Mismatch Repair of cis-Diamminedichloroplatinum(II)-Induced DNA Damage

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SUMMARY

Because cytotoxicity by an alkylating agent such as N-methyl-N'-nitrosoguanidine is markedly increased in adenine methylase-deficient dam-3 Escherichia coli, it was of interest to assess whether mismatch repair was similarly important in the repair of DNA damage induced by cis-diamminedichloroplatinum(II) (CDDP). The results demonstrate that after exposure to 5–40 μM CDDP, dam-3 E. coli are 2–15-fold more sensitive to the cytotoxic effects of this agent. Further, dam-3 mutL451 E. coli deficient in mismatch repair was as resistant as wild type. trans-Diamminedichloroplatinum(II) treatment did not cause marked increments in cytotoxicity in dam-3 E. coli compared to wild type. The rate of excision of platinum was significantly reduced in dam-3 E. coli compared to wild type, demonstrating that differences in the repair of CDDP-induced DNA damage underlie enhanced cytotoxicity by this agent. Lastly, mutagenesis by CDDP was abrogated in umuDC⁻ E. coli, showing that this gene product mediates mutagenesis by this agent.

INTRODUCTION

CDDP[1] is an effective agent in the treatment of a broad spectrum of neoplasms (1, 2). While biochemical mechanisms underlying the interaction of CDDP with DNA are reasonably well elucidated, relatively little is known with respect to the molecular mechanisms by which CDDP causes cytotoxicity and mutagenesis (3–6). The mechanisms which underlie the repair of CDDP-induced DNA damage are also poorly understood and are of great potential clinical relevance since the pharmacological modulation of these processes may ultimately enhance therapeutic efficacy of this important antineoplastic agent.

CDDP belongs to a group of agents which are SOS-dependent mutagens. Such agents trigger an inducible response which promotes cell survival at the expense of DNA fidelity, i.e., survival is accompanied by an increased mutation frequency. The SOS response has been studied in greatest detail in *Escherichia coli* (7). About 20 genes, including *uvrA*, *uvrB*, and *umuDC*, are derepressed after DNA damage. *uvrA* and *uvrB*, for example, are genes that specify an endonuclease that excises UV

irradiation-induced thymine dimers. This endonuclease also appears important in the repair of CDPP-induced adducts since mutants deficient at the *uvrA* and *uvrB* loci (*uvrA⁻* and *uvrB⁻*) are highly sensitive to CDDP (8, 9). Another genetic locus in *E. coli*, *umuDC*, mediates mutagenesis after UV irradiation (7). These loci as well as others involved in SOS-mediated repair are controlled primarily by the products of the regulatory elements *recA* and *lexA* (7). The mechanisms underlying the marked sensitivity of *recA*-deficient *E. coli* (*recA⁻*) to CDDP is not as clear as for *uvrA⁻* and *uvrB⁻* cells: it may be required for the repair of cross-linked DNA occurring after CDDP exposure (3–6) by enhancing recombinational events as well as by its role in regulating the SOS response.

E. coli mutants at *lexA* and *recA* loci are not mutable by CDDP (8). This indicates that error-prone repair is required for mutagenesis.

In addition to excision and recombinational repair, at least two other mechanisms have been described in bacteria. One of these is specific for methylating agents (the adaptive response) and does not seem to be involved in repair of CDDP-induced DNA damage.[2] The other DNA repair mechanism involves the correction of mismatched base pairs in DNA. Mismatch repair occurs at the replication fork and requires the products of the *mutL* and *mutS* genes (10). The biochemical function of these *mut* gene products is not known. Evidence that alkylating agents produce damage which is susceptible to mismatch

[1] The abbreviations used are: CDDP, cis-diamminedichloroplatinum (II); MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; TDDP, trans-diamminedichloroplatinum(II).

[2] Unpublished data.

repair has come from the study of *dam* mutants of *E. coli* which appear to be unable to discriminate mismatched bases or its equivalent in DNA during mismatch repair (11–13). These mutant strains are more sensitive to the cytotoxic action of, for example, an alkylating agent such as MNNG. If mismatch repair is prevented in *dam⁻* strains by a second mutation in either the *mutL* or *mutS* loci, then cytotoxicity to MNNG is markedly reduced (14).

