

MutS inhibits RecA-mediated strand exchange with platinated DNA substrates

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Human cell lines and *Escherichia coli* *dam* mutants are sensitive to the cytotoxic action of the anticancer agent, cisplatin. Introduction of mutations disabling DNA mismatch repair into these cell lines renders them resistant to the action of this drug. We used RecA-mediated strand exchange between homologous ϕ X174 molecules, one that was platinated and the other that was unmodified, to show that strand transfer is decreased in a dose-dependent manner. Transfer was severely decreased at 10 adducts per molecule (5,386 bp) and abolished with 24 adducts. At low levels of adduction, addition of MutS to the reaction further decreases the rate and yield in a dose-dependent manner. MutL addition was without effect even in the presence of MutS. The results suggest that although mismatch repair is beneficial for mutation avoidance, its antirecombination activity on inappropriate substrates can be lethal to the cell.

E*scherichia coli* cells mutant at the *dam* locus have decreased amounts of DNA adenine methyltransferase (Dam), which affects the physiology of the cell in a variety of ways, including mismatch repair (MMR) directionality (1). In wild-type cells, the MMR complex assembles on hemimethylated DNA trailing the replication fork and includes the MutS protein, which recognizes a variety of base mismatches and insertion/deletions; the MutH protein, whose latent endonuclease activity is activated in the ternary complex, and MutL, which acts as a bridge between the two proteins (2). MutH binds preferentially to hemimethylated DNA and introduces a nick 5' to the G in a -GATC- sequence in the newly synthesized unmethylated strand of hemimethylated DNA. The -GATC- sequences are also the substrate for Dam methyltransferase and fully Dam-methylated DNA is resistant to MutH action.

In *dam* mutants, the directionality of MMR is lost and MutH incision can occur at -GATC- sequences in either the parental or daughter strands (1). Unlike the wild-type, where MMR action is restricted to the hemimethylated DNA trailing the fork, in *dam* mutants, MutH incisions can occur at unmethylated -GATCs- anywhere in the chromosome. The presence of MMR-induced nicks or gaps results in the formation of double-strand breaks (DSBs) that require recombination to restore genomic integrity. Inactivation of recombination ability by mutation in various genes leads to a lethal phenotype in *dam* mutants (3).

E. coli dam mutants are more susceptible to the cytotoxic action of cisplatin, an antitumor drug, than wild-type (4). Inactivation of MMR, however, results in near wild-type levels of drug resistance. That is, MMR action provokes cell death in *dam* bacteria exposed to cisplatin, presumably through the recognition of cisplatin intrastrand crosslinks by MutS (5). Mammalian cell lines also show sensitivity to cisplatin and MMR-deficient lines derived from them are resistant (6, 7), although whether resistance is due specifically to MMR deficiency has recently been challenged (8). Cisplatin-resistant cells isolated from patients treated with this drug have also been shown to be deficient in MMR (6). As with bacterial MutS protein, the human MutS- α counterpart also binds to cisplatin intrastrand crosslinks (9). At present, the molecular mechanism of MMR-mediated drug resistance in *dam* bacteria and mammalian cells is not known.

In addition to preventing mutations (mutation avoidance), MMR in *E. coli* prevents recombination between similar, but not identical (homeologous), DNA sequences (10). Genetic crosses between *E. coli* and the closely related *Salmonella typhimurium* are sterile unless the recipient bacterium is MMR-deficient in which case the recombination frequency is increased by at least 1,000-fold to form chimeras containing genetic material from both organisms (10). Biochemical experiments employing the closely related bacteriophages M13 and fd, showed that MutS and MutL blocked RecA-mediated strand transfer of homeologous (M13-fd) but not homologous (M13-M13) DNA substrates (11, 12). ATPase-deficient MutS proteins that can still bind to mismatches also inhibit the reaction with homeologous substrates (13).

