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Dominant negative mutator mutations in the *mutL* gene of *Escherichia coli*

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ABSTRACT

The *mutL* gene product is part of the *dam*-directed mismatch repair system of *Escherichia coli* but has no known enzymatic function. It forms a complex on heteroduplex DNA with the mismatch recognition MutS protein and with MutH, which has latent endonuclease activity. An N-terminal hexahistidine-tagged MutL was constructed which was active *in vivo*. As a first step to determine the functional domains of MutL, we have isolated 72 hydroxylamine-induced plasmid-borne mutations which impart a dominant-negative phenotype to the wild-type strain for increased spontaneous mutagenesis. None of the mutations complement a *mutL* deletion mutant, indicating that the mutant proteins by themselves are inactive. All the dominant mutations but one could be complemented by the wild-type *mutL* at about the same gene dosage. DNA sequencing indicated that the mutations affected 22 amino acid residues located between positions 16 and 549 of the 615 amino acid protein. In the N-terminal half of the protein, 12 out of 15 amino acid replacements occur at positions conserved in various eukaryotic MutL homologs. All but one of the sequence changes affecting the C-terminal end of the protein are nonsense mutations.

INTRODUCTION

The MutL protein is part of the *dam*-directed MutHLS mismatch repair system which rectifies replication errors in newly synthesized daughter strands of DNA (1–3). The MutHLS system also acts on heteroduplex DNA recombination intermediates, which may result in drastic changes in expected recombination frequencies (1). The MutHLS proteins initiate the repair process. MutS binds to the mismatch in DNA, followed by recruitment of MutL. Subsequent binding of MutH to this complex activates its latent endonuclease activity which cleaves the newly synthesized strand, followed by excision repair (2,3).

The *mutL* gene of *Escherichia coli* was identified by the mutator phenotype of mutant alleles (4), which produce predominantly AT→GC and GC→ AT transitions (5–8). The inability of *mutL* mutants to correct base pair mismatches (9) or small loops (10) in heteroduplex DNA established a role for the MutL protein in *dam*-directed mismatch repair (reviewed in 2,3). The transient undermethylation at Dam recognition sites (-GATC-) of newly synthesized DNA chains directs repair to this strand (11).

Other known phenotypes of *mutL* mutants are consistent with its role in mismatch repair. These include: (i) extragenic suppression of *recA dam* bacteria (12,13); (ii) hypermutability to 5-bromouracil (14), 9-aminoacridine (15) and alkylating agents (16); (iii) resistance to the cytotoxic effects of certain alkylating agents of *dam mutL* bacteria compared with *dam* strains (17,18); (iv) increased homologous (19,20) and homeologous recombination (21); (v) increased excision from the chromosome of certain transposable elements (22); (vi) augmenting VSP (very short patch) repair (23); (vii) stability of nucleotide di- and tri-repeat sequences (24,25); (viii) stability of larger chromosomal directly repeated sequences (26).

The MutL protein (68 kDa) is a homodimer in solution and has no known catalytic function in mismatch repair. It increases the size of the heteroduplex DNA region protected by MutS from ~20 to ~100 bp (27). The MutL–MutS protein complex bound to DNA is required for activation of the endonuclease activity of MutH at the nearest hemimethylated -GATC- site (28). The role of MutL may be to stabilize and/or correctly position the MutS and MutH proteins. An additional role for MutL in the excision process is also possible.

Homologs of the *E.coli* MutL protein have been found in *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Saccharomyces cerevisiae*, mouse and human (29). Multiple homologs are found in eukaryotic organisms, which in certain cases form heterodimers (3,30). The region of greatest amino acid sequence similarity appears to be at the N-terminal end of the protein. In humans, mutation in the MutL homologs has been associated with non-polyposis colon cancer (31,32).

Little is known about the domain organization of MutL. In the absence of any structural information, we have used a new approach to identify functional domains which exploits the properties of dominant-negative mutations. Such mutations are used because: (i) the mutant proteins are expected to be relatively stable; (ii) the mutations should map throughout the gene, rather than be localized to one region (due to the multifunctional nature of MutL). This approach has been used successfully by Wu and Marinus (20; unpublished data) to identify mutations affecting all known enzymatic activities of the multifunctional MutS protein. A similar strategy should allow identification of the functional domains of MutL which interact with MutS and MutH.

