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SINGLE-STRAND BREAKS IN THE DNA OF THE *uvrA* AND *uvrB* STRAINS OF *ESCHERICHIA COLI* K-12 AFTER ULTRAVIOLET IRRADIATION

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Abstract—DNA single-strand breaks were produced in *uvrA* and *uvrB* strains of *E. coli* K-12 after UV (254 nm) irradiation. These breaks appear to be produced both directly by photochemical events, and by a temperature-dependent process. Cyclobutane-type pyrimidine dimers are probably not the photoproducts that lead to the temperature-dependent breaks, since photoreactivation had no detectable effect on the final yield of breaks. The DNA strand breaks appear to be repairable by a process that requires DNA polymerase I and polynucleotide ligase, but not the *recA*, *recB*, *recF*, *lexA*101 or *uvrD* gene products. We hypothesize that these temperature-dependent breaks occur either as a result of breakdown of a thermolabile photoproduct, or as the initial endonucleolytic event of a *uvrA*, *uvrB*-independent excision repair process that acts on a UV photoproduct other than the cyclobutane-type pyrimidine dimer.

INTRODUCTION

The *uvrA* and *uvrB* mutants of *Escherichia coli* K-12 were first described as strains that are sensitive to killing by UV radiation, and deficient in the excision of pyrimidine dimers from UV-irradiated DNA (Howard-Flanders *et al.*, 1966; Boyce and Howard-Flanders, 1964; Setlow, 1968). It has subsequently been found that the *uvrA* and *uvrB* strains both lack a particular endonuclease that acts on UV-irradiated DNA (Braun and Grossman, 1974). Thus, the incision break made by the *uvrA–uvrB* endonuclease constitutes the first step in the removal of pyrimidine dimers by the excision repair process.

Once the initial incision has been made, the dimer can be excised by exonucleolytic action, and the missing nucleotides can be replaced by repair resynthesis. The excision and resynthesis steps could use either DNA polymerase I or III, both of which possess 5′-3′ exonucleolytic activity capable of dimer excision, and polymerizing activity for the resynthesis step (e.g. Grossman, 1974; Livingston and Richardson, 1975). The final sealing step presumably is performed by polynucleotide ligase (e.g. Grossman, 1974).

At least a small proportion of the repair events are more complicated than this, since mutations in several other genes have been shown to adversely affect the excision repair process. These include *recA*, *recB*, *recC* (Cooper and Hanawalt, 1972; Youngs *et al.*, 1974) *lexA*101(Youngs and Smith, 1973b), *uvrD* (Ogawa *et al.*, 1968), *uvrE* (van Sluis *et al.*, 1974; Sinzinis *et al.*, 1973), *ras* (Walker, 1970), *polB* (Masker *et al.*, 1973; Tait *et al.*, 1974) and *polC* (Youngs and Smith, 1973c; Tait *et al.*, 1974). The mechanisms by which these various mutations inhibit the excision repair process are largely unknown, and in most instances the gene products have not been identified. In the case of the *lexA*101 (Mount *et al.*, 1972; Greenberg *et al.*, 1974) and *uvrD* (Ogawa *et al.*, 1968) mutations, the mutated allele is dominant to the wild-type form, indicating that the repair deficiency in these mutants is not simply due to the loss of some enzyme normally required for excision repair, but rather to a more complex interaction.

Several investigators have reported that no DNA incision breaks are produced in *uvrA* or *uvrB* strains after UV irradiation (Achey and Billen, 1969; Kato, 1972; Seeberg and Johansen, 1973). These results support the concept that the *uvrA-uvrB* endonuclease is the major enzyme producing incision breaks after UV irradiation. However, Braun and Grossman (1974) observed that a second UV specific endonuclease is present in extracts of *uvrA* and *uvrB* cells, suggesting that some DNA incision breaks should also be detectable in *uvrA* and *uvrB* strains. This prediction is further strengthened by the recent report by Radman (1976) of a UV endonuclease in *E. coli* cells that appears to be different from the *uvrA–uvrB* enzyme. Unpublished data of Setlow and Carrier cited in Setlow (1972) indicate that DNA single-strand breaks (or alkali-labile regions) are observed in *E. coli* B_{s-1} *uvrB* *lexA*101 after 200 and 2000 J m^{-2} at 265 nm. However, it is not clear from this report whether these breaks were the result of direct photochemistry or of endonuclease action.

We have reexamined the issue of DNA strand-breakage in *uvrA* and *uvrB* cells after UV irradiation, and have found that single-strand breaks are produced in these strains to a limited extent. Such breaks appear to result from both photochemical and temperature-dependent (possibly enzymatic) processes, and their repair depends upon DNA polymerase I and polynucleotide ligase. Photoreactivation had no effect on the extent of DNA strand breakage, suggest-

ing that the temperature-dependent breaks result from radiation product(s) other than cyclobutane-type pyrimidine dimers.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used for the present experiments are listed in Table 1. The transduction and mating techniques have been described (Youngs and Smith, 1973a). For the genetic crosses shown in Table 1, the first selection was for the nutritional marker indicated. The presence or absence of a *uvr* or *ligts*-7 mutation was determined by checking UV sensitivity or inability to grow at 42°C, respectively.

