Mutators and Long-Term Molecular Evolution of Pathogenic Escherichia coli O157:H7

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It has been proposed that an increased mutation rate (indicated by the frequency of hypermutable isolates) has facilitated the emergence of Escherichia coli O157:H7. Analysis of the divergence of 12 genes shows no evidence that the pathogen has undergone an unusually high rate of mutation and molecular evolution.

Escherichia coli O157:H7, a highly virulent organism first linked to infectious disease in 1982 (1) and now found worldwide, has caused serious foodborne epidemics in the United States, Japan, and Europe (2). One hypothesis for the emergence and rapid spread of this organism is that strong mutator alleles enhance genetic variability and accelerate adaptive evolution (3). LeClerc et al. (3) found that more than 1% of O157:H7 strains had spontaneous rates of mutation that were 1,000-fold higher than those of typical E. coli. These mutator strains were defective in methyl-directed mismatch repair (MMR) as a result of deletions in the intergenic region between the mutS and rpoS genes (3). According to the mutator hypothesis, a pathogen able to enter a transient hypermutable state could overcome the fitness costs of deleterious mutations by accruing new genetic variation at times critical for survival and colonization of new hosts.

Adaptive evolution by transient or prolonged states of hypermutation can cause neutral mutations to rapidly accumulate throughout the genome. To detect possible elevation in the rate of molecular evolution in the emergence of E. coli O157:H7, we compared 12 genes with housekeeping functions (Figure) that have been sequenced in both E. coli O157:H7 and E. coli K-12 (a commensal organism), as well as in an outgroup species, Salmonella enterica serotype Typhimurium. The evolutionary distance (expressed in point mutations per 100 sites) between Typhimurium and K-12 is shown against the distance between Typhimurium and O157:H7 for synonymous and nonsynonymous sites separately (Figure). The line indicates equal rates of evolution in the two

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lineages. An elevated mutation rate in O157:H7 over evolutionary time should result in greater divergence from Typhimurium than from K-12 and in the distribution of points above the equal-rate line. For both synonymous and nonsynonymous sites, most genes fall below or very near the equal-rate line with only two exceptions: tonB and trpA deviate in the direction expected under the mutator hypothesis. To test the significance of these deviations, we compared the observed degrees of divergence of K-12 and O157:H7 from Typhimurium and the expectations of the molecular evolutionary clock hypothesis (21). The basis of this test is that a constant rate of mutation results in equal numbers of substitutions in two sequences from an outgroup (21). Considering synonymous and nonsynonymous changes together with Typhimurium as an outgroup, we found that 11 of the 12 loci, including tonB and trpA deviate in the direction expected under the mutator hypothesis. To test the significance of these deviations, we compared the observed degrees of divergence of K-12 and O157:H7 from Typhimurium and the expectations of the molecular evolutionary clock hypothesis (21). The basis of this test is that a constant rate of mutation results in equal numbers of substitutions in two sequences from an outgroup (21). Considering synonymous and nonsynonymous changes together with Typhimurium as an outgroup, we found that 11 of the 12 loci, including tonB and trpA deviate in the direction expected under the mutator hypothesis. To test the significance of these deviations, we compared the observed degrees of divergence of K-12 and O157:H7 from Typhimurium and the expectations of the molecular evolutionary clock hypothesis (21).

Our findings do not conflict with the observation that MMR defects occur in relatively high frequency in emerging pathogens; however, the findings indicate no evidence of a genomewide elevation of the mutation rate in pathogenic E. coli O157:H7. The uniform rate of divergence of O157:H7 and K-12 suggests several possibilities. One is that the mutator state is transient and so brief that the impact on long-term rates of evolution is undetectable. This possibility is consistent with the view that mutators may generate favorable mutations in periods of intense selection and then revert to a nonmutator phenotype (22,23). Another possibility is that all bacterial populations experience brief episodes of adaptive evolution driven by hypermutation. Matic and co-workers (24) found equivalent frequencies of mutators among strains of commensal bacteria and both emerging and classical pathogenic E. coli.

Finally, defects in MMR that produce the mutator phenotype also relax the normal barriers to recombinational exchange between bacterial species (25). The enhanced recombina-

