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The Involvement of Polynucleotide Ligase in the Repair of UV-Induced DNA Damage in *Escherichia coli* K-12 Cells

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Summary. The effect of the *ligts-7* mutation on cell survival and the extent of DNA repair after UV (254 nm) irradiation was determined for wild-type and *uvrB5* cells of *E. coli* K-12 at 30° and 42° C. At the restrictive temperature (42° C) the *ligts-7* mutation resulted in (i) a decrease in the extent of repair of DNA incision breaks arising during the excision repair process, and (ii) a decrease in the extent of post-replicational repair of gaps in newly-synthesized DNA. These deficiencies in DNA repair correlated with increases in cellular sensitivity to killing by UV radiation. Thus, DNA ligase plays an important role in vivo in both the excision and post-replicational repair processes.

Introduction

The enzyme polynucleotide ligase catalyzes the covalent joining of juxtaposed 3′-OH and 5′-PO_4 groups at single-strand breaks in duplex DNA (Olivera and Lehman, 1967). Ligase activity has been considered essential for the final joining step in processes that repair single-strand breaks in DNA. Such breaks arise during normal DNA replication and DNA excision repair processes, and also as a result of direct injury to the DNA (e.g., after ionizing irradiation).

The isolation of a *ligts-7* mutant of *E. coli* 15 TAU deficient in DNA ligase was reported by Pauling and Hamm (1968). The mutant showed decreased DNA ligase activity and was unable to form colonies at 40° C. Konrad et al. (1973) and Gottesman et al. (1973) transferred the *ligts-7* mutation to *E. coli* K-12 and confirmed the observation that the *ligts-7* mutation results in a thermolabile DNA ligase activity and conditionally-lethal growth. These results establish DNA ligase as an enzyme essential for cell viability, and indicate that only one major DNA ligase activity is present in *E. coli* K-12 cells.

The *ligts-7* mutation has also been shown to increase cellular sensitivity to UV (Pauling and Hamm, 1968) and ionizing (Dean and Pauling, 1970) radiations, suggesting that DNA ligase is also required for rejoining steps in DNA repair processes. This argument is considerably strengthened by the observation that the UV sensitivity of the *ligts-7* strain is increased by incubation at the restrictive temperature (Pauling and Hamm, 1968; Gottesman et al., 1973), correlating with the temperature sensitivity of the DNA ligase activity. In addition, DNA ligase has been shown to participate in the in vitro repair of ionizing (Jacobs et al., 1972; Laipis and Ganesan, 1972; Noguti and Kada, 1972) and UV (Heijneker et al., 1971) radiation damage to DNA. Generally, repair processes utilize both DNA polymerase and DNA ligase to resynthesize and reseal damaged regions in DNA. These steps often follow endonucleolytic and exonucleolytic processes acting near damaged bases.

DNA ligase is able to directly reseal *uvrA-uvrB* endonuclease-induced incision breaks in DNA (Braun and Grossman, 1974; Seeberg and Strike, 1976), and controls the extent of DNA repair replication after UV (Pauling and Hamm, 1968) and ionizing irradiation (Billen and Hellerman, 1975; Billen et al., 1975). These functions of DNA ligase could act to limit exonucleolytic breakdown and thereby control the extent of lethal DNA double-strand breakage arising during attempted excision repair of damage in UV-irradiated cells (Bonura and Smith, 1975).

Thus, the available data suggest that DNA ligase is involved in DNA repair processes occurring after UV and ionizing irradiation. However, no direct demonstration of such involvement has been presented with an in vivo system. We have examined the survival and DNA repair capacity of *ligts-7* mutants of *E. coli* K-12. The results implicate DNA ligase in cell recovery and in the excision and post-replicational repair processes occurring after UV irradiation.

Materials and Methods

Bacterial Strains

The strains of *E. coli* K-12 used for the present experiments, and their source or derivation are given in Table 1. The transduction techniques have been described (Youngs and Smith, 1973).

Media and Growth Conditions

The media, labelling and growth conditions have been described (Youngs and Smith, 1976a, 1976b). The glucose minimal medium (MM) previously used was supplemented with Difco Casamino Acids (2 mg/ml) for the present experiments (MC medium). All strains were routinely grown at 30° C for DNA repair and cell survival experiments. Cell survival was determined by plating cells on YENB medium (Ganesan and Smith, 1970), solidified with 1.6% Difco Noble Agar (Van der Schueren et al., 1974). YENB agar was used since it gave a higher plating efficiency for unirradiated *ligts-7* cells than either MM or MC agar.

