A Transposon Insertion in the *Escherichia coli* *uvrC* Gene; UvrC Protein is Absolutely Required for the Incision Step in Excision Repair

By ROBIN G. WALTERS,¹ HAF O. WILBRAHAM,¹ PETER STRIKE^{1*} AND JOHN W. FORSTER²

¹Department of Genetics, Liverpool University, P.O. Box 147, Liverpool L69 3BX, UK

²Department of Agricultural Botany, School of Agricultural Sciences, Penglais, Aberystwyth, Dyfed SY23 3DD, UK

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The formation of single-stranded breaks in DNA following UV irradiation is assessed in *uvrC*34 mutants. By altering the SOS DNA-repair system, either by additional mutations or by using drugs affecting transcription or translation, it is shown that such single-stranded breaks require one or more DNA-damage-inducible functions. A UV-sensitive strain is characterized as carrying a Tn10 insertion into the *uvrC* gene. The absence of post-irradiation incision in this strain demonstrates that *uvrC* function is absolutely required *in vivo* for the incision stage of excision repair, and suggests that other *uvrC* mutants are 'leaky'.

INTRODUCTION

Excision repair of UV-irradiated DNA in *Escherichia coli* requires the products of the *uvrA*, *uvrB* and *uvrC* genes. The products of these genes combine *in vitro* to form UvrABC excinuclease, which makes single-stranded cuts at two sites, one each side of the damaged region of DNA (Sancar & Rupp, 1983). The efficiency of this process is enhanced by the presence of DNA helicase II (the *uvrD* gene product) and DNA polymerase I. These enzymes act together to release a damage-containing fragment of single-stranded DNA, 12–13 nucleotides in length, and to resynthesize that region of DNA, allowing turnover of UvrA, UvrB and UvrC proteins (Husain *et al.*, 1985; Caron *et al.*, 1985). Studies using purified enzyme preparations have indicated that all three *uvr* gene products are required for the initial incision process (Seeberg *et al.*, 1976; Sancar & Rupp, 1983).

In vivo and *in vitro* studies consistently show *uvrA* and *uvrB* mutants to be completely defective in incision (Seeberg *et al.*, 1980); in contrast, *uvrC* mutants show a measurable level of UV-dependent incision *in vivo*. Such UV-induced single-stranded breaks accumulate more slowly and to a lesser extent than in *uvr*⁺ strains. Incisions made in *uvrC* cells are not followed by dimer excision nor by repair synthesis (Kato, 1972).

Post-irradiation incision in *uvrC* mutants has been investigated *in vitro* using permeabilized cells; the results have been contradictory. In the presence of nicotinamide mononucleotide (NMN), which inhibits ligase-mediated resealing of single-stranded breaks, plasmolysed *uvrC* cells appear as incision defective as *uvrA* cells (Seeberg & Strike, 1976). However, when permeabilized by treatment with toluene, again in the presence of NMN, they have variously been reported as being either completely proficient (Sharma & Moses, 1979) or completely defective (Seeberg *et al.*, 1980) in *uvr*⁺-dependent incision. The reasons for the discrepancies are unclear, but may involve the observed wide variation in the efficiencies of *uvr*⁺-dependent and *uvr*⁺-independent incision between intact, plasmolysed and toluene-treated cells.

If ligase is inactivated in intact cells by the introduction of a *lig-7*(Ts) mutation and pre-incubation at the non-permissive temperature, the initial rate (but not the final level) of post-irradiation incision is increased in *uvrC* mutants (Seeberg & Rupp, 1975). It was originally

suggested that UvrC protein might have a role in blocking the action of ligase on the incisions made by the *uvrA* and *uvrB* gene products. This hypothesis is incompatible with further studies showing that incision capacity in *uvrC* mutants saturates at high UV doses (Seeberg *et al.*, 1980).

Ligase inactivation leads to efficient induction of the SOS regulon (Morse & Pauling, 1975). The increased rate of incision in a *lig-7*(Ts) mutant might therefore be due either to the resulting increased availability of UvrA and UvrB, or to a damage-inducible function capable of substituting for UvrC to some extent. An alternative is that those *uvrC* mutations studied (*uvrC34*, *uvrC56*) are 'leaky', and permit a low level of (UvrC-dependent) aberrant incision. These hypotheses have been tested by investigating strand incision: (i) in *uvrC34* cells in which the SOS response has been altered by additional mutations or by treatment with drugs, and (ii) in a *uvrC::Tn10* mutant.

