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MutS inhibits RecA-mediated strand transfer with methylated DNA substrates

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ABSTRACT

DNA mismatch repair (MMR) sensitizes human and Escherichia coli dam cells to the cytotoxic action of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) while abrogation of such repair results in drug resistance. In DNA methylated by MNNG, MMR action is the result of MutS recognition of O6-methylguanine base pairs. MutS and Ada methyltransferase compete for the MNNG-induced O6-methylguanine residues, and MMR-induced cytotoxicity is abrogated when Ada is present at higher concentrations than normal. To test the hypothesis that MMR sensitization is due to decreased recombinational repair, we used a RecA-mediated strand exchange assay between homologous phiX174 substrate molecules, one of which was methylated with MNNG. MutS inhibited strand transfer on such substrates in a concentration-dependent manner and its inhibitory effect was enhanced by MutL. There was no effect of these proteins on RecA activity with unmethylated substrates. We quantified the number of O6-methylguanine residues in methylated DNA by HPLC-MS/MS and 5–10 of these residues in phiX174 DNA (5386 bp) were sufficient to block the RecA reaction in the presence of MutS and MutL. These results are consistent with a model in which methylated DNA is perceived by the cell as homologous and prevented from recombining with homologous DNA by the MMR system.

INTRODUCTION

Methylating agents are found in the environment and are also used in cancer chemotherapy (1–3). These agents and laboratory versions, such as N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), can react with DNA to create a variety of lesions acted upon by different DNA repair pathways; such lesions include N7-methylguanine (7-meG) and O6-methylguanine (O6-meG), the latter being the subject of this investigation. In Escherichia coli, two methyltransferases can use O6-meG as a substrate: the constitutively produced Ogt protein and the inducible Ada protein (4). In order to demethylate an O6-meG, the Ada protein binds directly to the base, flips it out of the helix and into its substrate binding pocket, transferring the methyl group from the base to an internally located cysteine residue (5). The Ada protein also has a regulatory function to induce itself and other genes of the ada regulon, including alkA, alkB and aidB (1,3).

O6-meG paired with cytosine or thymine is also a substrate for the MutS protein of the E.coli DNA mismatch repair (MMR) system (6,7). The Dam-directed MMR system of E.coli acts to preserve the fidelity of the genome by removing base mismatches that arise as a result of replication (mutation avoidance) (8–10). During replication, the parental DNA strand is methylated at all adenines in the sequence GATC by the product of the dam gene. The newly synthesized strand is not yet methylated, due to a lag between replication and methylation by Dam, which leaves a hemi-methylated DNA state behind the replication fork. The MMR system exploits the hemi-methylated state of the DNA by allowing it to distinguish the new strand from the old strand for repair. When mismatches arise in the hemi-methylated region of DNA, they are first bound by the MutS protein. MutS then recruits MutL and MutH to form a ternary complex, which activates the latent endonuclease activity of MutH. Incision by MutH occurs at a nearby GATC sequence on the unmethylated strand, followed by exonucleolytic digestion to remove the mismatch. The gap produced by exonuclease action is re-synthesized by the replicative polymerase, DNA polymerase III, which restores the correct nucleotide sequence and the remaining nick is sealed by DNA ligase. Subsequently, the repaired strand will be methylated by the Dam methyltransferase at GATC sequences, and this methylation step prevents further action by the MMR system.

In E.coli mutant in the dam locus, both strands of DNA are unmethylated and the directionality of MMR is lost, allowing MutH to incise the daughter or parental DNA strands during...
repair (11). Thus, MutH endonuclease action can occur in both replicating and non-replicating DNA, anywhere in the chromosome, unlike in wild-type cells where MMR is tightly regulated to the region trailing the replication fork. MutH action can occur either at the same GATC on opposite strands (12) or by replication fork collapse (11,13). These actions result in the formation of double-strand breaks (DSBs), which require recombination to restore genomic integrity, as mutations in genes encoding recombination proteins in combination with dam results in a lethal phenotype (11).