In the present communication, we wished to determine if CDDP produces adducts susceptible to mismatch repair and if such damage can cause increased mutagenesis. We also clarify the mode of SOS-dependent CDDP-induced mutagenesis as well as the role of mismatch repair in the excision of platinum adducts from DNA.

MATERIALS AND METHODS

Bacterial strains. GM112 (*dam⁺*, wild-type *E. coli*, K12), GM113 (*dam-3* or *dam⁻*), and GM150 (*dam-3 mutL451* or *dam⁻ mut⁻*) have

TABLE 1
Temperature program

	Dry		Ash		Atomize
	I	II	I	II	
Temperature (°C)	100	120	600	1400	2600
Ramp (sec)	20	10	10	15	0
Hold (sec)	25	10	10	10	5
Gas flow (ml/min)	50				
Background correction	Yes				

been described previously (14). GW2100 (*umuC*::Tn5 or *umuDC⁻*) was a gift from Dr. Graham Walker (Massachusetts Institute of Technology). Complete media consisted of Difco brain-heart infusion broth and minimal media as described elsewhere (12).

Chemical reagents. CDDP and TDDP were obtained from Sigma Chemical Corp. (St. Louis, MO). Drug was dissolved in dimethylformamide just prior to use.

Cytotoxicity and mutagenesis studies. Cells were grown in brain-heart media to midexponential phase, harvested and resuspended in minimal salts with 1 mM thymine for 60 min at 37°. Cells were exposed to 0–80 μM CDDP in the dark for 2 hr at 37°, washed twice, and then plated on brain-heart media. Colonies were counted after incubation overnight at 37°.

Mutation frequency was assessed by resuspending cells at 10⁷/ml in brain-heart media, incubating overnight at 37°, and then plating onto nutrient agar containing rifampicin (100 μg/ml). Total viable bacteria were determined by plating onto nutrient agar plates.

Analysis of CDDP content in DNA by atomic absorption spectroscopy. Cells were exposed to 0–40 μM CDDP as previously described. After a 2-hr incubation, cells were spun, washed, and then resuspended at 2 × 10⁹/ml in 0.1 M Tris, 0.01 M EDTA, pH 8.0. Cells were lysed with lysozyme (100 μg/ml) and then sodium dodecyl sulfate (0.5% final concentration). The solution was extracted with phenol and chloroform/isoamyl alcohol (24:1) twice, treated with ribonuclease (50 μg/ml) and pronase (200 μg/ml) for 4 hr at 37°, extracted with chloroform/isoamyl alcohol (24:1), and precipitated with sodium chloride (0.4 M final concentration) and 2 volumes of 95% absolute ethanol at −20° overnight. The DNA was hydrolyzed with 1 N HCl and assessed spectrophotometrically at 260 and 280 nm.

Total platinum content was assessed with a Perkin-Elmer (Norwalk, CT) model 2380 atomic absorption spectrophotometer equipped with an HGA-400 graphite furnace. The approach is described elsewhere and only modifications are listed below (18). Since DNA hydrolysis
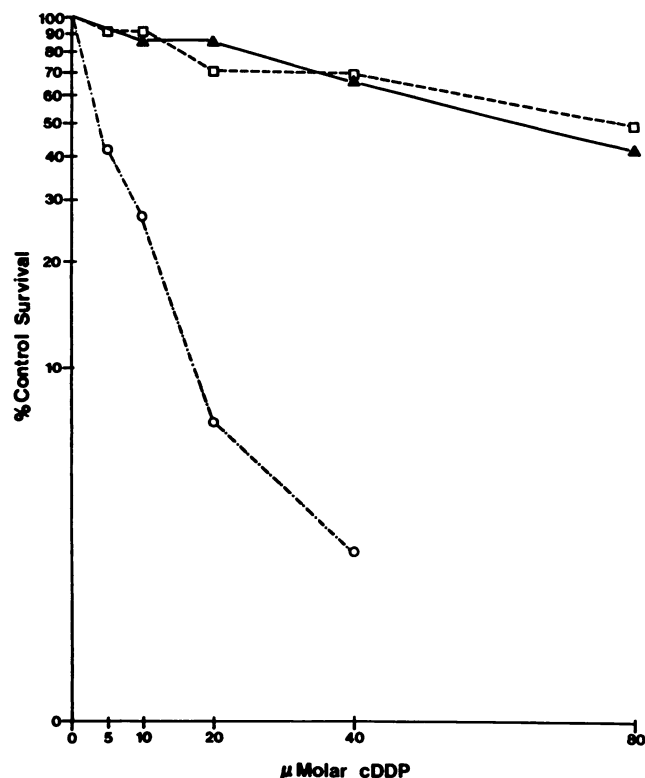

