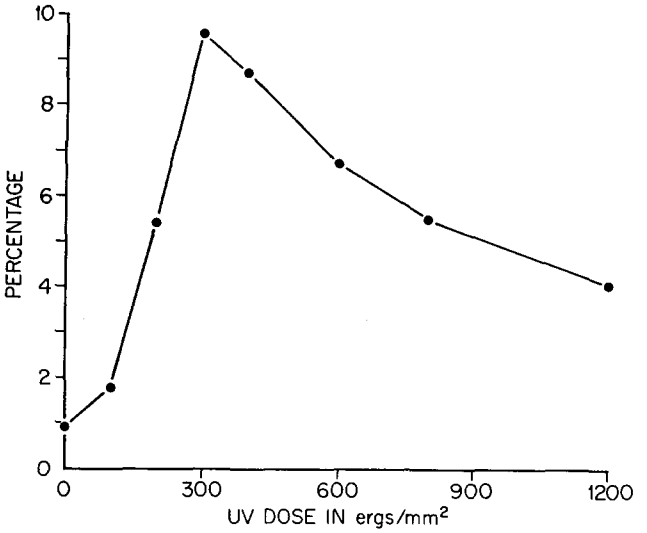


FIGURE 10.—Percentage of colonies of haploid recombinants which contained both *ara*+ *T1*^{s} ϕ^r*proA-B*$^-$ and *ara*+ *T1*^{r} ϕ^r*proA-B*$^-$ haploid recombinants as a function of UV dose. Data plotted are taken from Table 3.

further evidence that the apparent heterozygosity at the *T1* locus did not persist (see footnote to Table 3).

The data listed in Table 3 and illustrated in Figure 10 made it possible to calculate the frequency of *ara*+ haploid recombinant clones which arose by two independent recombinational events occurring in binucleate cells or in uninucleate cells with the diploid *ara* to *proB* region of the genome duplicated. This calculation for *ara*+ haploid recombinants formed after a UV dose of 300 ergs/mm² is presented in APPENDIX I. A value of 22% was obtained, which means that the remaining 78% of *ara*+ haploid recombinants arose from single recombinational events in uninucleate cells with the diploid *ara* to *proB* region unduplicated.

9. *Occurrence of regional heterozygosity as a result of recombination:* In addition to the occurrence of two independent haploidization events, there is another explanation, which might account for some of the clones containing both *T1*^{s} and *T1*^{r} haploid recombinants (Table 3, Figure 10). The recombinant clones on which this alternative explanation is based are listed in Table 4. Although the probabilities that these mixed clones were the result of single haploidization events are low, the probabilities that there were two independent events are exceedingly low in all eight instances (Table 4). These comparative probabilities suggest that the mixed clones resulted from single haploidization events in which the initial regional heterozygosity at the *T1* locus was due to inexact union of the two strands in the DNA helix. Such regional heterozygosis was discovered and described by HERSHEY and CHASE (1951) in crosses with phage T2 mutants. LEVINTHAL (1954) obtained evidence from genetic experiments with T2 that this regional heterozygosis was a consequence of genetic recombination. MESELSON (1964) has

TABLE 4

*Clones of haploid recombinants which suggest the occurrence of regional heterozygosity as a result of recombination**

Dose in ergs/mm²	Number	Genotype of both haploid recombinants occurring in the same mixed clone	Single event probability†	Double event probability‡
0	5	*ara*+ *leu*+ *T1*^{s} ϕ^r*proA-B*⁻ and *ara*+ *leu*+ *T1*^{r} ϕ^r*proA-B*⁻	1.2×10^{-3}	1.0×10^{-6}
300	1	*ara*⁻ *leu*+ *T1*^{s} ϕ^r*proA-B*⁻ and *ara*⁻ *leu*+ *T1*^{r} ϕ^r*proA-B*⁻	6.7×10^{-4}	1.4×10^{-7}
	1	*ara*+ *leu*⁻ *T1*^{s} ϕ^r*proA-B*⁻ and *ara*+ *leu*⁻ *T1*^{r} ϕ^r*proA-B*⁻	2.7×10^{-3}	1.7×10^{-6}
800	1	*ara*+ *leu*⁻ *T1*^{s} ϕ^r*proA-B*⁻ and *ara*+ *leu*⁻ *T1*^{r} ϕ^r*proA-B*⁻	6.3×10^{-3}	3.0×10^{-6}

* Recombinant clones obtained in experiments cited in Table 3.

† The probability that the two haploid recombinant types in each clone were due to one recombinational event was calculated by multiplying the frequency of haploidization per cell times the mean frequency between the two recombinant types from Table 2 times the frequency of viable uninucleate cells with the diploid *ara* to *proB* region unduplicated. These three values were 0.004, 0.445, and 0.67, respectively, for no UV; 0.25, 0.0035 (first example) or 0.014 (second example) and 0.77, respectively, for 300 ergs/mm² of UV; and 0.50, 0.014, and 0.9, respectively, for 800 ergs/mm² of UV. The spontaneous haploidization frequency was taken from CURTISS (1964) and for 300 and 800 ergs/mm² of UV from Figure 3 and Table 7. The frequencies of uninucleate cells with the diploid *ara* to *proB* region unduplicated were estimated by using equations in APPENDIX II (see DISCUSSION) and the assumption that the number of cells with two functional partially diploid genomes would decrease with increasing doses of UV (see RESULTS, section 8, and DISCUSSION).

‡ The probability that the two haploid recombinant types in each clone were due to two independent recombinational events occurring in binucleate cells or in uninucleate cells with the diploid *ara* to *proB* region duplicated was calculated by multiplying the frequency of haploidization per cell squared times the product of the frequencies of occurrence of the two recombinant types from Table 2 times the frequency of binucleate cells and uninucleate cells with the diploid *ara* to *proB* region duplicated. All values used were cited above or in Table 2. For simplicity it was assumed that the values for 300 ergs/mm² in Table 2 were valid for 800 ergs/mm².

recently confirmed this finding by employing density isotopes and genetic techniques with bacteriophage λ.

One requisite condition for concluding that the mixed clones listed in Table 4 are due to regional heterozygosis as a consequence of recombination is that the *T1*^{r} mutation must be a single nucleotide pair change rather than a deletion-type mutation. Experiments in our laboratory (unpublished) confirm this, since *T1*^{s} recombinants have arisen in matings between strains with different *T1*^{r} mutations, complementation between different *T1*^{r} mutations in partially diploid strains has resulted in a *T1*^{s} phenotype, and spontaneous reversions from *T1*^{r} to *T1*^{s} have been observed.

In section 8 of RESULTS and in APPENDIX I, calculations show that 22% of the *ara*+ haploid recombinants induced by 300 ergs/mm² of UV irradiation arose by two independent haploidization events. This value needs to be corrected to take into account those clones containing both *T1*^{s} and *T1*^{r} haploid recombinants which resulted from single recombinational events yielding heterozygosis at the *T1* locus. In order to make this correction the frequency of clones containing both *T1*^{s} and *T1*^{r} haploid recombinants spontaneously (0.92%, Table 3) is assumed to represent the frequency of mixed clones resulting from single haploidization events among survivors of other UV doses. In other words, we assume that regional heterozygosity for any marker will result from a single haploidization even about 1% of the time. When this value is subtracted from the frequency of clones containing both *T1*^{s} and *T1*^{r} haploid recombinants among survivors of 300 ergs/mm² of UV (9.5%–0.92%, Table 3), the remainder (8.65%) is equivalent to the frequency of mixed clones caused by two independent haploidization events. By using the equations in APPENDIX I to recalculate the values of p and q, it is found

that 19.5% of the *ara*$^+$ haploid recombinants induced by 300 ergs/mm^2 of UV arise by two independent haploidization events in binucleate cells and in uninucleate cells having the diploid *ara* to *proB* segment duplicated.

10. *Recombination with retention of the partially diploid state leading to homozygosity for one or more markers:* Recombinants which become homozygous for one or more alleles while remaining partially diploid were observed at appreciable frequencies among survivors of UV irradiation (RESULTS, sections 1 and 2). Since scoring of these recombinants involved random selection of colonies from surviving cells, and four to six streaking operations per isolate to determine the complete genotype, no attempt was made to enumerate and characterize them in all of the experiments cited in previous sections. In spite of these experimental difficulties, it was deemed worthwhile to study the nature of these recombinants more fully and to see if there was any segregational increase in their number by allowing for growth following UV.