Media and growth conditions. The media, labelling, and growth conditions have been described (Youngs and Smith, 1973b). For the present experiments, the glucose minimal medium (MM) was supplemented with Difco Casamino Acids (2 mg/mℓ). Strains containing the *ligts*-7 mutation were grown at 30°C.

For experimental use, an overnight culture was diluted 50- or 100-fold into fresh medium containing 100 μCi of [methyl-^{3}H] thymine (New England Nuclear Corp.; ~15 Ci/mmol) per mℓ, and incubated several hours to a cell density of $\sim 2 \times 10^8$ cells/mℓ. Cells were collected by filtration on Millipore filters (0.45 μm pore size), and resuspended in DTM buffer (MM medium without organic components) at approximately 3×10^7 cells/mℓ.

Irradiation and photoreactivation conditions. The methods used for UV irradiation and dosimetry have been described (Youngs *et al.*, 1974). The fluence rate was ~1 J m^{-2} s^{-1} and was checked before each experiment with a germicidal photometer (International Light, Inc., No. IL-254). Generally, the cells were irradiated in DTM buffer at room temperature, and were subsequently incubated at 37°C. For the experiments with the *uvrB lig* strain (Fig. 7 and 5c), and for certain control experiments with the *uvrB* strain (Fig. 3E), the cells were incubated for 30 min at the indicated temperature, irradiated on ice, and then incubated as desired. For the immediate lysis experiments (Fig. 2), the cells were irradiated on ice (UV fluence rate ~8 J m^{-2} s^{-1}), and were lysed immediately after irradiation.

For experiments involving photoreactivation (PR), samples were resuspended in DTM buffer containing 1 *M* glycerol, and were irradiated at room temperature using a low UV fluence rate (~0.05 J m^{-2} s^{-1}). The samples were exposed to PR light in 6 cm Pyrex Petri dishes placed on top of a 0.5-cm-thick plate of window glass supported ~0.2 cm above 3 black light bulbs (General Electric F15T8-BL, 15 W). Cells were exposed to PR light during and after the UV exposure, to give a total PR exposure of 50 or 100 min to all samples. The presence of glycerol had no apparent effect on the yield of UV-induced DNA strand breaks (Fig. 3B, C), but did protect against the production of breaks by the PR treatment (unpublished results).

The effectiveness of the PR treatment in enhancing survival was verified. The surviving fraction for *uvrB* (DY178) cells exposed to 20 J m^{-2} of UV radiation increased from ~10^{-4} to ~7×10^{-1} with PR exposures of 20 min or longer. The PR time required to give one-half the maximum protective effect was ~4 min (data not shown). Thus, the PR exposures of 50–100 min used for the gradient experiments should have been adequate to show a protective effect for DNA strand breakage if the causative lesion were photoreactivable.

Alkaline sucrose gradient techniques. The alkaline sucrose gradient techniques, and the procedure for measuring DNA degradation have been described (Youngs *et al.*, 1974). The centrifugation speeds ranged from 20,000 to 45,000 rpm with an SW50.1 rotor, depending upon the size of the DNA fragments being sedimented. The M_n ranges for single-stranded DNA and the corresponding centrifugation speeds were: 70–140 $\times 10^6$, 20,000 rpm;

Table 1. List of strains*

Designation	Genotype	Source
KH21†	F$^-$ *leuB metE bio thyA thyR rha lacZ malB str*	R. B. Helling
CHE30	Hfr KL16 *thi ptsILE3*	W. Epstein
SA420	F$^-$ *Δ(uvrB-chlA) his*	A. Campbell
KS252	F$^-$ *ligts-7 proC hsm str*	Konrad *et al.* (1973)
DY98	F$^-$ *metE thyA thyR lacZ str*	Youngs and Smith (1973a)
DY145	F$^-$ *uvrB5 leuB metE thyA thyR rha lacZ str*	Youngs and Smith (1976)
DY155	F$^-$ *uvrB5 recA56 leuB metE thyR rha lacZ str*	Youngs and Smith (1976)
DY157	F$^-$ *uvrB5 recB21 leuB metE thyR rha lacZ str*	Youngs and Smith (1976)
DY168	F$^-$ *Δ(uvrB-chlA) leuB metE thyA thyR rha lacZ malB str*	P_1·SA420 × KH21 (select Bio$^+$)
DY171	F$^-$ *ptsILE3 leuB metE bio thyR rha lacZ malB str*	CHE30 × KH21 (select Thy$^+$)
DY173	F$^-$ *ligts-7 leuB metE bio thyR rha lacZ malB str*	P_1·KS252 × DY171 (select Pts$^+$)§
DY178	F$^-$ *uvrB5 leuB thyA thyR rha lacZ str*	Youngs and Smith (1976)
DY179	F$^-$ *uvrB5 uvrD3 leuB thyA thyR rha lacZ str*	Youngs and Smith (1976)
DY180‡	F$^-$ *uvrB5 lexA101 leuB thyA thyR rha lacZ str*	Youngs and Smith (1976)
DY195	F$^-$ *uvrB5 ligts-7 leuB metE thyR rha lacZ malB str*	P_1·DY145 × DY173 (select Bio$^+$)
DY233	F$^-$ *uvrA6 Δ(uvrB-chlA) leuB metE thyA thyR rha lacZ str*	P_1·AB1886 × DY168 (select Mal$^+$)
AB1886	F$^-$ *uvrA6 arg pro his thr leu thi ara gal lac xyl mtl tsx str*	S. Linn
JG136	F$^-$ *uvrA6 polA1 thyA thyR rha lacZ str*	J. Gross
JG137	F$^-$ *uvrA6 thyA thyR rha lacZ str*	J. Gross
JC8909¶	F$^-$ *uvrB5 argE proA his thr leu thi thyA thyR galK lacY ara tsx supE*	R. H. Rothman and A. J. Clark
JC8910¶	F$^-$ *uvrB5 recF143 argE proA his thr leu thi thyA thyR galK lacY ara tsx supE*	R. H. Rothman and A. J. Clark