FIG. 1. *Effect of CDDP on cytotoxicity*

dam⁻, *dam⁻ mut⁻*, and *dam⁺ E. coli* were exposed to CDDP for 2 hr at 37°, washed twice, and plated. *dam⁻* (○); *dam⁻ mut⁻* (□); and *dam⁺* (▲). See text for details.
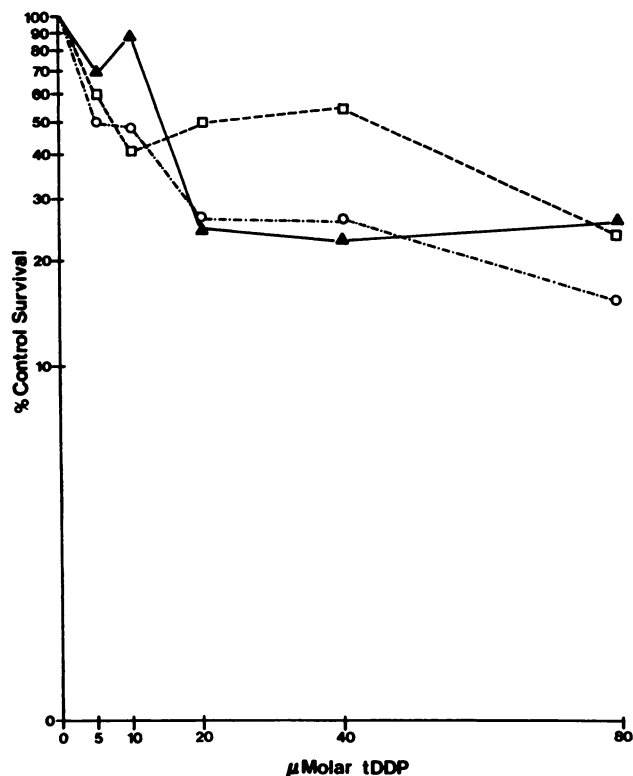

FIG. 2. *Effect of TDDP on cytotoxicity*

dam⁻, *dam⁻ mut⁻*, and *dam⁺ E. coli* were exposed to TDDP for 2 hr at 37°, washed twice, and plated. *dam⁻* (○); *dam⁻ mut⁻* (□); and *dam⁺* (▲). See text for details.

TABLE 2

Platinum content in DNA in wild-type, dam⁻, and dam⁻ mut⁻ E. coli

Platinum content was assessed by flameless atomic absorption spectroscopy. Cells were exposed to CDDP for 2 hr at 37°. Values represent the mean of results from two experiments.

CDDP concentration	Pt in strain DNA		
	dam^+	dam^-	$dam^- mut^-$
μM		ng/mg	
0	0	0	0
10	139	79	167
20	219	158	264
40	445	468	542

TABLE 3

CDDP-induced mutation to rifampicin resistance

Cells were exposed to CDDP for 2 hr at 37°, washed twice, and then resuspended at 10^7/ml in brain-heart media. After an incubation overnight at 37°, cells were plated onto nutrient agar containing rifampicin (100 μg/ml). Total viable bacteria were determined by plating onto nutrient agar. Mutation frequency is expressed as rifampicin-resistant mutants/10^8 viable bacteria.

