Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6443–6447, June 1996
Biochemistry

Human MutSα recognizes damaged DNA base pairs containing O^6-methylguanine, O^4-methylthymine, or the cisplatin-d(GpG) adduct

(DNA repair/mismatch repair/DNA alkylation)

DEREK R. DUCKETT*, JAMES T. DRUMMOND*, ALASTAIR I. H. MURCHIE†, JOYCE T. REARDON‡, AZIZ SANCAR‡, DAVID M. J. LILLEY†, AND PAUL MODRICH*§

*Howard Hughes Medical Institute and Department of Biochemistry, Duke University Medical Center, Durham, NC 27710; †Cancer Research Campaign Nucleic Acid Structure Research Group and Department of Biochemistry, The University, Dundee DD1 4HN, United Kingdom; and ‡Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599

Contributed by Paul Modrich, March 25, 1996

ABSTRACT **Bacterial and mammalian mismatch repair systems have been implicated in the cellular response to certain types of DNA damage, and genetic defects in this pathway are known to confer resistance to the cytotoxic effects of DNA-methylating agents. Such observations suggest that in addition to their ability to recognize DNA base-pairing errors, members of the MutS family may also respond to genetic lesions produced by DNA damage. We show that the human mismatch recognition activity MutSα recognizes several types of DNA lesion including the 1,2-intrastrand d(GpG) crosslink produced by *cis*-diamminedichloroplatinum(II), as well as base pairs between O^6-methylguanine and thymine or cytosine, or between O^4-methylthymine and adenine. However, the protein fails to recognize 1,3-intrastrand adduct produced by *trans*-diamminedichloroplatinum(II) at a d(GpTpG) sequence. These observations imply direct involvement of the mismatch repair system in the cytotoxic effects of DNA-methylating agents and suggest that recognition of 1,2-intrastrand *cis*-diamminedichloroplatinum(II) adducts by MutSα may be involved in the cytotoxic action of this chemotherapeutic agent.**

Strand-specific mismatch repair systems have been described in both bacteria and eukaryotic cells. The *Escherichia coli* methyl-directed repair system acts on newly replicated DNA to correct biosynthetic errors that have escaped the proofreading function of the replicative DNA polymerase (1, 2). Biochemical analysis of the methyl-directed reaction has demonstrated that products of the *mutS* and *mutL* genes play key roles in initiation of the excision reaction, which removes an incorrectly inserted nucleotide from the new DNA strand. The MutS protein is responsible for mismatch recognition (3, 4), with subsequent binding of MutL to the MutS–mismatch complex (5), probably serving to interface mismatch recognition by MutS to activities required for subsequent steps of repair (6, 7).

In addition to their roles in the processing of mismatched Watson–Crick bases, bacterial MutS and MutL have been implicated in cellular responses to certain types of DNA damage. *E. coli mutS* and *mutL* mutants are resistant to the cytotoxic effects of DNA damage produced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; refs. 8 and 9) and *cis*-diamminedichloroplatinum(II) (cisplatin or *cis*-DDP) (10). Products of the bacterial *mut* genes have also been implicated in UV-induced recombination (11, 12) and transcription-coupled repair of pyrimidine dimers (13). Although the simplest interpretation of these observations is that interaction of MutS and MutL with MNNG-, cisplatin-, or UV-induced DNA damage plays a role in cellular responses to such lesions, the ability of bacterial MutS to recognize damage produced by these agents has not been reported. Consequently the distinction between direct versus indirect participation of MutS and MutL in these effects has been uncertain.

Mammalian cells have a mismatch repair system that is similar to the bacterial pathway with respect to specificity, mechanism, and dependence on proteins homologous to MutS and MutL (2, 14). In human cells the MutSα heterodimer (hMutSα), comprised of the MutS homologs MSH2 and GTBP/p160, is apparently sufficient to provide the mismatch recognition function (15, 16), while MutL function is provided by the MutLα heterodimer of MLH1 and PMS2 (17). Defects in any of the four genes encoding these polypeptides results in a mutator phenotype (18–20) and mismatch repair deficiency (15, 20–25).

