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uvrC Gene Function Has No Specific Role in Repair of *N*-2-Aminofluorene Adducts

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In *Escherichia coli*, plasmid DNA modified with *N*-2-aminofluorene adducts survived equally well in wild-type, *uvrA*, or *uvrB* strains. Increased sensitivity was found in *uvrC* and *uvrD* strains. Moreover, *N*-2-aminofluorene-mediated toxicity in the *uvrC* background was reversed when an additional *uvrA* mutation was introduced into the strain.

The *Escherichia coli* UvrABC endonuclease is known to be involved in the repair of DNA damage induced by UV irradiation (6, 11, 23, 25) or a large number of chemical agents (1, 10, 12, 19, 24). This enzyme, termed excinuclease (12, 19), is constituted by three subunits. UvrA binds weakly to DNA in an ATP-dependent manner, UvrB binds to UvrA and DNA to form a stable complex which scans the DNA (14, 20, 27), and UvrC completes the excinuclease and is required in vitro for the incision step (26, 31). In vitro studies have shown that the UvrABC ternary complex binds tightly to DNA and that detachment of the enzyme needs the presence of two other proteins, namely the *uvrD* and *polA* gene products (12, 13, 15). It has also been demonstrated that the UvrABC complex incises a variety of DNA lesions such as UV dimers (6, 11, 23, 25), psoralen-light lesions (19, 24), *cis*-platinum adducts (1, 12), and guanine adducts formed by covalent binding of carcinogenic *N*-2-aminofluorene derivatives (10, 19).

These derivatives lead to two types of C(8)-guanine adduct, namely, the acetylaminofluorene adduct (AAF) and its deacetylated form, the aminofluorene (AF) adduct. It is possible to generate one or the other type of adduct in vitro by using different reactive intermediates of the carcinogen. The conformation of DNA in the vicinity of AAF adducts has been described as the insertion-denaturation model (7, 8, 9). In this model, the guanine is rotated from anti to syn with the concomitant insertion of the fluorene ring within the DNA helix. Local denaturation of the helix results from this conformational change (7, 8). Binding of AAF to alternating GC or GT sequences leads to a B → X transition (16, 17, 21, 22, 30). On the other hand, AF adducts bind to the C(8) of guanine residues without causing any major conformational change of the B-DNA structure (outside-binding model [4]).

Fuchs and Seeberg (10) have previously shown that plasmid pBR322 DNA samples modified with AAF to various extents (in vitro reaction with *N*-acetoxy-*N*-2-acetylaminofluorene) have a much lower transformation efficiency in a *uvrA* strain as compared with an isogenic wild-type strain. On the other hand, AF-modified plasmids (in vitro reaction with *N*-hydroxy-*N*-2-aminofluorene) survived equally well in *uvrA* and wild-type strains (10). Moreover, many more AF than AAF adducts are tolerated in a wild-type strain (37% survival at 60 and 18 adducts per plasmid, respectively) (10). On an adduct/nucleotide basis, AAF adducts and thymine dimers induced the *recA* protein synthesis with similar efficiency, whereas AF adducts did not trigger this response (18).

Taken together, these observations suggest two possible working hypotheses as far as the biological fate of AF adducts is concerned. (i) AF adducts, unlike AAF adducts, do not block the replication fork, and (ii) there is an efficient repair pathway for AF adducts different from the UvrABC pathway. Along these lines, it should be stressed that AF adducts were found to be toxic in *uvrC* strains but not in *uvrA* or *uvrB* strains (10, 29). To study the potential role of *uvrC* gene function in the repair of AF adducts, we compared the transformation efficiencies of AF-modified plasmids in single (*uvrA*, *uvrB*, and *uvrC*) and double (*uvrA-uvrC* and *uvrB-uvrC*) mutant strains.

Plasmid pBR322 DNA was grown in strain AB1157 and purified as described previously (10). Chemical modification of plasmid DNA with ^{3}H-ring-labeled *N*-OH-AF and quantification of the extent of modification were determined as described previously (5, 10). Wild-type strain AB1157 and isogenic Uvr$^-$ mutants AB1884 (*uvrC*34), AB1885 (*uvrB5*), AB1886 (*uvrA6*), AB2429 (*uvrC34-uvrA37*), and AB2430 (*uvrC34-uvrB45*) were originally isolated by Howard-Flanders et al. (11). Strain AB2429 (lambda *uvrA*$^+$) was constructed by lysogenization of strain AB2429 with a lambda phage containing the *uvrA*$^+$ gene (a gift from R. Alazard). Strain NR3951 (Δ*uvrB*) was a gift from R. Schaaper, and ES549 (*uvrD*) was from E. Siegel (28). *E. coli* cells were made competent for transformation by treatment with $CaCl_2$ (3). Transformants were selected on LB plates containing ampicillin (50 μg/ml).