MMR also plays an important role in preventing recombination between similar but non-identical (homeologous) DNA sequences, a function termed anti-recombination (14). Salmonella enterica serovar Typhimurium and E.coli share an 83% sequence identity, and genetic crosses between them are sterile unless a mutation in the mutS or mutL genes is present in the recipient (14). Recombination is increased by at least 1000-fold in recipients where MMR is inactivated and chimeras containing DNA from both species are formed. Biochemical experiments using homeologous DNA from the closely related M13 and fd phages (3% sequence divergence) showed that the addition of MutS and MutL protein block the progression of the RecA-mediated strand exchange reaction in vitro (15). There was no effect of MutS and MutL when homologous M13–M13 substrates were used. The genetic and biochemical results imply that MMR impedes or actively reverses recombination intermediates with the former having experimental support (9,16).

E.coli dam mutants are more susceptible to the cytotoxic action of MNNG and other chemical agents than wild-type (17–19). Mutations inactivating MMR (mutS, mutL) in a dam background confer a level of resistance to MNNG similar to wild-type (17,19). This indicates that MMR can act on chemically modified substrates through MutS binding specifically to O6-meG base pairs (6,7). MMR action at these base pairs may lead to the formation of nicks or gaps, which are converted to DSBs requiring recombination to repair them. We hypothesize that inhibition of recombinational repair by MMR would ensure because the homologous methylated DNA is perceived as homeologous DNA (20). Persistent unrepaired DSBs would eventually produce lethality. We have advanced a similar hypothesis to explain MMR sensitization of dam cells exposed to cisplatin in which dose- and MMR-dependent DSBs accumulate (13). MutS prevents RecA-mediated strand transfer with substrate DNAs containing platinated intrastrand crosslinks (21). In this work, we demonstrate a similar result with O6-meG base pairs.

### MATERIALS AND METHODS

#### Strains, media and chemicals

The E.coli K-12 strains used in this study are described in Table 1. Plasmid pBAR, a pEMBL derivative that contains the ada gene under control of an isopropyl-β-thiogalactopyranoside (IPTG) inducible promoter (22), was a gift from Dr Bruce Demple (Harvard School of Public Health, Boston). L medium contains 20 g tryptone (Difco), 10 g yeast extract (Difco), 0.5 g NaCl, 4 ml of 1 M NaOH per liter and solidified when required with 16 g of agar. Minimal medium was prepared as described by Davis and Mingioli (23). MNNG (Sigma–Aldrich) was prepared by dissolving 1 mg of MNNG in 100 µl of dimethyl sulfoxide and adding 900 µl of sterile water and aliquots were stored frozen at −20°C. Guanine, 7-methylguanine and O6-meG were obtained from Sigma–Aldrich.

#### Proteins and DNA

RecA protein was purified as described previously (21). MutS and MutL proteins were a gift from F. Lopez de Saro and M. O’Donnell (The Rockefeller University). Single-stranded binding protein (Ssb) was obtained from USBiological. PhiX174 RFI (covalently closed circular) and virion DNA molecules were reacted with various amounts of MNNG using a protocol adapted from Sussmuth et al. (24). The DNA (50 µg) was added to 0.1 M citric acid phosphate buffer, pH 6, containing 0.5 µM cysteine and varying amounts of MNNG. The solution was rocked gently at 37°C for 4 h, after which the DNA was ethanol precipitated four times to remove residual MNNG. For the phage survival, aliquots of methylated or unmethylated RFI were mixed with strains MV1161 (wild type) and MV3855 (alk tag), mixed with top agar and poured onto Luria–Bertani medium.

#### Methylation and survival of phiX174 DNA

PhiX174 RFI and linearized RFI DNA molecules were reacted with various amounts of MNNG using a protocol adapted from Sussmuth et al. (24). The DNA (50 µg) was added to 0.1 M citric acid phosphate buffer, pH 6, containing 0.5 µM cysteine and varying amounts of MNNG. The solution was rocked gently at 37°C for 4 h, after which the DNA was ethanol precipitated four times to remove residual MNNG. For the phage survival, aliquots of methylated or unmethylated RFI were mixed with strains MV1161 (wild type) and MV3855 (alk tag), mixed with top agar and poured onto Luria–Bertani medium.
plates. The plates were incubated overnight at 37°C and plaques scored.

