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Mismatch repair ensures fidelity of replication and recombination in the radioresistant organism *Deinococcus radiodurans*

Abstract We have characterized the mismatch repair system (MMR) of the highly radiation-resistant type strain of *Deinococcus radiodurans*, ATCC 13939. We show that the MMR system is functional in this organism, where it participates in ensuring the fidelity of DNA replication and recombination. The system relies on the activity of two key proteins, MutS1 and MutL, which constitute a conserved core involved in mismatch recognition. Inactivation of MutS1 or MutL resulted in a seven-fold increase in the frequency of spontaneous Rif[R] mutagenesis and a ten-fold increase in the efficiency of integration of a donor point-mutation marker during bacterial transformation. Inactivation of the mismatch repair-associated UvrD helicase increased the level of spontaneous mutagenesis, but had no effect on marker integration—suggesting that binding of MutS1 and MutL proteins to a mismatched heteroduplex suffices to inhibit recombination between non identical (homeologous) DNAs. In contrast, inactivation of MutS2, encoded by the second *mutS*-related gene present in *D. radiodurans*, had no effect on mutagenesis or recombination. Cells devoid of MutS1 or MutL proteins were as resistant to γ-rays, mitomycin C and UV-irradiation as wild-type bacteria, suggesting that the mismatch repair system is not essential for the reconstitution of a functional genome after DNA damage.

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Introduction

Mismatch repair (MMR) systems are evolutionarily conserved and play a primary role in mutation avoidance by correcting replication errors resulting from nucleotide misincorporation and polymerase slippage (for reviews see Modrich and Lahue 1996; Schofield and Hsieh 2003). In addition, MMR has an important role in ensuring the fidelity of homologous recombination. Indeed, MMR proteins are able to recognize mismatches in recombination intermediates and thus to inhibit recombination between moderately divergent (homeologous) sequences (Rayssiguier et al. 1989; Worth et al. 1994; Matic et al. 1995).

In *Escherichia coli*, the mismatch repair system requires the proteins MutS, MutL, MutH and MutU (formerly called UvrD), whose functions are quite well understood (Schofield and Hsieh 2003). The MutS protein recognizes and binds mismatches in DNA and recruits MutL. Together, the two proteins are thought to stimulate the nicking activity of MutH. MutH cleaves transiently unmethylated GATC sites in the nascent DNA strand, and provides entry sites for the MutU/UvrD helicase, which melts the nicked strand over a stretch of several kilobases to allow repair synthesis (Modrich and Lahue 1996).

The highly radiation-resistant bacterium *Deinococcus radiodurans* can reconstitute a functional genome from hundreds of radiation-induced chromosomal fragments. This process occurs mainly through homologous recombination and requires RecA function (for reviews see Battista 1997, 1999). The *D. radiodurans* genome contains numerous repeated sequences, including expanded families of transposons (Makarova et al. 2001). Since extensive intra- and inter-chromosomal recombination between

these sequences can be potentially highly deleterious, *D. radiodurans* must possess regulatory mechanisms that negatively control these recombination events, in particular, during the reconstruction of its genome after DNA damage. One such mechanism might involve a particular organization of its genome which serves to limit ectopic recombination events (Minton and Daly 1995; Levin-Zaidman et al. 2003). Another, and non mutually exclusive mechanism, may involve the operation of an efficient mismatch repair system (Myung et al. 2001).

Here we analyze the functional status of mismatch repair in this organism. Only the MutSL core of the mismatch repair system appears to have been conserved throughout evolution (for review see Kolodner 1996). A recent phylogenetic analysis of MutS homologs shows that this family can be divided into two major subfamilies, *MutS1* and *MutS2* (Eisen 1998) which can be aligned only in the central portion of their sequences containing the nucleotide-binding domain (Moreira and Philippe 1999). Only the members of the *MutS1* subfamily have been implicated in mismatch repair (Eisen and Hanawalt 1999). *D. radiodurans* possesses homologs of *mutL* (*DR1696*), *mutS1* (*DR1039*), *mutS2* (*DR1976*), and *uvrD* (*DR1775*). However, the sequence data (http://www.tigr.org/tigr-scripts/CMR2/GenePage.spl?locus = DR1039) indicate that the putative product of the *mutS1* gene is a truncated protein that is likely to be inactive in mismatch repair. Indeed, the *mutS1* homolog from *D. radiodurans* requires two translational frameshifts to encode a full length protein that aligns with the other members of the *MutS1* subfamily.

Here, we show that mismatch repair is functional in the reference wild-type strain of *D. radiodurans*, ATCC 13939. First, we re-examined the sequence of the *DR1039* gene (*mutS1*). We found that it contains two additional nucleotides relative to the published sequence, which restore a full-length MutS1 ORF. Second, we show that cells devoid of MutS1 or MutL have a mutator phenotype. These cells also display increased efficiency of integration, after bacterial transformation, of a donor DNA fragment containing a point mutation in the *rpoB* gene. In contrast, lack of the MutS2 protein had no effect on the levels of spontaneous mutagenesis or recombination. However, mismatch repair-deficient *D. radiodurans* cells were as resistant to γ-rays, mitomycin C or UV-light as wild-type bacteria, suggesting that the MMR system is not essential for the reconstitution of a functional genome after DNA damage.

