

## *Escherichia coli* Cells Exposed to Streptomycin Display a Mutator Phenotype

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**Mistranslation mediated by the *mutA* and *mutC* tRNA alleles elicits a strong mutator phenotype (H. S. Murphy and M. Z. Humayun, *J. Bacteriol.* 179:7507–7514, 1997; M. M. Slupska, C. Baikalov, R. Lloyd, and J. H. Miller, *Proc. Natl. Acad. Sci. USA* 93:4380–4385, 1996). Here, we show that exposure to streptomycin, an antibiotic known to promote mistranslation, induces a *recA*- and *umuDC*-independent mutator phenotype detected as enhanced mutagenesis at a 3,N<sup>4</sup>-ethenocytosine lesion borne on transfected M13 single-stranded DNA.**

Replication fidelity can be transiently altered by a number of environmental and physiological stimuli, and some of these so-called transient mutator responses are distinct from the classical *recA* and *umuDC*-dependent SOS mutagenesis pathway (2). One of the more provocative recent findings is that the expression of a mutant *glyV* or *glyW* tRNA gene (from the *mutA* or *mutC* allele, respectively) can confer a strong mutator phenotype (11) and that this phenotype is *recA* dependent but *umuDC* independent (4). The mutation in *mutA* (and *mutC*) alters the tRNA anticodon in such a way that cells expressing *mutA* are thought to have low but appreciable levels of asp→gly mistranslation. Two hypotheses have been proposed to account for how mistranslation can lead to a mutator phenotype. The first suggests that the phenotype arises from the specific mistranslation (targeting catalytically critical aspartates) of a specific protein—namely, the proofreading subunit epsilon of DNA polymerase III—so as to create a small pool of dominant-negative mutant epsilon proteins. The occasional recruitment of these mutant epsilon proteins into a holoenzyme assembly is presumed to create a transient mutator phenotype (11). The second hypothesis proposes that mistranslation, probably through elevated protein turnover, induces a *recA*-dependent mutagenic pathway that is constitutively expressed (4). The latter hypothesis (translational stress-induced mutagenesis [2]) makes the prediction that a variety of conditions that elevate mistranslation or otherwise increase protein turnover may be able to induce a similar mutator phenotype. In this study, we have asked whether streptomycin, an antibiotic known to increase the number of translational errors, can induce a mutator phenotype detectable as elevated mutagenesis at a site-specific 3,N<sup>4</sup>-ethenocytosine (εC) lesion borne on M13 single-stranded DNA (ssDNA) that is transfected into streptomycin-treated or untreated cells.

Luria-Bertani (LB) medium (100 ml) in a 250-ml culture flask was inoculated with 1 ml of a fresh overnight culture of an appropriate strain and then divided into 25-ml aliquots in sterile 125-ml culture flasks. Streptomycin (Sigma) was introduced to various final concentrations by adding appropriate volumes of freshly prepared stock solutions (1 and 10 mg/ml in sterile water), and the cultures were allowed to grow at 37°C with

vigorous aeration to an optical density at 600 nm of 0.3 to 0.4 ( $5 \times 10^7$  to  $1 \times 10^8$  cells/ml). Cells were pelleted by centrifugation at 4°C for 10 min in a Sovall SS-34 rotor at 3,000 rpm and washed by resuspension in an equal volume of ice-cold LB medium followed by centrifugation as described above. The final cell pellet was resuspended in transfection medium to render the cells transfection competent as described previously (6).

Competent cells (1 ml) were incubated on ice for 30 min with 50 ng of M13 ssDNA bearing a site-specific εC lesion (9). To determine ssDNA survival, two 0.1-ml aliquots of the transfected competent cells were plated with 0.2 ml of the overnight culture on LB-agar plates, and the average number of infectious centers was determined as the PFU count after overnight incubation. The remaining 0.8 ml of the transfected cells was transferred to 8 ml of fresh LB medium, together with 0.2 ml of a fresh overnight saturated culture, and allowed to grow overnight at 37°C with vigorous aeration to produce progeny phage (5, 7). Pooled progeny phage DNA from each transfection was prepared either as previously described (5, 7) or by the use of Qiaprep Spin M13 kits (Qiagen) in accordance with the instructions provided by the vendor. The frequency and specificity of mutations at the εC site were determined by the strategy shown in Fig. 1A and described in detail elsewhere (5, 7, 9).