Random selection of colonies from cells surviving 300 ergs/mm^2 of UV indicated the presence of partially diploid recombinants which had become homozygous for the *ara*$^-$, *leu*$^-$, and/or *T1*^{r} endogenote markers or for the *T1*^{s} exogenote marker (Table 5). Finding and proving the existence of recombinants homozygous for the exogenote *ara*$^+$ and/or *leu*$^+$ markers would have been exceedingly difficult, if not impossible. By suspending whole colonies grown up from surviving cells plated immediately after UV exposure, it was found that the reciprocal *T1*^{s}/*T1*^{s} and *T1*^{r}/*T1*^{r} recombinants never occurred in the same clone. This finding indicates that the two types are not formed by the same event. The data in Table 5 show that 127 out of the 134 diploid recombinants had become homozy-

TABLE 5

*Genotypes of UV-induced partially diploid recombinants homozygous for one or more markers**

Locus genotype (exogenote/endogenote)				Number	Percent
ara	*leu*	*T1*	*φproA-B*		
+/— or +	+/— or +	S/S	S+/R—	70	52.2
+/— or +	+/— or +	R/R	S+/R—	39	29.1
+/— or +	—/—	S/R	S+/R—	11	8.2
—/—	+/— or +	S/R	S+/R—	7	5.2
+/— or +	—/—	S/S	S+/R—	0	0
+/— or +	—/—	R/R	S+/R—	2	1.5
—/—	+/— or +	S/S	S+/R—	1	0.75
—/—	+/— or +	R/R	S+/R—	1	0.75
—/—	—/—	S/S	S+/R—	1	0.75
—/—	—/—	R/R	S+/R—	2	1.5
Total				134	99.95

* Data taken from eight experiments in which χ137Ex2 was grown to log phase in ML lacking proline prior to being given a dose of 300 ergs/mm^2. Plating on MA containing proline or on Penassay agar was done immediately after UV. The mean cell survival in the eight experiments was 32.3% (range of 29.0 to 37.6%). Colonies were picked at random into 2 ml of Penassay broth for testing. No attempt was made to determine the allelic state at the endogenote *ara* and *leu* loci when the exogenote carried the *ara*$^+$ and/or *leu*$^+$ markers.

gous for only one of the three loci tested. This lack of linkage, especially significant for the nearly adjacent *ara* and *leu* loci, coupled with the independent occurrence of the reciprocal $T1^s/T1^s$ and $T1^r/T1^r$ types, suggests that recombination leading to homozygosity with retention of the partially diploid state may be analogous to gene conversion as described by LINDEGREN (1953). Three of the partially diploid recombinants listed in Table 5 had to arise by quadruple recombination events, and two of these were homozygous for both endogenote and exogenote markers.

In regard to homozygosity for endogenote markers, homozygosity for $T1^r$ was most common (44 instances), for leu^- less frequent (16 instances), and for ara^- least frequent (12 instances). Although it was not possible to determine whether homozygosity for the exogenote marker ara^+ was more frequent than for leu^+ and homozygosity for leu^+ more common than for $T1^s$, the above results on homozygosity for endogenote markers suggest that the same factors responsible for the genotypes of UV-induced haploid recombinants (Table 2) are also operative for recombination leading to homozygosity with retention of the partially diploid state. Thus, among haploid segregants, the endogenote $T1^r$ marker was more commonly present than leu^-, and leu^- more commonly present than ara^- (Table 2). Therefore, an event akin to haploidization might be involved in forming these homozygous partially diploid recombinants.

Several tests were performed to show that partially diploid recombinants which were homozygous for endogenote markers had not lost part of the exogenote while retaining the $\phi^s proA\text{-}B^+$ exogenote marker. Twenty-one partially diploid recombinants, including all types found, were chosen for further study and each was made F^+ by infecting with F from $\chi 15$ (CURTISS 1964). These isolates were then mated with a series of appropriately marked cycloserine-resistant (CURTISS, CHARAMELLA, BERG, and HARRIS 1965) F^- strains which were closely related to $\chi 137$. For example, the ara^- mutation in $\chi 137$ is in the *araA* cistron and thus blocks the synthesis of L-arabinose isomerase. Therefore, when an F^+ partially diploid recombinant which was homozygous for this $araA^-$ marker was mated with an F^- with a mutation at the *araB* locus (deficient in L-ribulokinase) with selection for proline-independent cycloserine-resistant recombinants, it was observed that all of these recombinants were capable of fermenting arabinose. When some of these recombinants were then challenged with phage T7 and plated on EMB arabinose agar, more than 99% of the surviving haploid segregants were unable to ferment arabinose, thus proving that a complementation heterozygote had been formed. Similar tests on the other types of partially diploid recombinants which had become homozygous for one or more markers yielded results which indicated that these recombinants had not lost any part of the exogenote.

All 21 of the homozygous partially diploid recombinants chosen for further study were each grown up in ML deficient in proline and then challenged with T7 and plated on Penassay agar to determine the frequency of spontaneous haploid segregants (CURTISS 1964). This was done on the belief that a partially diploid strain which had lost most of the exogenote while retaining the $\phi^s proA\text{-}B^+$ marker might show a different stability in regard to spontaneous haploidization

TABLE 6

*Recombination with retention of the partially diploid state**

Conditions	Recombinants homozygous for:				Total number of colonies tested
	T1^r		*T1^s*		
	Number	Percent	Number	Percent	
No UV	2	0.11	6	0.33	1799
300 ergs/mm²; 0 generations	36	2.41	69	4.63	1491
300 ergs/mm²; 3.7 generations	25	1.53	64	3.92	1634

* Data taken from six experiments with χ137Ex2 by use of procedures as described in Table 5 footnote. Data from four of these experiments are included in the data in Table 5. The mean cell survival immediately after UV was 30.6% (range of 27.1 to 33.2%). Immediately following UV, the culture was diluted 5000-fold into prewarmed ML containing proline and allowed to grow for 5 hours at 37°C. The mean number of generations of growth following UV was 3.7 (range of 2.9 to 4.7 generations).

frequency. In all 21 strains, the spontaneous frequency of haploid segregants was between 0.35 and 0.65%, which is within the range found by CURTISS (1964) for the partially diploid strain having an exogenote with the entire *ara* to *proB* segment. Based on all the above described tests, it is concluded that these partially diploid recombinants are due to exchanges resulting in homozygosity and not to partial losses of the exogenote yielding hemizygosity.

To determine whether there was any segregational increase in the number of partially diploid recombinants which had become homozygous for either the *T1^s* or *T1^r* markers, several experiments were conducted in which several generations of growth were allowed following UV. The results of these experiments are summarized in Table 6. The frequency of recombinants which were homozygous for the endogenote *T1^r* marker were less frequent than those homozygous for the exogenote *T1^s* marker for each of the three conditions tested. This finding lends further credence to the conclusion that both types are not formed in the same event. It is obvious by inspection of the data in Table 6 that there was no segregational increase in the frequency of partially diploid recombinants by allowing for growth after UV irradiation. This result strongly suggests that these recombinants are formed in viable uninucleate cells and few, if any, in viable binucleate cells.

This conclusion was confirmed by analyzing partially diploid cells from sectored colonies formed on EMB arabinose agar in platings done immediately after UV. By analyzing 98 sectored colonies that contained nearly equal numbers of partially diploid and haploid cells, two instances were found in which the partially diploid cells were homozygous for the *T1^s* marker. Since this class of sectored colony occurred at a frequency of about 10 to 11% among survivors of 300 ergs/mm² of UV, then approximately 0.2% of the surviving cells would be binucleate cells in which recombination leading to homozygosity with retention of the partially diploid state had occurred in one nucleus and haploidization in the other nucleus. As mentioned in section 1 of RESULTS, about 0.5% of the surviving cells gave rise to sectored colonies that contained 95 to 99% haploid recombinant cells and 5 to 1% partially diploid cells. The partially diploid cells from 22 colonies of this type were analyzed, and surprisingly, eight of these colonies contained par-

tially diploid cells which were all recombinants homozygous for either the *T1*^{r} marker (2) or for the *T1*^{s} marker (6). In each of these cases the haploid recombinants in each clone had the same *T1* locus genotype as the partially diploid recombinants in that clone. Whether this indicates a causal relationship or not is difficult to conclude without further analysis. By assuming that this second type of sectored colony also arises from binucleate cells, it can be calculated that another 0.2% of the surviving cells would be binucleate with recombination leading to homozygosity at the *T1* locus having occurred in one nucleus and haploidization in the other. This last type of mixed clone, however, would not give any segregational increase in the number of partially diploid recombinants in the experiment presented in Table 6, because the number of haploid recombinant segregants during the generations immediately after UV was disproportionately high. By using (1) the frequency of sectored colony formers, (2) the calculated value for the frequency of binucleate cells and uninucleate cells with the diploid *ara* to *proB* region duplicated surviving 300 ergs/mm^2 of UV irradiation (see DISCUSSION and APPENDIX II), and (3) the frequency of sectored colonies having partially diploid cells that were homozygous at the *T1* locus, it can be estimated that between 0.5 and 1.0% of the surviving cells would contain homozygous partially diploid recombinants in mixed clones which could segregate by allowing for growth following 300 ergs/mm^2 of UV. Since 7% of the cells surviving this dose contain only partially diploid recombinants homozygous at the *T1* locus (Table 6), it is estimated that about 90% of these events did occur in uninucleate cells. It is therefore unlikely that the experiments presented in Table 6 would have shown any segregational increase in the number of partially diploid recombinants homozygous at the *T1* locus.

