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Induction and Inhibition of Ciprofloxacin Resistance-Conferring Mutations in Hypermutator Bacteria

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The emergence of drug-resistant bacteria poses a serious threat to human health. Bacteria often acquire resistance from a mutation of chromosomal genes during therapy. We have recently shown that the evolution of resistance to ciprofloxacin in vivo and in vitro requires the induction of a mutation that is mediated by the cleavage of the SOS repressor LexA and the associated derepression of three specialized DNA polymerases (polymerase II [Pol II], Pol IV, and Pol V). These results led us to suggest that it may be possible to design drugs to inhibit these proteins and that such drugs might be coadministered with antibiotics to prevent mutation and the evolution of resistance. For the approach to be feasible, there must not be any mechanisms through which bacteria can induce mutations and acquire antibiotic resistance that are independent of LexA and its repressed polymerases. Perhaps the most commonly cited mechanism to elevate bacterial mutation rates is the inactivation of methyl-directed mismatch repair (MMR). However, it is unclear whether this represents a LexA-independent mechanism or if the mutations that arise in MMR-deficient hypermutator strains are also dependent on LexA cleavage and polymerase derepression. In this work, we show that LexA cleavage and polymerase derepression are required for the evolution of clinically significant resistance in MMR-defective *Escherichia coli*. Thus, drugs that inhibit the proteins responsible for induced mutations are expected to efficiently prevent the evolution of resistance, even in MMR-deficient hypermutator strains.

An increasingly significant threat to public health today is the emergence of bacteria that are resistant to multiple antibiotics. One of the most important factors contributing to the evolution of resistance is the acquisition of mutations during therapy (7). For many antibiotics, including the fluoroquinolones (6), cephalosporins (23), and rifamycins (6), resistance typically results from the acquisition of point mutations in genes that encode the drug's molecular targets or proteins involved in drug inactivation (6) or drug efflux (4, 36). Mutation appears to be the only mechanism to acquire resistance in *Mycobacterium tuberculosis* (38, 46) and some *Pseudomonas aeruginosa* infections (31).

We are interested in understanding the mechanism(s) by which bacteria acquire resistance-conferring mutations in order to design therapies that intervene in the process and thereby protect the efficacy of currently available antibiotics. Conventionally, it has been thought that mutations are the inevitable consequence of imperfect DNA replication and repair. However, mounting evidence suggests that bacteria may play a more active role in the mutation of their own genomes by inducing proteins that actually promote mutation (5, 8–10, 20, 41, 44, 48). We recently demonstrated in *Escherichia coli* that the evolution of ciprofloxacin resistance both in vivo and in vitro requires the induction of stress response pathways that facilitate mutation (3). This response is controlled by the repressor protein LexA, whose cleavage in response to DNA damage or inhibited replication initiates the SOS response, which is the orderly derepression of many genes involved in DNA replication, repair, and mutation. In particular, sufficient cleavage of LexA results in the derepression of the SOS polymerases polymerase II (Pol II) (encoded by *polB*), Pol IV (encoded by *dinB*), and Pol V (encoded by *umuD* and *umuC*), which collaborate to introduce genomic mutations until the cell adapts to the stressful environment. *E. coli* is essentially unable to evolve resistance when LexA is rendered uncleavable by a mutation of Ser119 to Ala or when any of the three SOS polymerases is deleted. Based on these results, we suggested that drugs might be designed to inhibit LexA cleavage or SOS polymerase activity and thus prevent the emergence of resistant bacteria.

For this approach to be feasible, there must not be any mechanisms to mutate and acquire antibiotic resistance that act independently of LexA and its repressed polymerases. One of the most commonly cited mechanisms to elevate bacterial mutation rates is the acquisition of a hypermutable phenotype due to the defects in methyl-directed mismatch repair (MMR) (17, 22, 30, 35, 47) associated with the inactivation of MutS (17, 30, 31), which is a protein required for the detection of mutated DNA and for the recruitment of other MMR proteins that mediate repair. It has thus been suggested that a transient downregulation of MutS or other MMR proteins may be an important mechanism for elevating bacterial mutation rates during times of stress to facilitate adaptation (13, 33, 45). However, whether MMR deficiency alone is sufficient to confer cells with hypermutability depends on the origin of the mutations that persist when MMR is absent. Here, we show that MMR deficiency on its own is unlikely to be sufficient for the accelerated evolution of antibiotic resistance during therapy,

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as Δ*mutS E. coli* still requires LexA cleavage and SOS polymerase derepression to efficiently evolve resistance.

