Effect of Chromosome Location on Bacterial Mutation Rates

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In previous comparisons of enterobacterial sequences, synonymous substitution rates were higher in genes closer to the replication terminus, suggesting that mutation rates increase with distance from the replication origin. In order to directly test for the effects of chromosomal location on the rates of point mutations, we assayed the reversion rates of two *lacZ* alleles inserted at four positions in the *Salmonella enterica* chromosome. Mutation rates at an intermediate locus were significantly higher than those at loci nearer to and farther from the replication origin. The higher reversion rates at this locus were neither the result of an overall increase in mutation rates produced by the insertion at this location nor a function of the mutations' immediate neighbors, but rather a regional effect. At all loci, regardless of chromosome location, T·A → G·C transversions were more frequent than A·T → G·C transitions during the exponential phase.

Introduction

Models of molecular evolution are commonly based on the assumption that spontaneous mutations arise at random with respect to nucleotide type and position. However, evidence from experimental systems (Benzer 1961; Schaaper and Dunn 1991; Fujii et al. 1999) and from DNA sequence comparisons (Nussinov 1981; Blake, Hess, and Nicholson-Tuell 1992) show that mutation rates vary among sites. For example, A·T → T·A transversions are 80-fold more common than G·C → C·G transversions on an *Escherichia coli* plasmid (MacKay, Han, and Samson 1994), and the same trend is apparent in a mammalian in vitro system (Roberts et al. 1994; Izuta, Roberts, and Kunkel 1995).

Rates of replication errors, which underlie many spontaneous mutations, also vary among sites. Error rates can be affected by the specific bases adjacent to a base; for example, when the nucleotide adjacent to a thymidine is changed from a guanosine to an adenosine, T → G errors decrease more than 30-fold, and T → A errors increase more than 10-fold (Kunkel and Soni 1988). In addition, there are differences in the rates of errors originating on the leading and lagging strands. In a mismatch-repair–deficient strain of *E. coli*, G·C → A·T transitions were four times more frequent, and A·T → G·C transitions were two times less frequent, on the leading strand (Fijalkowska et al. 1998; Maliszewska-Tkaczyk et al. 2000), and errors attributable to misincorporations of dTTP were four times less frequent on the leading strand in the mammalian in vitro system (Roberts et al. 1994).

Rates of point mutations determined from sequence comparisons, as with those determined experimentally, depend on the particular type of mutation (Li, Wu, and Luo 1984), the adjacent nucleotides (Blake, Hess, and Nicholson-Tuell 1992), and the strand location (Lobry 1996; Francino and Ochman 1999). An additional factor influencing mutation rates, as revealed from comparisons of homologous genes of *E. coli* and *S. enterica*, is the distance from the replication origin: for genes of similar codon usage bias, those genes farthest from the origin have approximately twofold higher synonymous substitution rates than those nearest to it (Sharp et al. 1989). This difference may be solely because of the chromosomal position; for example, rates of postreplication recombinational repair may be higher for sequences closer to the origin because these sequences are more likely to be replicated and in higher copy numbers (Sharp et al. 1989). However, because different sets of genes are being compared, the inherent properties of the genes themselves, such as base composition and nearest-neighbor frequencies, might also contribute to variation in the mutation rates with chromosomal position.

To test how chromosomal location affects point mutation rates, we assayed mutation rates of *lacZ* alleles inserted at several positions on the chromosome. By assaying mutations that reside at identical positions within identical genes, but at different genomic locations, we were able to remove any confounding effects that neighboring bases or local base composition may have on mutation rates. We found that mutation rates do vary with chromosomal position, but do not increase with distance from the origin of replication, indicating that other factors underlie the observed trend in synonymous substitution frequencies.

