Cellular Responses and Repair of Single-strand Breaks Introduced by UV Damage Endonuclease in Mammalian Cells\*  

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Although single-strand breaks (SSBs) occur frequently, the cellular responses and repair of SSBs are not well understood. To address this, we established mammalian cell lines expressing Neurospora crassa UV damage endonuclease (UVDE), which introduces a SSB with a 3'-OH immediately 5' to UV-induced cyclobutane pyrimidine dimers or 6–4 photoproducts and initiates an alternative excision repair process. Xeroderma pigmentosum group A cells expressing UVDE show UV resistance of almost the wild-type level. In these cells SSBs are produced upon UV irradiation and then efficiently repaired. The repair patch size is about seven nucleotides, and repair synthesis is decreased to 30% by aphidicolin, suggesting the involvement of a DNA polymerase δ-dependent long-patch repair. Immediately after UV irradiation, cellular proteins are poly(ADP-ribose)ylated. The UV resistance of the cells is decreased in the presence of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase. Expression of UVDE in XRC1-defective EM9, a Chinese hamster ovary cell line, greatly sensitizes the host cells to UV, and addition of 3-aminobenzamide results in almost no further sensitization of the cells to UV. Thus, we show that XRC1 and PARP are involved in the same pathway for the repair of SSBs.

DNA single-strand breaks (SSBs) are frequently produced by environmental genotoxic agents and by endogenous cellular reactions. SSBs cause double-strand breaks when replication forks encounter SSBs and, thus, result in chromosomal rearrangements and instability (1). Despite the potentially harmful effects of SSBs, however, little is known about the details of the repair mechanisms and cellular responses to SSBs in mammalian cells. This may be due to the experimental difficulty to produce SSBs alone. Genotoxic agents that produce SSBs (ionizing radiation, oxidizing agents, and alkylating agents) generate a variety of DNA lesions (2). For instance, ionizing radiation and bleomycin produce not only SSBs but also base lesions and double-strand breaks (2, 3).

One of the immediate responses to SSBs in mammalian cells is thought to be the activation of poly(ADP-ribose) polymerase (PARP). PARP binds to SSBs and is activated (4). Although PARP has been considered to be involved in the repair of SSBs, especially in replicating cells (4), its precise role is still not well understood. Another player in the response to SSBs may be XRC1, which binds to DNA ligase III and DNA polymerase β (5), and is thought to be involved in a base excision repair (BER) pathway. Recently it was reported that XRC1 is also involved in an S-phase-specific repair pathway of SSBs (6). Since XRC1 binds to PARP (7) and some phenotypic characteristics of XRC1-deficient cells are similar to those of PARP-deficient cells (8), both proteins may be involved in the S phase-specific mode of SSB repair. However, the functional relationship between PARP and XRC1 is unknown and remains to be elucidated.

In addition to nucleotide excision repair (NER) for UV-induced DNA damage, the filamentous fungus, Neurospora crassa, and the fission yeast Schizosaccharomyces pombe, possess an alternative excision repair mechanism, which is initiated by an endonuclease called UV damage endonuclease (UVDE) and is referred to as UVDE-initiated excision repair (9–14). UVDE introduces a SSB immediately 5' to UV-induced cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts, leaving 3'-hydroxyl and 5'-phosphoryl groups at the site of cleavage (9–11). Until now UVDE has been found only in some eukaryotic microorganisms and in some bacteria including Bacillus subtilis (9) and Dierococcus radioduras (15), but neither a similar enzymatic activity nor any homologous genes have been found in mammalian cells.

To understand the cellular responses and repair of SSBs in mammalian cells, we have made use of UVDE. We introduced the N. crassa UVDE gene into a human and a Chinese hamster ovary (CHO) cell lines and analyzed the responses of the transfected cells to UV. Using these unique systems, we found the following. 1) Judging from the UV resistance of xeroderma pigmentosum group A (XPA) cell line expressing UVDE, UVDE-initiated alternative excision repair in human cells works almost as efficiently as NER. 2) The UVDE-initiated repair is mediated mainly by aphidicolin-sensitive DNA polymerase(s), and the repair patch size is about seven nucleotides. 3) XRC1 and PARP cooperate and contribute to cell survival after SSBs are produced.