It is concluded from these studies (1) that the partially diploid recombinants arise by recombinational events yielding homozygosity for one or more markers with no change in the length of the exogenote, (2) that events giving rise to these recombinants are similar to those giving rise to haploid recombinants, (3) that reciprocal *T1*^{s}/*T1*^{s} and *T1*^{r}/*T1*^{r} homozygous partially diploid recombinants arise by separate events in different cells, and (4) that 90% of these recombinants arise in uninucleate cells.

11. *Search for reciprocal recombinational events induced by UV-irradiation:* To facilitate the search for recombinants in which the position of exogenote and endogenote alleles had become reversed due to reciprocal recombination events, rapid screening techniques were devised. For example, if the endogenote *ara*$^-$ marker had become associated with the exogenote and the original exogenote *ara*$^+$ marker with the endogenote by reciprocal recombination events, then most haploid segregants from a recombinant clone of this type would be *ara*$^-$ in genotype. This is because exogenote markers farthest from the ϕ^s*proA-B*$^+$ end of the exogenote are integrated into haploid recombinants at highest frequency (see Table 2). Therefore, clones to be tested for reciprocal exchanges involving the *ara* markers were streaked against phage T7 on EMB arabinose agar. If reciprocal exchanges had occurred, growth to the left of the T7 streak would indicate fermentation of arabinose, whereas most of the T7-resistant haploid segregants to the right of the

T7 streak would be *ara*$^-$ and thus could not ferment arabinose. Similar reasoning can be applied to reciprocal exchanges occurring for the *leu* alleles; therefore, clones were cross-streaked against T7 on MA containing threonine, proline, and thiamine, but lacking leucine. In this case the presence or absence of growth to the right of the T7 streak would indicate the absence or presence, respectively, of reciprocal exchanges involving the *leu* markers. The detection of reciprocal exchanges involving *T1* markers was more difficult. It was reasoned, however, that if the *T1*^{r} marker had become the exogenote marker, then there would be a higher frequency of *T1*^{r} haploid segregants and also of partially diploid recombinants homozygous for *T1*^{r} (see Tables 2, 5, and 6). Thus, clones to be tested were streaked against T1 on EMB containing 0.1% glucose, and all clones having a higher than usual fraction of cells surviving T1 infection were further tested as follows. They were infected with T7 in broth, and then appropriate dilutions were plated to give a total of between 100 and 200 haploid segregants on four Penassay agar plates. After the colonies had grown up, these plates were replica-plated to EMB agar containing 0.1% glucose previously spread with T1. If the *T1*^{s} marker were still in the exogenote, then it would be expected that only 20 to 35% of the haploid segregants would be resistant to T1; but if the *T1*^{r} allele were in the exogenote, 80 to 65% of these haploid recombinants would be resistant to T1 (see Table 2).

Screening techniques described above were used to test 2418 clones picked at random from Penassay agar or MA containing proline. Of this total, 450 were from cells receiving no UV, 871 from cells plated immediately after receiving 300 ergs/mm^2 of UV irradiation, 950 from cells plated after 5 hours of growth following 300 ergs/mm^2 of UV, and 147 from cells plated immediately after receiving UV doses in excess of 300 ergs/mm^2. Not a single instance of reciprocal exchange was found in this search. Thus, either these recombinational events do not exist or exist but are lethal, or occur at a 100-fold lower frequency than the other types of recombinational events enumerated in this communication. While this result was totally unexpected, it must be realized that two reciprocal exchanges would be necessary to obtain the sought-after classes of recombinants.

DISCUSSION

Recombinant types formed in various cell types in log-phase, partially diploid populations: The cell types that give rise to the various recombinant types can be specified by considering the results obtained in this study. Diagrammatical sketches in Figure 11 illustrate the possible majority classes of cell types and the nuclear forms that each cell type contains in log-phase cultures of the partially diploid strain, $\times$ 137Ex2, growing in ML lacking proline. Type 5 cells could be divided into subtypes with various combinations of individual nuclei as represented for Types 1, 2, 3, and 4 cells. To simplify this discussion, this further subdivision has not been done.

The data presented in sections 3, 4, 6, 7, and 8 of RESULTS indicate that clones containing only haploid recombinants after UV irradiation arise by one recombi-

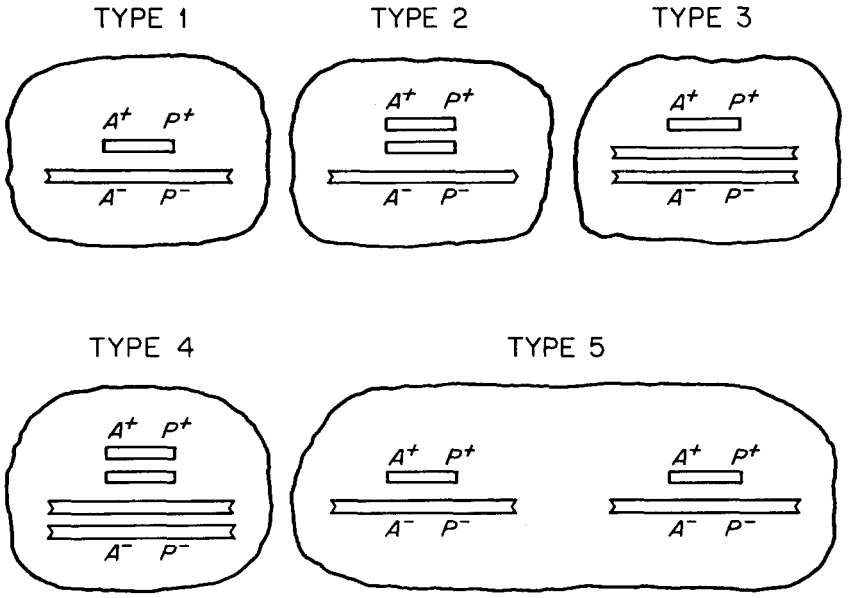


FIGURE 11.—Postulated cell types in log-phase cultures of the partially diploid strain, χ 137Ex2. The figure is diagrammatical in that only the partially diploid portion of the genome is shown in each nucleus. The exogenote is drawn as a linear element unattached to the endogenote as discussed in the MATERIALS AND METHODS. *A* is used to denote the *ara* alleles and *P* to denote the *φproA-B* alleles.

national event in Type 1 cells (Figure 11) and by two independent recombinational events in Type 4 and 5 cells (Figure 11). (A recombinational event, as used here, actually means any number of exchanges required to yield a recombinant structure within one partially diploid genome. It is evident that the integration of exogenote markers into the circular chromosome to yield a haploid recombinant always requires an even number of exchanges.) Type 3 cells do not yield haploid recombinants, since the result would be a sectored colony, composed half of ara^+ haploid recombinant cells and half of ara^- nonrecombinant cells. Such colonies have never been observed. Similarly, Type 2 cells cannot yield haploid recombinants because there would be a leftover exogenote. Therefore, Type 1, 4, and 5 cells can give rise to haploid recombinants. The data in sections 1, 7, and 8 of RESULTS indicate that one recombinational event in Types 4 and 5 cells yields sectored colony formers.

The data in section 10 (RESULTS) show that most partially diploid recombinants are homozygous at only one of the three loci tested (127/134), that the reciprocal types homozygous for $T1^r$ and $T1^s$ arise in separate cells, and that at least 90% of these recombinants arise in pure clones by plating immediately after UV. It was also reasoned (RESULTS, section 10) that the event giving rise to homozygous partially diploid recombinants was analogous to gene conversion and that the process might involve a recombinational event akin to that occurring during UV-induced haploidization.

In order to discuss the cell types which give rise to homozygous partially diploid recombinants, it is necessary to mention briefly the mechanisms of recombination. A more complete treatment of this problem is presented in the last section of the DISCUSSION. Homozygous partially diploid recombinants could arise in Type 1 cells by a conservative copy-choice type of recombination if UV always inactivated either the exogenote (for recombinants homozygous for an endogenote marker) or the endogenote (for recombinants homozygous for an exogenote marker). If such inactivation (i.e., prevention of further replication) did not occur, then homozygous partially diploid recombinants would not be found in pure clones by plating immediately after UV. A further requirement needed to invoke conservative copy-choice recombination to explain formation of homozygous partially diploid recombinants in Type 1 cells is that copy-choice recombination is obligatory when the exogenote is inactivated. If this condition were not met, inactivation of the exogenote without recombination would produce nonrecombinant haploid segregants; these are not found at appreciable frequency (Table 2). In the last section of the DISCUSSION, it is concluded that UV-induced haploidization does not occur by a conservative copy-choice mechanism. Due to the similar response of homozygous partially diploid and haploid recombinants to photoreactivation (RESULTS, section 5) and to the two obligate restrictions mentioned above, it is concluded that conservative copy-choice recombination in Type 1 cells is an unreasonable mechanism to obtain homozygous partially diploid recombinants.

WHITEHOUSE (1963) and HOLLIDAY (1964) have recently proposed that "repair replication" following reciprocal crossing over could account for gene conversion type events in higher organisms. Such a mechanism, regardless of the cell type in which it was operative, could not account for production of UV-induced homozygous partially diploid recombinants for two reasons. First, few homozygous partially diploid recombinants, if any, would be found in pure clones by plating immediately after UV, a prediction which is contrary to fact, (RESULTS, sections 1, 2, and 10). Second, recombinants having undergone reciprocal crossing over were undetectable before or after UV (RESULTS, section 11).