Cisplatin-induced DNA damage in *E. coli* is repaired by nucleotide excision repair (NER) and recombination, both systems being equally important (14, 15). Given that any impairment of recombination ability in *dam* bacteria is expected to be lethal and that MMR blocks homeologous recombination, we hypothesized that recombinational repair of platinated DNA is functionally identical to homeologous recombination. To test our hypothesis, we have used the RecA-catalyzed strand transfer reaction to show that MutS decreases transfer with platinated but not unmodified substrates.

Materials and Methods

Cell Survival. Survival after exposure to cisplatin was performed as described (4, 15). Strain MV1161 has the genotype *thr-1 araC14 leuB6(Am) Δ (gpt-proA)62 lacY1 tsx-33 supE44(AS) galK2(Oc) hisG4(Oc) rfbD1 mgl-51 rpoS396(Am) rpsL31(Str^R) kdgK51 xylA5 mtl-1 argE3(Oc) thi-1 rfa-550*; and strain MV3855 has the following additional mutations *uvrA6 alkA1 tagA1 zhb::Tn5*. Both strains were obtained from M. Volkert (University of Massachusetts Medical School, Worcester, MA).

Proteins and DNA. RecA protein was purified from strain GM7487 (*precA430/F-lacI^q lacZ Δ M15 pro A⁺B⁺/P90C [ara Δ (lac-pro)13*] as described (16), except that the DE-52 and Sephacryl S-1000 steps were replaced by Q Sepharose (Amersham Pharmacia, Piscataway, NJ) FPLC that was eluted with a linear gradient of 200–550 mM ammonium chloride. This procedure was followed by loading the fractions containing RecA onto a single-stranded DNA (ssDNA) cellulose column (Sigma), equilibrating with 25 mM NaCl, and eluting with a solution of 500 mM NaCl and 2 μ M ATP. Analysis of the purified protein by SDS/PAGE showed no visible contaminants. The concentration of RecA was determined by the ninhydrin protein assay (17). MutS protein was purified as described (18) with minor modifications (19). MutS Δ 680 protein was purified as described (20). MutL protein was a gift from F. J. Lopez de Saro and M. O'Donnell (The Rockefeller University, New York) and MutL

Abbreviations: MMR, mismatch repair; Dam, DNA adenine methyltransferase; DSB, double-strand break; NER, nucleotide excision repair; RFI, replicative form; NC, nicked circular; ssDNA, single-stranded DNA.

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and single-strand binding protein were obtained from United States Biological (Cleveland, OH). PhiX174 replicative form (RFI) and virion DNA forms were from New England Biolabs and the RFI form was digested with *Xho*I restriction endonuclease (New England Biolabs) to produce the linear duplex form.

Platination and Survival of phiX174 DNA. PhiX174 DNA was platinated by reacting various cisplatin molar equivalents to 1 molar equivalent of ssDNA or RFI DNA in 5 mM sodium phosphate buffer, pH 7.4. The reaction was allowed to proceed for 16–24 h at 37°C, after which the DNA was precipitated with ethanol and resuspended in water. The average number of cisplatin adducts was determined by flameless atomic absorption spectroscopy. Aliquots of double-stranded platinated RFI and unmodified DNA were mixed with strains MV1161 (wild-type) and MV3855 (*uvrA6*), mixed with top agar and poured onto L broth plates solidified with 1.6% agar. The plates were incubated overnight at 37°C before scoring the plaques.

Strand Exchange Assay. Reaction mixtures contained 25 mM Tris acetate (pH 7.5), 10 mM magnesium acetate, 5% glycerol, 1 mM DTT, 8 mM phosphocreatine, 10 units/ml creatine kinase, 1 nM circular ssDNA, and 6.7 μM RecA. Reaction mixtures were preincubated at 37°C for 10 min, and linear duplex DNA substrate was added to a concentration of 0.8 nM and incubated for an additional 10 min. Strand exchange was initiated by addition of a premixed solution containing 2 μM single-strand binding protein and 3 mM ATP. MutS and/or MutL were added 1 min before initiation of the reaction or 30 min later. Samples were taken at indicated times and strand transfer was terminated by 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 40 mM Tris acetate, 2 mM EDTA. Gels were processed by staining in Vistra green (Amersham Pharmacia) fluorescent stain (1:10,000) for 60 min and then analyzed by using IMAGE READER 1 LASER/1 IMAGE software at 473 nm on a Fuji FLA-5000 PhosphorImager. The gels were quantitated by using IMAGE GAUGE V. 3.1 software.

