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# Spontaneous modulation of a dynamic balance between bacterial genomic stability and mutability: roles and molecular mechanisms of the genetic switch

TANG Le[1†], LIU RuoWei[1†], JIN Gang[1], ZHAO ErYing[1], LIU GuiRong[1*] & LIU ShuLin[1,2,3,4*]

[1] *Genomics Research Center, Harbin Medical University, Harbin 150081, China;*
[2] *Department of Biopharmaceutics, Harbin Medical University, Harbin 150081, China;*
[3] *HMU-UCFM Center for Infection and Genomics, Harbin Medical University, Harbin 150081, China;*
[4] *Department of Microbiology and Infectious Diseases, University of Calgary, Calgary T2N 1N4, Canada*

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Bacteria need a high degree of genetic stability to maintain their species identities over long evolutionary times while retaining some mutability to adapt to the changing environment. It is a long unanswered question that how bacteria reconcile these seemingly contradictory biological properties. We hypothesized that certain mechanisms must maintain a dynamic balance between genetic stability and mutability for the survival and evolution of bacterial species. To identify such mechanisms, we analyzed bacterial genomes, focusing on the *Salmonella* mismatch repair (MMR) system. We found that the MMR gene *mutL* functions as a genetic switch through a slipped-strand mispairing mechanism, modulating and maintaining a dynamic balance between genetic stability and mutability during bacterial evolution. This mechanism allows bacteria to maintain their phylogenetic status, while also adapting to changing environments by acquiring novel traits. In this review, we outline the history of research into this genetic switch, from its discovery to the latest findings, and discuss its potential roles in the genomic evolution of bacteria.

**genetic switch, genomic evolution, mismatch repair, comparative genomics, *Salmonella* genome**

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Bacteria are among the earliest cellular life forms on earth, and have required two fundamental features for their long-term survival. The first is high genetic stability. A simple case is the comparison of *Salmonella* species and *Escherichia coli*. These two genera diverged about 150 million years ago [1–3], yet their overall genome structures remain very similar [4]. The other feature is certain mutability or the potential for evolution. A well-known example is the diversification of *Salmonella* into thousands of distinct biological and pathogenic types—to date, more than 2500 *Salmonella* serotypes have been documented [5,6], and much greater diversity may still exist. Theoretically, too great a capacity for mutation would be highly detrimental to the bacteria and, conversely, too little would reduce their adaptability to the changing environment. It is a long unanswered question that how bacteria reconcile these seemingly contradictory biological properties. We hypothesize that certain mechanisms must exist to maintain a dynamic balance between genetic stability and mutability for the survival and evolution of bacterial species. Our recent work indicates that the *Salmonella* mismatch repair (MMR) system functions as a genetic switch, through a slipped-strand mispairing mechanism, which modulates and maintains a

†Contributed equally to this work
*Corresponding author (email: grliu.natsumi@gmail.com; slliu@ucalgary.ca)

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dynamic balance between genetic stability and mutability during bacterial evolution. Here we review the main events that have led to the discovery of this genetic switch and discuss its roles in the genomic evolution of bacteria.

## 1 The diversifying bacterial colony morphology and genome

Bacterial genomes were once believed to be highly stable, and this property is the basis of bacterial classification and identification in the era of genetic and linkage analyses. Physical mapping provided the first molecular evidence of genomic stability, showing that bacterial strains of the same *Salmonella* species or serotype, isolated in different years or locations, have a common genome structure [7,8]. Little was known about the maintenance and modulation of such high levels of genomic conservation. We unexpectedly observed the phenomenon of genomic diversification during the analysis of a *Salmonella* mutator strain, *S. typhimurium* LT7, provided by Dr. Abraham Eisentark of the Cancer Research Center, USA. Dr. Eisentark had stored over 20000 *Salmonella* strains at room temperature for 40 years, including a large number of auxotrophic mutants of *S. typhimurium* LT7. In 1999, he communicated with us to suggest a collaborative study of these bacteria. At that time, we were focusing on comparative genomics of *Salmonella* using physical mapping methods [8–10], which can precisely reveal genomic sizes and structures as well as large-scale changes (inversions, translocations and duplications) [11–13]. We suggested using these methods to explore the genomic changes of the bacterial strains from the Eisenstark lab.