Toxicity of AF adducts in *uvrC* strains is abolished in a strain carrying an additional *uvrA* mutation. Plasmid pBR322 DNA samples modified to various extents with AF adducts were transformed into various *E. coli* strains, and the relative transformation efficiencies were plotted as a function of the number of AF adducts per plasmid molecule (Fig. 1A). The extent of modification at a relative transformation efficiency of 37% (i.e., corresponding to an average of one lethal hit per plasmid molecule) are given in Table 1. Strains carrying the *uvrA6*, Δ*uvrB*, or *uvrB5* allele produced results identical to those of the wild-type strain (i.e., ≈60 AF adducts at a 37% relative transformation efficiency), in agreement with previously reported results (11, 29). On the other hand, the isogenic *uvrC34* strain showed increased sensitivity toward AF-modified plasmid (37% relative transformation efficiency at 18 AF adducts per molecule) (Fig. 1A; Table 1). Surprisingly, the sensitivity of the strain

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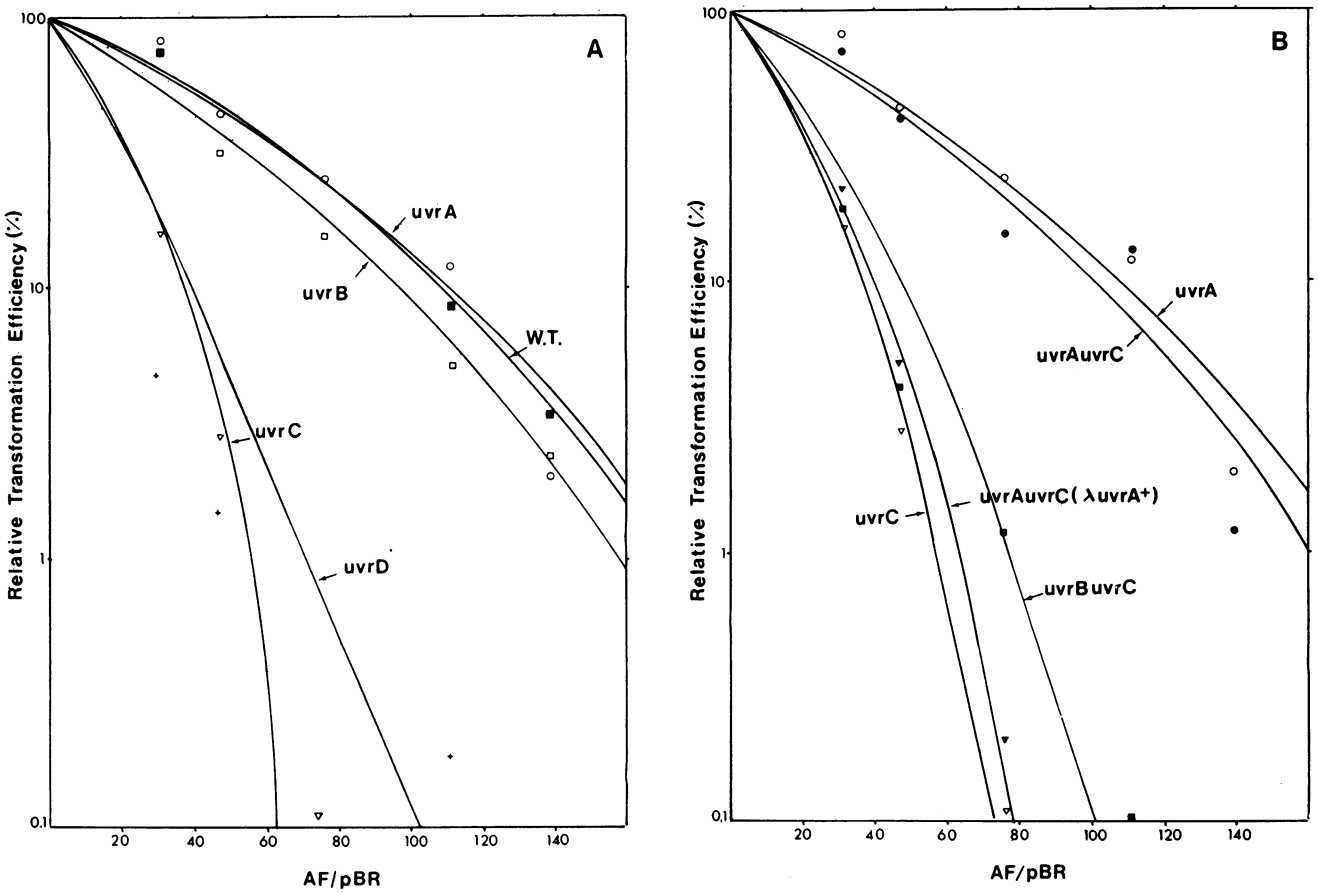