Results

Survival of Strains Exposed to Cisplatin. We have used deletion mutations in the *dam*, *mutS*, and *mutL* genes to measure survival of strains containing them to cisplatin exposure. The data in Fig. 1 show that the *dam* mutant is more sensitive to cisplatin than its isogenic *mutS* or *mutL* derivative. The *dam* *mut* strains are marginally, but consistently, more sensitive than wild type. These

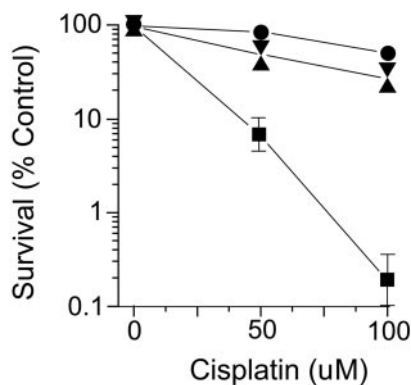


Fig. 1. Survival of *E. coli* strains after exposure to cisplatin. Logarithmic phase cells were treated for 60 min with the indicated concentration of cisplatin and plated for survival. ●, wild type; ▼, *mutS dam* deletion mutant; ▲, *mutL dam* deletion mutant; ■, *dam* mutant.

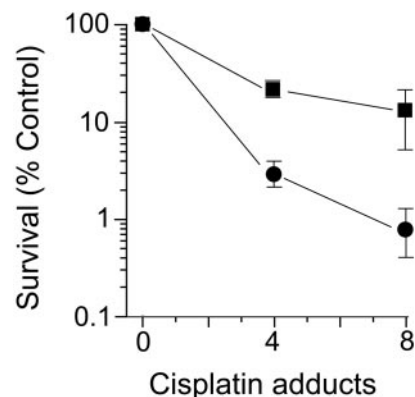


Fig. 2. Survival of platinated phiX174 RFI in wild-type and *NER*-deficient *E. coli*. RFI molecules with the indicated number of cisplatin adducts were mixed with an excess of wild-type (■) or *uvrA6* (●) bacteria and the number of plaque-forming units was determined.

results confirm our previous observation (4) using presumed base substitution mutations that inactivation of MMR in a *dam* background indeed results in drug resistance.

Platination and Survival of phiX174 DNA. Single-stranded phiX174 DNA molecules were reacted with various molar ratios of cisplatin to DNA and under the conditions of platination we have used, GG and AG intrastrand crosslinks constitute ≈65% and 25% of the adducts, respectively, and GNG intrastrand crosslinks constitute ≈5–10%. Interstrand crosslinks comprise ≈2% of the total adducts in double-stranded DNA and there are trace amounts of monoadducts (20, 21). At the levels of platination we have used, the contribution of interstrand crosslinks in ssDNA should be minimal.

The levels of adduction at the predicted ratios of four and eight adducts per DNA molecule were measured by flameless atomic absorption spectroscopy and were in good agreement with the predicted values. In the same experiment, we also platinated phiX174 RFI (covalently closed) DNA and the relationship between transfection efficiency of this platinated RFI DNA in wild-type and *NER*-deficient (*uvrA6*) bacteria is shown in Fig. 2. There was a lower survival of the treated phage DNA in the *NER*-deficient strain, which was expected given the known requirement for *NER* to remove cisplatin adducts (14, 22).

