Efficient Repair of 8-Oxo-7,8-dihydrodeoxyguanosine in Human and Hamster Xeroderma Pigmentosum D Cells†

Enrico Cappelli,‡,§ Paolo Degan,§ Larry H. Thompson,|| and Guido Frosina*,‡,§

DNA Repair Unit and Mutagenesis Laboratory, Istituto Nazionale Ricerca Cancro, 16132 Genova, Italy, and Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94551

Received November 11, 1999; Revised Manuscript Received May 17, 2000

ABSTRACT: The repair of the endogenous lesion 8-oxo-7,8-dihydrodeoxyguanosine (8-oxodG) was investigated in the nucleotide excision repair mutant xeroderma pigmentosum D (*XPD*), using human normal or transformed *XPD* fibroblasts and the Chinese hamster *XPD* cell line UV5. In vivo repair of 8-oxodG induced by hydrogen peroxide treatment and analyzed by high-performance liquid chromatography/electrochemical detection was normal in the *XPD* mutant fibroblasts XP15PV and GM434, as compared to normal human fibroblasts GM970, GM5757, and GM6114. Similar results were obtained with the human SV40-transformed *XPD* mutant cell line GM8207 in comparison to the control cell line GM637. Repair of 8-oxodG was even slightly (2−3-fold) but reproducibly increased in Chinese hamster *XPD* mutant UV5 cells, as compared to parental AA8 cells. This unexpected effect was reversed by transfection in UV5 cells of a wild-type *XPD* cDNA and confirmed in in vitro experiments in which a plasmid substrate containing a single 8-oxoG was repaired by UV5 cell extracts. The data show that repair of 8-oxodG is normal in *XPD* cells, thus indicating that the neurological complications of *XPD* patients may not be linked to in vivo accumulation of this lesion.

DNA damage of endogenous origin may significantly contribute to human degenerative diseases (*1*). One major endogenous lesion is 8-oxo-7,8-dihydrodeoxyguanosine (8-oxodG)[1] that is highly mutagenic and cytotoxic (*2*) and whose rate of spontaneous formation is elevated in human cells (*3*). DNA base excision repair (BER) is a major mechanism of repair of 8-oxodG. BER of 8-oxodG is initiated in mammalian cells by 8-oxodG DNA glycosylase 1 (OGG1), a bifunctional DNA glycosylase with associated abasic (AP) site lyase activity whose coding sequence has been cloned (*4*−*6*). A backup system for repair of oxidized lesions is nucleotide excision repair (NER), the main pathway involved in repair of ultraviolet damage (*7*). NER is defective in the hereditary disorder xeroderma pigmentosum (XP), an autosomal recessive syndrome characterized by elevated frequency of skin cancer in areas exposed to sunlight. About 25% of XP patients (particularly those of complementation groups A and D) are additionally characterized by neurological complications caused by neuronal death in the central and peripheral nervous system (*8*). It has been hypothesized that the XP neurological symptoms may be linked to inability to remove from DNA of neuronal cells oxidized lesions such as 8-oxodG and thymine glycol, with consequent accumulation of this kind of damage and eventual cell death (*9*). The importance of NER in repair of oxidized bases is an open question yet (*10*). We report here that repair of 8-oxodG is normal in human *XPD* cells and may even be slightly accelerated in an hamster *XPD* mutant.

EXPERIMENTAL PROCEDURES

Cell Culture. The following human cells were obtained from the Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ) and cultured as recommended: GM970, normal nonimmortalized fibroblasts; GM5757, normal nonimmortalized fibroblasts; GM6114, normal nonimmortalized fibroblasts; GM434, *XPD* mutant nonimmortalized fibroblasts; GM637 cells, an SV40-immortalized normal cell line; GM8207 [XP6BE(SV40)], an SV40-immortalized *XPD* mutant cell line. The *XPD* mutant nonimmortalized fibroblasts XP15PV were a gift from Dr. Miria Stefanini (Istituto di Genetica Biochimica ed Evoluzionistica, Consiglio Nazionale della Ricerche, Pavia, Italy). Passage numbers of GM970, GM5757, GM6114, GM434, and XP15PV fibroblasts were between 15 and 20.

