

DNA repair and sequence context affect $^1\text{O}_2$ -induced mutagenesis in bacteria

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ABSTRACT

Electronic excited molecular oxygen (singlet oxygen, $^1\text{O}_2$) is known to damage DNA, yielding mutations. In this work, the mutagenicity induced by $^1\text{O}_2$ in a defined sequence of DNA was investigated after replication in *Escherichia coli* mutants deficient for nucleotide and base excision DNA repair pathways. For this purpose a plasmid containing a $^1\text{O}_2$ -damaged 14 base oligonucleotide was introduced into *E. coli* by transfection and mutations were screened by hybridization with an oligonucleotide with the original sequence. Mutagenesis was observed in all strains tested, but it was especially high in the BH20 (*fpg*), AYM57 (*fpg mutY*) and AYM84 (*fpg mutY uvrC*) strains. The frequency of mutants in the *fpg mutY* strain was higher than in the triple mutant *fpg mutY uvrC*, suggesting that activity of the UvrABC excinuclease can favor the mutagenesis of these lesions. Additionally, most of the mutations were G→T and G→C transversions, but this was dependent on the position of the guanine in the sequence and on repair deficiency in the host bacteria. Thus, the kind of repair and the mutagenesis associated with $^1\text{O}_2$ -induced DNA damage are linked to the context of the damaged sequence.

INTRODUCTION

Oxidative DNA damage and mutagenesis have been proposed to be directly involved as possible causes of the aging process, neurological degenerative diseases and cancer (1). Reactive oxygen species, such as hydroxyl radicals, superoxide, hydrogen peroxide and singlet oxygen ($^1\text{O}_2$), are products of cell metabolism which may reach the genetic material yielding DNA damage. Cells have evolved different DNA repair systems that deal with these lesions, in order to maintain the integrity of this molecule. In some instances, however, mutations create a permanent sequel of oxidative DNA

damage (2). The DNA damage induced by $^1\text{O}_2$ is mainly at guanine sites and several DNA alterations have been identified when free 2'-deoxyguanosine (dG) or DNA is treated with $^1\text{O}_2$. The major type of DNA lesion induced by this oxidative agent is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), but other mutagenic and/or lethal lesions are also formed (3,4).

Many DNA repair pathways have been characterized as implicated in the removal of DNA damage induced by $^1\text{O}_2$. In *Escherichia coli*, formamidopyrimidine-DNA *N*-glycosylase (FPG) is probably the most important protein involved in oxidative DNA damage repair: this enzyme excises 8-oxodG, when paired with cytosine, and also recognizes several imidazole ring-opened purines. After removing the altered base by its glycosylase activity, it cleaves the phosphodiester bound at the abasic site formed, acting as an AP lyase (5,6). On the other hand, MutY glycosylase removes the unmodified base adenine when mispaired with 8-oxodG (7,8). That UvrABC-mediated nucleotide excision repair participates in removal of DNA oxidative lesions was first suggested after analysis of the transformation efficiency of plasmids treated with $^1\text{O}_2$. It was observed that plasmid inactivation by oxidative damage was more pronounced in bacteria deficient in the *fpg* and *uvrA* genes, compared to wild-type or single mutant strains, suggesting that FPG and UvrABC excinuclease complement each other in the repair of lethal damage induced by $^1\text{O}_2$ (9,10). Similar experiments indicated that exonuclease III (the *xth* gene) and endonuclease IV (the *nfo* gene) also participate in the repair of DNA damage induced by $^1\text{O}_2$ in independent and complementary pathways, probably acting on abasic sites and on deoxyriboses modified at the 3'-OH end causing blocks to DNA polymerase action (11).

The mutagenicity of $^1\text{O}_2$ -induced damage has been demonstrated in bacteria (12–14) and mammalian cells (15–18). As expected, mutations are mainly base substitutions affecting G:C base pairs, implicating mutations targeted to base damage. G:C→T:A transversions are interpreted as being due to the ability of 8-oxodG to mispair with dG, yielding these mutations after one round of DNA replication (19). The second class of common mutations are G:C→C:G transversions, but the

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lesion involved in induction of this kind of mutation is unknown (14,15,20,21).