EXPERIMENTAL PROCEDURES

Cell Lines, Vectors, and Transfection—A human cell line derived from an XPA patient, XP12ROSV, was obtained from Dr. K. Tanaka (Osaka University) and used as the host cell for complementation of UV sensitivity by the introduced UVDE gene. The CHO cell line EM9 was purchased from the American Type Culture Collection. Plasmid pCY4B, a derivative of the one described by Niwa et al. (16), contains a chicken β-actin promoter (obtained from Dr J. Miyazaki, Osaka University). This plasmid was used for expression of the N. crassa UVDE gene in XPA cells and EM9 cells. pCY4B-UVDE was made by inserting the
EcoRI fragment carrying the UVDE-coding region into the EcoRI site of pCY4B. XP12ROSV cells and EM9 cells were transfected with pCY4B-UVDE together with a plasmid harboring a G418 resistance marker by Lipofectin (Life Technologies, Inc.). We used actin as the control (monoclonal antibody to actin, clone C4, Roche Molecular Biochemicals). For detection of poly(ADP-ribose) (pADPr), cells were harvested from 35-mm culture dishes just before reaching confluence. Cells were washed with phosphate-buffered saline (PBS) and irradiated with 2.5, 7.5, and 20 J/m² UV. After UV irradiation, cells were incubated for various periods of time at room temperature. After incubation, cell extracts were prepared by adding 400 μl of SDS-polyacrylamide gel electrophoresis sample buffer (1% SDS, 1% β-mercaptoethanol, 5% glycerol, 25 mM Tris-HCl (pH 6.5), and 0.05% bromophenol blue) to each dish. In another experiment, cells were preincubated in medium containing 2 mM of proteinase of each, fluorostreous monolane (PALL Gelman Laboratories), and an antibody against XPA (Vector). The supernatants were recovered and used for immunoblot analysis as well as for the incision assay described below. Western analysis was done by standard methods using whole cell extracts (20 μg of protein of each), fluorostreous monolane (PALL Gelman Laboratories), and an antibody against XPA (Vector). Human cells were grown in Eagle's minimal essential medium (Nissui) containing 10% fetal calf serum, whereas CHO cells were grown in 10% fetal calf serum-supplemented Dulbecco’s modified medium (Nissui).

**Immunoblot Analysis—** Whole cell extracts were prepared from cultured cells by homogenizing cell pellets in a lysis buffer (50 mM Tris-HCl (pH 7.5), 0.3 mM KCl, 0.05% Nonidet P-40, 2 mM dithiothreitol) containing protease inhibitors (protease inhibitor mixture tablets, Roche Molecular Biochemicals). After centrifugation of the homogenate at 100,000 × g, supernatants were recovered and used for immunoblot analysis as well as for the incision assay described below. Western analysis was done by standard methods using whole cell extracts (20 μg of protein of each), fluorostreous monolane (PALL Gelman Laboratories), and an antibody against XPA (Vector). Human cells were grown in Eagle’s minimal essential medium (Nissui) containing 10% fetal calf serum-supplemented Dulbecco’s modified medium (Nissui).

**In Vitro Incision Assay—** Nicking activity of UVDE was measured as described (18). The whole cell extracts (40 μg of protein of each), and the synthetic oligonucleotides containing CPD were used.

UVDE was introduced behind the chicken β-actin promoter and was introduced into a human XPA cell line. Cell lines transfected with UVDE (XP[A(UVDE)]), wild-type XPA cDNA (XP[A(xXPA)]), and pCY4B vector (XP[A(VECTOR)]) were obtained. Immunoblotting using polyclonal anti-Neuro-

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**RESULTS**

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**Analysis of Patch Size by BrdUrd-induced Density Shift—** Repair patch size was also measured as described by Smith et al. (19). XP[A(UVDE)] cells were prelabeled with [3H] thymidine, incubated in medium containing FdUrd, BrdUrd, and hydroxyurea for 2 h, and irradiated with 20 J/m² UV as described above. Cells were then incubated in medium containing FdUrd, BrdUrd, hydroxyurea, and [3H] thymidine for 2 h, then lysed. The parental-density DNA was purified by two successive neutral CsCl gradient sedimentation processes. Two kinds of DNA were prepared for use as markers as follows. [3H] thymidine labeled DNA was prepared from cells immediately after irradiation with UV. Fully BrUrd-substituted hybrid DNA was prepared from unirradiated cells that were incubated for 3 h in medium containing FdUrd, BrdUrd, and [3H] thymidine. These DNA was sonicated and size distribution of the fragments of the sonicated DNA was detected by electrophoresis in a denaturing polyacrylamide gel as described (20). From the data, the number-average molecular size of the fragments was calculated as described (21). The size of the repair patches was measured in alkaline CsCl gradients using the parental-density DNA sonicated to a number-average molecular size of 188 nucleotides. The gradients were fractionated, and the radioactivity of each fraction was measured. The repair patch size was calculated as follows. First, the distance between the [3H] thymidine DNA distribution and that of the 7-H-repair label distribution was measured. This distance was then compared with the separation between the peak of the [3H] thymidine DNA and that of the 7-H-labeled fully BrUrd-substituted DNA, which was determined from a separate analysis of DNA markers in a similar gradient. The ratio of these two distances was then multiplied by the average fragment size to give the average repair patch size.