Therefore, a third mechanism for gene-conversion-type recombination for production of homozygous partially diploid recombinants in χ137Ex2 is proposed. In this mechanism, the recombinational event giving rise to homozygous partially diploid recombinants is identical to that which gives haploid recombinants in Type 1 cells (see last section of DISCUSSION). It is postulated that partially diploid recombinants homozygous for an exogenote marker arise by one recombinational event in Type 2 cells and those homozygous for an endogenote marker arise by one recombinational event in Type 3 cells (Figure 11). The 5 to 10% of the homozygous partially diploid recombinants which do occur in mixed clones can be accounted for by recombinational events in binucleate cells which contain at least one nucleus of the type possessed by either Type 2 or Type 3 cells. The infrequent partially diploid recombinant classes which were homozygous for both exogenote and endogenote markers (2/134, Table 5) might have arisen by two recombinational events in Type 4 cells.

TABLE 7

Summary of spontaneous and UV-induced recombinant types from log-phase, partially diploid populations

Recombinant type	Before UV			After UV			
	Number of recombinants	Number of colonies scored	Frequency	Number of recombinants	Number of colonies scored	Frequency	Corrected frequency‡
ara+ haploid*	154	13782	0.0112	879	6144	0.143	0.140
sectored colony formers*	22	13782	0.0016	666	6144	0.108	0.108
$T1^s/T1^s$ partially diploid†	6	1799	0.0033	102	2279	0.045	0.044
$T1^r/T1^r$ partially diploid†	2	1799	0.0011	56	2279	0.025	0.025

* Data taken from 12 experiments including those presented in Figures 2, 7, and 8 with plating on EMB arabinose agar immediately before and immediately after UV. The mean dose of UV for the 12 experiments was 302 ergs/mm² (range of 291 to 312 ergs/mm²), and the mean survival was 30.5% (range of 26.0 to 39.0%).

† Data taken from ten experiments given in Tables 5 and 6 with plating on Penassay agar or MA with proline immediately before (only six experiments) and immediately after UV. Testing was done by random picking of colonies. The mean dose of UV for the 10 experiments was 301 ergs/mm² (range of 291 to 312 ergs/mm²), and the mean survival was 31.4% (range of 27.1 to 37.6%).

‡ The corrected frequencies were calculated by assuming that the recombinant types in the unirradiated sample had the same chance of surviving the given UV dose as did the remaining cells in the population. This assumption was validated in the experiments cited in RESULTS, section 2.

In Figure 11 the exogenote in Type 2 cells and the endogenote in Type 3 cells are shown as fully replicated. This does not have to be, however, since it is believed that homozygous partially diploid recombinants are formed during the time when the exogenote and endogenote are replicating. In higher organisms, a slight asynchrony in meiotic chromosome replication could permit a replicated gene to "convert" a nonreplicated gene by a nonreciprocal breakage-reunion recombination event. Such recombinational events would be associated with the time of DNA synthesis and, if chromosome replication were sequential in that region, might result in a polarized segregation for outside markers, as has been observed in some instances.

Based on the above discussion and conclusions, it is possible to calculate the frequency of each cell type in the log-phase x 137Ex2 population surviving 300 ergs/mm² of UV by using the summary data in Table 7. The derivation of formulae are given in APPENDIX II, and the calculated frequencies are listed in Table 8.

The validity of the assumptions and calculations given in APPENDIX II can be

TABLE 8

Cell type frequencies in log-phase χ137Ex2 populations surviving 300 ergs/mm² of UV irradiation

Cell type	Cell type frequency*
1	0.48
2	0.185
3	0.105
4 + 5	0.23(0.02 + 0.21)†

* Calculated in APPENDIX II from data on frequency of UV-induced recombinant types given in last column of Table 7.
† See text.

partially evaluated by using two independent methods to compare the frequencies of *ara*$^+$ haploid recombinant clones which arise by two recombinational events in Types 4 and 5 cells. In section 8 (RESULTS), the frequency of haploid recombinant clones which contained both *ara*$^+$ *T1*^{s} ϕ^r*proA-B*$^-$ and *ara*$^+$ *T1*^{r} ϕ^r*proA-B*$^-$ haploid recombinants (Table 3) was used to calculate (APPENDIX I) that 22% of all haploid recombinant clones arose by two independent recombinational events in Types 4 and 5 cells after a UV dose of 300 ergs/mm^2. This value was decreased to 19.5% to account for single haploidization events resulting in regional heterozygosity at the *T1* locus (RESULTS, section 9). By using the data in Table 7 and the calculated cell-type frequencies (APPENDIX II), it was calculated that 18% of the haploid recombinant clones arose by two independent recombinational events in Types 4 and 5 cells. The similarity of these values for the frequency of haploid recombinant clones arising by two independent recombinational events strengthens the argument that the assumptions (APPENDIX II) are valid and that the calculated cell-type frequencies (Table 8) are reasonable approximations of the actual cell-type frequencies in the log-phase *x* 137Ex2 population surviving 300 ergs/mm^2 of UV.

As reported in section 3 of RESULTS, 33% of the cells in log phase *x* 137Ex2 cultures were binucleate. The calculated cell type frequencies given in Table 8 indicate that only 23% of the cells surviving 300 ergs/mm^2 of UV irradiation have two viable partially diploid genomes (Types 4 and 5). This finding indicates that UV irradiation can convert cells with two partially diploid genomes to cells with only one functional partially diploid genome as was suggested in section 8. The UV dose dependence of this conversion can be determined from recombination data from log phase cultures of *x* 137Ex2 receiving less than 300 ergs/mm^2 of UV irradiation by using the equations in APPENDIX II. Only recombination data from experiments in which all recombinant types were classified were used, and the spontaneous recombinant frequencies (Table 7) times the mean cell survivals for each given dose were used to obtain corrected recombinant frequencies before employing the APPENDIX II equations (see footnote to Table 7). The calculated frequencies of surviving cells with two viable partially diploid genomes (Types 4 and 5 cells) for each UV dose (in parentheses) are as follows: 0.28 (50 ergs/mm^2); 0.31 (100 ergs/mm^2); 0.30 (150 ergs/mm^2); and 0.23 (200 ergs/mm^2). Each of these values is based on only two or three experiments; thus, when these values along with the value for 300 ergs/mm^2 (Table 8) are plotted (on semilogarithmic paper), there is a wide scatter of points. However, the line of best fit, when extrapolated to 0 ergs/mm^2 of UV irradiation, indicates that the frequency of Types 4 and 5 cells in the unirradiated population is about 33%, a value which equals the frequency of binucleate cells determined by microscopy. It seems invalid to conclude from these data that Type 4 cells are indeed absent in the unirradiated *x* 137Ex2 population. Therefore, it is estimated that Type 4 cells can account for no more than 10% of the total frequency of cells having two viable partially diploid genomes in both unirradiated and irradiated populations (Table 8), since Types 4 and 5 cells should survive UV irradiation equally well.

Chromosome replication in partially diploid cells: The frequencies of UV-induced recombinant types (Table 7) and the calculated frequencies of various cell types in the χ 137Ex2 population surviving 300 ergs/mm² of UV can be used to infer the manner of vegetative chromosome replication in χ 137Ex2 growing in ML lacking proline. The following discussion will attempt to validate the conclusions (1) that the mean number of exogenotes per endogenote is very close to 1, (2) that the exogenote replicates either very soon before or very soon after the endogenote in any given cell, and (3) that the exogenote and endogenote replicate late (during the last third) of a presumably sequential chromosome replication cycle.

To use the cell-type frequencies (Table 8) in the following discussion on exogenote and endogenote replication, it is necessary to compute the frequencies of each nuclear type in the χ 137Ex2 population surviving 300 ergs/mm² of UV. These values are presented in Table 9.

a. Reasons for concluding that the mean number of exogenotes per endogenote is very close to 1. The exogenote must replicate at some time prior to cell division, since nonrecombinant haploid segregants have been observed only rarely at frequencies of 2×10^{-4} in unirradiated cultures and at 6×10^{-3} in irradiated cultures (Table 2; see also CURTISS 1964). Therefore, the mean number of exogenotes per endogenote for all cell types in the χ 137Ex2 population must be 1.0 or slightly greater so that almost all cells inherit an exogenote at cell division.