RecA Strand Transfer with Unmodified Substrates. The strand transfer reaction is shown schematically in Fig. 3 *Upper*. Under the conditions we have used, the reaction is ≈90% complete in 30 min as measured by the appearance of the nicked-circular (NC) product or the disappearance of duplex linear substrate (Fig. 3 *Lower*). This step is preceded by the formation of slowly migrating intermediate structures that persist throughout the reaction. In the absence of single-stranded substrate, no NC product was produced during 90 min of incubation, indicating that there was no ligase contamination in the RecA preparation (data not shown). Inclusion of MutS in the reaction up to 250 nM with unmodified substrates had no significant influence on the rate or yield of the reaction (Fig. 5). At concentrations higher than 250 nM, a slight inhibition was noted presumably due to nonspecific binding to the ssDNA substrate and/or intermediates (data not shown).

The linear duplex substrate was derived from the RFI (covalently closed) form by almost complete cleavage with *Xho*I. The small amount of the covalently closed form remaining was used as a standard to quantitate substrate and product bands

because it has no influence on the reaction and its concentration remains constant.

RecA Strand Transfer with Platinated Substrates. The effect of 0, 1, 10, and 24 cisplatin adducts in single-stranded substrate molecules (5,386 nt) on RecA-mediated strand transfer is shown in Fig. 4. Compared with the unmodified substrate where the reaction is 90% complete in 30 min, even a single adduct per genome resulted in a reproducible reduction in reaction rate and at 90 min the yield is $\approx 90\%$ compared with the unmodified substrate. At 10 or 24 adducts per genome, the yield was reduced to $\approx 50\%$ and $<5\%$, respectively. These experiments were carried out by using platinated ssDNA and unmodified linear duplex to reduce the effect of cisplatin interstrand crosslinks. Reversing the modification of these two species gave essentially the same results with respect to reduced RecA strand transfer (data not shown).

RecA Strand Transfer with Platinated Substrates and MutS. As described above, addition of MutS has no effect on the RecA-catalyzed strand transfer reaction with unmodified substrates. In contrast, an inhibition of strand exchange occurs in the presence of MutS if one of the substrate molecules contains cisplatin

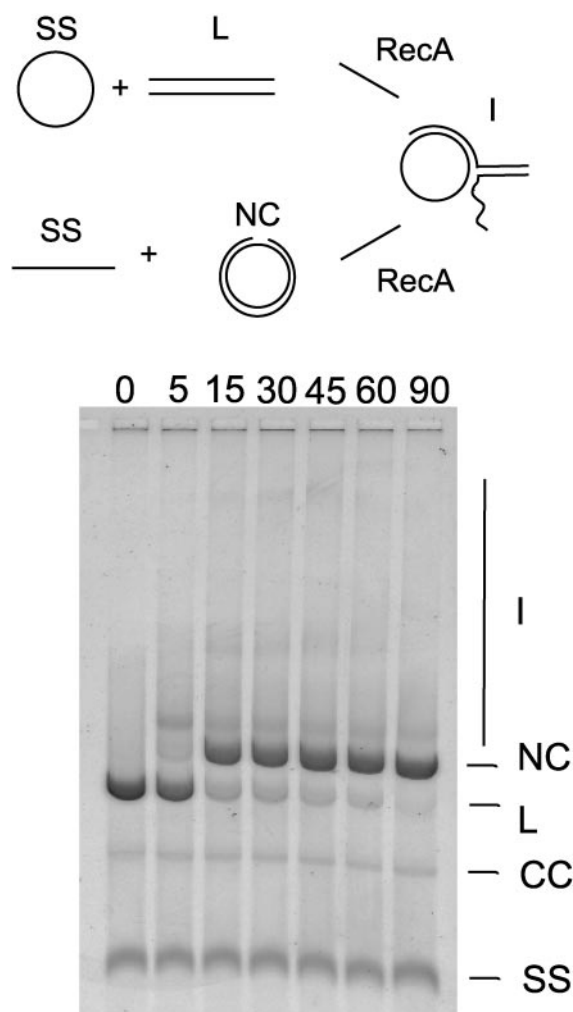


Fig. 3. RecA-mediated strand-exchange reaction. (Upper) Schematic representation of the reaction with ssDNA (SS) and linear (L) DNA substrates, intermediate structures (I), and the NC. (Lower) Time course of the RecA reaction in minutes with unmodified substrates. CC, covalently-closed DNA, which was used as a constant marker to quantitate substrates and products.