In this work, the frequency and spectra of mutations induced by $^1\text{O}_2$ were analyzed by damaging a specific DNA sequence within a plasmid, which was then introduced by transfection in *E.coli* bacterial strains with different DNA repair deficiencies. A sequence of 14 nt was treated with $^1\text{O}_2$, generated by thermolysis of disodium 3,3'-(1,4-naphthylidene) dipropionate endoperoxide (NDPO₂), and used to construct the vector pUC3GN. After vector replication in bacteria, mutations were screened by hybridization. The results indicate that both G→T and G→C transversions are frequent mutations in the $^1\text{O}_2$ -damaged oligonucleotide, with clear differences in the position within the sequence. As expected, DNA repair deficiencies (*fpg*, *mutY* and *uvrC*) affect $^1\text{O}_2$ -induced mutagenesis but, surprisingly, MutY also reduces G→C mutations. Moreover, the activity of UvrABC may increase mutagenesis in *fpg mutY* strains. Thus, $^1\text{O}_2$ -induced DNA mutagenesis is affected by these DNA repair pathways and is dependent on the sequence context neighboring the lesion.

MATERIALS AND METHODS

Oligonucleotide treatment with singlet oxygen

The target oligonucleotide (5'-CATACCCGGGACAT-3', 2 µg) was incubated with 50 mM NDPO₂. Pure $^1\text{O}_2$ is generated through thermal decomposition of NDPO₂ for 1 h at 37°C (22). During thermal decomposition of the water-soluble NDPO₂ to 3,3'-(1,4-naphthylidene) dipropionate (NDP) and molecular oxygen, half of the oxygen is in the excited state (23). The maximal rate of $^1\text{O}_2$ generation was calculated by measuring NDP formation (24). For example, at 6 min after addition of 10 mM endoperoxide the maximal rate of $^1\text{O}_2$ generation was 38 µM/min, decreasing to 10 µM after 30 min. Thus, the steady-state of $^1\text{O}_2$ in the incubation medium was calculated to be 31×10^{-12} M at 6 min and 8×10^{-12} M at 30 min after addition of NDPO₂ (24). The oligonucleotide was purified by ethanol precipitation before pUC3GN construction.

Plasmids and bacteria

pUC3GN is a derivative of pUC8, containing a sequence of 14 nt treated with NDPO₂ before insertion into the vector, through the gapped duplex technique, described by Koehl *et al.* (25) and Lambert *et al.* (26). The oligonucleotide contains only three contiguous dG, which are the major target for $^1\text{O}_2$ treatment. Plasmid pUC3G contains the untreated oligonucleotide sequence and was used as a control. The plasmids were used to transform wild-type and repair-deficient *E.coli* strains. The bacterial strains used were AB1157 (F⁻ *thr-1 leuB6 proA2 his-4 thi-1 argE2 lacY1, galK2 rpsL supE44 ara-14 xyl-15 ml-1 tsx-33*), as wild-type, and its derivatives BH20 (*fpg*), AB1886 (*uvrA*), BW9101 (*xth*), AYM57 (*fpg mutY*) and AYM84 (*fpg mutY uvrC*).

Mutagenesis assay

After DNA transfection, the bacterial cells were spread on LB-agar plates supplemented with antibiotics for plasmid selection (27). The colonies were grown overnight at 37°C and potential mutants were screened by colony differential hybridization, using an oligonucleotide (20 bases) which includes the wild-

type target sequence as probe. The probe was 5'-labeled with T4 polynucleotide kinase employing [γ -³²P]ATP (Amersham Pharmacia Biotech). After autoradiography, colonies with decreased radioactive signal were selected and plasmid DNA purified (28). For each strain tested approximately 10 independent transfections and hybridization assays (each with around 100 colonies tested) were performed. The potentially mutated plasmids were sequenced by the DNA polymerase termination method (29).