We streaked the bacteria on LB plates and incubated them at 37°C overnight. Seeing colonies of various sizes on the same plate the next morning, we picked single colonies and streaked them onto fresh LB plates to obtain pure cultures of the bacteria. To our surprise, while the LT7 strain stored at −80°C had relatively normal colony morphology, colonies obtained from a single colony each of several LT7 strains kept at room temperature showed variable morphologies. We repeated the streaking several times and observed the same colony-diversifying phenomenon in the LT7 strains stored at room temperature. We categorized the colonies into six groups, based on colony diameter: A, >4 mm; B, 2–4 mm; C, 1–2 mm; D, 0.5–1 mm; E, 0.3–0.5 mm; and F, <0.3 mm. By pulsed-field gel electrophoresis analysis, we observed different cleavage patterns with endonucleases *Xba* I, I-*Ceu* I, *Bln* I, and *Spe* I, and great diversity in the genome structure between the bacteria with different colony morphologies that were grown from the same single colony two days prior. In addition to small mutations, indicated by differences in endonuclease cleavage patterns (Figure 1), structural changes of the genome, including translocations (Figure 2), duplications (Figure 3), and inversions (Figure 4),

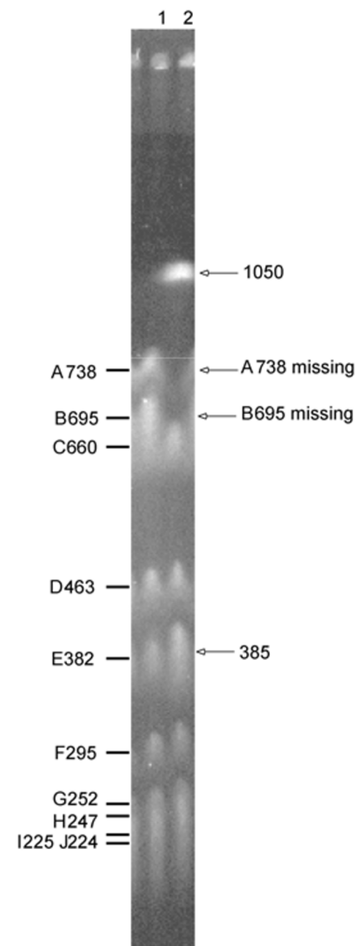


**Figure 1** Point mutations as inferred from the loss of *Xba* I endonuclease cleavage sites. Lane 1, LT7 stored at −80°C; lane 2, LT7 stored at room temperature for over four decades.

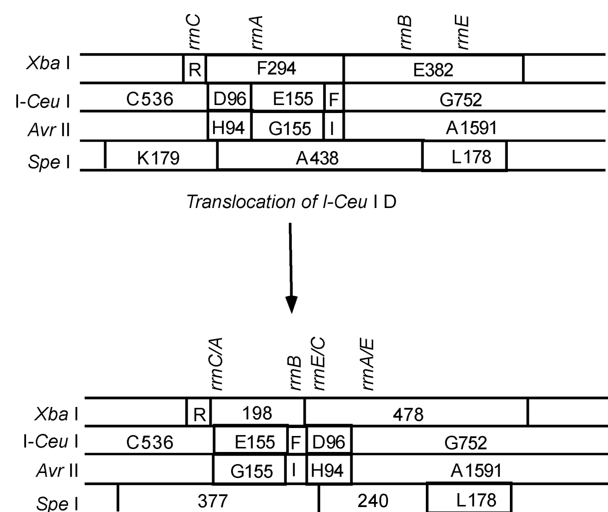


**Figure 2** Genomic translocation. Top, LT7 stored at −80°C; bottom, LT7 stored at room temperature for over four decades.

were observed. Such genomic changes, alone or in combination, made the overall genome structure considerably