For experimental use, an overnight culture was diluted 50- or 100-fold into fresh MC medium and incubated for several hours at 30° C to a cell density of $\sim 2 \times 10^8$ cells/ml. The cells were collected on Millipore filters (0.45 μm pore size), and resuspended in DTM buffer (MC medium without organic components).

The cells were incubated as desired, and were then UV-irradiated (254 nm) in DTM buffer either at room temperature or on ice. The UV fluences given have been corrected for sample absorption (Youngs and Smith, 1976a), and represent average fluences. After irradiation, samples were incubated as indicated in the figure legends, and then either plated on YENB agar to measure cell survival, or lysed on alkaline sucrose gradients for DNA molecular weight determinations.

Alkaline Sucrose Gradient Techniques

The techniques for determining DNA molecular weights have been described (Youngs and Smith, 1976b). Centrifugation speeds were chosen so as to avoid speed dependent sedimentation artifacts. The DNA number average molecular weights were either calculated directly from the gradient profiles, or by taking one-half the calculated weight average molecular weights. The former method was used for samples with number average molecular weights $> 5 \times 10^7$, and for DNA samples from post-replicational repair experiments, where the distributions appeared to be non-random.

Table 1. List of strains

Designation	Genotype[a]	Source
DY98	F *metE thyA thyR lacZ str*	Youngs and Smith (1973)
DY145	F⁻ *uvrB5 metE leuB thyA thyR rha lacZ str*	Youngs and Smith (1976a)
DY173	F⁻ *ligts-7 metE leuB bio thyR rha lacZ malB str*	Youngs and Smith (1976b)
DY178	F *uvrB5 leuB thyA thyR rha lacZ str*	Youngs and Smith (1976a)
DY194	F *ligts-7 metE leuB thyR rha lacZ malB str*	P_1 · DY145 X DY173 (Select Bio⁺)
DY195	F *uvrB5 ligts-7 metE leuB thyR rha lacZ malB str*	Youngs and Smith (1976b)

[a] Symbols are those used by Bachman et al. (1976)

Results

The results of cell survival and DNA repair experiments with the wild-type and *ligts-7* strains are shown in Figures 1 and 2. In these experiments the cells

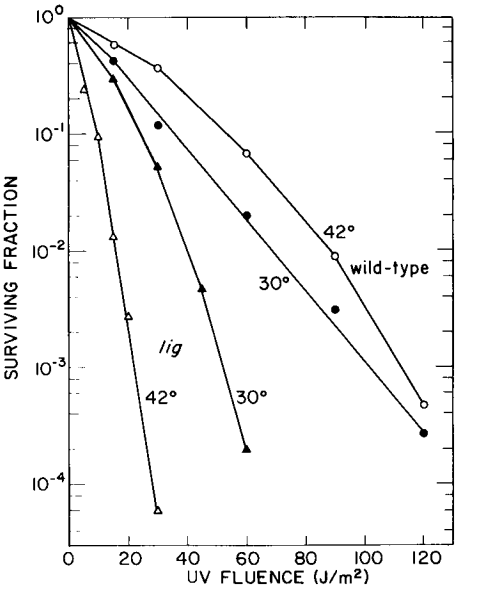

Fig. 1. Survival of wild-type (DY98) and *ligts-7* (DY173, DY194) cells of *E. coli* K-12 after UV (254 nm) irradiation. The cells were incubated in DTM buffer at 30° or 42° C for 30 min prior to, and 120 min after UV irradiation. The cells were irradiated in DTM buffer on ice. Colony-forming ability was determined on YENB agar at 30° C. Symbols are: wild-type 30° C (●), 42° C (○); *ligts-7* 30° C (▲), 42° C (△). Each point represents the average of three or more experiments