MATERIALS AND METHODS

Bacterial strains and growth. *E. coli* strains used in this study are listed in Table 1. Solid medium was Lennox LB (26) plus 1.6% agar; liquid medium was Miller LB (26). For selection, antibiotics were used at the following concentrations: kanamycin, 30 μg/ml; spectinomycin, 100 μg/ml; chloramphenicol, 20 μg/ml; and gentamicin, 15 μg/ml. All bacteria were grown at 37°C unless otherwise indicated.

Strain construction. The *mutS* deletion strain was constructed by first generating a disruption cassette using three-way PCR as described previously by Murphy et al. (29). Oligonucleotide primers used in the construction of the disruption cassette are listed in Table 2. The cassette contained flanking regions of ~500 nucleotides that are homologous to the DNA upstream and downstream of the *mutS* gene and a gentamicin resistance (Gm^r) cassette. *E. coli*-specific sequences were amplified from strain MG1655 (1) genomic DNA that was purified with the DNeasy Tissue kit (QIAGEN). The Gm^r cassette was amplified from pBBR1MCS-5 (15) with primers GatF and GatR. The disruption cassette was transformed by electroporation into strain PS6275, plated onto LB supplemented with gentamicin, and grown at 30°C. After confirmation of a correct chromosomal insertion by PCR, the Δ*mutS*::Gm^r cassette was transferred into the strains listed in Table 1 by P1 transduction (25) with selection on minimal medium lacking biotin and containing gentamicin. The presence of the allele in each strain was confirmed by PCR.

Determination of preexposure mutation rate. For each strain, 10 independent cultures were grown for 25 h in permissive media. For strains RTC0001 and RTC0011, 150 μl from each culture (~5×10^8 cells) was plated onto LB agar containing 0.045 μg/ml ciprofloxacin and onto LB agar containing 100 μg/ml rifampin. For all other strains (i.e., those containing Δ*mutS*), 15 μl from each culture (~5×10^7 cells) was plated onto 0.045 μg/ml ciprofloxacin, and an additional 3 μl from each culture (~1×10^7 cells) was plated onto 100 μg/ml rifampin. (The plating scheme was designed to obtain 30 to 300 colonies per plate.) Viable cell counts for each culture were determined by plating serial dilutions onto permissive media. Plates were incubated for 48 h, and the number of resistant colonies was determined. Mutation rates were calculated using the method of the median (16).

Determination of postexposure mutation rate. For each strain, five independent cultures were grown for 25 h without ciprofloxacin. Viable cell counts in these cultures were determined by plating serial dilutions onto permissive media. The number of ciprofloxacin-resistant colonies for each strain was determined by plating 15 μl from each culture (~5×10^7 cells) in duplicate onto LB agar containing 0.045 μg/ml ciprofloxacin. (Five additional aliquots from two cultures of each strain were also plated on the same medium for use in the "survival" assay [see below].) At 24-h intervals, visible colonies were counted, their location on the plate was marked, and they were stocked at −80°C for later use in the reconstruction assay (see below).

Cell viability was determined every 24 h for the Δ*mutS lexA*(S119A) strain as well as for the Δ*lacZ*, *lexA*(S119A), Δ*dinB*, Δ*umuDC*, Δ*dinB* Δ*umuDC*, and Δ*polB* Δ*dinB* Δ*umuDC* strains. All visible colonies were excised from plates designated for assaying survival (see above), the remaining agar was homogenized in saline, and dilutions were plated in duplicate onto LB agar to determine the total number of viable, ciprofloxacin-sensitive cells present as a function of time and onto LB agar containing 0.045 μg/ml ciprofloxacin to determine if any ciprofloxacin-resistant colonies remained after excision. An experimental validation of this method has been described previously (3).