*Symbols are as used by Taylor (1972). †Strain KH21 was derived from *E. coli* K-12 W3110 Thy$^-$ (Bachmann, 1972). ‡*lexA*101 refers to the *exrA* mutation from *E. coli* B_{s-1}. §The Pts$^-$ phenotype was scored as the ability to utilize galactose, but not glucose, as a carbon source. ¶Strains JC8909 and JC8910 were derived from AB2499, a *uvrB*5 mutant isolated from strain AB1157 (Howard-Flanders *et al.*, 1966).

40–70 × 10^6, 25,000 rpm; 20–40 × 10^6, 30,000 rpm; 9–20 × 10^6, 35,000 rpm; 5–9 × 10^6, 40,000 rpm; <5 × 10^6, 45,000 rpm. This alteration in the previous experimental procedure was made in order to avoid the possibility of a speed dependent decrease in the sedimentation rate of large molecular weight DNA samples, as has been described by other workers (e.g. Hutchinson, (1975).

The alkaline sucrose gradients were collected by pumping fixed-volume (~0.16 m*l*) fractions onto 1.75 × 1.65 cm sections of a Whatman No. 17 filter paper strip (Carrier and Setlow, 1971). The strips were processed as previously described for filter paper discs (Youngs and Smith, 1973a).

Molecular weight calculations. The methods used for the calculation of weight and number average molecular weight values for DNA profiles from alkaline sucrose gradients have been described (Roots and Smith, 1974; Bonura *et al.*, 1975; Youngs and Smith, 1976). They are presented here in greater detail to provide a more accurate and complete statement of our methodology.

The weight average, M_w, and number average, M_n, molecular weight values are defined (Charlesby, 1954) as:

$$M_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} \quad (1)$$

$$M_n = \frac{\Sigma n_i M_i}{\Sigma n_i} \quad (2)$$

where n_i is the number of molecules, and M_i is the molecular weight of the molecules in the i^{th} fraction of the gradient.

The number of molecules in each fraction, n_i, is proportional to the fractional amount of recovered radioactivity in a fraction, f_i, divided by the molecular weight associated with that fraction, if it is assumed that all molecules in a given fraction are of equal molecular weight. Thus, $n_i \propto f_i/M_i$. Substituting for n_i in Eqs. 1 and 2 gives

$$M_w = \frac{\Sigma f_i M_i}{\Sigma f_i} = \Sigma f_i M_i \quad (3)$$

$$M_n = \frac{\Sigma f_i}{\Sigma (f_i/M_i)} = \frac{1}{\Sigma (f_i/M_i)} \quad (4)$$

Studier (1965) has shown that $S \propto M^a$, where S is the sedimentation coefficient associated with molecules of molecular weight M, and a is a constant. We have used a value of 0.38 for a (Freifelder, 1970). In addition, Burgi and Hershey (1963) have shown that $S \propto d$, where d is the distance sedimented by molecules with sedimentation coefficient S. Thus, it follows that $M_i = kd_i^{2.63}$, where M_i is the molecular weight of molecules in the i^{th} fraction of the gradient and d_i is the distance sedimented by the molecules in that fraction. This assumes that molecules in a given fraction have a unique molecular weight. Substituting for M_i in Eqs. 3 and 4 gives

$$M_w = k\Sigma f_i d_i^{2.63} \quad (5)$$

$$M_n = \frac{k}{\Sigma (f_i/d_i^{2.63})} \quad (6)$$

The proportionality constant, k, can be eliminated by using a DNA sample of known molecular weight under identical sedimentation conditions. We have used bacteriophage T2 DNA, and assume a single-stranded molecular weight of 55 × 10^6 (Freifelder, 1970). Using the relationship $M = kd^{2.63}$, from above, and the molecular weight of T2 DNA

$$k = \frac{55 \times 10^6}{d_{T2}^{2.63}} \quad (7)$$

where d_{T2} is the distance sedimented by the T2 DNA.

Substituting for k in Eqs. 5 and 6 gives the formulas used for calculating the M_w and M_n values for *E. coli* DNA.

$$M_w = \left[\frac{55 \times 10^6}{d_{T2}^{2.63}}\right] \times [\Sigma f_i d_i^{2.63} \text{ for } E.\ coli \text{ DNA}] \quad (8)$$

$$M_n = \left[\frac{55 \times 10^6}{d_{T2}^{2.63}}\right] \times [1/\Sigma (f_i/d_i^{2.63}) \text{ for } E.\ coli \text{ DNA}] \quad (9)$$

The gradient limits used for *E. coli* DNA excluded material near the top and bottom of the gradient that was not a part of the main peak (e.g. see Fig. 1 in Bonura *et al.*, 1975). The d_{T2} value for T2 DNA samples was obtained either from the position of the peak fraction only, or as the first moment ($\Sigma f_i d_i$) of the largest two fractions, if the peak position appeared to be located between two fractions.