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MATERIALS AND METHODS

Bacterial strains and plasmids

Escherichia coli K-12 strains and the plasmids used are listed in Table 1. GM4250 (*mutL*::Km Δ*Stu*) was isolated as a kanamycin-resistant recombinant after P1*vir* transduction of CC106 with phage grown on strain NU747 (33). λ DE3 derivatives of strains CC106 and GM4250 were constructed as described by the suppliers (Novagen). A His-tagged *mutL*$^+$ derivative of pET15b, pMQ378, was constructed by PCR amplification of the gene on pNU127 (33) using primers MM155 (5′-cgggatccgatatcactcatctt-tcag-3′) and MM156 (5′-ccaaactaaggacgacatatgccaattcaggtc-3′) and Taq DNA polymerase in the buffer supplied by the manufacturer (Promega) with 25 mM $MgCl_2$ for 35 cycles. Cycle settings were 92, 52 and 72°C for 2 min each. The amplified fragment was purified using a QiaQuick (Qiagen) column, digested with *Nde*I and *Bam*HI and inserted into the corresponding sites of pET15b, to yield pMQ378. DNA sequence analysis indicated an A→G base change at position 225 compared with the GenBank Z11831 sequence (34) and presumably represents an error amplified during the PCR. The sequence change does not alter the coding for His75.

Media

L broth contained 10 g/l Bacto tryptone, 5 g/l Bacto yeast extract, 10 g/l NaCl and 2 ml/l 1 M NaOH, solidified when required with 16 g/l agar (Difco). Brain heart infusion broth (Difco) was used at 20 g/l. The minimal medium was that of Davis and Mingioli (35). MacConkey agar containing lactose was purchased from Difco. Ampicillin, chloramphenicol, kanamycin and rifampicin (all from Sigma) were added at 100, 20, 40 and 100 μg/ml respectively.

Mutagenesis of plasmid DNA and isolation of mutants

Plasmid DNA (1–2 μg) was exposed to 400 mM hydroxylamine (Kodak) in 50 mM sodium phosphate buffer, pH 6.0, at 70°C for 60 min, precipitated with ethanol and resuspended in 10 mM Tris, 1 mM EDTA buffer, pH 8.0. Strain CC106 was transformed to chloramphenicol resistance with portions of treated plasmid DNA on either MacConkey lactose medium or L agar. After overnight incubation, the colonies were replica-plated from L agar to L agar + rifampicin and incubated for 1–2 days at 37°C. The colonies on MacConkey agar were scored for papillation after 2–3 days incubation at 37°C. Plasmid DNA was extracted from rifampicin-resistant or papillating colonies and used to transform strain CC106. From plasmids retaining the dominant phenotype the promoter-proximal *Ava*I–*Apa*LI and promoter-distal *Apa*LI–*Bam*HI fragments were purified and used to replace the corresponding fragment of the wild-type plasmid. The replacement plasmid showing the dominant phenotype was used in all further work.

Genetic complementation

Each CC106 derivative containing a plasmid conferring a dominant-negative *mutL* phenotype was transformed with a pBR322 derivative containing the wild-type *mutL*, *mutH* or *mutS* gene. The extent of papillation on MacConkey agar was measured after 2–3 days incubation at 37°C. Alternatively, the frequency to rifampicin resistance was measured by plating aliquots of a saturated broth culture on L agar containing rifampicin and incubating overnight at 37°C.

Table 1. *Escherichia coli* K-12 strains and plasmids

	Genotype or description	Reference
Strains		
CC106	*ara thi* Δ*(gpt-lac)*5/F-*lacI*^{q} *lacZ proAB*$^+$	40
GM4250	As CC106 but *mutL*Δ (Kan *Stu*)	
GM5861	As CC106 but (λDE3)	
GM5862	As GM4250 but (λDE3)	
Plasmids		
p613	Amp^R; synthetic glutamic acid inserting UAG suppressor gene in pGF1B1	39
pACYC184	Tet^R Cam^R	41
pET15b	Amp^R; N-terminal fusion to hexahistidine and thrombin cleavage peptide vector (Novagen)	
pMQ350	Amp^R; *Bam*HI (*mutL*) fragment from pNU127 into *Bam*HI site of pZ150	42
pMQ378	Amp^R; as pET15b but *mutL*$^+$ on an *Nde*I–*Bam*HI fragment insert obtained from pNU127 by PCR	
pMQ393	Cam^R; pACYC184 with a *Bgl*II–*Hin*dIII fragment from pMQ378 (includes the T7 promoter) replacing the *Bam*HI–*Hin*dIII region	
pMQ396	Cam^R; pACYC184 with a replacement fragment from pMQ378 (does not include the T7 promoter; Fig. 1c)	
pNU127	Tet^R Cam^R; *mutL*$^+$ on a 6.5 kb *Pst*I fragment in pBR325	33
pSTL113	Amp^R, Tet^S; pBR322 with mismatched duplication in the *tet* gene	26

