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Differential effects of cisplatin and MNNG on *dna* mutants of *Escherichia coli*

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Abstract

DNA mismatch repair (MMR) in mammalian cells or *Escherichia coli* *dam* mutants increases the cytotoxic effects of cisplatin and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). We found that, unlike wildtype, the *dnaE486* (alpha catalytic subunit of DNA polymerase III holoenzyme) mutant, and a DnaX (clamp loader subunits) over-producer, are sensitive to cisplatin but resistant to MNNG at the permissive temperature for growth. Survival of *dam-13 dnaN159* (beta sliding clamp) bacteria to cisplatin was significantly less than *dam* cells, suggesting decreased MMR, which may be due to reduced MutS-beta clamp interaction. We also found an elevated spontaneous mutant frequency to rifampicin resistance in *dnaE486* (10-fold), *dnaN159* (35-fold) and *dnaX36* (10-fold) strains. The mutation spectrum in the *dnaN159* strain was consistent with increased SOS induction and not indicative of MMR deficiency.

Keywords

Cisplatin; MNNG; DNA repair; DNA replication; mutation

1. Introduction

Cisplatin (*cis*-diaminodichloroplatinum (II)) is an antitumor agent which has a cure rate of greater than 90% for testicular cancer [1]. It reacts with DNA to produce mostly intrastrand crosslinks between adjacent guanines (65% of the total), adjacent guanine and adenines (25%), and guanines separated by a base (1,3-GNG, 5–10%) [2,3]. Interstrand crosslinks comprise about 2% of the total adducts and small amounts of monoadducts are also formed. The biologically inactive *trans* isomer of cisplatin also produces 1,3-GNG adducts, interstrand crosslinks and monoadducts [4,5], suggesting that intrastrand crosslinks between adjacent purines are the biologically important adducts of cisplatin since they efficiently block progression of DNA polymerases *in vitro* and *in vivo* [6].

Nucleotide excision repair (NER) removes platinated intrastrand crosslinks but the 1,3-GNG lesions are removed at a rate 50-fold faster than those between adjacent purines [7]. The importance of NER is manifested by the increased sensitivity of NER-deficient mutants of *Escherichia coli* and mammalian cells to cisplatin [8–10]. In addition, however, recombinational repair mechanisms are as important as NER in allowing cells to survive cisplatin damage since *E. coli* strains in which recombination is defective show increased susceptibility to cisplatin [11,12]. Cisplatin is also a potent inducer of recombination [13].

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Methylating agents are used in cancer chemotherapy and laboratory versions, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), react with DNA and cause a signature type of damage, producing a variety of lesions which include O⁶-methylguanine (O⁶-meG), 3-methyladenine and 7-methylguanine [14]. These lesions are usually removed either directly, in the first case by a methyltransferase, or through base excision repair using specific glycosylases.

GATC sequences in *E. coli* DNA are methylated directly after replication at the N6 position of adenine by Dam methyltransferase a product of the *dam* gene [15]. The DNA behind the replication fork is already methylated on the parental strand but not on the newly-synthesized daughter strand to give hemimethylated DNA. Replication errors are present in the unmethylated strand and MMR is initiated when MutS binds to base mismatches [16]. Recruitment of MutL and MutH forms a ternary complex which activates the latent endonuclease activity of MutH to cleave 5' to the G at an unmethylated GATC sequence. The UvrD helicase unwinds the unmethylated strand in either the 3' or 5' direction and which is digested by exonucleases with a distinct polarity. Resynthesis is accomplished by the polymerase III holoenzyme complex and the resulting nick is ligated by DNA ligase [16]. In wildtype cells, mismatch correction is restricted to the hemimethylated region as MutH has little, if any, activity on fully methylated DNA [17]. In *dam* mutants, which lack DNA adenine methyltransferase, no methylation is present and MMR can occur in newly replicated as well as in unreplicated DNA because MutH can utilize unmethylated DNA as a substrate [18].

E. coli dam mutants are more sensitive to the cytotoxic effects of cisplatin [19] and MNNG [20,21] than wildtype. Mutations disabling the MMR system (*mutS*, *mutL*) in a *dam* cell, however, render it as resistant to these agents as wildtype [19,20]. The MutS protein from *E. coli* specifically recognizes the platinated GG intrastrand crosslink opposite CC or CT [22,23] and O⁶-meG-cytosine and O⁶-meG-thymine base pairs [23,24] and the latter can be formed by replicative or bypass polymerase action [25]. MMR action on cisplatin intrastrand crosslinks in *dam* bacteria leads to the formation of double-strand breaks that require recombination to repair them [26]. MutS interferes with RecA-mediated strand exchange during the recombinational repair process [27].