For large molecular weight DNA samples, the M_n values were calculated directly from the gradient profiles. M_n values smaller than 50 × 10^6 were estimated by taking one-half the calculated M_w value.

It should be noted that the term "DNA single-strand break", as used in this paper, includes breaks resulting from the scission of alkali-labile bonds, and from DNA double-strand breaks, as well as true single-strand breaks.

RESULTS

Figure 1 shows the kinetics of the production of DNA single-strand breaks in *uvrB* cells after a UV fluence of 258 J m^{-2}. The cells were irradiated on ice, and then incubated on ice or at 37°C in DTM buffer. Samples taken immediately after irradiation already showed a significant amount of DNA strand breakage compared to the unirradiated control samples. A further rapid increase in the extent of strand breakage occurred during the first few minutes of incubation at 37°C. Little or no additional strand

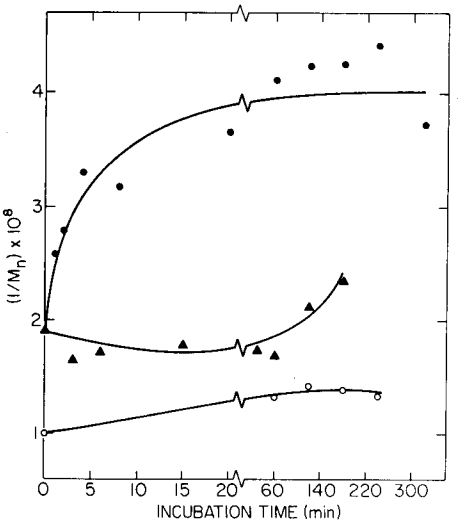

Figure 1. Kinetics of DNA single-strand break production in *E. coli* K-12 *uvrB* cells (DY178) after UV irradiation. The cells were irradiated in DTM buffer at ice temperature and then either held on ice (▲), or transferred to a 37°C bath (●) for the indicated period before lysis on alkaline sucrose gradients. The UV fluence was 258 J m^{-2}. Unirradiated controls were incubated at 37°C (○). Each point represents the average of three or more experimental values.

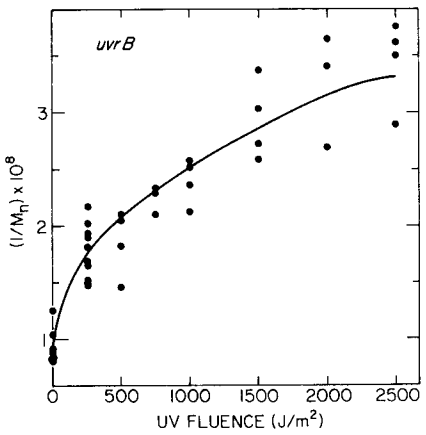

Figure 2. Photochemical production of DNA strand breaks by UV radiation. The cells (DY178 *uvrB*) were irradiated at 0°C as described in Fig. 1, and were lysed immediately after irradiation to avoid the temperature-dependent production of DNA strand breaks.

breakage occurred after 20 min of incubation; samples incubated for times ranging from 20 to 320 min showed similar extents of DNA strand breakage. Cells that were incubated on ice after irradiation did not show the rapid increase in DNA strand breakage observed during incubation at 37°C.

The extent of DNA strand breakage in cells irradiated at 0°C, and lysed immediately after UV irradiation, is shown in Fig. 2 as a function of the UV fluence. The slope of the fluence response curve decreases with increasing UV fluence. These data are suggestive of strand breakage resulting from photochemical events *per se*.

The results shown in Fig. 3 indicate the extent of DNA strand breakage observed with the *uvrA*, *uvrB*, Δ(*uvrB-chlA*), and *uvrA* Δ(*uvrB-chlA*) strains after incubation for 80 or 180 min in DTM buffer. The results obtained with each of these strains were quite similar (Table 2). The final extent of DNA strand breakage was not significantly changed by incubation at 42 or 46°C (Fig. 3E, Table 2), or by photoreactivation (Fig. 3F, Table 2). The high temperature experiments were done as controls for the experiments with the *uvrB lig* strain described below.

The final extent of DNA strand breakage was also determined for the *uvrB recA*, *uvrB recB*, *uvrB uvrD*, and *uvrB lexA*101 strains at different UV fluences (Table 2). The yield of breaks observed with these strains was slightly greater than found for the *uvrA* and *uvrB* strains.

The *uvrB* and *uvrB recF* strains (derived from strain AB1157) showed break yields that were lower than for any other strain examined (Table 2). This may be related to the greater radioresistance of strains derived from AB1157 rather than W3110 (e.g. compare survival data in Kapp and Smith, 1970, and Youngs and Smith, 1973a).

