ULTRAVIOLET LIGHT-INDUCED RECOMBINATION

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Received October 8,1973

Stimulation of transduction in Escherichia coli by ultraviolet irradiation of the transducing phage P1 requires the uvrA-uvrB nuclease but not the uvrC product or DNA polymerase I. It is hypothesized that the first step in "normal" recombination can be bypassed by any procedure generating single-stranded ends of DNA (as, for example, by uvrA-uvrB nuclease activity).

Stimulation of recombination by ultraviolet (UV) irradiation was discovered by Jacob and Wollman[1] using the phage λ. Subsequently recombination in both prokaryotic and eukaryotic organisms has been shown to be stimulated by UV light and other agents acting on DNA.

A close relationship between the repair of damaged DNA and recombination was established directly by the isolation of rec mutants of Escherichia coli which are defective in both functions[2]. Other types of mutants deficient in DNA-repair have been isolated, but most of these exhibit normal recombination frequencies. The role of each of these "repair" genes in UV-stimulated recombination has not been determined.

In my work the effect of UV light on recombination in mutants of E. coli deficient in excision-repair of DNA has been examined. uvrA and uvrB mutants lack an endonuclease which makes a single-strand break in DNA containing a UV-induced pyrimidine dimer[3]. uvrC mutants have this activity[3] but are unable to make the second break which releases an oligonucleotide containing the dimer[4]. DNA polymerase I is believed to replace the DNA lost by excision; this enzyme is lacking in polA mutants[5]. DNA ligase is believed to complete excision-repair by making the last phosphodiester linkage.

The conversion of abortive (potential) to complete transductants provides a convenient and sensitive assay for UV-stimulated recombination[6]. Usually less than 10% of the cells which receive a given gene from a transducing phage form stable transductants containing that gene. Following UV-irradiation, the number of complete transductants increases. This is a consequence of increased recombination which converts abortive to stable transductants[7]. Certain mutants of E. coli[6], Salmonella typhimurium[8], and Streptococcus pyogenes[9] defective in DNA-repair fail to exhibit this UV-stimulated conversion.

The results of the experiments presented here show that in transduction,

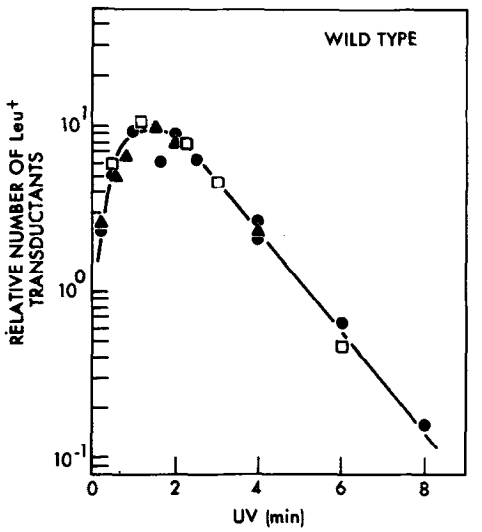

Figure 1. Stimulation of transduction of KH21 to Leu^+ by UV-irradiation of the transducing phage. Cells were harvested from exponential growth in minimal glycerol medium and infected at a multiplicity of 0.2 in medium X supplemented with 0.005% thymine and 0.0001% biotin. (Medium X consists of 0.012 M $MgSO_4$, 0.025 M $CaCl_2$, 2% ammonium acetate, 4% tryptone broth and 0.4% glycerol.) After 40 min in an ice bath, followed by 40 min at room temperature, aliquots were plated in melted agar medium selective for Leu^+ transductants. After hardening the plates were incubated 3 days at 37 C before counting. Results from 3 experiments are plotted.

UV-induced recombination is initiated by the first step in the excision-repair pathway. The product of this step, DNA with a single-strand nick near a dimer, can either enter the reaction sequence terminating in recombination, or instead, continue in the excision-repair pathway.

MATERIALS AND METHODS

KH21 is leuB1 lacY bio thyA strA metE rha malB (a K12 strain). Derivatives containing the mutations uvrA6[10], polA1[5], or the uvrB mutation from E. coli Bs1[6] were obtained by cotransduction with malB, metE or bio. The mutant transductants were identified by UV-sensitivity and verified by transducing the mutation back to KH21. AB2497 is thi-1 his-4 proA2 leu-6 thr-1 thyA17 thyR11 mt1-1 xyl-5 ara-14 galK2 lacY1 str-31 tsx-33 sup-37 (amber). Ab2498 is its uvrC34 derivative[10]. These two strains came from the collection of K.C. Smith via D. Youngs.

