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Double-Strand Breaks in DNA Caused by Repair of Damage Due to Ultraviolet Light

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DNA DSBs are formed in normal human IMR-90 cells during repair incubation after 100 and 300 $J \cdot m^{-2}$ of UVL. By contrast, no DSBs are formed after UVL in human XPA cells that are unable to excise pyrimidine dimers. The DSBs are not due to immediate cell death since all the cells excluded trypan blue at the time of assay and because XPA cells, which are much more UVL-sensitive than IMR-90, did not form DSBs after UVL. We suggest that these repair-induced DSBs should be potent lesions that might lead to cytotoxicity, chromosome aberrations, deletion mutations, and perhaps cellular transformation. transformation.

Key words: neutral filter elution, human cells, carcinogenesis, excision repair, DNA, double-strand breaks, S1 nuclease

Mammalian cells repair pyrimidine dimers by excising between 35 [1, 2] and 85 [3] nucleotides from the DNA strand that contains the dimers. Since excision occurs 5′ to 3′, excision gaps on opposite strands would move toward each other and might overlap. Harm [4] and Setlow [5] first suggested that in E coli, DNA double-strand breaks (DSBs) might be formed enzymatically during excision repair. In fact Bonura and Smith [6, 7] subsequently showed that DSBs are introduced into the DNA of E coli during the repair of ultraviolet light (UVL) damage. DSBs were formed in wild-type, polA1, recB21, recA, and exrA strains but not in strain uvrA6, which is deficient in the incision step of excision repair.

Utilizing a neutral filter elution method for measuring small numbers of DNA DSBs in mammalian cells [8], we have recently studied whether DSBs are induced in human cells during repair of UVL damage [9].

Abbreviations: DSB, DNA double strand break; UVL, ultraviolet light; XPA, Xeroderma pigmentosum complementation group A.

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RESULTS

IMR-90 Cells, UVL

Ultraviolet light at doses up to 300 J•m^{-2} did not directly induce any detectable DNA DSBs in IMR-90 cells irradiated and held on ice until lysis (Fig. 1). However, when IMR-90 cells were irradiated with 100 or 300 J•m^{-2} of UVL at room temperature and allowed to incubate in fresh medium at 37°C, a time and dose-related increase occurs in the rates of pH 9.6 elution (Fig. 1) and therefore in the number of DNA DSBs [8]. DSBs began to appear by 3 hr after irradiation and continued to increase up to 51 h.

The cells remain attached to the dishes up to 51 hr after either 100 or 300 J•m^{-2} of UVL, and all of the attached cells exclude trypan blue at both 24 and 51 h. Although the cells will eventually detach and die, up until the point they are lysed for elution they appear to possess an intact metabolism. These data suggest that the DSBs are not a secondary result of cell death.