Figure 4 shows the extent of DNA strand breakage in *uvrB lig* cells incubated in DTM buffer at various temperatures for 30 min before and 180 min after UV irradiation. Since the *ligts*-7 mutation results in the production of a thermolabile polynucleotide ligase molecule (Gottesman *et al.*, 1973; Konrad *et al.*, 1973), the amount of ligase activity remaining in the cells should decrease as the incubation temperature is increased. This should result in decreased repair, and, therefore, increased DNA strand breakage at the higher temperatures. The results shown in Fig. 4 indicate that the extent of DNA strand breakage in irradiated cells increased with respect to incubation temperature in the range of 30°–52°C, as predicted. However, DNA strand breaks also began to accumulate in unirradiated cells at temperatures above 40°C. Thus, only in the range of 30°–42°C was there an actual increase in the extent of UV-induced DNA

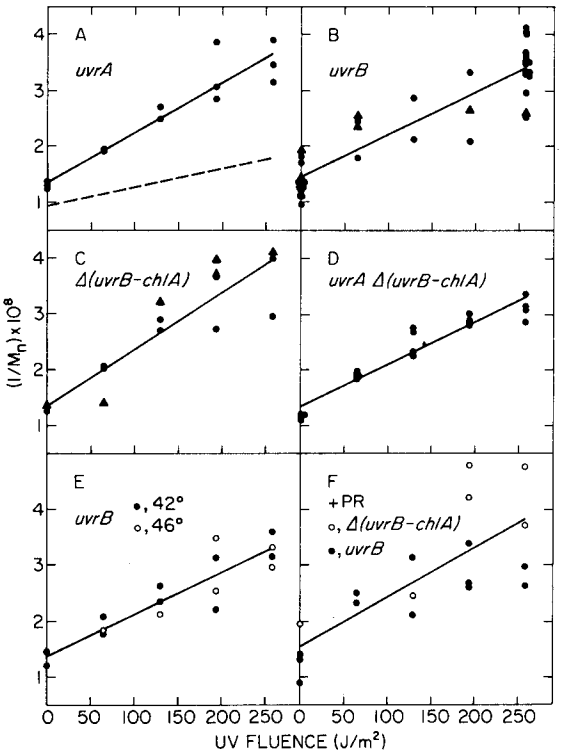

Figure 3. The yield of unrepairable DNA single-strand breaks after UV irradiation of *uvrA* and *uvrB* strains of *E. coli* K-12. The cells were irradiated in DTM buffer at room temperature, and then incubated at 37°C for 80 min before lysis. The procedures for photoreactivation are given in the text. For the 42° and 46°C experiments (Fig. 3E), the cells were incubated for 30 min at the indicated temperature, irradiated at ice temperature, and then incubated at the indicated temperature for either 80 or 180 min. (Early experiments used 180 min; later experiments used 80 min since the same results were obtained. See Fig. 1.). The triangular symbols in Fig. 3B and 3C indicate that the cells were irradiated in DTM buffer containing 1 *M* glycerol. The strains used were *uvrA* (JG137), *uvrB* DY178), Δ(*uvrB-chlA*) (DY168), and *uvrA* Δ(*uvrB-chlA*) (DY233). The lines shown were fitted to the data by linear regression analysis. The dashed line in Fig. 3A indicates the extent of DNA strand breakage in samples taken immediately after irradiation at 0°C, using the 0 and 250 J m^{-2} data points from Fig. 2.

Table 2. DNA strand breakage in *E. coli* K-12 cells after UV irradiation

Strain	DNA single-strand breaks remaining after repair [(half genome)$^{-1}$ J^{-1} m^2]*
uvrA	0.124 (0.013)†
uvrB	0.106 (0.008)†
Δ(*uvrB-chlA*)	0.142 (0.018)†
uvrA Δ(*uvrB-chlA*)	0.105 (0.007)†
uvrB (42°C, 46°C)	0.104 (0.013)†
uvrB, Δ(*uvrB-chlA*) + PR	0.122 (0.026)†
uvrB recA	0.155 (0.017)‡
uvrB recB	0.152 (0.022)‡
uvrB uvrD	0.134 (0.018)‡
*uvrB lexA*101	0.129 (0.012)‡
uvrB (JC8909)	0.068 (0.010)‡
uvrB recF	0.076 (0.014)‡
uvrA polA	0.284 (0.033)§
uvrA polA + PR	0.261 (0.048)§
uvrB lig (42°C)	0.399 (0.035)§
wild-type	0.655 (0.026)¶
wild-type + PR	0.216 (0.021)¶

*The single-strand break yields were calculated from the slopes of the lines in Figs. 3–6, as indicated. The values represent the extent of DNA strand breakage after incubation for 80 min or longer in DTM buffer to allow temperature-dependent events to occur. The standard deviation of each value is shown in parentheses. †Data from Fig. 3. ‡Data not shown. The cells were irradiated in DTM buffer at room temperature, and then incubated at 37°C for 80 min before lysis. The fluences used were the same as in Fig. 3. The values listed above were obtained by linear regression analysis of either two or three data points at each fluence. §Data from Fig. 5. ¶Data from Fig. 6.

strand breakage due to the temperature-dependent inactivation of the thermolabile polynucleotide ligase. The maximum extent of UV-induced DNA strand breakage in the *uvrB lig* strain occurred after incubation at temperatures in the range of 38°–42°C. A temperature of 42°C was chosen for further experimentation.

