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The MutS C Terminus Is Essential for Mismatch Repair Activity In Vivo

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An *Escherichia coli* K-12 strain was constructed with a chromosomal deletion (*mutSΔ800*) in the *mutS* gene that produced the removal of the C-terminal 53 amino acids which are not present in the MutS crystal structure. This strain has a MutS null phenotype for mutation avoidance, antirecombination, and sensitivity to cytotoxic agents in a *dam* mutant background.

DNA mismatch repair (MMR) plays an important role in two distinct processes, mutation avoidance and antirecombination (11, 12, 16). Mutation avoidance corrects mismatches in hemimethylated DNA behind the replication fork, and the MMR proteins MutS and MutL prevent recombination between similar but not identical (homeologous) sequences.

The crystal structure of *Escherichia coli* MutS bound to an oligonucleotide with a G-T mismatch has been determined using a derivative of the MutS protein, MutSΔ800, which lacks the C-terminal 53 amino acids (10). The MutSΔ800 mutant crystallizes as a dimer and retains the ability to bind DNA and ATP, just as full-length MutS (853 amino acids) does. The atomic structure of a truncated MutS from *Thermus aquaticus* has also been determined and is very similar to that of *E. coli* MutS (14). The physiological effects of the *mutSΔ800* mutation have so far been studied only in multicopy (2, 4, 10), and we show below that, in single copy, it imparts a MutS null phenotype.

The procedure used to construct the *mutSΔ800* chromosomal mutation is outlined in Fig. 1. The sequence around and including the *bla* gene (ampicillin resistance) and its promoter was amplified by PCR (Fig. 1A) from strain TP879 to produce a product (Fig. 1B) bearing the *bla* region flanked by 50-bp regions. The 5′ flanking region has the DNA sequence immediately upstream of residue 800 of *mutS* plus a termination codon (Fig. 1B), and the 3′ flanking region has the downstream sequence immediately following the termination codon of *mutS*. The PCR product was electroporated (13) into strain TP798, which constitutively expresses the products of the *exo* (exonuclease) and *bet* (beta protein) recombination genes of bacteriophage lambda (15). Recombination between the homologous regions of the PCR product and the *mutS* gene and its flanking sequence (Fig. 1C) produces a recombinant sequence in which the *mutS* gene is truncated at residue 800 and has an adjacent *bla* gene (Fig. 1D). By changing the upstream PCR primer sequence, we also constructed *mutSΔ2*, in which all but the first two and last codons of *mutS* were deleted, and the control *mutS*$^+$ construct with the flanking *bla* gene.

We measured the levels of native and mutant MutS, by Western blotting (5, 7), in strains with the chromosomal constructs as well as multicopy plasmids which were in a *mutS* null host (Fig. 2 and Table 1). The levels in GM8311 (*mutS*$^+$) were the same as those in AB1157 (*mutS*$^+$) and were increased fourfold in GM7451, which harbors pMQ372 (*mutS*$^+$), but no MutS was detected in GM8313 (*mutSΔ2*). Strain GM8315 (*mutS*Δ800) contained 2.5-fold less MutS than that contained by GM8311 (*mutS*$^+$), but in multicopy (GM7453), the level was the same as that for the wild-type strain.

The strains bearing the *mutSΔ2*, *mutSΔ800*, and wild-type alleles were tested for reversions of the *argE3* marker and for mutations to rifampin resistance as described elsewhere (4). The results in Table 2 show that with the wild-type strain, a low