The mean number of exogenotes per endogenote calculated by using the frequencies of nuclear types (Table 9) that were computed from the recombination data (Table 7) is 1.06. The mean number of exogenotes per endogenote can also be computed from data on rates of spontaneous recombination. CURTISS (1964) found that the spontaneous rate of haploidization was 0.4%/bacterium/generation and that the spontaneous rate of formation of $T1^r/T1^r$ homozygous partially diploid recombinants was 0.009%/bacterium/generation. Spontaneous $T1^s/T1^s$ partially diploid recombinants are three times more frequent than are $T1^r/T1^r$ recombinants (Table 7). Therefore, the spontaneous rate of formation of $T1^s/T1^s$ recombinants should be about 0.03%/bacterium/generation. By using these

TABLE 9

*Nuclear-type frequencies in log-phase χ137Ex2 populations surviving 300 ergs/mm² of UV irradiation**

Nuclear type similar to that in cell type:	Calculated nuclear-type frequencies
1	0.72
2	0.165
3	0.095
4	0.02

* The nuclear-type frequencies were calculated from the cell-type frequencies listed in Table 8 by making use of two factors. First, it was assumed that the frequency of Type 4 cells equalled 10% of the frequency of Types 4 and 5 cells (see text). Second, it was assumed that Type 5 cells contained nuclei equivalent to those in Types 2 and 3 cells at a frequency of 7.5% of the frequency of Types 2 and 3 cells. This would account for the 5 to 10% of partially diploid recombinants which arose in mixed clones (see RESULTS, section 10). The remaining nuclei in Type 5 cells were then assumed to be equivalent to the nuclei in Type 1 cells.

values and the assignment of recombinant types to cell types as discussed in the preceeding section, it is found that the mean number of exogenotes per endogenote is 1.04 (0.40 + 0.03 + 0.03 + 0.01 ÷ 0.40 + 0.03 + 0.01 + 0.01).

b. Reasons for concluding that the exogenote replicates either very soon before or very soon after the endogenote. In considering the timing of exogenote replication in terms of endogenote replication, it is assumed that the exogenote replicates at the same rate per unit length as does the chromosome. (If replication of the exogenote is continuous and at one-tenth the rate of chromosome replication, then it would be necessary that the exogenote always commence replication from the *proB* end. This would only partially account for the recombinant types observed. Also, the conclusion that the mean number of exogenotes per endogenote is very nearly 1 is not compatible with continuous exogenote replication.)

Replication of the exogenote could be (1) simultaneous with endogenote replication, (2) random in time with respect to endogenote replication, or it could (3) sequentially precede or follow endogenote replication. Each of these possible replication schemes allows predictions which can be compared with the recombination data obtained.

The exogenote and endogenote are each about 10 minutes long, which is equivalent to 10% of the chromosome length; or, in the case of the partially diploid strain, the exogenote and endogenote would account for 18% of the total genomic length. If the exogenote and endogenote replicated with perfect synchrony, then the frequency of Types 2 and 3 cells should approach 0%. As an intermediate value, it would be predicted that nuclei like those in Types 2 and 3 cells would occur at a frequency of 18% if the exogenote always replicated immediately before or immediately after the endogenote. With complete randomness in the timing of exogenote and endogenote replication, the frequency of nuclei as in Types 2 and 3 cells would approach 50% if the rates of exogenote and endogenote replication (per unit length) are the same and there is no preferential origin and/or direction for the initiation of chromosome replication. If there were a fixed origin and direction for chromosome replication, then this value of 50% would increase or decrease by an amount dependent on the site of this origin for the initiation of chromosome replication.

The data on the relative frequencies of $T1^s/T1^s$ and $T1^r/T1^r$ homozygous partially diploid recombinants (Table 7) would indicate that the exogenote replicates twice as often before the endogenote as after. The calculated values given in Table 9 suggest that 26% of the nuclei have either two exogenotes and one endogenote or one exogenote and two endogenotes. However, if exogenote-exogenote interactions in Type 2 cells and endogenote-endogenote interactions in Type 3 cells do not occur as was assumed in APPENDIX II, then this value of 26% would have to be decreased to 19%. These values of 19 or 26% are closer to those expected for sequential replication of the exogenote and endogenote (18%) than for complete randomness in time of exogenote and endogenote replication (50%). Therefore, based on this discussion, it seems reasonably permissible to conclude that the exogenote and endogenote replicate close in time to one another.

The above conclusion is based on an analysis of UV-induced recombinant frequencies and the cell type and nuclear type frequencies calculated from them for cells surviving 300 ergs/mm^2 of UV irradiation. The spontaneous rates of haploidization (0.4%/bacterium/generation), of formation of *T1^r/T1^r* homozygous partially diploid recombinants (0.01%/bacterium/generation) and of *T1^s/T1^s* homozygous partially diploid recombinants ($\sim$ 0.03%/bacterium/generation) can also be used to corroborate the conclusion that the exogenote and endogenote replicate close in time to one another. By assuming that the frequency of each cell type which gives rise to recombinants is proportional to the frequencies of each cell type which do not, the frequency of cells with nuclei like those in Types 2 and 3 cells can be estimated. This computation ($0.03 + 0.01 \div 0.03 + 0.01 + 0.4$) indicates that the frequency of cells containing nuclei with either two exogenotes and one endogenote or one exogenote and two endogenotes is about 0.09 in the unirradiated x 137Ex2 population. This value, even if low by a factor of two, supports the conclusion that the exogenote replicates either very soon before or very soon after the endogenote.

The simplest hypothesis to permit exogenote replication soon before or soon after endogenote replication would be to postulate that the exogenote is attached to the endogenote. This attachment of the exogenote to the endogenote could not be by any stable covalent bonds, since the exogenote and endogenote are transferred independently during conjugation (CURTISS 1964). More likely, the attachment would be by synaptic homology in the region including the *ara*, *leu*, and *T1* loci. Synapsis of the exogenote with the endogenote throughout the chromosome replication cycle would account for the finding that up to 75% of the survivors of UV irradiation can be recombinants (Figure 3) and might permit exogenote replication to be controlled by the same system which controls chromosome replication.

c. *Reasons for concluding that the exogenote and endogenote replicate late (during the last third) of a presumably sequential chromosome replication cycle.* The previous discussion validated the conclusion that the exogenote and endogenote replicated close in time to each other during the vegetative chromosome replication cycle. It was also suggested that the exogenote was synapsed with the endogenote and that the timing of exogenote replication was somehow controlled by the timing of endogenote replication. The timing of endogenote replication would depend on whether the origin for initiation of chromosome replication was genetically fixed or random; and if fixed, it would depend on the distance from the origin of replication.

As was amply shown in many of the experiments presented in the RESULTS (also Table 7), the frequency of *ara*$^+$ haploid recombinants *always* exceeded the frequency of sectored colony formers in platings done immediately after a UV dose of 300 ergs/mm^2. This finding is also true for all UV doses up to 400 ergs/mm^2 (Figure 3). These results indicate that the number of Type 1 cells (Figure 11), which give rise to pure clones of haploid recombinants, must be about twice as frequent as the combined number of Types 4 and 5 cells, which give rise to sectored colonies, in the population surviving 300 ergs/mm^2 of UV. This state-

ment is based on the reasonable assumption that the probability of UV-induced haploidization of a single diploid region should be twice as great for cells containing two diploid regions (as in Types 4 and 5 cells) as for those containing one (as in Type 1 cells). The analysis of the frequencies of recombinant types obtained and of the cell type (Table 8) and nuclear type (Table 9) frequencies calculated from them partially validates the conclusion that the endogenote, and thus the exogenote, replicate late in a presumably sequential chromosome replication cycle.

The above conclusion is based on an analysis of cells surviving 300 ergs/mm^2 of UV. In section 3 of RESULTS it was stated that one-third of the cells in an unirradiated χ 137Ex2 population were binucleate, whereas the *maximum* frequency of viable binucleate cells in the population surviving 300 ergs/mm^2 of UV is about 23% (Table 8). UV irradiation inactivates single partially diploid genomes in Types 4 and 5 cells and converts them to functional uninucleate partially diploid cells (see section 8, RESULTS and preceding section of DISCUSSION). Thus, with increasing UV dose, all surviving cells should functionally behave as uninucleate partially diploid cells, and the frequency of sectored colony formers should decrease relative to the frequency of *ara*$^+$ haploid recombinants. Yet this is not the case (Figure 3). In section 1 (RESULTS), it was mentioned that the scoring of sectored colonies was somewhat inaccurate and that some of the recombination events giving rise to them could be due to delayed effects of UV expressed in cell divisions after the first (section 7, RESULTS). To avoid these potential difficulties encountered with UV treatment, the frequencies of recombinant types formed spontaneously can be evaluated. The frequency of spontaneous *ara*$^+$ haploid recombinants (0.0112) is seven times the spontaneous frequency of sectored colony formers (0.0016) (Table 7). However, *ara*$^+$ haploid recombinants can grow and divide for one or two generations after their formation in ML lacking proline (CURTISS 1964). Therefore, the frequency of spontaneous *ara*$^+$ haploid recombinants (Table 7) should be reduced by about a factor of 4 (to 0.0025 to 0.003) to eliminate those recombinants formed in preceeding generations. Also, the sum of the frequencies of *ara*$^+$ haploid recombinant clones and sectored colony formers spontaneously occurring in one generation should be nearly equal to the spontaneous rate of haploidization of 0.004/bacterium/generation (CURTISS 1964). Therefore, subtracting the frequency of sectored colony formers (0.0016) from 0.004 yields a value of 0.0024 for the spontaneous frequency of pure clones of *ara*$^+$ haploid recombinants, a value which is similar to the values 0.0025 to 0.003 computed above. Thus, the frequency of pure clones of spontaneous haploid recombinants is 1.5 to 2 times greater than the frequency of sectored colony formers, and it is therefore concluded that the endogenote and exogenote replicate very late in a presumably sequential chromosome replication cycle. In fact, it is suggested that cells of Type 4 (Figure 11) do not exist at an appreciable frequency and that the exogenote and endogenote are the last segments of the partially diploid genome to replicate.