Strain	Mutation frequency after exposure to CDDP				
	$0\ \mu M$	$10\ \mu M$	$20\ \mu M$	$40\ \mu M$	$80\ \mu M$
GM112 (dam^+)	<0.1	26	46	33	127
Gm113 (dam^-)	1	68	86	140	109
GM150 ($dam^- mut^-$)	20	119	93	148	139

FIG. 4. *Effect of CDDP on umuDC⁻ E. coli*

umuDC⁻ E. coli was exposed to CDDP for 2 hr, washed, and plated. *umuDC⁻* (O); *umuDC⁺* (●). See text for details.
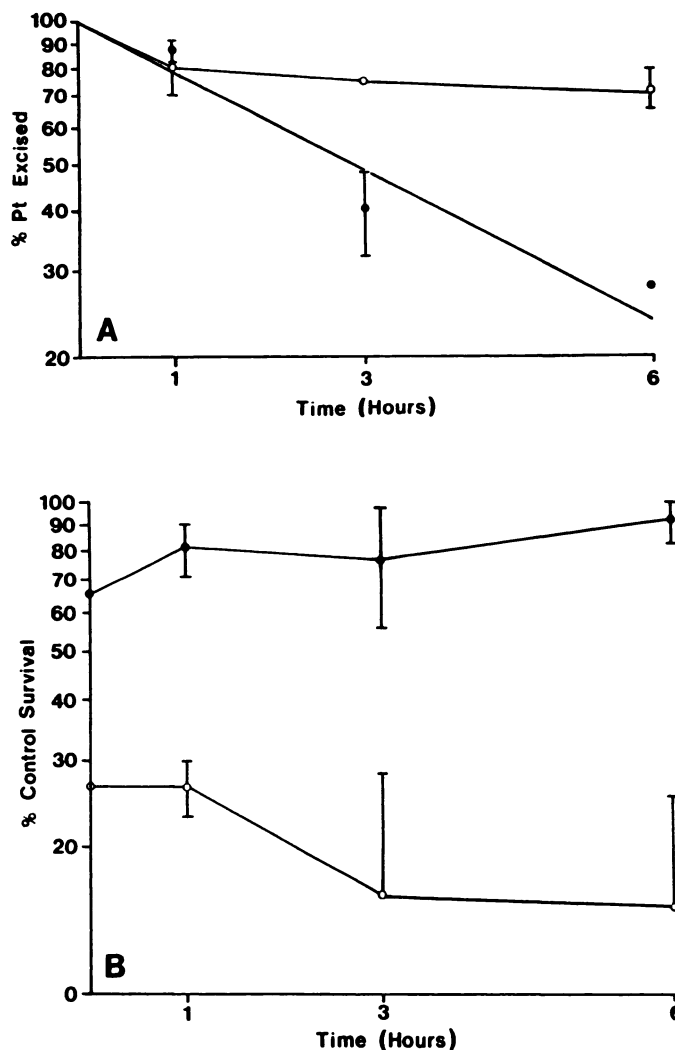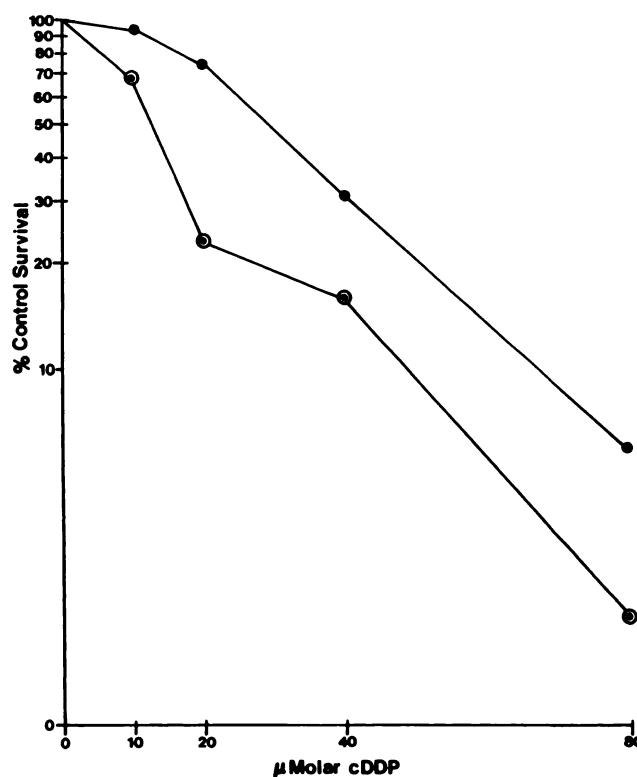
FIG. 3. *Excision of CDDP and cell viability*

