RESEARCH LETTER

Mutation rate is reduced by increased dosage of *mutL* gene in *Escherichia coli* K-12

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Abstract

A variable but substantial proportion of wild *Escherichia coli* isolates present consistently lower mutation frequencies than that found in the ensemble of strains. The genetic mechanisms responsible for the hypo-mutation phenotype are much less known than those involved in hyper-mutation. Changes in *E. coli* mutation frequencies derived from the gene-copy effect of *mutS*, *mutL*, *mutH*, *uvrD*, *mutT*, *mutY*, *mutM*, *mutA*, *dnaE*, *dnaQ*, and *rpoS* are explored. When present in a very high copy number ($\sim$300 copies cell^{-1}), *mutL*, *mutH*, and *mutA* gene copies yielded $\geq$twofold decrease in mutation rates determined by Luria–Delbrück fluctuation tests. Nevertheless, when the copy number was not such high ($\sim$15 copies cell^{-1}), only *mutL* results in a consistent twofold decrease in the mutation rate. This reduction seems to be independent from the RecA background, phase of growth, or from the presence of proficient MutS. An increase in *mutL* gene copies was also able to partially compensate the hypermutator phenotype of a *mutS*-defective *E. coli* derivative.

Introduction

Bacterial mutation frequencies (ratio of mutants/total cells in the population) vary not only among bacterial species but also within species, and even within particular clones (Martinez & Baquero, 2000). Mutation frequencies of a given bacterial species are usually clustered around discrete values (Baquero *et al.*, 2004), with most isolates (frequently named normo-mutators) presenting very similar mutation frequencies that define the modal value for this species. Mutation frequencies lower and higher than this modal value have been observed among natural isolates of the same species, indicating the existence of hyper and hypomutable variants (Matic *et al.*, 1997). It has been suggested that these changes in mutation frequencies might be associated with the lifestyle of the particular strains, and can be influenced by population sizes, frequency of stressful events, and recombinatorial activity (Tenaillon *et al.*, 1999; Oliver *et al.*, 2000; Richardson & Stojiljkovic, 2001; Falush *et al.*, 2006). As different mutation frequencies might influence the different speeds of bacterial adaptation to stressful situations, including host-tissues or antibiotic environments, research on mutation frequencies in natural populations has recently received considerable interest in clinical microbiology and public health (Giraud *et al.*, 2002; Baquero *et al.*, 2004). Strong mutators, which increase the average mutation frequency by 100–1000-fold (Horst *et al.*, 1999), might be prevalent in around 1% of *Escherichia coli* human-associated strains (LeClerc *et al.*, 1996), but this proportion increases if the strains are isolated from clinical environments in which selective pressures are severe. This is the case for *Pseudomonas aeruginosa* strains recovered from the respiratory tract in patients with chronic infections submitted to intensive antibiotic therapy (Oliver *et al.*, 2000; Maciá *et al.*, 2005). Relatively modest but consistent increases in mutation frequency (weak mutators) occur in a high proportion (> 10%) of strains in both *E. coli* and *P. aeruginosa* involved in clinical infections (Baquero *et al.*, 2005).

Much less attention has been paid to the natural strains exhibiting mutation frequencies lower than the modal ones for the species, hypomutators, or antimutators.

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Table 1. Primers and PCR conditions used to amplify the different genes involved in this study

