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Properties of F' Factor Deoxyribonucleic Acid Transferred from Ultraviolet-Irradiated Donors: Photoreactivation in the Recipient and the Influence of *recA*, *recB*, *recC*, and *uvr* Genes

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Ultraviolet (UV)-irradiated *Escherichia coli* K-12 F *lac*$^+$ donors transfer damaged F' factors when mated with female cells. Exposure of the zygotes to white light after mating can cause the photoreactivation of the damaged transferred F' factors. In so far as the photoreactivation is specific for pyrimidine dimers, these experiments indicate the presence of UV-induced dimers in the transferred F' factor deoxyribonucleic acid (DNA). These damaged F' factors are remarkably stable in *recA* recipients, having a half-life for susceptibility to photoreactivation of 1.5 to 2 hr. This is judged by the formation of Lac$^+$ colonies, all of which are male secondary donors. In crosses with wild type and *uvrA*, *recB*, or *recC* mutant recipients, however, the Lac$^+$ colonies are predominantly recombinants and photoreactivation is not detected. The extent of DNA synthesis resulting from transfer of F' episomes from irradiated donors suggests that a complementary strand is formed on the damaged template. Photoreactivation behavior and sedimentation properties are used to deduce properties of such damaged episomes. We conclude that the complementary strand is discontinuous directly opposite dimers in the transferred strand. This structure may be an intermediate in the recombinational event sequence in Rec$^+$ recipients.

When cells are exposed to ultraviolet (UV) light, photoproducts, mainly pyrimidine dimers, are formed in the deoxyribonucleic acid (DNA). These products interfere with the ability of DNA to act as a template for ribonucleic acid (RNA) polymerase or DNA synthesis.

It is also known that strains of *Escherichia coli* K-12, carrying F' factors, transfer a single strand of F' factor DNA to the female during mating. The transferred strand is normally replicated in the recipient, where it becomes circular (6, 12, 20). If an F *lac*$^+$ donor is exposed to UV light and mated, F' factors are still transferred, as is shown by the appearance of the *lac*$^+$ gene in the recipient (14); however, the F' factor DNA is damaged, and Lac$^+$ colonies so obtained appear to be recombinants rather than secondary F *lac*$^+$ donors (10, 14).

This paper is concerned with the properties of the damaged transferred episome, particularly with regard to its susceptibility to photoreactivation (PR) in recipients carrying various mutations affecting DNA repair. By using the F *lac*$^+$ episome transfer system, we have shown biologically that UV photoproducts are transferred during bacterial conjugation (10). We now report experiments designed to provide further information about the nature of the structural defects in such damaged episomes. Our results indicate that a complementary strand is synthesized on the photoproduct-containing strand, and that this newly synthesized strand is discontinuous opposite positions of pyrimidine dimers in the template. Although excision-repair enzymes appear not to act on such damaged episomes, we find that these structures are treated quite differently by the various recombination functions.

MATERIALS AND METHODS

Bacterial strains. Genetic characteristics of *E. coli* K-12 strains are as follows. AB1157 carries the markers: *arg*, *his*, *leu*, *pro*, *thr*, *ara*, *gal*, *lac*, *mtl*, *xyl*, *thi*, Str^R, T1^S, T4^S, T6^R, λ^S, F$^-$ (obtained from E. Adelberg). AB2463 is a *recA13* mutant of AB1157 (reference 11), and AB3114 is a *uvr*$^-$ mutant of AB2463 isolated by M. Tepper in these laboratories. AB3071 and AB3058

are, respectively, *recB21* and *recC22* *thy*$^+$ transductants of a *thyR thyA* mutant of AB1157 (reference 2). AB2496 is a MS2^R derivative of AB2480 *pro*, *lac*, *thi*, Str^R, T1^S, *uvrA6*, *recA13*. NH4547 (obtained from K. B. Low) has markers *pro*, *leu*, *drm*, *recA1*, *recB21*, F$^-$. The F *lac*$^+$ episome donor, provided by R. Devoret, has the markers *leu*, *thr*, *thyA*, *thyR*, *ura*, *lacY*, *mal*, *thi*, Str^S, T1^R, T5^S, T6^S, *uvrB*, F *lac*$^+$. NH4104 and AB2500 are, respecitvely, Arg$^+$, *uvrA*/F *lac*$^+$, and *uvrA*, *thyA*, *thyR* derivatives of AB1157.