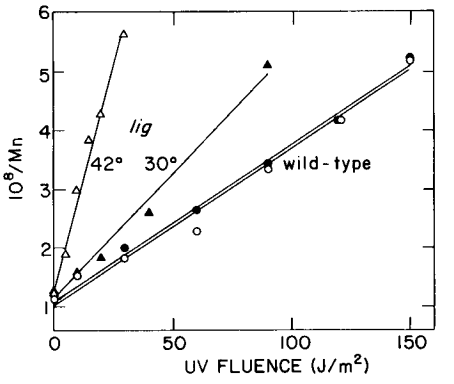

Fig. 2. Repair of UV-induced incision breaks in the DNA of wild-type (DY98) and *ligts-7* (DY173, DY194) strains of *E. coli* K-12. Procedures are as in the legend to Figure 1 except that the cells were prelabelled with ^{3}H-thymine, and the post-irradiation incubation was for 40 min in DTM buffer. The number average molecular weights (M_n) were determined using alkaline sucrose gradient techniques. Symbols are: wild-type 30° C (●), 42° C (○); *ligts-7* 30° C (▲), 42° C (△). The lines were fitted to the data by linear regression analysis, and have slopes (±standard deviation) of 0.026±0.001, 0.026±0.002, 0.042±0.003, and 0.15±0.01, respectively. Each point represents the average of two or more experiments

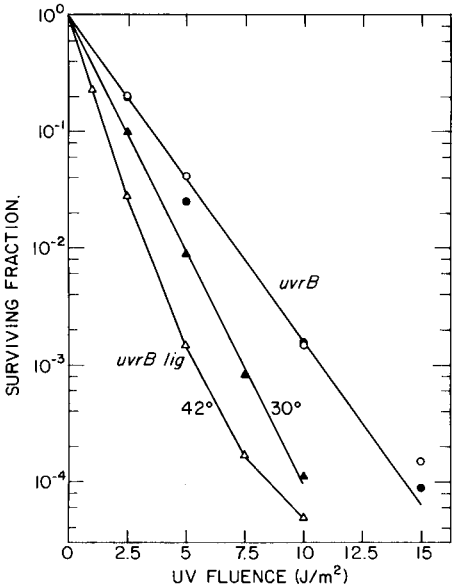

Fig. 3. Survival of *uvrB5* (DY178) and *uvrB5 ligts-7* (DY195) strains of *E. coli* K-12 after UV (254 nm) irradiation. The cells were irradiated in DTM buffer at room temperature, incubated for 30 min in MC medium at 30° C, and then for 120 min in DTM buffer at either 30° or 42° C. Symbols are: *uvrB5* 30° C (●), 42° C (○); *uvrB5 ligts-7* 30° C (▲), 42° C (△). Each point represents the average of three experiments

were incubated for 30 min at either 30° or 42° C, UV irradiated at ice temperature, and then incubated for an additional period at 30° or 42° C.

In the survival experiments shown in Figure 1, a post-irradiation incubation period of 120 min was allowed before the cells were plated on YENB agar to measure colony-forming ability. Similar results were also obtained with a post-irradiation incubation period of 40 min (data not shown). The wild-type cells were more resistant to UV radiation after treatment at 42° than at 30° C. The *ligts-7* strain was more sensitive than the wild-type strain at 30° C, and was further sensitized by treatment at 42° C.

In the DNA repair experiments shown in Figure 2, the extent of strand breakage in parental DNA was determined after a 40 min post-irradiation incubation period, using alkaline sucrose gradient techniques. The results generally correlate with the survival data described above. The *ligts-7* strains showed greater DNA strand breakage (the $1/M_n$ values are proportional to the number of DNA strand breaks) at 42° than at 30° C, and more breakage at either temperature than the wild-type strain. The same extent of DNA strand breakage was observed in samples of the wild-type cells, whether they were incubated at 42° or 30° C.

We also examined the involvement of DNA ligase in post-replicational repair processes. The survival results comparing the *uvrB5* and *uvrB5 ligts-7* strains