Selection of mammalian cell lines resistant to the cytotoxic effects of MNNG or *N*-methyl-*N*-nitrosourea (MNU) frequently yields mutants deficient in mismatch repair (21, 26, 27). Conversely, at least some mismatch repair-deficient cell lines are resistant to killing by these agents (28). Genetic analysis of several MNU- and MNNG-resistant cell lines has demonstrated that resistance is associated with deficiency of MLH1 or GTBP/p160 (20, 28), but cell lines deficient in the other subunits of MutLα or MutSα have not been tested for this phenotype. The alkylation-tolerant phenotype associated with mismatch repair deficiency has been interpreted in terms of direct participation of the repair system in the sequence of events that results in cell death upon exposure to MNNG or MNU (21, 29). However, because recognition of MNNG or MNU damage by mammalian MutSα has not been demonstrated, an indirect role of the repair system in the cytotoxic response to these agents has not been excluded.

The involvement of MutS and MutL activities in cellular responses to DNA damage coupled with their established role in rectification of DNA metabolic errors has led to the suggestion that these proteins may serve as general sensors of genetic damage (21, 28). Indeed, repair-deficient, alkylation-tolerant cell lines display a G2 checkpoint defect upon challenge with MNNG (18) or exposure to the base analog 6-thioguanine (30). We report here that human MutSα efficiently recognizes several forms of DNA damage, including base pairs involving O^6-methylguanine (O^6meG), O^4-methylthymine (O^4meT), and the cisplatin-(GpG) adduct. Our findings are consistent with a direct role of the human mis-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; O^6meG, O^6-methylguanine; O^6meT, O^4-methylthymine; DDP, diamminedichloroplatinum(II); S^6G, 6-thioguanine; HMG, high mobility group.

§To whom reprint requests should be addressed.

Table 1. Oligonucleotides used in this paper

Oligonucleotide no.	Sequence
1	5′-GCTAGCAAGCT**O⁶meG**TCGATTCTAGAAATTCGGC
2	5′-GCTAGCAAGCT**O⁴meT**TCGATTCTAGAAATTCGGC
3	5′-GCTAGCAAGCT**S⁶G**TCGATTCTAGAAATTCGGC
4	5′-GCTAGCAAGCT**G**TCGATTCTAGAAATTCGGC
5	5′-GCCGAATTTCTAGAATCGA**A**AGCTTGCTAGC
6	5′-GCCGAATTTCTAGAATCGA**G**AGCTTGCTAGC
7	5′-GCCGAATTTCTAGAATCGA**C**AGCTTGCTAGC
8	5′-GCCGAATTTCTAGAATCGA**T**AGCTTGCTAGC
9	5′-TCCCTCCTTCCTTCC**GG**CCCTCCTTCCCCTTC
10	5′-TCCCTCCTTCCTTCC**AG**CCCTCCTTCCCCTTC
11	5′-TCCCTCCTTCCTTCT**GTG**TCCTCCTTCCCCTTC
12	5′-GAAGGGGAAGGAGGG**CC**GGAAGGAAGGAGGGA
13	5′-GAAGGGGAAGGAGGG**CT**GGAAGGAAGGAGGGA
14	5′-GAAGGGGAAGGAGGA**CA**CAGAAGGAAGGAGGGA

Nucleotides shown in boldface type indicate the location of a G-T mispair or DNA lesion after hybridization with the appropriate complementary strand.

match recognition activity in the cytotoxic response provoked by certain types of DNA damage.

MATERIALS AND METHODS

Proteins and Oligonucleotides. Human MutSα was purified as described (15). Oligonucleotides used in this study (Table 1; purchased from Oligos Etc., Wilsonville, OR) were purified by gel electrophoresis through 20% polyacrylamide (wt/vol) in the presence of 7 M urea. Oligonucleotides were recovered by excision of bands, electroelution, and ethanol precipitation. Platinated derivatives of oligonucleotides 9, 10, and 11 (Table 1) were prepared by reaction with 0.1 mM *cis*-DDP (cisplatin; Aldrich) or *trans*-DDP (transplatin; Aldrich) in 100 μl of 10 mM Tris·HCl (pH 7.5) for 24 h at 37°C (31, 32). Unreacted cisplatin or transplatin was removed by chromatography on Sephadex G25 (Pharmacia), platinated DNA precipitated with three volumes of ethanol, and purified by denaturing gel electrophoresis (32). The cis- and transplatinated oligonucleotides were significantly retarded relative to unreacted oligonucleotide. Oligonucleotides were labeled using [γ-^{32}P]ATP (3000 Ci/mmol; DuPont/NEN) and T4 polynucleotide kinase (Amersham), and unincorporated label was removed by chromatography on Sephadex G25.