Experimental procedures: growth, mating, and PR methods. Cells were grown exponentially in a complete medium (YET broth), the details being similar to those described for Hfr $\times$ F$^-$ matings (22). At a density of about 2×10^8/ml, F *lac*$^+$ donor cells were harvested by sedimentation and resuspended in an equal volume of saline buffer (2.7 g of $Na_2HPO_4 \cdot 7H_2O$, 1.3 g of KH_2PO_4, and 0.5 g of NaCl per liter of distilled water) for UV-irradiation. The source of UV light was a 15-w General Electric (G.E.) germicidal lamp emitting radiation principally at 254 nm. The detailed procedures and calibrations were previously reported (22). Treated cells were sedimented and resuspended in an equal volume of YET broth, and 2×10^8 male cells were mixed with an equivalent number of female cells in 2.0 ml of YET broth in a 125-ml Erlenmeyer flask. The mixture was slowly swirled in a 37 C water bath for 30 min. Conjugation was then interrupted by addition of T7 phage (ca. 100 phage/male) and this solution was diluted with nine volumes of cold saline buffer, followed by vigorous agitation with a Vortex mixer. After serial dilution in saline buffer, the mating mixture was plated on medium selective for Lac$^+$, Ura$^+$, Thy$^+$, Str^R colonies.

PR of the plated bacteria was carried out at 36 to 38 C under a bank of G.E. F20T12CW fluorescent lamps, 5 cm from the surface of the plates. The light was filtered by a 3/8-inch thick Pyrex plate, eliminating light of wavelengths less than 300 nm. A 1-hr exposure time was used in all experiments, although a large PR effect could be achieved with a 15-min exposure, and a 30-min exposure gave a near maximum effect. After PR, the plates were incubated in the dark for 2 days before counting visible colonies.

The disappearance of photoreactivable species was followed by first incubating the plated mating mixture in the dark and then periodically withdrawing samples for white-light treatment, followed by a 2-day dark incubation before counting colonies.

The Lac$^+$ colonies were picked, purified by restreaking, and then tested for the ability to act as secondary F *lac*$^+$ donors when crossed with a suitable F$^-$*lac*$^-$ strain.

Specific labeling of F lac$^+$ episomes. The labeling procedure used is essentially that developed by D. R. Freifelder (4, 5). It consists of mating *thy*$^+$ F *lac*$^+$ donors with heavily irradiated (ca. 9,000 ergs/mm^2) *thy*$^-$ females in medium containing thymine-methyl-^{3}H. The female chosen, AB2500, is also *uvrA*, thus reducing possible complications from excision-repair of the female chromosome. Sedimentation of specifically labeled F *lac*$^+$ DNA was accomplished by preparing spheroplasts of the mating mixture and lysing them on top of an alkaline sucrose gradient. The detailed procedure was similar to that previously reported (16).

RESULTS

Survival of the Lac$^+$ colony-forming ability. *E. coli* strain NH4395 is the F *lac*$^+$ episome donor for all experiments reported here. Figure 1 shows the relative yield of Lac$^+$ colonies for the female strains, AB2463 *recA* and AB3114 *recA uvr*$^-$, as a function of UV dose to the donor before conjugation. PR of the females after interruption of the mating results in an increased yield of Lac$^+$ recipients. Exposure of the recipients to white light before conjugation did not affect survival of the episome. Thus, the effect we observe is a PR rather than one of photoprotection (13). To demonstrate that the Lac$^+$ recipients harbored an intact F *lac*$^+$ episome, a test was made on their ability to retransmit the F *lac*$^+$ property to a secondary female. Twenty colonies each were picked from plates derived from males receiving 0 to 100 ergs/mm^2, both with and without PR. All such colonies were able to act as secondary F *lac*$^+$ donors. Strains AB2496 *recA13 uvrA6* and NH4547 *recA1 recB21* displayed survival and PR characteristics similar to the two mentioned *recA* recipients.