It was also determined whether colonies isolated after plating onto ciprofloxacin formed as a result of mutation during growth in liquid culture (preexposure mutation) or after being plated onto medium containing ciprofloxacin (postexposure mutation). Liquid cultures of permissive media were inoculated with ciprofloxacin-resistant clones stocked during the mutation assay (see above) and grown to saturation overnight. Cultures were diluted and plated in duplicate on both LB agar, to confirm viability, and LB agar containing 0.045 μg/ml ciprofloxacin, to confirm resistance. Clones that were resistant before exposure were defined as those clones that formed colonies on the ciprofloxacin-containing media in the same number of days in the reconstruction assay as they did in the original mutation assay. Conversely, clones that mutated after exposure to ciprofloxacin were defined as those that formed colonies at least 2 days faster in the reconstruction assay. Additional control experiments validating this method were described previously (3). The postexposure mutation rate was defined as the number of postexposure ciprofloxacin-resistant mutants per viable cell as a function of time. As discussed in Results, the number of viable cells as a function of time was approximated using the corresponding mutant strain in the MMR-proficient background. The postexposure mutation rates exhibited the expected Poisson distribution (19).

MIC determination. For each strain, two independent cultures were grown for 25 h at 37°C in LB containing no antibiotic. From each culture, ~10^4 CFU were spotted in duplicate onto LB agar containing ciprofloxacin at 0, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, 0.05, or 0.055 μg/ml. After 24 h of incubation at 37°C, the MIC was determined to be the concentration at which no visible growth was observed. Single colonies were not counted as growth and represent ciprofloxacin-resistant mutants. We chose to use this agar dilution method, as opposed to the broth microdilution method, to avoid the effects of selection and clonal expansion (32).

TABLE 1. *E. coli* strains used in this study

Strain	Relevant genotype	Reference or source
MG1655	F^- λ^- *ilvG rfb*-50 *rph*-1	2
PS6275	MG1655 Δ*bio nadA*::Tn*10* cI1857 Δ(*cro-bioA*)	6
RTC0001	MG1655 Δ*lacZ*::Km^r	6
RTC0004	MG1655 Δ*dinB*::Km^r	6
RTC0005	MG1655 Δ*umuDC*::Km^r	6
RTC0009	MG1655 Δ*dinB*::Km^r Δ*umuDC*::Cm^r	6
RTC0010	MG1655 Δ*polB*::$Spec^r$ Δ*dinB*::Km^r Δ*umuDC*::Cm^r	6
RTC0011	MG1655 *lexA*(S119A)::Km^r	6
RTC0086	MG1655 Δ*mutS*::Gm^r	This work
RTC0104	MG1655 Δ*mutS*::Gm^r Δ*dinB*::Km^r	This work
RTC0105	MG1655 Δ*mutS*::Gm^r Δ*umuDC*::Km^r	This work
RTC0087	MG1655 Δ*mutS*::Gm^r Δ*dinB*::Km^r Δ*umuDC*::Cm^r	This work
RTC0088	MG1655 Δ*mutS*::Gm^r Δ*polB*::$Spec^r$ Δ*dinB*::Km^r Δ*umuDC*::Cm^r	This work
RTC0089	MG1655 Δ*mutS*::Gm^r *lexA*(S119A)::Km^r	This work
RTC0135	MG1655 Δ*mutS*::Gm^r Δ*lacZ*::Km^r	This work

TABLE 2. Primers used in this study

Primer	Sequence (5′→3′)
Gat_IntConfF	GTG ATG CAC TTT GAT ATC GAC
GatF	GCT CAC TGC CCG CTT TCC AG
GatR	GCT TGA ACG AAT TGT TAG GTG
mutS_CF-Gat	CT GGA AAG CGG GCA GTG AGC CTG GTG TAA TAA CAA TTC CCG
mutS_CR	GG ATA ATC TGC ATG TGC GAT GGC
mutS_IntConfR	CTG CCA GAT AGC CGC CAG CAG
mutS_Nconf	GGC ACC GGA CGC TTG TCA TCG
mutS_NF	GCA GGC TGG TAA CAG TGC ACC
mutS_NR-Gat	CAC CTA ACA ATT CGT TCA AGC GAA ATT TTC TAT TGC ACT CAT GG