Gene	Primer	Sequence primer (5′ → 3′)	PCR conditions
mutS	*mutS*-F1	CTCCACCTCATTAAGATGTAT	94 °C/12′ (94 °C/1′+57 °C/1′+72 °C/3′) × 35
	mutS-R1	ATTCGTCAGTTGTCGTTAATATT	
mutL	*mutL*-F1	ACCAACAACAGCTGGCAGAA	94 °C/12′ (94 °C/1′+59 °C/1′+72 °C/3′) × 35
	mutL-R1	TACGCAGCTCAATGGCTAAC	
mutH	*mutH*-F1	CGTTGCAGTGTTGCGCAACT	94 °C/12′ (94 °C/1′+58 °C/1′+72 °C/1′) × 35
	mutH-R1	CGATGGCTACTGGATCAGAA	
uvrD	*uvrD*-F1	TCTGTATATATACCCAGCTTT	94 °C/12′ (94 °C/1′+56 °C/1′+72 °C/3′) × 35
	uvrD-R1	GCCTACATGACGTTGCAATT	
mutT	*mutT*-F1	GAGCGCAAAGTAGGACGTAA	94 °C/12′ (94 °C/1′+58 °C/1′+72 °C/1′) × 35
	*mutT-R*1	ACATTCGCACACGATGCCTGA	
mutY	*mutY*-F1	CGTCGCTGTGCTGCAATCT	94 °C/12′ (94 °C/1′+58 °C/1′+72 °C/1′) × 35
	mutY-R1	GATAAAGAGGACGATTTATGAG	
mutM	*mutM*-F1	CGCCAGCACGTGATCTACAAA	94 °C/12′ (94 °C/1′+58 °C/1′+72 °C/1′) × 35
	mutM-R1	ATACCATCCGGCATAAACGCT	
mutA	*mutA*-F1	GATACGCCGAACGACACACCTGGAA	94 °C/12′ (94 °C/1′+50 °C/1′+72 °C/1′) × 30
	mutA-R1	AAAGAAACTTCGCACGGTGAATAGT	
rpoS	*rpoS*-F1	TGCTTGAATGTTCCGTCAA	94 °C/12′ (94 °C/1′+56 °C/1′+72 °C/1′) × 35
	rpoS-R1	TCAGAAAGGCCAGTCTCAAG	
dnaE	*dnaE*-F1	CTGGGACTTGCGTCCTGATTCTT	94 °C/12′ (94 °C/1′+70 °C/4′) × 40
	dnaE-R1	GAACTGGAGTTTGACTAATACAGGAA	
dnaQ	*dnaQ*-F1	AAGCATCTCTGGTAGACTT	94 °C/12′ (94 °C/1′+50 °C/1′+72 °C/2′) × 35
	dnaQ-R1	AATACCTGTGAAAGGCGC	

Unexpectedly, strains with lower-than-modal frequencies were found in 2% of *E. coli* clinical strains, but this proportion can reach 20% among commensal strains (Baquero *et al*., 2004), suggesting that stable environments might select strains presenting low mutation frequencies. Genetic alterations in the genes involved in mismatch repair system genes [methyl-mismatch repair (MMR)] seem to account for most cases of strong mutators in clinical strains (LeClerc *et al*., 1996; Oliver *et al*., 2002; Prunier & Leclercq, 2005), while the mechanisms involved in hypo-mutation remain scarcely explored. It is hypothesize that the increase in efficiency of DNA-repairing systems might reduce bacterial mutation frequencies. To explore this possibility, mutation rates (number of mutants per cell division) and mutation frequencies of different isogenic *E. coli* K-12 strains containing multiple copies of genes involved in the mismatch repair system, in the correction of mutations generated by oxidative damage, and in the generation of error-prone DNA polymerase by mutant mistranslator glyV tRNA (Al Mamun *et al*., 1999; Horst *et al*., 1999) were studied.

Materials and methods

Bacterial strains

The following *E. coli* K-12 strains were used: RYC1000 (*araD139* Δ*lacU169 rpsL* Δ*rib7 thiA gyrA recA56*) (Genilloud *et al*., 1984), DH5α [*supE*44, Δ*lacU*169(ϕ80 *lacZ*ΔM15), *hsdR*17, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*)] (Sambrook *et al*., 1989), MC4100 [F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150* (Str^r) *relA1 flbB5301 deoC1 ptsF25 rbsR*] (Casadaban, 1976), MI1443 (Δ*frdABCD* Δ*ampC recA Sm*), and its isogenic GB20 (MI1443 *mutS*::Tn*10*) (Galán *et al*., 2003).

