Effect of *tif* Expression, Irradiation of Recipient and Presence of Plasmid pKM101 on Recovery of a Marker from a Donor Exposed to Ultraviolet Light Prior to Conjugation

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To detect the effect of the postulated inducible error-prone repair system ('SOS repair') on the bacterial chromosome, an Hfr *Escherichia coli* strain JC5088 *recA* was u.v.-irradiated immediately before mating it with recipients in which SOS repair was supposed to be functioning either through *tif* expression, u.v. irradiation or the presence of plasmid pKM101. The recombinant yields of these crosses were compared with those obtained in corresponding crosses with recipients in which SOS repair either was not induced or was totally eliminated by the *lexA* mutation. No difference in marker recovery efficiency could be detected between these two sets of recipients and thus no induced repair process acting on donor DNA could be demonstrated. The possible reasons for this finding are discussed.

INTRODUCTION

The enhanced survival of u.v.-irradiated bacteriophage (Weigle reactivation) when plated on a u.v.-irradiated *Escherichia coli* host (Weigle, 1953) has been postulated to reflect the activity of an inducible repair system ('SOS repair') acting on irradiated DNA (Radman, 1974, 1975). According to this hypothesis, various treatments affecting DNA metabolism, such as u.v. irradiation, thymine starvation, nalidixic acid, chemical mutagens, etc., may trigger an inducible error-prone DNA repair system dependent upon the *recA*⁺ and *lexA*⁺ genes, which is responsible not only for the increased survival of irradiated phage but also for phage and bacterial mutagenesis. One of the most convincing pieces of evidence in support of this theory is the existence of a certain group of mutants (*tif*) altered at the *recA* locus of *Escherichia coli*. These mutants, when grown at 43 °C, express constitutively some of the supposed 'SOS functions', such as increased survival of irradiated phage lambda and high mutability of both phage and bacterial genes (Castellazzi *et al.*, 1972; Witkin, 1974). SOS repair is also assumed to be constitutively expressed in bacterial strains harbouring an ampicillin resistance plasmid pKM101 (Mortelmans & Stocker, 1976). These strains show high spontaneous and induced mutability and are also more resistant to u.v. radiation than corresponding isogenic strains without the plasmid.

There has been, however, no direct evidence of any inducible repair system acting to enhance the survival of the bacterial chromosome and the theory is largely based on phage experiments. To provide a more direct test it is necessary to arrange a system analogous to that of Weigle reactivation of bacteriophage, whereby the bacterial chromosome may be damaged outside the cell in which SOS induction takes place. We have therefore attempted to detect possible SOS repair of the bacterial chromosome by mating a u.v.-irradiated donor with recipients in which the repair system was activated either by expression of the *tif*

mutation, by u.v. irradiation of the recipient before the mating, or by the presence of the plasmid pKM101. The number of recombinants obtained from these crosses was compared with that obtained from corresponding crosses with near-isogenic *tif*⁺, unirradiated, or non-plasmid-carrying recipients.

METHODS

Bacteria. *Escherichia coli* JC5088 *recA* str⁺ Hfr (Green *et al.*, 1971) was used as the donor in all the experiments. Its origin of transfer is at 62 min and the *his* gene (at 44 min) enters after about 20 % of the genome has been introduced. The strains JM12 *tif-1* (Castellazzi *et al.*, 1972), AB1157 (Howard-Flanders *et al.*, 1966) with or without the plasmid pKM101, and PAM5717 *lexA* (Donch *et al.*, 1968) were used as recipients. All the recipient strains carry *thr leu thi pro his arg str lac gal* as additional markers. The plasmid pKM101 was transferred into AB1157 from an *Escherichia coli* B strain CM891 *uvrA trp*, constructed previously in this laboratory (Doubleday *et al.*, 1977). The bacteria were grown routinely in L broth [containing (per litre): 5 g glucose, 10 g Bacto Tryptone, 5 g Bacto yeast extract, 5 g NaCl; pH 7.0], in which the mating experiments were also performed. For each experiment overnight cultures of the strains were diluted into fresh medium. The donor was grown with aeration into the exponential growth phase (around 1×10^8 to 2×10^8 ml⁻¹) and the recipients to late-exponential phase (around 10^9 ml⁻¹). The incubation temperature was 32 °C for all the strains if JM12 was used in the experiment, otherwise it was 37 °C.