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References

1. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med 1983;308:681-5.
2. Doyle MP, Zhao T, Meng J, Zhao S. Escherichia coli O157:H7. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington: American Society for Microbiology; 1997. p. 171-91.
3. LeClerc JE, Li B, Payne WL, Cebula TA. High mutation frequencies among Escherichia coli and Salmonella pathogens. Science 1996;274:1208-11.
4. Kumar S, Tamura K, Nei M. MEGA: molecular evolutionary genetics analysis [computer program]. Version 1.0. University Park (PA): The Pennsylvania State University; 1993.
5. Nelson K, Wang F-S, Boyd EF, Selander RK. Size and sequence polymorphism in the isocitrate dehydrogenase kinase/phosphatase gene (aceK) and flanking regions in Salmonella enterica and Escherichia coli. Genetics 1997;147:1509-20.
6. Hall BG, Sharp PM. Molecular population genetics of Escherichia coli: DNA sequence diversity at the celC, crf, and gutB loci of natural isolates. Mol Biol Evol 1992;9:654-65.
7. Nelson SO, Schuitema AR, Benne R, van der Ploeg LH, Plijter JS, Aan F, et al. Molecular cloning, sequencing, and expression of the cfr gene: the structural gene for IIIGlc of the bacterial PEP:glucose phosphotransferase system. EMBOJ 1984;3:1587-93.
8. Saffen DW, Presper KA, Doering TL, Roseman S. Sugar transport by the bacterial phosphotransferase system. Molecular cloning and structural analysis of the Escherichia coli ptsH, ptsI, and cfr genes. J Biol Chem 1987;262:16241-53.
9. Li J, Nelson K, McWhorter AC, Whittam TS, Selander RK. Recombinational basis of serovar diversity in *Salmonella enterica*. Proc Natl Acad Sci U S A 1994;91:2552-6.

10. Nelson K, Whittam TS, Selander RK. Nucleotide polymorphism and evolution in the glyceraldehyde-3-phosphate dehydrogenase gene (gapA) in natural populations of *Salmonella* and *Escherichia coli*. Proc Natl Acad Sci U S A 1991;88:6667-71.

11. Wang FS, Whittam TS, Selander RK. Evolutionary genetics of the isocitrate dehydrogenase gene (icd) in *Escherichia coli* and *Salmonella enterica*. J Bacteriol 1997;179:6551-9.

12. Boyd EF, Nelson K, Wang F-S, Whittam TS, Selander RK. Molecular genetic basis of allelic polymorphism in malate dehydrogenase (mdh) in natural populations of *Escherichia coli* and *Salmonella enterica*. Proc Natl Acad Sci U S A 1994;91:1280-4.

13. Haber LT, Pang PP, Sobell DI, Mankovich JA, Walker GC. Nucleotide sequence of the *Salmonella typhimurium mutS* gene required for mismatch repair: homology of MutS and HexA of *Streptococcus pneumoniae*. J Bacteriol 1988;170:197-202.

14. Schlesnog V, Bock A. The *Escherichia coli* fdv gene probably encodes mutS and is located at minute 58.8 adjacent to the hyc-hyp gene cluster. J Bacteriol 1991;173:414-5.

15. Nelson K, Selander RK. Evolutionary genetics of the proline permease gene (putP) and the control region of the proline utilization operon in populations of *Salmonella* and *Escherichia coli*. J Bacteriol 1992;174:6886-95.

16. Hannavy K, Barr GC, Dorman CJ, Adamson J, Mazenger LA, Gallagher MP, et al. TonB protein of *Salmonella typhimurium*. A model for signal transduction between membranes. J Mol Biol 1990;216:897-910.

17. Milkman R. Recombinational exchange among clonal populations. In: Neidhardt FC, Curtiss IR, Ingraham JL, Lin ECC, Low KB, Magasanik B, et al, editors. *Escherichia coli* and *Salmonella*: cellular and molecular biology. 2nd ed. Washington: American Society for Microbiology; 1996. p. 2663-84.

18. Crawford IP, Nichols BP, Yanofsky C. Nucleotide sequence of the trpB gene in *Escherichia coli* and *Salmonella typhimurium*. J Mol Biol 1980;142:489-502.

19. Horowitz H, Van Arsdell J, Platt T. Nucleotide sequence of the trpD and trpC genes of *Salmonella typhimurium*. J Mol Biol 1983;169:775-97.

20. Nichols BP, Yanofsky C. Nucleotide sequences of trpA of *Salmonella typhimurium* and *Escherichia coli*; an evolutionary comparison. Proc Natl Acad Sci U S A 1979;76:5244-8.

21. Tajima F. Simple methods for testing the molecular evolutionary clock hypothesis. Genetics 1993;135:599-607.

22. Rosenberg SM, Thulin C, Harris RS. Transient and heritable mutators in adaptive evolution in the lab and in nature. Genetics 1998;148:1559-66.

23. Taddei F, Radman M, Maynard-Smith J, Toupane B, Gouyon PH, Godelle B. Role of mutator alleles in adaptive evolution. Nature 1997;387:700-2.

24. Matie I, Radman M, Taddei F, Picard B, Doit C, Bingen E, et al. Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. Science 1997;277:1833-4.

25. Rayssiguier C, Thaler DS, Radman M. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. Nature 1989;342:396-401.

26. Whittam TS, McGraw EA, Reid SD. Pathogenic *Escherichia coli* O157:H7: a model for emerging infectious diseases. In: Krause RM, editor. Emerging infections. New York: Academic Press; 1998. p. 163-83.

27. Rodrigues J, Scaletsky ICA, Campos LC, Gomes Tat, Whittam TS, Trabulsi LR. Clonal structure and virulence factors in strains of *Escherichia coli* of the classic serogroup O55. Infect Immun 1996;64:2680-6.