In another experiment, [3H]BrdUrd (Moravek) was used as the isotopic label in place of [3H] thymidine. Hydroxyurea was not used. The number-average molecular size of the sonicated parental-density DNA was 211 nucleotides in this case, and the experiment was performed in the same way as above.

**Alkaline Gel Analysis—** The relative number of SSBs in the genomic DNA from cells collected at various periods of time after UV irradiation was determined by using alkaline gel analysis. Briefly, DNA was transferred to a nitrocellulose membrane. After hybridization, the membrane was washed in 2× SSPE, 0.1% SDS, and 0.1% sodium dodecyl sulfate (SDS) and then incubated in a 3.5% alkaline gel (10 mM Tris, 1 mM EDTA (pH 8) at 37 °C overnight. DNA was isolated by incubating in 0.5% SDS, 100 μg/ml proteinase K (Wako), 10 μg/ml aphidicolin, 10 μg/ml EDTA (pH 8) at 37 °C overnight. Genomic DNA was isolated by phenol/chloroform extraction and ethanol precipitation. Each 5-μg DNA sample was mixed with alkaline loading buffer (22) and electrophoresed in a 3.5% alkaline agarose gel (Agarose H, Wako, Ltd.), and dried and analyzed using FLA-2000 (Fujifilm). As a measure of the relative amount of SSBs, we took the following ratio: the radioactivity of the 5-μg DNA sample in the 5.6- to 14.3-kilobase area/the total radioactivity.

**RESULTS**

Establishment of XPA Cell Line Expressing cDNA of Neurospora UVDE and Nicking Activity of the Cell Extracts—cDNA of *N. crassa* UVDE was introduced behind the chicken β-actin promoter and was introduced into a human XPA cell line. Cell lines transfected with UVDE (XP[A(UVDE)]), wild-type XPA cDNA (XP[A(xXPA)]), and pCY4B vector (XP[A(VECTOR)]) were obtained. Immunoblotting using polyclonal anti-Neuro-

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pore UVDE antibody shows a single band only in XPA[UVDE] cells (Fig. 1A). The nicking activity of the extract prepared from XPA[UVDE] cells to UV damage is shown in Fig. 1C. The extract introduced an incision immediately 5' to the CPD, as judged by the decrease of a 49-mer band and concomitant strong appearance of the 20-mer band. This incision activity is the same as previously reported for recombinant UVDE (18). These data indicated that XPA[UVDE] cells express UVDE and retain its nicking activity.

Survival of XPA Transfectants—The colony-forming ability of the XPA transfectants after UV irradiation was assessed. XPA[Vector] cells were extremely sensitive to UV irradiation, whereas XPA[cXPA] cells exhibited UV resistance (Fig. 2). XPA[UVDE] cells showed almost the same level of UV resistance as XPA[cXPA] cells at low UV doses (Fig. 2). As the UV dose increased, XPA[cXPA] cells became more UV-resistant than XPA[UVDE] cells (Fig. 2). Thus, the alternative excision repair found in eukaryotic microorganisms provided NER-deficient human cells with UV resistance of almost wild-type level. Now the question is how UV-induced DNA damage is repaired in these cells.

SSBs in Genomic DNA of XPA[UVDE] Cell after UV Irradiation—To examine whether SSBs are actually produced after UV irradiation in XPA[UVDE] cells, we conducted alkaline gel electrophoresis analysis of the genomic DNA of UV-irradiated XPA[UVDE] cells. After 20 J/m² UV irradiation, cells were incubated in buffer for various periods of time before genomic DNA was isolated and electrophoresed on an alkaline-agarose gel. UV-irradiated DNA migrated as a discrete band near the origin, whereas the DNA isolated after UV irradiation showed a broad smeared band on the gel (Fig. 3A). These results indicate that the SSBs are actually produced by UVDE in intact XPA[UVDE] cells immediately after UV irradiation. To quantify the SSBs, we measured the amount of DNA between 5.6- and 14.3-kilobase DNA. The amount of smeared DNA gradually increased up to 40 min after UV irradiation and reached a plateau level (Fig. 3A). Two hours after UV irradiation, the amount of the SSBs was significantly decreased (Fig. 3B), indicating repair of SSBs. Under the conditions used, no significant difference in the extent of the smear was observed in XPA[Vector] and XPA[cXPA] cells (Fig. 3B). These data suggest that in XPA[UVDE], SSBs are produced by UVDE, which initiates an alternative excision repair in human cells.

