The Effects of Ultra-violet Irradiation on the Fertility of F^+ and Hfr Strains of *Escherichia coli* K12 Defective for the Repair of Damaged DNA

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SUMMARY

The effect of irradiating donor cells, immediately before mating, upon the yield of recombinants has been investigated for male (F^+ + Hfr) strains of *Escherichia coli*, which, owing to a mutational block (*uvr*), are unable to excise pyrimidine photoproducts. Despite the extreme sensitivity of the *uvr*⁻ strains employed, as judged by colony formation, the yield of recombinants was surprisingly little affected by u.v. In particular, using an Hfr *uvr*_B⁻ strain it was found that, after an initial fall to about 30-40% of that given by the unirradiated control, the yield of recombinants for both early and late markers declined with increasing dose at about the same rate as for the parent *uvr*⁺ strain. There was evidence of damage in the DNA transferred from irradiated males in that normal linkage of unselected markers was reduced, but the decline in linkage with increasing dose was the same for both *uvr*⁺ and *uvr*⁻ strains. The yield of recombinants was nearly independent of the *uvr* phenotype of the F^- parent. Thus although fertility and survival are closely correlated in the *uvr*⁺ Hfr, this correlation disappears in the *uvr*⁻ male. Instead the u.v. sensitivity of the processes involved in chromosome transfer appears only slightly altered in these mutants despite the considerable change in sensitivity as judged by colony-formation.

INTRODUCTION

Irradiation of F^+ cultures of *Escherichia coli* K12 with small doses of u.v. light stimulates the transfer of chromosomal material to F^- recipients, increasing the yield of recombinants 10 to 100 fold (Hayes, 1952). In the course of experiments exploring the requirements for this stimulation of fertility we examined the effect of u.v. upon F^+ donors which carried mutational defects (*uvr*⁻) in the repair mechanisms for u.v. and other forms of damage to DNA (Evenchik, Stacey & Hayes, 1969). Surprisingly, the yield of recombinants remained similar to that of the unirradiated control even when the survival of the donor population, as judged by colony formation, had fallen to 10^{-3} . Because a number of observations concerning the effects of u.v. on the fertility of Hfr strains had shown that fertility and the capacity to form colonies are lost more or less in parallel (Joset & Wood, 1966; Doudney & Bruce, 1966; Evenchik, Stacey & Hayes, 1969), we investigated the fertility of Hfr strains defective in one of the genes governing the excision repair process, in particular an Hfr H strain carrying

an uvr_B^- mutation. These experiments showed that, as for the F^+ strains, fertility was lost much more slowly than viability; the yield of recombinants fell only to 40% of the unirradiated control for a colony-forming fraction less than 10^{-3} .

This paper reports our preliminary observations on this system. They are very similar to the results obtained by Wilkins & Howard-Flanders (1968).

EXPERIMENTAL

Strains. All strains were derivatives of *Escherichia coli* K12.

W1655 F^+ $met^- Sm^S \lambda^- \lambda^R$ (Scaife & Gross, 1963).

W1655 F^+ uvr^- isolated by us using the technique of Howard-Flanders & Theriot (1962).

KMBL 49 $F^- thr^- leu^- thi^- lac^- thy^- ura^- Sm^R$ (van de Putte *et al.* 1965).

KMBL 49 F^+ isolated by us after conjugation with W1655.

KMBL 90 $F^- uvr_B^-$ mutant of KMBL 49 (van de Putte *et al.* 1965).

KMBL 90 F^+ isolated by us after conjugation with W1655.

Hfr H Sm^S (Evenchik *et al.* 1969).

Hfr Z48 (Hfr H $gal^+ uvr_B^-$, made by transduction of Hfr H gal^-) using phage P1 grown upon AB1885 $gal^+ uvr_B^-$ and selected from among the gal^+ recombinants for the co-transduction of uvr_B^- .

F^- W945 $pro^- Sm^R$ (Evenchik *et al.* 1969).

F^- AB1157 $thr^- leu^- pro^- his^- arg^- thi^- ara^- Sm^R$ (Adelberg & Burns, 1960).

F^- AB1884 uvr_A^-
 AB1885 uvr_B^-
 AB1886 uvr_O^- } derivatives of AB1157, isolated by Howard-Flanders, Boyce & Theriot (1966).