Materials and methods

Bacterial strains, cultures, media, and transformation

Bacterial strains are listed in Table 1. The *Escherichia coli* strains used were DH5α and GM2163. Strain GM2163 was used to propagate plasmids prior to transformation of *D. radiodurans,* since this process is sensitive to the methylation status of the transforming DNA (Meima et al. 2001). The *D. radiodurans* R1 strain used in this work was ATCC 13939. Strains GY11713 (Δ *mutS1*:: *tet*), GY11712 (Δ *mutS2*:: *tet*) and GY11708 (Δ*mutL*:: *tet*) were constructed by transformation of wild-type *D. radiodurans* R1 cells with a PCR fragment containing the mutated allele amplified from plasmid pGY11635, pGY11624 or pGY11625, followed by selection for Tet[R]. Gene replacement in the Tet[R] transformants, and their genetic purity, were verified by PCR using appropriate primer pairs to demonstrate the presence of the two predicted junction fragments and the absence of an internal fragment of the wild-type allele (Supplementary Fig. S1, Supplementary Table S1).

Strains GY11724 (Δ *mutS2*:: *kan*) and GY11725 (Δ *mutS2*:: *kan* Δ *mutS1*:: *tet*) were constructed as follows. A *kan* cassette (Bonacossa de Almeida et al. 2002) and two 500-bp genomic fragments from upstream

Table 1 Bacterial strains

Name	Relevant markers[a]	Source/reference, alias
Escherichia coli		
DH5α	*hsdR17 recA1 endA1 lacZ*ΔM15	Hanahan (1983)
GM2163	*dam::*Tn9 *dcm6*	Biolabs
Deinococcus radiodurans		
R1 (ATCC 13939)	Wild type	Anderson et al. (1956), GY9613
NS3113	*uvrD::*Tn *DrCat*	Kim et al. (2004), GY12307
GY11713	*mutS1::tet*	This work
GY11708	*mutL::tet*	This work
GY11712	*mutS2::tet*	This work
GY11724	*mutS2::kan*	This work
GY11725	*mutS1::tet mutS2::kan*	This work
GY10916	Wild type	This work, GY9613 (pI8)
GY11723	*mutS1::tet*	This work, GY11713 (pI8)
GY11721	*mutL::tet*	This work, GY11708 (pI8)
GY11718	*mutS1::tet/mutS1*[+]	This work, GY11713 (pGY11637)
GY11717	*mutS2::tet/mutS2*[+]	This work, GY11724 (pGY11633)
GY11716	*mutL::tet/mutL*[+]	This work, GY11708 (pGY11634)
GY11730	Rif[R]	This work, rif1
GY11731	Rif[R]	This work, rif2
GY11732	Rif[R]	This work, rif3
GY11733	Rif[R]	This work, rifΔ9

[a]Alleles on the right of the slash are contained in a plasmid. Names of *D. radiodurans* genes are from their *E. coli* orthologs (Makarova et al. 2001) except for *mutS1* and *mutS2* named according to Eisen (1998)

and downstream of the coding region of *mutS2* were amplified by PCR using primer pairs that introduced *Bam* HI and *Xba* I restriction sites. The three fragments were ligated together so that the *kan* cassette was flanked by the two genomic fragments, and the tripartite ligation product was used to transform *D. radiodurans* R1 to replace the wild-type allele by homologous recombination. This gave rise to strain GY11724. The DNA from this strain was then used to transform strain GY11713 containing the Δ *mutS1*:: *tet* allele, to generate strain GY11725. The genetic structure of GY11725 was checked by PCR as described previously (see Supplementary Table S1 for the diagnostic primer pairs used).

Growth of *D. radiodurans* and *E. coli* was as described in Bonacossa de Almeida et al. (2002). When necessary, media were supplemented with the appropriate antibiotics used at the following final concentrations: ampicillin, 100 μg/ml for *E. coli*; chloramphenicol, 20 μg/ml for *E. coli* and 3 μg/ml for *D. radiodurans*; tetracycline, 10 μg/ml for *E. coli* and 2.5 μg/ml for *D. radiodurans*; rifampicin, 25 μg/ml for *D. radiodurans*; kanamycin 6 μg/ml for *D. radiodurans*; spectinomycin, 40 μg/ml for *E. coli* and 75 μg/ml for *D. radiodurans*.

Transformation of *D. radiodurans* with genomic DNA, PCR products, or plasmid DNA was performed as described by Bonacossa de Almeida et al. (2002).

PCR, oligonucleotide primers and plasmids

PCR amplification of plasmid or genomic DNA was performed with DyNAzyme EXT DNA polymerase (Finnzyme). Oligonucleotide primers used for cloning and diagnostic PCR experiments are listed in Supplementary Table S1.

Plasmids are listed in Table 2. Plasmids pGY11618, pGY11619 and pGY11617, containing the Δ *mutS1*:: *tet*, Δ *mutS2*:: *tet*, and Δ*muL*:: *tet* alleles used to inactivate the corresponding chromosomal genes, were constructed as follows. First, *D. radiodurans* genomic fragments spanning *mutS1*, *mutS2* and *mutL*, respectively, were cloned into the vector pBCSKP to generate the plasmids pGY11618, pGY11619 and pGY11617. Second, the genes were inactivated in vitro by replacing an internal fragment (a *Sex* AI-*Apa*I fragment for *mutS1,* a *Spl* I-*Bst*EII fragment for *mutS2* and a *Pvu* II fragment for *mutL*) with a *tet* cassette. Plasmid pGY11615 containing a *tet* cassette, which confers tetracycline resistance on *D. radiodurans,* was constructed by inserting, upstream of the *tet* gene, between the *Pst* I and *Eco* RI sites of plasmid pBR322, a 213-bp PCR fragment spanning the Deinococcal *groESL* promoter amplified from plasmid pRADZ3.