Repair Process for SSBs Produced by UVDE in XPA[UVDE] Cells—First we characterized the repair synthesis in XPA[UVDE] cells after UV irradiation. Unscheduled DNA synthesis after UV irradiation was observed in XPA[UVDE] cells but not in XPA[Vector] cells (Fig. 4). The unscheduled DNA synthesis in XPA[UVDE] was slightly less than unscheduled DNA synthesis determined in HeLa cells and increased with UV doses (Fig. 4). We next examined the sensitivity of the repair synthesis to aphidicolin, a specific inhibitor of DNA polymerase α, δ, and ε. We measured repair synthesis using the BrdUrd density shift technique (see “Experimental Procedures”). Exponentially growing cells were exposed to 20 J/m² and 40 J/m² UV. Hydroxyurea was added to the growth medium to reduce the level of semi-conservative DNA synthesis. At both doses used, most of UV-induced repair synthesis was aphidicolin-sensitive (Table 1). These data suggest that repair synthesis is mediated mainly by aphidicolin-sensitive DNA polymerase(s); presumably by DNA polymerase δ and/or ε (see “Discussion”).

Estimation of Repair Patch Size in XPA[UVDE] Cells—The measurement of repair patch size is an extension of the method used to measure the repair synthesis. 32P-Prelabeled XPA[UVDE] cells were irradiated with 20 J/m² UV and incubated in medium containing [3H]dThd, hydroxyurea, and BrdUrd for 3 h. Parental-density DNA was isolated by two successive processes of neutral CsCl gradient sedimentation. This DNA was sonicated to an average size of 188 nucleotides and then centrifuged to equilibrium in alkaline CsCl gradients. Under these conditions, the increase in density of DNA fragments that contain repair patches (synthesized in the presence of BrdUrd) is large enough to be measured and can be compared with the increase in density of DNA completely substituted with BrUra. Gradients were fractionated, and the radioactivity profiles of 3H and 32P were determined (Fig. 5). The density of the DNA molecules containing repair patches (shown by the profile of 3H) was clearly larger than that of bulk genomic DNA (shown by the profile of 32P) (Fig. 5). Based on the shift between the
profiles of $^{3}$H and $^{32}$P and referring to the position of fully BrUra-substituted DNA, the patch size was determined as 8 ± 2 nucleotides. In the second experiment we did not add hydroxyurea, and $[^{3}H]$BrdUrd was used as the isotopic label. In this case, the patch size was determined as 7 ± 2 nucleotides (radioactivity profiles were not shown).

Involvement of PARP Activation in the UV Resistance of XPA$^{[UVDE]}$—We further investigated whether PARP and XRCC1 are involved in the repair process. In the presence of 3AB, a widely used inhibitor of PARP, enhanced lethality after UV irradiation was observed in XPA$^{[UVDE]}$ cells (Fig. 2). By contrast, in XPA$^{[Vector]}$ cells and XPA$^{[cXPA]}$ cells, no significant increase in sensitivity to UV was observed in the presence of 3AB (Fig. 2). These results demonstrate the involvement of PARP in the repair of the SSBs introduced by UVDE. By immunoblot analysis with monoclonal antibody to pADPr, we examined whether the activation of PARP occurs in XPA$^{[UVDE]}$ in response to UVDE-induced SSBs. Thirty seconds after 20 J/m² UV irradiation in XPA$^{[UVDE]}$ cells, a significant amount of pADPr was synthesized (Fig. 6). This is consistent with the result that the SSBs had already been introduced in
the genomic DNA of XPA[UVDE] after 30 s of 20 J/m² UV irradiation (Fig. 3A). A peak for the poly(ADP-ribose)lation of cellular proteins was found only 2 min after UV irradiation in the cells. After 10 min, no significant pADPr was observed (Fig. 6A) in the presence of considerable SSBs at this time (Fig. 3). Under the conditions used, no significant pADPr was detected in XPA[Vector] cells (Fig. 6A) and XPA[Vector] cells (data not shown) until 10 min after UV irradiation. In the presence of 3AB, the inhibition of pADPr synthesis was shown (Fig. 6A) even in the presence of considerable SSBs at this time (Fig. 3).