METHODS

Bacteria and bacteriophages. All the *E. coli* and bacteriophage λ strains, and plasmids, used are listed in Table 1.

Media. M9 buffer contained (per litre of water): NH_4Cl , 1 g; KH_2PO_4 , 3 g; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 7.5 g; NaCl, 5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.246 g; CaCl_2 , 0.011 g. K medium was M9 buffer supplemented with 1% (w/v) glucose, 1% (w/v) Casamino acids, and 0.0001% thiamin. L agar contained (per litre of water): Bacto-tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; agar, 15 g.

UV survival. Fresh stationary-phase cultures of bacteria were resuspended in M9 buffer to a cell density of approximately 10^9 cells ml^{-1} . Samples (0.1 ml) of appropriate dilutions were spread on L agar plus antibiotics, and irradiated with UV light at 254 nm. Survivors were scored after incubation at 37 °C overnight. Each result is the mean of up to four replicates.

Growth and irradiation of ^3H -labelled cells. Cells were grown for several generations to mid-exponential phase in 5 ml K medium supplemented with uridine (50 $\mu\text{g ml}^{-1}$) and [^3H]thymidine (5 μCi , 70–90 Ci mmol^{-1}) (185 kBq, 2.59–3.33 TBq mmol^{-1}). After washing twice in 40 mM-Tris/HCl, pH 8.0, cells were resuspended in 5 ml M9 buffer and irradiated (24 J m^{-2}) on ice. K medium (5 ml, containing rifampicin or chloramphenicol if necessary at 200 $\mu\text{g ml}^{-1}$) was added and the cells were incubated with aeration at 37 or 42 °C to allow strand-break formation.

Measurement of DNA strand breaks. Samples (0.1 ml) of irradiated cells were incubated on ice for 10 min with 20 μl spheroplast mix (10%, w/v, sucrose; 2 mg lysozyme ml^{-1} ; 0.02 M-EDTA; 0.2 M-Tris/HCl, pH 8.0) and mixed with equal volumes of lysis mix (1%, w/v, Sarkosyl NL-97; 0.01 M-EDTA) on 5 ml 5–20% (w/v) alkaline sucrose density gradients. ^{14}C -labelled T4 DNA was added as a size marker, and gradients were spun at 35000 r.p.m. for 90 min at 20 °C in an SW50 rotor. Fractions (0.25 ml) were collected onto GF/C filters using a peristaltic pump, and washed in ice-cold 10% (w/v) TCA, 0.1 M-HCl, and finally ethanol. Filters were then dried and counted in a scintillation counter. Values for the number of strand breaks per 10^8 atomic mass units of DNA ($10^8/M_n$) were obtained as described previously (Seeberg & Strike, 1976).

Preparation of chromosomal DNA. Samples (200 ml) of a fresh stationary-phase culture were washed in M9 buffer and resuspended in 8.5 ml 25% (w/v) sucrose, 0.05 M-Tris/HCl, pH 8.0. Lysozyme (0.65 ml of a 10 mg ml^{-1} solution) was added, and the cells were incubated on ice for 15 min. After addition of 3.3 ml 0.2 M-EDTA, pH 7.8, and a further 5 min on ice, the cells were lysed with 3.3 ml 1% (w/v) Brij-58, 0.4% sodium deoxycholate, 0.05 M-Tris/HCl, pH 8.0, 0.002 M-EDTA. Cellular protein was degraded by the addition of proteinase K to 100 $\mu\text{g ml}^{-1}$ and incubation overnight at 4 °C. Any remaining protein was extracted by heating at 60 °C for 10 min, followed by several extractions with phenol/chloroform (1:1, v/v). Nucleic acids were precipitated with 0.1 vol. 5 M-NaCl and 2 vols ethanol, washed in 70% (v/v) ethanol, dried, redissolved in TE (0.01 M-Tris/HCl, 0.001 M-EDTA, pH 7.5), and treated with 100 μg RNAase ml^{-1} at 37 °C for 60 min. DNA was reprecipitated as before.