Plasmids pGY11637, pGY11633 and pGY11634, used in complementation experiments, were constructed by inserting, between the *Bam* HI and *Hin*dIII sites of the shuttle vector pI8, the Deinococcal *mutS1*, *mutS2*, or *mutL* genes, respectively.

Plasmid pGY11559, used to assess bacterial competence, was constructed by replacing the *cat* gene of the shuttle vector pGY11530 with the *spec* gene from plasmid pGB2 in order to express spectinomycin resistance in *D. radiodurans*.

Sequencing

To sequence the *DR0139* gene of *D. radiodurans*, a segment (coordinates 1051459 to 1054168 on chromosome I) encompassing the *DR0139* gene was amplified

Table 2 Plasmids

Name	Vector	Relevant features	Source/reference
Plasmids that replicate in E. coli			
pBCSKP		Phagemid cloning vector Cam^R	Stratagene
pGB2		Source of a *spec* cassette in *D. radiodurans*	Churchward et al. (1984)
pGY 11618	pBCSKP	*mutS1*$^+$ (3372-bp insert)	This work
pGY 11619	pBCSKP	*mutS2*$^+$ (3306-bp insert)	This work
pGY 11617	pBCSKP	*mutL* $^+$ (2595-bp insert)	This work
pBR322		Amp^R Tet^R	Bolivar et al. (1977)
pGY 11615	pBR322	Source of a *tet* cassette in *D. radiodurans*	This work
pGY 11635	pBCSKP	Derived from pGY 11618 by allelic replacement of *mutS1* by the *tet* cassette	This work
pGY 11624	pBCSKP	Derived from pGY 11619 by allelic replacement of *mutS2* by the *tet* cassette	This work
pGY 11625	pBCSKP	Derived from pGY 11617 by allelic replacement of *mutL* by the *tet* cassette	This work
Plasmids that replicate in E. coli and D. radiodurans			
pI8		Shuttle vector Amp^R in *E. coli*; Cam^R in *D. radiodurans*	Meima and Lidstrom (2000)
pRADZ3		Shuttle vector Amp^R in *E. coli*; Cam^R in *D. radiodurans*, and source of *groESL* promoter	Meima and Lidstrom (2000)
pGY 11530	pI8	Expression vector Cam^R in *D. radiodurans* and *E. coli*	Lecointe et al. (2004)
pGY 11559	pI8	Spec^R derivative of pGY 11530	This work
pGY 11637	pI8	*mutS1*$^+$	This work
pGY 11633	pI8	*mutS2*$^+$	This work
pGY 11634	pI8	*mutL* $^+$	This work

from genomic DNA purified from eight independent cultures of strain ATCC 13939. To sequence the *rpoB* gene for Rif[R] mutations, a segment (coordinates 924608 to 925464 on chromosome I) encompassing a region of the *rpoB* gene in which most of the Rif[R] mutations occur (Kim et al. 2004), was amplified by PCR from genomic DNA purified from independent Rif[R] mutants. The primers used are described in Supplementary Table S1. Sequencing of the PCR fragments was performed by Genome Express (Grenoble, France).

Determination of the frequency of spontaneous Rif[R] mutants

Single colonies from each strain were inoculated into 3 ml of TGYX2 and grown to an $A_{650} = 3$. The cells were then harvested by centrifugation, resuspended in 300 μl of 20 mM potassium phosphate buffer (pH 7.0) and plated on TGY plates containing 25 μg/ml rifampicin to determine the number of Rif[R] mutants, and on TGY plates without rifampicin to determine the total number of viable cells. The frequencies of the Rif[R] mutants per viable cell from at least 11 independent measurements (from 11 to 47) were used to calculate a median value.

Analysis of mutation rates

The mutation rate (μ) per replication was calculated as the ratio m/N, where m is the number of mutational events per culture and N the total number of cells in the culture. The m value was calculated by the maximal likelihood method described by Lea and Coulson (1949) using the statistical fluctuation analysis program Salvador (Zheng 2002). This program takes into account a theoretical value of ρ (ρ being the ratio of the averaged growth rate of the mutants to the growth rate of the wild type) that is calculated from the distribution of the numbers of mutants in cultures of the same size using the Mandelbrot law. The ρ parameter might be of particular importance in evaluating the μ value in *D. radiodurans*, since an important segregational lag due to the multiple genome copies present in this organism, as well as a possibly reduced growth rate of the mutants, may lead to an underestimation of the mutational events. However, an evaluation of the rate of spontaneous Rif[R] mutagenesis in wild-type *D. radiodurans* from a set of 47 independent experiments using the Salvador program gave a μ value (μ = 3.78) that was of the same order of magnitude as that obtained (μ = 3.54) from the same set of data by the method of Drake (1991) using the formula $\mu = f/\ln(N\mu)$ where f is the median Rif[R] frequency and N is the average number of cells in a culture. Note that the ρ parameter is especially important for mutational events arising early in a culture. If the frequency of mutation is low, it is expected that mutational events essentially occur late in the culture, when the size of the culture is high enough. Under these conditions, the effect of ρ on the μ value could be very weak. This effect could be more important in mismatch repair-deficient strains in which the frequency of mutations is higher than in the wild-type strain.