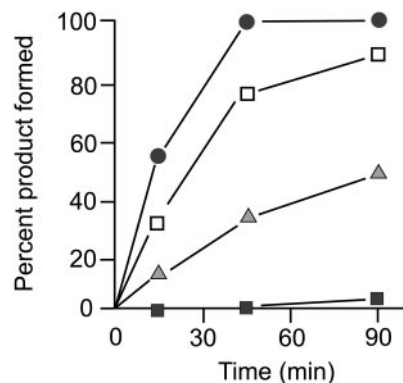


Fig. 4. Effect of cisplatin adducts on RecA-mediated strand transfer to form NC product. Filled circles, unmodified single-stranded substrate; open squares, 1 adduct per molecule; shaded triangles, 10 adducts per molecule; filled squares, 24 adducts per molecule.

adducts. As shown in Fig. 5, addition of 25, 125, and 250 nM MutS results in a concentration-dependent inhibition of strand transfer when the single-stranded substrate molecule is modified with 1, 4, 8, or 10 adducts. In these experiments, MutS was added at the beginning of the reaction. We have also added MutS 30 min after starting the reaction and this step has the effect of slowing considerably further product formation compared with its absence (data not shown). The population of molecules modified to four or eight adducts each is expected to contain 3% or 0.02%, respectively, unmodified genomes as calculated by using the Poisson distribution. Because the amount of product formation at these adduct concentrations exceeded these values, RecA can perform strand exchange with adducted DNA but at a slower rate.

MutS $\Delta 680$ Does Not Affect Strand Exchange. We have described (23) a deletion mutation of *E. coli* MutS in which the terminal amino acid residues 680–853 are deleted from the protein and that confers a mutator phenotype on host cells, indicating inactivation of the MMR process. The mutant protein hydrolyzes ATP at the same rate as the wild-type protein but has reduced ability to bind specifically to mismatched DNA, to form tetramers and dimers, and to interact with MutL (23). This mutant protein did not affect RecA-mediated strand exchange at concentration up to and including 250 nM with either modified or unmodified substrate (data not shown).

Addition of MutL to the Reaction. We next added active MutL protein to the reaction, expecting that there would be enhanced inhibition of strand exchange in the presence of MutS. However, we have not been able to show any specific inhibitory effect of MutL on the reaction except at high MutL concentrations at which nonspecific DNA binding occurs. We used two different preparations of MutL, each of which was active in an *in vitro* MMR repair assay. No effect of MutL was found when various suboptimal concentrations of MutS were used in the reaction (data not shown).

Discussion

The mechanism by which MMR sensitizes human cells and *E. coli dam* mutants to the cytotoxic action of cisplatin is not known. The sensitization is undoubtedly related to the ability of MutS from *E. coli* and human cells to bind specifically to the platinated GG intrastrand crosslink (5, 9), but not to any other adduct, including the interstrand crosslink (5). The affinity of MutS for the intrastrand crosslink is 10- to 40-fold less than that for a G-T mismatch for the human and *E. coli* enzyme, respectively (5, 9).