RESULTS

A plasmid containing $^1\text{O}_2$ -induced damage in a specific sequence (pUC3GN) was transfected into different DNA repair-deficient strains and potentially mutated colonies were sequenced. The mutation frequency in each strain is shown in Table 1. There was an increase in mutagenesis with the damaged vector (pUC3GN) in all strains tested, when compared with the unmodified vector (pUC3G). This confirms the mutagenic consequences of $^1\text{O}_2$ -induced DNA damage. Although UvrABC (9,10) and exonuclease III, the product of the *xth* gene (14), have been implicated in the repair of $^1\text{O}_2$ -induced damage, the mutation frequencies in the single mutant strains BW9109 (*xth*) and AB1886 (*uvrA*) were similar to that found in the wild-type strain, AB1157. Thus, it is likely that these oxidative damage repair pathways are redundant. The BH20 (*fpg*), AYM57 (*fpg mutY*) and AYM84 (*fpg mutY uvrC*) strains exhibited mutation frequencies for the treated vector significantly higher than the wild-type strain. Curiously, the triple mutant AYM84 had a lower mutation frequency when compared to the double mutant AYM57. These data suggest that, in the *fpg mutY* background, UvrABC activity contributes to the increase in mutagenesis.

Table 1. Mutation frequency in the pUC3GN vector after replication in wild-type and DNA repair-deficient *E.coli* strains

Strain (plasmid) ^a	Genotype	Number of colonies	Number of mutants	Frequency (%)
AB1157 (pUC3G)	wt	1152	0	<0.087
AYM84 (pUC3G)	<i>fpg mutY uvrC</i>	1110	0	<0.09
AYM57 (pUC3G)	<i>fpg mutY</i>	984	0	<0.1
AB1157 (pUC3GN)	wt	1300	8	0.6
BW9109 (pUC3GN)	<i>xthA</i>	1846	9	0.5
AB1886 (pUC3GN)	<i>uvrA</i>	661	2	0.3
BH20 (pUC3GN)	<i>fpg</i>	1041	15	1.4 ^b
AYM84 (pUC3GN)	<i>fpg mutY uvrC</i>	1052	19	1.8 ^b
AYM57 (pUC3GN)	<i>fpg mutY</i>	1059	35	3.1 ^{b,c}

^apUC3G and pUC3GN correspond to control and $^1\text{O}_2$ -damaged plasmids, respectively.

^bValues statistically significant ($P < 0.05$) when compared to wild-type bacteria and ^cvalues significantly higher ($P < 0.05$) when compared with all other strains. Statistics were performed with the Bross test (42), which is used for rare events. Mutant proportions were also assessed by G -tests (log likelihood ratio tests) with Bonferroni's corrected levels of significance, with identical results (43).

Table 2. Frequency of G→T and G→C in pUC3GN after replication in wild-type and DNA repair-deficient *E.coli* strains

Strain	Number of colonies	Number of G→T	G→T frequency (%)	Number of G→C	G→C frequency (%)
AB1157 (wt)	1300	4	0.3	2	0.15
BW9109 (<i>xth</i>)	1846	5	0.27	1	0.05
BH20 (<i>fpg</i>)	1041	12	1.1	1	0.1
AYM84 (<i>fpg mutY uvrC</i>)	1059	12	1.1	7	0.66
AYM57 (<i>fpg mutY</i>)	1052	19	1.8	12	1.1

when compared with AYM57 (*fpg mutY*) (Tables 1 and 2). The overall decrease in mutagenesis was 1.7-fold, but the reduction was more pronounced at the third dG base (3.5-fold). Involvement of the UvrABC enzymes in the repair of ¹O₂-induced DNA damage has been proposed, but the lesion repaired by this system is unknown (9). The results shown in this work, however, indicate that UvrABC activity in strain AYM57 (*fpg mutY*) contributes to mutation fixation. Similar data were obtained for ionizing radiation-induced mutagenesis in double-stranded M13 DNA (39), i.e. G:C→C:G transversions were observed in DNA repair-proficient *E.coli* and not in *uvrA*-deficient cells. The authors also suggest that the nucleotide excision repair (UvrABC) pathway is implicated in the generation of this particular kind of mutation, by removing the damaged base when mispaired with an undamaged dG. The final product would be a transversion mutation due to excision of the damaged base. This model can also account for the G→C transversions at position G3 observed in the *fpg mutY* strain, described in this work. In this case, a deficiency in the ability to recognize the damaged dG (dG*) by FPG or the mispair dG*:dG by MutY would enrich substrates for nucleotide excision repair, which originates the mutation. Otherwise, in the triple mutant other DNA repair pathway(s) would remove the mispaired dG or a second round of replication would allow correct insertion of a dC pairing with the damaged base and the final result would be a decreased frequency of mutations in the absence of the UvrABC pathway.