Results and discussion

The *DR1039* gene of the *D. radiodurans* ATCC 13939 reference strain encodes a full length MutS1 protein without the need for translational frameshifts

An analysis of the annotated *D. radiodurans* genome sequence (White et al. 1999; http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gdr) reveals that this organism possesses key genes pertaining to the core of the mismatch repair system, *mutS* (*DR1039* and *DR1976*), *mutL* (*DR1696*), and *mutU/uvrD* (*DR1775*). Of the two Deinococcal *mutS* genes, *DR1976* encodes a putative protein belonging to the *MutS2* subfamily, members of which are found in some bacterial species but are not currently known to play a role in mismatch repair (Eisen 1998). The other, *DR1039*, requires two translational frameshifts to encode a putative MutS protein that can be aligned with the other proteins of the MutS1 subfamily known to play a key role in mismatch recognition (Eisen 1998). In the absence of these frameshifts, *DR1039* is predicted to encode a truncated, 237-amino acid MutS protein, which is probably inactive.

The data just mentioned would imply that mismatch repair does not operate in wild-type *D. radiodurans* unless *DR1039* is subjected to a particular type of translational regulation. Alternatively, the product of the *mutS2* gene, *DR1976*, might be unusually active in mismatch repair in *D. radiodurans*. On the other hand, the recent finding that disruption of the *uvrD/mutU* (*DR1775*) gene results in a mutator phenotype (Kim et al. 2004) suggests that the mismatch repair system may indeed function in *D. radiodurans*. Given these contrasting data, we sequenced the *DR1039* gene in the reference *D. radiodurans* strain ATCC 13939.

We found that the sequence of *DR1039* in strain ATCC 13939 differs at two positions from the published sequence (http://www.tigr.org/tigr-scripts/CMR2/GenePage.spl?locus=DR1039): an additional cytosine is present at position 711 and an additional guanine is found at position 887. These two changes make it possible to construct a full-length alignment of the predicted *DR1039* product with MutS1 proteins from *Thermus thermophilus* and *E. coli,* with which it shares 49% and 34% sequence identity, respectively (Fig. 1). Accordingly, we named the *DR1039* gene *mutS1,* following the nomenclature of Eisen (1998).

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Fig. 1 Alignment of the
D. radiodurans MutS1 protein
with the corresponding E. coli
and T. thermophilus MutS
proteins. The alignment was
generated using the
CLUSTALW program.
Shading was based on amino
acid identity (black boxes) or
similarity (grey boxes) using the
BioEdit program
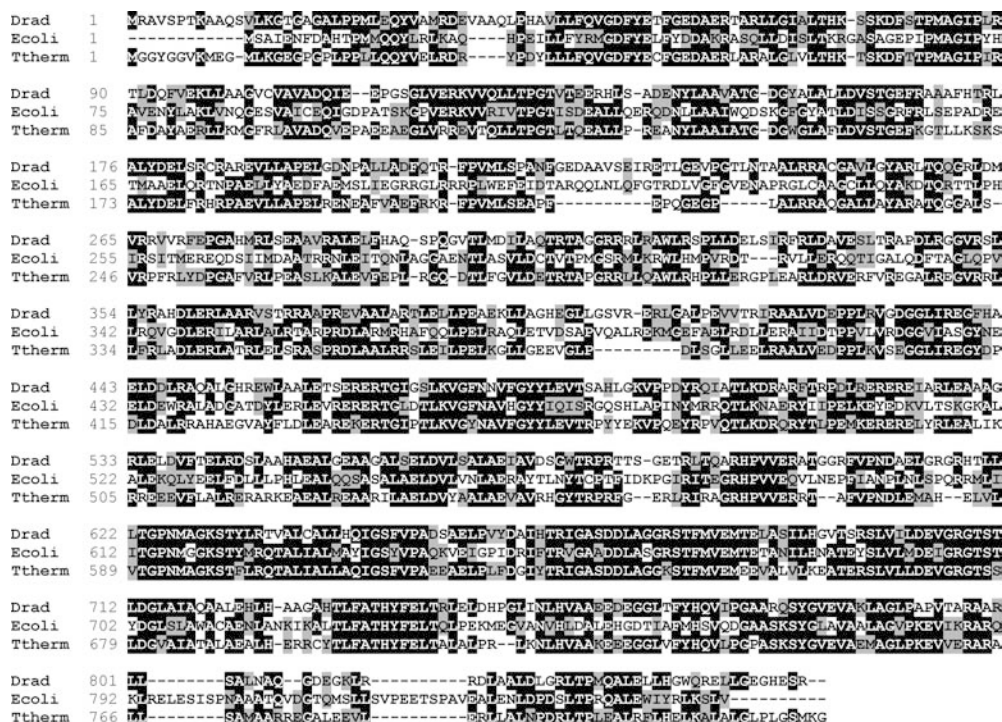
The *mutS1* and *mutL* genes are required for efficient mismatch repair in *D. radiodurans*

To determine the phenotype of cells lacking the *mutS1*, *mutS2* or *mutL* gene, and to identify the cellular function of the proteins encoded by these genes, we constructed deletion mutants. For this purpose, the central parts of *mutS1*, *mutS2* or *mutL* were replaced by a *tet* cassette in vitro (Supplementary Fig. S1). The mutated alleles were then used to transform *D. radiodurans*, selecting for tetracycline resistance in order to inactivate the corresponding chromosomal alleles via homologous recombination. The genetic structure of the inactivated genes was verified by PCR (Supplementary Fig. S1).