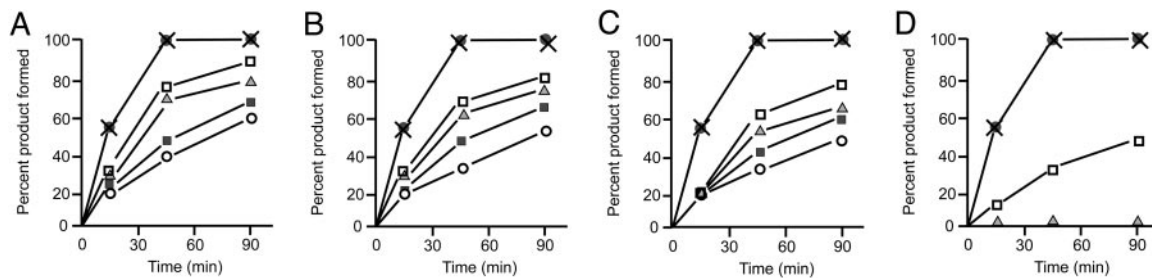


Fig. 5. Inhibition of RecA-mediated strand transfer by MutS. ssDNA molecules modified with 1 (A), 4 (B), 8 (C), or 10 (D) cisplatin adducts were used as substrates for RecA-catalyzed strand exchange. In each image, the reaction with unmodified substrate is shown for comparison (shaded circles) with the modified substrate (open squares) and for the latter with 25 (shaded triangles), 125 (filled squares), and 250 nM (open circles) MutS. The effect of adding MutS, up to and including 250 nM to unmodified substrate, is shown by the crosses.

It is possible that after exposure to cisplatin, MutS is bound to these adducts in the chromosome and is effectively titrated out, leading to a temporary MMR deficiency. If this interpretation were correct, however, we would expect to see drug resistance in a *dam* mutant at low doses or at short exposure times and this outcome is not observed (Fig. 1). As an alternative, we favor the idea that MutS binding to platinated DNA during RecA-mediated recombinational repair is the critical mode of action of MutS. We propose that inhibition of recombinational repair in *dam* mutants treated with cisplatin is central to MMR-mediated cytotoxicity. The results described in this paper suggest that it is both the reduction of RecA strand-exchange activity by relatively few cisplatin adducts and the inhibition caused by the addition of MutS that blocks recombinational repair.

In *dam mutS* or *dam mutL* bacteria, there are few DSBs due to MMR inactivation, the cells are not SOS-stressed, and so there is adequate reserve recombinational capacity upon exposure to cisplatin. Although the MutS inhibition of RecA strand transfer is relieved in these strains, due to MMR inactivation, the reduction in the rate of RecA activity with platinated substrate remains. With excess recombinational capacity, however, a reduction in rate may be inconsequential and the repair process simply takes longer to complete. This delay may favor removal of adducts by NER that otherwise would be substrates for recombinational repair. When a *dam* mutant, with limiting or no spare recombinational capacity, is overwhelmed with endogenous and cisplatin-induced DSBs, the recombinational repair process cannot be completed in the presence of MutS inhibition.

In wild-type strains, there are only the cisplatin-induced DSBs to repair and, although MutS inhibition of RecA strand transfer should occur at the same frequency as in a *dam* strain, the cells are resistant to the action of cisplatin. This finding suggests that MMR inhibition of strand transfer is reversible and *in vitro* results by using M13-fd heteroduplexes indicate that the RuvAB proteins are capable of this action (12). The reverse reaction produces the substrates and allows RecA to try again, and, with the low affinity of MutS for the adduct, strand exchange will eventually occur. In *dam* mutants, however, we propose that the reverse reaction occurs infrequently, if at all, due to limited availability of RuvAB proteins (3) that are engaged in endogenous DSB repair.