Irradiations. Broth cultures of F^+ and Hfr strains were grown, from 1 in 20 dilutions of saturated over-night cultures in minimal media, to 1×10^8 bacteria/ml., filtered and resuspended in buffer at 5×10^8 bacteria/ml., and irradiated 50 cm. from a Hanovia lamp at dose rates of approximately 2 ergs/mm.²/sec.

Crosses. After irradiation the bacteria were diluted with broth, mixed with an equal volume of a culture of the appropriate F^- strain and incubated for 45 min. Recombinants were, in most cases, scored on minimal media containing all but one of the requirements of the F^- strain and 200 μ g./ml. of streptomycin. In the crosses involving the F^+ KMBL strains which are streptomycin resistant, the males were counter-selected by omission of their several requirements. In the analysis of the unselected markers recombinants were picked onto the same selective medium and these plates used as master plates for replica plating. The inheritance of arabinose fermentation was scored on EMB-arabinose plates.

RESULTS

$F^+ \times F^-$ crosses

Comparisons of recombinants by the F^+ derivatives of strains KMBL 49 and 90 before and after doses of u.v. which reduced their survival to between 1 and 0.1%, showed little diminution in the yield of recombinants. Since this yield was in any case small, we isolated a uvr^- strain of the relatively fertile F^+ used in previous studies (Evenchik *et al.* 1969). The loss with increasing u.v. dose of the capacity of this mutant, W1655 $F^+ uvr^-$, to form colonies, and its ability to reactivate u.v. irradiated phage were com-

parable with those of the most sensitive of the known *uvr*⁻ strains available to us for comparison. Again, after u.v. irradiation its capacity to transfer chromosomal material was still 40% of the control value even when its colony forming ability had been reduced to 10⁻³. There was, however, none of the stimulation of chromosomal transfer at low doses (50% survival) which is shown by the parent, *uvr*⁺, strain (Evenchik *et al.* 1969). This apparent u.v. resistance of the transfer capacity prompted us to examine the effect of u.v. on the transfer of chromosomal material by a *uvr*⁻ mutant of an Hfr strain.

Fig. 1

Fig. 2

Fig. 1. The effect of u.v. light on the survival of Hfr H *uvr*⁺ $\times$ — $\times$, and on the yield of recombinants in a standard cross (see Methods) with AB1157 F⁻ selected for the inheritance of the donor markers threonine and leucine Δ — Δ , proline $\circ$ — $\circ$, and arginine $\square$ — $\square$.

Fig. 2. The effect of u.v. light on the survival of Hfr H *uvr*⁻ (z48) $\times$ — $\times$ and on the yield of recombinants in a standard cross (see Methods) with AB1157 F⁻ selected for the inheritance of the donor markers threonine and leucine Δ — Δ , proline $\circ$ — $\circ$, and arginine $\square$ — $\square$.

Hfr $\times$ F⁻ crosses

We had already repeated some of the observations of Joset & Wood (1966), which showed that survival and the transfer of early markers by an Hfr *uvr*⁺ strain were affected by u.v. to about the same extent, in the course of the earlier experiments on the effect of u.v. on chromosomal transfer (Evenchik *et al.* 1969). These experiments were repeated over a wider range of doses and for a greater number of markers using as F⁻ recipients AB1157 and three *uvr*⁻ strains derived from it (AB1884 A⁻, AB1885 B⁻ and AB1886 C⁻) all kindly given by Dr P. Howard-Flanders. The results in Fig. 1 for the crosses with AB1157 (confirming our earlier results) show that u.v. irradiation had a very similar effect on both viability and fertility even for the late

marker (*arg*). These results differ in detail from those of Joset & Wood, who reported that the yield of recombinants was more sensitive to irradiation, the further the selected marker from the origin. It is possible that this discrepancy may be explained by differences between the strains used, since Jacob & Wollman (1958) obtained results very similar to ours. The $F^- uvr^+$ parent and its three *uvr*⁻ derivatives were equally good recipients irrespective of the irradiation dosage to the Hfr strain.