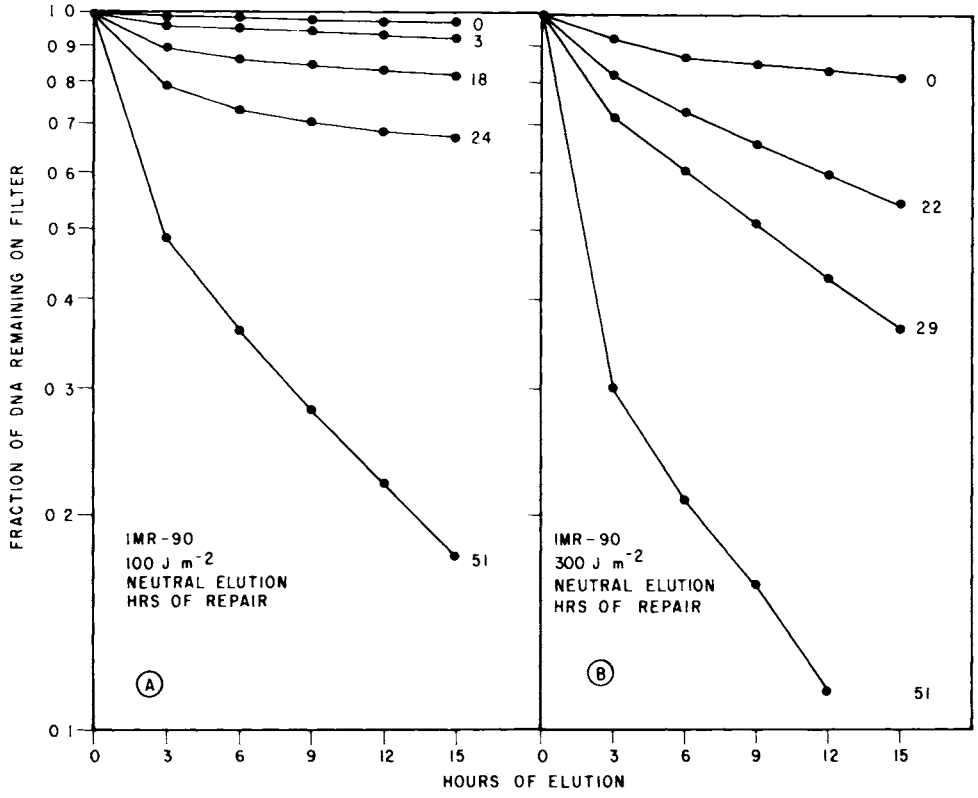

Fig. 1. Induction of DNA DSBs during the repair of UVL damage. IMR-90 cells were cultured, radioactively labeled, and irradiated with UVL as described in the text. The number of hours of repair incubation is shown next to each elution curve. 1a) Left panel, 100 J•m^{-2} UVL; 1b) right panel, 300 J•m^{-2} UVL.

XPA Cells, UVL

XPA cells are unable to incise pyrimidine dimers and so cannot initiate excision repair; they are much more sensitive to killing by UVL than are normal human cells. As seen in Figure 2, XPA cells do not induce DSBs after UVL even though they are more sensitive to the cytotoxic effects of UVL. Since normal human cells are excision-competent and form DNA DSBs after UVL, whereas XPA cells do neither, excision repair is implicated as a mechanism for DNA DSB formation after UVL.

DSBs Formed in Proliferating and Nonproliferating Cells

In all of the above experiments the cells were dividing at the time of UVL irradiation. DNA replication across pyrimidine dimers may leave gaps in the strand opposite the dimer [10]. DNA DSBs might result if a cellular single-strand endonuclease were to digest the strand opposite the gap. To test this hypothesis, we performed experiments with contact inhibited IMR-90 cells that were left unstimulated in conditioned medium or that were stimulated into DNA synthesis and mitosis by a fresh medium change 24 and 48 h before UVL. One-hour pulse incorporations of ^{3}H-thymidine showed that the unstimulated cells incorporated between 5% and 10% of the amount of thymidine as the stimulated cells at 24 and 48 h after medium change. Both serum-stimulated and unstimulated (non-

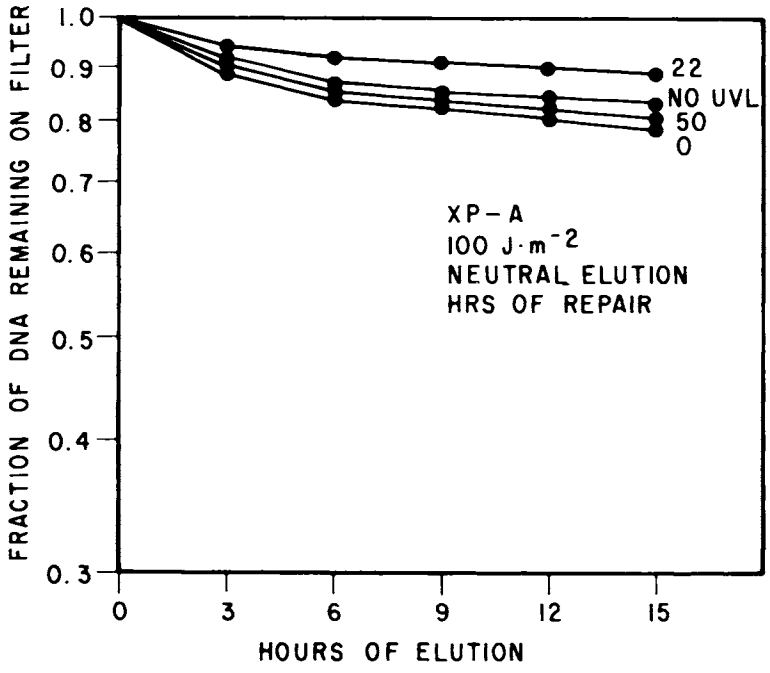

Fig. 2. DSBs are not induced in XPA cells after UVL. XPABE cells were cultured, irradiated, and eluted as described in the text. The number of hours of repair incubation is shown next to each elution curve.

dividing) IMR-90 formed the same number of DNA DSBs after UVL. Therefore, the DSBs are not due to DNA replication occurring after UVL.