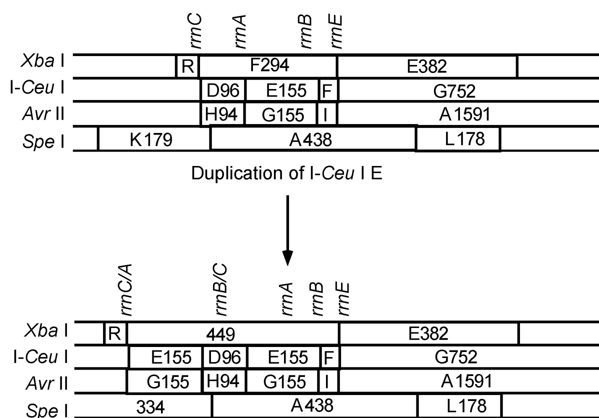


**Figure 3** Genomic duplication. Top, LT7 stored at −80°C; bottom, LT7 stored at room temperature for over four decades.

diverse (Figure 5). Although we reported this phenomenon of genomic diversification [14], we did not understand the genetic basis at that time.

## 2 Genetic basis of genomic diversification

We focused on the genes of the MMR system, as the mutator phenotype of *S. typhimurium* LT7 was previously associated with a gene from this system [15,16]. MMR genes are highly conserved from bacteria to humans, and play a central role in maintaining genetic stability by correcting DNA biosynthetic errors, inhibiting homologous recombination events, and repairing genetic damage [17]. The basic components of the MMR system include MutS, MutL, MutH, UvrD, DNA ligase, exonuclease, and single strand binding protein. We began by analyzing the genes coding for MutS, MutL, and MutH, as these are the main components in the initiation of mismatch repair.

We sequenced *mutL*, *mutS*, and *mutH* from *S. typhimurium* LT7 strains stored at room temperature and compared them with those from the *S. typhimurium* LT7 strain stored at −80°C, which did not show the genome diversification phenomenon. We found that *mutS* and *mutH* were unchanged; however, *mutL* had a 6-bp deletion (GCTGGC). This sequence was found as three tandem repeats in the *S. typhimurium* LT7 strain stored at −80°C, but only two repeats in the strains stored at room temperature (Figure 3). We analyzed the sequences of *mutL* genes from *Salmonella* genomes deposited in GenBank, and all had three copies of the GCTGGC sequence. We reported this finding and designated the defective *mutL* allele 6bpΔ*mutL* [18]. However, we did not know whether this 6-bp deletion was associated with the genome structure diversification phenomenon or