The Chinese hamster ovary (CHO) AA8 cell line, its *XPD* mutant derivative UV5, and the CXPD-5 clone, obtained by transfection of a wild-type *XPD* cDNA into the UV5 cell line (*11*), were cultured in Alpha-MEM with 10% foetal calf serum. The CHO-9 cell line and its *ERCC1* mutant derivative 43-3B were cultured in F10/DMEM 1:1 with 10% fetal calf serum.

† This work was partially supported by Telethon Italy [Grant E728], Italian Association for Cancer Research (AIRC), National Research Council [Grant 99.02487.CT04 (*)], and Italian Ministry of Health.

* Corresponding author: DNA Repair Unit, Mutagenesis Laboratory, Istituto Nazionale Ricerca Cancro, Largo Rosanna Benzi n. 10, 16132 Genova, Italy. Tel +39.010.5600292; fax +39.010.5600992; e-mail: gfrosina@hp380.ist.unige.it.

‡ DNA Repair Unit, Istituto Nazionale Ricerca Cancro.

§ Mutagenesis Laboratory, Istituto Nazionale Ricerca Cancro.

|| Lawrence Livermore National Laboratory.

[1] Abbreviations: 8-oxodG, 8-oxo-7,8-dihydrodeoxyguanosine; 8-oxoG, 8-oxo-7,8-dihydroguanine; BER, DNA base excision repair; OGG1, 8-oxoguanine DNA glycosylase 1; AP, abasic; NER, nucleotide excision repair; XP, xeroderma pigmentosum; HPLC/ED, high performance liquid chromatography/electrochemical detection; CHO, Chinese hamster ovary; TFIIH, transcription factor IIH; CS, Cockayne syndrome.

10.1021/bi992610l CCC: $19.00
Published on Web 08/03/2000

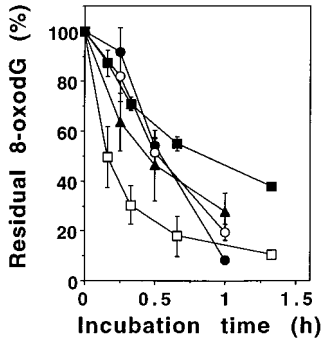

FIGURE 1: Efficient in vivo repair of 8-oxodG in human *XPD* nonimmortalized fibroblasts. Exponentially growing cells were harvested by trypsinization, resuspended in medium with serum, and treated with 10 mM hydrogen peroxide for 30 min at 37 °C. Thereafter cells were rinsed, incubated in fresh medium for the indicated repair times, pelleted by centrifugation, and lysed. DNA was extracted and enzymatically hydrolyzed and 8-oxodG and deoxyguanosine content determined by HPLC coupled to an electrochemical detector. Data are the mean ± S.E.M. of three experiments. Error bars are not indicated when smaller than symbols. GM970 (normal, ■); GM5757 (normal, ▲); GM6114 (normal, ●); GM434 (*XPD*, □); XP15PV (*XPD*, ○).

In Vivo Repair of 8-OxodG. The DNA extraction and purification procedures as well as quantification of 8-oxodG by high performance liquid chromatography/electrochemical detection (HPLC/ED) were performed following published methods (*12*, *13*). Briefly, exponentially growing cells were harvested by trypsinization, resuspended in medium with serum, and treated with 10 mM hydrogen peroxide for 30 min at 37 °C. Thereafter cells were rinsed with PBS, incubated in fresh medium for the indicated repair times, pelleted by centrifugation, and lysed. DNA was extracted and enzymatically hydrolyzed, and 8-oxodG and deoxyguanosine content were determined by HPLC/ED.