A, kinetics of excision of CDDP from DNA in dam^- (O) and dam^+ (●) *E. coli*. Cells were exposed to CDDP at 40 μM for 2 hr at 37°, washed twice, and then placed in media for 0, 1, 3, and 6 hr at 37°. Cells were spun and DNA isolated, and platinum content and per cent platinum excised were determined as described in the text. Values represent the mean ± standard deviation. B, viability of cells was assessed at 0, 1, 3, and 6 hr after exposure to CDDP by plating of cells on brain-heart infusion. Values represent the mean ± standard deviation. Symbols are as in A.

TABLE 4

CDDP-induced mutation to rifampicin resistance in umuDC⁻ E. coli

Cells were exposed to CDDP for 2 hr at 37°, washed twice, and resuspended at 10^7/ml in brain-heart media. Mutation frequency was assessed by plating cells onto nutrient agar containing rifampicin as described in the text. Results are expressed as rifampicin-resistant mutants/10^8 viable bacteria.

CDDP	Mutation frequency after exposure to CDDP				
	$0\ \mu M$	$10\ \mu M$	$20\ \mu M$	$40\ \mu M$	$80\ \mu M$
AB1157 (wild type)	0	56	78	72	126
GW2100 ($umuDC^-$)	0	1	1	2	1

involves the use of a mineral acid in its final stage, platinum can be analyzed by a single 40-μl injection of the DNA hydrolysate directly into the furnace. The temperature program is presented in Table 1. The assay is linear to 300 ng/ml and can be used to measure concentrations accurately to 10 ng/ml with a precision at 100 ng/ml of 4%. The effect of matrix was checked by spiking DNA hydrolysates with aqueous standards and no matrix effect was observed. Total platinum is expressed as micrograms of Pt/mg of DNA.

Analysis of platinum excision from DNA. Cells were exposed to 40 μM CDDP for 2 hr at 37° as previously described. Cells were then washed twice and resuspended in media for 0, 1, 3, and 6 hr at 37°. DNA was then isolated as previously described and platinum content was assessed with atomic absorption spectroscopy. Platinum content expressed as nanograms of Pt/mg of DNA was then corrected for any potential dilutional effects of DNA replication by dividing by the fractional increase in viable cells during the repair period after exposure to Pt. Viable cell number was assessed by plating cells after 0, 1, 3, and 6 hr after exposure to 40 μM Pt.

RESULTS

Because cytotoxicity by an alkylating agent such as MNNG is markedly increased in adenine methylase-deficient (*dam⁻*) *E. coli*, it was of interest to assess whether mismatch repair was similarly important in the repair of DNA damage induced by CDDP (13). Cytotoxicity was assessed in *dam⁻*, *dam⁻ mut⁻*, and wild-type (*dam⁺*) *E. coli* after a 2-hr exposure to 0–80 μM CDDP. Fig. 1 demonstrates a 2–15-fold increase in cytotoxicity in *dam⁻ E. coli*. Further, the introduction into the *dam⁻* strain of a *mutL* mutation, which eliminates mismatch correction, abolished sensitivity to CDDP (19).

To assess the importance of stereochemistry, cells from the three strains were exposed to TDDP, the *trans* isomer of CDDP. No marked differences in cytotoxicity between *dam⁻*, *dam⁻ mut⁻*, and wild-type cells was noted (Fig. 2).