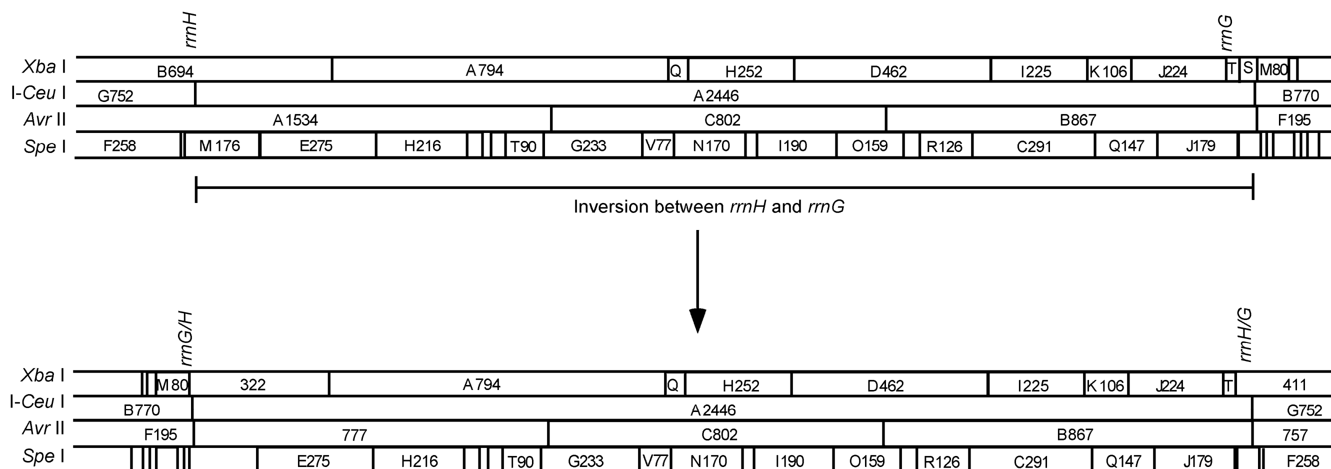


**Figure 4** Genomic inversion. Top, LT7 stored at −80°C; bottom, LT7 stored at room temperature for over four decades.

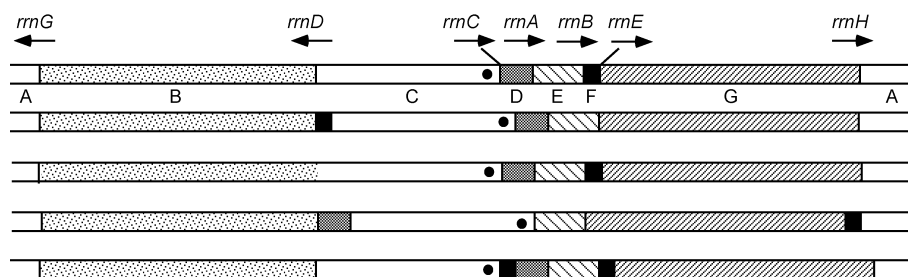


**Figure 5** I-*Ceu* I maps of *S. typhimurium* LT7 to illustrate the diverse genome structures among *S. typhimurium* LT7 mutants. The top map is LT7 stored at −80°C, and the lower maps are four representative LT7 mutants.

**Table 1** Substitutions made in *mutL* to disrupt the 6-bp repeats to test for the genetic switch

| Gene | Location | DNA repeats[a)] | Allele | Substituted bases[b)] | Protein |
|---|---|---|---|---|---|
| | | | *mutL* wild-type | No change | |
| *mutL* | 201–218 | GCTGGCGCTGGCGCTGGCC | $mutL^{Locked\text{-}1}$ | GCT*T*GC*C*CTGGCGCTGGCC | LALALA |
| | | | $mutL^{L1\text{-}UL}$ | GCT*G*GC*G*CTGGCGCTGGCC | |

a) Underlined bases denote a codon. b) Italicized bases denote substituted nucleotides.

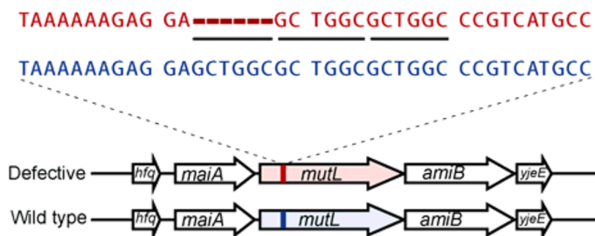


**Figure 6** Comparison of the 6-bp repeats between the wild-type and defective *mutL* gene alleles.

was merely a coincidence.

To answer this question, we first used the *S. typhimurium* LT7 strain stored at −80°C to construct two defective MMR strains with genotypes of 6bpΔ*mutL* and Δ*mutL* (*mutL* gene knocked out completely), and then compared their phenotype with wild-type LT7 stored at –80°C (hereafter called wLT7), focusing on their mutation rates and recombination frequencies. In both the 6bpΔ*mutL* and Δ*mutL* strains, the mutability was elevated more than 100-fold, demonstrating that the 6-bp deletion completely abolished the MutL function. Combined with the findings that all of the strains that exhibited genomic diversification had the same 6bpΔ*mutL* genotype, and the isogenic wLT7 strain had a relatively stable genome, we concluded that the 6bpΔ*mutL* genotype was responsible for the genomic diversification phenomenon in the *S. typhimurium* LT7 strains stored at room temperature [18].