In Vitro Repair of 8-OxoG. The procedures for preparation of plasmids carrying a single lesion at a defined location have been described elsewhere (*14*−*16*). Briefly, pGEM3Zf-(+) single-stranded DNA was annealed with a 22-mer oligonucleotide containing a single 8-oxoG in position 12 [TIBMOLBIOL, Genova, Italy (pGEM 8-oxoG)] thus generating a single 8-oxoG/cytosine base pair. Closed circular double-stranded DNA was obtained by incubating with T4 DNA polymerase, gene 32 protein, and DNA ligase (*15*). Control pGEM T plasmids were prepared with an oligonucleotide carrying the normal base guanine in the same position (*15*).

Plasmid substrate (300 ng) was incubated with 30 μg of extract protein prepared by the procedure described in refs *17* and *18* for the indicated times at 30 °C in the presence of [^{32}P]dGTP (*16*). Extracts from mutant and control cell lines were always prepared in parallel. After the repair reaction, plasmid DNA was purified and treated with the restriction endonucleases *Xba*I and *Hinc*II (20 units each). The resulting 8-mer fragments were resolved by polyacrylamide gel electrophoresis in the presence of 7 M urea at 30 mA.

RESULTS

Human Cells. Two types of *XPD* nonimmortalized fibroblatsts (XP15PV and GM434) and three types of nonimmortalized wild-type fibroblasts (GM970, GM5757, and GM6114) were examined for their ability to repair 8-oxodG in vivo (Figure 1). Cells were treated with H_2O_2 and induced 8-oxodG was determined by HPLC/ED at different post-treatment times. The levels of induced 8-oxodG determined at time 0 were similar in all cell strains. No evident defect in removal of 8-oxodG could be observed in *XPD* cells, in comparison to control cells. In particular, the half-life of 8-oxodG in XP15PV (0.52 h, ○) was similar to that of control GM6114 (0.54 h, ●) and GM5757 (0.45 h, ▲) cells and slightly lower than that of control GM970 (0.86 h, ■) cells. In the case of *XPD* GM434 cells (□), the rate of 8-oxodG removal was even accelerated in comparison to control cells GM970, GM5757, and GM6114, with a half-life of 8-oxodG of 0.16 h. Similarly, no difference was found in the rate of 8-oxodG repair in transformed *XPD* cells (Figure 2). The SV40-transformed GM8207 (*XPD* mutant, ○) and GM637 (control, ●) cell lines were analyzed under the same conditions used for nonimmortalized fibroblasts. No difference in the rate of repair of 8-oxodG in vivo could be observed between GM8207 and GM637 cells, with half-lives of 0.77 and 0.66 h, respectively.

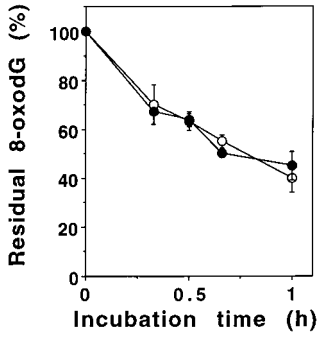

FIGURE 2: Efficient in vivo repair of 8-oxodG in human *XPD* SV40-transformed fibroblasts. The experiments were performed as described under Figure 1. Data are mean ±SEM of three experiments. GM637 (normal; ●); GM8207 (*XPD*; ○).

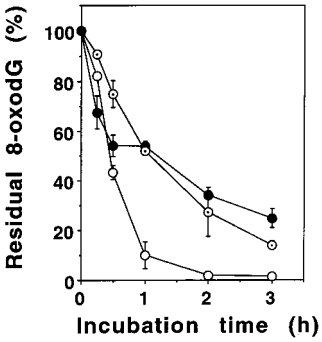

FIGURE 3: In vivo repair of 8-oxodG in the Chinese hamster *XPD* UV5 cell line. The experiments were performed as described under Figure 1. Data are the mean ±SEM of three experiments. AA8 (control; ●), UV5 (*XPD*; ○), and CXPD-5 (corrected UV5 cells; ⊙).