PCR conditions and construction of plasmids with multiple *mut* copies

The multiple copy effect of genes involved in the MMR system (*mutS*, *mutL*, *mutH*, and *uvrD*), oxidative damage (GO) repair (*mutT*, *mutY*, and *mutM*), replication (*dnaE*, *dnaQ*), *mutA* generation of error-prone polymerase (*glyV*), and the general stress response gene (*rpoS*) was studied. The primers and PCR conditions used to amplify the different *mut* genes are shown in Table 1. The amplified products were purified, cloned in the multicopy plasmid pGEMt-easy (Ap^r), and transformed into DH5α the *E. coli* strains. Recombinant clones were selected in plates containing ampicillin (50 μg mL^{-1}), Xgal (100 μg mL^{-1}), and isopropyl-β-D-thiogalactopyranoside (40 μg mL^{-1}) and then rechecked by sequencing the entire gene to assess that they contained the appropriate insert. The different hybrid plasmids were then transformed into *E. coli* RYC1000 strain. According to customer's information (www.promega.com), the constructions with pGEMt-easy have 300–500 copies cell^{-1}. Those MMR genes yielding a ≥2-fold reduction in the mutation rate were also cloned in pACYC184

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(Tet^R and Cm^R), which has a number of copies ranging from 10 to 20 (Chang & Cohen, 1978).

Estimation of mutation rates

Mutation rates were determined by the modified Luria–Delbrück fluctuation test (Luria & Delbruck, 1943). In short, 600 μL of Luria–Bertani (LB) broth containing $\sim 5 \times 10^2$ bacterial cells from overnight cultures were inoculated into 25 tubes and submitted to overnight incubation at 37 °C with strong shaking. Then, 500 μL of overnight cultures from 20 tubes were mixed in soft agar and plated in LB agar plates containing rifampicin (100 μg mL) and 100 μL of a 10^{-6} dilution from the remaining five tubes were used to estimate the total viable count in drug-free LB plates and were incubated at 37 °C. The number of colonies growing on drug-free and rifampicin agar plates was determined after 24 and 48 h, respectively. This period of time was selected after performing several parallel tests, using well-characterized normo-mutable and hypo-mutable strains to determine whether the number of mutants could eventually increase significantly under extended incubation (until 5 days). The mutation rate (μ) was estimated by means of the Ma-Sandri-Sarkar (MSS)-maximum likehood method, using the MSS algorithm (Sarkar *et al.*, 1992) for estimating the number of mutants (m), and the Luria–Delbrück estimation of mutation rate as $\mu = m/N_t$, N_t being the final number of cells in a culture (Luria & Delbruck, 1943). The recursive equation used in this estimator was calculated using values up to $r = 190$, r being the number of mutants per plate. This guarantees the reliability of the estimations, because it has been established that r values higher that 150 can be lumped into one category, because their contribution to the estimated value of m is very low (Foster, 2006). The MSS-maximum likehood method allows the statistical analysis of the obtained estimations of μ (Stewart, 1994). Using this approach, confidence intervals of the estimated mutation rates were calculated at the 95% level (Stewart, 1994).

Estimation of mutation frequencies

Mutation frequencies were calculated as described previously (Baquero *et al.*, 2004). A 1 : 200 dilution of three different overnight cultures were seeded in LB broth and submitted to gentle agitation at 37 °C. The same experiment was repeated in three different times. The estimated mutation frequency of each strain was the mean of these nine values. The values were considered to be significantly different for P values ≤ 0.05. For estimating mutation frequencies along growth cycle, samples were obtained in the exponential phase (OD = 0.5 at 3:30 h), early stationary phase (OD = 2 at 9 h), and late stationary phase (OD = 1.8 at 24 h).

