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Resident Enhanced Repair: Novel Repair Process Action on Plasmid DNA Transformed into *Escherichia coli* K-12

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The survival of UV-irradiated DNA of plasmid NTP16 was monitored after its transformation into recipient cells containing an essentially homologous undamaged plasmid, pLV9. The presence of pLV9 resulted in a substantial increase in the fraction of damaged NTP16 molecules which survived in the recipient cells. This enhanced survival requires the host *uvrA*$^+$ and *uvrB*$^+$ gene products, but not the host *recA*$^+$ gene product. The requirement for both homologous DNA and the *uvrA*$^+$ and *uvrB*$^+$ gene products suggests that a novel repair process may act on plasmid DNA. Possible mechanisms for this process are considered.

The repair of DNA damaged by UV irradiation has been well studied in *Escherichia coli* and involves a complex series of pathways (1, 4). Two main groups of repair pathways have been identified which operate in the dark: namely, the excision repair pathways which characteristically require the *uvrA*$^+$, *uvrB*$^+$, and *uvrC*$^+$ gene products; and the postreplicational repair pathways which require the *recA*$^+$ gene product. The sensitivities to UV irradiation displayed by mutant strains indicate that excision repair and post-replicational repair are of roughly equal importance for the survival of cells.

We previously studied the repair of plasmid DNA in *E. coli* (9, 13) by transforming UV-damaged DNA of the plasmid NTP16 into calcium-treated bacterial cells and showed that *recA*$^+$-dependent repair contributes little to plasmid survival, whereas excision repair acts effectively on plasmid DNA. NTP16 is a non-conjugative plasmid (molecular weight, 5.6×10^6) which encodes resistance to ampicillin and kanamycin (5).

To find out whether *recA*$^+$-dependent repair of NTP16 was limited by the absence of homologous DNA, we measured the survival of UV-irradiated NTP16 in cells already containing the closely related plasmid pLV9. This latter plasmid was derived from NTP16 by hydroxylamine mutagenesis and is thought to carry a point mutation in the ampicillin resistance gene (G. O. Humphreys, personal communication). Thus, the survival of NTP16 may be gauged by following the inheritance of ampicillin resistance.

The resident pLV9 did increase the survival of the incoming NTP16 in wild-type cells and in *recA* and *uvrC* mutant cells (Fig. 1a, b, and c), but not in *uvrA* or *uvrB* mutant cells (Fig. 1b and c). Additional experiments with pLV9-carrying derivatives of the recombination- or repair-deficient mutants revealed no other strains which failed to give enhanced survival of NTP16. In contrast, cells containing the plasmid pBR313, which is unrelated to NTP16, did not give enhanced survival (selecting in this instance for the stable acquisition of kanamycin resistance [Fig. 1a]).

Increased survival has been observed in similar conditions with phage (prophage reactivation, multiplicity reactivation, and marker rescue) (2) and with transducing phage-plasmid hybrids (6). In each of these systems, the increase in survival appears to be because of recombination between the damaged molecule and its undamaged homolog. However, since the enhancement of NTP16 survival by pLV9 is a *recA*$^+$-independent phenomenon, this cannot be a recombination repair mechanism unless there is a plasmid-coded recombination or repair function which can substitute for the *recA*$^+$ gene product in this instance. The presence of such a function appears unlikely since (i) there was no increase in recombination frequency in an Hfr cross in which the recipient (either *recA*$^+$ or *recA*$^-$) carried resident pLV9 (data not shown); (ii) resident pLV9 did not enhance the survival of irradiated wild-type or *recA* mutant cells (Fig. 2a); and (iii) resident pLV9 did not enhance the survival of incoming damaged pBR325, a plasmid to which it is not related. These results appear to rule out a plasmid-coded recombination or repair function, unless for some reason it acts only upon NTP16 and its derivatives.

If the ampicillin resistance marker of NTP16 is carried on a transposon, then transposition from the damaged NTP16 into pLV9 might occur, resulting in stable acquisition of this resistance. However, agarose gel electrophoresis of cleared lysates from 12 ampicillin-resistant survivors revealed that the plasmids present in these cells