Media and general methods including UV-irradiation (incident dose-rate 1330 ergs/mm^2/min) and transduction with phage P1bt have been described[6].

RESULTS

Stimulation of recombination by irradiation of the transducing phage is shown in Fig. 1. Assuming 0.13 pyrimidine dimers per phage genome (10^5 nucleo-

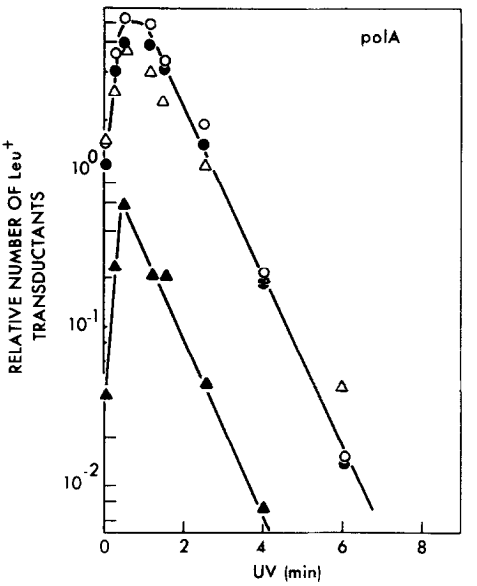

Figure 2. Influence of recipient's growth condition on frequency of normal and UV-induced recombination. The recipient was polA metE$^+$ KH21. The influence of growth medium on KH21 was similar. The recipient was harvested from exponential growth ($3x10^8$ cells/ml) in minimal-glycerol medium (●) or L-broth (▲), or from stationary phase (about 1.5×10^9 cells/ml) in minimal-glycerol (○), or L-broth (△).

tide pairs) per erg per mm^2 [4,6,11,12] the maximum number of transductants is obtained at a UV dose equivalent to about 250 dimers per phage particle (1 1/2 min) or about 1 dimer per 400 nucleotide pairs.

Influence of the growth medium. The medium in which the recipient was grown prior to infection had no effect on the shapes of the dose-response curves. However, the number of transductants recovered was much lower when cells exponentially growing in a rich medium were used rather than cells from minimal medium or cells grown into stationary phase in rich medium (Fig. 2). In subsequent experiments cells were harvested from exponential growth in minimal medium.

UV-stimulated recombination requires uvrA$^+$ and uvrB$^+$ but not uvrC$^+$ or polA$^+$. Cells deficient in the dimer-specific uvrA-uvrB endonuclease are also deficient in UV-stimulated recombination (Fig. 3). The small stimulus observed shows that some other enzyme can partially replace the uvrA-uvrB nuclease, but most UV-induced recombination is dependent on this function. The response of the double mutant uvrA-uvrB was similar to that of the single mutants (not shown), so the small stimulus is unlikely to be due to leakiness of the individual uvrA and uvrB mutations.

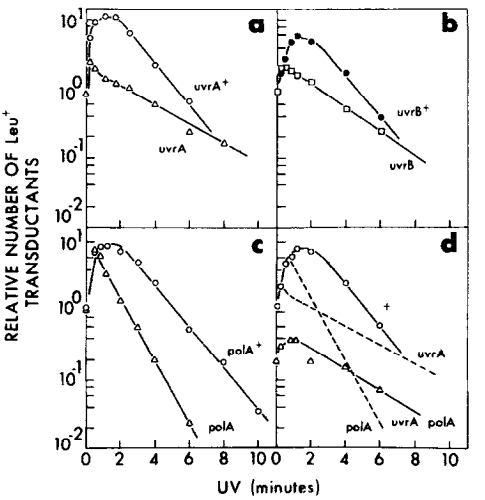

Figure 3. The roles of uvrA+, uvrB+ and polA+ genes in induced transduction. Mutant recipients were a) uvrA mal+ KH21; b) uvrB bio+ KH21; c) polA met+ KH21; d) uvrA polA met+ mal+ KH21. Numbers plotted are relative to those of the wild-type recipient.

Contrary to the behavior of the mutants lacking the first step in excision-repair, recombination in polA mutants is greatly stimulated by UV-irradiation to the transducing phage (Fig. 3). In the uvrC mutant and its uvrC+ parent, the frequency of transduction using untreated phage is higher than in KH21 and derivatives of KH21 (Fig.4). The basis for the difference has not been determined. However, UV-treatment of the phage increases the frequency 3- to 5-fold in both the mutant and the uvrC+ parent. In the mutant, maximal stimulation is achieved by a UV dose lower than required with wild type cells.