DNA sequencing

Double-stranded plasmid DNA was sequenced by the dideoxy chain termination technique (36) with Sequenase 2.0 (US Biochemical Co.) using a series of synthetic oligonucleotides of 15–17 residues corresponding to various sites in the *mutL* gene as primers. Either the *Nde*I–*Apa*LI or the *Apa*LI–*Bam*HI region was completely sequenced for each dominant mutant.

Purification of His-tagged MutL, antisera preparation and Western blotting

His-tagged MutL was isolated as recommended by the manufacturer (Novagen). Two milliliters of a saturated culture of the plasmid-containing strain was inoculated into 1 l of L broth and grown to an optical density (A_{600}) of 0.8, IPTG (Sigma) was added to 0.5 mM and the culture incubated for an additional 90 min. The cells were harvested by centrifugation, resuspended in 20 ml binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris–HCl, pH 7.9) and sonicated for 5 min using 1 s pulses at 1 s intervals. After centrifugation in a Ti70 rotor for 30 min at 39 000 r.p.m. at 4°C in a Beckman L5-50 ultracentrifuge, the supernatant was loaded on a 2.5 ml Ni^{2+} resin column. The column was washed with 60 mM imidazole and MutL eluted with 500 mM imidazole and frozen at –70°C.

Rabbit polyclonal antiserum to His-tagged MutL was prepared by BAbCO (Richmond, CA). Polyclonal antiserum was pre-adsorbed using a cell extract made from the *mutL* deletion strain GM4250. The strain was grown at 37°C in L broth to an optical density (A_{600}) of 1.2, centrifuged and resuspended in a 1/10th volume of 100 mM sodium phosphate, pH 7.4. A portion of 500 µl was sonicated on ice for 60 s using 1 s pulses at 1 s intervals, followed by centrifugation at 10 000 *g* for 5 min. Fifty microliters of antiserum were mixed with the supernatant and incubated at room temperature with gentle shaking for 2 h. After diluting 1000-fold, the solution was used for Western blotting. Cells were grown to an optical density (A_{600}) of 1.0 and 200 µl portions were centrifuged and resuspended in 10 µl water, diluted with an equal volume of 2× loading buffer [135 mM Tris–HCl, pH 6.8, 20% glycerol, 4% SDS (Gibco BRL), 0.0025% bromophenol blue, 10% 2-mercaptoethanol], boiled for 10 min and loaded on SDS–8% polyacrylamide gels in a Hoeffer SE 250 slab gel electrophoresis unit (22). After electrophoresis, the proteins were transferred to Immobilon-P membranes (Millipore) using a semi-dry electroblotter (Owl Scientific). MutL was detected using chemiluminescence according to the manufacturer's (Tropix Inc.) instructions. Bradford protein assay kits and standards were obtained from BioRad Laboratories.

Stability of tandem repeats

CC106 cells containing plasmid-borne dominant mutations were transformed to ampicillin resistance with plasmid pSTL113 (26) and the transformants were purified by streaking for single colonies. Logarithmic phase cultures (~10^8 cells/ml) were prepared and portions plated on L ampicillin agar and L ampicillin plus tetracycline agar to determine the number of viable cells and cells with *tet* gene rearrangments respectively (26).