Although *dam* bacteria are sensitive to the cytotoxic action of both MNNG and cisplatin via specific binding by MutS, the recognition of adducts by this enzyme is complex. We recently described a mutated MutS protein, MutSΔ800, produced from a multicopy plasmid, that sensitizes *dam* cells to MNNG but not to cisplatin, and was found to bind to O⁶-meG mismatches but not platinated GG intrastrand crosslinks opposite CC [23]. MMR in wild type cells occurs near the replication fork and the MutS protein has been reported to interact with the DNA polymerase III beta-clamp [28] which confers processivity on the holoenzyme by "clamping" the catalytic subunit, DnaE, to DNA. Sensitivity of *dam* cells to cisplatin and MNNG could, therefore, reflect an interaction between mismatch repair and DNA polymerase III holoenzyme and associated replication proteins. We have examined various DNA replication mutants and we describe two instances where *dna* gene products alter the cellular response to the two agents. Furthermore, if the interaction between the beta clamp, a product of the *dnaN* gene, and MutS is diminished it might lead to a mutator phenotype due to ineffective MMR. We show that the *dnaN159* mutant does indeed have a mutator phenotype but it is not due to lack of MMR.

2. Materials and methods

2.1. Bacterial strains and plasmid

The bacterial strains used in this study are listed in Table 1. J. R. Walker's laboratory [29] constructed and designated strain GM36 (*dnaX36*), but it is called GM8051 in this paper to avoid confusion with the strain of the same name but different genotype derived in this laboratory. The *dnaX* plasmid, obtained from J.R. Walker (University of Texas, Austin), is a derivative of pBR322 [30].

2.2 Media

L medium contains 20 g tryptone (Difco), 10 g yeast extract (Difco), 0.5 g NaCl, 4 ml of 1M NaOH per liter and solidified when required with 16 g of agar. Brain Heart Infusion (Difco) broth was prepared using 20 g of powder per liter of water and solidified, when required, with 16 g of agar. Minimal medium was prepared as described by Davis and Mingioli [31] and supplemented with amino acids (80 µg/ml) as required. Ampicillin and rifampicin were included in media at 100 µg/ml while, tetracycline and chloramphenicol were included in media at 10 µg/ml. Kanamycin was included, when required, at 20 µg/ml. Media for the reversion assays contained 10% lactose and Xgal (40 ng/ml) in minimal media.

2.3 Estimation of rifampicin-resistant mutant frequency

Multiple cultures (generally 10) of the deletion strains and their isogenic partners were generated from an inoculum of a few hundred cells and grown to saturation. Portions of the cultures were plated on BH medium with rifampicin and incubated at 30°C and 34°C or 37°C depending on cell viability, until colonies appeared. Media were also supplemented with 100 µg ampicillin /ml when required.

2.4 Lac reversion assay

CC101–107 *dnaN159* derivatives were constructed by P1*vir* transduction using the closely-linked *zid-501::Tn10* marker. CC101–106 *mutS458* derivatives have been described [32] and CC107 *mutS458* was constructed by P1 *vir* transduction. Ten cultures of these strains and the CC101–107 parental strains were grown to saturation in minimal media. For Fig. 6, 10 µl of the culture was spotted on a minimal media plate containing lactose and Xgal and incubated at 30°C until colonies formed and papillae appeared. For Table 3, portions of the cultures were plated and incubated at 30°C on the same type of plates and blue colonies counted to calculate the reversion rate. Viable counts were determined on glucose minimal plates.

2.5 Cisplatin and MNNG survival

Cisplatin (Sigma) was dissolved in water and incubated at 37°C for at least 2 hours. The molar concentration was measured by taking an absorbance spectrum and reading a maximal absorbance at 301nm and dividing by the extinction coefficient, 131 M⁻¹ cm⁻¹. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG (Sigma)) was prepared by dissolving 1 mg of MNNG in 100 µl of DMSO and adding 900 µl of sterile water. Cells were grown in 10 ml L medium to an OD₆₀₀ of 0.35–0.45, harvested and resuspended in the same volume of minimal salts. Varying concentrations of cisplatin were added and incubated at 30°C for 1 hour. Serial dilutions of cisplatin-exposed cells were plated on L media plus ampicillin and incubated overnight. For MNNG exposure, the logarithmic phase cells in L broth were exposed to various concentrations of MNNG for 10 minutes at 30°C followed by dilution for plating as described above.