Chinese Hamster Cells. The in vivo repair of 8-oxodG in the *XPD* Chinese hamster ovary cell line UV5 is shown in Figure 3. Longer incubation times were considered here, because of the slower rate of 8-oxodG repair in hamster cells with respect to human cells. No defective 8-oxodG repair was observed in UV5 cells. On the contrary, the kinetics of removal were faster in UV5 cells (○) as compared to AA8 (●). The maximum difference was detectable at 1 h incubation time. Half-life for 8-oxodG in AA8 cells was 1.23 h, and 25% of induced 8-oxodG was still present after 3 h. In UV5 cells, half-life of 8-oxodG was 0.45 h (2.7-fold lower

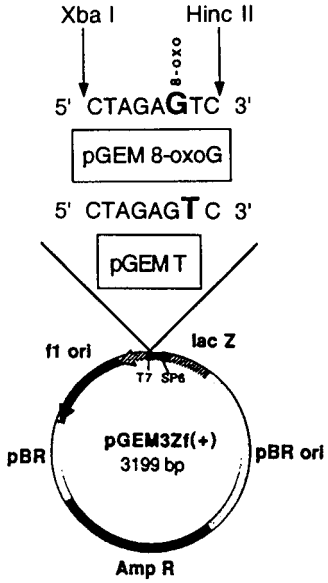

FIGURE 4: DNA plasmid substrates. Schematic of plasmid substrates pGEM-8oxoG (carrying a single 8-oxoG) and pGEM-T (control) plasmid substrates. The location of the single lesion and the *Xba*I−*Hin*cII restriction sites are shown.

with respect to AA8) and no lesions were left after 2 h. The 8-oxodG in vivo repair kinetics of UV5 cells corrected with a wild type *XPD* cDNA [CXPD-5 cells, ⊙ (*11*)] were essentially superimposable on that of AA8 cells [8-oxodG half-life = 1.09 h (1.1-fold lower as compared to AA8) and 14% of lesions remaining at 3 h].

A slight improvement of 8-oxoG repair in UV5 cells could also be observed in vitro. The capacity of hamster cell extracts to perform BER on 8-oxoG was evaluated with plasmid substrates containing single lesions at a defined position (*14*−*16*). Figure 4 shows a schematic representation of closed circular double-stranded DNA constructs pGEM 8-oxoG and pGEM T. Only single nucleotide insertion events are detected in the 8-mer resulting from treatment of repaired products with restriction endonucleases *Xba*I and *Hin*cII as indicated by previous determinations of repair patch length (*19*) and lack of sensitivity of repair synthesis to anti-PCNA antibodies (*14*).

We have recently observed that 8-oxoG is repaired by mammalian cell extracts with low efficiency, in comparison to other endogenous lesions (*16*). In particular, repair replication of an 8-oxoG/cytosine base pair performed by normal Chinese hamster extracts is 3-fold less efficient than repair of a uracil/adenine base pair, and in turn, the latter is repaired 3-fold less efficiently than a natural AP site placed in front of an adenine (*16*). In absolute terms, the repair incorporation stimulated by 30 μg of protein of normal AA8 or CHO-9 cell extracts incubated for 3 h with plasmids containing a single AP site, U, or 8-oxoG lesion is ∼200, 67, and 22 net cpm, respectively, as determined by liquid scintillation counting of excised bands of the gel. 8-OxoG is preferentially repaired via single nucleotide insertion (*20*).