The importance of sequence context to the mutagenesis induced by ¹O₂ is clear from the results presented here. In all strains studied the mutations occurred preferentially in guanine sites, as expected for ¹O₂ action (14). There is a distinct mutation pattern at the three guanines present in the target. Mutations were much more frequent at G1 and G3, with only 6 (of 77) mutations found at G2. Another interesting observation is the occurrence of G→T and G→C transversions predominantly at the G1 and G3 positions, respectively. These data are consistent with the idea that DNA structure at specific sequence contexts may influence the DNA repair/tolerance pathways that deal with damaged bases. Alternatively, different lesions may be produced at different positions of the DNA target. The lower frequency of mutations at G2 may simply be due to the induction of a lower frequency of damage at this position. The heterogeneous distribution of this damage in DNA has already been observed in ssDNA treated with NDPO₂ (40). However, the ability to deal with lesions at G2 by a more error-free DNA repair or replication mechanism may also account for the low frequency of mutagenesis at this position.

Mutations at the cytosine preceding G1 were also observed at low frequency (5 of 77, 6.5%). The presence of 8-oxodG in the G1 position could facilitate the occurrence of mutagenesis in the 5'-flanking nucleotide. In fact, experiments reporting *in vitro* replication assays, using an oligonucleotide with a single 8-oxodG as template, indicated that DNA polymerases could make an error at the 5' neighbor pyrimidine (41). Mutations at the 5'-flanking sites were also observed when a monomodified vector carrying 8-oxodG was transfected and replicated in mammalian cells (37). Thus, 8-oxodG at position G1 may be responsible not only for the targeted G→T transversions but also for these mutations at the 5'-flanking pyrimidine.

The effects of DNA sequence context on mutation induction by ¹O₂ indicate a strong role of DNA repair and replication mechanisms that confront DNA lesions. According to the data reported in this work, the participation of base excision repair, with FPG and MutY glycosylase, and nucleotide excision repair, with UvrABC, in ¹O₂-induced lesion repair may depend upon the position of the damage. A DNA sequence effect may be one of the explanations for the high frequency of G→C mutations found in several ¹O₂-induced (and other oxidative damage) mutational spectra studies (14,15,20). As analogous and/or homologous genes to these bacterial proteins have been found in human cells, it would be expected that the related mammalian proteins would also have their activity influenced by DNA sequence context, influencing mutational fingerprinting of tumor-inducing agents.

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REFERENCES

1. Briviba, K., Klotz, L.O. and Sies, H. (1997) Toxic and signaling effects of chemically or photochemically generated singlet oxygen in biological systems. *Biol. Chem.*, **378**, 1259–1265.
2. Sies, H. and Menck, C.F.M. (1992) Singlet oxygen induced DNA damage. *Mutat. Res.*, **275**, 367–375.
3. Ravanat, J.L. and Cadet, J. (1995) Reaction of singlet oxygen with 2'-deoxyguanosine and DNA. Isolation and characterization of the main oxidation products. *Chem. Res. Toxicol.*, **8**, 379–388.
4. Cadet, J., Berger, M., Douki, T., Morin, B., Raoul, S., Ravanat, J.L. and Spinelli, S. (1997) Effects of UV and visible radiation on DNA—final base damage. *Biol. Chem.*, **378**, 1275–1286.