Results

Multiple-copy effect of candidate genes for hypomutation

The results of the multiple copy effect of genes involved in the MMR, GO, replication, error-prone polymerase, and general stress response on the mutation rate are shown in Table 2. Among genes involved in the MMR system, only the increase in the copy number ($\sim$300 copies cell^{-1}) of *mutL* and *mutH* genes significantly reduced the mutation rate by at least twofold in the RYC1000 strain. Increased dosage of

Table 2. Mutation rates to rifampicin-resistance of *Escherichia coli* strains containing high copy numbers of genes with a potential role in modulating mutations

Repair pathway	Strain	Mutation rate	Confidence intervals*	Ratio†
Controls	RYC1000	6.63×10^{-9}	$(4.64–8.89) \times 10^{-9}$	1.02
	RYC1000 (pGEMT)‡	6.51×10^{-9}	$(4.97–8.28) \times 10^{-9}$	1
MMR	RYC1000 (pGEMT-mutS)	3.65×10^{-9}	$(2.66–4.82) \times 10^{-9}$	0.56
	RYC1000 (pGEMT-mutL)	**3.14** $\times 10^{-9}$	**(1.86–4.68)** $\times 10^{-9}$	**0.48**
	RYC1000 (pGEMT-mutH)	**2.68** $\times 10^{-9}$	**(1.83–3.66)** $\times 10^{-9}$	**0.41**
	RYC1000 (pGEMT-uvrD)	*12.8* $\times 10^{-9}$	*(9.31–16.7)* $\times 10^{-9}$	*1.96*
GO	RYC1000 (pGEMT-mutT)	5.12×10^{-9}	$(3.20–7.38) \times 10^{-9}$	0.78
	RYC1000 (pGEMT-mutY)	6.00×10^{-9}	$(3.65–8.65) \times 10^{-9}$	0.92
	RYC1000 (pGEMT-mutM)	8.68×10^{-9}	$(5.54–12.3) \times 10^{-9}$	1.33
glyV tRNA	**RYC1000 (pGEMT-mutA)**	**2.92** $\times 10^{-9}$	**(2.05–3.92)** $\times 10^{-9}$	**0.44**
Replication	RYC1000 (pGEMT-dnaE)	3.49×10^{-9}	$(2.16–5.05) \times 10^{-9}$	0.53
	RYC1000 (pGEMT-dnaQ)	4.71×10^{-9}	$(3.34–6.23) \times 10^{-9}$	0.72
General stress	*RYC1000 (pGEMT-rpoS)*	*20.5* $\times 10^{-9}$	*(15.9–25.3)* $\times 10^{-9}$	*3.15*

Boldcase: Genes yielding at least a twofold reduction in mutation rates. Italics: genes yielding increases in mutation rates.

*Estimated at the 95% level of confidence as described (Stewart, 1994).

†Mutation rate of control normo-mutator strain/mutation rate of tested strain.

‡Control strain for this experiment.

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genes involved in oxidative damage repair (*mutT*, *mutY*, and *mutM*) did not result in any clear change in the mutation rate. For the other tested genes, only *mutA* yielded a significant twofold decrease in the mutation rate when present in high-copy number. The copy number of the plasmids used in this first screening was very high. To perform a more physiologically sounded analysis, those genes producing a two-fold decrease in the mutation rate were also cloned in the low copy-number ($\sim$15 copies cell^{-1}) plasmid, pACYC184. Meanwhile the control strain, RYC1000 (pACYC184), showed a mutation rate of 6.53×10^{-9} mutants cell^{-1} division (confidence interval at 95% of 5.09×10^{-9}–8.11×10^{-9}); only RYC1000 (pACYC-*mutL*) among the tested strains yielded a twofold reduction in mutation rate (2.41×10^{-9}, with a confidence interval at 95% of 1.69×10^{-9}–3.19×10^{-9}). These results indicate that *mutL* confers a hypomutator phenotype to *E. coli* even when present in low copy number. Thus, further work has been focused on this gene.

It has been stated that a reduced growth rate might produce artifactual results because of different generation numbers during pregrowth of cultures (Drake, 1993). To ascertain this possibility, the growth rates of the different strains, as well as changes in filamentation or clumping were measured. In all assays, the strain *E. coli* RYC1000 (pGEMt-*mutL*) behaved exactly the same as its parental isogenic *E. coli* strains RYC1000 and RYC1000 (pGEMt) containing the cloning vector without any insertion (data not shown). All this indicates that the observed reduction in the mutation rate is not the consequence of any growth defect.