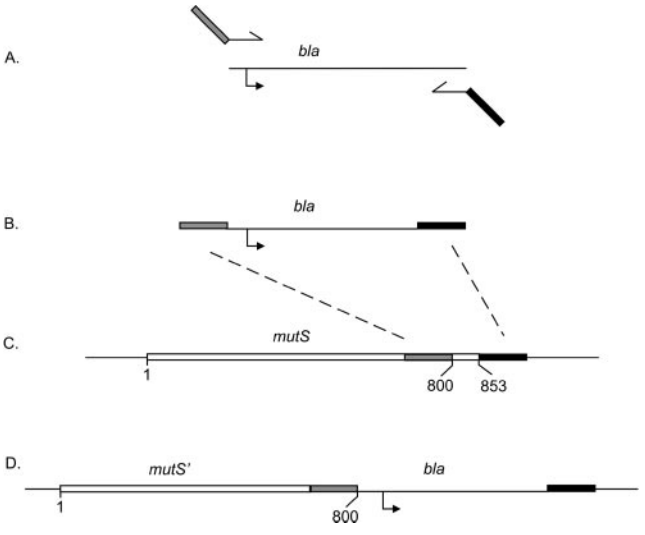

FIG. 1. Construction of chromosomal *mutS* alleles. The *bla* region was amplified by PCR (A) to yield a product with flanking sequences homologous to the distal end of codon 853 of the *mutS* gene (B). Recombination along the dotted lines between the PCR fragment and the homologous regions in the chromosome (C) yields an ampicillin-resistant truncated *mutS* gene at codon 800. The gray rectangle indicates 50 bp of sequence immediately upstream of codon 800, and the black rectangle indicates 50 bp of sequence immediately downstream of the *mutS* termination codon. The bent arrow denotes the promoter region for the *bla* gene.

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FIG. 2. Cellular levels of MutS. The amount of MutS, as determined by Western blotting, is shown for (lane a) AB1157, wild-type control; (lane b) GM4799 (*mutS458*)/pMQ372 (*mutS*$^+$); (lane c) GM8311 (*mutS*$^+$-*bla*); (lane d) GM8313 (*mutSΔ2-bla*); (lane e) GM8315 (*mutSΔ800-bla*); and (lane f) GM4799 (*mutS458*)/p*mutSΔ800*. Equal amounts of cell extract were loaded in each lane. There was no detectable MutS in extracts of strain GM4799 (data not shown).

rate of resistance or reversion was observed. However, for the strains with *mutSΔ2* and *mutSΔ800*, the number of rifampin-resistant mutants increased about 60-fold for both, and the increases for Arg$^+$ revertants were about 60- and 40-fold, respectively.

Each of the chromosomal *mutS* mutants was used as a recipient in conjugal crosses with *E. coli* (homologous) or *Salmonella enterica* serovar Typhimurium (homeologous) donors as described previously (4). For the homologous crosses with the *E. coli* donor, recombinants are formed at the same frequency, indicating that there is no effect on homologous recombination stemming from the construction of the chromosomal *mutS* mutations (Table 3). With the *Salmonella* donor and a wild-type *mutS* recipient, no recombinants were detected. In contrast, the *mutSΔ2* and *mutSΔ800* recipients increased the ability to form recombinants 11,000- and 27,000-fold, respectively, with the same *Salmonella* donor (Table 3). These increases were abrogated in crosses with *recA* deletion derivatives of the *mutSΔ2* and *mutSΔ800* mutant recipients (data not shown), indicating that the *mutS* mutations reduce antirecombination function in a *recA*-dependent manner.

E. coli dam mutS$^+$ mutants are more sensitive to exposure to MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) (Fig. 3A) and cisplatin (Fig. 3B) than the wild type (6, 8, 9) is, but the *dam mutSΔ2* deletion strain is as resistant to both treatments as the wild type is (Fig. 3), based on determinations using the protocol described previously (4). Figure 3 also shows that *dam mutSΔ800* bacteria are as resistant to MNNG and cisplatin as the *dam mutSΔ2* cells are.

We conclude that the *mutSΔ800* mutation in a single copy on the chromosome confers a *mutS* null phenotype to a cell to the same degree as the *mutSΔ2* deletion mutation does for mutation avoidance, antirecombination, and resistance to cytotoxic agents. On a multicopy plasmid, the *mutSΔ800* mutation in a *dam mutS* host confers a "split" phenotype, where mutation avoidance (2, 4, 10) and MNNG sensitivity are at the wild-type levels but antirecombination and resistance to cisplatin are severely diminished (4).

The MutS null phenotype of the *mutSΔ800* strain is due in part to the decreased cellular level of MutSΔ800 compared to that of MutS (Fig. 2), indicating that the C-terminal 53 amino acids impart stability to the protein. Even when corrected so that the levels of the proteins are similar, as in strains with the multicopy plasmid *mutSΔ800* and single-copy *mutS*, there is still the deficiency of antirecombination and resistance to cisplatin (4). Furthermore, purified MutSΔ800 protein has a lower affinity than MutS does for certain oligonucleotides with base pair mismatches and, in the presence of other MMR components, reduces the efficiency of MutH-induced incision at hemimethylated GATC sequences in vitro (3). The lower cellular amount of MutSΔ800 protein, therefore, cannot be the sole explanation for the phenotypic differences between wild-type and *mutSΔ800* strains.