Figure 1B (lanes 1 to 4) shows the effect of exposing *Escherichia coli* KH2 [ $\Delta(lac-pro) trpE9777$  F' *lacI<sup>a</sup> ZΔM15 pro<sup>+</sup>*] (*recA<sup>+</sup> umuD<sup>+</sup> umuC<sup>+</sup>* [10]) cells to streptomycin at concentrations of 0.5, 2, and 5 μg/ml (lanes 2 to 4, respectively) compared to no streptomycin exposure (lane 1). There was a dose-dependent increase in mutagenesis at εC, as indicated by the increased signal in the 22-mer band and, to a much smaller extent, in the 21-mer band. Quantitation of the signal by densitometry (Table 1, experiment A) showed that in the absence of streptomycin, the level of mutagenesis was low (about 2%). The level of mutagenesis increased with increasing streptomycin concentration, such that at the maximum concentration used (5 μg/ml), the mutation frequency was about 24%. An essentially similar pattern was given by *E. coli* KH2R [ $\Delta(srIR-recA)306::Tn10(Tet^r)$ ] in KH2 (8)] cells (Fig. 1, lanes 5 to 8; Table 1, experiment B), suggesting that increased mutagenesis does not require a functional *recA* gene. The data for experiment C in Table 1 show that the same pattern is observed in *E. coli* SR100 ( $\Delta umuDC$  in KH2 [4]) cells, indicating that the streptomycin effect is also independent of the *umuDC* genes. In all cases, the C→A mutation level is elevated to a

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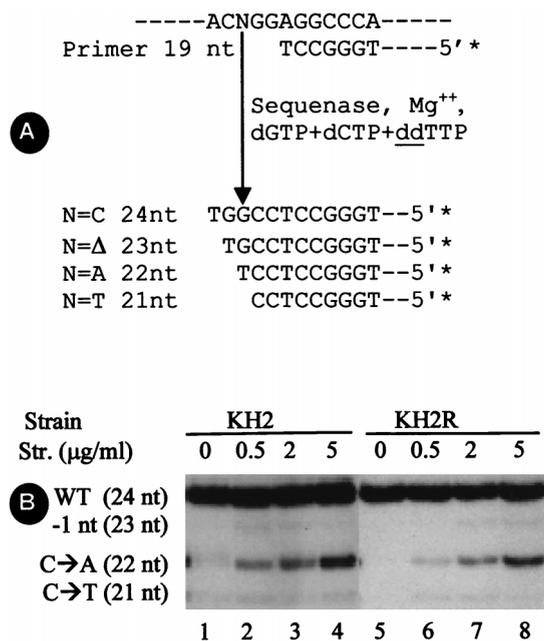


FIG. 1. (A) Principles of multiplex sequence analysis. A pre-labeled 19-mer primer was annealed to the pooled progeny phage ssDNA and allowed to elongate in the presence of dGTP, dCTP, and dideoxy-TTP. Depending on the base at position N, limited-elongation products of characteristic length were produced, and they were fractionated by high-resolution electrophoresis and quantitated as described previously (5, 7). C→A transversions yield a 22-mer, and C→T transitions result in a 21-mer. εC is also known to induce -1-nucleotide (nt) deletions that give rise to a 23-mer. Wild-type sequence gives rise to 24-mers. (Note that any C→G transversions can also give rise to a 24-mer, but εC does not induce C→G mutations at appreciable levels [3, 8].) (B) Effect of streptomycin treatment of wild-type (WT; KH2) (lanes 1 to 4) or *DrecA* (KH2R) (lanes 5 to 8) cells on mutation fixation at an εC residue borne on transfected M13 ssDNA. Procedures are described in the text. Elongation product lengths and identities are shown on the left. The level of mutagenesis was low in uninduced cells (lanes 1 and 5), but there was an increase in mutagenesis in response to pretreatment with streptomycin (Str.) at 0.5, 2, or 5 μg/ml (lanes 2 to 4 and 6 to 8). These results are expressed quantitatively in Table 1.