Transfer of plasmid pKM101 into *E. coli* AB1157. Portions (1.0 ml) of overnight cultures of both CM891 and AB1157 were mixed together with 10 ml fresh medium. After overnight incubation at 37 °C the mating mixture was diluted 10^{-5} into buffer and plated on Davis-Mingioli (1950) minimal agar supplemented with Casamino acids (Difco, 4.0 g l⁻¹) and ampicillin (0.2 g l⁻¹). The plates were incubated at 37 °C for 48 h. Several colonies were then picked, cultured and tested for both spontaneous and u.v.-induced mutability. The strain that showed the highest spontaneous and induced mutation frequency was also tested for u.v. resistance and found to be about twice as resistant as the parent AB1157. This strain was designated CM1112 and subsequently used as one of the recipients.

Ultraviolet irradiation of the donor. Exponentially growing cells were centrifuged and suspended in phosphate buffer. This suspension was divided into portions which were then u.v.-irradiated with different doses. Irradiated cells were again centrifuged and resuspended in fresh medium.

Induction of SOS functions in the recipients. In the temperature-sensitive strain JM12, *tif* expression was induced by performing the crosses at 43 °C. In the strain AB1157, SOS functions were triggered by irradiating the cells (suspended in 0.01 M-MgSO₄) with u.v. light (30 J m⁻²) immediately before mating. The success of the induction was checked by microscopic examination of the JM12 cells to detect the characteristic filamentous growth (Kirby *et al.*, 1967) after 2 to 3 h growth at 43 °C and, in case of the strain AB1157, by plating u.v.-irradiated phage lambda with either u.v.-irradiated or unirradiated bacteria to detect Weigle reactivation.

In the plasmid-carrying strain CM1112 the expression of SOS function was assumed to be constitutive and consequently no special inducing treatment was given.

Mating. Recipient cultures were centrifuged and suspended in fresh medium (one-third of the original volume). Donor and recipient cultures were then mixed in a ratio of 1 to 4. With the strain JM12 as recipient this mating mixture was, in some experiments, divided into three subcultures one of which was left as a control, one supplemented with adenine (75 µg ml⁻¹) to enhance *tif* expression and one with guanosine and cytidine (100 µg ml⁻¹) to suppress it (Witkin, 1974). After 45 min the mating was interrupted by vigorous agitation and the cells were plated on selective media [Davis-Mingioli minimal medium supplemented with threonine (20 µg ml⁻¹), leucine (10 µg ml⁻¹), proline (10 µg ml⁻¹), arginine (20 µg ml⁻¹), thiamin (0.1 µg ml⁻¹) and streptomycin (0.3 mg ml⁻¹); when the mating mixture had contained adenine or guanosine and cytidine, the corresponding additions were also made to the selective medium]. The plates were incubated at 32 °C for 48 h and then *his*⁺ *str* recombinants were counted.

RESULTS AND DISCUSSION

The results are expressed in Fig. 1. Although there were great differences between the recipient strains in their abilities to form recombinants in the crosses, the curves obtained by plotting the numbers of recombinants against u.v. dose given to the donor were almost parallel for each donor-recipient pair. Neither *tif* expression, u.v. irradiation of the recipient nor the presence of the plasmid pKM101 affected the slope of the curves. Expression of Weigle reactivation in irradiated recipients was confirmed by the observation of enhanced (4- to 9-fold) survival of u.v.-irradiated phage lambda.