3. Results

3.3 Survival of the *dnaE486* mutant and DnaX36 overproducing strain after treatment with cisplatin and MNNG

At the permissive temperature for growth, 30°C, the *dnaE486* (encodes the alpha-catalytic subunit of PolIII) strain is more sensitive than wildtype to cisplatin, but not MNNG (Fig. 1). The degree of cisplatin sensitivity of the *dnaE486* strain is the same as that for a *dam-13* mutant (Fig. 1A). The *dnaE486 dam-13* double mutant strain is more sensitive to both cisplatin and MNNG than either parent.

The *dnaX36* (tau and gamma subunits of PolIII) bacteria show the same survival to cisplatin as wildtype at 30°C but are more sensitive at 37°C to both cisplatin and MNNG (Fig. 2) although the viable counts of these cultures were the same at the two temperatures. An unusual feature of DnaX is that when expressed from a multicopy plasmid at 30°C, the *dnaX36* host strain bearing it becomes sensitive to cisplatin (Fig. 2A), to the same degree as a *dam* mutant, but remains resistant to MNNG (Fig. 2B). No sensitivity to cisplatin was detected when the same plasmid is expressed in a wildtype strain (Fig. 2A) or with the vector plasmid (data not shown). We could not test the effect of the *dam-13 dnaX36* combination because although the strain was viable at 30°C, it cannot form colonies at 37°C in contrast to the parental strains.

3.4 Survival of *dnaN159* bacteria after treatment with cisplatin and MNNG

There was no differential effect on the *dnaN159* (beta subunit of PolIII holoenzyme) strain to the cytotoxic action of cisplatin and MNNG (Fig. 3). The *dam-13 dnaN159* double mutant, however, is more resistant to MNNG than the *dam-13* strain alone (Fig. 3B). This increased resistance could be due to a reduction in MMR since null mutations in the *mutS* or *mutL* genes in a *dam* mutant increase resistance to MNNG to the wildtype level. To monitor the effect of MMR on *dnaN159 dam-13* survival, a *dnaN159 dam-13 mutS458* triple mutant was constructed. The survival of the double and triple mutants after MNNG exposure was the same (Fig. 3B) suggesting a reduction of MMR in the *dnaN159 dam-13* strain (see Discussion).

3.5 Survival of *dnaB43* and *dnaG3* bacteria after treatment with cisplatin and MNNG

Neither *dnaB43* (helicase) nor *dnaG3* (primase) mutants showed a differential sensitivity to cisplatin or MNNG (Figs. 4, 5). The *dam* derivatives of these strains showed no additional increase in sensitivity to both agents indicating that the *dna* strains had a wildtype phenotype at the temperature (30°C) at which the experiments were conducted. The exception was the *dam dnaG3* strain which showed increased sensitivity to MNNG but not cisplatin (Fig. 4B).

3.1 Spontaneous mutant frequency to rifampicin-resistance in wild type and *dna* strains

Bacteria with an inactive MutS protein exhibit a mutator phenotype [33]. If the interaction between the MutS protein and the beta-clamp, a product of the *dnaN* gene [34], is required for efficient MMR, then if it is diminished a mutator phenotype should result. We therefore tested the parental and *dnaN* temperature sensitive mutant strain as well as *dnaB*, *dnaE*, *dnaG* and *dnaX* bacteria for spontaneous mutant frequency to rifampicin-resistance at the permissive temperature, 30°C. The results in Table 2 show that *dnaN* has the highest mutation frequency, a 35-fold increase, in comparison to the parental strain. The *dnaX* mutant has a wildtype level of spontaneous mutagenesis at 30°C but this is increased to 10-fold at 37°C, a temperature at which full viability is retained. A strain with a *holD* mutation, which encodes the *psi* subunit of the holoenzyme, has been reported to show a seven-fold increase in spontaneous mutation frequency using rifampicin-resistance as an assay [35]. Bacteria with the *dnaE486* allele show a 10-fold increase in mutation frequency to

rifampicin resistance (Table 2) confirming a previous 40-fold increase with this marker [36] and a 6-fold increase in *his-4* reversion [37]. The *dnaB* and *dnaG* mutants do not exhibit a mutator phenotype (Table 2), as there is less than a 2-fold difference when comparing these strains with the wild type strain.