To determine the impact of the inactivation of *mutS1*, *mutS2* and *mutL* genes on the rate of spontaneous mutagenesis, we used the Rif^R forward mutation assay. This assay detects essentially base substitutions that occur at specific positions in the chromosomal *rpoB* gene which alter the β subunit of RNA polymerase, rendering the enzyme resistant to rifampicin (Campbell et al. 2001). The Rif^R/ *rpoB* system has recently been used to evaluate the frequency of spontaneous mutagenesis in *D. radiodurans* (Kim et al. 2004).

A comprehensive analysis of spontaneous mutation frequencies and rates in the wild type and in strains in which the putative mismatch repair genes have been inactivated is shown in Table 3. In addition to the Δ *mutS1*, Δ *mutS2* and Δ*mutL* mutants constructed here, we also included in the analysis, as an internal standard, the *uvrD*::Tn *DrCat* mutant previously shown to possess a mutator phenotype (Kim et al. 2004).

We observed a seven-fold increase in the mutation frequencies, and a five-fold increase in the mutation rates, in cells lacking MutS1, MutL or UvrD proteins as compared to the wild type. Furthermore, when the mutated proteins were restored in Δ *mutS1* or Δ*mutL* bacteria by transformation with the wild-type genes cloned in the shuttle vector pI8, the mutation frequencies and rates shifted to approximately wild-type levels. Since both *mutS1* and *mutL* are the first genes of putative operons, this result eliminates the possibility that the mutator phenotype observed in Δ *mutS1* or Δ*mutL* bacteria might be due to a polar effect on the expression of downstream genes.

In contrast to the above results, we did not observe any increase in the level of spontaneous mutagenesis upon deletion of the *mutS2* gene (Table 3). However, if

Table 3 Effect of mismatch repair genes on spontaneous mutagenesis

Strain[a]	Parameter[b]		
	f (10^{-8})	μ (10^{-8})	n
Wild type	9.4 ($\pm$1.08)	3.78 ($\pm$0.08)	47
Δ*mutS1*	70.5 ($\pm$4.83)	20.12 ($\pm$1.82)	36
Δ*mutS2*	10.8 ($\pm$1.24)	4.55 ($\pm$0.86)	12
Δ*mutS1* Δ *mutS2*	72.2 ($\pm$4.62)	21.30 ($\pm$4.63)	12
Δ*mutL*	70.7 ($\pm$5.16)	20.76 ($\pm$4.81)	12
uvrD::Tn *DrCat*	73.8 ($\pm$6.36)	22.60 ($\pm$4.19)	11
Δ*mutS1*/ *mutS1*$^+$	10.2 ($\pm$1.01)	4.55 ($\pm$1.07)	12
Δ*mutL*/*mutL* +	12.9 ($\pm$1.41)	4.37 ($\pm$0.85)	12

[a] Alleles to the *right* of the *slash* are contained in a plasmid
[b] Each f value (mutation frequency) is the median of at least ten independent measurements. The mutation rates (μ) were determined by the method of maximum likelihood (Lea and Coulson 1949). Values in *parentheses* are standard deviations for f and 95% confidence intervals limits for μ, calculated according to Zheng (2002). n is the number of independent cultures used

MutS1 and MutS2 play a somewhat redundant function in mismatch repair, the phenotype of Δ mutS2 bacteria might be masked by the activity of the MutS1 protein. We thus constructed a mutant lacking both MutS proteins. The mutation frequency in these Δ mutS1 Δ mutS2 bacteria was of the same order of magnitude as that observed in bacteria deleted for the mutS1 gene alone (Table 3).

We conclude that mismatch repair is functional in D. radiodurans. Both MutS1 and MutL proteins, together with the associated UvrD helicase, are required for this process, but MutS2 appears to play no major role.

MutS1 and MutL proteins inhibit recombination between DNAs that differ in only one base pair

To investigate the action of the mismatch repair proteins in ensuring the fidelity of homologous recombination in D. radiodurans, we took advantage of the natural competence of this organism to use genetic transformation as a recombination assay. Although the mechanism of genetic transformation in D. radiodurans has not yet been elucidated, it seems likely to involve processing of donor DNA into single-stranded DNA fragments that are subsequently assimilated into homologous double-stranded DNA through the action of the RecA protein. This process directly produces heteroduplex DNA containing one strand from each parent. In many transformable species, the presence of even a single mismatch within otherwise identical sequences can inhibit transformation (Mejean and Claverys 1984; Claverys and Lacks 1986).

To determine whether a single base pair difference between donor and recipient DNAs also inhibits genetic transformation in D. radiodurans, we used three donor DNAs isolated from spontaneous Rif[R] mutants, each containing a single point mutation in rpoB: an AT → GC transition at position 1259 (mutant rif1), an AT → GC transition at position 1304 (mutant rif2), and a GC → AT transition at position 1319 (mutant rif3). The DNA purified from another spontaneous Rif[R] mutant bearing an in frame 9-bp deletion ($\Delta_{1250-1258}$) in rpoB (mutant rifΔ9) was chosen as the reference donor DNA. In making this choice, we assumed, by analogy with the recognition specificities of the MutS protein from E. coli (Parker and Marinus 1992; Joshi and Rao 2001), that the Deinococcal mismatch repair proteins do not recognize a large 9-nt DNA loop.