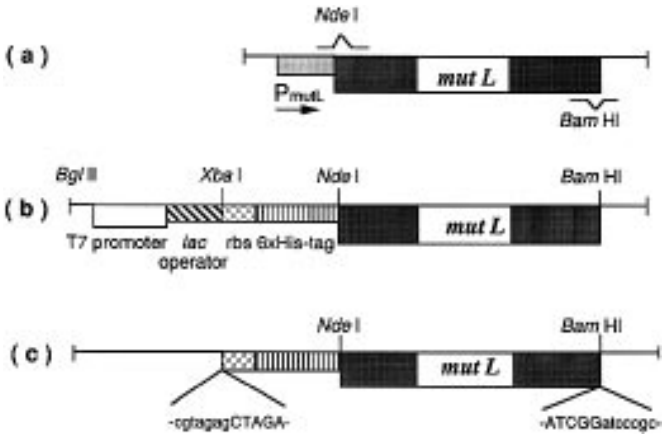

Figure 1. Sub-cloning of DNA fragments. (**a**) The *mutL* gene was amplified by PCR using primers which introduce *Nde*I and *Bam*HI sites as shown. The location of the endogenous promoter (P_{mutL}) is also shown. (**b**) The DNA fragment from (a) was inserted into pET15b to give pMQ378, generating a hexahistidine tag (6×His-tag). The ribosome binding site (rbs) is also shown. The *Bgl*II–*Hin*dIII fragment was used to construct pMQ393. (**c**) pMQ396 was constructed by ligating an *Xba*I (filled in)–*Bam*HI fragment from pMQ378 into the backbone of pACYC184 digested with *Eco*RV and *Bam*HI. All the resulting plasmids had *mutL* in the opposite orientation to that expected and the sequence at the fragment junctions is shown. Bases in lower and upper case are from pACYC184 and *mutL* respectively.

RESULTS AND DISCUSSION

Isolation of mutants

In order to facilitate processing of the anticipated mutant MutL proteins we constructed plasmid pMQ378, a pET15b derivative containing the *mutL* gene which produces MutL with a hexahistidine tag at the N-terminal end (Fig. 1). The results in Table 2 show that this plasmid complements the *mutL* defect of strain GM4250 (*mutL*Δ), indicating that the extra amino acids at the N-terminal end do not interfere with MutL function *in vivo*. A similar result has been reported by Feng and Winkler (37) while this work was in progress.

Table 2. Complementation of a *mutL* deletion strain with pMQ378

Strain	Mutation frequency (rifampicin resistance/10^{-8})
CC106 (*mutL*$^+$)	1
GM4250 (*mutL*$^-$Δ)	160
GM4250/pET15b	210
GM4250/pMQ378	4
GM4250/pMQ393	1
GM4250/pMQ396	2

Plasmid pMQ378 is a *mutL*$^+$ derivative of pET15b and pMQ393 and pMQ396 are derivatives of pACYC184. The bacterial strains were grown overnight at 37°C in L broth with or without ampicillin. The following day portions of the culture were spread on L agar plates (with or without rifampicin) and incubated overnight at 37°C before counting.

The *mutL* gene, with the His-tagged coding region, was subcloned from pMQ378 into vector pACYC184 to produce a

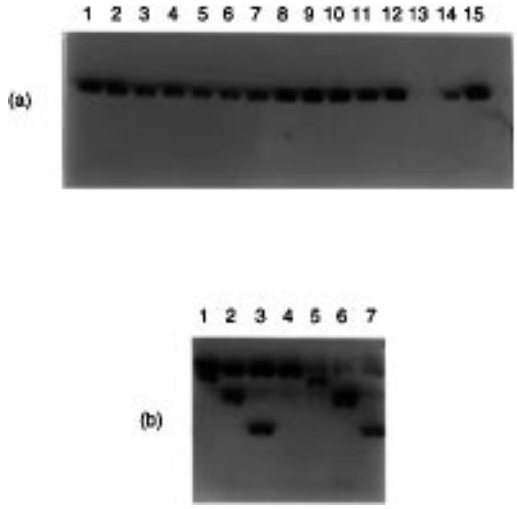

Figure 2. Steady-state level of MutL in selected dominant-negative mutant strains. Cell extracts of strain CC106 with plasmid-borne wild-type and dominant mutations were assayed by immunoblotting. (**a**) Proteins with single amino acid substitutions. Lanes 1–12, *mutL713–mutL702*. Lanes 13–15, pMQ350 (wild-type *mutL*) extract diluted 25- and 5-fold and undiluted respectively. (**b**) Truncated proteins. Lanes 1 and 5, *mutL725*; lanes 2 and 6, *mutL723*; lanes 3 and 7, *mutL722*. Lane 4, pMQ350 (wild-type *mutL*, 68 kDa). Lanes 1–3, with pMQ350; lanes 5–7, without pMQ350.