The next interesting question is how the UVDE-initiated repair proceeds in human cells. The repair synthesis was shown to be mostly dependent on aphidicolin-sensitive DNA polymerase, and the determined patch size of the repair was about seven nucleotides. Since the repair patch size of the proliferating cell nuclear antigen-dependent pathway of BER has been reported as between 7 and 14 nucleotides (26), or less than 10 nucleotides in length (27, 28), the patch size for UVDE-initiated excision repair fits in reasonably well with that of the BER pathway. It has also been reported that the repair synthesis of the proliferating cell nuclear antigen-dependent pathway is not catalyzed by DNA polymerase α (29) and is catalyzed by DNA polymerase δ or ε (27, 28, 29). By in vitro assays with purified recombinant proteins, we and other groups (30, 31) showed that the SSBs produced by UVDE became substrates for cleavage by FEN1 (flap endonuclease 1), which has already been shown to be a factor involved in the proliferating cell nuclear antigen-dependent BER pathway (27, 28, 32). The SSBs produced by UVDE in XPA[UVDE] cells may be processed by DNA polymerase δ and/or ε and components that are common with long patch repair pathway of BER.

The third interesting question about UVDE-initiated repair concerns the cellular responses to the induced SSBs in mammalian cells. Western blot analysis showed that immediately after irradiation with a high dose of UV, cellular proteins were poly(ADP-ribose)lated in XPA[UVDE] cells (Fig. 6). This is in contrast to the response in XPA[Vector] cells, which showed no significant synthesis of pADPr (Fig. 6A). By adding 3AB, a competitive inhibitor of PARP, to XPA[UVDE] cells, pADPr synthesis was suppressed (Fig. 6A). These results gave additional clear evidence for PARP activation by SSBs in human cells. 10 min after UV irradiation, pADPr was no longer observed (Fig. 6A). This is explained by reports that, after excessive activation of PARP, pADPr has a short half-life close to 1 min (34), and the levels of NAD, which is a substrate of PARP, are depleted (4).

We have shown here that 3AB enhanced the UV lethality of XPA[UVDE], whereas 3AB did not make any significant differ-
suggest that SSBs are one of the major risk factors for genome instability induced by oxidative DNA damage. We consider that UVDE-expressing cell lines offer a unique experimental system for the analysis of the cellular response to SSBs in mammalian cells.