5. Boiteux, S., O'Connor, T.R., Lederer, F., Gouyett, A. and Laval, J. (1990) Homogenous *Escherichia coli* FPG protein. *J. Biol. Chem.*, **265**, 3916–3922.
6. Boiteux, S.; Gajewski, E., Laval, J. and Dizdaroglu, M. (1992) Substrate specificity of *Escherichia coli* FPG protein (formamidopyrimidine-DNA glycosylase): excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, **31**, 106–110.
7. Michaels, M.L., Cruz, C., Grollman, A.P. and Miller, J.H. (1992) Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. *Proc. Natl Acad. Sci. USA*, **89**, 7022–7025.
8. Bulych, N.V., Varaprasad, C.V., Dorman, G., Miller, J.H., Eisenberg, M., Grollman, A.P. and Johnson, F. (1996) Substrate specificity of *Escherichia coli* MutY protein. *Biochemistry*, **35**, 13147–13156.
9. Czebot, H., Tudek, B., Lambert, B., Laval, J. and Boiteux, S. (1991) *Escherichia coli* FPG protein and UvrABC endonuclease repair DNA damage induced by methylene blue plus light *in vivo* and *in vitro*. *J. Bacteriol.*, **173**, 3419–3424.
10. Tudek, B., Laval, J. and Boiteux, S. (1992) SOS-independent mutagenesis in *lacZ* induced by methylene blue plus visible light. *Mol. Gen. Genet.*, **236**, 433–439.
11. Agnez, L.F., Costa de Oliveira, R.L., Di Mascio, P. and Menck, C.F.M. (1996) Involvement of *Escherichia coli* exonuclease III and endonuclease IV on the repair of singlet oxygen-induced DNA damage. *Carcinogenesis*, **17**, 1183–1185.
12. Decuyper-Debergh, D., Piette, J. and Van den Vorst, A. (1987) Singlet oxygen-induced mutations in M13 *lacZ* phage DNA. *EMBO J.*, **6**, 3155–3161.
13. Retél, J., Hoebbe, B., Braun, J.E.F., Lutgerink, J.T., Van den Akker, E., Wanamarta, A.H., Joenje, H. and Lafleur, M.V.M. (1993) Mutational specificity of oxidative DNA damage. *Mutat. Res.*, **299**, 165–182.
14. Agnez-Lima, L.F., Di Mascio, P., Napolitano, R.L., Fuchs, R.P.P. and Menck, C.F.M. (1999) Mutation spectrum induced by singlet oxygen in *Escherichia coli* deficient in exonuclease III. *Photochem. Photobiol.*, **70**, 505–511.
15. Costa de Oliveira, R., Ribeiro, D.T., Nigro, R.G., Di Mascio, P. and Menck, C.F.M. (1992) Singlet oxygen induced mutation spectrum in mammalian cells. *Nucleic Acids Res.*, **20**, 4319–4323.
16. Ribeiro, D.T., Madzak, C., Sarasin, A., Di Mascio, P., Sies, H. and Menck, C.F.M. (1992) Singlet oxygen induced DNA damage and mutagenicity in a single-strand SV40-based shuttle vector. *Photochem. Photobiol.*, **55**, 39–45.
17. Moriya, M. (1993) Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G:C→T:A transversions in simian kidney cells. *Proc. Natl Acad. Sci. USA*, **90**, 1122–1126.
18. Ribeiro, D.T., Costa de Oliveira, R., Di Mascio, P. and Menck, C.F.M. (1994) Singlet oxygen induces predominantly G to T transversions on a single-stranded shuttle vector replicated in monkey cells. *Free Radic. Res. Commun.*, **21**, 75–83.
19. Menck, C.F.M., Di Mascio, P., Agnez, L.F., Ribeiro, D.T. and Costa de Oliveira, R. (1993) Genetic deleterious effects of singlet oxygen. *Química Nova*, **16**, 328–336.
20. McBride, T.J., Schneider, J.E., Floyd, R.A. and Loeb, L.A. (1992) Mutations induced by methylene blue plus light in single-stranded M13mp2. *Proc. Natl Acad. Sci. USA*, **89**, 6866–6870.
21. Duarte, V., Muller, J.G. and Burrows, C.J. (1999) Insertion of dGMP and dAMP during *in vitro* DNA synthesis opposite an oxidized form of 7,8-dihydro-8-oxoguanine. *Nucleic Acids Res.*, **27**, 490–502.
22. Pierlot, C., Aubry, J.-M., Briviba, K., Sies, H. and Di Mascio, P. (2000) Naphthalene endoperoxides as generators of singlet oxygen in biological media. *Methods Enzymol.*, **319**, 3–20.