Duplex molecules were constructed by mixing approximate molar equivalents of a [5′-^{32}P]-oligonucleotide with an unlabeled complementary sequence. The solution was heated to 90°C in 10 mM Tris·HCl (pH 7.5) and 100 mM KCl and slow cooled to 15°C over 90-min period using a Perkin–Elmer Gene Amp 9600 thermocycler.

Gel Electrophoretic Retardation Assay. Reactions (15 μl) contained 10 mM Hepes·KOH (pH 7.6), 100 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 50 mg of bovine serum albumin per ml, [5′-^{32}P]oligonucleotide duplex (10 fmol), unlabeled 31-bp duplex competitor (200 fmol, oligonucleotides 4 and 7 in Table 1), and hMutSα heterodimer as indicated. After 10-min incubation on ice, reactions were supplemented with 1.5 μl of 10 mM Hepes, pH 7.6/50% sucrose. Samples were immediately loaded onto a 6% polyacrylamide gel [36:1 acrylamide/bisacrylamide (wt/wt)] and subjected to electrophoresis at 4°C in 20 mM Tris-acetate (pH 7.6), 1 mM $MgCl_2$ at 8 V/cm with buffer recirculation (1 liter/h). Gels were dried onto Whatman 3-MM paper and autoradiographed at −70°C using Kodak Biomax x-ray film with Ilford fast tungstate intensifier screens. In some cases the relative amounts of DNA species were quantitated using a Molecular Dynamics PhosphorImager.

RESULTS

hMutSα Binds to Base Pairs Containing O⁶-Methylguanine or O⁴-Methylthymine. The DNA-methylating agents MNNG and MNU are both mutagenic and cytotoxic. Because inactivation of the O⁶-methylguanine-DNA methyltransferase renders cells hypersensitive to mutagenesis and killing by these agents (33), both effects have been attributed to DNA lesions processed by this activity, namely O⁶meG and, to a lesser degree, O⁴meT in the case of the human protein (21, 29, 34). Because a functional mismatch repair system has been implicated in the cytotoxic effect of MNNG and MNU on mammalian cells (21, 26–28), we have used gel retardation assay to test human MutSα for its ability to recognize base pairs containing O⁶meG and O⁴meT. In the presence of excess homoduplex competitor DNA, hMutSα binds specifically and with similar affinities to 31-bp oligonucleotide duplexes containing either an O⁶meG-C or an O⁶meG-T base pair (Fig. 1). The latter nucleotide pair expected to occur at significant frequency upon replication of a DNA template containing O⁶meG (35). We estimate the binding constants that govern interaction of protein with these O⁶meG-containing base pairs to be about an order of magnitude less than that for a G-T mismatch (compare Fig. 1 with lane 2 of Fig. 2). Human MutSα binds with similar affinity to an oligonucleotide duplex containing an O⁴meT-A base pair, but binds only weakly to an oligonucleotide duplex containing O⁴meT-G (Fig. 2). Of the

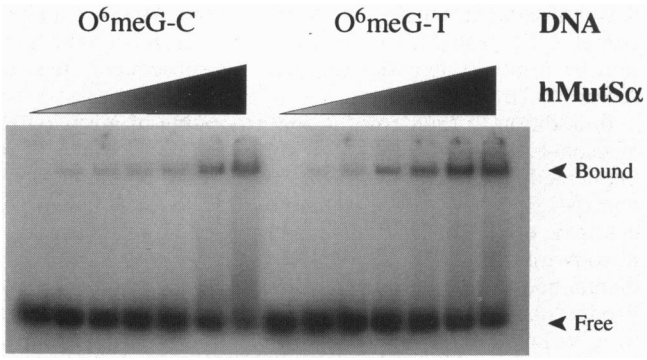

FIG. 1. MutSα binds to O⁶meG-C and O⁶meG-T base pairs. Gel shift binding assays were performed in reactions containing 12 nM G-C homoduplex competitor (oligonucleotides 4 and 7) and 0.6 nM 5′-^{32}P-labeled O⁶meG-C heteroduplex (oligonucleotides 1 and 7) or O⁶meG-T heteroduplex (oligonucleotides 1 and 8). The concentration of hMutSα heterodimer was varied in each series (0 nM, 0.8 nM, 1.6 nM, 3.3 nM, 6.6 nM, 13 nM, or 26 nM) as indicated.