To determine the mutation spectrum in the *dnaN159* strain, we have used the *lacZ* reversion assay described by Cupples and Miller [38,39]. The *dnaN159* mutation was introduced into each of the seven tester strains and the number of Lac⁺ revertants was measured at 30°C and compared to the wildtype strains. The results are shown in Table 3 and Fig. 6. There is a 7-fold increase in AT to TA transversions which is probably due to increased SOS induction in this strain [36,40]. This change is not expected in MMR-deficient strains which show increases in transition and +1 and -1 frameshift mutations [32,38,39] and the pattern of reversion of a *mutS* strain is clearly different from that of *dnaN159* (Fig. 6). We did not observe an increase in GC to AT revertants in the *mutS* strain which was expected based on previous results [33,38,41]. We conclude that the increased spontaneous mutation frequency in the *dnaN159* mutant is not due to diminished MMR.

4. Discussion

We showed previously that the *mutS*Δ800 mutation on a multicopy plasmid in a *mutS* null mutation host conferred resistance to cisplatin, but not MNNG, and that there was reduced binding of MutSΔ800 to platinated, but not methylated, oligonucleotides [23]. The contrasting types of modifications produced in DNA by these agents prompted us to examine if DNA polymerase III holoenzyme mutants also showed such a differential sensitivity. The results are summarized in Table 4. We found that unlike wildtype, *dnaE486* bacteria (Fig. 1) and those overproducing DnaX (Fig. 2) were more sensitive to cisplatin but resistant to MNNG. Possible explanations are that the DnaE486 holoenzyme (at the permissive temperature) is less capable of restarting replication after removal of a blocking cisplatin crosslink than a DnaE holoenzyme during chromosome replication. This holoenzyme might not be as efficient as DnaE in gap filling during MMR. Alternatively, the DnaE486 holoenzyme progression may not be blocked as efficiently as DnaE by cisplatin adducts leading to fork disintegration. On the other hand, an O⁶-meG base pair, which is a miscoding but not a blocking lesion, would be replicated by both DnaE486 and DnaE holoenzymes with, presumably, similar efficiencies.

The DnaX overproducing plasmid in *adnaX36* host restores the ability to grow at 43°C indicating complementation of the mutant phenotype by the wildtype gene product (Fig. 2). Although complementation by DnaX overproduction also restores MNNG resistance to the wildtype level, the strain is still sensitive to cisplatin. There are at least two possible explanations. First, since nothing is known about the regulation of DNA polymerase III holoenzyme assembly, it is possible that overproduction of the tau and gamma subunits, the products of the *dnaX* gene, disrupts holoenzyme function. This option is unlikely because overproduction of DnaX in the wildtype does not lead to cisplatin sensitivity. Second, it is possible that the DnaX36 clamp loader complex has a higher affinity for other holoenzyme proteins, such as DnaN (beta clamp), and although DnaX is overproduced clamp loader complexes of DnaX and DnaX36 are formed. Since DnaX36 is probably slightly defective, the mixed DnaX-DnaX36 complex is proficient for clamp loading onto methylated but not platinated DNA.

Dam mutS bacteria are resistant to cisplatin and MNNG. MutS interacts with the beta clamp [28] and this interaction may be important for the re-synthesis step of MMR by DNA polymerase III holoenzyme. If this interaction is critical for MMR sensitization, then an increase in resistance to cisplatin and MNNG might be expected in the *dam-13 dnaN159*

mutant. At the permissive temperature, there was no difference in survival between the double mutant and the *dam-13* strain to cisplatin (Fig. 3). The *dam-13 dnaN159* strain, however, is more resistant to the cytotoxic effect of MNNG than *dam-13* bacteria which indicates reduced MMR perhaps due to a reduction in MutS-beta clamp interaction. The triple *dam-13 dnaN159 mutS458* mutant has the same survival as the *dam-13 dnaN159* double mutant suggesting that indeed there is reduced MMR (Fig. 3). The triple mutant is expected to have the same resistance as wild type because that is observed in *dam mutS* strains. This is not the case indicating that there must also be an MMR-independent sensitivity to MNNG in the *dnaN159* strains as well as an MMR impairment.