The Lac$^+$ colony-forming abilities of zygotes from the recipients, AB1157 (wild type), AB3071 *recB21*, and AB3058 *recC22*, however, are dif-

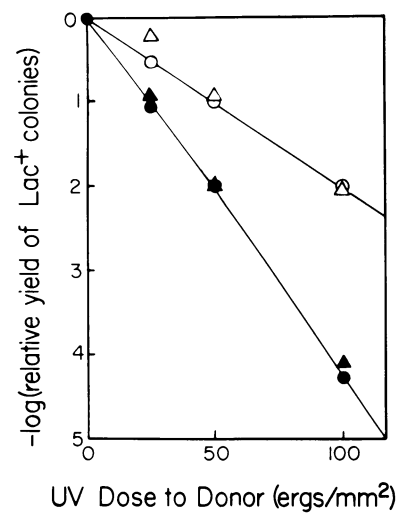

FIG. 1. *Relative Lac$^+$ colony-forming abilities of recA and recA uvr$^-$ recipients receiving F lac$^+$ episomes from an ultraviolet (UV)-irradiated, excision-defective donor, as a function of UV dose to donor before mating. Symbols: AB2463 recA (●) and AB3114 recA uvr$^-$ (▲) with dark incubations, and AB2463 recA (○) and AB3114 recA uvr$^-$ (△) with photoreactivation treatment after interruption of mating and destruction of males with T6 phage. Exposure to white light after transfer of defective F lac$^+$ episomes increases the yield of intact episomes in these strains.*

ferent from those of *recA* mutants, since exposure to light after mating does not appreciably change the yield of Lac$^+$ colonies (*see* Fig. 2). When AB1157 was mated with a donor exposed to 400 ergs/mm^2, most of the Lac$^+$ colonies were F$^-$*lac*$^+$. Only $^2/_{20}$ and $^1/_{20}$ of the purified colonies were able to act as secondary F *lac*$^+$ donors, with light and dark incubation, respectively.

Life-time for PR in recombination-deficient zygotes. The rate of conversion of damaged transferred episomes to a form not susceptible to PR by visible light was measured. This was accomplished by first plating the mating mixture from males exposed to 100 ergs/mm^2 on the selective agar, followed by dark incubation at 37 C. Samples were periodically withdrawn from incubation, exposed to white light for 1 hr, and then reincubated for 2 days. Typical results from such an experiment are shown in Fig. 3 for the *recA uvrA* recipient, AB3114. The half-life for reactivation by white light in this case is 1.4 ± 0.2 hr, and a detectable PR is observed even after 6 hr of dark incubation. A 10-hr, or longer, dark incubation before white-light exposure reduces the Lac$^+$ colony yield to the value observed for portions incubated solely in the dark. Table 1 lists similarly measured values for the other strains. The approximate 2-hr half-life in NH4547 *recA recB* was difficult to measure accurately, as the exposure to white-light kills about 60% of these light-sensitive cells. Compensating for this effect, we estimate the actual half-life to be about 1.5 hr. The corresponding values for strains AB3071 *recB*, AB3058 *recC*, and wild-type AB1157 are less than 0.25 hr, too short to be measured in our system.

DNA synthesis resulting from conjugation with UV-irradiated donors. It is well established that a newly transferred episome has one strand derived from the donor and a complementary strand which was synthesized in the female as a result of the transfer process (6, 12, 20). Although UV-ir-