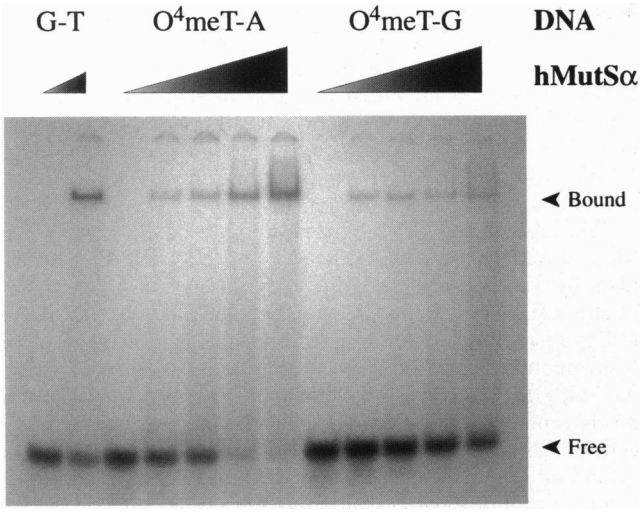

FIG. 2. hMutSα binds to the O⁴meT-A base pair. Binding reactions contained 12 nM G-C homoduplex competitor (oligonucleotides 4 and 7) and 0.6 nM 5′-^{32}P-labeled G-T (oligonucleotides 4 and 8), O⁴meT-A (oligonucleotides 2 and 5), or O⁴meT-G (oligonucleoties 2 and 6) heteroduplexes as indicated. In the G-T reactions shown in the left two lanes, hMutSα was either absent or present at 2.6 nM. In the O⁴meT-A and O⁴meT-G reaction series, hMutSα concentration was varied (0 nM, 3.3 nM, 6.6 nM, 13 nM, or 26 nM) as indicated.

eight possible base mispairs, hMutSα binds with highest affinity to the G-T mismatch, which we have used here as the basis for comparison, but exhibits reduced affinity for other mispairs that are nevertheless corrected by the human repair system (D.R.D., J.T.D., and P.M., unpublished data). Consequently, binding affinities for O⁶meG-C, O⁶meG-T, and O⁴meT-A pairs described here would be consistent with elicitation of a biological response.

In addition to their resistance to MNNG and MNU, mismatch repair-deficient mammalian cells are also resistant to the cytotoxic effects of the base analog 6-thioguanine (S⁶G; refs. 30 and 36), an effect that may be due to structural similarities between S⁶G and O⁶meG (30, 37). Experiments similar to those shown in Figs. 1 and 2 have demonstrated that hMutSα binds to a 31-bp duplex that contains one S⁶G-T base pair (oligonucleotides 3 and 8 in Table 1) with an affinity similar to that for DNAs containing O⁶meG (data not shown). However, the protein binds only weakly to an otherwise identical 31-bp duplex containing a S⁶G-C base pair.

hMutSα Binds to Cisplatinated GpG. The *cis* isomer of DDP (cisplatin or *cis*-DDP) displays antibacterial and anticancer activity, but the *trans* isomer (transplatin or *trans*-DDP) does not (reviewed in refs. 38 and 39). The major products of cisplatination of DNA are 1,2-intrastrand crosslinks between purine N7 atoms in the dinucleotide sequences d(GpG) (65%) and d(ApG) (25%), with minor products including the 1,3-crosslink between guanines in trinucleotide sequence d(GpNpG) (6%) and monoadducts (40). Steric constraints preclude formation of 1,2-intrastrand crosslinks by transplatin, although this isomer can form 1,3-crosslinks (41).

E. coli mutL mutations confer resistance to the cytotoxic effects of cisplatin (10), and human cell lines resistant to cisplatin have been found to display frequent mutations in microsatellite sequences and attenuation of p53 function (42). Because the former phenotype is often associated with mismatch repair deficiency (43), we have tested the affinity of hMutSα for several cisplatin adducts as well as the transplatin 1,3-intrastrand-d(GpTpG) crosslink. As shown in Fig. 3, hMutSα binds tightly to the cisplatin 1,2-d(GpG) crosslink, but little if any binding was observed with the transplatin 1,3-d(GpTpG) adduct.