Materials and Methods

Strain Construction and Verification

Strains used for mutation rate assays contain one of two mutant *lacZ* alleles—one reverting by a transition and the other by a transversion—inserted into one of four locations of the *S. enterica* sv. Typhimurium LT2 chromosome (table 1). These strains were derived from those listed in table 2 as follows: F′ episomes containing the mutant *lacZ* alleles were moved into Typhimurium strain TT18300 by conjugation (Zinder 1960) to produce strains TT22260 and TT22265. To add a selectable marker, the *lacA* genes of these strains were replaced with *lacA::Tn10d*(Cm) from Typhimurium strain TT10604 by phage P22 (HT105/1 *int-201*)–mediated transduction (Schmieger 1972), producing strains

Key words: chromosomal location, molecular evolution, *Salmonella enterica*, spontaneous mutations, base substitutions.

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Mol. Biol. Evol. 19(1):85–92. 2002

Table 1
Strains of *S. enterica* sv. Typhimurium Used in Reversion Assays

Strain	Sequence at Residue 461[a]	Reversion[b]	Chromosomal Location (locus)	Distance from Origin[c]
LT2 (wild type)...	GAG (Glu)			
TT22907	TAG (Amber)	T·A → G·C	85.3′ (*ilvA*)	0.4′ R
TT22908	TAG (Amber)	T·A → G·C	86.1′ (*metE*)	1.2′ R
TT22909	TAG (Amber)	T·A → G·C	76.0′ (*envZ*)	8.9′ L
TT22910	TAG (Amber)	T·A → G·C	40.2′ (*treA*)	44.7′ L
TT22911	AAG (Lys)	A·T → G·C	85.3′ (*ilvA*)	0.4′ R
TT22912	AAG (Lys)	A·T → G·C	86.1′ (*metE*)	1.2′ R
TT22913	AAG (Lys)	A·T → G·C	76.0′ (*envZ*)	8.9′ L
TT22914	AAG (Lys)	A·T → G·C	40.2′ (*treA*)	44.7′ L

[a] Coding strand.
[b] Reversion to wild type involves a transversion for strains TT22907, TT22908, TT22909, and TT22910 and a transition for strains TT22911, TT22912, TT22913, and TT22914.
[c] Distances given in minutes clockwise (R) or counterclockwise (L) from the replication origin.

TT22269 and TT22274. The chromosomal integration sites for these *lacZ* constructs were supplied by the Mu*d*P elements of Typhimurium strains TT17165, TT15269, TT15270, TT10604. These elements were converted into Mu*d*F elements, which contain the complete *lac* operon and a kanamycin (*kan*) resistance cassette (Sonti, Keating, and Roth 1993), by transduction from Typhimurium TT12116, yielding strains BGA through BGD. Next, the *lacZlacA::Tn10d*(Cm) constructs of strains TT22269 and TT22274 were transduced into the Mu*d*F elements of strains BGA through BGD, producing Mu*d*F[*lacZ*$^-$*lacA::Tn10d*(Cm)] constructs. Finally, these constructs were transduced into the wild-type background supplied by Typhimurium TR10000.

To confirm that the constructs possessed the appropriate mutations, a 613-bp portion of each mutant *lacZ* allele was amplified by the polymerase chain reaction and sequenced, using primers that span the active site of this gene: 5′ AAA ATC ACC GCC GTA AGC CGA CCA 3′, and 5′ GCT GTT CGC ATT ATC CGA ACC ATC C 3′ (positions 72322 through 72345, and 72935 through 72911, respectively, GenBank sequence U73857). To determine whether the *lac* operons of these strains were inducible, revertants were isolated, grown to stationary phase, and then plated with and without isopropyl-β-D-thiogalactoside (IPTG) on minimal glycerol media supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). All strains produced blue colonies on plates with IPTG, and white or pale blue colonies on plates lacking IPTG.