Preparation of ^{32}P -labelled DNA probes. Bacteriophage λ strains were isolated as described in Maniatis *et al.* (1982): λCA1 by temperature inducing an exponentially growing culture of CA148 (a bacterial strain lysogenic for the phage), λNK55 by large-scale liquid culture. After degradation of cellular nucleic acids, and concentration of the phage by precipitation with polyethylene glycol (PEG 6000), phage DNA was prepared by phenol/chloroform extraction and ethanol precipitation.

Radioactive probes were prepared according to the method of Feinberg & Vogelstein (1984). The 1.95 kb *Bg*II fragment of λCA1 and 2.8 kb *AccI* fragment of λNK55 were cut out of 1% (w/v) low-melting-point agarose gels, diluted, and used as the template for the labelling reaction. The probe was separated from unincorporated label by running it down a Sephadex G-50 column.

Southern blotting and hybridization. Chromosomal DNA was digested with appropriate restriction enzymes (buffer conditions as recommended by suppliers). Samples (5 μg) were run alongside *Bg*II-digested λCA1 and *AccI*-digested λNK55 DNA on a 1.0% agarose gel in TBE electrophoresis buffer (0.089 M-Tris/borate, 0.089 M-boric acid, 0.002 M-EDTA, pH 8.0). The gel was denatured (0.2 M-NaOH, 0.6 M-NaCl, 30 min at room temperature) and washed in three changes of sodium phosphate transfer buffer (0.025 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$,

Table 1. *Bacteria, bacteriophages and plasmids*

Bacteria	Relevant genotype	Source or reference
AB1157	Wild-type	Our collection
AB1884	<i>uvrC34</i>	Our collection
AB1886	<i>uvrA6</i>	Our collection
AB2405	AB1157 <i>uvrC33</i>	Our collection
AB2433	<i>uvrC34</i> HfrH	B. Bachmann
CA148	AB1884 (λ CA1)	Lysogeny
CA159	<i>uvrC34 recA441 lexA51</i> (Def)	DM1187 nal ^r $\times$ AB2433 his ⁺
CA173	DM1187 <i>uvrA277::Tn10</i>	Transduction from N3055
DM1187	<i>recA441 lexA51</i> (Def) His ⁻	Our collection
N3055	<i>uvrA277::Tn10</i>	R. G. Lloyd
N3124	<i>uvrC279::Tn10</i>	R. G. Lloyd
PE136	<i>polA12 endA26</i>	P. Emmerson
Bacteriophages		
λ CA1	p <i>uvrC</i> ⁺ <i>cI857</i> (ind ⁻)	E. Seeberg
λ NK55	<i>b221 cIII167::Tn10 cI857</i> (ind ⁺) <i>Oam29</i>	Kleckner <i>et al.</i> (1978a)
Plasmids		
pJL26	<i>lexA3</i>	Little (1980)
pJWF10	<i>uvrC'</i>	Forster & Strike (1984)
pPE25	<i>lexA3</i>	Emmerson <i>et al.</i> (1981)

pH 6.5) for 60 min at room temperature. DNA was transferred from the gel onto a GeneScreen hybridization membrane (New England Nuclear) using the capillary transfer method of Southern (1975). The membrane was gently washed in buffer, dried, and baked at 90 °C for 3–4 h.

Hybridization was done as follows. The membrane was incubated with constant agitation overnight at 60 °C in prehybridization solution (5 $\times$ Denhardt's solution, 250 μ g denatured salmon sperm DNA ml⁻¹) and was transferred to hybridization solution (1 $\times$ Denhardt's solution, 0.5% SDS, 250 μ g denatured salmon sperm DNA ml⁻¹, < 10 μ g denatured radioactive DNA probe ml⁻¹, 0.3 M-NaCl, 0.002 M-EDTA, 0.06 M-Tris/HCl, pH 8.0) and again incubated with constant agitation overnight at 60 °C. The stock Denhardt's solution was 50 $\times$ concentration and contained 1% (w/v) BSA, 1% (w/v) Ficoll (*M_r* 400 000), 1% (w/v) polyvinyl pyrrolidone (*M_r* 400 000).

After hybridization, the membrane was washed twice in 0.3 M-NaCl, 0.002 M-EDTA, 0.06 M-Tris/HCl, pH 8.0, at room temperature for 5 min, twice in the above plus 0.5% SDS at 60 °C for 30 min, and twice in 0.003 M-Tris base at room temperature for 30 min. The membrane was not allowed to dry before autoradiography; subsequently the probe was stripped from the membrane by washing in 0.5 M-KOH at room temperature for 60 min. The removal of all radioactive probe was confirmed by autoradiography.