Finally, in accordance with available data (Yang *et al.*, 2004), an increase in the copy number ($\sim$300 copies cell^{-1}) of *rpoS* and *uvrD* genes led to an increase in the mutation rate, statistically significant, of around twofold under the present experimental conditions, yielding weak mutator phenotypes in both cases (Table 2).

Multiple *mutL* gene copies decreases the mutation rate both in RecA$^+$ and RecA$^-$ backgrounds

The results presented in the former section were obtained using a K-12 RecA$^-$ *E. coli* strain, in order to avoid any potential effect on mutation rates due to increased transcription of RecA-regulated error-prone DNA-polymerases (Walker *et al.*, 2000; Cirz *et al.*, 2005). To analyze the effect of *mutL* multicopy in a genetic background closer to that of the wild-type strains, the plasmid pGEMt-*mutL* was introduced into the K-12 RecA$^+$ *E. coli* strain MC4100. Following the introduction of pGEMt-*mutL*, the mutation rate was estimated in comparison with its parental isogenic *E. coli* strains MC4100 and MC4100 (pGEMt). The estimated mutation rate in MC4100 (pGEMt-*mutL*) was 1.15×10^{-9}mutants cell^{-1} division (confidence interval at 95% of 0.75×10^{-9}–1.61×10^{-9}), 5.9-fold lower than its parental strain MC4100 (pGEMt), and 6.81×10^{-9} (confidence interval at 95% of 4.96×10^{-9}–8.85×10^{-9}) and 4.4-fold with respect to MC4100 (5.07×10^{-9} with a confidence interval at 95% of 4.00×10^{-9}–6.27×10^{-9}). This confirms that the effect of a *mutL*-mediated decrease in mutation rates also occurs in a RecA-proficient genetic background.

MutL-derived hypomutation is not dependent on the growth phase

The decrease in mutation rate mediated by multiple *mutL* gene copies might be expected to be particularly relevant at the stationary phase of growth, when *mutL* becomes a limiting factor for MMR (Harris *et al.*, 1997). To check the effect of *mutL* on hypomutation along the cell cycle, estimation of mutation rates is a cumbersome methodology. Thus, in this study the mutation frequencies have been measured (see 'Materials and methods'). The results are shown in Table 3. The mutation frequencies of the RYC1000 (pGEMt-*mutL*) strain were consistently lower ($\geq$3.5-fold,

Table 3. Rifampicin mutation frequency values along the growth phases in a *recA* background

Growth phase	Strains	MF (mean)*	SD†	*P*	Ratio‡
Exponential phase (OD = 0.5)	RYC1000 (pGEMt)§	3.60×10^{-8}	1.27×10^{-8}		
	RYC1000 (pGEM-mutL)	0.98×10^{-8}	0.65×10^{-8}	0.007	0.27
	RYC1000 (pGEM-rpoS)	7.60×10^{-8}	2.77×10^{-8}	NS	2.11
Stationary phase (OD = 2.0)	RYC1000 (pGEMt)	3.63×10^{-8}	0.54×10^{-8}		
	RYC1000 (pGEM-mutL)	0.65×10^{-9}	0.46×10^{-8}	0.05	0.18
	RYC1000 (pGEM-rpoS)	$\mathbf{5.60 \times 10^{-8}}$	2.90×10^{-8}	NS	1.54
Late stationary phase (OD = 1.8)	RYC1000 (pGEMt)	2.90×10^{-8}	1.44×10^{-8}		
	RYC1000 (pGEM-mutL)	0.81×10^{-8}	0.64×10^{-8}	0.01	0.28
	RYC1000 (pGEM-rpoS)	6.00×10^{-8}	1.65×10^{-8}	< 0.0001	2.07

The mutation frequency was measured at exponential phase (at 3:30 h of incubation), early stationary phase (9 h), and late stationary phase (24 h).
*MF, Mutation frequencies were the means of nine independent values (see 'Materials and methods').
†SD.
‡Ratio: mutation rate of control normo-mutator strain/mutation rate of tested strain.
§Control strain for this experiment. NS, not significant.