Mutation Assays

For each strain, four estimates of *lacZ* reversion rates were derived from batches of 15–70 cultures. To make estimates statistically independent, each batch was derived from a different colony, but all cultures within a batch were from the same colony. Cultures were grown in 20 ml of M9 minimal glycerol media (Miller 1992) supplemented with 300 μM amino acids when appropriate. Each culture was inoculated with 2.0 μl of

Table 2
Strains Used in Constructions

Strain[a]	Salient Feature(s)	Chromosomal Genotype (episomal genotype)
E. coli CC101	*lacZ* residue 461 = TAG	*ara*Δ (*lac proB*)$_{XIII}$ (F′ *lacI lacZ proB*$^+$)
E. coli CC106	*lacZ* residue 461 = AAG	*ara*Δ(*lac proB*)$_{XIII}$ (F′ *lacI lacZ proB*$^+$)
Typhimurium BGA.....	*treA::*Mu*d*F	*kan* [Lac$^+$ Cm^S]
Typhimurium BGB.....	*envZ::*Mu*d*F	*kan* [Lac$^+$ Cm^S]
Typhimurium BGC.....	*ilvA::*Mu*d*F	*kan* [Lac$^+$ Cm^S]
Typhimurium BGD.....	*metE::*Mu*d*F	*kan* [Lac$^+$ Cm^S]
Typhimurium TT10604 ..	*lacA::Tn10d*(Cm)	*Cm*
Typhimurium TT12116 ..	Mu*d*F in episome	*nadA* (F′ *152 nadA*$^+$ *zzf*$^-$::Mu*d*F)
Typhimurium TT15269 ..	*ilvA::*Mu*d*P	*Cm*[Lac$^-$ Kan^S]
Typhimurium TT15270 ..	*metE::*Mu*d*P	*Cm*[Lac$^-$ Kan^S]
Typhimurium TT15630 ..	*treA::*Mu*d*P	*Cm*[Lac$^-$ Kan^S]
Typhimurium TT17165 ..	*envZ::*Mu*d*P	*Cm*[Lac$^-$ Kan^S]
Typhimurium TT18300 ..	*ProB*	*proB*
Typhimurium TT22260 ..	*lacZ* residue 461 = TAG	*proB* (F′ *lacI lacZ proB*$^+$)
Typhimurium TT22265 ..	*lacZ* residue 461 = AAG	*proB* (F′ *lacI lacZ proB*$^+$)
Typhimurium TT22269 ..	*lacZ* residue 461 = TAG; Cm^R	*proB* (F′ *lacI lacZlacA::Tn10d*(Cm) *proB*)
Typhimurium TT22274 ..	*lacZ* residue 461 = AAG; Cm^R	*proB* (F′ *lacI lacZlacA::Tn10d*(Cm) *proB*)
Typhimurium TR10000 ..	LT2 background	wild type

[a] *E. coli* strains described in Cupples and Miller (1988) and Typhimurium strains in Benson and Goldman (1992).

an overnight culture grown in Luria-Bertani (LB) medium. (Inocula of this size rarely contain Lac revertants and allow cultures to reach the stationary phase before sampling.) Cultures were incubated at 37°C for 24 h under moderate shaking.

Serial dilutions of one to three cultures per batch were plated on minimal glycerol (0.2%) plates to obtain total cell counts. To enumerate revertants, cultures were centrifuged for 20 min at 3000 *g*, then cells were resuspended in 0.4 ml of M9 salts and plated onto minimal lactose plates (0.2%) supplemented with 100 μM IPTG, 20 μg/ml X-gal, and 0.3 mM amino acids. Blue colonies visible after 3-day incubation at 37°C were scored as revertants.

To reduce the survival of nonrevertants, minimal lactose plates were pretreated overnight with $\sim 10^{10}$ scavengers (*S. enterica* serovar Typhimurium strain 14028s), i.e., Lac$^-$ cells added to consume residual nutrients. Scavenger cells from an overnight culture were resuspended in 1/10 volume M9 salt solution, and 100 μl of the suspension was spread onto each plate.