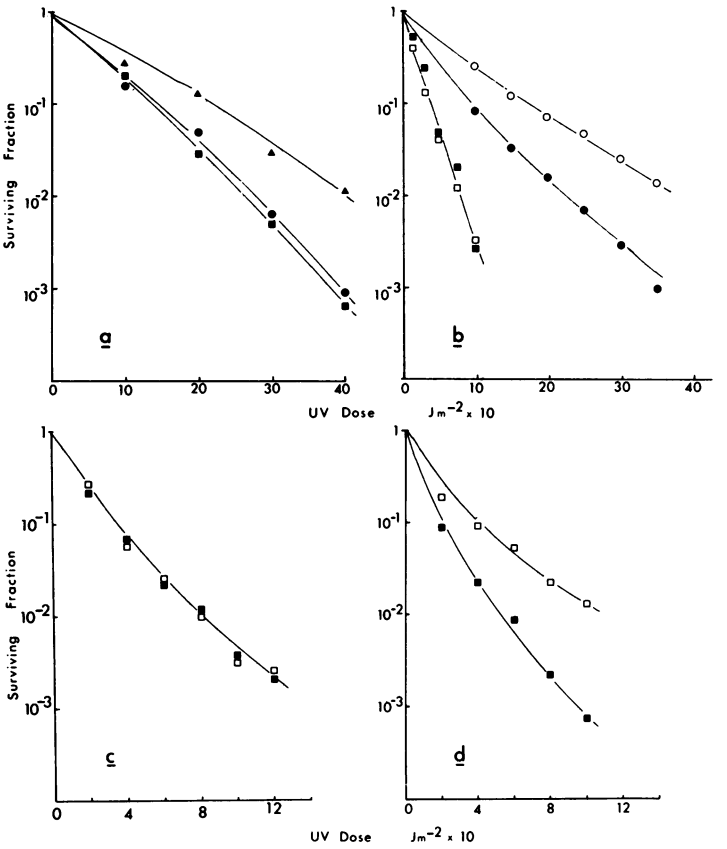

FIG. 1. Survival of UV-irradiated NTP16 DNA transformed into calcium-treated cells of the following: (a) AB1157 *uvr*$^+$ *rec*$^+$, ●; AB1157(pBR313), ■; and AB1157(pLV9), ▲. (b) AB1886 *uvrA6*, ■; AB1886(pLV9), □; BW41 *recA13*, ●; and BW41(pLV9), ○. (c) AB1885 *uvrB5*, ■; and AB1885(pLV9), □. (d) AB1884 *uvrC34*, ■; and AB1884(pLV9), □. Preparation of DNA, transformation of calcium-treated cells, and scoring of transformants were as described previously (5, 9, 13). In most cases, NTP16 survival was measured by scoring ampicillin transformants. When pBR313 was present as the resident plasmid, however, kanamycin was used as the selective marker. In experiments transforming NTP16 into plasmid noncontaining strains, the ampicillin resistance marker was inherited with a frequency identical to that of the kanamycin resistance marker. To maintain strict comparability, the results shown in each section of the figure are for cells transformed at the same time with the same irradiated sample of DNA. DNA was irradiated as a 100-μl drop at a concentration of 20 to 50 μg/ml in 10^{-2} M Tris–10^{-3} M EDTA, pH8.0, and subsequently diluted for transformation. The sample was at ice temperature and was shaken throughout irradiation. The final DNA concentration in the transformation mix was 0.5 mg/ml, which is just below saturation. The absolute transformation frequencies (i.e., the fraction of viable cells transformed) were routinely between 10^{-3} and 5×10^{-3} for all plasmid noncontaining strains and between 2×10^{-4} and 7×10^{-4} for strains containing incompatible resident plasmids.

were of the same sizes as those of NTP16 and pLV9 and did not show the increased size which would have resulted from transposition. Transposition also seems an unlikely explanation for other reasons. A transposon might be expected to move with equal facility into both related and unrelated plasmids, and we demonstrated previously that unrelated plasmids are not capable of enhancing the survival of NTP16. We have also demonstrated that enhanced survival is a *uvrA*$^+$ *uvrB*$^+$-dependent process, and there have been no reports that these gene products are required for transposition. Moreover, to give a significant increase in survival, transposition of the ampicillin resistance determinant would have to occur at a much higher frequency than has been previously observed.

A *uvr*$^+$-dependent and *recA*$^+$-independent repair process has been identified in bacterial cells, namely, liquid holding recovery (8). This process may operate by allowing more extensive excision repair than usual to occur before DNA