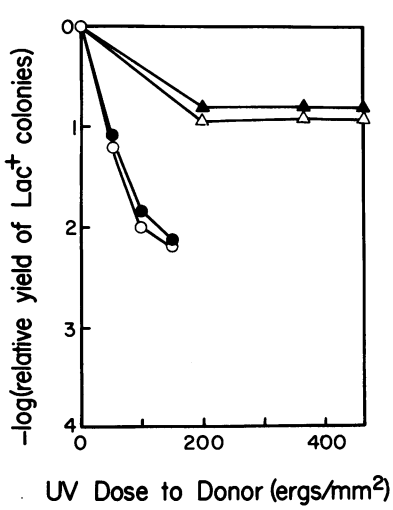

FIG. 2. *Relative yield of Lac$^+$ colony-forming units when an ultraviolet-irradiated F lac$^+$ donor is mated with wild-type AB1157 (▲, △) and AB3071 recB (●, ○). The closed and open symbols respectively denote dark incubation and white light treatment after interruption of mating. The small difference shown here is within the reproducibility of the experiment, indicating that white light does not appreciably affect the survival of defective F lac$^+$ in these strains.*

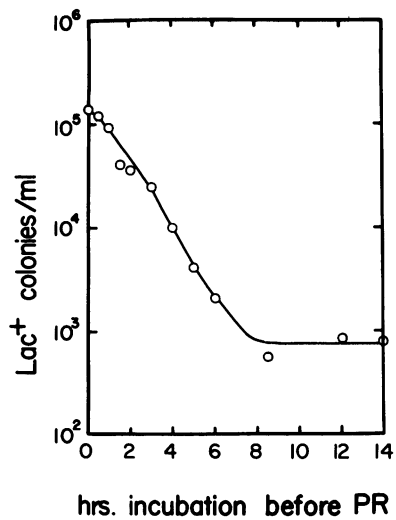

FIG. 3. *Disappearance of damaged F lac$^+$ episomes which can be repaired by photoreactivation in strain AB3114 recA uvr$^-$. After interruption of mating and destruction of the males, the mating mixture was plated and incubated in the dark. Samples were then exposed to white light at the various times on the ordinate and returned to dark incubation for development of visible colonies. The half-life is 1.4 ± 0.2 hr, and a significant recovery is observed even 6 hr after transfer.*

TABLE 1. *Half-life for photoreactivation (PR) of damaged F lac$^+$ episomes in various recipient strains*

Strain	Pertinent genotype	Half-life for susceptibility to PR of damaged episomes in recipients (hr)
AB1157	Wild type	ND[a]
AB3058	*recC*	ND
AB3071	*recB*	ND
AB2463	*recA*	1.4 ± 0.2
AB2496	*recA uvrA*	1.4 ± 0.2
AB3114	*recA uvr*$^-$	1.4 ± 0.2
NH4547	*recA recB*	2.0 ± 0.5[b]

[a] None detected.

[b] Correcting for killing of the recipient by white light, this value is estimated to be 1.5 hr.

radiation of males before mating only slightly inhibits transfer of genetic markers (10, 22), it remains to be shown that a complementary strand is indeed synthesized for a transferred template containing UV-photoproducts. Demonstration of this permits a meaningful discussion of the complementary strand properties inferred by the PR behavior in various recipients. The specific labeling of episomes was done by a procedure developed by D. R. Freifelder (4).

UV-irradiated *thy*$^+$ F *lac*$^+$ donors were mated with *thy*$^-$ recipients exposed to 9,000 ergs/mm^2. The heavy dose given to the females inhibits uptake of ^{3}H-thymine by chromosomal DNA synthesis, enabling detection of the small amount of synthesis associated with conjugation and transfer.

Results of a typical labeling during mating experiments are given in Table 2 for NH4104 (F *lac*$^+$) × AB2500 (*uvrA*). Two types of controls were used. (i) The F *lac*$^+$ donor and the heavily irradiated female were incubated separately in the mating broth, or (ii) an appropriate F$^-$ strain (closely related to the F *lac*$^+$ donor, although not carrying the episome) is used in place of the F *lac*$^+$ donor. Both controls gave similar results. The uptake of ^{3}H-thymine is considerably enhanced by mating an F *lac*$^+$ donor with a suitable recipient, and this incorporation is not greatly inhibited by production of about two pyrimidine dimers in the strand derived from the male. Larger doses (100 to 200 ergs/mm^2) did decrease the extent of uptake, but the results were frequently variable and thus difficult to interpret meaningfully. However, an important point can be made. It appears that a complementary strand is synthesized for the DNA strand transferred from UV-irradiated F *lac*$^+$ donors.