To test for differences in overall mutation rates among the strains, we assayed for *rpsL* and *rpoB* mutations that confer resistance to 500 μg/ml streptomycin sulfate and 100 μg/ml rifampicin, respectively. Estimates of the rates of the streptomycin-resistant mutations were derived from two or more batches of 17–70 cultures per strain grown and plated as described. Estimates of the rates of mutations to rifampicin resistance for each strain were derived from four batches of 18 cultures grown and plated as follows: tubes containing 0.3 ml LB media were inoculated with approximately 10^6 cells and incubated at 37°C for 6.5 h with moderate shaking. Fifteen cultures per batch were plated directly onto LB-rifampicin plates, and the remaining three were plated to enumerate the numbers of total cells.

Calculation of Mutation Rates

When calculating mutation rates, it is necessary to distinguish between the frequencies of mutants and those of mutations because each early exponential-phase mutation gives rise to several mutants. Furthermore, determination of mutation rates from information about the frequencies of mutants requires mathematical models of all the relevant mutational processes. Standard models for mutations occurring in the exponential phase predict that mutants follow a Luria-Delbrück (LD) distribution (Luria and Delbrück 1943), whereas models for stationary-phase and postplating mutations predict a Poisson distribution. As our procedures yielded cultures that were predominantly, but not exclusively, in the exponential phase, we expected that the mutants followed some particular combined Poisson-LD distribution. The distribution parameters, m, the overall mutation rate and θ, the proportion of the mutations that occurred in the stationary phase and after plating, were estimated for the given batches from the data (to estimate the latter with sufficient accuracy, batches of 45–70 cultures were required).

Data were analyzed using a program made available by P. Gerrish (ftp-t10.lanl.gov). This program calculates the likelihood of distributions based on different values of θ. Because few post–exponential-phase mutations were expected, we assumed that mutations followed a pure LD distribution (θ = 0), unless the best-fitting combined Poisson-LD distribution described the observed distribution much better, as indicated by a likelihood 10-fold greater than that of the pure LD distribution. When the pure LD distribution was rejected, we employed the value of θ that yielded the maximum likelihood. Because the estimates of θ for strains containing the same *lacZ* mutation were similar, we grouped the strains by mutation type and produced one estimate for each group by averaging those derived from two different strains.

Estimates of overall (LD + Poisson) mutations per culture for each batch were generated by a maximum-likelihood method. Batch mutation rates were used in the following formula to estimate the mutation rate of each strain:

$$\ln(\mu_{\text{strain}}) = \left[\sum_{i}^{N} (\ln(m_i/n_i))\right] \Big/ N$$

where μ_{strain} is the strain overall mutation rate (i.e., overall mutations per cell), m_i and n_i are the estimates for overall mutations and for total cells per culture of batch i, respectively, and *N* is the total number of batches. Confidence intervals were calculated assuming that the log-transformed data followed a normal distribution, as suggested by experiments (data not shown) and simulation studies (Stewart 1994).

Estimates of exponential-phase (LD) mutation rates and their confidence intervals were derived using the same procedures. Estimates of LD mutations per culture for each batch were generated by determining the value of this parameter that maximized likelihood, using the value of θ chosen for that strain.

Results

Mutation Rates at Different Locations

To test if chromosomal location affects point mutation rates, rates of reversions were calculated for two *lacZ* alleles inserted at four chromosomal loci. Figure 1 shows reversion rates plotted as a function of mutation type and chromosomal location. Chromosomal location has a statistically significant effect on both exponential-phase (LD) and overall (LD + Poisson) mutation rates (table 3). However, this effect is the result not of an increase in mutation rates with distance from the replication origin, but rather of elevated mutation rates for alleles inserted at an intermediate locus, *envZ*. Rates of T·A → G·C mutations are significantly higher in the *envZ* insert than in those at any other locus tested, for both overall and exponential-phase mutations ($P < 0.01$, Tukey-Kramer test for a posteriori comparisons of means). Although the *treA* locus is over 30 min farther from the replication origin than *envZ*, the mutation rate for the *envZ*-strain is 230% higher. Rates of A·T → G·C mutations are also significantly higher for inserts at the