At this point, we asked the fundamental questions of what benefits did the 6bpΔ*mutL* genotype bring the bacteria, and by what mechanisms, over the four decades of storage.

## 3 The genetic switch model of bacterial adaptation and evolution: modulating a dynamic balance between genetic stability and mutability

The findings that 6bpΔ*mutL* may spontaneously convert to the *mutL* genotype, and that the strains undergoing this conversion regained a level of genomic stability similar to that of wLT7, prompted us to postulate that *mutL* was a genetic switch for conversion between the 6bpΔ*mutL* and *mutL* genotypes [18]. We speculated that the three identical repeats may produce errors during replication, gaining or losing one copy of the GCTGGC sequence through slipped-strand mispairing [19]. To test this hypothesis, we converted the 6-bp perfect repeats (GCTGGC) to GCTTGC, CCTGGC, and GCTGGC (the underlined bases are modified) through molecular manipulation. Although the modified sequence still encoded the amino acids Leu-Ala-Leu-Ala-Leu-Ala (Table 1), the three repeats were no longer identical and hence should inhibit slipped-strand mispairing. We called this experimental process "locking the genetic switch". When the genetic switch was "locked", the genomic stability remained high and mutability was minimized, considerably limiting genomic diversification events, with much lowered genomic mutation rates and recombination frequencies [20,21].

## 4 DNA repeat sequences within MMR genes in other bacterial species

Previous work demonstrated similar changes in *mutL* repeats in *E. coli*, such as changes in 6-bp repeats in *E. coli* B [22]. The reported repeat sequence in *E. coli* was CTGGCGGTGGCGGCGGCG, which is seemingly different from the $(GCTGGC)_3$ perfect repeats in *Salmonella*. However, when the upstream G was counted, the sequence would be GCTGGCGGTGGCGGCGGCG, which is identical to that in *Salmonella*. Interestingly, although both of the 6-bp repeat sequences in *E. coli* and *S. typhimurium* LT7 code for the amino acid sequence Leu-Ala-Leu-Ala-Leu-Ala, the last Ala is encoded by GCG in *E. coli* but GCC in *S. typhimurium* LT7. According to a computer simulation of the MutL molecular model, the region of the protein that this 6-bp repeat corresponds to is in the top of the ATP-binding pocket. Thus, deletion of one 6-bp repeat would delete one of the three Leu-Ala dipeptides and abolish the ATPase activity.

In addition to *Salmonella* and *E. coli*, the genome sequences of *Shigella* species published in GenBank contain these 6-bp repeats in the *mutL* gene. We also analyzed the genomic sequences of other bacteria and found that, although the 6-bp *mutL* repeats are not highly conserved, many species have short repeats in other MMR genes such as *mutS* [20,21]. Some of these repeat sequences might also act as genetic switches, and further investigation may provide insights into bacterial evolution and, especially, the acquisition of virulence, drug resistance, or novel evolutionary pathways.

## 5 Prospects

Bacterial evolution is driven by genetic novelty through lateral gene transfer and accumulation of point mutations. Although mechanisms such as DNA mismatch repair would inhibit and minimize such processes, genomic diversification events do occur. The mechanisms to establish and maintain a dynamic balance between genomic stability and mutability have been largely unstudied to date. Our work demonstrates the existence of molecular mechanisms that work as genetic switches, turning on and off spontaneously to modulate the genomic status for or against genetic changes. Future studies to determine to what extent the genetic switch benefits the bacteria are necessary. To this end, we are conducting competition experiments using the wild-type strain and strains with a locked genetic switch to determine whether an active genetic switch contributes to survival in challenging environments (antibiotics, starvation, etc.) or establishing host infection. Based on the genetic switch model, we predict that pathogenic organisms would require transient MMR "off" periods to gain novel traits or allow genomic changes that lead to adaptation. If the genetic switch was locked and genomic changes therefore could not take place, the pathogens would have much reduced adaptability compared to isogenic bacteria with wild-type MMR genes.