The data presented here indicate that the C-terminal 53 amino acids are essential for MutS function in vivo. At present, the only known feature associated with this region comes from equilibrium sedimentation and gel filtration studies showing that MutS dimers can assemble into higher-order oligomeric structures, while MutSΔ800 is restricted to dimer formation only (3). A similar oligomeric composition occurs with the MutS protein from *Thermus* species (1). At present, the loca-

TABLE 1. *E. coli* and *Salmonella* strains used in the study

Strain	Description	Reference or source
AB259	*relA1 spoT1 thi-1 supQ80*	E. A. Adelberg
AB1157	*thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2 hisG4-rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	E. A. Adelberg
GM3819	AB1157 but Δ*dam-16*::Kan	Lab stock
GM4799	AB1157 but *mutS458*::mTn*10*Kan	Lab stock
GM7451	GM4799/pMQ372(*mutS*$^+$)	Lab stock
GM7453	GM4799/p*mutSΔ800*	Lab stock
GM8311	AB1157 but *mutS*$^+$-*bla*[a]	Lab stock
GM8313	AB1157 but *mutSΔ2-bla*[a]	Lab stock
GM8315	AB1157 but *mutSΔ800-bla*[a]	Lab stock
GM8317	GM3819 but *mutS*$^+$-*bla*	Lab stock
GM8319	GM3819 but *mutSΔ2-bla*	Lab stock
GM8321	GM3819 but *mutSΔ800-bla*	Lab stock
SA534[b]	*serA13 rfa-3058*	K. E. Sanderson
TP798	MG1655 Δ(*recC-ptr-recB-recD*)::P_{tac}-*gam-bet-exo-cat*	A. R. Poteete et al. (15)
TP879	AB1157 but Δ(*recC-ptr-recB-recD*)::P_{tac}-*gam-bet-exo-pae-recA*$^+$-*bla*	A. R. Poteete

[a] Referred to as *mutS*$^+$, *mutS*Δ2, and *mutSΔ800* in the text.
[b] *Salmonella enterica* serovar Typhimurium.

TABLE 2. Spontaneous mutant frequencies

Strain	Frequency (fold increase) of:	
	Rif^r	Arg$^+$
GM8311 (wild type)	1	3
GM8313 (*mutSΔ2*)	63	66
GM8315 (*mutSΔ800*)	55	38

TABLE 3. Recombination frequencies in homologous and homeologous crosses[a]

Donor strain	Recipient mutant	Frequency
AB259	GM8311 (*mutS*$^+$)	2.0×10^6
	GM8313 (*mutSΔ2*)	1.8×10^6
	GM1315 (*mutSΔ800*)	1.9×10^6
SA534	GM8311 (*mutS*$^+$)	<1
	GM8313 (*mutSΔ2*)	11.1×10^3
	GM1315 (*mutSΔ800*)	27.1×10^3

[a] Thr$^+$ Leu$^+$ Str^r Amp^r recombinants were selected, and the frequency is expressed per 10^8 donors.

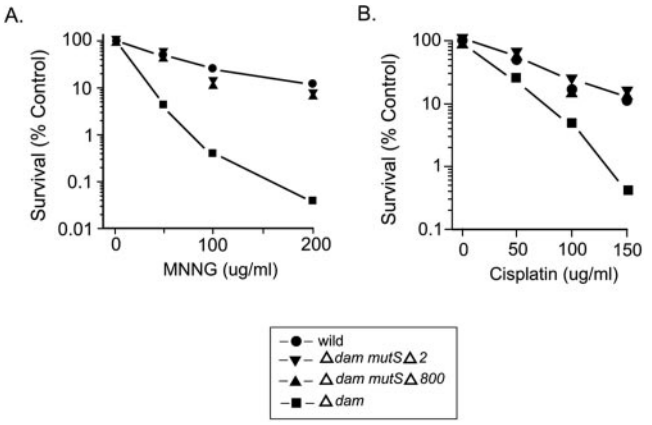

FIG. 3. Cell survival after exposure to (A) MNNG and (B) cisplatin. Cells in the logarithmic phase of growth were exposed to MNNG for 10 min or cisplatin for 60 min, and survival levels were determined as a function of dose. Circles, wild type (GM8311); inverted triangles, *dam mutSΔ2* mutant (GM8319); triangles, *dam mutSΔ800* mutant (GM8321); squares, *dam* mutant (GM3819).

tion of the tetramerization sequence is not known, but we are currently attempting to localize it.

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