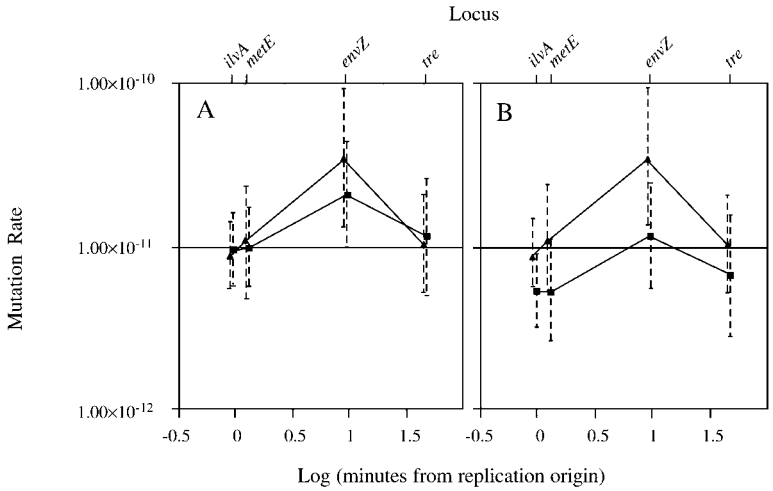

FIG. 1.—Mutation rates at different locations of the *Salmonella* chromosome. Distance from the origin of replication is given as the log of the number of minutes. Overall mutation rates (*A*) and exponential-phase mutation rates (*B*) at each position, calculated for the T·A → G·C transversion (▲) and the A·T → G·C transition (■), are averages of four replicates shown with 95% confidence intervals.

envZ locus than for those at *ilvA* and *metE*. The average rate of this mutation at *envZ* is 62% higher than at *treA*, though this difference is not statistically significant ($P = 0.30$).

Despite differences in mutation rates at certain chromosomal locations, there is no systematic increase in mutation rates with distance from the replication origin. Mutation rates for the *treA* inserts were not significantly different from those of the *ilvA* inserts, or from those of the *metE* inserts, although the distance hypothesis predicts that the mutation rate at *treA* should be about twice that at *ilvA* and should exceed that at *metE* by about 75%. Such differences, given the number of revertants recovered in these experiments, should be readily evident: calculations of power indicate that the differences among mutation rates of 65% or more should achieve significance more than 95% of the time.

Effects of Growth Phase and Mutation Type

The majority of A·T → G·C mutations, but few if any of T·A → G·C mutations, occur after the exponential phase: θ, the frequency of mutations arising postexponentially, is 0.52 for A·T → G·C mutations and 0.00 for T·A → G·C mutations. For strains reverting by A·T → G·C, the likelihood is maximized at $\theta = 0.51$ for a batch of 70 cultures of strain TT22912 and at $\theta = 0.53$ for a batch of 69 cultures of strain TT22911 (fig. 2); θ was 0 for strains reverting by all other mutations tested. The combined Poisson-LD distributions based on these θ values fit the data much better than the pure LD distributions, with likelihood ratios exceeding 22. Conversely, the pure LD distributions fit the data well for strains reverting by T·A → G·C, yielding an estimate for θ of 0 for both a batch of 70 cultures of strain TT22907 and a batch of 48 cultures of strain TT22910. In the former case, the pure LD distribution had the highest likelihood, but in the latter, θ was set to 0 because the likelihood of the optimum combined distribution exceeded that of the pure distribution by only a very small amount. To investigate the reliability of our procedures for estimating θ, we analyzed the distribution of revertant colonies arising on the fourth through the

Table 3
Analysis of Variance (ANOVA) of Reversion Rates as a Function of Location and Mutation Type

Source of Variation	df	Sum of Squares	Mean Squared	F Ratio[a]
Overall reversion rates				
Locus	3	5.76	1.92	13.93***
Mutation type	1	0.09	0.09	0.64
Locus × mutation type	3	0.51	0.17	1.28
Error	24	3.72	0.13	
Exponential-phase reversion rates				
Locus	3	6.02	2.01	13.98***
Mutation type	1	3.81	3.81	26.60***
Locus × mutation type	3	0.49	0.17	1.15
Error	24	3.39	0.14	

[a] F ratios test the degree to which a factor makes a significant contribution to the overall variation; Location × mutation type refers to the interaction effect of these two factors.