Figure 5 shows the extent of DNA strand breakage as a function of UV fluence for the *uvrA polA* strain, and the *uvrB lig* strain after incubation at 37° and 42°C, respectively. The slope values for the lines fitted to the data are given in Table 2. The results for the related *uvrA* and *uvrB* strains (from Fig. 3A and E, respectively) are shown as dashed lines for comparison. The slopes of the regressed lines for the *uvrA polA* and *uvrB lig* strains are significantly greater than observed for the *uvrA* and *uvrB* strains. In addition, the *uvrB lig* strain showed more extensive DNA strand breakage than the *uvrA polA* strain. Exposure to PR light did not significantly alter the final yield of DNA strand breaks in the *uvrA polA* strain.

As a control for the PR experiments shown in Fig. 3F and 5B, the extent of unrepaired DNA strand breakage was also measured for a closely-related wild-type strain, with and without exposure to PR

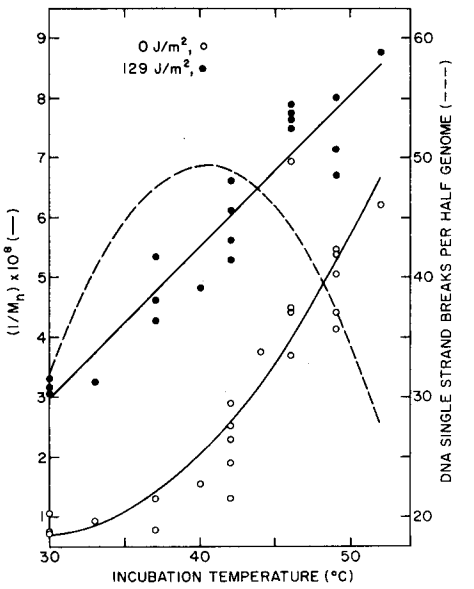

Figure 4. Inactivation of DNA strand break repair in *E. coli* K-12 *uvrB lig* (DY195) cells at different temperatures. The cells were incubated 30 min at the indicated temperature, irradiated at ice temperature, and then incubated an additional 180 min at the temperature indicated. Symbols are: ○, unirradiated controls; ●, samples irradiated with a UV fluence of 129 J m^{-2}. The line fitted to the data points from irradiated samples was derived by linear regression analysis, and has the formula $10^8/M_n = 0.254\ F - 4.64$, where F is the UV fluence. The data from unirradiated samples were fitted by polynomial regression analysis, and the line shown has the formula $10^8/M_n = 0.115\ F^2 - 0.675\ F + 10.6$. The dashed line showing the number of single-strand breaks was obtained by substituting these two formulas into the equation for DNA single-strand breakage; $(1.4 \times 10^9)(10^8/M_{nx} - 10^8/M_{no})$, where M_{nx} and M_{no} indicate the molecular weights of irradiated and control samples, respectively.

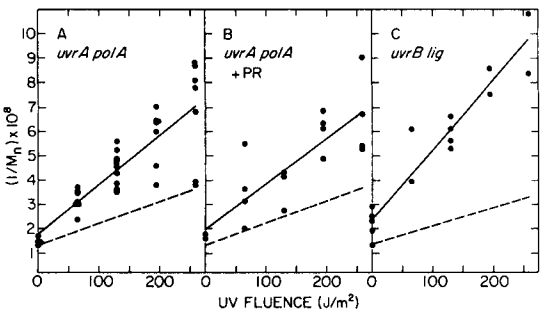

Figure 5. DNA strand breakage in the *uvrA polA* (JG136) and *uvrB lig* (DY195) strains of *E. coli* K-12 after UV irradiation. The *uvrA polA* cells were irradiated at room temperature with or without PR, as indicated in the text, and then incubated for 80 or 180 min at 37°C. The *uvrB lig* cells were incubated at 42°C for 30 min, irradiated at ice temperature, and then incubated for 180 min at 42°C. The lines shown were fitted to the data by linear regression analysis. For panels A and B, the dashed line is identical to that shown in Fig. 3A for *uvrA* cells. For panel C, the dashed line is identical to that shown in Fig. 3E for *uvrB* cells.

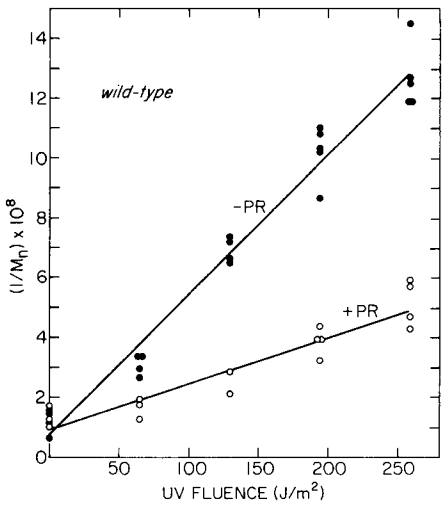

Figure 6. The yield of unrepairable DNA single-strand breaks in the DNA of *E. coli* K-12 wild-type cells (DY98). The cells were irradiated in DTM buffer at room temperature at a low UV fluence rate, with or without simultaneous exposure to PR light, as described in the text. After irradiation, the cells were incubated for 80 min at 37°C before lysis on alkaline sucrose gradients. The lines shown were fitted to the data by linear regression analysis.

light (Fig. 6). The final extent of DNA strand breakage was 5- to 6-fold greater for wild-type cells than for *uvrA* or *uvrB* cells (Table 2), and was in agreement with previous results (Youngs *et al.*, 1975). Exposure to PR light decreased the number of DNA strand breaks present in wild-type cells after incubation, in contrast to the lack of any PR effect in *uvrA* or *uvrB* cells (Figs. 3 and 5). However, even after PR treatment, approximately twice as many strand breaks remained unrepaired in wild-type cells as in the *uvrA* or *uvrB* cells (Table 2).