FIG. 1. Survival in different strains of plasmid pBR322 reacted with *N*-OH-AF to various extents. (A) ■, AB1157 (wild type [W.T.]), ○, AB1886 (*uvrA6*), □, NR3951 (Δ*uvrB*), +, ES549 (*uvrD*), ▽, AB1884 (*uvrC34*). (B) ○, AB1886 (*uvrA6*); ▽, AB1884 (*uvrC34*); ●, AB2429 (*uvrC34-uvrA37*); ■, AB2430 (*uvrC34-uvrB45*); ▼, AB2429 lambda *uvrA*$^+$.

carrying the *uvrC34* allele was suppressed when associated with the *uvrA37* allele. In fact, in a strain carrying both the *uvrC34* and *uvrA37* alleles, AF-modified plasmids survived equally as well as in a wild-type strain (Fig. 1B; Table 1). To ascertain that this effect was really associated with the double mutation, we constructd a phage lambda *uvrA*$^+$ lysogen of this strain. The resulting *uvrC34-uvrA37* lambda *uvrA*$^+$ strain showed sensitivity toward AF-modified plasmid similar to that of the *uvrC34* strain (Fig. 1B; Table 1). The *uvrC*-associated toxicity toward AF adducts could not be rescued by the presence of a *uvrB* allele. In fact, a double (*uvrC34-uvrB45*) mutant strain exhibited sensitivity toward AF-modified plasmid similar to that observed in the *uvrC* background. A strain carrying a *uvrD* mutation showed a response toward AF adducts similar to that observed in a *uvrC* strain (Fig. 1B, Table 1).

TABLE 1. Survival of AF-modified pBR322 in various *uvr* strains

Strain	Relevant genotype	AF adduct/plasmid ratio at 37% survival
AB1157	Wild type	60
AB1886	*uvrA6*	60
AB1885	*uvrB5*	60
NR3951	Δ*uvrB*	50
AB1884	*urvC34*	18
ES549	*uvrD* (*mutU*)	18
AB2430	*uvrC34 uvrB45*	24
AB2429	*uvrC34 uvrA37*	55
AB2429 (lambda *uvrA*$^+$)	*uvrC34 uvrA37* lambda *uvrA*$^+$	20

[a] Wild-type AB1157 and the isogenic Uvr$^-$ mutants AB1884 (*uvrC34*), AB1885 (*uvrB5*), AB1886 (*uvrA6*), AB2429 (*uvrC34-uvrA37*), and AB2430 (*uvrC34-uvrB45*) were originally isolated by Howard-Flanders et al. (11). Construction of strain AB2429 (lambda *uvrA*$^+$) is described in the text. Strain NR3951 (ΔuvrB) was a gift from R. Schaaper, and ES549 (uvrD) was from E. Siegel (28).

These observations tend to rule out the possibility of an active role of the *uvrC* gene product in the repair of AF adducts other than its known role as part of the UvrABC excinuclease but point out that the toxicity that was observed in *uvrC* strains was due to impaired functioning of the mutant form of the UvrABC excinuclease (i.e., the excinuclease containing the mutant form of the UvrC protein). In vitro experiments have shown that AF-modified plasmid is a substrate for the purified UvrABC excinuclease (10). Since AF-modified plasmids transformed *uvrA* and wild-type strains equally well, it was concluded that in vivo UvrABC-mediated excision repair of AF adducts is not useful for survival of the modified plasmids (10). An explanation for the toxicity of AF adducts in the *uvrC* strain might be that, like the wild-type UvrABC excinuclease, its mutant form (i.e., in the *uvrC* strain) is able to bind at the site of AF adducts. Although the molecular basis of the toxicity is unknown, two models can be proposed. (i) The mutant form of the excinuclease creates abnormal breaks in the vicinity of

the lesion. This hypothesis is supported by the observation of slow accumulation of single-strand breaks in a UV-irradiated *uvrC* strain (26). These breaks can be qualified as abnormal since they are not repaired in the presence of an active *polA* allele (26). (ii) The mutant excinuclease is such that it cannot be detached from its binding site, thus creating a block to the replication machinery. Husain et. al. (13) have recently demonstrated in vitro that turnover of the UvrABC excinuclease needs active participation of both the *uvrD* and the *polI* gene products. They propose that the *uvrD* gene product (i.e., helicase II) is necessary for release of the UvrC protein and that the combined action of PolA and UvrD proteins acts to dissociate the remaining complex. This model would also account for the high sensitivity toward AF adducts that is observed in *uvrD* strains.