CURTISS (1965, 1966) presented genetic evidence that the ϕ,*4*^{r}*proA-B*$^-$ mutation (a pleiotropic mutation causing resistance to bacteriophages T3, T7, λ, P1, and T4 and deleting the information for the first two enzymes in proline bio-

synthesis) caused the circular chromosome to become linear. It was postulated that the replication of such linear chromosomes commenced from one or the other end of the break. Although it was shown that when T4 sensitivity was regained the chromosome became circular again (CURTISS 1965), it is still conceivable that the origin of chromosome replication in χ 137Ex2 would still be at the ϕ^r*proA-B*$^-$ mutation. It is concluded in this communication that χ 137Ex2 has a sequential chromosome replication cycle with replication of exogenote and endogenote late in the cycle. This would be compatible with the origin for replication being either in the *ara-thr region* (with replication proceeding counterclockwise) or in the *proB-lac* segment (with replication proceeding clockwise) (see Figure 1). In view of what has been stated above, it is reasonable to assume that chromosome replication in χ 137Ex2 is initiated between the ϕ*proA-B* and *lac* loci and proceeds clockwise with *lac* being first replicated.

The manner of chromosome replication in the partially diploid strain χ 137Ex2 is unique and is not accounted for by previously proposed models of chromosome replication in *E. coli* K-12 (NAGATA 1963; JACOB and BRENNER 1963; BERG 1966). It is my contention that the chromosome of the original *E. coli* K-12 prototroph possessed many potential sites for the initiation of vegetative chromosome replication and that during the development of the various K-12 substrains now in use, mutations or mutation-like events occurred which imparted unique origins for initiation of chromosome replication in some, but not in all, of these substrains. The mutation to ϕ^r*proA-B*$^-$ would be one type of change imparting such a unique origin for replication, as would be the ϕ,4^r mutation (CURTISS 1965) and possibly F integration in some (NAGATA 1963), but not in other strains (BERG 1966). My belief that there has been evolutionary divergence during the development of K-12 substrains is supported by evidence that F$^+$ strains are either able or unable to give rise to Hfr donors (CURTISS and RENSHAW 1968) and by evidence of differences in mutability at specific loci (CURTISS and CHARAMELLA 1968).

Failure to obtain reciprocal recombinational events: As noted in section 11 (RESULTS), all attempts to demonstrate reciprocal recombinational events failed. It was thus concluded that either such events did not occur or occurred but were lethal or occurred at frequencies 100 times lower than recombinational events that caused other classes of recombinants. CURTISS (1964) showed that the exogenote in χ 137Ex2 is linearly transferred during conjugation and therefore not physically attached by covalent bonds to the endogenote. Also the *ara*$^+$ and *proB*$^+$ exogenote markers were not capable of being cotransduced by P1*kc* (see MATERIALS AND METHODS). Thus, a single reciprocal exchange between a linear exogenote and the endogenote which is part of the circular chromosome would result in a single linear duplication chromosome. Based on evidence presented by CURTISS (1965) and the fact that the ends of this duplication chromosome would be the ends of the exogenote, it is reasonable to assume that this structure might be viable. However, if chromosome replication only commenced between the *proB* and *lac* loci in a clockwise manner, it is also conceivable that part of this linear duplication chromosome might not be replicated and would thus be lost. If such a strain were viable, it is not known what the segregational behavior would be,

but no isolates from x 137Ex2 have been obtained which show greater stability or instability than the parental strains in terms of spontaneous haploidization, etc. To obtain the looked-for recombinant, a second reciprocal exchange would be necessary. Therefore, to have found any recombinants in which reciprocal recombinational events had occurred, the two reciprocal exchanges would have had to occur in the same generation for screenings done for platings immediately after UV or during successive generations for screenings done for platings made after several generations of growth following UV. Several complications are now evident. First, the occurrence of two reciprocal exchanges in the same generation may be unlikely because of the possibility of positive interference. Second, UV might not increase the frequency of reciprocal exchanges the way it increases the frequency of nonreciprocal exchanges. Third, even if UV did increase reciprocal exchange, it is evident that the spontaneous frequency would be operative if the second event had to occur in a generation following the generation of the UV-induced exchange. In view of these considerations, it is not surprising that no example of reciprocal exchange was detected.

In terms of the successful demonstration of reciprocal exchange between an F′ exogenote and endogenote by HERMAN (1965), slightly different aspects are apparent. If, as is believed, the F′ exogenote is circular (see BERG and CURTISS, 1967), then two simultaneous reciprocal exchanges between two circular elements will probably give rise about 50% of the time to two interlocked rings that might or might not be viable. If the exchanges are separated in time, then the first product will be a duplication circular chromosome which by a second reciprocal exchange can give rise to the separate F′ exogenote and reform the nonduplication chromosome. Thus, it is probable that the linear exogenote in the partially diploid strain used in this study prevented the detection of reciprocal recombinational events.

Mechanisms of genetic recombination in the partially diploid strain: The basic intent of this study was to elucidate the nature of the recombination event by employing a partially diploid strain in which all viable products of the recombinational events could be recovered. The system employed is unique in that no complications due to mating and chromosome transfer were involved. It was hoped than an analysis of the recombinant types formed and the timing of their appearance following UV irradiation would help to distinguish between the three principal models for genetic recombination, namely, conservative copy-choice, breakage-reunion-copy, and breakage-reunion. As noted in section 3 of RESULTS, the partially diploid strain x 137Ex2, when grown in synthetic medium lacking proline, divided by the formation of uninucleate cells from binucleate cells. This fact greatly enhanced the success of this study.

Since the data on UV-induced haploidization are most extensive, this type of recombinational event will be discussed first. In section 1 of RESULTS, a variety of techniques proved that UV-irradiated log-phase cultures of partially diploid cells plated immediately after UV gave rise to pure clones containing only haploid recombinants; that is, neither partially diploid cells nor any gene fragments excluded from the majority haploid recombinant type were found in these clones.

In section 4, it was shown that 97.7% of the spontaneous and 95.5% of the UV-induced haploid segregants were recombinant for one or more exogenote markers (see also CURTISS 1964). It was reasoned that the low frequency of nonrecombinant haploid segregants was due to recombinational events in genetically unmarked regions, and it was therefore concluded that genetic recombination was obligatory to obtain haploidization. This conclusion led to the postulate that during spontaneous or UV-induced haploidization the exogenote is physically consumed and portions of the exogenote and endogenote not included in the recombinant are lost. The above experimental observations strongly favored a breakage-reunion type or breakage-reunion-copy type of recombination. For a conservative copy-choice recombination mechanism to give rise to a high frequency of pure clones of haploid recombinants, it would be necessary to inactivate the replication of both the exogenote *and* endogenote. At a dose of 300 ergs/mm^2 of UV, which produces about 1800 pyrimidine dimers per partially diploid genome (SWENSON and SETLOW 1966) and which leaves 30% of the cells surviving, this idea of two "lethal" events is unreasonable. Besides, single inactivation of either the exogenote or the endogenote should occur more frequently than joint inactivation of both. Inactivation of the exogenote could lead either to nonrecombinant haploid segregants or to partially diploid recombinants homzoygous for one or more endogenote markers by a copy-choice type of event. Inactivation of the endogenote would either lead to lethality or to partially diploid recombinants homozygous for one or more exogenote markers. Since nonrecombinant haploid segregants are very rare (Table 2) and since the frequency of homozygous partially diploid recombinants is only half that of haploid recombinant segregants (see Table 7), the whole idea of haploid recombinants arising by a conservative copy-choice mechanism involving joint inactivation of exogenote and endogenote is untenable.

The experiments on photoreactivation (RESULTS, section 5) indicated that within 30 minutes after UV an event occurs that produces a recombinant structure no longer sensitive to photoreactivation. Experiments on the effects of starvation on UV-induced recombination (RESULTS, section 6) showed that even 6 hours of starvation before UV and 2 hours of starvation after UV had no appreciable effect on the frequency of haploid recombinants among the survivors. This could mean that the UV-induced lesion that ultimately leads to recombinant formation was stable during starvation or that the preliminary recombinant structure was formed in the absence of an energy source. In view of results obtained in photoreactivation experiments, it is concluded that the latter explanation is correct and that the formation of the initial haploid recombinant structure does not require any DNA synthesis. Although these conclusions rule out copy-choice-type recombination, they do not preclude the probability that after growth conditions are restored, a small amount of DNA synthesis occurs in which patching of phosphodiester bonds finalizes the recombination process.

The above-discussed results eliminate conservative copy-choice-type recombination as a mechanism for UV-induced haploidization. While the experimental results strongly favor a pure breakage-reunion-type recombination, they do not rule out the occurrence of copying along with breakage and reunion, provided

that the copying does not have to occur in the first 2 hours after UV. Otherwise, the experiments on starvation after UV (RESULTS, section 6) would rule out such copying.