Fig. 1. (a) Effect of *tif* expression on the ability of recipients to form recombinants in crosses with a u.v.-irradiated donor. The results are expressed as a fraction of the number of recombinants obtained in crosses between an unirradiated donor and a *tif*-induced recipient (JM12). All the matings were performed at 43 °C and the selection plates were incubated at that temperature for a further 45 min before being transferred to 32 °C. ○, JM12; ●, JM12 with guanosine and cytidine in the mating medium; □, AB1157; ■, PAM5717. The vertical lines represent standard errors of the means of three or more experiments.

(b) Effect of u.v. irradiation of the recipient on the yield of recombinants in crosses with a u.v.-irradiated donor. The results are expressed as a fraction of the number of recombinants in crosses with unirradiated donors and recipients. □, Unirradiated AB1157; ▲, u.v.-irradiated AB1157 (dose 30 J m^{-2}).

(c) Effect of the presence of plasmid pKM101 in the recipient on the yield of recombinants in crosses with a u.v.-irradiated donor. The results are expressed as a fraction of the number of recombinants in crosses with unirradiated donors and non-plasmid-carrying recipients. □, AB1157; △, CM1112 (i.e. AB1157 carrying the plasmid pKM101).

Expression of *tif* in JM12 bacteria at 43 °C was clearly shown in these experiments by the development of the characteristic filamentation and by an increase in the number of recombinants with both irradiated and unirradiated donors compared with AB1157. Guanosine and cytidine, which are inhibitors of *tif* expression, somewhat reduced the number of recombinants in JM12. The results are in agreement with a previous report which noted an increase in the number of recombinants in *tif* recipients (Lloyd, 1978). Irradiation of the recipient did not affect the number of recombinants obtained. The plasmid pKM101 did not greatly affect the number of recombinants, but their growth was somewhat slower compared with the rest of the recipients. Our data therefore provide no evidence for an inducible SOS repair system acting on incoming donor DNA that can enhance the recovery of a donor marker as a recombinant.

We have considered the possibility that repair systems might have been induced indirectly by the irradiated donor DNA itself. This possibility seems to be excluded, however, by the result with PAM5717. This *lexA* derivative of AB1157 would not be expected to express any of the SOS inducible functions. *LexA* strains do not show *tif* phenomena (Castellazzi *et al.*, 1973), Weigle reactivation (Defais *et al.*, 1971) or pKM101-mediated repair (Walker, 1977).

The incoming donor chromosome is, of course, to some extent unrepresentative of the complete bacterial chromosome as it normally exists, most chiefly in that it is believed to enter as a single strand of DNA. Weigle reactivation is, nonetheless, able to operate on single-stranded phage DNA (Ono & Shimazu, 1966) and one might *a priori* have expected

the u.v.-irradiated donor strand to be a valid homologue of this. The fact that the presence or absence of functional excision repair in the recipient has no influence on the yield of recombinants with *Flac*⁺ donor DNA (Howard-Flanders *et al.*, 1968) argues that irradiated donor DNA is not integrated into the recipient chromosome as an unrepaired single strand. It is clear from the data of Howard-Flanders *et al.* (1968), however, that a considerable amount of the incoming strand repair must occur in the recipient, presumably to generate a continuous duplex molecule. One implication of our negative finding is that this does not occur by a *lexA*⁺-dependent inducible process such as SOS repair.

There seems to be no reason why a constitutive recombinational process, similar to that postulated by Rupp & Howard-Flanders (1968) to occur at daughter strand gaps produced during replication of u.v.-irradiated DNA, should not be able to act on donor DNA. The *lexA* mutation, although depressing daughter strand repair, does not constitute an absolute block. Be that as it may, our results require the conclusion that inducible SOS repair is either non-existent or insignificant in extent compared with constitutive processes leading to the recovery of viable recombinants from u.v.-irradiated donor DNA. It may well be that this difference from what is observed with irradiated bacteriophage is a reflection of the fact that phage does not need to undergo a recombination step in order to survive and be detected. To this extent, therefore, the Hfr system that we have used may be an imperfect model.

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