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$P \leq 0.05$) than that of the control strain throughout all the growth phases, suggesting that MutL could also be limited during all bacterial growth cycles. On the other hand, the effect of *rpoS* in multicopy [RYC1000 (pGEMt-*rpoS*) strain] that has shown a significant increase in the mutation rate (see Table 2) was also analyzed along the growth curve. Although the mutation frequency was always higher than its isogenic strain (Table 3), the differences were only significant in the stationary phase ($P < 0.0001$).

Multiple *mutL* gene copies partially revert a hypermutator phenotype due to a *mutS* defect

Plasmids pGEMt-*mutS*, pGEMt-*mutL*, pGEMt-*mutH*, pGEMt-*uvrD*, pGEMt-*mutT*, and pGEMt-*mutY* were introduced into the MutS-proficient *E. coli* K-12 MI1443, and in its *mutS*$^-$ isogenic derivative, the GB20 strain. The results are shown in Table 4. Only the presence of multiple *mutL* gene copies among all the tested genes (except to *mutS* gene in deficient MutS strain) significantly decreased the mutation frequency ($P < 0.01$) in the *mutS*-proficient (2.5-fold) as well as deficient strain (3.7-fold), suggesting that MutS is not involved in the observed reduction of the mutation frequency due to MutL, at least in those strains.

Discussion

Considerable effort has been devoted during the last years to elucidate the emergence, molecular causes, and impact of hyper-mutation in bacterial adaptation. However, the extent and mechanisms related to the hypo-mutation phenotype have received little attention. The first reported hypo-mutable organism, a variant of bacteriophage T4 carrying a mutation in the DNA polymerase, was studied in 1969 (Drake *et al.*, 1969). The first hypo-mutable 'mud' strain in *E. coli* was described in 1977 (Geiger & Speyer, 1977), but it has been suggested that 'mud' hypo-mutation was only apparent and dependent on the delayed appearance of mutants (Schaaper & Dunn, 2001). In 1985, during a direct search for hypo-mutable *E. coli*, eleven different variants with reduced spontaneous mutability (reduced papillation on lactose plates containing 2-aminopurine) were found, mapping at ten different sites around the bacterial chromosome (Quiñones & Piechocki, 1985). As the loss of MMR genes' expression generates stable mutator phenotypes, it was suspected that the increase in some of these might eventually confer hypo-mutable phenotypes, particularly those expressed in limiting amounts under natural conditions or during certain phases of the growth cycle (Tsui *et al.*, 1996; Harris *et al.*, 1997).

The results of the present study indicate that an increased number of *mutL* gene copies encoding the *MutL* protein results in a small but consistent decrease in mutation rates. This reduction seems to be independent of the *RecA* background, phase of growth, or the presence of proficient *MutS*. The expected increase in the MutL protein was mediated in the present experiments by multiple *mutL* gene copies derived from multicopy plasmids, and such a mechanism

Table 4. Effect of the presence of high copy number of different genes involved in DNA-damage repair on the alleviation of the strong mutator phenotype displayed by a *mutS*-deficient *Escherichia coli* (GB20)

Strains	Plasmid	MF (mean)*	SD†	*P*	Ratio‡
GB20	pGEMt	1.38×10^{-6}	2.35×10^{-7}		
	pGEM-mutS	$\underline{2.34 \times 10^{-8}}$	1.10×10^{-8}	< 0.0001	0.01
	pGEM-mutL	$\mathbf{0.36 \times 10^{-6}}$	1.00×10^{-7}	0.01	0.26
	pGEM-mutH	1.03×10^{-6}	1.83×10^{-7}	NS	0.44
	pGEM-uvrD	1.56×10^{-6}	1.82×10^{-7}	NS	0.67
	pGEM-mutT	1.27×10^{-6}	7.42×10^{-7}	NS	0.54
	pGEM-mutY	1.18×10^{-6}	1.77×10^{-7}	NS	0.50
MI1443	pGEMt	1.20×10^{-8}	1.05×10^{-8}		
	pGEM-mutS	1.00×10^{-8}	1.04×10^{-8}	NS	0.83
	pGEM-mutL	$\mathbf{0.47 \times 10^{-8}}$	2.41×10^{-9}	0.008	0.34
	pGEM-mutH	3.44×10^{-8}	6.54×10^{-10}	NS	2.8
	pGEM-uvrD	1.30×10^{-8}	1.05×10^{-8}	NS	1.08
	pGEM-mutT	ND			
	pGEM-mutY	ND			