Table 1. *Effect of u.v. irradiation of Hfr H uvr⁻ upon the yield of recombinants*

u.v. dose (sec.)	Survival (%)	Recombinants					
		F ⁻ <i>thr⁺ leu⁺</i>	% of control	<i>pro⁺</i>	% of control	<i>arg⁺</i>	% of control
		10 ⁸ /ml.		10 ⁸ /ml.		10 ⁴ /ml.	
0	100	1884 <i>uvr_A⁻</i> 17	100	3.7	100	4.0	100
5	12.3	12	69	3.2	86	1.8	45
10	1.2	6.1	36	1.6	43	1.4	35
0	100	1885 <i>uvr_B⁻</i> 25	100	7.4	100	17	100
5	14.6	13.6	54	4.9	66	—	—
10	2.1	13	52	4.8	65	10	59
0	100	1886 <i>uvr_G⁻</i> 26	100	8.2	100	7.4	100
5	11	23	88	5.7	70	6.1	82
10	0.94	12	46	3.1	58	—	—
20	0.4	5.2	20	1.5	18	—	—

Crosses under the same conditions with the same four recipients were then made with the u.v. sensitive strain Hfr Z48 carrying the *uvr_B⁻* mutation, transferred by P1 transduction from AB1885. Some of the results with AB1157 as recipient are shown in Fig. 2: comparable results were obtained with the *uvr⁻* recipients (Table 1). Despite the great difference in sensitivity between the two Hfr strains, the only striking difference between the recombination data in Figs. 1 and 2 is that quite low doses cause about a halving in the number of recombinant given by the *uvr⁻* donor. It seems possible that there might be two populations, one very sensitive to u.v. and the other no more so, with respect to chromosome transfer, than a *uvr⁺* strain, but this is not reflected in the survival curve. In many *uvr⁺* strains there is a 3- to 20-fold increase in survival if, after irradiation, bacteria are incubated in a growth medium before plating. (Barner & Cohen, 1956 and Stacey & Atkinson, unpublished observations). To check that the 45 min. incubation with the F^- did not 'rescue' any substantial fraction of the irradiated donor bacteria, the survival of the Hfr *uvr⁻* strain was measured in control cultures both before and after a period of incubation equivalent to those used for mating. There was no increase in viable count for periods up to an hour.

The most obvious explanation of these results is that damaged DNA can be transferred and integrated into the chromosome of the F^- although neither donor nor recipient carry the full set of functional genes necessary for excision repair. By analogy with 'marker rescue' one might anticipate that crossing-over is more frequent in recombination involving damaged chromosomes. Such an effect has been observed in the region of the chromosome carrying the closely linked loci *thr*, *ara*, and *leu*. The recombinants selected for the distal marker, *pro*, were examined for the inheritance of both *thr⁺* and *leu⁺* and among these the inheritance of the interjacent *ara⁺* locus was about 98% in the control crosses; in a cross after a dose of 40 ergs/mm.² which reduced the survival of Hfr Z48 to 0.1%, the percentage of *thr⁺ leu⁺* recombinants that were

also *ara*⁺ had been reduced to 78%. The linkage between *pro* and the *thr* and *leu* markers was also drastically reduced (Table 2). However, just such a breakdown in linkage was reported by Jacob & Wollman (1958) and confirmed by Joset & Wood (1966) in normal, *uvr*⁺ Hfr strains. We therefore compared the linkage of unselected markers in the two types of cross at the same absolute doses. Table 2 shows that the difference between the two strains was very small.

These observations confirm a more extensive study by Wilkins & Howard-Flanders (1968) who propose that the explanation lies in the transfer of DNA which has been replicated with gaps opposite some of the DNA lesions, which in a *uvr*⁺ strain would have been repaired by excision (Rupp & Howard-Flanders, 1968). Although our data do not permit any very direct comment on this idea, we have not observed any marked differences in the time of entry of early markers, which implies that if DNA synthesis is impeded under these conditions then this is not the rate-limiting step. Comparable experiments in which the *uvr*⁻ F⁻ parent has been irradiated, have, in a limited number of observations, shown that zygotes are more resistant than the survival curve would lead one to expect.