The extent of UV-induced DNA degradation was examined for each of the strains over the UV fluence range used for the gradient experiments. A significant amount of breakdown of DNA to acid-soluble material occurred during incubation after these large UV fluences (Table 3). Two exceptions, the *uvrB recF* and the related *uvrB* strains, showed very little DNA degradation. These strains were derived from the relatively resistant AB1157 strain.

DISCUSSION

Our results suggest that DNA single-strand breaks are produced in *uvrA* and *uvrB* cells by direct photochemical action, and by temperature-dependent processes after UV irradiation (Figs. 1 and 2). Direct photochemical breakage of DNA by 254 nm UV light has previously been reported for DNA irradiated *in vitro* (e.g. Moroson and Alexander, 1961; Marmur *et al.*, 1961). Hutchinson and Hales (1970) observed no DNA strand breakage in DNA irradiated *in vitro*, but their procedure precluded the detection of DNA strand breaks if the yield was less than 0.2 breaks per *E. coli* half genome per J m^{-2}. Setlow (1972) observed DNA strand breakage *in vivo* in *E. coli* B_{s-1} cells immediately after UV irradiation (265 nm), which may have resulted from either direct photochemical processes or enzymatic processes; results were shown for 200 and 2000 J m^{-2} and the calculated yield of strand breaks was 0.025 per half genome per

Table 3. DNA degradation in Uvr^- strains of *E. coli* K-12 after UV irradiation

Strain	% of radioactivity remaining TCA insoluble after indicated UV fluence*			
	64 J m^{-2}	129 J m^{-2}	194 J m^{-2}	258 J m^{-2}
uvrA (JG137)	87	80	76	68
uvrB (DY178), 30°C	91	87	91	76
uvrB (DY178), 42°C	94	88	86	82
Δ(*uvrB-chlA*) (DY168)	90	88	88	84
uvrA Δ(*uvrB-chlA*) (DY233)	94	99	96	91
uvrB recA (DY155)	92	93	90	89
uvrB recB (DY157)	97	92	90	88
uvrB uvrD (DY179)	80	75	77	72
*uvrB lexA*101 (DY180)	91	92	88	85
uvrB (JC8909)	96	96	96	98
uvrB recF (JC 8910)	104	98	103	101
uvrA polA (JG136)	85	77	72	63
uvrB lig (DY195), 30°C	92	88	85	80
uvrB lig (DY195), 42°C	90	84	76	76

*Samples were irradiated at room temperature and then incubated 80 min at 37°C in DTM buffer. The *uvrB* (DY178) and *uvrB lig* (DY195) strains were incubated at 30° or 42°C for 30 min prior to irradiation (at 0°C) and samples were subsequently incubated for 80 min at 30° or 42°C. In each experiment, triplicate samples were then placed on filter paper discs which had been soaked in 10% trichloroacetic acid (TCA) and dried. The samples were processed as previously indicated (Youngs *et al.*, 1974). The percentage of TCA-insoluble radioactivity is given, with the value for unirradiated cells incubated under identical conditions taken as 100%. The values above are averages of data from two experiments.

J m^{-2}. Our results indicate that the yield of photochemically-induced strand breaks *in vivo* is ~0.05 per half genome per J m^{-2}, as calculated from the mean of the data points in Fig. 2 for a UV fluence of 250 J m^{-2}. At higher UV fluences, the yield of breaks decreased (Fig. 2). This could be due to DNA–DNA or DNA–protein cross-linking, which would result in a compensatory increase in the DNA molecular weight. In addition, the yield of breaks would also decrease as the number of sites remaining susceptible to breakage approaches zero.

DNA single-strand breaks also appear to be produced by a temperature-dependent process in *uvrA* and *uvrB* cells after UV irradiation (Fig. 1). Our results are in contrast to several earlier reports, which indicated that no detectable DNA strand breakage occurred in *uvrA* and *uvrB* strains after UV irradiation (Kato, 1972; Achey and Billen, 1969; Seeberg and Johansen, 1973). We attribute these differences in results to the generally much lower UV fluences used previously, and also to the problem of a centrifugation speed effect (e.g. Hutchinson, 1975) that may have masked low levels of DNA strand breakage in the earlier experiments.

The *uvrA*, *uvrB*, Δ(*uvrB-chlA*), and *uvrA* Δ(*uvrB-chlA*) strains all showed about the same final extent of DNA strand breakage (Table 2). Thus, it seems reasonable to conclude that the temperature-dependent DNA strand breaks are either produced by some nuclease other than the *uvrA-uvrB* enzyme or they result from non-enzymatic breakdown of a thermolabile photoproduct. The results of Braun and Grossman (1974) and Radman (1976) indicate that *E. coli* cells contain one or more UV endonucleases, in addition to the *uvrA-uvrB* enzyme, which could produce such breaks.