It was proposed (3) that recombination in *dam* mutants is performing near its maximal capacity to repair MMR-induced DSBs. Exposure of *dam* mutants to cisplatin would increase recombinational demand, which could not be met resulting in unrepaired DSBs and subsequent cell death. This idea is supported by data showing that recombination is as critical as NER for survival in *E. coli* after cisplatin challenge (15). That MMR has an antirecombination function in homeologous recombination, and that MutS binds specifically to cisplatin lesions, suggested that this mechanism might be responsible for inhibiting

recombinational repair (10, 11). In contrast to homeologous recombination, antirecombination action by MMR occurs on homologous DNA in which one of the recombining partner strands contains cisplatin adducts. This model is shown schematically in Fig. 6, where after the replication complex encounters the adduct, replication fork progression is blocked followed by regression, where the newly synthesized complementary strands pair to form a duplex with a flush end (24). This end becomes a substrate for RecBCD digestion, which, after encountering a Chi sequence to activate its 5' exonuclease activity and promote the release of the RecD subunit, RecA strand exchange can occur (24, 25). This strand-exchange reaction can occur opposite a cisplatin lesion as shown in Fig. 6, albeit at a slower rate. It is the heteroduplex region formed at this step to which MutS binds and decreases strand exchange activity even further. Although MutS must dissociate from this complex, given the relatively low affinity for the cisplatin intrastrand crosslink, inhibition occurs *in vitro* (Fig. 5). In the absence of MutS, strand exchange can occur past the adduct on the complementary strand and the Holliday junction intermediates are recognized and are cleaved by the RuvABC complex. At this stage, the adduct has been effectively bypassed by the replication fork and the PriA-dependent pathway can reload the replication complex at the fork (26, 27). The adduct can now be removed by NER. Support for this model includes the high sensitivity of *priA* and *ruvABC* mutants to cisplatin as well as *recA* and *recBC* strains (15, 22, 28).

An alternative to the above is the futile-cycle model originally developed to explain the role of MMR sensitization of cells to the cytotoxic effect of methylating agents (29). In this case, the replication machinery stalls at a cisplatin adduct and the replicative polymerase is temporarily replaced by a translesion polymerase (30), followed by restoration of the replicative polymerase after bypassing the lesion. Replication across the adduct is error-prone, resulting in the insertion of the incorrect base opposite the platinum adduct and subsequent binding of MutS to the base mismatch to initiate repair. Because no "good" base match exists for the adduct, the MMR system continually removes and replaces bases in a futile cycle (29, 31). The stalled fork is unable to progress and eventually disintegrates. Evidence supporting the involvement of translesion polymerases includes bypass of the AG cisplatin crosslink by DNA polymerase V (the *umuDC* product) (4) and that SOS induction also promotes translesion synthesis across GG cisplatin adducts (32).

Both models can be incorporated into the SOS stress response that occurs after challenge with cisplatin (28, 33). The initial phase of the SOS response is the rapid induction of the Uvr proteins to remove cisplatin lesions. However, NER has a 50-fold lower affinity for GG and AG intrastrand crosslinks than GNG intrastrand crosslinks, increasing the chance that the replication fork encounters one of the GG or AG lesions (34). The second