RESULTS

Pre- and postexposure mutation in wild-type *E. coli*. For this study, we define the mutation rate as the number of cells that acquire resistance to 0.045 μg/ml ciprofloxacin per viable cell per unit of time. We chose 0.045 μg/ml ciprofloxacin as it was found to be the lowest concentration of drug that required mutation for growth. Typically, under these conditions, a single mutation in *gyrA* confers drug resistance (3). In addition, we found that 0.045 μg/ml killed ~99% of the cells within 24 h of plating, while ~1% of the population persisted for several weeks, allowing for the characterization of mutations both in the absence and in the presence of the antibiotic. We refer to

TABLE 3. MICs and pre- and postexposure mutation rates for strains tested

Strain	Ciprofloxacin MIC (ng/ml)		Preexposure mutation rate (10^{-8})		Postexposure mutation rate (10^{-8})	
	MutS$^+$	MutS$^-$	MutS$^+$	MutS$^-$	MutS$^+$	MutS$^-$
MG1655	25	25	0.09 (±0.09)[a]	98 (±37)	1,550 (±499)[a]	4,380 (±62)
Δ*lacZ*	25	25	0.15 (±0.01)	118 (±27)	927 (±184)	4,440 (±68)
Δ*dinB*	25	25	0.12 (±0.18)[a]	87 (±17)	266 (±153)[a]	332 (±32)
Δ*umuDC*	25	25	0.03 (±0.01)[a]	87 (±5)	264 (±234)[a]	−[b]
Δ*dinB* Δ*umuDC*	25	25	0.04 (±0.02)[a]	63 (±11)	40 (±82)[a]	−[b]
Δ*polB* Δ*dinB* Δ*umuDC*	25	25	0.25 (±0.32)[a]	104 (±40)	50 (±87)[a]	261 (±80)
lexA(S119A)	20	20	0.02 (±0.02)[a]	55 (±32)	11 (±22)[a]	−[b]

[a] Data provided from reference 6 for comparison.
[b] −, no measurable rate.

mutations that occur during growth in ciprofloxacin-free liquid culture as preexposure mutations and those that occur after plating on ciprofloxacin-containing media as postexposure mutations (see Materials and Methods). Resistant colonies were counted in 24-h intervals over 14 days and confirmed as postexposure mutants in reconstruction assays that were designed to determine when the mutations occurred (see Materials and Methods). In the Δ*lacZ* control strain, the preexposure mutation rate was $(1.5 \pm 0.1) \times 10^{-9}$ mutants/viable cell/day, while the postexposure rate was $(9.3 \pm 1.8) \times 10^{-6}$ mutants/viable cell/day (Table 3), in agreement with our previous results that ciprofloxacin induces resistance by a factor of 10^4 (3).

Deletion of *mutS* results in elevated pre- and postexposure mutation. Deletion of the *mutS* gene was carried out as described in Material and Methods. In agreement with previous reports (37), deletion of *mutS* resulted in a significantly elevated rate of preexposure mutation (Table 3 and Fig. 1A). The rate of evolution of resistance to rifampin and ciprofloxacin was elevated 200- and 800-fold, respectively, confirming that the MMR system is functioning to repair spontaneous replication errors during exponential growth in liquid medium. We also determined the rate of postexposure mutation to ciprofloxacin resistance in the Δ*mutS* strain and found that it is approximately fourfold higher than that in the control strain (Fig. 1B). This increase in mutation rate agrees with the three- to sixfold-elevated rate of adaptive or stationary-phase mutation observed in MMR-deficient strains during prolonged starvation (33) and indicates that MMR is functioning to repair resistance-conferring mutations in the presence of ciprofloxacin.

The increase in preexposure mutation associated with Δ*mutS* does not depend on either LexA cleavage or the derepression of the SOS polymerases. We demonstrated previously that LexA and the SOS polymerases do not play a role in preexposure mutation to ciprofloxacin resistance (3). To determine whether the increased rate of the preexposure mutation in Δ*mutS* strains depends on induced mutation, we examined the effect of deleting *mutS* in Δ*lacZ* (control) as well as in *lexA*(S119A), Δ*dinB*, Δ*umuDC*, Δ*dinB* Δ*umuDC*, and Δ*polB* Δ*dinB* Δ*umuDC* (Table 1) strains. All of the resulting strains exhibited a significantly elevated preexposure mutation rate to both rifampin and ciprofloxacin (Table 3) that was not significantly different than that of the single Δ*mutS* mutant. These results indicate that even in the absence of MMR, the SOS-regulated polymerases do not contribute to spontaneous, preexposure mutation.