**HPLC-MS/MS**

Calculation of the number of methylated purine bases in each of the DNA substrates was determined by reverse phase high-performance liquid chromatography (HPLC) with online positive ion electrospray multiple reaction monitoring MS/MS (HPLC-ESI-MRM-MS/MS). HPLC was performed using a Rheos microgradient pumping system (Leap Technologies, Inc.) with a 150 × 1 mm ID BetaBasic-18 (3 μm particle size, 150 Å pore, Keystone Scientific, Inc.) in the isocratic mode with 2% acetonitrile, 0.1% formic acid as the mobile phase at 50 μl/min. The column outlet was directly connected to the Z-Spray™ ion source on a Quattro-II triple quadrupole mass spectrometer (Waters). Optimal MS conditions for each analyte were determined by adjustment of conditions during infusion of solutions of each analyte in mobile phase with the capillary at 2.4 kV and collision cell argon pressure at 1 mBar. Guanine was measured by MRM of the transition from the m/z 136 (MH⁺) to m/z 92 with the cone at 30 V, collision energy at 30 V and a dwell time of 0.05 s. 7-meG was measured by MRM of the transition from the m/z 166 (MH⁺) to m/z 124 with the cone at 35 V, collision energy at 23 V and a dwell time of 0.5 s. O⁶-meG was measured by MRM of the transition from the m/z 166 (MH⁺) to m/z 134 with the cone at 35 V, collision energy at 24 V and a dwell time of 5 s. Amounts of each analyte in the samples were calculated by comparison of sample peak areas with those measured from injection of external standards. The methylated DNA was subject to formic acid hydrolysis using 65% formic acid for 30 min at 130°C.

**Strand exchange assay**

Reaction mixtures contained 25 mM Tris-acetate, pH 7.5, 10 mM MgOAc, 5% glycerol, 1 mM DTT, 8 mM phosphocreatine, 10 U/ml creatine kinase, 1 nM single-stranded circular DNA and 6.7 μM RecA. Reaction mixtures were pre-incubated at 37°C for 10 min, linear duplex DNA substrate was added to a concentration of 0.8 nM and incubated an additional 10 min. Strand exchange was initiated by the addition of a preincubated solution containing 2 μM Ssb and 3 mM ATP. MutS and/or MutL were added 1 min prior to initiation of the reaction. Samples were taken at indicated times and strand transfer was terminated by the addition of 2 μl of buffer containing 5% SDS, 20% glycerol, 60 mM EDTA and Proteinase K to a concentration of 1 mg/ml. After incubation at 42°C for 30 min, samples were analyzed by electrophoresis in a 0.8% agarose gel with 20 μM Tris-acetate, 2 mM EDTA. Gels were processed by staining in Vistra Green (Amersham) fluorescent stain (1:10 000) for 60 min and then analyzed by Image Reader 1 Laser/1 Image at 473 nm on a Fuji FLA-5000 phosphorImager. The gels were photographed and analyzed using Multi-gauge V.2.3 software.

**RESULTS**

**Survival of dam strains exposed to MNNG**

We have used deletion mutations in the dam, mutS, mutL, and mutH genes to measure survival of strains containing them to MNNG exposure. The data in Figure 1 show that the dam mutS mutant is more sensitive to MNNG than wild type. The double mutants, dam mutS, dam mutL, and dam mutH, have a level of resistance equivalent to wild type. These results confirm previous data where inactivation of MMR by presumed base substitution mutations mutS and mutL in a dam background results in drug resistance. In contrast to a previous study (19), we find that the dam mutH strain is as resistant to MNNG as wild type.

**The effect of Ada protein on cell survival**

We also examined the role of the Ada protein on dam cell survival by using strains in which it was overproduced from a multicopy plasmid or was absent due to an ada gene deletion. The dam ada double mutant is more sensitive to MNNG than dam alone (Figure 2A), but overexpression of the Ada protein in both these strains results in a wild-type level of resistance (Figure 2B), indicating that Ada methyltransferase can prevent MMR-induced cytotoxicity. The survival of Ada overproducing wild-type and dam mutS strains was not significantly different from the Ada-overproducing strains shown in Figure 2B (data not shown). We conclude that these results are consistent with competition between Ada and MutS for substrate, which we assume is O⁶-meG base pairs.