When samples containing 2 or 20 ng of each genomic DNA were used to transform wild-type D. radiodurans, the frequencies of the Rif[R] transformants differed greatly, depending on whether the donor DNA contained a point mutation or the Δ9 deletion. Indeed, as can be seen in Fig. 2a, all three point-mutation DNAs were much less efficient in transforming a wild-type host than the DNA containing the 9-bp deletion. The same difference was observed when PCR fragments (850 bp) encompassing each mutation were used for
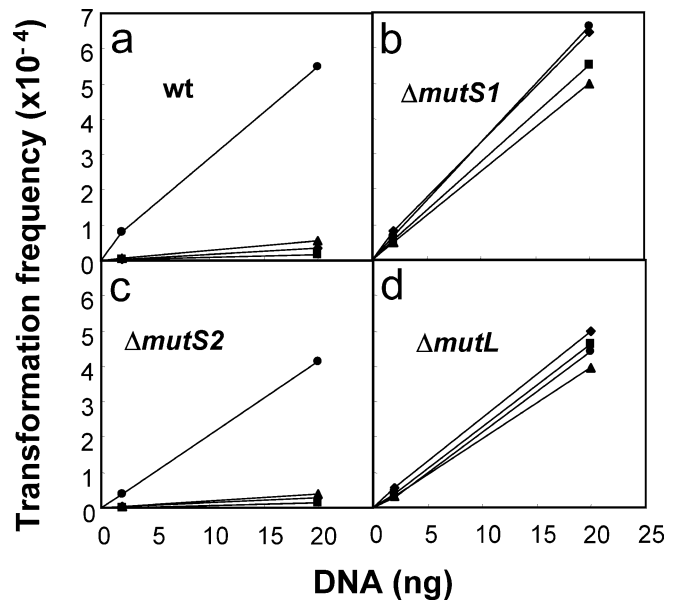

Fig. 2a–d Effects of MutS and MutL on the inhibition of transformation by a single point mutation in the donor DNA. GY9613 (wild type, panel a), GY11713 (Δ mutS1:: tet, panel b), GY11712 (Δ mutS2:: tet, panel c) and GY11708 (ΔmutL:: tet, panel d) bacteria were transformed with 2 ng and 20 ng of genomic DNA purified from D. radiodurans Rif[R] mutants (rif1, diamonds; rif2, squares; rif3, triangles; rifΔ9, circles) and appropriate dilutions were plated on TGY plates to measure numbers of viable cells and on TGY plates containing 25 µg/ml rifampicin to select Rif[R] transformants. Transformation frequencies were expressed as the number of transformants divided by the total number of viable cells in the transformation mixture

transformation. In this case, using 300 ng of donor DNA in the transformation mixture, the frequencies of Rif[R] transformants were 1.6×10^{-5}, 0.6×10^{-5}, 2.4×10^{-5}, and 20.5×10^{-5} for the rif1, rif2, rif3 point mutants and the rifΔ9 deletion mutant DNA, respectively.

To relate the observed marker effect to the activity of the mismatch repair proteins, the same set of genomic DNAs was used to transform Δ mutS1, Δ mutS2, or ΔmutL recipients. As shown in Fig. 2, the three DNAs with point mutations (rif1, rif2, and rif3) transformed recipient bacteria devoid of MutS1 or MutL with approximately the same elevated frequency as the deletion mutant DNA (rifΔ9) (Fig. 2b and d), while they showed a reduced transformation frequency in recipient bacteria devoid of MutS2, which appear to behave in a manner identical to wild-type bacteria (Fig. 2a and c).

We interpret the above data as indicating that an efficient mismatch repair system can abort recombination intermediates containing a single mismatched base, while it tolerates intermediates containing a 9-bp DNA loop. In this process, MutS1 and MutL play a fundamental role, while MutS2 appears to have no major effect. Thus, the requirement for these mismatch repair proteins in the control of recombination fidelity parallels that observed in the process of error avoidance during DNA replication.

Two types of models could account for the MutS1 and MutL-mediated inhibition of recombination

between divergent DNA sequences: (1) preferential correction of the donor strand could occur through a strand targeting mechanism involving the presence of single-strand breaks in the initial integration product (Claverys and Lacks 1986), or (2) binding of MutS1 and MutL proteins to the nascent mismatched heteroduplex recombination intermediate directly could prevent further steps in the recombination process, possibly by reducing the rate and the extent of heteroduplex formation, as shown in vitro for the MutS and MutL proteins from *E. coli* (Worth et al. 1994).

To discriminate between these two models, we analyzed the effect of inactivating the UvrD helicase on the inhibition of recombination between divergent sequences. For this purpose, we measured the efficiencies of transformation of a *uvrD*::Tn *DrCat* mutant by the previously described set of DNAs containing a point mutation or a 9-bp deletion in *rpoB*.