Although reduced MMR in the *dnaN159* strain affects survival after exposure to MNNG, it is not sufficient to affect the mutant frequencies shown in Tables 2 and 3 and Fig. 6. That is, the pattern of reversion is clearly not that expected for an MMR-deficient strain. The *dnaN159* mutant displays constitutive SOS induction and this is the likely explanation for the increase in AT to TA transversions [40]. The reduction in +1 frameshift mutations in CC107 *dnaN159* and GC to TA transversions in CC104 *dnaN159* could be due to slower DNA replication resulting in more efficient repair of replication errors (frameshifts) and spontaneous oxidative lesions (8-oxoguanine) [33]. For the latter, it is also possible that SOS-induced translesion polymerases in the *dnaN159* strain efficiently bypass this modified base, while in the wildtype it is mutagenic when replicated by DNA polymerase III holoenzyme.

Mammalian cells are sensitive to the cytotoxic action of MNNG [42] and cisplatin [43] but MMR deficient cell lines derived from them are resistant to the action of these agents, although this association for cisplatin has recently been questioned [44,45]. The MutS protein from human cells specifically recognizes the platinated GG intrastrand crosslink and O⁶-meG/cytosine and thymine base pairs [46]. 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 double-strand break formation and subsequent signaling for cell cycle arrest and apoptosis [42]. An alternative model posits a direct link between lesion recognition by MutS and a signal transduction cascade leading to cell death [47,48]. Insofar as the work reported here with *E. coli* can be extrapolated to human cells, our results would favor the double-strand break model.

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Fig. 1. Survival at 30°C of wildtype (CR34), *dnaE486* (JW130), *dam-13* (GM2927) and *dam-13 dnaE486* (GM8096) strains after treatment with cisplatin and MNNG.

Fig. 2.
Survival of strains GM8051 (*dnaX36*) at 30°C and 37°C, GM2927 (*dam-13*) at 37°C, GM8128 (*pdnaX*⁺/*dnaX36*) at 30°C and GM8137 (*pdnaX*⁺/AB1157) at 30°C after treatment with cisplatin and MNNG.

Fig. 3. Survival at 30°C of wildtype (AB1157), *dnaN159* (GM8020), *dam-13* (GM2927), *dam-13 dnaN159* (GM8024) and *dam-13 dnaN159 mutS458* (GM8060) strains after treatment with cisplatin and MNNG.

Fig. 4. Survival at 30°C of wildtype (CR34), *dnaG3* (JW177), *dam-13* (GM2927) and *dam-13 dnaG3* (GM8047) strains after treatment with cisplatin and MNNG.

Fig. 5. Survival at 30°C of wildtype (AB1157), *dnaB43* (GM534), *dam-13* (GM2927) and *dam-13 dnaB43* (GM8026) strains after treatment with cisplatin and MNNG.

Fig. 6. Reversion of *lacZ* alleles in CC101–CC107 (top row), and their *dnaN159* (middle row) and *mutS215* (bottom row) derivatives.