Because cytotoxicity is correlated with the extent of platinum bound to DNA in eukaryotic cells, experiments were performed to assess the total platinum content in DNA after exposure to CDDP (20). Although the total platinum content in DNA was not significantly different in the three strains after exposure to 10–40 μM CDDP (Table 2), the excision of CDDP from DNA is significantly slower in *dam⁻* compared to wild-type cells (Fig. 3A). Further, a significant reduction in cellular replication after exposure to 40 μM CDDP was noted in *dam⁻* cells compared to the wild type throughout a 6-hr repair period (Fig. 3B). Thus, differences in the excision of CDDP underlie increased cytotoxicity in *dam⁻ E. coli*.

The role of mutagenesis in causing cytotoxic events also was evaluated. Mutation frequency was assessed after a 2-hr exposure to CDDP in the three strains. CDDP did not significantly enhance mutation frequency in *dam⁻* compared to *dam⁺* cells (Table 3). And while spontaneous mutation rates are increased in *dam⁻ mut⁻ E. coli*, this strain was not hypermutable after exposure to CDDP.

The role of SOS-mediated error-prone repair also was assessed with respect to cytotoxicity and mutagenesis. While cytotoxicity was not substantially enhanced in *umuDC⁻ E. coli*, mutation frequency after exposure to CDDP was markedly reduced (Fig. 4 and Table 4).

DISCUSSION

dam⁻ E. coli have decreased adenine methylase activity and a pleiotropic phenotype which includes increased spontaneous mutability, hyperrecombination, increased spontaneous induction of phage λ from lysogens, and increased sensitivity to UV irradiation, base analogues, and alkylating agents such as MNNG (13, 14). The present study demonstrates that mismatch repair is important in the repair of CDDP-induced DNA damage. The involvement of abortive mismatch repair is suggested by the abrogation of enhanced cytotoxicity in *dam⁻ mut⁻ E. coli*, a strain unable to engage in mismatch repair. Further, the inability of *dam⁻* cells to excise platinum adducts efficiently also confirms that repair of CDDP-induced DNA damage is defective in these cells, although the precise molecular mechanism(s) by which this particular defect in repair causes inefficient excision of platinum and delays in cellular and DNA replication remains unclear. Adenine methylation of the parental DNA strand specified by the *dam* gene may enhance the efficiency of recognition and, ultimately, the excision of platinum adducts from DNA by repair enzymes. The excision of CDDP and subsequent repair of intrastrand and interstrand DNA cross-links, lesions that are likely important in CDDP-induced cytotoxicity, might then allow a resumption of DNA replication.

Mutagenesis after exposure to CDDP, in contrast to a methylating agent such as MNNG, was markedly decreased in *umuDC⁻ E. coli*. This finding demonstrates that the *umuDC* gene product mediates mutagenesis by CDDP. Differences between CDDP and MNNG with respect to the involvement of *umuDC* in the induction of mutations most likely results from the types of lesions caused by each agent. For example, CDDP unlike MNNG, in addition to forming a very different adduct with DNA, also causes a broad spectrum of lesions such as DNA strand breaks as well as intrastrand and interstrand cross-links (3–6). CDDP resembles UV irradiation in its requirement for *umuDC* gene product for the induction of mutants. The contribution of *umuDC* gene product to mutation induction by CDDP is consistent with enhanced mutation induction by CDDP in *E. coli* K12 with the plasmid pKM101; this plasmid carries two genes, *mucA* and *mucB*, that resemble *umuC* and *umuD* in *E. coli* (21). *umuDC⁻ E. coli* in contrast with *recA⁻*, *uvrA⁻*, and *uvrB⁻ E. coli* was not significantly more sensitive to the cytotoxic effects of CDDP. This finding is consistent with the heterogeneous effects resulting from *recA* deficiency as well as the distinct role of *uvrA* and *uvrB* from *umuC* in SOS-mediated repair (17).

Prior studies demonstrated both decreased and increased mutation induction in *recA⁻ E. coli* (8, 9). These varying results may reflect differences in methodology as well as of the bacterial strains employed. The use of a *umuDC⁻* strain in the current report obviates the heterogeneous effects that occur with deficiency in *recA* and clearly demonstrates involvement of *umuDC* gene product in mutation induction by CDDP.