Escherichia coli strain MI1443, harboring a wild-type *mutS* gene, was used as control. The underlined result shows the reversion of mutator phenotype in GB20 (MI1443 *mutS*-minus derivative) when a wild-type *mutS* was introduced. The mutation frequencies in GB20 and MI1443 strains with multiple *mutL* gene copies are in bold.

*MF, Mutation frequencies represent the mean of nine independent values (see 'Materials and methods').

†SD.

‡Ratio was calculated as in Table 3.

NS, not significant; ND, not determined.

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seems unlikely to occur in nature. However, other possibilities might be investigated that might result in *MutL* overexpression, for instance mutations or insertions increasing the efficiency of the weak σ^{70} and σ^{32} *mutL* promoters. Tsui & Winkler (1994) described an *E. coli* mutant, called rne3071, where the transcription of the *mutL* gene was increased eightfold.

The MMR system seems to require the co-ordinated activity of *MutL*, *MutH*, and *MutS* in a stoichiometric proportion. There are *c.* 113 *MutL* dimers, 135 *MutH* monomers, and 186 *MutS* dimers per cell in stationary growing *E. coli* cultures (Feng *et al.*, 1996). With the precautions derived from the difficulties in these types of quantitative studies, these results might indicate that *MutL*, the protein in the lowest proportion, might constitute the first bottleneck of the system. Interestingly, the present data suggest that increased *MutL* might reduce mutation rates even in the absence of functional *MutS*. *MutL* has an apparently nonspecific DNA-binding activity (Guarné *et al.*, 2004), which may suggest the preservation of a certain *MutS*-independent effect in repair, a question of potentially deep significance for the evolution of the MMR system. In a seminal paper, multicopy-plasmid containing *MutL* was shown to reduce the extremely high mutation rates of *E. coli mutD5* (Schaaper & Radman, 1989). Another possibility is that hyper-expression of *MutL* might reduce mutation rates by enhancing the very short patch (VSP) repair pathway by binding the Vsr endonuclease (Lieb & Bhagwat, 1996; Bhagwat & Lieb, 2002).

The phenomenon of hyper-mutation has quickly attracted the attention of evolutionary biologists. Hypermutable organisms are selected by a hitch-hiking effect with advantageous mutations that are facilitated by enhanced mutation rates, particularly if the population is exposed to consecutive bottlenecks (Mao *et al.*, 1997). The adaptive advantages provided by a hypo-mutable phenotype seem less evident Drake, 1993) but may well exist, as a substantial number of *E. coli* strains (ranging from 2% to 20%, depending on the clinical origin of the strain) display low mutation frequencies (Baquero *et al.*, 2004). Low rates of mutation might be particularly advantageous in complex bacterial communities (as the intestine) where cooperation between populations might be broken by the emergence of noncooperating cheats (Harrison & Bucking, 2007). In fact, there is a significantly higher frequency of hypo-mutators in fecal samples strains from human volunteers than in isolates from blood cultures of infected hospital-based patients (Baquero *et al.*, 2004). This suggests that the hypo-mutation phenotype might be sufficient to optimize the balance between conservation and adaptation in the more stable environments, and thereby prevent the fixation of deleterious alleles (Tanaka *et al.*, 2003). Note that high-density populations of hypo-mutable bacterial strains might still be able to mutate with low frequency and therefore be selected because of the acquired advantageous mutation. Consequently, it is proposed that the misleading term 'antimutator' should be avoided for these variants.

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