*** $P < 0.0001$, otherwise $P > 0.05$.

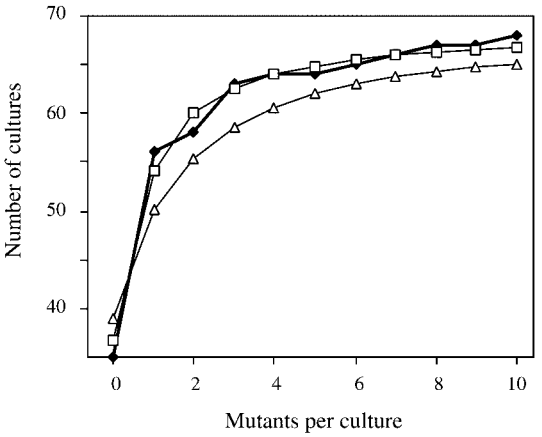

FIG. 2.—Actual versus theoretical distributions of revertants per culture for strain TT22911. Cumulative number of cultures based on experimental data (♦), theoretical LD distribution (△), and combined LD-Poisson distribution (□) are plotted. The LD distribution accounts only for mutations occurring in the exponential phase; the combined distribution accounts for those arising in the exponential phase, in the stationary phase, and after plating. The combined distribution fits the data better: it has a likelihood 32 times greater than that of the pure LD distribution, and yields a higher value for goodness of fit (*G* test; $P = 0.75$ for combined LD-Poisson, 0.18 for pure LD; data are pooled such that the expected number of each category >3).

eighth day of plate incubation, which most likely reflect postplating mutations; as expected, the pure Poisson distribution ($\theta = 1.00$) best fit these data.

As the overall rates of the two mutation types are similar, but the proportion of T·A → G·C mutations occurring in the exponential phase is higher, the exponential-phase mutation rates are significantly higher for the T·A → G·C mutation (fig. 1 and table 3). The higher postexponential rates of the transition can be attributed to a higher stationary-phase mutation rate or a greater production of postplating (adaptive) revertants. To distinguish between these alternatives, we compared the mutation rate of TT22914 cultures grown for 17 h (i.e., not reaching stationary phase) with that of TT22914 cultures grown for 24 h. The Poisson mutation rate of the 17-h cultures was virtually identical to that of the 24-h cultures (data not shown), indicating that post–exponential-phase mutations are predominantly caused by postplating mutations.

Discussion

To test for the effects of chromosomal location and mutation type on the mutation rate, we assayed the reversion rates of two *lacZ* alleles inserted at four positions in the *S. enterica* chromosome. Reversion rates for inserts at a locus 8.6′ from the replication origin, *envZ*, were significantly higher than those at loci nearer to and farther from it. Although it was originally observed that the evolutionary rates of synonymous substitutions increased with distance from the replication origin (Sharp et al. 1989), T·A → G·C mutation rates for the insert at *treA*, which is very close to the replication terminus, are significantly lower than those for inserts at *envZ* and similar to those at two loci close to the origin. Moreover, the same pattern was observed for the rates of A·T → G·C mutations at different locations.

The higher reversion rates of alleles inserted at the *envZ* locus could reflect either a genome-wide increase in mutation rates caused by the inactivation of *envZ* or a locally elevated mutation rate at *envZ*. These alternatives were distinguished by determining the rates of streptomycin- and rifampicin-resistance mutations, which arise via mutations within the *rpsL* and the *rpoB* genes, respectively (Jin and Gross 1988; Lisitsyn, Monastyrskaya, and Sverdlov 1988; Timms et al. 1992; Ito et al. 1994), in each of the *lacZ* strains. Neither of the mutations conferring antibiotic resistance occurred at a significantly higher rate in strains harboring inserts at the *envZ* locus (table 4), although power calculations indicate differences equal to or greater than 50% and 30%, respectively, which would have been significant at the 5% level. Thus, the increase in reversion rates in these strains was specific to the inserts at *envZ*.