Sedimentation properties of damaged transferred episomes. We also examined some alkaline sucrose sedimentation properties of F *lac*$^+$ DNA transferred from UV-irradiated donors. Figure 4 shows the sedimentation profiles after 0, 15, and 25 ergs/mm^2. The fast sedimenting component at fraction 6 has been previously identified as covalently closed double-stranded circular F *lac*$^+$ DNA, and the main peak found in fractions 20 to 25 is reported to be a mixture of linear, covalent circular single-stranded, and fragmented F *lac*$^+$ DNA (5). We identified the shoulder near frac-

TABLE 2. *Uptake of ^{3}H-thymine during mating of ultraviolet (UV)-irradiated NH4104 (F lac$^+$) with AB2500*

UV dose to donor or to control (ergs/mm^2)	Acid-precipitable ^{3}H from mating mixture (counts/min)[a]	Acid-precipitable ^{3}H of control (counts/min)[b]	^{3}H uptake as a result of mating (counts/min)
0	2,852	888	1,964
15	2,056	561	1,495
25	2,000	348	1,652

[a] Thymine concentration in the mating mixture was 1 μg/ml with an activity of 50 μCi/ml. Ten μliters was counted to determine the uptake.

[b] These values were determined by using strain AB1886 (an F$^-$ close relative of the NH4104) in place of the F *lac*$^+$ donor.

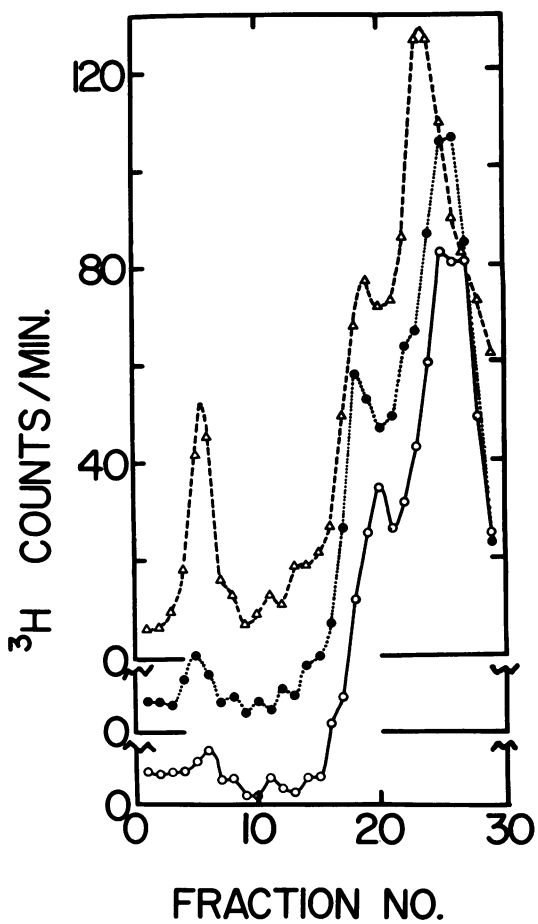

FIG. 4. *Sedimentation profiles of F lac$^+$ episome DNA labeled with ^{3}H-thymine during conjugation. Ultraviolet doses to donor are: 0 ergs/mm^2, △; 15 ergs/mm^2, ●; and 25 ergs/mm^2, ○. After 30 min of mating, cells were washed twice with saline buffer, and spheroplasts were formed at 0 C with lysozyme. Spheroplasts (ca. 7 × 10^8) were placed in a 0.1 ml of 0.5 N NaOH overlayer on a linear 5 to 20% alkaline sucrose gradient and sedimented at 40,000 rev/min for 30 min at 24 C in a SW65 rotor. Fractions were collected from the tube bottom (left to right) on 2-cm filter discs, and, when dry, washed several times with cold 5% trichloroacetic acid solution before counting for ^{3}H in a scintillation counter.*

tion 18 as being chromosomal DNA from the transfer partners. It usually accounts for 15 to 30% of the incorporated ^3H radioactivity. The area under the main peak does not change greatly when the transferred strand contains one to two dimers, and this is consistent with the extent of uptake of ^3H-thymine during mating. However, radioactivity associated with the covalently closed, double-stranded circle peak decreases exponentially with a D_{37} of 15 ergs/mm^2. These results (Fig. 5) are taken from three separate determinations, one of which is shown in Fig. 4.