Table 2. *Effect of u.v. irradiation of the donor upon linkage*

Cross	u.v. dose (sec.)	Survival (%)	% Linkage of <i>leu</i> ⁺ to <i>pro</i> ⁺	% Linkage of <i>ara</i> ⁺ to <i>thr</i> ⁺ <i>leu</i> ⁺
Hfr H <i>uvr</i> ⁺ × AB 1157	0	100	73	96
	10	80	47	88
	80	5	19	78
Hfr H <i>uvr</i> ⁻ × AB 1157	0	100	70	96
	10	1	69	89
	80	< 0.1	36	74
Hfr H <i>uvr</i> ⁻ × AB 1886	0	100	86	98
	10	1	22	63

DISCUSSION

Irradiation of normal Hfr strains with ultra-violet light reduces their fertility to much the same extent as it renders them incapable of forming colonies. In contrast an Hfr strain which is incapable of excision-repair as a result of a mutation in the *uvr*_B locus, continues to yield recombinants at a high rate at doses of u.v. which have reduced viability to a low level, even when the recipient is also *uvr*⁻. However, it is clear from analysis of the unselected markers in these recombinants that the transferred chromosome fragment is damaged because it appears to be involved in more frequent recombination, but the magnitude of this effect is the same among recombinants from crosses with the *uvr*⁺ Hfr strain at the same dose.

There seem to be two tenable hypotheses to explain these effects. The first is that DNA synthesis is possible even when there are many pyrimidine dimers present in the chromosome involved in transfer. This is the suggestion of Wilkins & Howard-Flanders (1968) based on the observations of Rupp & Howard-Flanders (1968) that DNA synthesis in irradiated *uvr*⁻ bacteria leads to the formation of DNA which, when examined by the alkaline sucrose gradient technique of McGrath & Williams (1966), appears to contain many single strand breaks. This, they suggest, is due to interruptions in the newly synthesized strand caused by pyrimidine dimers in the template strand.

If this view is correct the transferred DNA contains gaps opposite dimers: since this damage cannot be repaired by the excision mechanism it would explain why the presence of *uvr*⁺ genes in the recipient is irrelevant. It is then not clear why the DNA transferred by the Hfr *uvr*⁺ seems to contain an equal amount of damage as judged by the linkage data.

The second hypothesis is that after irradiation DNA can be transferred without synthesis. If this occurred by transfer of the double strand then the capacity of the female to repair should be manifest, but if the transfer were of only one strand then again excision repair would be impossible. This idea, too, does not explain why the excision repair system does not reduce the degree of damage to DNA before transfer by the *uvr*⁺ donor, and one is led to enquire whether conjugation in some way interferes with the normal process of repair. Experiments on this point are being made.

An attempt is also being made to investigate the nature of the DNA transferred by irradiated Hfr bacteria, but the experiments of Vielmetter, Bonhoeffer & Shütte (1968) appear to support very strongly the idea that only one DNA strand is transferred in the normal course of bacterial conjugation. They interpret their observations on the segregation of recombinants containing mutations generated in the Hfr parent immediately prior to transfer as being most consistent with single strand transfer (see also Kunicki-Goldfinger, Piekarowicz & Włodarczyk, 1968). It is clear from the results of Rupp & Ihler (1968) that a particular strand in the Hfr chromosome is transferred, although their technique does not permit them to determine whether a complementary strand must be synthesized in the donor for transfer to take place.

One test of the hypothesis that u.v. damage is not repaired in the female because the DNA is transferred as a single strand, would seem at first sight to be a study of the effect of the *uvr* loci on transduction by u.v.-irradiated phage, because there is good evidence that the DNA of the transducing particle is double-stranded (Ikeda & Tomizawa, 1965). However the effect of u.v. on generalized transduction by phages P 22 (Garen & Zinder, 1955) and P 1 (Arber, 1960), and on specialized transduction by phage λ (Arber, 1958) is first to increase the efficiency of transduction, presumably by increasing the efficiency of recombination, well above the level obtained with un-irradiated phage. In an *uvr*⁻ recipient the efficiency of transduction by phage P 22 falls with increasing irradiation (Takebe, 1968), so that a direct comparison is impossible. One can perhaps draw a parallel between the effects of u.v. on chromosomal mobilization by the F episome and transduction, for in both cases in *uvr*⁺ strains there is an increase in recombination which is not seen in *uvr*⁻ strains, and argue as we have previously (Evenchik *et al.* 1969), that the increase in recombination is the result of excision giving rise to single-stranded regions, which facilitate the pairing necessary to initiate recombination. This seems to be different from the situation studied here, where the recovery of recombinants fell with increasing u.v. dose, regardless of the possession by donor or recipient of the excision-repair system.

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