REFERENCES

1. Wolpert-DeFilippes, M. K. Antitumor activity of cis-dichlorodiammine-platinum (II). *Cancer Treat. Rep.* **63**:1453–1458 (1979).

2. Einhorn, L. H. and J. Donohue. Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann. Intern. Med.* **87**:293–298 (1977).
3. Zwelling, L. A., and K. W. Kohn. Mechanism of action of cis-dichlorodiammineplatinum (II). *Cancer Treat. Rep.* **63**:1439–1444 (1979).
4. Lippard, S. J. New chemistry of an old molecule: cis-[Pt(NH3)$_2$Cl$_2$]. *Science* **218**:1075–1082 (1982).
5. Roberts, J. J., and A. J. Thomson. The mechanism of action of antitumor platinum compounds. *Progress Nucleic Acid Res. Mol. Biol.* **22**:71–129 (1979).
6. Wing, R. M., P. Pjura, H. R. Drew, and R. E. Dickerson. The primary mode of binding of cisplatin to a B-DNA dodecamer C-G-C-G-A-A-T-T-C-G-C-G. *EMBO J.* **3**:1201–1206 (1984).
7. Walker, G. C. Mutagenesis and inducible responses in deoxyribonucleic acid damage in *Escherichia coli. Microbiol. Rev.* **48**:60–93 (1984).
8. Brouwer, J., P. van de Putte, A. M. J. Fichtunger-Schepman, and J. Reedijk. Base-pair substitution hotspots in GAG and GCG nucleotide sequences in *Escherichia coli* K-12 induced by cis-diamminedichloro platinum (II). *Proc. Natl. Acad. Sci. U. S. A.* **78**:7010–7014 (1981).
9. Konishi, H., T. Usui, H. Sawada, H. Uchino, and Y. Kidani. Effects of anticancer platinum compounds on *Escherichia coli* strains with normal and defective repair capacity. *Gann* **72**:627–630 (1981).
10. Pukkila, P. J., J. Peterson, G. Herman, P. Modrich, and M. Meselson. Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *Escherichia coli. Genetics* **104**:571–582 (1983).
11. Lien-Lu, A., S. Clark, and P. Modrich. Methyl-directed repair of DNA base-pair mismatches in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **80**:4639–4643 (1983).
12. Marinus, M. G., and R. N. Morris. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. *J. Bacteriol.* **114**:1143–1150 (1973).
13. Bale, A., M. d'Alarcao, and M. G. Marinus. Characterization of DNA adenine methylation mutants of *Escherichia coli* K-12. *Mutat. Res.* **59**:157–165 (1979).
14. Karran, P., and M. G. Marinus. Mismatch correction O^6-methylguanine residues in *E. coli* DNA. *Nature* **296**:868–869 (1982).
15. Schendel, P. F., and M. Defais. The role of *umuC* gene product in mutagenesis by simple alkylating agents. *Mol. Gen. Genet.* **177**:661–665 (1980).
16. Kato, T. and Y. Shinoura. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol. Gen. Genet.* **156**:121–131 (1977).
17. Elledge, S. J., and G. C. Walker. Proteins required for ultraviolet light and chemical mutagenesis. *J. Mol. Biol.* **164**:175–192 (1983).
18. Smeyers-Verbere, J., M. R. Detaevernier, L. Denis, and D. L. Massart. The determination of platinum in biological fluids by means of graphite furnace atomic absorption spectroscopy. *Clin. Chim. Acta* **113**:329–333 (1981).
19. Rydberg, B. Bromouracil mutagenesis and mismatch repair in mutator strains of *Escherichia coli. Mutat. Res.* **52**:11–24 (1978).
20. Fraval, H. N. A., and J. J. Roberts. Excision repair of cis-diamminedichloroplatinum (II)-induced damage to DNA of Chinese hamster cells. *Cancer Res.* **39**:1793–1797 (1979).
21. Brouwer, J., M. R. Adhen, and P. van de Putte. Effect of pkM 101 on cell killing and specificity of mutation induction by cis-diamminedichloroplatinum (II) in *Escherichia coli* K-12. *J. Bacteriol.* **156**:1275–1281 (1983).

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