To account for the different results obtained in the double *uvr*$^-$ strains (*uvrC34-uvrB45* and *uvrC34-uvrA37*), one can suggest that the mutated UvrABC excinuclease present in the former strain ($UvrA^+B^-C^-$) is still able to bind to its DNA substrate, thus creating a block to replication, whereas in the latter strain the mutated $UvrA^-B^+C^-$ excinuclease is unable to bind to DNA. This might reflect the fact that the UvrA protein is a DNA-binding protein, whereas UvrB is not.

In conclusion, one might speculate that (i) the *uvrC* gene product does not play an active role in repair of AF adducts, and (ii) impaired functioning of the mutated form of the UvrABC excinuclease bound to AF adducts containing DNA creates a lesion that blocks replication.

Therefore, we stress that increased sensitivity of a mutant strain when transformed with a plasmid containing a given type of lesion does not necessarily imply that the mutated gene has a specific function in the repair of that lesion.

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LITERATURE CITED

1. **Beck, D. J., S. Popoff, A. Sancar, and D. W. Rupp.** 1985. Reaction of the UvrABC excision nuclease with DNA damaged by diammine dichloroplatinum II. Nucleic Acids Res. **13:** 7395–7412.
2. **Caron, P. C., S. R. Kushner, and L. Grossman.** 1985. Involvement of helicase II (*uvrD*, gene product) and DNA polymerase I in excision mediated by the UvrABC protein complex. Proc. Natl. Acad. Sci. USA **82:**4925–4929.
3. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibody resistance in bacteria: genetic transformation of *E. coli* by F-factor DNA. Proc. Natl. Acad. Sci. USA **69:**2110–2114.
4. **Daune, M. P., R. P. P. Fuchs, and M. Leng.** 1981. Structural modification and protein recognition of DNA modified by *N*-2-fluorenylacetamide, its 7-iodo derivative, and by *N*-2-fluorenamine. Natl. Cancer Inst. Monogr. **58:**201–210.
5. **de Murcia, G., M. C. Lang, A. M. Freund, R. P. P. Fuchs, M. P. Daune, and M. Leng.** 1979. Electron microscopic visualization of *N*-acetoxy-*N*-2-acetyl aminofluorene binding sites in ColE1 DNA by means of specific antibodies. Proc. Natl. Acad. Sci. USA **76:**6076–6080.
6. **Franklin, W. A., and W. A. Haseltine.** 1984. Removal of UV light-induced pyrimidine-pyrimidone (604) products from *Escherichia coli* DNA requires the *uvrA*, *uvrB* and *uvrC* gene products. Proc. Natl. Acad. Sci. USA **81:**3821–3824.
7. **Fuchs, R., and M. Daune.** 1972. Physical studies on deoxyribonucleic acid after covalent binding of a carcinogen. Biochemistry **11:**2659–2666.
8. **Fuchs, R. P. P.** 1975. In vitro recognition of carcinogen-induced local denaturation sites in native DNA by S1 endonuclease from *Aspergillus oryzae*. Nature (London) **257:**151–152.
9. **Fuchs, R. P. P., J. F. Lefèvre, J. Pouyet, and M. P. Daune.** 1976. Comparative orientation of the fluorene residue in native DNA modified by *N*-aco-*N*-2-acetylaminofluorene and two 7-halogeno derivatives. Biochemistry **15:**3347–3351.
10. **Fuchs, R. P. P. and E. Seeberg.** 1984. pBR322 plasmid DNA modified with 2-acetylaminofluorene derivatives: transforming activity and in vitro strand cleavage by the *Escherichia coli* UvrABC endonuclease. EMBO J. **3:**757–760.
11. **Howard-Flanders, P., R. P. Boyce, L. Theriot.** 1965. Three loci in *E. coli* K12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53:**1119–1136.
12. **Husain, I., S. G. Chancy, and A. Sancar.** 1985. Repair of *cis*-platinum DNA adducts by ABC excinuclease in vivo and in vitro. J. Bacteriol. **163:**817–823.
13. **Husain, I., B. Van Houten, D. C. Thomas, M. Abdel-Monem, and A. Sancar.** 1985. Effect of DNA polymerase I and DNA helicase II on the turnover of UvrABC excision nuclease. Proc. Natl. Acad. Sci. USA **82:**6774–6778.