The first steps in the formation of a recombinant structure must involve recognition and then effective homologous pairing between the exogenote and endogenote. I feel that the specificity for effective pairing must reside in the base sequence of DNA and that two homologous DNA sequences can neither recognize nor pair with each other if both helices remain tightly entwined with all hydrogen bonding intact. We can now ask how UV increases recombination. As first thought (see HERSHEY 1958; ROMAN and JACOB 1958), UV was believed to place lesions in the DNA that caused copying of one homolog to switch to another homolog. Such copy-choice recombination induced by UV has been ruled out by the evidence given in this report, and therefore another explanation for UV stimulation of recombination must be found. As shown in section 5 of RESULTS, the data from photoreactivation experiments directly implicated pyrimidine dimers as playing an important role in the recombination process. Dimer formation between adjacent thymines, adjacent cytosines, and between adjacent thymine and cytosine will all cause disruption of hydrogen bonding between the complementary DNA strands. At 300 ergs/mm^2 the dose used in most experiments, there would be about 300 dimers distributed in the exogenote and endogenote. (This and the following calculations make use of data provided by CAIRNS 1963 and SWENSON and SETLOW 1966.) It is thus believed that there would be sufficient change (estimated probability of 0.1 to 1) for pyrimidine dimers to be juxtapositioned at some point in the exogenote and endogenote so as to cause regional melting of both DNA molecules, followed by the opportunity for local recognition and then effective pairing of much longer homologous segments. Once effective pairing had been achieved, breakage by some as yet undefined force could ensue, with the initial joining occurring solely by hydrogen bonding. It is thus felt that the sole function of UV is to increase the frequency of recognition and thus of effective pairing. A model similar to that proposed above has been expounded by KELLENBERGER, ZICHICHI and EPSTEIN (1962).

The above proposed mechanism for relating recombination frequency to the frequency of effective homologous pairing can be used to explain the high frequencies of inheritance of the *ara*$^+$ exogenote markers in χ 137Ex2 and of the *ara*$^+$ and *proB*$^+$ exogenote markers in partially diploid strains with a *proB*$^-$ point mutation in the endogenote (RESULTS, section 4), provided that the exogenote is a linear structure. The reasons for believing that the exogenote is linear have been adequately discussed. Regional melting with separation of the complementary strands of DNA should occur more frequently at the ends than in the middle of DNA molecules. This should be true for regional melting occurring spontaneously or following formation of pyrimidine dimers by UV irradiation. Thus, the probability for local recognition followed by effective homologous pairing between the ends of the exogenote and the endogenote should be greater than the probability for such recognition and pairing between the middle of the exogenote and the endogenote, even though the probability of regional melting in the endo-

genote is most likely constant throughout its length. Since the frequency of inheritance of a marker should be directly proportional to the probability of involving the region containing it in effective homologous pairing, the high frequency of inheritance of markers on the ends of the exogenote by haploid recombinants is to be expected. The presence of the ϕ^r*proA-B*$^-$ deletion mutation in the endogenote of χ 137Ex2 prevents homologous pairing of the right end of the exogenote with the endogenote. Therefore, the data in Table 2, which show that the *ara*$^+$ exogenote marker is inherited by haploid recombinants more often than the *leu*$^+$ exogenote marker and *leu*$^+$ more often than *T1*^{s}, only reflect the changes in probabilities of effective homologous pairing between portions of the left half of the exogenote and the comparable portions of the endogenote.

Evidence, although statistical in nature (RESULTS, section 9), suggests that in bacteria, as in phage, marker heterozygosity can result from recombination.

Based on the above discussion, a proposal can be presented to explain the UV-induced recombination process (Figure 12) that is similar in some respects to events proposed by a number of investigators working on the mechanism of recombination in phage (MESELSON and WEIGLE 1961; KELLENBERGER *et al.* 1962; MESELSON 1964, 1967; and TOMIZAWA and ANRAKU 1964). In this model, UV-induced pyrimidine dimers cause regional disruption of the hydrogen bonding between the complementary DNA strands (Figure 12B), which provides an opportunity for recognition between exogenote and endogenote that initially may be for only a short sequence of nucleotides (Figure 12C). This triggers further separation of the original complementary strands leading to the gradual formation of an effective pairing site (Figure 12D). (The above events would also occur spontaneously but at lower frequencies in the absence of UV.) At this stage, breakage of the phosphodiester bonds would occur with the formation of a 4-strand overlap region (Figure 12E). If breakage were due to an enzymatic step, then the enzyme must be pre-existent in the cells and active in the absence of energy metabolism. The 4-strand overlap structure formed after breakage would be the initial recombinant structure, which is unaffected by photoreactivation, and which might also be stable to starvation conditions. The extra pieces of exogenote and endogenote would be eventually degraded. The initial recombinant structure could now take either or both of two paths. In a breakage-reunion-type recombination (Figure 12F, G), two of the DNA strands would be enzymatically digested to produce a 2-strand overlap region which, if a marker were present, would result in heterozygosity for that marker (this process might also occur in the absence of energy metabolism). One or two other enzymes would fill in the gaps and re-establish the phosphodiester linkages of the DNA chains. MESELSON (1964) has shown that these last steps occur during recombination in phage. In a breakage-reunion-copy type of recombination (Figure 12H), the 4-strand overlap structure would commence replication in opposite directions, which would result in the eventual production of two recombinants from two parental structures. Rotational problems would be involved in replications proceeding in two directions unless the enzyme(s) performing this replication is different from the one normally replicating the chromosome and/or there are multiple swivels in the

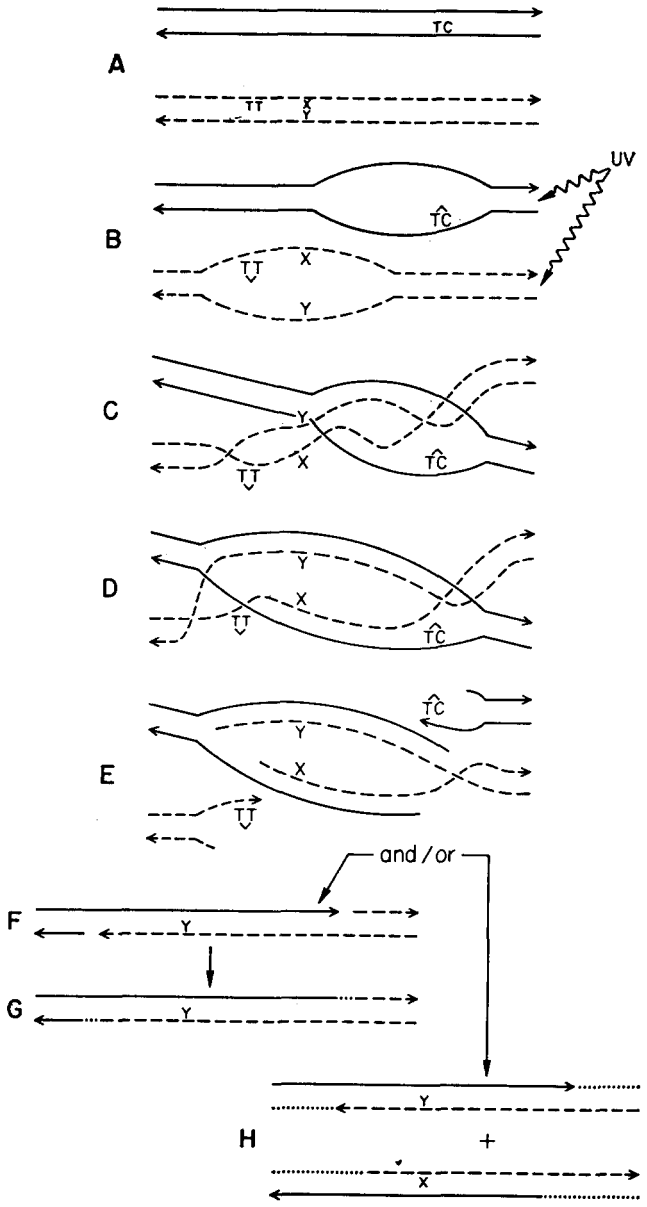


FIGURE 12.—Proposed model for mechanism of UV-induced genetic recombination. A. Exogenote (solid lines) and endogenote (dashed lines). Arrows denote polarity of DNA strands; T and C stand for thymine and cytosine, respectively; X and Y are base pairs in a mutant endogenote. B. UV with production of pyrimidine dimers ($\widehat{TT}$ and $\widehat{TC}$) with regional melting of base pairing. C. Recognition. D. Formation of effectively paired region. E. Breakage with eventual loss of left-over fragments by degradation. F and G. Terminal steps in breakage-reunion recombination with clipping off of excess DNA and patching of phosphodiester linkages. Note formation of heterozygotes in terms of mutant site. Dots refer to newly synthesized DNA. H. Terminal steps in breakage-reunion-copy recombination with formation of two recombinants. See text for full description of steps.

chromosome. While these restrictions on the breakage-reunion-copy model make it somewhat unattractive, no convincing proof has been provided either for or against it.