derivative with (pMQ393) or without (pMQ396) the T7 promoter region. Both these plasmids complemented the *mutL* defect of strain GM4250 (*mutL*Δ) (Table 2). The construction of pMQ393 was straightforward, resulting in the *mutL* gene being inserted into the pACYC184 backbone in a counterclockwise direction. For pMQ396, on the other hand, we expected the *mutL* gene to be inserted in a clockwise direction. All the transformants examined, however, had *mutL* in a counterclockwise direction, altering the expected sequence at the fragment junctions (Fig. 1c). Apparently only counterclockwise insertions of *mutL* are tolerated in this region of pACYC184, perhaps because it is in the vicinity of the replication origin.

Mutations were induced in pMQ393 and pMQ396 by hydroxylamine and surviving plasmids were introduced into strain CC106, which is *mutL*$^+$ and has a mutation in the *lacZ* gene. The transformed colonies were screened for increased spontaneous mutagenesis in two ways. First, dominant mutations were isolated essentially as described previously for *mutS* (20). Wild-type or recessive mutations in the *mutL* plasmid lead to normal levels of spontaneous mutagenesis, which is monitored by a low level of reversion from Lac$^-$ to Lac$^+$, i.e. by lack of papillation in the white colonies on MacConkey plates. Dominant mutations, however, increase the frequency of Lac$^-$ to Lac$^+$ reversion due to inhibition of mismatch repair. This results in the formation of Lac$^+$ (red) papillae in the Lac$^-$ (white) colonies. The extent of papillation is variable, depending on the mutation.

Second, we have also isolated dominant mutations on the basis of rifampicin-resistant colonies. Wild-type or recessive mutations in the *mutL* plasmid result in a low frequency of rifampicin resistance and colonies containing such plasmids show no growth on rifampicin plates after replica-plating. Dominant mutations increase the frequency of mutation to rifampicin resistance and show growth on rifampicin plates after replica-plating. Using both approaches we have isolated a spectrum of mutational responses ranging from just above background to at least a 100-fold increase.

Plasmid DNA was extracted from the putative dominant mutants and was used to re-transform the wild-type strain CC106. A total of 72 independently isolated mutant plasmids were retained for further characterization, since these showed increased Lac reversion and simultaneous resistance to rifampicin.

Expression levels of mutant proteins

We tested the assumption that the dominant-negative MutL proteins are relatively stable *in vivo* by measuring the level of these proteins in extracts of late log phase cells by immunoblotting. Figure 2a shows that the full-length mutant proteins show variable steady-state levels compared to wild-type, but not less than 2- to 3-fold. Figure 2b indicates that truncated nonsense fragments can be detected by immunoblotting at the approximate expected molecular weight positions deduced from the DNA sequence. The difference in MutL level in wild-type cells (lanes 5–7) compared with those with pMQ350 (lanes 1–4) is also shown. Diluting the plasmid-derived extract (Fig. 2a) shows that the MutL level is increased ~25-fold in cells bearing plasmid pMQ396 compared to a single copy wild-type strain. A faint cross-reacting band is present at the same amount in all samples in Figure 2b, suggesting that it is not a MutL degradation product.

Instability of tandemly repeated DNA sequences

Tandemly repeated DNA sequences are unstable in *E.coli* (26). Lovett and Feschenko (26) have examined the frequency of deletion between two 101 bp repeats in the tetracycline (*tet*) resistance gene of pBR322. One of the repeats contains four silent mutations in the *tet* gene, leading to 4% heterology between the repeated sequences. Tetracycline-resistant colonies which have resolved the duplication by deletion arise at high frequency by a mechanism that does not involve *recA*-dependent homologous recombination. Instead, the frequency to tetracycline resistance is increased in mutants defective in *dam*-directed mismatch repair and in a *dam* mutant strain, suggesting that these genetic realignments occur during replication by the resolution of heteroduplex DNA.