The increased sensitivity of the dam ada double mutant compared with the dam strain alone suggests an ada-dependent mechanism of sensitization distinct from that caused by MMR. Further evidence for this idea is that the survival of the dam ada mutS triple mutant is less than that for the dam mutS double (Figure 2A). As expected, the ada derivative of the wild type also shows increased sensitivity (Figure 2A) under the same experimental conditions.

**Methylation of DNA by MNNG and determination of the number of lesions by HPLC-ESI-MRM-MS**

The double-stranded linear form of phiX174 DNA (5386 bp) was reacted with 0, 150, 300, and 600 μM MNNG. After formic acid hydrolysis, the levels of guanine, 7-meG and O⁶-meG for each substrate were measured by HPLC-ESI-MRM-MS.
Survival of methylated phiX174 DNA

We methylated phiX174 RFI (covalently closed) DNA to examine the relationship between transfection efficiency of RFI DNA methylated with 0, 150, 300 and 600 μM MNNG in wild-type and alkA tag bacteria. The alk tag strain was chosen because it is a direct measure of survival of methylated phage DNA since potentially lethal types of base methylation, such as 3-methyladenine, are not removed by the AlkA and TagA glycosylases. The data in Figure 4 show that at concentrations above 150 μM, there was a lower survival of the treated phage DNA in the alkA tag strain, indicating the presence of inhibitory methylated lesions.

ReCA strand exchange with unmodified and methylated substrates

The strand exchange reaction is schematically represented in the top panel of Figure 5. An example of a typical assay result showing the separation of the various substrates and products using linear duplex (DS) methylated with 600 μM MNNG is shown in the bottom panel of Figure 5. We used DS methylated DNA because the types and abundance of methylated bases differ from that in single-stranded (SS) DNA and DS DNA is the probable target in vivo. The results of the RecA assay with unmethylated and methylated DNA and various concentrations of MutS and MutL are shown in Figure 6. The first four lanes in Figure 5 show the reaction with methylated DNA but the identical result was obtained with unmethylated DNA (data not shown), indicating that methylated bases do not interfere with RecA action at any concentration of MNNG used (Figure 6). In the absence of MutS, the reaction is complete within 45 min (Figures 5 and 6) by measuring the appearance of nicked circle (NC) product. Over time, there is also an increase in slowly migrating intermediate (I) structures, the step which precedes the formation of the NC product. Inclusion of MutS in the reaction with unmodified substrates has no significant effect on the rate or yield of the reaction up to concentrations of 250 nM (data not shown). At higher concentrations, MutS begins to bind nonspecifically to DNA, causing a slight inhibition of the reaction.

The addition of MutS to the RecA strand exchange reaction

As described above, no change in rate or yield of product is observed upon the addition of MutS to a strand transfer reaction between unmethylated substrates. In contrast, inhibition of strand transfer occurs when MutS is added to a reaction where the linear duplex substrate contains methylated bases (Figure 5, 25 μM MutS). As shown in Figure 6, addition of 25, 125 and 250 μM MutS results in a concentration-dependent inhibition of strand exchange, when the linear duplex substrate is modified by 150, 300 or 600 μM MNNG corresponding to between 5–10, 10–20 and 20–40 O6-meG residues, respectively. At higher concentrations of MNNG, the binding of MutS to the substrate is saturated, as the percent NC formation is equal to that of the 600 μM modified substrate (data not shown). At modifying concentrations of MNNG below 150 μM, the binding of MutS has no effect on product formation and is analogous to that of the unmodified substrate as measured by product formation (data not shown). Note that phiX174 RFI DNA treated with 150 μM MNNG shows no reduction in plaque formation in transfection assays (Figure 4) but a reduction was seen at 300 and 600 μM MNNG-treated DNA.

Addition of MutL to the reaction

Previous studies with homeologous substrates (M13 and fd DNA) have shown that MutL stimulates MutS binding to mismatches during strand exchange when MutS is at suboptimal concentrations (15). The last four lanes in Figure 5 show the inhibitory effect of 50 μM MutL addition to a strand
exchange reaction in the presence of a lower concentration (25 μM) of MutS. Inclusion of 50 or 100 μM MutL in the reactions in the presence of MutS with methylated substrates severely reduces product formation by RecA (Figure 6). In the absence of MutS, inclusion of up to 200 μM MutL has no effect on the rate of product formation or yield of the reaction with modified or unmodified substrates (data not shown).

