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PYRIMIDINE DIMER EXCISION IN EXONUCLEASE DEFICIENT MUTANTS OF *ESCHERICHIA COLI*[1]

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DISCLAIMER

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ABSTRACT The rate of pyrimidine dimer excision has been measured in *Escherichia coli* strains deficient in exonuclease V, exonuclease VII, and the 5'→3' exonuclease of DNA polymerase I. The results suggest that a reduced level of the 5'→3' exonuclease of DNA polymerase I diminishes the rate of dimer excision and that an additional deficiency in exonuclease VII causes a significantly greater reduction in the cell's ability to remove dimers.

INTRODUCTION

Three 5'→3' exonucleases are currently known in *Escherichia coli* which may be capable of removing pyrimidine dimers in the excision repair process since they have been shown to excise pyrimidine dimers *in vitro* (1,2,3). These are the 5'→3' exonuclease activities associated with DNA polymerases I and III and exonuclease VII. We have extended our previous studies (4) on the exonucleases of *E. coli* involved in excision repair by examining the effect of combined mutations in *recB,C*, *xseA*, and *polAex* (deficient in exonuclease V, exonuclease VII, and the 5'→3' exonuclease activity of DNA polymerase I, respectively) (5,6,7) on UV survival and on the rate and extent of dimer excision. Our earlier study (4) demonstrated that mutants deficient in exonuclease VII and the 5'→3' exonuclease activity of DNA polymerase I are nearly as efficient as wild-type strains at excising dimers produced by up to 40 J/m^2 UV. At higher doses strains containing only the *polA546ex* mutation and the *polA546ex xseA7* mutations show reduced ability to excise dimers; however, the interpretation of dimer excision data at these doses is complicated by extreme postirradiation DNA degradation in these strains. We

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have attempted to reduce postirradiation DNA degradation by reducing the level of exonuclease V (the *recB,C* gene product) in order to evaluate the apparent deficiency in dimer excision at high UV doses observed in the *polA546ex* single mutant and the *polA546ex xseA7* double mutant strains. Although there is evidence against the direct involvement of exonuclease V in the excision repair process (8), the enzyme might play a more important role in dimer excision when other exonucleases are missing. This fact should be borne in mind in the interpretation of the data presented here.

RESULTS

We were unable to construct the *recB21* derivative of strains containing the *polA546ex* mutation suggesting that this may be an inviable combination. Derivatives of these strains were therefore constructed containing the *recB270 recC271* temperature-sensitive mutations (5). As expected, these strains were found to be conditionally lethal. Strains containing only the *recB270 recC271 xseA7* mutations show slightly reduced survival at temperatures restrictive to derivatives containing *polA546ex*.

DNA degradation studies performed after low doses of UV reveal no significant differences between the wild-type and mutant strains after doses of 20 J/m^2. After higher UV doses (90 J/m^2) which caused 50-80% DNA degradation after 60 min of 43°C incubation in *polA546ex* and *polA546ex xseA7* strains, the *recB270 recC271* derivatives of these strains show only about 20% DNA degradation. DNA degradation in the *recB270 recC271 xseA7* strain was indistinguishable from wild-type at all UV doses. It is therefore possible to reduce postirradiation DNA degradation in these *polA546ex* derivatives by reducing the levels of exonuclease V.

UV survival studies show that the *recB270 recC271* derivatives of strains containing the *polA546ex* and *polA546ex xseA7* mutations are considerably more sensitive to UV irradiation than any of the strains from which they were constructed. The strain containing the mutations *recB270 recC271 xseA7* is only slightly more UV sensitive than strains containing the *recB270 recC271* mutation alone (data not shown).

Thymine dimer excision studies were performed on KLC124 (wild-type), KLC333 (*recB270 recC271 polA546ex*), and KLC341 (*recB270 recC271 polA546ex xseA7*). Cultures labeled with [^{3}H]-thymine were irradiated, warmed to 43°C, and samples were examined for thymine-containing dimers as a function of incubation time (Fig. 1). Our results thus far show that KLC333 (*recB270 recC271 polA546ex*) is slightly reduced in its ability to remove thymine dimers compared to the wild-type. This may mean that the apparent deficiency in dimer excision in the