The elevated reversion rates of mutations of inserts situated within *envZ* cannot be caused by their immediate nucleotide neighbors because they are located within the same inserted construct as are the other mutations: sequences of the 224 bases downstream and the 385 bases upstream of all mutations subject to reversion

Table 4
Control Mutation Rates as a Function of Chromosome Location

Strain(s)[a]	Construct Location	Mutant Type	Mutation Rate (N)[b]	LN (Mutation Rate)[c]	*P*[d]
TT22907 and TT22911 . .	*ilvA*	$Strp^R$	1.16×10^{-11} (4)	−25.17 ± 0.18	0.48
TT22908 and TT22912 . .	*metE*	$Strp^R$	1.97×10^{-11} (6)	−24.65 ± 0.37	0.82
TT22909 and TT22913 . .	*envZ*	$Strp^R$	1.64×10^{-11} (6)	−24.83 ± 0.51	NA
TT22910 and TT22914 . .	*treA*	$Strp^R$	1.15×10^{-11} (5)	−25.18 ± 0.40	0.40
TT22909.	*envZ*	Rif^R	3.28×10^{-9} (4)	−19.53 ± 0.34	NA
TT22913.	*envZ*	Rif^R	3.24×10^{-9} (4)	−19.55 ± 0.39	NA
TT22910.	*treA*	Rif^R	3.08×10^{-9} (4)	−19.60 ± 0.20	0.99
TT22914.	*treA*	Rif^R	3.32×10^{-9} (4)	−19.53 ± 0.52	0.99

[a] Streptomycin mutation rates pooled for strains with constructs at the same locus.
[b] Confidence intervals are not shown because they are asymmetric.
[c] Mean ± 95% confidence interval.
[d] Comparisons between LN (mutation rate) of the given strains versus that of the corresponding *envZ* strain(s) using the Tukey-Kramer test.

assays are identical. Therefore, the elevated rate is a regional effect, caused either by sequences not immediately adjacent to the mutation site or by the relative position of this site with respect to other chromosomal locations. Moreover, in strains of *E. coli* harboring *lac* operons inserted at one of the nine chromosomal positions, there was a regional elevation in the mutagen-induced frequency of Lac^- mutants at 58′ and 60′ (Van Brunt and Edlin 1975). This regional effect is not apparent from the sequence comparisons: the rates of divergence at synonymous sites of the *envZ* gene and the neighboring genes, as calculated from comparisons of *Salmonella* and *E. coli* homologs, are no higher than average ($K_s = 0.85$). A local elevation in mutation rates could occur if mismatch repair was less efficient in the region, which might be associated, for example, with a relative lack of Dam methylation sites.

Whereas mutation rates are influenced by the orientation of a gene (Fijalkowska et al. 1998), this does not contribute to the variation in mutation rates observed in this study. At each locus, the orientation of the inserted constructs, the disrupted gene, and the leading-strand replication all coincide. Although it is also possible that the increased mutation rates observed in the inserts at the *envZ* locus were caused by elevated transcription from the disrupted gene's promoter, this hypothesis is inconsistent with other data. Transcription increases the rate of this A·T → G·C reversion over twofold more than that of the T·A → G·C reversion (unpublished data). However, the increased rates observed for the *envZ* inserts follow a different pattern, with the former being augmented only about one-third as much as the latter.

Although we detected no systematic increase in mutation rates with distance from the replication origin, the frequency of synonymous substitutions among enterobacterial genes, as determined from sequence comparisons, increases with distance (Sharp et al. 1989). This disparity could be the result of the differences in mutation rates under certain conditions, such as prolonged starvation or anaerobiosis, which might be evolutionarily relevant but not tested in our experiments. Alternatively, the differences in the inherent properties of the genes included in the substitution-rate comparisons might underlie the variation in these rates. The analysis of Sharp et al. (1989) controlled for the effects of codon usage bias, thereby eliminating this factor as the cause of the variation in substitution rates with chromosomal position; however, genes closer to the origin may more often possess other properties associated with more stringent selective constraints or lower mutation rates.