Table 1*E. coli* K-12 strains used in this study

Number	Description	Source of mutation/strain
AB1157	<i>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</i>	E.A Adelberg
CC101– CC107	<i>ara-600 delta (gpt-lac)5 relA1 spoT1 thi-1/ F128 lacI373 lacZ</i>	C. Cupples
CR34	<i>thr-1 leuB6(Am) fhuA21 lacY1 supE44(AS) rfbD1 thyA6 thi-1 deoC1</i>	CGSC
GM534	As AB1157 but <i>dnaB43 thyA6 drm-6</i>	
GM2927	As AB1157 but <i>dam-13::Tn9</i>	
GM4252– GM4257	As CC106 to CC107 but <i>mutS215::Tn10</i>	
GM4799	As AB1157 but <i>mutS458::mTn10Kan</i>	
GM8020	As AB1157 but <i>dnaN159 zid-501::Tn10</i>	
GM8024	As GM8020 but <i>dam-13::Tn9</i>	
GM8026	As GM534 but <i>dam-13::Tn9</i>	
GM8047	As JW177 but <i>dam-13::Tn9</i>	
GM8051	<i>dnaX36(Ts) thr-1 leuB6(Am) fhuA2 pro-33 lacY1 supE44(AS) gal-6 uidA2 galP63 xylA7 mtlA2 delta rplL9(L?) thi-1 argH1</i>	J.R Walker
GM8056	As GM4799 but <i>dnaN159 zid-501::Tn10</i>	
GM8058	As GM4799 but <i>dam-13::Tn9</i>	
GM8060	As GM8058 but <i>dnaN159 zid-501::Tn10</i>	
GM8067	As GM8051 but <i>dam-13::Tn9</i>	
GM8096	As JW130 but <i>dam-13::Tn9</i>	
GM8128	GM8051/ <i>pdnaX</i> ⁺	
GM8137	AB1157/ <i>pdnaX</i> ⁺	
GM8301	As CC101 but <i>dnaN159 zid-501::Tn10</i>	
GM 8303	As CC102 but <i>dnaN159 zid-501::Tn10</i>	
GM 8304	As CC103 but <i>dnaN159 zid-501::Tn10</i>	
GM 8306	As CC104 but <i>dnaN159 zid-501::Tn10</i>	
GM 8308	As CC105 but <i>dnaN159 zid-501::Tn10</i>	
GM 8310	As CC106 but <i>dnaN159 zid-501::Tn10</i>	
JW130	As CR34 but <i>dnaE486</i>	J. Wechsler
JW177	As CR34 but <i>dnaG3</i>	J. Wechsler

Unless otherwise stated, strains are laboratory stocks. Abbreviations:Am, *amber* mutation; AS, *amber* suppressor; delta , deletion; Fs, frameshift; mTn10, miniTn10; Oc, ochre mutation; Str, streptomycin; Kan, kanamycin; Tn9 and Tn10 encode chloramphenicol and tetracycline resistance respectively; Ts, temperature-sensitive.

Table 2Mutant frequencies ($\times 10^{-8}$) to rifampicin resistance

Allele	Temp	Ratio
<i>dnaB43</i>	30°C	1.6
<i>dnaE486</i>	30°C	9.6
<i>dnaG3</i>	30°C	0.8
<i>dnaN159</i>	30°C	35
<i>dnaX36</i>	30°C	1.9
	37°C	10.1

The mutant frequency to rifampicin resistance was measured by simultaneously cultivating ten independent cultures of each *dna* mutant with its isogenic wildtype and expressing the result as a ratio. The average mutant frequency for the wildtype was 2×10^{-8} . Viability was the same at both temperatures for *dnaX36* bacteria.

Table 3Reversion frequencies ($\times 10^{-8}$) of *lacZ* alleles

Base change	Wildtype	<i>dnaN159</i>	Ratio
AT to CG	1.7	1.8	1.1
GC to AT	2.8	2.2	0.8
GC to CG	<0.5	<0.5	—
GC to TA	46.2	3.1	0.07
AT to TA	2.0	14.3	7
AT to GC	<0.5	<0.5	—
6G to 7G	133.0	4.0	0.03

Measurements of *lacZ* reversion were carried out at 30°C. The column describing the base changes in descending order represent the wildtype strains, CC101 to CC107. The values in the third column are for the *dnaN159* derivatives of CC101–CC107.

Table 4

Summary of cellular responses to MNNG and cisplatin

Strain	Response to	
	MNNG	Cisplatin
<i>dnaE486</i>	–	+
<i>dnaX36</i> at 30° C	–	–
<i>dnaX36</i> at 37° C	+	+
<i>pdnaX/dnaX36</i>	–	+
<i>dnaN159</i>	–	–
<i>dnaG3</i>	–	–
<i>dnaB43</i>	–	–
<i>dam-13</i>	+	+
<i>dam-13 dnaE486</i>	>	>
<i>dam-13 dnaX36</i> at 30°C	=	=
<i>dam-13 dnaX36</i> at 37°C	nd	nd
<i>dam-13 dnaN159</i>	<	=
<i>dam-13 dnaN159 mutS458</i>	<	nd
<i>dam-13 dnaG3</i>	>	=
<i>dam-13 dnaB43</i>	=	=

A score of (–) indicates a response similar to the isogenic partner of the specified strain.

A score of (+) indicates a sensitive response upon exposure to the agent.

A score of (=) indicates the response is equivalent to *dam* alone.

A score of (>) indicates a response which is more sensitive than *dam* alone.

A score of (<) indicates a response which is more resistant than *dam* alone.

nd indicates not determined.

Unless otherwise stated, all responses were measured at 30°C.