The increase in postexposure mutations associated with Δ*mutS* requires both LexA cleavage and the derepression of the SOS polymerases. We demonstrated previously that the vast majority of mutations that confer ciprofloxacin resistance arise after exposure to the drug and are mediated by the SOS polymerases Pol II, Pol IV, and Pol V. To determine whether this is also true in MMR-defective cells, we examined postexposure mutations in Δ*mutS lexA*(S119A) and Δ*mutS* Δ*pol* strains.

As described in Materials and Methods, postexposure mutation rates were calculated based on the number of resistant colonies that arise as a function of time normalized by the

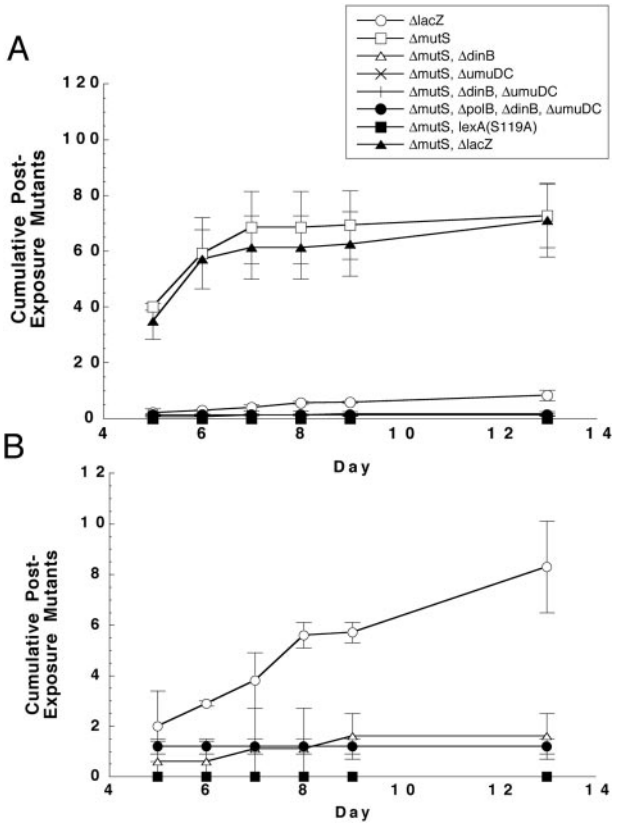

FIG. 1. Cumulative number of postexposure mutants per day. (A) All strains. (B) The Δ*mutS* strain has been omitted and the *y* axis has been expanded for clarity.

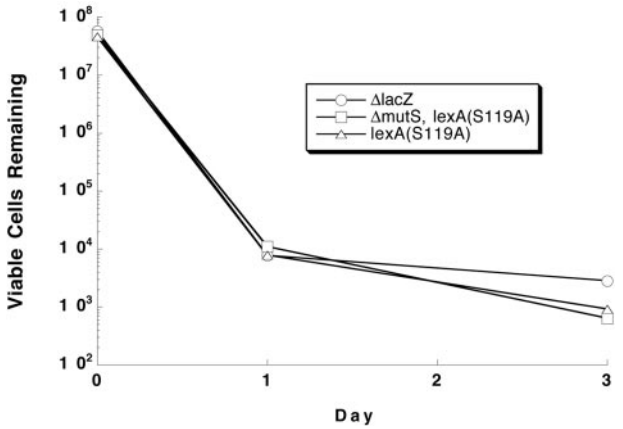

FIG. 2. Cellular persistence of the Δ*lacZ* control, *lexA*(S119A), and Δ*mutS lexA*(S119A) strains on media containing ciprofloxacin as a function of time.