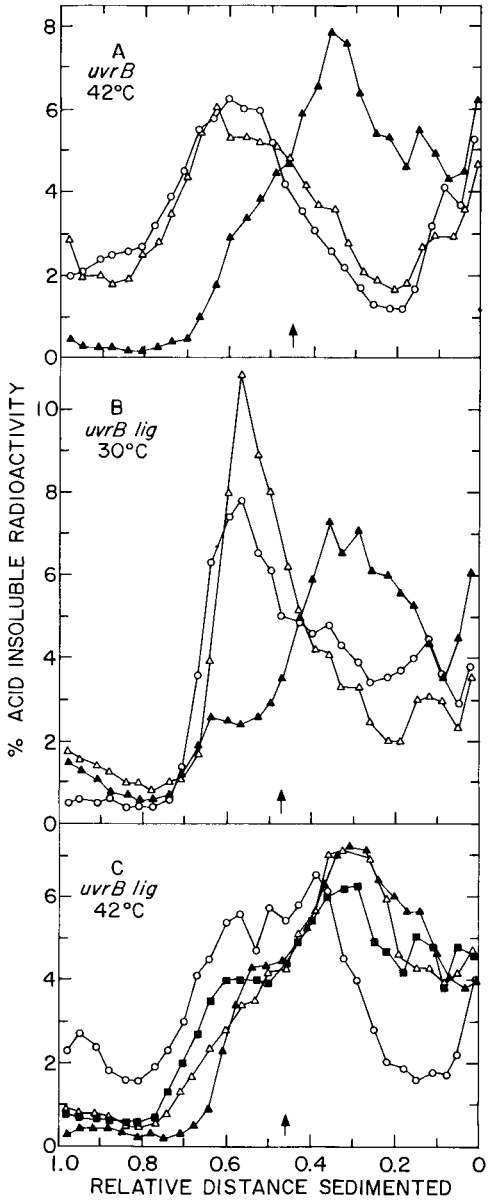

Fig. 4. Repair of gaps in newly-synthesized DNA after UV (254 nm) irradiation in *uvrB5* (DY178) and *uvrB5 ligts-7* (DY195) cells of *E. coli* K-12. The cells were irradiated at room temperature in DTM buffer and then pulse labelled with ^{3}H-thymine in MC medium at 30° C for 10 min. Subsequently, the cells were incubated in DTM buffer at 30° or 42° C. The arrows indicate the distance sedimented by T2 DNA. Symbols are: A. Unirradiated cells incubated for 120 min (○); irradiated (3.0 J/m^2) cells incubated for 0 min (▲) or 120 min (△). B. Unirradiated cells incubated for 0 min (○); irradiated (3.0 J/m^2) cells incubated for 0 min (▲) or 120 min (△). C. Unirradiated cells incubated for 120 min (○); irradiated (3.0 J/m^2) cells incubated for 0 min (▲), 60 min (■), or 120 min (△)

are shown in Figure 3. The cells were incubated for 30 min at 30° C in MC medium after UV irradiation to allow DNA replication past UV-induced base damage to occur. The cells were then filtered, resuspended in DTM buffer, and incubated for 120 min at

either 30° or 42° C before plating to measure cell survival. We found that unirradiated *uvrB5 ligts-7* cells lose colony-forming ability when incubated in growth medium at 42° C (the surviving fraction was $\sim 10^{-2}$ after 120 min). For this reason all incubations at 42° C were performed with the cells suspended in DTM buffer. The data shown in Figure 3 indicate that the *uvrB5* cells had the same UV sensitivity whether the samples were incubated at 42° or 30° C. The *uvrB5 ligts-7* cells were more sensitive than the *uvrB5* cells at 30° C, and were further sensitized by the 42° C treatment.

Figure 4 shows the results obtained with alkaline sucrose gradients in experiments designed to measure the extent of post-replicational repair. The conditions were similar to those used for the survival experiments shown in Figure 3. The only difference was that the post-irradiation incubation in MC medium at 30° C was for 10 min, and the cells were pulse-labelled with [methyl-^{3}H]-thymine during this period. The data indicate that complete repair of gaps in the DNA synthesized during the pulse-labelling period occurred in *uvrB5* cells at 30° (data not shown) or 42° C (Fig. 4A), and in the *uvrB5 ligts-7* cells at 30° C (Fig. 4B). However, the *uvrB5 ligts-7* cells showed little repair of such gaps when incubated at 42° C after the pulse-labelling period (Fig. 4C).

Discussion

Our results clearly implicate DNA ligase in cell recovery and DNA repair processes occurring in vivo after UV irradiation. At the restrictive temperature (42° C), the *ligts-7* mutation sensitized wild-type *E. coli* K-12 cells to the lethal effects of UV radiation (Fig. 1). These data are consistent with the survival results of Pauling and Hamm (1968) and Gottesman et al. (1973). This effect on cell survival correlated with the inhibition of DNA repair by the *ligts-7* mutation. Incubation of *ligts-7* cells at the restrictive temperature markedly decreased the extent of repair of single-strand breaks (Fig. 2), which are produced in DNA as the first step of excision repair processes (e.g., Grossman et al., 1975). Therefore, these data indicate that DNA ligase is required for the completion of excision repair processes.