Curiously, the *uvrD*::Tn *DrCat* strain displayed a general reduction in transformation efficiency as compared to the other strains (note the changes in the abscissa and the ordinate between Fig. 2 and Fig. 3). Since the *uvrD*::Tn *DrCat* strain also displayed reduced efficiency in plasmid transformation (data not shown), it may have a defect in DNA uptake or processing. Whatever the exact reasons for the reduced transformation efficiency in the *uvrD*::Tn *DrCat* strain, the relative frequency of transformation by the point mutations (rif1, rif2 or rif3) and the deletion (rifΔ9) in the *uvrD* host was similar to that observed in the wild-type strain. For instance, the ratio of transformation frequencies of the rif1 to rifΔ9 transformants was 0.02 in the wild type (Fig. 2a, 20 ng of input DNA) and 0.02 in the *uvrD*::Tn *DrCat* recipient (Fig. 3, 200 ng of input DNA).
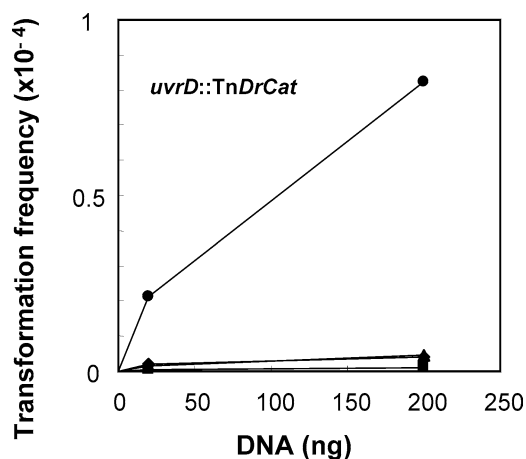
The above results suggest that the interaction of the MutS1 and MutL proteins with a mismatched heteroduplex is sufficient to abort recombination in the absence of the UvrD helicase, and that the inhibition of transformation by DNA containing a single point mutation is not due to a preferential mismatch correction in the donor strand.

Cells devoid of MutS1 or MutL proteins are resistant to DNA-damaging agents

To analyze the possible involvement of the *D. radiodurans* mismatch repair proteins in other repair pathways, we measured the survival of mismatch repair-deficient bacteria after γ-ray irradiation, treatment with mitomycin C or UV irradiation.

As can be seen in Fig. 4, Δ*mutS1* and Δ*mutL* bacteria displayed wild-type levels of resistance to the three DNA-damaging treatments, indicating that the MMR system is not required for overall cell survival after DNA damage. Bacteria devoid of MutS2 protein were also damage resistant, yielding no clues to the biological function, if any, of this Deinococcal protein.

In contrast, the UvrD-deficient bacteria were moderately sensitive to γ-rays, extremely sensitive to mitomycin C, and resistant to UV irradiation (Fig. 4). In *E. coli*, the UvrD helicase, in addition to participating in mismatch repair, is also a component of the UvrABC-dependent nucleotide excision repair pathway. The Deinococcal UvrD enzyme may have a similarly broad range of function. The UV resistance of the *uvrD* mutant (Fig. 4c) can be accounted for by the fact that *D. radiodurans* possesses a functionally redundant pathway dependent upon the UV-specific endonuclease UvsE, that compensates for the loss of nucleotide excision repair to restore resistance to UV irradiation (Minton 1994; Earl et al. 2002). Our results thus suggest that the UvrD protein does not participate in the UvsE pathway and that, in the absence of UvrD, the UvsE pathway might be more efficient in eliminating UV-induced DNA damage.

Finally, it should be noted that, although prokaryotic and eukaryotic MMR proteins are known to recognize numerous types of DNA damage (for reviews see Schofield and Hsieh 2003; Harfe and Jinks-Robertson 2000), the damage-resistant phenotype of bacteria lacking MutS1 or MutL sheds no light on the capacity of these Deinococcal proteins for lesion recognition. Indeed, the genetic outcomes of an interaction of the MMR proteins with damaged DNA might be largely dependent on the relative affinity for a given lesion of the MMR proteins versus specific DNA repair proteins, and on the capacity of the MMR system to initiate or to interfere with other repair pathways. For example, inactivation of the MMR system in *E. coli* results in a slight sensitization of the cells to UV-light, but also to increased tolerance to many drugs, since the mismatch repair system stimulates the transcription-coupled repair
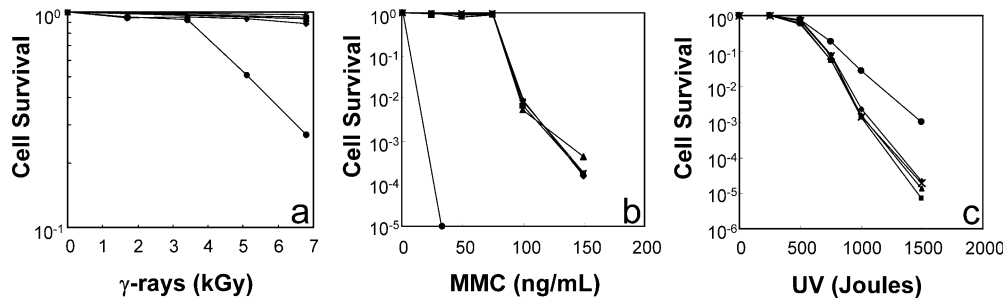

Fig. 3 UvrD is not involved in transformation inhibition by a single point mutation in donor DNA. GY12307 (*uvrD*::Tn *DrCat*) bacteria were transformed with 20 ng or 200 ng of genomic DNA purified from *D. radiodurans* Rif[R] mutants (rif1, *diamonds*; rif2, *squares*; rif3, *triangles*; rifΔ9, *circles*) and transformation frequencies were measured as described in the legend to Fig. 2