number of viable cells (at the time the mutation arose). For most Δ*mutS* strains, it is not possible to accurately determine the number of viable, ciprofloxacin-sensitive cells remaining as a function of time due to the large number of resistant cells that arise on the plate. However, we were able to measure cell viability over the first 3 days of the experiment for the Δ*mutS lexA*(S119A), Δ*lacZ*, and *lexA*(S119A) strains (Fig. 2). Over the 3 days measured, all three strains persisted equally well on medium containing ciprofloxacin. This result suggests that Δ*mutS* does not affect persistence in the *lexA*(S119A) strain. Because LexA represses all three polymerases, a *mutS* deletion is also not expected to affect persistence in any of the polymerase deletion backgrounds. To further support the idea that Δ*mutS* does not impart the cell with increased sensitivity to ciprofloxacin, we measured ciprofloxacin MICs for each strain using the agar dilution method (Table 3). Deletion of *mutS* did not alter the MICs. This is in agreement with results reported previously which demonstrate that the deletion of *mutS* does not impart the cell with altered sensitivity to ciprofloxacin (32). Thus, we used the persistence of each of the deletion strains in an MMR-proficient background to normalize the resistant-cell count for the corresponding MMR-deficient mutants.

We determined the postexposure mutation rate of the Δ*mutS* Δ*lacZ* control strain as well as the Δ*mutS lexA*(S119A), Δ*mutS* Δ*dinB*, Δ*mutS* Δ*umuDC*, Δ*mutS* Δ*dinB* Δ*umuDC*, and Δ*mutS* Δ*polB* Δ*dinB* Δ*umuDC* strains. All of the mutants grew approximately equally in the absence of ciprofloxacin. However, unlike preexposure mutations, rendering LexA uncleavable or deleting the SOS polymerases alone, or in any combination, dramatically suppressed the postexposure mutation rate of a Δ*mutS* strain to levels lower than those of the wild type (Fig. 1A and B and Table 3). In fact, the mutation rates of these strains were near or below the limits of detection. Remarkably, the MMR-deficient double mutants showed virtually the same mutability as their MMR-proficient single-mutant counterparts. (Differences between the MMR-proficient and -deficient strains, as well as the differences between the Δ*dinB* or Δ*polB* Δ*dinB* Δ*umuDC* strains, for which we were able to detect a rate, and the other hypomutators, for which we were unable to measure a rate, are unlikely to be significant. We ascribe these differences to our limit of detection, which required optimization for both the hyper- and hypomutators.) The data indicate that while MMR is functioning to repair postexposure mutations, the hypermutability of the MMR-defective strains absolutely requires LexA cleavage and derepression of Pol II, Pol IV, and Pol V.

DISCUSSION

The evolution of antibiotic resistance by the accumulation of mutations during treatment is one of the most important factors contributing to therapy failure (7). Thus, the mechanism(s) by which mutations arise and are repaired is of paramount importance in the effort to combat bacterial pathogens. In *E. coli*, MMR is the most important mechanism by which polymerase errors are repaired (27, 37), and it is conserved throughout all kingdoms of life (14, 28, 39). The central role played by MMR in correcting polymerase errors makes its regulation an attractive mechanism to control mutability. Indeed, in addition to evidence that MMR-dependent hypermutability may provide a selective advantage for bacteria (11, 12, 21, 42, 43), it has been demonstrated that MMR may be modulated during the stationary phase, where its reduction allows the accumulation of mutations (13, 33, 45).

As a mechanism to induce mutations, the depletion of MMR alone would only be sufficient if a significant percentage of the resistance-conferring mutations that are allowed to persist are the unavoidable result of normal DNA synthesis. We recently demonstrated in MMR-proficient cells that the vast majority of the bacteria that evolve resistance to ciprofloxacin or rifampin do so by inducing the cleavage of LexA, which derepresses the error-prone SOS DNA polymerases that collaborate to introduce mutations (3). Because MutS deficiencies are the most common mechanism to deactivate MMR, we determined whether the resistance-conferring mutations that accrue in a Δ*mutS* strain require induction by LexA cleavage and SOS polymerase derepression.