We have tested the effect of representative dominant *mutL* mutations on the stability of tandem repeats using the system described by Lovett and Feschenko (26). The data in Table 3 confirm that a >30-fold increase occurs in an otherwise isogenic *mutL*Δ strain compared with wild-type. The various dominant mutations alter the frequency to tetracycline resistance over a spectrum of values ranging from wild-type to the *mutL* control value. One mutant allele (*mutL712)* gave a value four times higher than the *mutL* control, but this is probably a statistical outlier due to the low number of samples (five) used. The spectrum of values in Table 3 indicates that the dominant mutations are heterogeneous with respect to duplication resolution. The correlation between duplication resolution and spontaneous mutation is variable, depending on the mutation (Table 2). The correlation is high, for example, for both *mutL711* and *712* (at about the *mutL*Δ level) and *715* and *725* (at about the wild-type level). For *mutL720*, however, the correlation is poor, with a high level of spontaneous mutation but a moderately low duplication resolution frequency. Similarly, *mutL726* shows a low correlation with a low spontaneous mutation frequency but a moderately high duplication resolution frequency. At present the implication of

these results is unclear, given the qualitative measurements and the absence of biochemical characterization of the mutant proteins.

Identification of mutational sites

To facilitate localizing the mutations, we took advantage of the centrally located *Apa*LI site (bp 903) in the *mutL* gene (1848 bp) to replace the promoter-proximal or promoter-distal fragments of the wild-type plasmid with those from the mutant plasmid. The majority (75%) of the mutations were located in the promoter-proximal fragment.

Table 3. Duplication and spontaneous mutation frequencies in the presence of mutant and wild-type *mutL* alleles

mutL allele	Duplication frequency	Spontaneous mutation frequency
Wild-type	0.8	0
Δ *(Stu)*	30	3+
702	11	2+
703	10	2+
704	17	2+
705	nd	3+
706	5	1+
707	nd	1+
708	nd	1+
709	nd	2+
710	19	2+
711	30	3+
712	120	3+
713	10	3+
714	9	3+
715	1	1+
716	7	1+
717	nd	2+
718	12	3+
719	2	1+
720	7	3+
721	3	1+
722	nd	1+
723	19	1+
724	23	1+
725	0.9	1+
726	14	1+

Plasmid pSTL113 (a pBR322 derivative) was introduced into wild-type cells containing each dominant *mutL* mutation or the wild-type allele on a pACYC184 backbone. Logarithmic phase cultures (~10^8 cells/ml) of the strains were plated on media containing ampicillin plus tetracycline and ampicillin. The ratio of the viable counts on these plates ($\times 10^{-4}$) is given for the wild-type and mutant alleles. The last column gives a qualitative estimate of the strength of dominance, where 0 denotes a wild-type phenotype (based on the level of rifampicin resistance) and 3+ a *mutL*$^-$ phenotype.
nd, not done.

DNA sequencing was carried out on either the promoter-proximal or promoter-distal regions of *mutL*. The locations of the 72 mutations together with the predicted amino acid substitutions are shown in Table 4. All but one of the mutations are GC→AT base changes consistent with the mutagenic action of hydroxylamine. The mutations occur throughout the gene, affecting amino acids A16–Q549 of the 615 amino acid MutL protein. A mutational hotspot is present at base 317, since the same mutation was recovered 23 times. Most of the mutations affect amino acids in the N-terminal end of the protein, which we define as residues 1–308. Of the 15 amino acid replacements, 12 occur at identical positions in various eukaryotic MutL homologs (bold type amino acids in Table 4) and the remaining three (S106, S112 and G238) are conserved in bacterial species. This suggests that the results obtained with our experimental approach for the *E.coli* MutL protein will also be applicable to bacterial and eukaryotic homologs.

Table 4. Location of dominant mutations in the *mutL* gene

mutL allele	Base	Amino acid substitution	Number
702	46	**A16**T	5
703	47	**A16**V	2
704	85	**E29**K	5
705	94	**E32**K	1
706	109	**A37**T	2
707	172	**D58**N	1
708	178	**G60**S	1
709	277	**G93**S	5
710	278	**G93**D	1
711	283	**R95**C	4
712	286	**G96**S	4
713	287	**G96**D	1
714	317	S106F	23
715	335	S112L	1
716	713	G238D	1
717	812	**A271**V	1
718	914	**P305**L	2
719	922	**H308**Y	1
721	991	Q331*ochre*	3
722	994	Q332*amber*	2
723	1198	codon417*ochre*	1
724	1201	Q401*ochre*	1
	(1591	R531C)	
720	1552	A518T	1
725	1555	Q519*amber*	2
726	1645	Q549*amber*	1