The *ligts-7* mutation also sensitized *uvrB5* cells to killing by UV radiation (Fig. 3). Since *uvrB5* cells lack excision repair, these data suggest ligase involvement in the post-replicational mode of repair. The DNA repair data (Fig. 4) show that the *uvrB5 ligts-7* strain was deficient in the repair of gaps in newly-synthesized DNA at the restrictive temperature. Thus, these data support the hypothesis that DNA ligase is required for the completion of post-replicational repair processes.

The survival experiments with wild-type cells (Fig. 1) showed higher cell survival after post-irradiation incubation at 42° than 30° C. This effect appears to be dependent on the presence of excision repair processes, since *uvrB5* cells showed identical UV sensitivities whether incubated at 30° or 42° C (Fig. 3). A possible explanation is that the rate of normal replicative DNA synthesis is decreased in wild-type cells at 42° C relative to 30° C (e.g., Wechsler et al., 1973). This would allow more time for excision repair to take place before DNA replication past dimers could occur.

The survival data in Figures 1 and 3, indicate that cells containing the *ligts-7* mutation show enhanced UV sensitivity even at the permissive temperature (30° C). This is in agreement with earlier results, both for UV (Pauling and Hamm, 1968; Gottesman et al., 1973; Konrad et al., 1973) and ionizing (Dean and Pauling, 1970) radiations. The *ligts-7* cells were also somewhat deficient in the repair of UV-induced incision breaks at 30° C (Fig. 2). These results correlate with the decreased level of DNA ligase activity present in extracts of *ligts-7* cells at 30° C, as compared to wild-type cells (Gottesman et al., 1973; Konrad et al., 1973).

The yield of unrepaired breaks in the DNA of *ligts-7* cells at 42° C was 0.15 per 10^8 daltons per J/m^2 (Fig. 2). This is much lower than the initial yield of pyrimidine dimers in the DNA, ~ 2.3 per 10^8 daltons per J/m^2 (Rupp and Howard-Flanders, 1968), There appear to be at least two explanations for this difference: (i) the ligase activity in *ligts-7* cells is not completely absent at 42° C. This is indicated by kinetic strand break repair data in the *uvrB5 ligts-7* (Fig. 4C) and *ligts-7* (unpublished results) strains, and also by the data of Gottesman et al. (1973) showing slow resealing of Okazaki fragments in *ligts-7* cells at 42° C. (ii) The ATP requirement of the *uvrA,B* endonuclease (Waldstein et al., 1974; Deutsch et al., 1976; Seeberg and Strike, 1976) could limit the extent of endonucleolytic breakage, since the *ligts-7* cells are incubated in buffer after irradiation rather than in complete growth medium. In fact, the excision of pyrimidine dimers from the DNA of UV-irradiated cells occurs at a greatly reduced rate when incubation is in growth medium lacking glucose (Setlow and Carrier, 1964) or in buffer (unpublished results) rather than in complete growth medium.

Although the extent of DNA strand breakage in *ligts-7* cells at 42° C (Fig. 2) was much less than the initial yield of dimers, it appears that most of these breaks do result from *uvrA,B*-dependent incision events. This is indicated by the fact that the yield

of breaks in *ligts-7* cells at 42° C, 0.15 per 10^8 daltons per J/m^2, is several-fold greater than that found with *uvrB5 ligts-7* cells at 42° C, 0.029 per 10^8 daltons per J/m^2 (Youngs and Smith, 1976b).

In summary, our results support the often made assumption that DNA ligase is required as the final step in the repair of UV-damaged DNA in vivo by the excision and post-replicational repair processes. It is also probable that DNA ligase is required for other repair processes that affect cell survival after UV irradiation. Two possibilities are: (i) excision repair of DNA damage other than pyrimidine dimers (Youngs and Smith, 1976b), and (ii) the repair of strand breaks produced in parental DNA during post-replicational repair. Thus, it appears that DNA ligase commands a key position in cellular DNA repair processes as well as in normal DNA replication.

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