RESULTS AND DISCUSSION

SOS dependence of incision in uvrC34 mutants

We have compared the extent of post-irradiation incision observed in *uvrC34* cells in both the presence and the absence of the transcription-inhibiting drug rifampicin (Fig. 1). Incision is reduced by the drug to similar levels to those observed in a *uvrA* mutant. The simplest explanation is that incision in *uvrC34* mutants requires the induction of damage-inducible genes, and that the amounts of the gene products present in uninduced cells are insufficient to allow readily detectable levels of incision when induction is prevented by rifampicin. Similar experiments (results not shown) were done using chloramphenicol, which has its effects at the translational level. A slight decrease in strand incision was observed, but the effect was not as great as that with rifampicin. It has previously been observed that rifampicin is particularly effective in repressing the induction of the *recA* gene product by DNA damage (Satta *et al.*, 1979).

In an attempt to confirm the SOS dependence of incision in *uvrC* mutants, a *recA56* mutation, which prevents induction of the SOS regulon, was introduced into a *uvrC34* mutant; it appears that incision in the *uvrC34 recA56* strain (not shown) is only slightly reduced from the levels observed in the *uvrC34* single mutant. However, the absence of a functional RecA protein makes

Fig. 1

Fig. 1. Formation of single-stranded breaks in *E. coli* DNA following UV irradiation. ●, PE136 (*uvr⁺ polA12 endA26*); Δ, AB1884 (*uvrC34*); ▲, CA159 (*uvrC34 recA441 lexA51*); ○, AB1884 + rifampicin (200 μg ml⁻¹); □, AB1884 + pPE25 (*lexA3*); ▽, AB1886 (*uvrA6*); ▼, CA173 (*uvrA277::Tn10 recA441 lexA51*). M_n , average mass number.

Fig. 2

Fig. 2. Complementation of UV-repair defects in *E. coli*. ●, AB1157 (*uvr⁺*); Δ, AB1884 (*uvrC34*); ▲, AB1884 + pJWF10 (*uvrC⁺*); ○, CA148 (*uvrC34 Δp uvrC⁺*); □, N3124 (*uvrC279::Tn10*); ▽, N3124 + pJWF10.

the double mutant deficient in not only excision repair but also recombination repair (Rupp *et al.*, 1971). This deficiency would lead to the production at replication forks of single-stranded gaps, which are indistinguishable by this method from single-stranded nicks. Similarly, the addition of a *recB* mutation to a *uvrC34* mutant leads to only a modest reduction in incision; the *recB* mutation has been shown to have little effect on the efficiency with which the *recA* gene product is induced by UV irradiation (Little & Hanawalt, 1977).

A different approach to repressing the SOS system involved introducing a multicopy plasmid carrying the mutant *lexA3* gene into the *uvrC* strain. The presence of such plasmids in bacterial cells has been shown to sensitize them to UV irradiation by effectively switching off the cells' SOS response, the mutated LexA protein being resistant to the cleavage activity of the RecA protein (Little *et al.*, 1981). The effect of introducing the plasmid pPE25 into AB1884 (*uvrC34*) was to markedly reduce the amount of observed strand breakage. In contrast, the presence of the plasmid had little effect on incision in AB1886 (*uvrA6*, not shown). Similar results were obtained using pJL26, which also encodes the uncleavable LexA3 protein. It proved impossible to assess the effect of these plasmids on *uvr⁺* cells, since in order to effectively detect incisions in such strains, rapid completion of excision repair must be blocked by a mutation in either of the genes *polA* or *lig*. pPE25 and pJL26 are both based on the ColE1 replicon, and therefore have an absolute requirement for DNA polymerase I for their replication (Cooper & Hanawalt, 1972). They proved to be unstable in *polA12* strains, even at 30 °C, and were also rapidly lost from *lig-7(Ts)* strains.