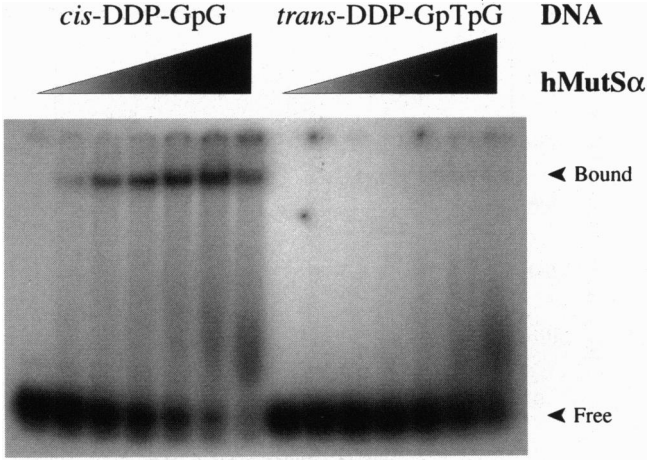

FIG. 3. hMutSα binds to cisplatin 1,2-d(GpG) but not transplatin 1,3-d(GpTpG) adducts. Binding reactions contained 12 nM G-C homoduplex competitor (oligonucleotides 4 and 7), 0.6 nM 5′-^{32}P-labeled duplex DNA containing a single cisplatin 1,2-d(GpG) crosslink (cisplatinated oligonucleotide 9 hybridized to oligonucleotide 12) or a single transplatin 1,3-d(GpTpG) adduct (transplatinated oligonucleotide 11 hybridized to oligonucleotide 14). Concentration of hMutSα was varied (0 nM, 0.8 nM, 1.6 nM, 3.3 nM, 6.6 nM, 13 nM, or 26 nM) as indicated.

The specificity of hMutSα for the cisplatin 1,2-d(GpG) adduct was further assessed by competition methods. Complexes of the human protein with a 31-bp G-T heteroduplex or a 32-bp duplex containing a cisplatin 1,2-d(GpG) crosslink were similarly resistant to competition by a 31-bp homoduplex competitor, with binding reduced only 20–40% by a 160-fold molar excess of the competing DNA (Fig. 4, *Upper*). Furthermore, the oligonucleotide duplex containing a cisplatin 1,2-d(GpG) crosslink effectively competed with the G-T heteroduplex for hMutSα binding, whereas a similar duplex containing a transplatin 1,3-d(GpTpG) adduct did not (Fig. 4, *Lower*). Fifty percent inhibition of G-T heteroduplex binding occurred at a ≈20:1 molar ratio of the cisplatin 1,2-d(GpG) duplex to the G-T substrate, indicating a binding constant for the cisplatin 1,2-d(GpG) crosslink about an order of magnitude less than that for a G-T mispair. As mentioned above, binding constants of this magnitude are consistent with production of a biological response.

As observed with the transplatin 1,3-crosslink, we have found that hMutSα binds poorly to the cisplatin 1,2-d(ApG) or 1,3-d(GpTpG) adducts. In the sequence contexts employed here, the human protein thus displays high specificity for the 1,2-intrastrand crosslink produced by cisplatination of the d(GpG) dinucleotide.

DISCUSSION

Observations implicating mismatch repair in the cellular response to certain types of DNA damage have been attributed to recognition of DNA lesions by components of this repair system (8–10, 21, 29), but direct evidence on this point has been lacking. Our results show that human MutSα efficiently recognizes O⁶meG-C, O⁶meG-T, and O⁴meT-A base pairs. Because the cytotoxicity of MNNG and MNU are largely attributable to the production of O⁶meG (18, 33), this binding specificity is consistent with direct participation of the human mismatch repair system in the cytotoxic response that occurs upon cellular exposure to these DNA-alkylating agents.

We have also shown that hMutSα binds with high specificity to the cisplatin 1,2-d(GpG) crosslink, the major product produced upon cisplatination of duplex DNA (42). Nevertheless, the protein fails to bind with detectable affinity to the