The nucleotide sequence is taken from GenBank Z11831 (30). Base 1 is the A of the ATG codon. All mutations except *mutL723* were GC→AT transitions. A frameshift mutation due to deletion of a G was identified in *mutL723*. *MutL724* contains two mutations at nt 1201 and 1591. The fourth column is the number of times the mutation was present in the collection. Amino acids in bold are conserved in eukaryotic homologs.

All but one (*mutL720*) of the mutations affecting the C-terminal end of the protein (residues 309–615) are nonsense mutations. *MutL722*, *725* and *726* are *amber* mutations, while *mutL721* and *724* are *ochre* mutations. The *amber* mutations were suppressed in the presence of plasmid p613, which encodes a synthetic glutamic acid-inserting tRNA (39), forming mostly full-length protein as measured by immunoblotting (data not shown). The presence of p613 also converted the phenotype of the *amber* mutants from MutL$^-$ to MutL$^+$ in strain GM4250 (*mutL*Δ), indicating that substituting glutamic acid for Gln332, Gln519 and Gln549 does not appreciably affect enzyme function *in vivo*. The truncated nonsense fragments will be useful in future biochemical analysis to delineate the functional domains of the protein.

MutL723 is a frameshift mutation due to loss of a G residue at bp 1198 which leads to a protein fragment with the correct sequence of amino acids to codon 399 and out of frame amino acids until the *ochre* termination codon at position 417. Two mutations were identified in *mutL724*; one at bp 1201, which generates an *ochre* mutation, and a second at bp 1591, which must be inconsequential since a MutL fragment with the anticipated

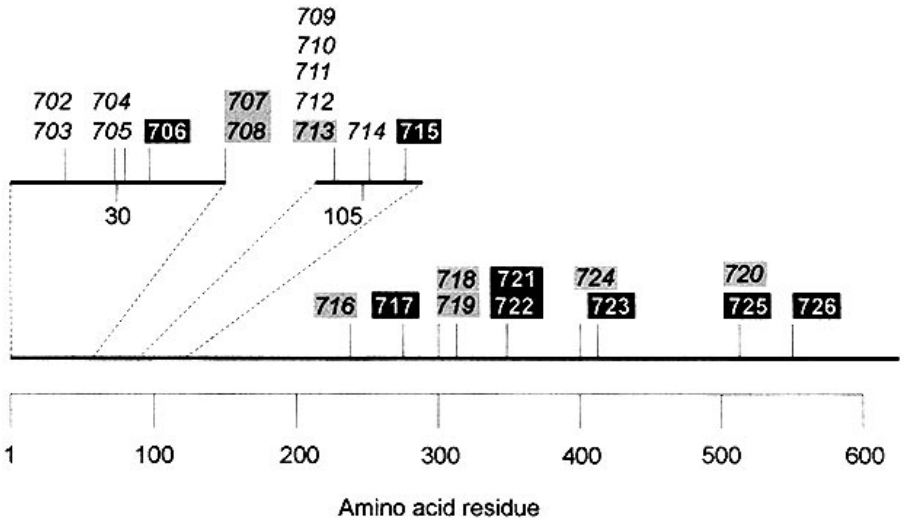

Figure 3. Linear representation of the *mutL* gene with the relative positions of the mutations. Amino acid regions 1–60 and 90–120 have been expanded to accomodate the higher density of mutations in these regions. Mutations on shaded or black backgounds are complemented by *mutS*$^+$ and *mutS*$^+$ and *mutH*$^+$ respectively.

molecular weight is produced in cells with this mutation (data not shown).

Complementation in a *mutL* strain

The dominant-negative plasmids were identified by their phenotypic effect on spontaneous mutation frequency in the wild-type strain CC106. To test if the dominant mutants were defective for MutL function, strain GM4250, a *mutL* deletion derivative of CC106, was transformed with each mutant plasmid and the frequency to rifampicin resistance was measured. In every case the frequency was the same as that for the untransformed GM4250 (data not shown), indicating that the dominant mutations cannot complement the *mutL* defect and are defective in MutL function.