Fig. 4a–c *mutS1* -, *mutS2* - or *mutL* -deficient bacteria are resistant to DNA-damaging agents. GY9613 (wild type, *diamonds*), GY11713 (Δ *mutS1*:: *tet*, *squares*), GY11712 (Δ *mutS2*:: *tet*, *triangles*), GY11708 (Δ*mutL*:: *tet*, *crosses*) and GY12307 (*uvrD*::Tn *DrCat*, *circles*) bacteria were exposed to γ-rays (panel **a**), mitomycin C (**b**) or UV-irradiation (**c**) at the doses indicated on the *abscissa*. **a** Bacteria were grown in TGY2X to an A_{650} value of 2–3. The cultures were concentrated ten times in TGY2X and irradiated on ice with a ^{137}Cs irradiation system (Institut Curie, Orsay, France) at a dose rate of 56.6 Gy/min. Following irradiation, diluted samples were plated on TGY plates and incubated for 3–4 days at 30°C before the colonies were counted. **b** Bacterial cultures (A_{650}=2–3) were serially diluted in TGY2X and plated on TGY plates supplemented with increasing concentrations of mitomycin C. **c** Bacterial cultures (A_{650}=2–3) were serially diluted in TGY medium, and 10 μl drops of each dilution were deposited on TGY plates and exposed to UV light at a dose rate of 3.5 J/m^2/s

pathway for UV lesions while it interferes with the repair of drug-induced lesions (for reviews see Harfe and Jinks-Robertson 2000; Schofield and Hsieh 2003). These MMR-related effects on DNA repair may fail to take place or go undetected in *D. radiodurans* since this organism may possess regulatory mechanisms that favor pre-replicative processing of DNA lesions (Battista 1997), while the MMR proteins mainly recognize mismatches that result from misincorporation opposite DNA damage (Mu et al. 1997). Moreover, *D. radiodurans* possesses highly redundant repair activities (Makarova et al. 2001) that may mask a possible involvement of the mismatch repair system in a given repair pathway.

Conclusions

Here, we have characterized the mismatch repair system of the *D. radiodurans* type strain ATCC 13939 and demonstrated that it participates in ensuring the fidelity of DNA replication and recombination. We also showed that the *mutS1* gene in this strain contains two additional nucleotides relative to the published sequence, thus yielding a full length and functional MutS1 protein. In a recent report, the groups that published the *D. radiodurans* genome sequence (White et al. 1999) acknowledged that they had actually sequenced a *D. radiodurans* strain from the Minton laboratory collection and not the ATCC 13939 type strain, as initially indicated (see Science vol 303, p 766; 2004). If the published sequence data are correct, the sequenced strain is likely to be a mutator strain, as its *mutS1* gene encodes a

truncated protein. This could explain why it accumulated many mutations, as suggested by the many base-pair changes and nucleotide deletions/insertions found between the ATCC 13939 strain and the sequenced strain in several recent reports (this paper; Science vol 303, p 766, 2004; Eggington et al. 2004; Southworth and Perler 2002).

Inactivation of MutS1 or MutL proteins in *D. radiodurans* resulted in a seven-fold increase in spontaneous Rif^R mutagenesis, whereas the data of Glickman and Radman (1980), who used the same mutagenesis assay, showed that inactivation of the corresponding proteins in *E. coli* resulted in an 80- to 100-fold increase. Since the mutation rate per cell per generation in wild-type *D. radiodurans* was 15-fold higher than that reported for *E. coli* (Glickman and Radman 1980), the modest mutator effect of inactivating the MutS1 and MutL proteins in *D. radiodurans* might be related to an inefficient mismatch repair system, rather than to a highly accurate replication apparatus. *D. radiodurans* does not possess homologs of the MutH and Dam proteins that ensure methylation-directed targeting of mismatch repair to the newly synthesized DNA strand in *E. coli*. However, the ends present in the leading strand, or the nicks located between Okazaki fragments in the lagging strand of a replication fork, could provide a strand discrimination mechanism in *D. radiodurans* in which the mismatch repair system may be coupled to DNA replication. Thus, a possible cause of the low efficiency of the mismatch repair system in this organism may be the short time window during which the system can act. Nevertheless, it should be noted that other bacterial species, such as *Bacillus subtilis* and *Streptococcus pneumoniae*, which also lack a methylation-directed strand discrimination mechanism, possess mismatch repair systems as effective as that of *E. coli* (Tiraby and Fox 1973; Rossolillo and Albertini 2001).

Inactivation of the MutS1 or MutL protein in *D. radiodurans* also resulted in a 10-fold increase in recombination between DNAs that differed by a single base pair, indicating that the two Deinococcal proteins are required to inhibit recombination between non-identical (homeologous) DNA sequences. However, a comparison of the efficiency of the Deinococcal mismatch repair system in inhibition of homeologous recombination with that of other species is complicated by the fact that the recombination efficiencies can vary widely, depending upon the genetic assay used to mea-

sure recombination, the type of mismatches present in heteroduplex recombination intermediates, and the degree of divergence between the recombining DNAs (Rayssiguier et al. 1989; Petit et al. 1991; Humbert et al. 1995; Abdulkarim and Hughes 1996; Zahrt et al. 1994; Majewski and Cohan 1998).

The MMR system has been implicated in the prevention of potential lethal rearrangements mediated by dispersed repetitive elements (for review see Harfe and Jinks-Robertson 2000) and in the control of damage-inducible chromosomal duplications (Hill and Combriato 1973; Petit et al. 1991). The *D. radiodurans* genome contains numerous repeated sequences, including expanded families of transposons (Makarova et al. 2001) that may constitute a source of genome instability upon recombination. By its capacity to inhibit recombination between partially divergent DNA sequences, the Deinococcal MMR system might contribute to the maintenance of genome stability. However, further elucidation of this issue awaits the construction of adequate tester strains to detect damage-induced rearrangements that may occur at a frequency that is too low to significantly affect overall cell survival after DNA damage.

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