Repair replication performed by *XPD* mutant UV5 extracts and *ERCC1* mutant 43-3B extracts on pGEM 8-oxoG is shown in Figure 5. In vitro BER of 8-oxoG was increased 2.0- and 3.6-fold in UV5 extracts at incubation times 3 and 6 h, respectively (lanes 6 and 7), as compared to AA8 extracts (lanes 2 and 3). The visual effect was partially

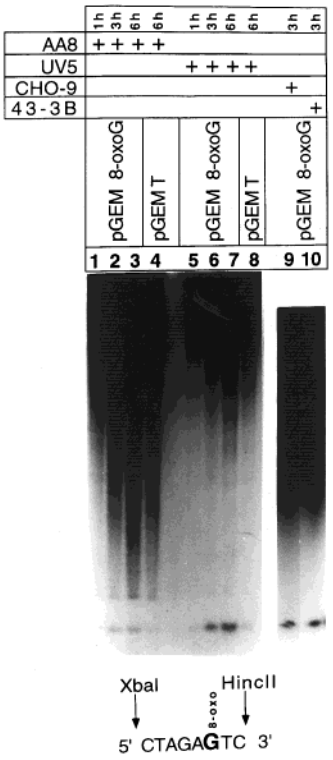

FIGURE 5: BER of 8-oxoG in *XPD* mutant UV5 and *ERCC1* mutant 43-3B cells. pGEM 8-oxoG (containing a single 8-oxoG; lanes 1−3, 5−7, 9−10) or pGEM T (control; lanes 4 and 8) plasmid substrates were incubated with 30 μg of protein of AA8 parental wild-type cell extract (lanes 1−4), UV5 *XPD* mutant cell extract (lanes 5−8), CHO-9 parental wild-type extract (lane 9), or 43-3B *ERCC1* mutant cell extract (lane 10) at 30 °C for the indicated times. After extraction with phenol−chloroform, plasmid DNAs were digested with *Xba*I−*Hin*cII.

flattened by the very low repair incorporation stimulated in vitro by 8-oxoG, even after 6 h of incubation (lanes 3 and 7) but was reproducible with different sets of extracts independently prepared. The higher unspecific incorporation in the AA8 as compared to the UV5 extract was probably linked to different intensity of nick translation of plasmid substrates at sites of DNA breakage. This difference was opposite to that of repair incorporation on pGEM 8-oxoG and unlikely affected the damage-dependent repair synthesis. No increase in BER of 8-oxoG was observed in the *ERCC1* mutant 43−3B extracts (lane 10) as compared to extracts prepared from the parental cell line CHO-9 (lane 9). The latter showed a basal 8-oxoG repair ability that was higher than that observed with AA8. This was probably linked to variations in activity of extracts prepared at different times, the pair of extracts CHO-9 and 43-3B being prepared independently with respect to the pair AA8−UV5. Negligible unspecific incorporation was detectable on pGEM T control substrate (lanes 4, 8).

DISCUSSION

Neurological abnormalities occur in ∼25% of XP patients. In these cases, sometimes termed de Sanctis-Cacchione syndrome, patients exhibit progressive neurological deterioration with massive loss of neurons (*8*, *21*). The origins of these symptoms cannot be readily explained as being due to UV-induced DNA damage and it has been hypothesized that they could arise because of accumulation of DNA damage generated by reactive oxygen species (*8*). In particular, the

oxidized base 8-oxoG has been repeatedly suggested as a contributing factor to human degenerative disorders for its marked miscoding properties and elevated rate of formation (*1*). The complementation group D of XP is one of the most frequently accompanied by neurological complications (*8*, *21*). The XPD protein, one of the seven subunits of transcription factor IIH (TFIIH), is a 5′ → 3′ DNA helicase that creates during NER a local opening of DNA around the site of damage, that will serve as substrate for the subsequent action of repair endonucleases *ERCC1*/XPF and XPG (*22*). It has been reported that repair of 8-oxoG and thymine glycol is defective in different complementation groups of XP, including *XPD* (*9*). We could not find yet in our experiments any defect of 8-oxodG repair in different *XPD* human cells, nonimmortalized or SV40-transformed. The inconsistency with previous data may likely be explained with the different repair pathways under observation. The excision assay used in ref *9* specifically detects NER, that to a limited extent can act on 8-oxodG and is of course defective in *XPD* mutants. However, it is well established that the primary pathway for repair of 8-oxodG in mammalian cells is not NER but rather the hOGG1-initiated BER pathway (*23*, *24*). The in vivo repair assay used in our experiments most likely detects mainly glycosylase-initiated BER events, although a limited contribution of NER to base removal cannot be ruled out. Hence, while NER is of course defective in *XPD* cell extracts, when removing either pyrimidine dimers or oxidized bases, the hOGG1-inititated BER process is not affected at all.