DISCUSSION

The survival of intact F *lac*$^+$ episomes in *recA* recipients decreases exponentially with exposure of the episome donor to UV light before mating. The D_{37} dose of 13 ergs/mm^2 corresponds to production of about one pyrimidine dimer per 40 $\times$ 10^6 dalton molecular weight [based on estimation used by Rupp and Howard-Flanders (16)], which is approximately the size of a single strand of the F *lac*$^+$ episome (3). Since pyrimidine dimers are the most frequent photoproduct, it is likely that a single pyrimidine dimer renders a transmitted episome defective in *rec*$^-$ recipients. However, a proportion of such damaged episomes can be photoreactivated in *recA* recipients after interruption of mating. This effect is observed with *recA* recipients, but not with *recB*, *recC*, or wild type. Evidently, UV photoproducts repairable by exposure to white light are transferred during conjugation with irradiated donors. Several lines of evidence can be used to deduce characteristics of damaged episomes: their PR behavior, template activity, and sedimentation properties.

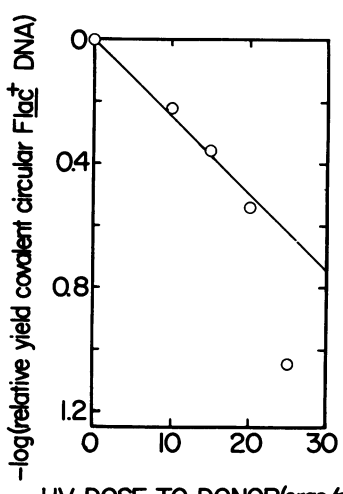

FIG. 5. *Relative amount of ^3H radioactivity in the fast sedimenting peak shown in Fig. 4, as a function of ultraviolet dose to the F lac$^+$ donor. Although the experimental uncertainty increases rapidly for doses greater than 15 ergs/mm^2, a D_{37} of 15 $\pm$ 2 ergs/mm^2 was derived from results of three separate determinations.*

We now consider the effect of transferred UV photoproducts on the complementary episome strand. Results of "labeling during mating" experiments indicate that UV doses sufficient to inactivate 90% of the transferred F *lac*$^+$ episome do not greatly inhibit incorporation of ^3H-thymine into episomal DNA. It thus appears that a complement is synthesized on a template containing UV photoproducts. However, such damaged episomes are apparently not able to form covalent circles in the recipient. This is suggested by the decreased yield of the rapidly sedimenting component from F$^-$ cells mated with UV-irradiated males. The D_{37} (from Fig. 5) of 15 ergs/mm^2 for this decrease is also close to the value which would produce one pyrimidine dimer per male strand.

We interpret these results in terms of the Rupp and Howard-Flanders model for DNA synthesis on a damaged template (16). By this model, the complementary strand should be discontinuous at the position of each dimer in the transferred strand. Such a discontinuity would prevent formation of covalent circular episomes, as is observed.

The PR behavior of damaged episomes in various recipient strains can also be used to infer details about their structure and about processes in which they are involved. These damaged episomes are remarkably stable in *recA* recipients, and a significant PR is observed even 6 hr after interruption of conjugation. So far as is known, PR is a reaction specific for pyrimidine dimers splitting the cyclobutane ring in situ and restoring the intact DNA sequence (17, 18). Thus, PR of the proposed dimer-gap defect should yield a structure suitable for DNA synthesis and strand joining necessary for formation of an intact episome duplex, as shown in the suggested scheme of Fig. 6.