S1 and Cellular Endonucleases

Another explanation for these results is that a cellular single-strand endonuclease might be released by the cell after UVL to digest any single-strand regions opposite excision gaps. To test this possibility we irradiated IMR-90 with 300 $J \cdot m^{-2}$ and allowed them to repair for 3 h in order to make excision gaps. We then lysed these cells as usual for the elution procedure except that the lysis solution was removed with four 5-ml rinses of 0.01 M Tris HCl, pH 7.4, to make DNA that was free of protein and held on the filter. This DNA was incubated with either a total cell homogenate from IMR-90 cells or with S1 nuclease. The results showed that the S1 nuclease produced DNA DSBs in irradiated 3 h repair DNA, but that the cell extract did not. The S1 results support the idea that single-stranded gaps or other S1-sensitive sites do remain open for some period of time during UVL repair in human cells. We conclude that if single-strand endonucleases similar to S1 were present in IMR-90 cells they might contribute to the induction of DSBs; however, we found no direct evidence for the presence of such endonucleases.

DISCUSSION

These data implicate one or more of the processes involved in excision repair as the mechanism for producing DNA DSBs after UVL. The DSBs do not seem to be due to immediate cell death or to cellular endonucleases, or to be associated with replication of UVL-irradiated DNA. Perhaps the strongest evidence that these are excision repair-induced DSBs is that XPA, an excision-defective and UVL-sensitive cell, does not produce DSBs after UVL, whereas IMR-90, an excision-competent and relatively more UVL-resistant cell, does produce DSBs following UVL.

DSBs could be formed during excision repair by the sort of mechanisms outlined in Figure 3. These models are based in part on those of Bonura and Smith [7], who described mechanisms for the formation of DNA DSBs during excision repair in E coli. Model 1 proposes that there is a certain low probability that the mammalian enzymes for incision and excision mistakenly attack both the dimer-containing and the opposite dimer-free DNA strand forming a DSB. Model 2 proposes that DSBs result from simultaneous excision gap overlap on opposite strands. Assuming that 3 dimers/10^8 daltons/$J \cdot m^{-2}$ are formed in human cellular DNA [11] and that the molecular weight of one base pair is 656, then 100 $J \cdot m^{-2}$ forms 1 dimer/507 base pairs, and 300 $J \cdot m^{-2}$ forms 1 dimer/169 base pairs. With between 35 [1, 2] and 85 bases [3] removed from each dimer-containing strand during excision repair, it is likely that excision gaps on opposite strands would overlap. If nonrandom dimer clustering occurs, then the probability for excision gap overlap on opposite strands would be even higher. Because the excision gaps would have to be open simultaneously for a DSB to be formed, this criterion would substantially reduce the number of such occurrences. However, to the extent that localized regions of the DNA are repaired preferentially and simultaneously, then the probability of excision gap overlap increases greatly.