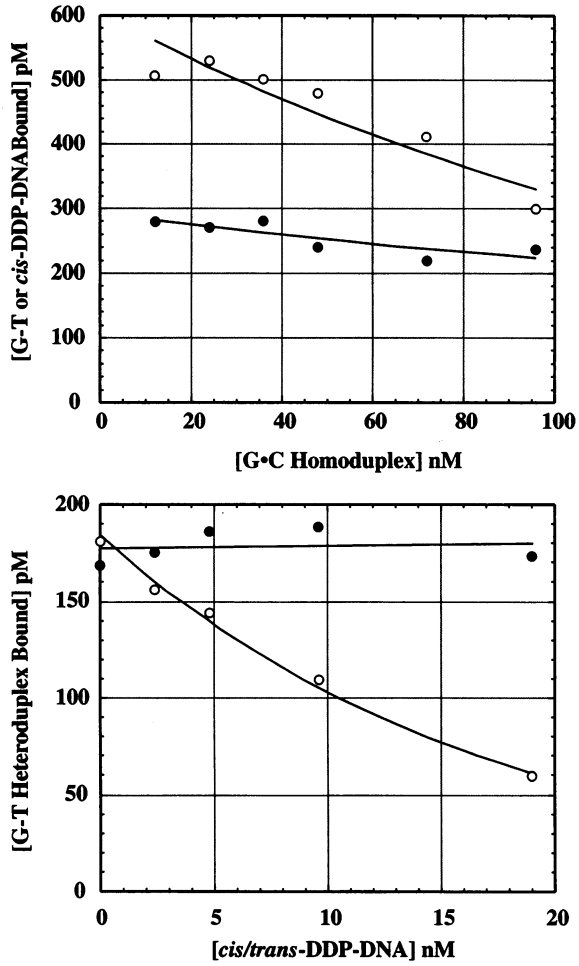

FIG. 4. Specificity of hMutSα for a cisplatin 1,2-d(GpG) adduct by competition assay. (*Upper*) Binding of hMutSα to a G-T heteroduplex (oligonucleotides 4 and 8) or to a duplex containing a single cisplatin 1,2-d(GpG) crosslink (cisplatinated oligonucleotide 9 and oligonucleotide 12) in the presence of competitor G-C homoduplex DNA (oligonucleotides 4 and 7). Binding reactions contained 0.6 nM 5′-^{32}P-labeled G-T heteroduplex or cisplatinated duplex, and 4 nM (G-T reactions) or 10 nM [cisplatin 1, 2-d(GpG) reactions] hMutSα. Unlabeled homoduplex competitor was present as indicated on the abscissa [-○-, hMutSα·G-T complexes; and -●-, hMutSα–cisplatin 1,2-d(GpG) complexes]. (*Lower*) Binding of hMutSα to G-T heteroduplex in the presence of unlabeled competitor DNA containing a single cisplatin 1,2-d(GpG) or a single transplatin 1,3-d(GpTpG) adduct (transplatinated oligonucleotide 11 hybridized to oligonucleotide 14). Binding reactions contained 0.6 nM 5′-^{32}P-labeled G-T heteroduplex, 2 nM hMutSα, and unlabeled cisplatinated or transplatinated competitor as indicated [-○-, cisplatin 1, 2-d(GpG) competitor; and -●-, transplatin 1,3-d(GpTpG) competitor].

1,3-intrastrand adduct produced by transplatin, a compound that cannot produce 1,2-crosslinks and displays little cytotoxicity and no anticancer activity (10, 38, 41). As noted above, *E. coli* mutants deficient in mismatch repair are resistant to the cytotoxic effects of cisplatin, and human cell lines resistant to cisplatin exhibit frequent mutations in microsatellite sequences and apparent attenuation of p53 function (42), with the former phenotype characteristic of mismatch repair deficiency (43). In fact, we have recently found several cisplatin-resistant human cell lines to be deficient in mismatch repair (J.T.D., R. Brown, and P.M., unpublished data). Recognition of the cisplatin 1,2-d(GpG) adduct by hMutSα may therefore play a role in the cytotoxic action of this anticancer compound. It is important to note that proteins of the high mobility group (HMG) family also bind to cisplatin 1,2-crosslinks (44, 45). Binding of HMG domain proteins has been found to inhibit *in vitro* repair of 1,2-d(GpG) adducts by the human excision repair system (46), and deficiency of the *Saccharomyces cerevisiae IXR1* product, which contains an HMG box, is associated with increased resistance to cisplatin (47). To our knowledge, however, genetic alteration of the intracellular levels of HMG proteins has not been described in cisplatin-resistant mammalian cells.