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REFERENCES

1. Paulovich, A. G., Tyczynski, D. P., and Hartwell, L. H. (1997) Cell 88, 315–321
2. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) DNA Repair and Mutagenesis, American Society for Microbiology, Washington, D. C.
3. Povirk, L. F., Wubker, W., Kohnleim, W., and Hutchinson, F. (1977) Nucleic Acids Res. 4, 4573–4580
4. Chatterjee, S., and Berger, N. A. (1998) in DNA Damage and Repair (Nicklolloff, J. A. and Hoeckstra, M. F., eds) Vol. 2, pp. 487–515, Humana Press Inc., Totowa, NJ
5. Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E., and Lindahl, T. (1999) EMBO J. 18, 6662–6670
6. Taylor, R. M., Moore, J. D., Whitehouse, J., Johnson, P., and Caldecott, K. W. (2000) Mol. Cell. Biol. 20, 735–740
7. Masson, M., Niedergang, C., Schreiber, V., Muller, S., de Murcia, J. M., and de Murcia, G. (1998) Mol. Cell. Biol. 18, 3563–3571
8. Thompson, L. H., and West, M. G. (2000) Mutat. Res. 459, 1–18
9. Takao, M., Yonemasu, R., Yamamoto, K., and Yasui, A. (1996) Nucleic Acids Res. 24, 1267–1271
10. Bowman, K. K., Sidik, K., Smith, C. A., Taylor, J. S., Doetsch, P. W., and Freyer, G. A. (1994) Nucleic Acids Res. 22, 3926–3032
11. Yajima, H., Takao, M., Yasuhira, S., Zhao, J. H., Ishii, C., Inoue, H., and Yasui, A. (1995) EMBO J. 14, 2393–2399
12. Freyer, G. A., Davey, S., Ferrer, J. V., Martin, A. M., Beach, D., and Doetsch, P. W. (1995) Mol. Cell. Biol. 15, 4572–4577
13. Yonemasu, R., McCready, S. J., Murray, J. M., Osman, F., Takao, M., Yamamoto, K., Lehmann, A. R., and Yasui, A. (1997) Nucleic Acids Res. 25, 1553–1558
14. Yasui, A., and McCready, S. J. (1998) Bioessays 20, 291–297
15. White, O., Eisen, J. A., Heidelberg, J. F., Hickey, E. K., Peterson, J. D., Dodson, R. J., Haft, D. H., Omin, M. L., Nelson, W. C., Richardson, D. L., Moffat, K. S., Qin, H., Johnson, P., Pampel, W., Crosby, M., Shen, M., Vamathevan, J. J., Lam, P., McDonald, L., Utterback, T., Zalewski, C., Makarova, K. S., Aravind, L., Daly, M. J., Minton, K. W., Fleischmann, R. D., Ketchum, K. A., Nelson, K. K., Salzberg, S., Smith, H. O., Venter, J. C., and Fraser, C. M. (1999) Science 286, 1651–1677
16. Niwa, H., Yamamura, K., and Miyazaki, A. (1991) Gene 108, 193–200
17. Miyamoto, I., Miura, N., Niwa, H., Miyazaki, J., and Tanaka, K. (1992) J. Biol. Chem. 267, 12182–12187
18. Kanno, S., Iwai, S., Takao, M., and Yasui, A. (1999) Nucleic Acids Res. 27, 3096–3103
19. Smith, C. A., Cooper, P. K., and Hanawalt, P. C. (1981) in DNA Repair: A Laboratory Manual of Research Procedures (Friedberg, E. C., and Hanawalt, P. C., eds) pp. 289–305, Marcel Dekker, Inc., New York
20. Bowman, K. K., Smith, C. A., and Hanawalt, P. C. (1997) Mutat. Res. 383, 95–105
21. Lehman, A. R. (1981) in DNA Repair: A Laboratory Manual of Research Procedures (Friedberg, E. C., and Hanawalt, P. C., eds) pp. 471–485, Marcel Dekker, Inc., New York
22. Sambrook, J., Frisch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. B.24, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Shen, M. R., Zdzieciak, M. Z., Mohrenweiser, H., Thompson, L. H., and
Mammalian DNA Repair of UVDE-induced Single-strand Break

Thelen, M. P. (1998) Nucleic Acids Res. 26, 1032–1037
24. Yasuhira, S., Morimyo, M., and Yasui, A. (1999) J. Biol. Chem. 274, 26822–26827
25. Valerie, K., Green, A. P., de Riel, J. K., and Henderson, E. E. (1987) Cancer Res. 47, 2967–2971
26. Frosina, G., Fortini, P., Rossi, O., Carozzino, F., Raspaglio, G., Cox, L. S., Lane, D. P., Abbondandolo, A., and Dogliotti, E. (1996) J. Biol. Chem. 271, 9573–9578
27. Pascucci, B., Stucki, M., Jonsson, Z. O., Dogliotti, E., and Hubsher, U. (1999) J. Biol. Chem. 274, 33696–33702
28. Matsumoto, Y., Kim, K., Hurwitz, J., Gary, R., Levin, D. S., Tomkinson, A. E., and Park, M. S. (1999) J. Biol. Chem. 274, 33703–33708
29. Matsumoto, Y., Kim, K., and Bogenhagen, D. F. (1994) Mol. Cell. Biol. 14, 6187–6197
30. Yoon, J. H., Swiderski, P. M., Kaplan, B. E., Takao, M., Yasui, A., Shen, B., and Pfeifer, G. P. (1999) Biochemistry 38, 4809–4817
31. Alleva, J. L., Zuo, S., Hurwitz, J., and Doetsch, P. W. (2000) Biochemistry 14, 2659–2666
32. Kim, K., Biade, S., and Matsumoto, Y. (1998) J. Biol. Chem. 273, 8842–8848
33. Gary, R., Kim, K., Cornelius, H. L., Park, M. S., and Matsumoto, Y. (1999) J. Biol. Chem. 274, 4354–4363
34. Alvarez-Gonzalez, R., and Althaus, F. R. (1989) Mutat. Res. 218, 67–74
35. Boorstein, R. J., and Pardee, A. B. (1984) J. Cell. Physiol. 120, 345–353
36. Ben-Hur, E., Utsumi, H., and Elkind, M. M. (1984) Radiat. Res. 97 546–555