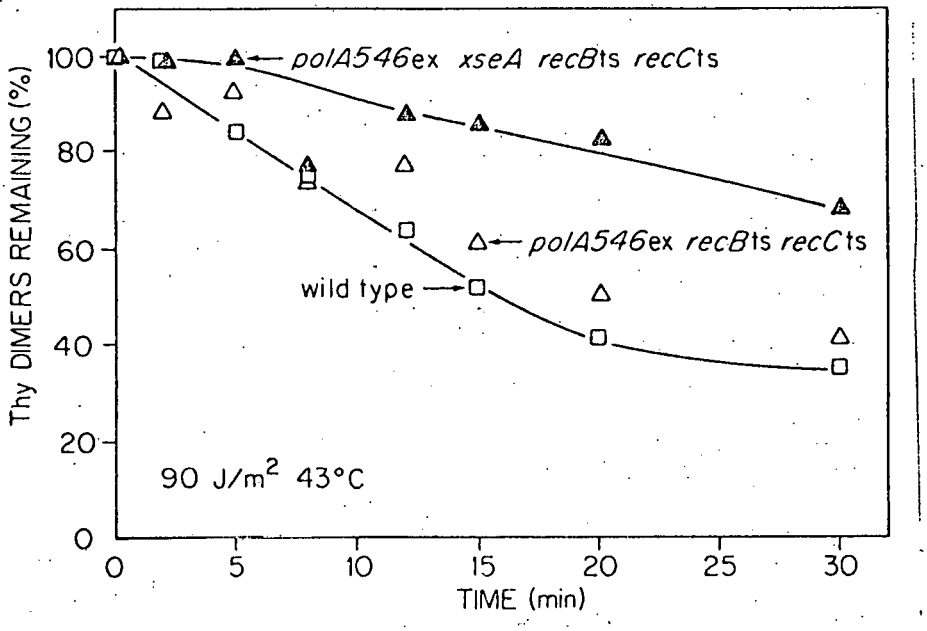

FIGURE 1. *Rate of Dimer Excision in Exonuclease Deficient Mutants.* Cultures of *E. coli* strains KLC124: *trpA33 rha thyA;* KLC333: *polA546ex recB270 recC271 trpA33 thyA;* and KLC341: *polA546ex recB270 recC271 xseA7 trpA33 thyA* were grown at 32°C to mid-log phase in M9 medium supplemented with required nutrients and ^{3}H-thymidine. The cells were chilled, harvested, and washed with M9 salts (without glucose or other additives) before irradiation with 90 J/m^2 UV light. The cells were warmed to 43°C for 5 min, nutrients were added (at t = 0) and samples were withdrawn at the indicated times. The cells were immediately chilled and ice cold NET buffer (0.1 M Tris HCl, pH 8, 0.01 M EDTA, 0.1 M NaCl) was added. The samples were collected by centrifugation, frozen, thawed and lysed by incubation with 400 µg/ml lysozyme for 30 min at 43°C and 0.2% sarkosyl for 10 min at 43°C. After 30 min of digestion with 250 µg/ml protease K at 50°C, the samples were chilled, TCA was added and the acid insoluble fraction digested with formic acid. Content of thymine dimers was determined by two-dimensional paper chromatography (9). At zero time 0.29% of the thymine was found as dimers. □ = KLC124; Δ = KLC333; ▲ KLC341.

polA546ex single mutant shown in our previous study (4) was largely due to postirradiation DNA degradation. Strain KLC341, however, is significantly deficient in dimer excision compared to wild-type and KLC333, suggesting that the additional deficiency in exonuclease VII in KLC341 is responsible

for a major reduction in the ability of this strain to excise pyrimidine dimers. A more exhaustive study (to be published) of the extent of dimer excision in this series of mutants over the dose range 0 to 160 J/m^2 confirmed our finding that although the *recB270 recC271 polAex* strain shows some deficiency in dimer excision, the additional mutation *xseA* causes a significant further reduction in the cell's ability to cope with dimers.

DISCUSSION

We have investigated in some detail the role of two 5'→3' exonuclease activities (exonuclease VII and 5'→3' exonuclease of DNA polymerase I) thought to be involved in excision repair. Compared to wild-type strains, mutants deficient in only one of these activities show either no deficiency or slight deficiency in removing pyrimidine dimers when postirradiation DNA degradation is minimal; strains deficient in both activities show significantly less ability to excise pyrimidine dimers. These results are consistent with the interpretation that both of these enzymes function in the excision process and are able to substitute for one another. Although excision is reduced in strains deficient in both exonuclease VII and the 5'→3' exonuclease of DNA polymerase I, it still occurs at a measurable rate. This may be due to residual enzyme levels of either or both of these activities or to other nucleases capable of dimer excision. The only other exonuclease activity of *E. coli* known to be capable of pyrimidine dimer excision *in vitro*, the 5'→3' exonuclease of DNA polymerase III, has not yet been evaluated *in vivo*. The possibility also exists that as yet unidentified nucleases participate in the excision step.

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