The survival of these damaged episomes after transfer is not influenced by *uvr* genes of the recipient, which control the excision-repair enzyme system (9). This observation can be used to identify the relative positions of the gap, or discontinuity, and the dimer. Our reasoning is based on an understanding of how the excision-repair process works (1, 8, 19). Summarized briefly, it is thought to occur by enzymatic removal of the dimer containing segment from one strand, followed by nucleolytic widening of the gap. A DNA polymerase then resynthesizes a new strand in the gap, using the intact complementary strand as a template. The free ends can then be joined

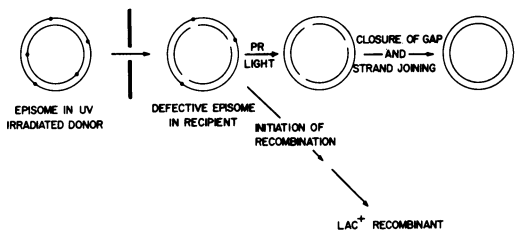

FIG. 6. *Suggested scheme for the transfer of F lac⁺ episomes from an ultraviolet-irradiated donor and the subsequent recovery and recombination events in the recipient. One strand of F lac⁺ containing photoproducts (●) is transferred and its newly synthesized complement is discontinuous at the positions of the dimers. Recipients then act on this damaged structure according to the capabilities of their repair systems. The life-time of damaged F lac⁺ in recA recipients is long, and photoreactivation (PR) can increase the yield of intact episomes. This might occur by initial cleavage of pyrimidine dimers to monomers. The transferred strand can then act as a template for DNA synthesis to close the gap, followed by a ligase joining of the strands to form an intact episome. Competing with PR repair is a slow degradation in recA strains. In wild-type and recB recipients (both recA⁺), the defective episome is acted on quickly by recombination enzymes, converting it to a species not recoverable by PR treatment.*

by a ligase. Since excision-repair is thought to depend on the presence of an intact strand opposite the dimer, a simple explanation of the lack of repair is that the transferred dimers are in single-stranded regions, as would be the case if the damaged F *lac*⁺ is discontinuous directly opposite the dimer.

In addition to being unable to contribute to the survival of damaged F *lac*⁺, excision enzymes also appear to be unable to make cuts adjacent to pyrimidine dimers in the strand transferred from the male. Such an incision would result in a double-strand break, yielding a product not rescuable by PR, thereby decreasing the measurable half-life. We find, however, that the half-life is the same in *recA uvr*⁺, *recA uvr*⁻, and *recA uvrA* strains, indicating that excision enzymes do not act on the structure repairable by white light. This latter conclusion contrasts with results reported by Ono and Shimazu (15), which suggest that excision enzymes cut ϕR single strands containing UV photoproducts. Further work may resolve this apparent conflict.

The suggested dimer discontinuity appears to be an intermediate in the recombination sequence (16). A similar long-lived recombinational intermediate is reported by Hertman and Luria (7), as a result of UV-induced damage in a *recA* strain. Their experiment consisted of transducing the *recA*⁺ gene into UV-irradiated *recA* cells at various times. Even done several hours after irradiation, this transduction led to an increased cell survival, suggesting the presence of a long-lived intermediate which also could be acted upon by a *recA*⁺ function.

In wild-type cells, a large fraction of the episomes (ca. 10 to 30%) from UV-irradiated donors undergo recombinational events, yielding F⁻ *lac*⁺ recombinants (10). Considering the apparent high efficiency with which this process takes place, it seems reasonable that the *recA*⁺-controlled modification of defective episomes to a form not rescuable by white light may be one of the steps in the sequence of events leading to recombinant formation. If this is indeed the case, from our failure to detect damaged episomes in *recA*⁺ cells, we estimate that initiation of this process occurs within 15 min after transfer. The operation order of recombinational functions can also be considered and compared to that suggested by other properties of *rec*⁻ strains. Wilkins (21) reports that chromosome mobilization in *recB* and *recC* mutants occurs with an efficiency similar to that measured for wild-type cells. He suggests that, if chromosome mobilization involves DNA strand cutting and subsequent joining between an F′ episome and the bacterial chromosome, the *recA*⁺ function apparently precedes those of *recB*⁺ and *recC*⁺. Defective episomes appear to be rapidly modified in all *recA*⁺ recipients tested, but seemingly not so in *recA* mutants. These observations are also consistent with the above order for recombination functions.

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