23. Di Mascio, P. and Sies, H. (1989) Quantification of singlet oxygen generated by thermolysis of 3,3'-(1,4-naphthylidene) dipropionate. Monomer and dimol photoemission and effects of 1,4-diazabicyclo[2.2.2]octane. *J. Am. Chem. Soc.*, **111**, 2909–2914.
24. Di Mascio, P., Wefers, H., Do-Thi, H.P., Lafleur, M.V.H. and Sies, H. (1989) Singlet molecular oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. *Biochim. Biophys. Acta*, **1007**, 151–157.
25. Koehl, P., Burnouf, D. and Fuchs, R.P.P. (1989) Construction of plasmids containing a unique acetylaminofluorene adduct located within a mutation hot spot: a new probe for frameshift mutagenesis. *J. Mol. Biol.*, **207**, 355–364.
26. Lambert, I.B., Napolitano, R.L. and Fuchs, R.P.P. (1992) Carcinogen-induced frameshift mutagenesis in repetitive sequences. *Proc. Natl Acad. Sci. USA*, **89**, 1310–1314.
27. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
28. Birboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.*, **7**, 1513–1523.
29. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA*, **74**, 5463–5467.
30. Shibutani, S., Takeshita, M. and Grollman, A.P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.
31. Wood, M.L., Dizdaroglu, M., Gajewski, E. and Essigmann, J. (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry*, **29**, 7024–7032.
32. Floyd, R.A., West, M.S., Eneff, K.L. and Schneider, J.E. (1989) Methylene blue plus light mediates 8-hydroxyguanine formation in DNA. *Arch. Biochem. Biophys.*, **273**, 106–111.
33. Schneider, J.E., Price, S., Maldt, L., Gutteridge, J.M.C. and Floyd, R.A. (1990) Methylene blue plus light mediates 8-hydroxy-2'-deoxyguanosine formation in DNA preferentially over strand breakage. *Nucleic Acids Res.*, **18**, 631–635.
34. Devasagayam, T.P.A., Steenken, S., Obendorf, M.S.W., Schulz, W.A. and Sies, H. (1991) Formation of 8-hydroxy-deoxyguanine and generation of strand breaks at guanine residues in DNA by singlet oxygen. *Biochemistry*, **30**, 6283–6289.
35. Zhang, Q.-M., Ishikawa, N., Nakahara, T. and Yonei, S. (1998) *Escherichia coli* MutY protein has a guanine-DNA glycosylase that acts on 7,8-dihydro-8-oxoguanine: guanine mispair to prevent spontaneous G:C to C:G transversions. *Nucleic Acids Res.*, **26**, 4669–4675.
36. Kamiya, H., Murata-Kamiya, N., Koizume, S., Inoue, H., Nishimura, S. and Ohtsuka, E. (1995) 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-ras gene: effects of sequence contexts on mutation spectra. *Carcinogenesis*, **16**, 883–889.
37. Kuipers, G.K., Slotman, B.J., Poldevaart, H.A., Reitsma-Wijker, C.A. and Lafleur, V.M. (2000) The influence of combined FPG- and MutY-deficiency on the spontaneous and γ -radiation-induced mutation spectrum in the *lacZ* α gene of M13mp10. *Mutat. Res.*, **461**, 189–195.
38. Schulz, I., Mahler, H.C., Boiteux, S. and Epe, B. (2000) Oxidative DNA base damage induced by singlet oxygen and photosensitization: recognition by repair endonucleases and mutagenicity. *Mutat. Res.*, **461**, 145–156.
39. Braun, J.E., Wanamarta, A.H., Westmijze, E.J., Wientjes, N.M., Wijker, C.A., Lafleur, M.V.H. and Retél, J. (1997) Influence of nucleotide excision repair of *Escherichia coli* on radiation-induced mutagenesis of double-stranded M13 DNA. *Mutat. Res.*, **384**, 45–53.
40. Ribeiro, D.T., Bourre, F., Sarasin, A., Di Mascio, P. and Menck, C.F.M. (1992) DNA synthesis blocking lesions induced by singlet oxygen are targeted to deoxyguanosines. *Nucleic Acids Res.*, **20**, 2465–2469.
41. Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S. (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature*, **327**, 77–79.
42. Bross, I. (1954) A confidence interval for a percentage increase. *Biometrics*, **10**, 245–250.
43. Sokal, R.R. and Rohlf, F.J. (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd Edn. W.H. Freeman & Co., New York, NY.