Based on our estimates for θ, T·A → G·C transversions are more frequent than A·T → G·C transitions during the exponential phase, but less frequent in other phases. In the exponential phase, chromosomal T·A → G·C transversions occur approximately twofold more frequently than A·T → G·C transitions, in agreement with the mutation rates determined using plasmids (MacKay, Han, and Samson 1994) and with the analyses of spontaneous mutational spectra (Hutchinson 1996). Because in mismatch-repair–deficient cells, T·A → G·C transversions are generated at a lower frequency than are A·T → G·C transitions (Fujii et al. 1999), these results suggest either that mismatch repair is more efficient at correcting the mismatches that lead to this transition or that DNA repair creates more T·A → G·C than A·T → G·C mutations.

Although half of the A·T → G·C transitions detected in this study were attributed to postplating mutations, none of the T·A → G·C transversions were. The strains reverting by A·T → G·C transitions have higher postplating reversion rates because they are leakier, that is, they produce more wild-type *lacZ* proteins via mis-transcription or mis-translation (Cupples and Miller 1988; Andersson, Slechta, and Roth 1998). In contrast to these results, postplating $T_{\text{coding strand}}\cdot A \rightarrow G_{\text{coding strand}}\cdot C$ mutations were detected using a *hisG428* reversion assay; however, these arose on plates lacking scavengers after prolonged incubation (Prival and Cebula 1992).

The spontaneous mutation rates that we estimated for the *lacZ* reversions on the *S. enterica* chromosome are lower than those previously reported for the same alleles. Reversion rates on an *E. coli* episome are approximately 10-fold higher than those observed in this study (MacKay, Han, and Samson 1994), and the reversion rate of the A·T → G·C transversion on the *E. coli* chromosome is 100-fold higher (Fijalkowska et al. 1998). The differences are consistent with data reporting higher mutation rates in nutrient-rich media (Smith 1992) and lower mutation rates in media that enable cells to skip the mutagenic steps of glycolysis (Lee and Cerami 1990). The low mutation rates that we observed are not the result of plating high numbers of Lac^- cells because the average number of colonies formed on minimal lactose plates by $lacZ^+$ revertants plated in the presence or absence of 10^{10} Lac^- cells was similar (36.2 vs. 33.6 per plate, respectively; difference was not significant by Student's t test: $t = 1.30$, $P = 0.23$, df = 8).

The volume of cultures that we employed in these studies permitted the detection of frequencies of mutants well below the threshold of other protocols. In addition, our methods yielded more accurate mutation rates by providing the following advantages: (1) statistical power was enhanced by using a maximum-likelihood–estimation procedure (Stewart 1994); (2) the confounding effects of post–exponential-phase mutations on the estimates of exponential-phase mutation rates were removed by partitioning the mutations into LD and Poisson fractions; and (3) spurious significant differences, which can arise, for example, when different strains have different carrying capacities, were avoided by the use of multiple batches to estimate the mutation rate of each strain and by enumerating the total cells.

Applying an experimental approach, we have shown that both the chromosomal location and the type of mutation affect the rates of spontaneous point mutations. However, a general increase in mutation rate with distance from the origin of replication, as suggested by sequence divergence of homologous genes in *E. coli* and *S. enterica*, is not supported by our results. Variation in

mutation rates among sites can also be influenced by other factors, such as frequency of transcription and direction of DNA replication (Beletskii and Bhagwat 1996, 1998; Francino and Ochman 1997); therefore, future work will exploit this system to establish the effects of additional factors on the rates of different point mutations.

Acknowledgments

The authors thank Frank Stewart and Phil Gerrish for assistance and for providing computer programs. This research was supported by NIH grants GM 55535 (to H.O.) and GM 27068 to (J.R.R.).

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JULIAN ADAMS, reviewing editor

Accepted September 7, 2001