As a further way of investigating the SOS dependence of incision, the *lexA51* (*spr*) mutation was introduced into a *uvrC34* strain. This mutation inactivates the LexA protein (Pacelli *et al.*,

1979) and thus allows constitutive expression of those functions which it normally represses. If incision capacity were normally induced by DNA damage in *uvrC* mutants, the introduction of such a mutation would be expected to alter the kinetics of incision, with the breaks being introduced much more rapidly. Fig. 1 shows this to be the case; the speed with which strand breaks are produced is very much greater in the double mutant than in the *uvrC* strain (an effect which is reversed by the introduction into the strain of pJL26, not shown). Indeed, most of the breaks are made within the first 5 min after irradiation, exactly as in *uvr*⁺ strains. However, the extent of incision is still limited compared to wild-type strains, and does not exceed the level normally seen in *uvrC* cells after prolonged incubation. In these respects, the behaviour of the *uvrC34 lexA51* strain is very similar to that of a *uvrC34 lig-7(Ts)* strain, provided the latter has been incubated at the non-permissive temperature for at least 60 min (Seeberg & Rupp, 1975). This similarity might well be a reflection of the effective induction of SOS functions by ligase inactivation (Morse & Pauling, 1975). In contrast, a *uvrA6 lexA51* double mutant, when compared with the *uvrA* single mutant, showed little difference in either the extent or the kinetics of incision.

Preventing the expression of damage-inducible genes after irradiation slows or prevents the formation of single-stranded breaks in *uvrC34* mutants. Conversely, prior expression of SOS genes leads to an increase in the rate of incision. In all cases, altering the expression of SOS functions also alters post-irradiation incision in *uvrC34* strains, but has little or no effect on incision in *uvrA* mutants. These data support the hypothesis that an SOS-inducible gene is a rate-limiting factor in incision in *uvrC34* mutants. Early results suggested that *uvrC* might be damage-inducible (van Sluis *et al.*, 1983), but later work has shown this not to be the case (Forster & Strike, 1985, Granger-Schnaar *et al.*, 1986, Moolenaar *et al.*, 1987). The non-inducibility of *uvrC* implies that the inducible component must be either UvrA and UvrB proteins, or some other gene product able to substitute for UvrC but which still interacts with UvrA (a *uvrA uvrC* double mutant shows no incision, data not shown).

One possible explanation might be that the *uvrC34* mutation is 'leaky', and that increasing the availability of UvrA and UvrB proteins allows the defective protein to produce a limited number of aberrant incisions more rapidly. Turning off the expression of SOS genes would have the opposite effect.

Characterization of a transposon insertion in *uvrC*

Strain N3124 is a derivative of AB1157 constructed by P1-mediated transduction from strain N3024 of the *Tn10*-encoded tetracycline-resistance marker. N3024 was selected as a mitomycin-C-sensitive, UV-sensitive, host-cell-reactivation-deficient derivative of W3110, produced by *Tn10* mutagenesis according to the method of Kleckner *et al.* (1978a), namely infection with λ NK55 and selection for tetracycline resistance (R. G. Lloyd, personal communication).

N3124 is also highly sensitive to UV irradiation (Fig. 2) and mitomycin C. UV sensitivity is partially complemented by pJWF10 (a high-copy-number plasmid encoding a slightly truncated *uvrC* gene product: Forster & Strike, 1985), and is completely complemented by a lysogenic λ CA1 (a derivative of λ puvC⁺ constructed by *in vivo* recombination with λ cI857: Blingsmo *et al.*, 1981; E. Seeberg, personal communication). It is also approximately 100% cotransducible (by T4gt7, Wilson *et al.*, 1979) with tetracycline resistance (the cotransducibility can only be estimated, since some or all of any Tet^r UV^r colonies may be a consequence of transposition of the *Tn10* element). These data strongly suggest that the UV sensitivity of N3124 is due to a *Tn10*-associated *uvrC* mutation, and that it carries no additional copies of *Tn10*.

To further characterize the putative mutation in *uvrC* carried by N3124, ³²P-labelled *uvrC* DNA was used to probe Southern blots of chromosomal DNA from AB1157 and N3124, digested with a variety of restriction enzymes. In this way, any changes in the *uvrC* region of the chromosome could be assessed. Preliminary results (not shown) using a heterogeneous probe (isolated from a *Hind*III/*Eco*RI digest of λ CA1 DNA) showed the *uvrC* region to be disrupted in N3124, and suggested that this was due to a *Tn10* insertion directly into the *uvrC* structural gene.