much higher degree than is the level of C→T mutations. This mutational specificity is similar to that observed in *mutA* cells and in cells induced for the UVM response (4). The transfection efficiency data in Table 1 show that streptomycin treatment does not appear to dramatically affect survival of DNA bearing εC, which ranges from 28 to 44% of the control values without a consistent pattern (Table 1, column 3; compare with the parenthetical numbers, which represent DNA bearing normal cytosine residues). It is possible that exposure to streptomycin alters the relative proportions of different bases inserted opposite the lesion without increasing the magnitude of the bypass events. It should be noted that all three strains used here are streptomycin sensitive and essentially stop growing at a streptomycin concentration of 50 μg/ml or greater.

These data indicate that streptomycin does induce a mutator phenotype, as predicted by the hypothesis that mistranslational stress induces a mutagenic pathway. This finding is also consistent with a previous report showing that streptomycin treatment elevates background mutagenesis by an appreciable margin (1). However, the streptomycin effect does not require the *recA* gene, whereas the *mutA* effect does (4), suggesting differences in the underlying induction mechanisms. The genetic requirement profile for the streptomycin effect resembles that for the UVM response in that neither requires the *recA* or the *umuDC* genes. Nevertheless, the findings reported here raise interesting questions with regard to the role of mistranslation

TABLE 1. Effect of streptomycin treatment of *E. coli* cells on mutation fixation at an εC residue borne on transfected M13 ssDNA

Expt	Host strain <sup>a</sup>	Streptomycin concn (μg/ml) <sup>b</sup>	Survival of εC DNA (C-DNA) <sup>c</sup>	Mutation frequency ± SD (%) <sup>d</sup>		
				Total	C→A	C→T
A	KH2	0	1,470 (4,410)	2 ± 1	2 ± 1	1 ± 0
		0.5	1,510 (4,630)	8 ± 2	7 ± 1	1 ± 0
		2	1,630 (5,079)	15 ± 2	15 ± 1	1 ± 0
		5	1,230 (4,359)	24 ± 3	22 ± 3	2 ± 1
B	KH2R	0	1,250 (4,224)	2 ± 1	1 ± 1	1 ± 0
		0.5	1,500 (3,928)	8 ± 1	6 ± 1	2 ± 1
		2	1,410 (3,648)	14 ± 1	11 ± 1	3 ± 1
		5	1,010 (3,584)	26 ± 1	24 ± 1	1 ± 1
C	SR100	0	400 (1,044)	5 ± 2	4 ± 1	1 ± 0
		2	1,040 (2,500)	13 ± 2	12 ± 1	2 ± 1
		5	1,120 (2,870)	23 ± 2	20 ± 2	2 ± 0

<sup>a</sup> KH2, wild type; KH2R, *DrecA*; SR100, *ΔumuDC*.

<sup>b</sup> Streptomycin was added to LB cultures inoculated with fresh overnight cell cultures, the cultures were allowed to grow to an optical density at 600 nm of 0.3 to 0.4 (about 100 min at 37°C with aeration), the streptomycin in the medium was removed by centrifugation and washing steps as described in the text, and the cells were processed for transfection.

<sup>c</sup> Values are numbers of infectious centers per transfection (50 ng of ssDNA); data shown are averages of results from three transfections of DNA bearing εC. The parenthetical numbers represent values from a single transfection of control DNA (ssDNA bearing a normal cytosine in place of εC).

<sup>d</sup> Multiplex sequence analysis data shown are averages of results from three to six independent elongation assays; the numbers were rounded to the nearest integer.

in mutagenesis and suggest that exposure to mistranslation-promoting antibiotics may accelerate genetic variability in bacteria.

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