DISCUSSION

The cytotoxic effects of both cisplatin and MNNG are enhanced by MMR in dam bacteria (17–19). With cisplatin, we have shown that it promotes dose- and MMR-dependent DSB formation in dam cells (13). Genetic studies indicate that recombination is required for the repair of such breaks (25,26) and the initiation of recombination by RecA-mediated strand transfer is inhibited by MutS if one of the recombining partners contains platinated diguanosyl intrastrand cross-links (21). MMR anti-recombination with platinated DNA occurs because such DNA is recognized as homeologous (20) in the same manner as that formed between E.coli and Salmonella typhimurium, which are 17% divergent in sequence (14). MMR-mediated inhibition of DSB repair should lead to lethality since even a single unrepaired DSB is expected to be lethal.

The results reported in this study begin to form a unified hypothesis explaining why cisplatin and MNNG, compounds that produce damage acted upon by different repair systems, cause a similar sensitization by MMR in dam cells. MutS prevents RecA strand transfer when one of the substrates is either methylated or platinated. Since cisplatin has been shown to induce DSB formation in dam cells (13), we predict that MNNG will do so as well and experiments to test this
prediction are in progress. If, indeed, this is the case, then inhibition of DSB repair by anti-recombination underlies MMR sensitization.

The data reported here and for cisplatin (21) begin to answer how much modification in E.coli homologous DNA is required before it is converted to homeologous DNA. Currently, it is known that 192 mismatches, or 3% divergence, between M13 and fd (6407 nt) are sufficient for recognition as homeologous DNA (15). The data in Figure 6 show that 5–10 O6-meG residues in the 5386 bp phiX174 molecule (1–2 modified bases per 1000 bp) are sufficient to provoke MutS and MutL inhibition of strand exchange. With cisplatin, 4–8 platinated intrastrand crosslinks in phiX174 are sufficient for a substantial MutS inhibitory effect although, unlike the case with O6-meG mismatches (Figure 6), intrastrand crosslinks reduce the ability of RecA to perform strand transfer. With this caveat in mind, the number of lesions required to effect MutS inhibition of strand transfer is about the same (~1–2 per 1000 bp).

The strand transfer experiments show that MutS must recognize O6-meG paired with cytosine confirming the binding to synthetic oligonucleotides containing such a base pair (6,7). MutS affinity for O6-meG mismatches was at least 2-fold lower than for G/T base pairs. We wish to emphasize that even though MutS has a lower affinity for DNA containing O6-meG/C mismatches, it is sufficient to produce a striking reduction in strand transfer when a low number of such mismatches are present. This argument also holds for platinated DNA where platinated GG/CC crosslinks must be the adducts recognized by MutS and which have lower affinity for MutS than GG/CT crosslinks (7).

The E.coli dam mutant was shown to be more sensitive than wild type to MNNG, but not dimethylsulfate, suggesting that O6-meG was the lesion recognized by MMR (17). Subsequently, it was shown that MutS was able to bind specifically to O6-meG base pairs in vitro (6,7). Overexpression of Ada methyltransferase abrogates MMR sensitization of dam mutants (Figure 2B) and in its absence, the sensitization is enhanced (Figure 2A). A simple explanation for these results is that the two proteins compete for the same substrate. Given that Ada methyltransferase acts on O6-meG residues (among others) and that MutS can also bind to O6-meG base pairs, it is reasonable to assume that this is the modified base in question and which is recognized in vivo.

Mammalian cells are sensitive to the cytotoxic action of MNNG and cisplatin but MMR-deficient cell lines derived from them are resistant to the action of these agents (27–29), although this association for cisplatin has recently been questioned (30,31). The MutS protein from human cells specifically recognizes the platinated GG intrastrand crosslink.
and O6-methylguanine-cytosine and thymine base pairs (32). The sensitization by MMR to cisplatin or MNNG has been proposed to occur by several models, including futile repair cycles by MMR at lesions followed by DSB formation and subsequent signaling for cell cycle arrest and apoptosis (33). An alternative model postulates a direct link between lesion recognition by MutS and a signal transduction cascade leading to cell death (34,35). Insofar as the work reported here with E.coli can be extrapolated to human cells, our results would favor the DSB model.