As observed for other systems, the Δ*mutS* strain was a strong hypermutator (37). In this work, the preexposure or spontaneous mutation rates to ciprofloxacin resistance were elevated ~800-fold with the deletion of *mutS*. We reported previously that preexposure mutations occur during normal Pol I- or Pol III-mediated synthesis, since they are not affected by the mutation of LexA or by the deletion of the SOS polymerases. Consistent with this result, we find that the preexposure hypermutability associated with defective MMR is also independent of LexA cleavage and the derepression of the SOS polymerases. Thus, MMR deficiency is sufficient for preexposure hypermutability.

In the case of the postexposure mutation rates, deletion of *mutS* results in a ~4-fold increase, indicating that MMR is functioning to repair postexposure mutations (Table 3). However, rendering LexA uncleavable or deleting the SOS polymerases reduces the rate of mutation of the Δ*mutS* strain below that of the wild-type strain. In fact, the rate of evolution to ciprofloxacin resistance in the Δ*mutS lexA*(S119A) or the Δ*mutS* Δ*pol* strain is identical to those of the corresponding MMR-proficient strains. This implies that in the absence of SOS polymerase derepression, the contribution of MMR to the repair of ciprofloxacin resistance-conferring mutations is

negligible. In addition, this demonstrates that MMR deficiency alone is not sufficient for hypermutability after the bacteria are exposed to the antibiotic. The increased mutation observed in the hypermutator strains requires the induction of LexA cleavage and is mediated by the SOS polymerases.

The data suggest that the inhibition of LexA cleavage or SOS polymerase activity should prevent postexposure mutations but not preexposure mutations. Thus, it is critical to consider whether clinically resistant bacteria are likely to be present at the start of therapy. As has been described previously for Δ*mutS* of *Pseudomonas aeruginosa* (32), a significant number of bacteria with single resistance-conferring mutations are expected to be present prior to therapy. However, in the case of ciprofloxacin, these single resistance-conferring mutations are expected to confer only low to moderate resistance. Clinically significant levels of resistance in all bacteria require more than one resistance-conferring mutation (18). The probability of a single bacterium independently acquiring two resistance-conferring mutations is approximately the product of the individual probabilities and approaches zero in even the largest infections.

Upon exposure to the selective pressure of the antibiotic, the probability of evolving clinically significant levels of resistance increases for two reasons. First, we and others (2, 3, 40) have demonstrated that rates of mutation are significantly elevated in the presence of an antibiotic. Second, antibiotic selection results in the clonal expansion of mutants with single resistance-conferring mutations, which then serve as a background upon which a second resistance-conferring mutation may be acquired. This stepwise evolution significantly increases the probability that a single bacterium will acquire two resistance-conferring mutations. This is consistent with the conclusions of others that the acquisition of mutations during treatment is one of the most important factors contributing to therapy failure (7) and suggests that even hypermutator strains of bacteria are likely to require postexposure, induced mutations to evolve resistance during ciprofloxacin or multidrug therapy, which also requires multiple mutations. Thus, for wild-type and Δ*mutS* MMR-deficient strains alike, inhibition of LexA cleavage or inhibition of the inducible polymerases should be an effective means of preventing the evolution of antibiotic resistance.

The traditional paradigms of DNA replication and mutation suggest that resistance-conferring mutations are the inevitable consequence of polymerase errors and offer no obvious means for intervention. From this perspective, MMR deficiencies would be an independent mechanism for elevating mutation rates by simply allowing more of the always-present mutations to persist. In stark contrast to this model, the data presented above demonstrate that MMR deficiencies alone are in fact not sufficient for the elevation of mutation rates during therapy; the mutations that escape repair must still be induced by LexA cleavage and derepression of Pol II, Pol IV, and Pol V. This suggests that suitably designed inhibitors of LexA cleavage or of the SOS polymerases would have a profound effect on the treatment of even hypermutator strains of bacteria, potentially minimizing or eliminating the threat of antibiotic resistance. The elevated rates of mutation in the presence of ciprofloxacin might also induce mutations that confer resistance to other unrelated antibiotics, and thus, LexA or SOS polymerase inhibitors might also help combat the evolution of multidrug-resistant bacteria during therapy. In addition, it has been demonstrated recently that cells respond to β-lactams by inducing SOS genes that enhance their survival (24, 34), and therefore, inhibiting LexA cleavage may also enhance the activity of these antibiotics.

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