Recombination with retention of the partially diploid state leading to homozygosity for one or more markers occurs most often in uninucleate cells by a process analogous to haploidization. In the photoreactivation experiments, homozygous partially diploid recombinants responded in a manner similar to that of the UV-induced haploid recombinants. Therefore, pyrimidine dimers are probably involved in providing for greater changes of recognition and effective pairing between double-stranded DNA homologs, as proposed above. As has been previously discussed a conservative copy-choice type of recombination mechanism has been ruled out as a probable mechanism for producing homozygous partially diploid recombinants. Thus, it is concluded that homozygous partially diploid recombinants arise by breakage-reunion or breakage-reunion-copy recombination in cells of Types 2 and 3 (Figure 11) by a series of events similar to that occurring during UV-induced haploidization, as diagrammed in Figure 12. Since the partially diploid strain replicates the exogenote and endogenote late in a presumably sequential chromosome replication cycle, it should be possible (by using synchronized cultures) to further corroborate this conclusion by showing that partially diploid recombinants can be induced by UV only late in this chromosome replication cycle.

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SUMMARY

Studies on UV-induced genetic recombination in log-phase cultures of a stable partially diploid strain of *Escherichia coli* K-12 provided the following results: (1) The strain used divided by the formation of uninucleate cells from binucleate cells. (2) The frequency of all recombinant types among survivors increased linearly up to a UV dose of 500 ergs/mm^2; and at 1200 ergs/mm^2, 75% of the survivors were recombinants. There was an absolute net increase in recombinant

number after UV treatment, and UV did not select pre-existent recombinants from the unirradiated population. (3) Plating cells immediately after a UV dose of 300 ergs/mm^2 resulted in the formation of pure clones of haploid recombinants, pure clones of homozygous partially diploid recombinants, and mixed clones containing equal numbers of haploid recombinants and partially diploid cells. The cells giving rise to these mixed clones segregated at a mean of one generation of growth after UV to yield cells giving rise to pure clones of haploid recombinants and pure clones of partially diploid cells. (4) It was proven that one haploidization event in binucleate cells gave rise to these sectored colonies (mixed clones) and that two independent haploidization events in binucleate cells did occur giving rise to pure clones of haploid recombinants. (5) 90% of the partially diploid recombinants homozygous for one or more markers appeared in pure clones and were thus formed in uninucleate cells. Partially diploid recombinants homozygous for an exogenote marker occurred at different frequencies and in different clones from those homozygous for an endogenote marker. (6) Reciprocal recombinational events were either lethal or occurred at frequencies 100 times lower than other recombinational events. (7) Recombination was obligatory for both spontaneous and UV-induced haploidization. This suggested that the exogenote had to be physically consumed during the recombinational event to achieve the haploid state. (8) Efficient, although only partial, photoreactivation of UV-induced recombination could be achieved when the maximum photoreactivating dose of light was administered within 5 minutes after UV. When the photoreactivating treatment was not administered until 30 minutes after UV, very little reversal of the effect of UV on recombination could be demonstrated, even though photoreactivation of colony-forming ability was very efficient. These experiments show that cells initially killed by UV did contain recombinants. (9) Up to 6 hours of starvation of cells before UV and 2 hours of starvation after UV had no effect on the frequency of UV-induced recombination. (10) During the course of this study, evidence was obtained that regional or molecular heterozygotes analogous to those found in phage crosses were formed as a result of the recombinational event.—These results are discussed in terms of the types of cells and nuclei giving rise to each recombinant type. It is concluded that the exogenote and endogenote in the partially diploid strain replicated late in a presumably sequential chromosome replication cycle. This conclusion is discussed in terms of the existing models for chromosome replication. The data are discussed in terms of the mechanism of genetic recombination, with a conservative copy-choice process being ruled out for both UV-induced haploidization and recombination with retention of the partially diploid state leading to homozygosity for one or more markers. The data favor breakage-reunion and/or breakage-reunion-copy recombination mechanism(s), provided that in the latter process the copying could occur some time after the initial recombinational event and that the nature of this replication could be somewhat different from "normal" chromosome replication. It is concluded that recombinational events occur very soon after UV and in the absence of net synthesis and energy metabolism. A stepwise model for the stimulation of recombination by UV is presented in which the pyrimidine dimers

induced by UV cause disruption of hydrogen bonding between complementary DNA strands, which then increases the probability of recognition between homologs and the eventual formation of effective pairing sites.

APPENDIX I

Calculation of the Frequency of Haploid Recombinant Clones Arising by Two Independent Recombinational Events in Cells with Two Copies of the Partially Diploid Chromosome Segment

These calculations are based on the data for the haploid recombinants induced by 300 ergs/mm^2 of UV (Table 3). The following assumption is made: That clones which contain both *ara*+ *T1*^{s} ϕ^r*proA-B*$^-$ and *ara*+ *T1*^{r} ϕ^r*proA-B*$^-$ haploid recombinants arise by two independent recombinational events occurring in binucleate cells and in uninucleate cells with the diploid *ara* to *proB* segment of the genome duplicated.

To calculate the frequency of binucleate cells and uninucleate cells with the diploid *ara* to *proB* region duplicated giving rise to haploid recombinants at a UV dose of 300 ergs/mm^2, the Hardy-Weinberg equation will be used—$p^2 + 2pq + q^2 = 1$—in which p equals the frequency of exchanges between the *ara* and *T1* loci ($p = 0.32$; Table 3) and q the frequency of exchanges between the *T1* and ϕ*proA-B* loci ($q = 0.68$; Table 3). The expected frequency of clones containing both *T1*^{s} and *T1*^{r} haploid recombinants ($2pq$) can be calculated for the ideal case in which all cells were either binucleate or uninucleate with the diploid *ara* to *proB* region duplicated. This expected $2pq$ equals 0.44, whereas the observed $2pq$ equals 0.096 (Table 3). By dividing the observed $2pq$ by the expected $2pq$, it can be calculated that 22% of the cells giving rise to haploid recombinant clones after 300 ergs/mm^2 of UV were either binucleate or uninucleate with the diploid *ara* to *proB* region of the chromosome duplicated.

APPENDIX II

Calculation of Cell-Type Frequencies in Log-Phase Populations of χ 137Ex2 Surviving 300 ergs/mm^2 of UV irradiation

These calculations are based on the corrected frequencies of each recombinant type presented in Table 7. To make these calculations it is necessary to use the following assumptions: (1) That *ara*+ haploid recombinants arise by one recombinational event in Type 1 cells (Figure 11) and by two independent recombinational events in Types 4 and 5 cells (Figure 11). (2) That *T1*^{s}/*T1*^{s} homozygous partially diploid recombinants arise by one recombinational event in Type 2 cells (Figure 11). (3) That *T1*^{r}/*T1*^{r} homozygous partially diploid recombinants arise by one recombinational event in Type 3 cells (Figure 11). (4) That sectored colony formers arise by single recombinational events in Types 4 and 5 cells (Figure 11). (5) That the probability of recovery of UV-induced recombinational events as recombinants in cells of Types 4 and 5 is twice that in cells of Types 1, 2, and 3. (6) That the frequencies of each cell type which do give rise to recombinants are proportional to the frequencies of each cell type which do not give rise to recombinants.

Assumptions 1 and 4 have been proven valid, and the reasons for assumptions 2 and 3 were presented in the DISCUSSION. In assumption 5 the probabilities of recovery of UV-induced recombinants from cells of Types 1, 2, and 3 are stated to be equal. Types 2 and 3 cells contain 1.5 times as much genetic material in the diploid segment as do Type 1 cells, and might therefore be expected to give 1.5 times the number of UV-induced recombinants. However, exogenote-exogenote recombinational events in Type 2 cells and endogenote-endogenote recombinational events in Type 3 cells would not be detected as recombinants, thus allowing the recovery of only two-thirds of the UV-induced recombinational events. These two factors cancel each other out. Assumption 6 is necessary for simplicity of calculation and may or may not be valid.

In order to derive the formulae needed for these calculations, let:

x = probability of detected recombinational events induced by UV

a = frequency of Type 1 cells
b = frequency of Type 2 cells
c = frequency of Type 3 cells
d = Frequency of Types 4 and 5 cells
A = frequency of *ara*+ haploid recombinants (0.140)
B = frequency of $T1^s/T1^s$ recombinants (0.044)
C = frequency of $T1^r/T1^r$ recombinants (0.025)
D = frequency of sectored colony formers (0.108)

Then:

$$A = ax + 2dx^2 \quad (1)$$
$$B = bx \quad (2)$$
$$C = cx \quad (3)$$
$$D = 2dx \quad (4)$$
$$a + b + c + d = 1 \quad (5)$$

By summing the above and substituting,

$$A + B + C + \tfrac{1}{2} D = x(1 + D) \quad (6)$$

which gives a value of x = 0.237. Calculations with this value yield a = 0.48, b = 0.185, c = 0.105 and d = 0.23 (Table 8). The calculation $2dx^2/A$ yields the frequency of *ara*+ haploid recombinants arising by two recombinational events in cells of Types 4 and 5. This value equals 0.18.

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