Although mismatch repair defects have been implicated in the cytotoxicity of MNNG, MNU, and cisplatin, the mechanism by which lesion recognition by this system leads to cell death is still uncertain. The favored model suggests that cell killing results from translesion synthesis when the replication fork encounters a miscoding adduct in the template strand (18, 33), with the presence of the ensuing base pair anomaly in the newly replicated region provoking mismatch repair. Because action of this system is restricted to the new DNA strand, the offending lesion remains in the parental template leading to turnover of newly synthesized DNA by a reiterative process of excision and resynthesis, an effect postulated to result in cell death. It is important to note, however, that alternate mechanisms have not been excluded. For example, it is possible that recognition of damaged base pairs by hMutSα may lead to cell death by interface with systems other than the excision pathway involved in processing of replication errors. It is interesting to note that precedent for differential fates of a mismatch–MutS complex is available in the *E. coli* system. For example, the MutS- and MutL-dependent fate of G-T mismatch depends on its origin as a replication error or as the product of deamination of 5-methylcytosine in 5-methylcytosine-guanine base pair in resting DNA (reviewed in refs. 6 and 48). A similar phenomenon has been described with respect to function of MutS and MutL in recombination fidelity where the two proteins act in concert to block recombinational exchanges between DNA sequences that have diverged at the nucleotide level (49, 50). This effect, which has been attributed to recognition of mismatched base pairs within the recombination heteroduplex (51, 52), is apparently independent of the excision system that removes replication errors (49, 50). The fate of DNA lesion recognized by bacterial MutS family can therefore depend on the context in which it was produced.

This work was supported in part by Grants GM45190 (to P.M.) and GM32833 (to A.S.) from the National Institute of General Medical Sciences and by the Cancer Research Campaign (to D.M.J.L.)

1. Meselson, M. (1988) in *Recombination of the Genetic Material*, ed. Low, K. B. (Academic, San Diego), pp. 91–113.
2. Modrich, P. & Lahue, R. (1996) *Annu. Rev. Biochem.* **65,** 101–133.
3. Su, S.-S. & Modrich, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5057–5061.
4. Su, S.-S., Lahue, R. S., Au, K. G. & Modrich, P. (1988) *J. Biol. Chem.* **263,** 6829–6835.
5. Grilley, M., Welsh, K. M., Su, S.-S. & Modrich, P. (1989) *J. Biol. Chem.* **264,** 1000–1004.
6. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253.
7. Sancar, A. & Hearst, J. E. (1993) *Science* **259,** 1415–1420.
8. Jones, M. & Wagner, R. (1981) *Mol. Gen. Genet.* **184,** 562–563.
9. Karran, P. & Marinus, M. (1982) *Nature (London)* **296,** 868–869.
10. Fram, R. J., Cusick, P. S., Wilson, J. M. & Marinus, M. G. (1985) *Mol. Pharmacol.* **28,** 51–55.
11. Feng, W. Y., Lee, E. H. & Hays, J. B. (1991) *Genetics* **129,** 1007–1020.
12. Feng, W.-Y. & Hays, J. B. (1995) *Genetics* **140,** 1175–1186.
13. Mellon, I. & Champe, G. N. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1292–1297.
14. Kolodner, R. D. & Alani, E. (1994) *Curr. Opin. Biotech.* **5,** 585–594.
15. Drummond, J. T., Li, G.-M., Longley, M. J. & Modrich, P. (1995) *Science* **268,** 1909–1912.