These results show that UV-induced recombination requires a functional uvrA-uvrB nuclease, but neither the uvrC+ function nor DNA polymerase I. Double mutants uvrA polA and uvrB polA were constructed and tested in recombination to check this conclusion. As expected the uvrA polA recipient behaves like a uvrA mutant in that little recombination is induced by UV light (Fig. 3). (Fewer recombinants were obtained even without UV treatment, probably reflecting the high proportion of nonviable cells usually seen in uvrA polA strains[13].) Unexpectedly, the uvrB polA strain did not grow on complex media, although it could grow on minimal media. Shizuya and Dykhuizen[14] found a similar result, using uvrB point mutations; uvrB polA double mutants constructed from uvrB deletions, and a temperature-sensitive polA mutation are invariably lethal at the nonpermissive temperature. In my experiments, when uvrB polA mutants were used as recipients, no transductants were recovered. In view of the viability effects noted above it is plausible that this is a secondary effect,

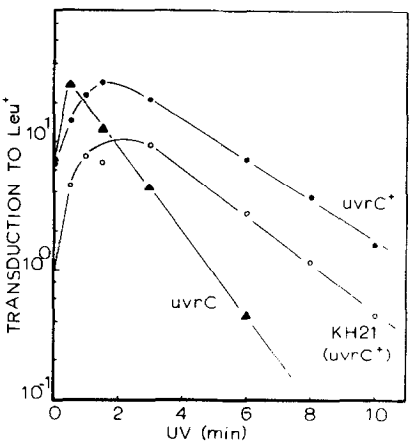

Figure 4. The role of the uvrC$^+$ gene in induced transduction. Strains were AB2497, AB2498, and KH21. An uncalibrated UV-source was used for this experiment.

resulting from lethality in cells undergoing recombination rather than inadequacy in the recombination process itself.

The loss of transductants. Comparison of the final inactivation rates shows that inactivation by UV-irradiation of the phage is greatest in polA and uvrC strains and least in uvrA and uvrB strains. I provisionally explain the differences in final slopes as follows. In the wild-type cell, the product of the uvrA-uvrB enzyme can a) enter a pathway leading to recombination; or b) continue in the excision-repair pathway, thus restoring the normal DNA structure but failing to form a UV-induced recombinant; or c) undergo degradation by other nucleases, thus failing to produce any recombinants whatsoever.

Although uvrA or uvrB mutants show little stimulation of recombination by UV light, the "normal" formation of recombinants is not inhibited by excision-repair, nor are normal recombinants lost by degradation during such repair. Thus the number of transductants in uvrA and uvrB cells shows a slower decline with increasing UV-dose because a pathway leading to degradation as well as stimulation is blocked.

The uvrC and polA strains show an increased rate of inactivation of transduction relative both to the uvrA or uvrB strains and to the wild-type. Thus as the distance between adjacent dimers decreases with increasing dose rate, more of the potential recombinant strands produced by the uvrA-uvrB endonuclease are destroyed without completing either UV-induced recombination or normal recombination than in the wild-type.

DISCUSSION

The system for studying recombination described here has several advan-

tages. The DNA in the phage can be irradiated and subsequently used to infect an undamaged host cell. Recombination is limited to a defined segment of bacterial DNA carried by the transducing phage and the homologous region of the chromosome. The recombination depends entirely on host enzymes because a transducing particle contains no phage genes[15].

What is the nature of the product of the uvrA-uvrB endonuclease which makes the DNA active in recombination? Is the single-strand nick sufficient? No, because single nicks made by ^{32}P-decay[16,17], or X-irradiation[18] fail to stimulate transduction. Instead recombination may result from the appearance of a single-stranded end. The end with the dimer may not pair properly, and the region of single-strandedness may be extended by transcription. This possibility is intuitively appealing for it provides a logical mechanism for the pairing of homologous strands. The free single-strand can anneal with a complementary strand in the chromosome which is unpaired as the result of replication, transcription, repair, or unwinding by an enzyme of normal recombination.

This paper has shown that the excision-repair genes uvrA$^+$ and uvrB$^+$ are required for most UV-stimulated transduction. Experiments involving repair genes not in the excision-repair pathway show that the recA$^+$ product (but not the recB-recC nuclease) is also needed. The roles of these and other genes in UV-stimulated recombination will be discussed in a subsequent paper. All of the results are consistent with the concept that the initial step (s) in normal recombination can be bypassed by any procedure generating single-stranded ends, but the overall process is still dependent on subsequent steps involving recA$^+$.

I thank J. Cairns and D. Youngs for strains, and H. Boyer for his hospitality while this was written. This work was supported by a grant from the Michigan Memorial-Phoenix Project.

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