Tn10 has been restriction mapped (Kleckner *et al.*, 1978b; our unpublished data) and the right-hand half has been sequenced (Halling *et al.*, 1982, Hillen & Schollmeier, 1983; N.

Fig. 3. Restriction analysis of the *uvrC* region of strain N3124 chromosomal DNA by Southern blotting, probed with (a) *uvrC*⁺ DNA or (b) Tn10 DNA (see main text). 1, λ CA1 *Bgl*II; 2, AB1157 *Bgl*II; 3, AB1157 *Pst*I/*Hind*III; 4, AB1157 *Pst*I/*Hind*III/*Eco*RI; 5, N3124 *Bgl*II; 6, N3124 *Hind*III/*Eco*RI; 7, N3124 *Hind*III/*Eco*RI/*Pst*I; 8, λ NK55 *Acc*I.

Kleckner, personal communication). This enabled enzymes to be chosen which would facilitate accurate determination of the position of the presumed insertion. The procedure was repeated, this time using a probe isolated from a *Bgl*II digest of λ CA1 DNA (Fig. 3a). It is immediately apparent that the 1.95 kb *Bgl*II fragment carrying the *uvrC* gene (track 2) has been rearranged in N3124 to give two fragments hybridizing to *uvrC* DNA, 3.55 and 4.75 kb in length (track 5); similarly, a comparison of tracks 3 and 7 also shows rearrangement of *uvrC*. In each case, the change in fragment length is fully in agreement with that expected from an insertion of Tn10. The position and orientation of this insertion into the *uvrC* gene can be unambiguously determined from the sequence and restriction data; 350 ± 20 bp upstream from the 3' *Bgl*II site, with IS10-R on the 5' side (Fig. 4).

The only simple alternative to an insertion directly into the *uvrC* gene is a chromosomal rearrangement associated with an insertion of Tn10 nearby – Tn10 readily generates deletions and inversions of neighbouring chromosomal material, with one endpoint at or within the Tn10 element itself (Kleckner *et al.*, 1979). To be consistent with the data from track 5, a Tn10-associated chromosomal inversion would have to start from a point between the two *Bgl*II sites, and extend upstream beyond the *Pst*I sites, or downstream beyond the *Eco*RI site. No such inversion can be reconciled with the data from both of tracks 6 and 7, suggesting that *uvrC* has not been subjected to a significant chromosomal rearrangement.

To confirm that the rearrangement of *uvrC* was due to the addition of Tn10 material, the nylon membrane was stripped of *uvrC* probe, using 0.5 M-KOH, and was reprobed with the 2.8 kb *Acc*I

Fig. 4. Restriction maps of the *uvrC* region of the *E. coli* chromosome, and of transposon Tn10, showing the site of insertion of Tn10 in strain N3124. The two solid bars indicate the DNA fragments used as probes in Fig. 3(a) and (b), respectively. The broken bar indicates a region homologous to part of the probe used in Fig. 3(b).

fragment of Tn10 DNA indicated in Fig. 4. The AB1157 tracks showed no sequences homologous to the Tn10 probe (Fig. 3b). In contrast, the N3124 tracks showed the same bands as in Fig. 3(a), plus additional bands corresponding in size to fragments internal to Tn10. The exact correspondence of restriction fragments hybridizing to *uvrC* and Tn10 DNA in N3124 confirms that the strain carries a Tn10 insertion mutation of *uvrC*.

Post-irradiation incision in a *uvrC* null mutant

It has been suggested that the carboxy-terminus of UvrC protein is important in excision repair (Sharma *et al.*, 1986), a hypothesis consistent with the incomplete complementation by pJWF10 of *uvrC* defects (Fig. 2). The insertion mutation in N3124 is likely to result in a UvrC protein truncated by approximately 100 amino acid residues at its carboxy-terminus, so it might be expected that N3124 encodes a *uvrC* gene product which has lost most, if not all, of its function, at least with respect to excision repair – a *uvrC* null mutant.