We tested the hypothesis that the strand breaks that are introduced into the DNA of UV-irradiated *uvrA* and *uvrB* cells are susceptible to repair processes similar to those known to act on other types of strand breaks (e.g. Youngs *et al.*, 1974) by determining the final extent of DNA strand breakage in radiation-sensitive mutant strains known to be deficient in repair processes. A strain that is deficient in the repair of these breaks would be expected to show increased DNA strand breakage after incubation, since the final extent of DNA strand breakage reflects the end result of initial photochemical breakage, temperature-dependent breakage, and repair.

The *recA*, *recB*, *uvrD*, *lexA*101, and *recF* mutations did not appear to severely interfere with the repair of DNA strand breaks in *uvrB* cells, since the additional presence of any one of these mutations had only a small effect on the final yield of breaks (Table 2). In contrast, a markedly-increased number of unrepaired DNA strand breaks was observed with cells deficient in either DNA polymerase I or polynucleotide ligase (Table 2). Thus, both DNA polymerase I and polynucleotide ligase appear to be involved in the repair of the temperature-dependent DNA strand breaks that are induced in UV-irradiated *uvrA* and *uvrB* cells.

Since the yield of photochemically-induced breaks is much smaller than the final yield of breaks (Fig. 3A), we can only interpret the results as indicative of repair of the temperature-dependent breaks. The photochemical breaks may also be repairable, but our data allow no conclusion in this regard.

Several considerations suggest that the temperature-dependent DNA single-strand breaks we observe with *uvrA* and *uvrB* cells arise from radiation products other than pyrimidine dimers. Earlier work has shown that pyrimidine dimers are not excised from the DNA of UV-irradiated *uvrA* and *uvrB* cells (Howard-Flanders *et al.*, 1966; Boyce and Howard-Flanders, 1964). In addition, the yield of DNA single-strand breaks was not reduced by photoreactivation (Table 2), as would be expected if the breaks had resulted from incisions made near pyrimidine dimers (Setlow *et al.*, 1965).

As a control, the effect of photoreactivation on the yield of unrepaired strand breaks in wild-type cells was also examined (Fig. 6). In this case, photoreactivation markedly reduced the extent of DNA strand breakage, indicating that most of the unrepairable breaks remaining in wild-type cells do result from dimer-specific incision events (see also Bonura and Smith, 1975).

A reasonable interpretation of our data is that breaks are produced in DNA at or near UV-induced photoproducts other than cyclobutane-type pyrimidine dimers. This could occur through the action of an endonuclease other than the *uvrA-uvrB* enzyme or by alteration of a thermolabile radiation product. These photoproducts may be removed by excision processes, and the missing nucleotides replaced by repair resynthesis. There is evidence for excision repair processes that act on lesions other than pyrimidine dimers, e.g. Paterson *et al.* (1974) have observed such a repair process in UV-irradiated chick embryo cells, and Hariharan *et al.* (1975) have observed the excision repair of base damage produced by ionizing radiation in bacterial and mammalian cells.

An estimate of the total yield of non-dimer lesions that result in DNA strand breaks can be made from the yield of breaks observed with the *uvrB lig* strain, if three assumptions are valid: (i) polynucleotide ligase is completely inactivated under the conditions used, (ii) ligase activity is required for the repair of each break produced, and (iii) one break is produced for each susceptible lesion in the DNA. Since none of these assumptions is likely to be completely true, the number of unrepaired strand breaks present in *uvrB lig* cells, 0.40 per half genome per J m^{-2}, is a minimum estimate of the yield of non-dimer photoproducts that result in the production of DNA strand breaks. This value can be compared with the yield of pyrimidine dimers, ~32 per half genome per J m^{-2} (Rupp and Howard-Flanders, 1968), to indicate that the strand breaks observed in *uvrA* and *uvrB* cells

may result from lesions that are formed at a minimum relative frequency of 1 per 80 dimers.* The unidentified photochemical lesions would probably be of little importance in the killing of *uvrA* or *uvrB* cells that are unable to excise the more abundant pyrimidine dimers. However, non-dimer lesions could make a significant contribution to the killing of wild-type cells, which might excise pyrimidine dimers much more efficiently than other lesions.

In conclusion, the results indicate that DNA single-strand breaks are produced by both photochemical and temperature-dependent processes in *uvrA* and *uvrB* cells after UV irradiation. The latter breaks may be formed either enzymatically, or through the chemical alteration of thermolabile photoproducts, and are substrates for cellular repair processes involving DNA polymerase I and polynucleotide ligase. The damage leading to the temperature-dependent breaks is not photoreactivable, suggesting that these breaks result from UV photoproducts other than cyclobutane-type pyrimidine dimers. We hypothesize that the temperature-dependent breaks may result from an endonucleolytic incision event initiating the excision repair of non-photoreactivable photoproducts.

*These lesions do not appear to be pyrimidine hydrates since incubation at 42° or 46°C, a treatment that would cause an increased rate of reversal of the hydrates compared to incubation at 37°C, had no effect on the final extent of DNA strand breakage (Table 2).

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