14. **Kacinsky, B. M., and W. D. Rupp.** 1983. *E. coli* uvrB protein binds to DNA in the presence of uvrA protein. Nature (London) **294:**480–481.
15. **Kumura, K., M. Sekiguchi, A. L. Steinum, and E. Seeberg.** 1985. Stimulation of the UvrABC enzyme-catalysed repair reaction by the uvrD protein (DNA helicase II). Nucleic Acids Res. **13:**1483–1492.
16. **Sage, E., and M. Leng.** 1980. Conformation of poly(dG-dC) · poly(dG-dC) modified by the carcinogen *N*-acetoxy-*N*-acetyl-2-aminofluorene and *N*-hydroxy-*N*-2-aminofluorene. Proc. Natl. Acad. Sci. USA **77:**4597–4601.
17. **Sage, E., and M. Leng.** 1981. Conformational changes of poly(dG-dC) · poly(dG-dC) modified by the carcinogen *N*-acetoxy-*N*-acetyl-2-aminofluorene. Nucleic Acids Res. **9:** 1241–1250.
18. **Salles, B., M. C. Lang, A. M. Freund, C. Paoletti, M. Daune, and R. P. P. Fuchs.** 1983. Different levels of induction of RecA protein in *E. coli* (PQ10) after treatment with two related carcinogens. Nucleic Acids Res. **11:**5235–5242.
19. **Sancar, A., K. A. Franklin, G. Sancar, and M. S. Tang.** 1985. Repair of psoralen and acetylaminofluorene DNA adducts by ABC excinuclease. J. Mol. Biol. **184:**725–734.
20. **Sancar, A., P. R. Wharton, S. Soltzer, B. M. Kacinsky, N. D. Clarke, and W. D. Rupp.** 1981. Identification of the uvrA gene product. J. Mol. Biol. **148:**45–62.
21. **Santella, R. M., D. Grunberger, S. Broyde, and B. E. Hingerty.** 1981. Z DNA conformation of *N*-2-acetylaminofluorene-modified poly(dG-dC) · poly(dG-dC) determined by reactivity with anticytidine antibodies and minimized potential energy calculation. Nucleic Acids Res. **9:**5459–5467.
22. **Santella, R. M., D. Grunberger, I. B. Weinstein, and A. Rich.** 1981. Induction of the Z conformation in poly(dG-dC) · poly(dG-dC) by binding of *N*-2-acetylaminofluorene to guanine residues. Proc. Natl. Acad. Sci. USA **78:**1451–1455.
23. **Seeberg, E.** 1978. Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated $uvrA^+$ and $uvrB^+/uvrC^+$ gene products. Proc. Natl. Acad. Sci. USA **75:** 2569–2573.
24. **Seeberg, E.** 1981. Strand cleavage at psoralen adducts and pyrimidine dimers in DNA caused by interaction between semi-purified uvr^+ gene products from *Escherichia coli*. Mutat. Res. **82:**11–22.
25. **Seeberg, E., J. Nissen-Meyer, and P. Strike.** 1976. Incision of ultraviolet-irradiated DNA by extracts of *E. coli* requires three different gene products. Nature (London) **263:**524–526.
26. **Seeburg, E., W. D. Rupp, and P. Strike.** 1980. Impaired incision of ultraviolet-irradiated deoxyribonucleic acid in *uvrC* mutants of *Echerichia coli*. J. Bacteriol. **144:**97–104.
27. **Seeberg, E., and A. L. Steinum.** 1982. Purification and properties of the uvrA protein from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **79:**988–992.
28. **Siegel, E. C., and F. Kamel.** 1974. Reversion of frameshift

mutations by mutator genes in *Escherichia coli*. J. Bacteriol. **117**:994–1001.
29. **Tang, M. S., M. W. Lieberman, and C. M. King.** 1982. uvr genes function differently in repair of acetylaminofluorene and aminofluorene DNA adducts. Nature (London) **299**:646–648.
30. **Wells, R. D., J. J. Mighetta, J. Klysik, J. E. Larson, S. M. Stirdivant, and W. Zacharias.** 1982. Spectroscopic studies on acetylaminofluorene-modified (dT-dG_n · $(dC\text{-}dA)_n$ suggest a left-handed conformation. J. Biol. Chem. **257**:10166–10171.
31. **Yeung, A. T., W. B. Mattes, E. Y. Oh, and L. Grossman.** 1983. Enzymatic properties of purified *Escherichia coli* UvrABC proteins. Proc. Natl. Acad. Sci. USA **80**:6157–6161.