During revision of our manuscript, a paper has been published by Le Page et al. (*25*) showing that *XPD* human cells are normal in repair of 8-oxoG in transcribed or nontranscribed sequences, while cells from *XPD* patients with additional symptoms of Cockayne syndrome (*XPD*/*CS*) are defective in transcription-coupled repair of 8-oxoG. Hence, defective repair of 8-oxoG may be limited to transcribed regions in *XPD*/*CS* cells, thus explaining why no defect could be observed in our experiments that were performed analyzing global genome repair in *XPD* cells with mutations that do not confer CS.

Repair of 8-oxoG may even be slightly accelerated in some *XPD* mutants. We have found that repair of 8-oxodG is increased 2−3-fold in the *XPD* Chinese hamster cell line UV5 with respect to the parental cell line AA8 (Figure 3). Normal and mutant cells showed similar doubling times (data not shown), so that the increased repair ability of *XPD* cells was unlikely linked to differences in cell cycle. A limited acceleration of repair had also been observed in the human mutant GM434 (Figure 1), but whether this was genuinely linked to the *XPD* phenotype was unclear, given that no corrected GM434 cells were available. On the contrary, corrected UV5 (CXPD-5) cells have been developed (*11*) and showed a rate of 8-oxodG repair similar to that of control AA8 cells (Figure 3). Enhanced repair of 8-oxoG in UV5 was also observed in vitro by determining the repair replication capacities of cell extracts on substrates containing a single lesion. In these experiments no significant 8-oxoG BER improvement could be observed in *ERCC1* mutant cell extracts, thus suggesting that this effect may not be a general characteristic of hamster NER mutants (Figure 5). The presence of a slight acceleration of 8-oxodG repair in UV5 cells but not in human *XPD* cells may be linked to some influences of the hamster genetic background or, alternatively, to the type of mutation involved. Major differences may indeed occur in cellular phenotype according to the types of the *XPD* mutation *(26)*. Human GM434, GM 8207, and XP15PV *XPD* cells all have mutations in the XPD C-terminal domain, at the level of R_{683} that is substituted to W (in GM434 and GM8207) or Q (in XP15PV) (*27*). It has been demonstrated that these C-terminal mutations disrupt the interaction of XPD protein with p44, another subunit of TFIIH, with loss of the stimulatory effect of p44 on the helicase activity of XPD (*28*). As a consequence, the DNA helicase activity of the XPD protein is heavily impaired in the human mutants that we have analyzed. On the contrary, UV5 cells harbor a G → A transition mutation resulting in substitution of C_{116} by Y (*11*). The latter represents a nonconservative amino acid change that severely affects the repair function but does not fall in the C-terminal domain nor affects the DNA helicase activity of the protein (*11*). It may be hypothesized that improvement in 8-oxoG repair is associated with those rare mutations that involve the N-terminal domain of the protein and do not affect its DNA helicase activity, but certainly this point requires further investigations. In conclusion, the complementation group D of XP is proficient for repair of 8-oxodG. This does not rule out the possibility that accumulation of other endogenous lesions may be responsible for the increased neuronal death observed in XPD patients.