Post-irradiation incision in N3124 was assessed, and compared with that in AB1884 (*uvrC34*). Fig. 5 shows that single-stranded break formation was reduced to levels comparable to those seen in *uvrA* mutants. A *uvrA*::Tn10 mutant, N3055, showed little difference in incision capability from a *uvrA6* mutant. The implication is that complete loss of *uvrC* function is accompanied by complete loss of incision capability, the formation of single-stranded breaks in *uvrC34* mutants being due to an altered UvrC protein; i.e. *uvrC34* is 'leaky', at least with respect to post-irradiation incision. A comparison of the UV sensitivities of N3124 and AB1884 shows the *uvrC34* mutant to be more UV resistant by a small but significant amount, again suggesting that it is a 'leaky' mutation. The variable levels of incision observed in various other *uvrC* mutants suggest that such mutations are also incomplete: *uvrC56* (Sharma & Moses, 1979; Tang & Ross, 1985) and *uvrC33* (Fig. 5) have similar incision kinetics to *uvrC34*, but the extent of incision varies, *uvrC33* leading to a markedly greater level of single-stranded breaks.

The events leading to incision *in vitro* are now well understood. UvrA protein binds to DNA in an ATP-dependent, damage-dependent manner (Seeberg & Steinum, 1982); UvrB stabilizes such an association, binding to form a stable complex (Kacinski & Rupp, 1981; Strike & Rupp, 1985); finally, UvrC completes the excinuclease, resulting in incision *in vitro* (Young *et al.*,

Fig. 5. Effect of *uvrC* alleles on post-irradiation incision in *E. coli*. ●, PE136 (*uvr*⁺ *polA12 endA26*); △, AB1884 (*uvrC34*); □, N3124 (*uvrC279::Tn10*); ■, AB2405 (*uvrC33*); ▽, AB1886 (*uvrA6*); ▼, N3055 (*uvrA277::Tn10*). M_n , average mass number.

1986). The above data suggest that the same is true *in vivo*. The UvrABC complex remains tightly bound following incision, requiring the combined action of helicase II and DNA polymerase I to be released (Husain *et al.*, 1985; Caron *et al.*, 1985). In an SOS-induced cell, UvrA and UvrB proteins are present in vast excess over the low level of UvrC (Yoakum & Grossman, 1981). A defect at any stage following incision which prevents UvrC turnover would result in the rapid depletion of UvrC. As a consequence there would be a low level of incision rapidly reaching a plateau, exactly as observed *in vivo* in *uvrC* mutants. The defect in repair in a *uvrC34* mutant might be either in incision leading to a block in turnover, or in turnover itself.

The unusual phenotype of *uvrC34* mutants, compared with *uvrA* and *uvrB* mutants, extends to the repair of chemically damaged transforming plasmids. *N*-2-Aminofluorene (AF) adducts are removed *in vitro* by the UvrABC excinuclease and yet are readily tolerated by both *uvr*⁺ and *uvrA* strains (Fuchs & Seeberg, 1984); *uvrC34* mutants, however, are sensitive to AF lesions, although this is suppressed by an additional *uvrA* mutation. Bichara & Fuchs (1987) suggest the sensitivity of *uvrC34* strains is due to impaired or defective UvrABC function, a conclusion supported by the sensitivity of *uvrD* cells. These effects are consistent with a *uvrC34* mutation causing a block in UvrABC turnover, which in turn would block replication of the plasmid, leading to plasmid loss.

The effects of *uvr* mutations on resident enhanced repair of UV-irradiated transforming plasmids (Strike & Roberts, 1982) also suggests that the basis for the UV sensitivity of *uvrC34* mutants lies in the block in turnover, not in incision. *uvrC34* and *uvrD* mutations have no effect on resident enhanced repair, while *uvrA* and *uvrB* mutations completely eliminate repair, resident-enhanced or otherwise (Roberts & Strike, 1981). The incompatible resident plasmid seems to provide a temporary block on replication, allowing repair to be completed (unpublished observations). As a consequence, when replication does eventually occur, the

replication fork does not encounter any lethal lesions. In the case of a *uvrC34* or *uvrD* mutant host cell, the UvrABC complex is presumably not irreversibly bound to the DNA and eventually dissociates, enabling other enzymes to complete the repair process.

The evidence shows that UvrC protein is absolutely required for the incision stage in excision repair *in vivo* as well as *in vitro*, and suggests that it may be involved in the subsequent release of the 12–13 bp single-stranded fragment containing the damage. A defective UvrC protein might be unable to turn over, resulting in a loss of incision capability, and as a consequence having toxic effects in some circumstances. Further study of the products of mutant *uvrC* genes may be of use in the study of the enzymology of excision repair.

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