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REFERENCES

1. Sedgwick,B. (2004) Repairing DNA-methylation damage. Nature Rev. Mol. Cell Biol., 5, 148–157.
2. Drablos,F., Feizi,E., Aas,P.A., Vaagbo,C.B., Kavli,B., Bratlie,M.S., Pena-Diaz,J., Otterlei,M., Slupphaug,G. and Krokan,H.E. (2004) Alkylation damage in DNA and RNA-repair mechanisms and medical significance. DNA Repair (Amst), 3, 1389–1407.
3. Kleib,K. (2002) Molecular mechanisms of adaptive response to alkylating agents in Escherichia coli and some remarks on O6-methylguanine-DNA-methyltransferase in other organisms. Mutat. Res., 512, 67–84.
4. Lindahl,T., Sedgwick,B., Sekiguchi,M. and Nakabeppu,Y. (1988) Regulation and expression of the adaptive response to alkylating agents. Annu. Rev. Biochem., 57, 133–157.
5. Moore,M.H., Gulbis,J.M., Dodson,E.J., Demple,B. and Moody,P.C. (1994) Crystal structure of a suicidal DNA repair protein: the Ada methyltransferase from E.coli. EMBO J., 13, 1495–1501.
6. Rasmussen,L.J. and Samson,L. (1996) The Escherichia coli MutS DNA mismatch binding protein specifically binds O(6)-methylguanine DNA lesions. Carcinogenesis, 17, 2085–2088.
7. Calmann,M.A., Nowosielska,A. and Marinus,M.G. (2005) Separation of mutation avoidance and antirecombination functions in an Escherichia coli muts mutant. Nucleic Acids Res., 33, 1193–1200.
8. Modrich,P. and Lahue,R. (1996) Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu. Rev. Biochem., 65, 101–133.
9. Schofield,M.J. and Hsieh,P. (2003) DNA mismatch repair: molecular mechanisms and biological function. Annu. Rev. Microbiol., 57, 579–608.
10. Marti,T.M., Kunz,C. and Fleck,O. (2002) DNA mismatch repair and mutation avoidance pathways. J. Cell. Physiol., 191, 28–41.
11. Marinus,M.G. (2000) Recombination is essential for viability of an Escherichia coli dam (DNA adenine methyltransferase) mutant. J. Bacteriol., 182, 463–468.
12. Au,K.G., Welsh,K. and Modrich,P. (1992) Initiation of methyl-directed mismatch repair. J. Biol. Chem., 267, 12142–12148.
13. Nowosielska,A. and Marinus,M.G. (2005) Cisplatin induces DNA double-strand break formation in Escherichia coli dam mutants. DNA Repair (Amst), in press (available online 31 May 2005).
14. Rayssiguier,C., Thaler,D.S. and Radman,M. (1989) The barrier to recombination between Escherichia coli and Salmonella typhimurium is disrupted in mismatch-repair mutants. Nature, 342, 396–401.
15. Worth,L., Clark,S.R.M. and Modrich,P. (1994) Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. Proc. Natl Acad. Sci. USA, 91, 3238–3241.
16. Harfe,B.D. and Jinks-Robertson,S. (2000) DNA mismatch repair and genetic instability. Annu. Rev. Genet., 34, 359–399.
17. Karran,P. and Marinus,M.G. (1982) Mismatch correction at O6-methylguanine residues in E.coli DNA. Nature, 296, 868–869.
18. Fram,R.J., Cusick,P.S., Wilson,J.M. and Marinus,M.G. (1985) Mismatch repair of cis-diaminedichloroplatinum(II)-induced DNA damage. Mol. Pharmacol., 28, 51–55.
19. Jones,M. and Wagner,R. (1981) N-Methyl-N-nitro-nitrosoguanidine sensitivity of E.coli mutants deficient in DNA methylation and mismatch repair. Mol. Gen. Genet., 184, 562–563.