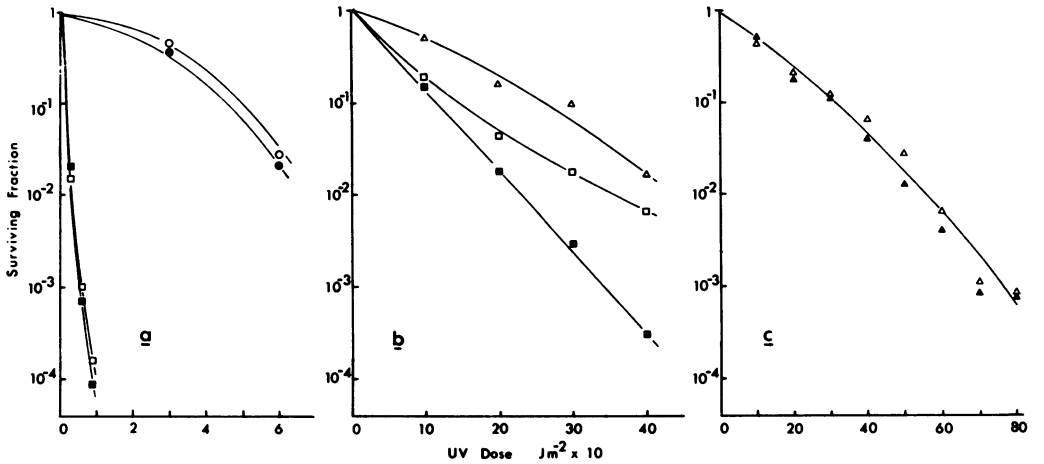

FIG. 2. (a) Survival of UV-irradiated bacterial cells of (□) BW41 *recA13*, (■) BW41 (pLV9), (○) AB1157 *uvr*$^+$ *rec*$^+$, and (●) AB1157(pLV9). Cells were irradiated as a 2-mm layer in M9 buffer at a concentration of 10^{-7} cells per ml. The sample was at ice temperature and was shaken throughout irradiation. Cells were plated on Luria broth plates to assess survival. (b) Survival of UV-irradiated NTP16 transformed into (■) AB1157 *uvr*$^+$ *rec*$^+$, (□) AB1157(pLV9), and (△) AB1157(NTP1). (c) Survival of UV-irradiated pBR325 transformed into (▲) AB1157 and (△) AB1157(pBR313). NTP16 survivors were scored as ampicillin-resistant colonies when transformed into AB1157(pLV9) and as kanamycin-resistant transformants when transformed into AB1157(NTP1). pBR325 survivors were always scored as chloramphenicol-resistant colonies. Details of DNA preparation, transformation procedure, and scoring of transformants are given in the legend to Fig. 1. To ensure strict comparability of results, the data in each section of the figure represent bacterial cells transformed at the same time with the same batch of irradiated DNA.

replication (3). In the case of plasmid DNA, if a resident plasmid is present, the mechanism which controls plasmid copy number might delay the replication of an incoming irradiated plasmid and thus allow more extensive excision repair than would normally be the case. Such a system, which might be considered analogous to liquid holding recovery, should only occur if both resident and incoming plasmids share the same copy number control system. The data in Fig. 2b show the survival of irradiated NTP16 transformed into cells having as resident the incompatible plasmid NTP1. It is apparent that the presence of NTP1 results in a substantial increase in the number of NTP16 molecules which survive. However, this is not conclusive evidence that delayed replication is the explanation of the enhanced survival, since NTP1 and NTP16 share partial homology (G. O. Humphreys, personal communication; Strike and Wilbraham, unpublished data) so that interactions other than copy number control could occur. Other evidence against the delayed replication idea comes from the observation that two alternative pairs of incompatible plasmids failed to show any enhanced survival (pBR325 and pBR313, Fig. 2c; pML2 and pDS120, data not shown). Moreover, if extensive excision repair was the simple explanation of the phenomenon, the *uvrC*$^+$ gene product would also be expected to be required since this protein is essential for the incision step of excision repair (10–12).

One further interaction between the plasmid molecules which might explain the repair enhancement is the interwinding of regions of the damaged plasmid with homologous regions of the undamaged plasmid. Such an interwound structure might allow excision repair of pyrimidine dimers which were formerly too closely spaced to allow repair. An enzyme activity capable of promoting such interwinding has been isolated from *recA* mutant cells (7), which would explain the *recA* independence of the process. The enhanced survival with resident NTP1 could result from its partial homology with NTP16, although other pairs of plasmids which failed to show resident enhanced survival (pBR325 and pBR313; pML2 and pDS120) also share homology and would be expected to interact in this way. The spacing of the pyrimidine dimers formed in NTP16 at the doses used would appear to be too great to interfere with short patch excision repair, but since exonuclease V appears to be necessary for NTP16 repair (9), repair patches may be longer than in the bacterial chromosome. The number of closely spaced dimers would not, however, rise linearly with UV dose, whereas the contribution of resi-

dent-enhanced repair does. This is, therefore, a strong argument against this hypothesis, unless interwinding is able in some as yet unidentified way to increase the efficiency of the excision repair processes. There is certainly scope for such improved efficiency since we previously demonstrated that excision repair works less efficiently on plasmid DNA than on bacterial chromosomal DNA (9).

Thus, the enhancement of survival does not appear to be either the result of a legitimate recombination repair process, since it is independent of the *recA*$^+$ gene product, or the result of normal excision repair, since the enhancement is not dependent upon the *uvrC*$^+$ gene product. We believe, therefore, that this may represent a novel repair process acting upon plasmid DNA.

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