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1 Ochman H, Wilson AC. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J Mol Evol, 1987, 26: 74–86

2 Doolittle RF, Feng DF, Tsang S, Cho G, Little E. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science, 1996, 271: 470–477

3 Feng DF, Cho G, Doolittle RF. Determining divergence times with a protein clock: update and reevaluation. Proc Natl Acad Sci USA, 1997, 94: 13028–13033

4 Liu SL, Hessel A, Sanderson KE. Genomic mapping with I-*Ceu* I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc Natl Acad Sci USA, 1993, 90: 6874–6878

5 Popoff MY, Bockemuhl J, Gheesling LL. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol, 2004, 155: 568–570

6 Popoff MY, Le Minor LE. Genus xxxiii. *Salmonella*. In: Brenner DJ, Krieg NR, Stanley JT, eds. Bergey's Mannual of Systematic Bacteriology. Springer, 2005. 764–799

7 Liu SL, Sanderson KE. I-*Ceu* I reveals conservation of the genome of independent strains of *Salmonella typhimurium*. J Bacteriol, 1995, 177: 3355–3357

8 Liu SL, Schryvers AB, Sanderson KE, Johnston RN. Bacterial phylogenetic clusters revealed by genome structure. J Bacteriol, 1999, 181: 6747–6755

9 Liu SL, Sanderson KE. A physical map of the *Salmonella typhimurium* LT2 genome made by using Xbai analysis. J Bacteriol, 1992, 174: 1662–1672

10 Liu SL, Sanderson KE. Homologous recombination between rrn operons rearranges the chromosome in host-specialized species of *Salmonella*. FEMS Microbiol Lett, 1998, 164: 275–281

11 Liu SL, Sanderson KE. Rearrangements in the genome of the bacterium *Salmonella* typhi. Proc Natl Acad Sci USA, 1995, 92: 1018–1022

12 Liu SL, Sanderson KE. Genomic cleavage map of *Salmonella* typhi Ty2. J Bacteriol, 1995, 177: 5099–5107

13 Liu SL, Sanderson KE. Highly plastic chromosomal organization in *Salmonella* typhi. Proc Natl Acad Sci USA, 1996, 93: 10303–10308

14 Liu GR, Edwards K, Eisenstark A, Fu YM, Liu WQ, Sanderson KE, Johnston RN, Liu SL. Genomic diversification among archival strains of *Salmonella* enterica serovar *typhimurium* LT7. J Bacteriol, 2003, 185: 2131–2142

15 Kirchner CE, Rudden MJ. Location of a mutator gene in *Salmonella typhimurium* by cotransduction. J Bacteriol, 1966, 92: 1453–1456

16 Miyake T. Mutator factor in *Salmonella typhimurium*. Genetics, 1960, 45: 11–14

17 Miller JH. Mutators in *Escherichia coli*. Mutat Res, 1998, 409: 99–106

18 Gong J, Liu WQ, Liu GR, Chen F, Li JQ, Xu GM, Wang L, Johnston RN, Eisenstark A, Liu SL. Spontaneous conversion between mutL and 6 bpDeltamutL in *Salmonella typhimurium* LT7: association with genome diversification and possible roles in bacterial adaptation. Genomics, 2007, 90: 542–549

19 Levinson G, Gutman GA. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol, 1987, 4: 203–221

20 Chen F, Liu WQ, Liu ZH, Zou QH, Wang Y, Li YG, Zhou J, Eisenstark A, Johnston RN, Liu GR, Yang BF, Liu SL. MutL as a genetic switch of bacterial mutability: turned on or off through repeat copy number changes. FEMS Microbiol Lett, 2010, 312: 126–132

21 Chen F, Liu WQ, Eisenstark A, Johnston RN, Liu GR, Liu SL. Multiple genetic switches spontaneously modulating bacterial mutability. BMC Evol Biol, 2010, 10: 277

22 Shaver AC, Sniegowski PD. Spontaneously arising mutL mutators in evolving *Escherichia coli* populations are the result of changes in repeat length. J Bacteriol, 2003, 185: 6076–6082

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