REFERENCES

1. Beckman, K. B., and Ames., B. N. (1997) *J. Biol. Chem*. *272*, 19633−19636.
2. Grollman, A. P., and Moriya, M. (1993) *Trends Genet. 9*, 246−249.
3. Kunkel, T. A. (1999) *Trends Genet*. *15*, 93−94.
4. Radicella, J. P., Dherin, C., Desmaze, C., Fox, M. S., and Boiteux, S. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8010−8015.
5. Roldàn-Arjona, T., Wei, W.-F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R.-P., Augustus, M., and Lindahl, T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8016−8020.
6. Prieto Alamo, M. J., Jurado, J., Francastel, E., and Laval, F. (1998) *Nucleic Acids Res. 26*, 5199−5202.
7. Satoh, M. S., and Lindahl, T. (1994) *Cancer Res. 54*, 1899s−1901s.
8. Robbins, J. H. (1989) *J. Child Neurol*. *4*, 143−146.
9. Reardon, J. T., Bessho, T., Kung, H. C., Bolton, P. H., and Sancar, A. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 9463−9468.
10. Dianov, G., Bischoff, C., Piotrowski, J., and Bohr, V. (1998) *J. Biol. Chem. 273*, 33811−33816.
11. Kadkhodayan, S., Salazar, E. P., Lamerdin, J. E., and Weber, C. A. (1996) *Somatic Cell Mol. Genet. 22*, 453−460.
12. Fraga, C. G., Shigenaga, M. K., Park, J.-W., Degan, P., and Ames, B. N. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 4533−4537.
13. Degan, P., Bonassi, S., DeCaterina, M., Korkina, L. G., Pinto, L., Scopacasa, F., Zatterale, A., Calzone, R., and Pagano, G. (1995) *Carcinogenesis 16*, 735−742.
14. Frosina G., Fortini, P., Rossi, O., Carrozzino, F., Raspaglio, G., Cox, L. S., Lane, D. P., Abbondandolo, A., and Dogliotti, E. (1996) *J. Biol. Chem. 271*, 9573−9578.
15. Frosina, G., Cappelli, E., Fortini, P., and Dogliotti, E. (1999) *Methods in Molecular Biology, Volume 113: DNA Repair Protocols − Eukaryotic Systems* (Henderson, D. S., Ed.) pp 301−315, Humana Press, Totowa, NJ.
16. Cappelli, E., Degan, P., and Frosina, G. (2000) *Carcinogenesis 21*, 1135−1141.

17. Tanaka, M., Lai, J. S., and Herr, W. (1992) *Cell 68*, 755−767.
18. Biade, S., Sobol, R. W., Wilson, S. H., and Matsumoto, Y. (1998) *J. Biol. Chem. 273*, 898−902.
19. Dianov, G., Price, A., and Lindahl, T. (1992) *Mol. Cell. Biol. 12*, 1605−1612.
20. Fortini, P., Parlanti, E., Sidorkina, O. M., Laval, J., and Dogliotti, E. (1999) *J. Biol. Chem. 274*, 15230−15236.
21. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, American Society for Microbiology Press, Washington, D.C.
22. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A., and Reinberg, D. (1994) *Nature 368*, 769−772.
23. Demple, B., and Harrison, L. (1994) *Annu. Rev. Biochem. 63*, 915−948.
24. Boiteux, S., and Radicella, J. P. (1999) *Biochimie 81*, 59−67.
25. Le Page, F., Kwoh, E. E., Avrutskaya, A., Gentil, A., Leadon, S. A., Sarasin, A., and Cooper, P. K. (2000) *Cell 101*, 159−171.
26. Berneburg, M., Clingen, P. H., Harcourt, S. A., Lowe, J. E., Taylor, E. M., Green, M. H. L., Krutmann, J., Arlett, C. F., and Lehmann, A. R. (2000) *Cancer Res. 60*, 431−438.
27. Taylor, E. M., Broughton, B. C., Botta, E., Stefanini, M., Sarasin, A., Jaspers, N. G. J., Fawcett, H., Harcourt, S. A., Arlett, C. F., and Lehmann, A. R. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8658−8663.
28. Coin, F., Marinoni, J.-C., Rodolfo, C., Fribourg, S., Pedrini, A. M., and Egly, J.-M. (1998) *Nature Genet. 20*, 184−188.

BI992610L