Complementation by wild-type *mut* plasmids

A dominant-negative phenotype for MutL in a wild-type background could occur, for example, through the formation of inactive heteromultimers composed of wild-type and mutant MutL monomers. Alternatively, the defective mutant MutL could sequester a vital component of the repair complex (e.g. MutS or MutH). When the mutant protein is expressed from a multicopy plasmid and the wild-type from a single chromosomal copy gene, all of the wild type protein is expected to be in the form of heteromultimers or deprived of an essential component on the sequestration model. Increasing the gene dosage of the wild-type, however, should allow for the formation of sufficient wild-type MutHLS complex to alter the phenotype from mutant to wild-type. We have transformed wild-type cells containing each of 25 plasmid-borne dominant mutants (representing all mutated sites) with a compatible (pBR322 origin) plasmid bearing the wild-type *mutL* gene. In all but one case, the phenotype was converted from mutant to wild-type (Table 5), consistent with either the heteromultimer or sequestration hypotheses. The level of complementation was at about the wild-type level for all mutants with the exception of *mutL707*, which showed a marginal but consistent decrease relative to the control *mutL*$^-$ level. The *mutL705* mutant, which was not complemented by wild-type, might represent a protein with a higher affinity for its target site than wild-type.

Table 5. Complementation of *mutL* dominant mutations by *mutL*, *mutS* and *mutH*

mutL allele	Complementation by		
	mutL$^+$	*mutS*$^+$	*mutH*$^+$
*mutL*Δ	3+	–	–
702	2+	–	–
703	2+	–	–
704	2+	–	–
705	–	–	–
706	3+	1+	2+
707	1+	–	1+
708	3+	–	1+
709	3+	–	–
710	3+	–	–
711	3+	–	–
712	2+	–	–
713	2+	–	1+
714	2+	–	–
715	3+	1+	3+
716	3+	–	3+
717	3+	1+	3+
718	3+	–	2+
719	3+	–	2+
721	3+	2+	2+
722	3+	2+	2+
723	3+	2+	2+
724	3+	–	2+
720	3+	–	2+
725	3+	1+	1+
726	3+	1+	1+

Complementation was measured qualitatively by the level of rifampicin resistance in cell cultures containing compatible wild-type and mutant plasmids. Not all the mutations in the collection were tested. A wild-type level is indicated by 3+, while – is the *mutL*$^-$ level.

In addition to testing complementation with wild-type *mutL*, we have also used multicopy *mutS*$^+$ and *mutH*$^+$ plasmids. The levels of complementation were variable, ranging from 1+ (weak) to 3+ (strong) depending on the mutant (Table 5). In general, the *mutH*$^+$ plasmid complemented the *mutL* mutations in the gene coding for the C-terminal but not the N-terminal end of the protein (Table 5 and Fig. 3), suggesting a functional difference between the two parts of the protein.

Weak complementation was also observed in eight mutant strains with *mutS*$^+$, which in all instances occurred with mutations also complemented by *mutH*$^+$ (Table 5 and Fig. 3). At present we do not know the mechanistic basis for the complementation by *mutH*$^+$ or *mutS*$^+$. For those mutations complemented by *mutH*$^+$ but not *mutS*$^+$, the mutant MutL proteins may act to sequester the limited amount of MutH in an inactive complex. Supplying either MutH or MutL in excess *in trans* should then restore the normal phenotype, as observed. We have no satisfying explanation for those *mutL* mutations complemented by any of the *mut* genes. Perhaps the currently unexplained requirement for the hydrolysis of two ATP molecules to form MutS–MutL and an active MutHLS ternary complex respectively is affected by these MutL mutations, even though only the MutS protein has ATPase activity. Whatever the inhibitory mechanism is, it suggests these classes of mutants will be interesting to characterize at the biochemical level.

CONCLUSIONS

The dominant-negative mutations we have isolated map throughout the gene and are heterogeneous with respect to their phenotypic traits. The mutant proteins are relatively stable. The histidine-tagged mutant and wild-type proteins can be rapidly purified (data not shown), allowing for the development of new assays to measure MutL–MutS and MutL–MutH interactions on heteroduplex DNA and how these are impaired by the mutant proteins and to identify the functional domains of the MutL protein.

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