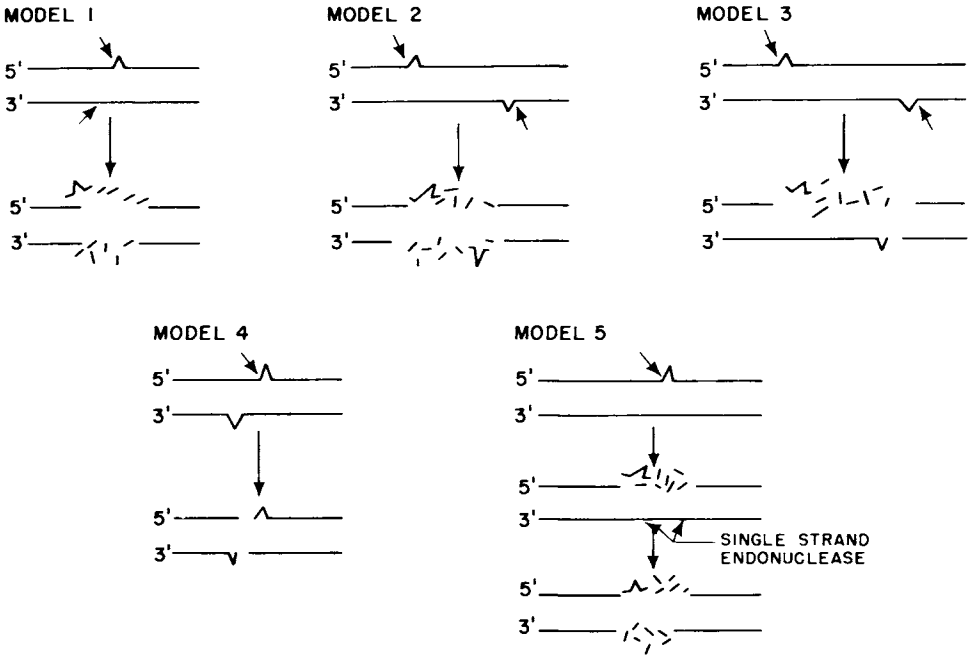

Fig. 3. Models for the formation of DNA DSBs during repair incubation after UVL. Model 1) Incision and excision on the dimer-containing strand followed by a low probability of incorrect incision and excision of the undamaged opposite strand. Model 2) Incision and excision occur simultaneously on opposite strands. The excision gaps overlap to form a DSB. The probability of overlap is increased if nonrandom clustering of DNA damage occurs and if such regions are repaired simultaneously by assemblies of enzymes. Model 3) An excision gap passes an incision break on the opposite strand. Model 4) Simultaneous incision near almost oppositely placed lesions. Model 5) Excision repair occurs on the dimer-containing strand while a cellular single-strand endonuclease digests the strand opposite the dimer and forms a DSB.

Furthermore, the time that each gap remains open would affect the probability of gap overlap. There is, as yet, no direct evidence for such nonrandom damage and repair, although a number of chemicals have been shown to selectively damage nuclease-sensitive (linker) regions of nucleosomes [10–12]. Likewise, repair of both chemical [13, 14] and UVL [15, 16] damage may occur selectively in linker regions. If either spontaneous or chemically induced DNA damage occurs preferentially in certain regions, and if repair enzymes work precessively clustered together, then these sorts of repair-induced DNA gaps may be of biological importance.

Model 3 proposes that incision near a dimer on the strand opposite an excision gap will led to a DSB. The same probabilistic arguments raised for Model 2 also apply here. Model 4 proposes that DSBs can form by simultaneous incision of two closely spaced lesions on opposite strands where hydrogen bonding and base stacking forces are not sufficient to hold the opposite strands together. These conditions are more stringent than those for Models 2 and 3 and would be expected to occur less frequently. Model 5 proposes that the strand opposite an

excision gap is attacked by a cellular single strand endonuclease. Since we showed that an exogenous endonuclease (S1) induced DSBs in the DNA of 3-h repair-incubated cells, either "S1-like" single-strand endonucleases do not exist in mammalian cells or our experimental conditions inactivated any endogenous endonculeases.

The cellular effects of even one DNA DSB of the sort outlined in Figure 3 (especially Models 1, 2, or 5) could be profound. Cytotoxicity might be one major consequence because large double-strand gaps may not be easily repaired so that fragments of chromosomal DNA, required for viability, might be lost without centromeric attachment. A number of cytogenetic consequences might also occur depending upon the stage of the cell cycle in which an unrepaired DSB was formed: in G1, chromosome breaks or losses; in G2, chromatid breaks or gaps; in S, a mixture of both chromosome and chromatid events. In addition, chromosome fragmentation and deletions might occur. If the strands on each side of a gap were resealed (although we have seen no evidence for that), then a deletion or frameshift mutation would occur. Whether any of this is important in certain mechanisms of carcinogenesis remains open for speculation; however, the cells in many human tumors contain chromosome anomalies [17, 18] of the sort that could be formed by overlapping excision repair on opposite DNA strands.

We have recently shown that the DNA intercalators adriamycin, actinomycin D, and ellipticine induce double-strand breaks in the DNA of mouse L1210 cells by a process that requires a viable cell and perhaps a topoisomerase activity [19]. Methylnitrosourea (1 mm) also induces DSBs in human cell DNA (Bradley, unpublished data); so repair-induced DSBs occur after chemical damage as well as UVL damage and may be a general phenomenon. More experiments are needed to verify these later results, to decide the appropriate model for repair-induced DSBs, to find the agents and cells in which the process occurs, and to determine whether it occurs frequently enough at low doses to be of biological importance. We hope to answer these questions by using the sensitive technique of filter elution at nondenaturing pH [8] to study cell lines with repair deficiencies.

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