16. Palombo, F., Gallinari, P., Iaccarino, I., Lettieri, T., Hughes, M., D'Arrigo, A., Truong, O., Hsuan, J. J. & Jiricny, J. (1995) *Science* **268,** 1912–1914.
17. Li, G. M. & Modrich, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1950–1954.
18. Goldmacher, V. S., Cuzick, R. A. & Thilly, W. G. (1986) *J. Biol. Chem.* **261,** 12462–12471.
19. Eshleman, J. R. & Markowitz, S. D. (1995) *Curr. Opin. Oncol.* **7,** 83–89.
20. Papadopoulos, N., Nicolaides, N. C., Liu, B., Parsons, R., Lengauer, C., Palombo, F., D'Arrigo, A., Markowitz, S., Willson, J. K. V., Kinzler, K., Jiricny, J. & Vogelstein, B. (1995) *Science* **268,** 1915–1917.
21. Kat, A., Thilly, W. G., Fang, W. H., Longley, M. J., Li, G. M. & Modrich, P. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6424–6428.
22. Parsons, R., Li, G. M., Longley, M. J., Fang, W. H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B. & Modrich, P. (1993) *Cell* **75,** 1227–1236.
23. Papadopoulos, N., Nicolaides, N. C., Wei, Y.-F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Peterson, G. M., Watson, P., Lynch, H. T., Peltomäki, P., Mecklin, J.-P., de la Chapelle, A., Kinzler, K. W. & Vogelstein, B. (1994) *Science* **263,** 1625–1629.
24. Umar, A., Boyer, J. C., Thomas, D. C., Nguyen, D. C., Risinger, J. I., Boyd, J., Ionov, Y., Perucho, M. & Kunkel, T. A. (1994) *J. Biol. Chem.* **269,** 14367–14370.
25. Risinger, J. I., Umar, A., Barrett, J. C. & Kunkel, T. A. (1995) *J. Biol. Chem.* **270,** 18183–18186.
26. Branch, P., Aquilina, G., Bignami, M. & Karran, P. (1993) *Nature (London)* **362,** 652–654.
27. Aquilina, G., Hess, P., Fiumicino, S., Ceccotti, S. & Bignami, M. (1995) *Cancer Res.* **55,** 2569–2575.
28. Koi, M., Umar, A., Chauhan, D. P., Cherian, S. P., Carethers, J. M., Kunkel, T. A. & Boland, C. R. (1994) *Cancer Res.* **54,** 4308–4312.
29. Karran, P. & Bignami, M. (1994) *Bioessays* **16,** 833–839.
30. Hawn, M. T., Umar, A., Carethers, J. M., Marra, G., Kunkel, T. A., Boland, C. R. & Koi, M. (1995) *Cancer Res.* **55,** 3721–3725.
31. Pinto, A. L., Naser, L. J., Essigmann, J. M. & Lippard, S. J. (1986) *J. Am. Chem. Soc.* **108,** 7405–7407.
32. Murchie, A. I. H. & Lilley, D. M. J. (1993) *J. Mol. Biol.* **233,** 77–85.
33. Karran, P. & Bignami, M. (1992) *Nucleic Acids Res.* **20,** 2933–2940.
34. Koike, G., Maki, H., Takeya, H., Hayakawa, H. & Sekiguchi, M. (1990) *J. Biol. Chem.* **265,** 14754–14762.
35. Singer, B., Chavez, F., Goodman, M. F., Essigmann, J. M. & Dosanjh, M. K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8271–8274.
36. Aquilina, G., Giammarioli, A. M., Zijno, A., DiMuccio, A., Dogliotti, E. & Bignami, M. (1990) *Cancer Res.* **50,** 4248–4253.
37. Rappaport, H. P. (1988) *Nucleic Acids Res.* **16,** 7253–7267.
38. Sherman, S. E. & Lippard, S. J. (1987) *Chem. Rev.* **87,** 1153–1181.
39. Chu, G. (1994) *J. Biol. Chem.* **269,** 787–790.
40. Eastman, A. (1986) *Biochemistry* **25,** 3912–3915.
41. Lippard, S. J. (1993) in *The Robert A. Welch Foundation 37th Conference on Chemical Research* (The Robert A. Welch Foundation, Houston), pp. 49–60.
42. Anthoney, A., McIlwrath, A., Gallagher, W., Edlin, A. & Brown, R. (1996) *Cancer Res.* **56,** 1374–1381.
43. Loeb, L. A. (1994) *Cancer Res.* **54,** 5059–5063.
44. Pil, P. M. & Lippard, S. J. (1992) *Science* **256,** 234–237.
45. Hughes, E. N., Engelsberg, B. N. & Billings, P. C. (1992) *J. Biol. Chem.* **267,** 13520–13527.
46. Huang, J. C., Zamble, D. B., Reardon, J. T., Lippard, S. J. & Sancar, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10394–10398.
47. Brown, S. J., Kellett, P. J. & Lippard, S. J. (1993) *Science* **261,** 603–605.
48. Lieb, M. & Bhagwat, A. S. (1996) *Mol. Microbiol.*, in press.
49. Rayssiguier, C., Thaler, D. S. & Radman, M. (1989) *Nature (London)* **342,** 396–401.
50. Petit, M.-A., Dimpfl, J., Radman, M. & Echols, H. (1991) *Genetics* **129,** 327–332.
51. Radman, M. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington, DC), pp. 169–192.
52. Worth, L., Jr., Clark, S., Radman, M. & Modrich, P. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3238–3241.