20. Marinus,M.G. (2005) Dr Jekyll and Mr Hyde: how the MutSLH repair system kills the cell. In Higgins,N.P. (ed.), The Bacterial Chromosome. ASM Press, Washington, DC, pp. 413–430.
21. Calmann,M.A. and Marinus,M.G. (2004) MutS inhibits RecA-mediated strand exchange with platinumated DNA substrates. Proc. Natl Acad. Sci. USA, 101, 14174–14179.
22. Demple,B., Sedgwick,B., Robins,P., Tottt,N., Waterfield,M.D. and Lindahl,T. (1985) Active site and complete sequence of the suicidal methyltransferase that counters alkyl mutagenesis. Proc. Natl Acad. Sci. USA, 82, 2688–2692.
23. Davis,B.D. and Mingioli,E.S. (1951) Mutants of Salmonella typhimurium disrupted in mismatch-repair mutants. J. Cell. Physiol., 57, 342–350.
24. Sussmuth,R., Haerlin,R. and Lingens,F. (1972) The mode of action of N-methyl-N-nitro-N-nitrosoguanidine in mutagenesis. VII. The transfer of the methyl group of N-methyl-N-nitro-N-nitrosoguanidine. Biochim. Biophys. Acta, 269, 276–286.
25. Zdraveski,Z.Z., Mello,J.A., Marinus,M.G. and Essigmann,J.M. (2000) Multiple pathways of recombination define cellular responses to cisplatin. Chem. Biol., 7, 39–50.
26. Nowosielska,A., Calmann,M.A., Zdraveski,Z., Essigmann,J.M. and Marinus,M.G. (2004) Spontaneous and cisplatin-induced recombination in Escherichia coli: DNA Repair (Amst), 3, 719–728.
27. Branch,P., Masson,M., Aquilina,G., Bignami,M. and Karran,P. (2000) Spontaneous development of drug resistance: mismatch repair and p53 defects in resistance to cisplatin in human tumor cells. Oncogene, 19, 3138–3145.
28. Kartalou,M. and Essigmann,J.M. (2001) Mechanisms of resistance to cisplatin. Mutat. Res., 478, 23–43.
29. Stojic,L., Brun,R. and Jiricny,J. (2004) Mismatch repair and DNA damage signalling. DNA Repair (Amst), 3, 1091–1101.
30. Massey,A., Offman,J., Macpherson,P. and Karran,P. (2003) DNA mismatch repair and acquired cisplatin resistance in E.coli and human ovarian carcinoma cells. DNA Repair (Amst), 2, 73–89.
31. Caij,N. and Te Riele,H. (2004) Msh2 deficiency does not contribute to cisplatin resistance in mouse embryonic stem cells. Oncogene, 23, 2660–2666.
32. Duckett,D.R., Drummond,J.T., Murchie,A.I., Reardon,J.T., Sancar,A., Lilley,D.M. and Modrich,P. (1996) Human MutSalpha recognizes O(6)-methylguanine-DNA adducts. Proc. Natl Acad. Sci. USA, 93, 6443–6447.
33. Bignami,M., O’Driscoll,M., Aquilina,G. and Karran,P. (2000) Unmasking a killer: DNA O6-methylguanine and the cytotoxicity of mismatching agents. Mutat. Res., 462, 71–82.
34. LIN.D.P., Wang,Y., Scherer,S.J., Clark,A.B., Yang,K., Avdievich,E., Jin,B., Werling,U., Parris,T., Kurthara,N. et al. (2004) An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. Cancer Res., 64, 517–522.
35. Yang,G., Scherer,S.J., Shell,S.S., Yang,K., Kim,M., Lipkin,M., Kucherlapati,R., Kolodner,R.D. and Edelmann,W. (2004) Dominant effects of an Msh6 missense mutation on DNA repair and cancer susceptibility. Cancer Cell, 6, 139–150.
36. Loh,T., Murphy,K.C. and Marinus,M.G. (2001) Mutational analysis of the MutH protein from Escherichia coli. J. Biol. Chem., 276, 12113–12119.
37. Takano,K., Nakamura,T. and Sekiguchi,M. (1991) Roles of two types of O6-methylguanine-DNA methyltransferases in DNA repair. Mutat. Res., 254, 35–44.