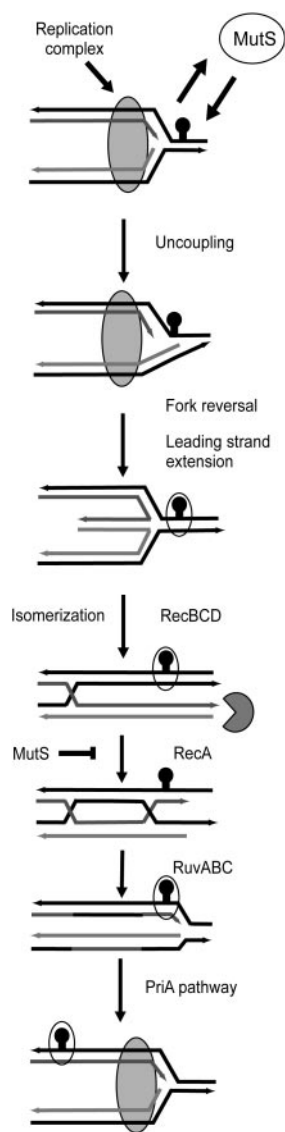


Fig. 6. Proposed model for recombinational tolerance of cisplatin adducts and its inhibition by MutS. The replication complex encounters a cisplatin adduct (filled symbol, to which MutS may or may not be bound) and stalls after uncoupling of leading and lagging strand replication, followed by fork reversal. The duplex end formed by the new strands is digested by RecBCD which, after activation at a Chi site, promotes RecA strand exchange and the Holliday junctions are acted on by RuvABC. The replication complex is reloaded onto the fork by the PriA pathway and the adduct is effectively bypassed. MutS inhibits the process at the RecA strand exchange step. Only a replication blocking lesion in the leading strand is shown, although gap repair in the lagging strand would follow the same scheme and inhibition by MutS.

phase of the SOS response is recombinational repair of stalled or collapsed forks at these lesions as described in the first model above (33). The last phase of the SOS response is translesion synthesis across the lesions, as described in the futile-cycle model. In this scenario, there are two points in the SOS response at which inhibition of repair by MMR can occur.

MutL is clearly implicated *in vivo* in conferring cisplatin resistance to *dam* cells (Fig. 1) and in preventing homeologous recombination between related bacteria *in vivo* as well as preventing RecA-mediated strand exchange between closely related M13-fd bacteriophage DNAs *in vitro* (10–12). In the M13-fd experiments, MutL alone is without effect but it potentiates the inhibition caused by MutS. MutS alone inhibited the reaction by $\approx 50\%$ and this increased to $\approx 90\%$ in the presence of MutL (11). One difference between the M13-fd and platinated DNA experiments described here is the level of adducts or base mismatches per genome; four or eight cisplatin adducts versus 192 base mismatches in the M13-fd strand-exchange reaction (35). The difference in results between mismatches and platinum adducts may reflect a dose–response relationship, where at low levels of adducts or mismatches, MutS does not need the potentiating effect of MutL for antirecombination and the inhibition of strand transfer is not as drastic. On this model, MutL is not required to cooperate with MutS to inhibit strand exchange in the *in vitro* assay where the few cisplatin crosslinks are present, but MutL is required *in vivo* where a larger number of adducts are predicted to be present (Fig. 1). The inhibitory effect of a cisplatin adduct on RecA action is presumably due to the introduction of a 50° bend toward the major groove and concomitant widening of the minor groove (36). In contrast, many base mismatches induce little deformation of the DNA (2). We know of no *in vitro* data where fewer mismatches have been used than the 192 in the M13-fd strand-exchange reactions. Alternatively, both the number of adducts or mismatches and their distribution may be important for efficient inhibition by MutS. Whereas the mismatches are scattered throughout the genomes of M13 and fd heteroduplexes (35), the distribution of cisplatin adducts in the phiX174 DNA is not known. Single-strand annealing between homeologous substrates in budding yeast has been shown to be reduced by the MutS homologues, Msh2 and Msh6, but not by the MutL homologue Pms1, an outcome similar to the result we have described here (37).

It may be that the *in vitro* RecA-mediated strand-exchange reaction does not contain all of the components to accurately reflect antirecombination *in vivo* and that an additional factor(s) is required for interaction of MutL with MutS and platinated DNA. A possible candidate protein is the DNA polymerase III holoenzyme beta clamp (proliferating cell nuclear antigen is the eukaryotic homolog) encoded by the *dnaN* gene. The β -clamp has been shown to interact with MutS (38) and the holoenzyme is required for MMR resynthesis after exonucleolytic digestion to remove mismatches (2). Future work exploring whether *dnaN* mutations affect recombinational repair is warranted.

Given the conservation of the mechanism and proteins of MMR between *E. coli* and humans, it is possible that the same mechanism of cisplatin-induced cytotoxicity may also apply in human cells. We note that cisplatin has been most strikingly successful in the treatment of testicular tumors (39) that occur in a tissue undergoing obligatory meiotic recombination and in which abrogation of recombinational repair is expected to promote apoptosis.

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