to the gut lumen. Further studies with other mucosal pathogens are needed to determine the general applicability of this protective mechanism.

The mAbs 7D9 and 10C10 inhibited replication of at least two separate murine rotavirus strains (EC and EW; Fig. 1, A and B) and reacted with virtually all other mammalian strains in ELISA (*15*). Immunization with VP6-encoding DNA has also been shown to protect mice from rotavirus challenge in recent studies (*20*). If VP6-specific IgA antibodies with similar protective activity are generated after natural rotavirus infection or vaccination, they are likely to play a role in the heterotypic immunity observed in a variety of vaccine field trials and epidemiologic studies.

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12. Four to six days after oral immunization with infectious wild-type murine rotavirus strains EC, EHP, or EW (*9*), Peyer's patch lymphocytes were obtained from 4-month-old mice and fused with FOX-NY myeloma cells (*21*), as previously described (*22*). Rotavirus-positive clones were identified with an immunohistochemical staining procedure and were isolated and characterized as previously described (*23*). The protein specificity of the clones was determined by ELISA with the use of recombinant baculovirus antigen or solid-phase immunoisolation techniques (or both) using radiolabeled rotavirus-infected cell lysates (*22, 24*). The isotype of the individual mAbs was determined as previously described (*22*), and the molecular weight was determined on non-denaturing PAGE gels as previously described (*25*). In vitro neutralization assays of individual mAbs were carried out as previously described (*23*).
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17. Three nonimmune adult BALB/c mice (6 to 8 weeks old) were fed a 1:2 dilution of ascites containing either mAb 7D9 or 60 (*26*) 1 hour before challenge (10^4 shedding dose 50s of wild-type EC murine rotavirus) and 1 hour after challenge (100 μl per animal). Subsequently, ascites were fed every 8 hours for 8 days. Stool samples were collected and antigen shedding was measured by ELISA.
18. The closed intestinal loop model was as described by J. L. Wolf *et al.* [*Science* **212**, 471 (1981)]. Closed loops of ileum (approximately 1 cm in length) were constructed in anesthetized BALB/c mice (6 to 8 weeks old) by suture ligation. Animals were anesthetized with equal volumes of xylazine (20 mg per kilogram of body weight) and ketamine (100 mg/kg) diluted in sterile physiologic saline (50 μl per animal). Supplemental anesthesia was administered with halothane inhaled via the open drop method. Fifty microliters of the following solutions were injected into the loops of four mice: (i) 25 μl of activated wild-type EC (10^6 shedding dose 50s) plus 25 μl of 7D9 ascites (1:4 dilution); (ii) 25 μl of activated EC plus 25 μl of a cocktail of four high-titer neutralizing IgG antibodies directed at VP4 and VP7 (*27, 28*) (mAbs 4F8, 159, 2G4, and 57-4, each 1:4 diluted); (iii) 25 μl of activated EC plus 25 μl of phosphate-buffered saline (PBS); and (iv) 25 μl of mouse myeloma IgA (undiluted, 1 mg/ml) (Sigma) plus 25 μl of activated EC. After injection, the loops were returned to the abdominal cavity and the abdominal wall was closed. The loops were removed 12 hours later and immunostained for EC with a rabbit hyperimmune serum against rotaviruses (Fig. 4).
19. Twenty-five microliters of activated EC plus 25 μl of PBS were injected into the intestinal loops of a mouse that had received the 7D9 hybridoma transplant 2 weeks before. The loop was removed and immunostained for EC 12 hours after inoculation as described [(*18*) and Fig. 4 legend].
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Asymmetries Generated by Transcription-Coupled Repair in Enterobacterial Genes

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Although certain replication errors occur at different frequencies on each of the complementary strands of DNA, it remains unclear whether this bias is prevalent enough during chromosome replication to affect sequence evolution. Here, nucleotide substitutions in enteric bacteria were examined, and no difference in mutation rates was detected between the leading and lagging strands, but in comparing the coding and noncoding strands, an excess of C→T changes was observed on the coding strand. This asymmetry is best explained by transcription-coupled repair on the noncoding strand. Although the vast majority of mutations are thought to arise from spontaneous errors during replication, this result implicates DNA damage as a substantial source of mutations in the wild.

One of the fundamental assumptions in molecular evolution is that mutations are equally likely at any site of the genome. Evidence indicates that the probability of a nucleotide substitution may depend on positional factors, including the DNA strand on which the nucleotide is located (*1–4*). Because of the complementary and antiparallel nature of the DNA double helix, each strand is replicated in a very different manner. On one strand, the leading strand, replication proceeds continuously, whereas on the other strand, the lagging strand, replication occurs discontinuously by the synthesis and joining of short Okazaki fragments (*5*). Several experimental systems have revealed that the lagging strand undergoes a higher incidence of certain replication errors, such as mismatches induced by an excess of deoxythymidine triphosphate or deoxyguanosine triphosphate during in vitro replication in human cell extracts (*1, 2*) and deletions during plasmid replication in *Escherichia coli* (*3, 4*). On an evolutionary timescale, a consistent strand-bias in the introduction of mutations would strongly affect the patterns of change in DNA sequences, and such an asymmetry would be detected by reconstructing the substitutions that have occurred among homologous regions (*6*).

Not all replication errors are equally frequent. The introduction of a pyrimidine opposite a template pyrimidine is a very rare event relative to other mismatches (*7*); therefore, most transversions, that is, mutations from a purine (R) to a pyrimidine (Y) or vice versa arise through R:R mismatches. Thus, a Y→R transversion on a given strand of DNA results from an R:R mismatch introduced during the synthesis of

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that strand, but an R→Y transversion on that same strand is induced by an R:R mismatch originating on the complementary strand during the previous round of replication. Consequently, by comparing the rates of Y→R and R→Y substitutions among homologous sequences, it is possible to test whether error rates differ significantly for the leading and lagging strands during chromosomal replication in vivo.

In an analysis of intergenic regions from the β-globin complex of primates, Wu and Maeda (6) concluded that a difference existed between Y→R and R→Y substitution rates. However, when the same sequences are analyzed more conservatively by considering only those substitutions that can be unambiguously inferred by parsimony, the asymmetry disappears (8). Eukaryotic sequences are not particularly well suited for such an analysis of mutational asymmetries because the positions of most of the origins of replication along a chromosome are not known, making the distinction between leading and lagging strands impossible (9). However, this problem is eliminated when examining bacterial genomes because their single replication origin allows one to unequivocally establish if a gene is coded on the leading or lagging strand.

We first examined the patterns of substitutions in four genes, *mdh*, *putP*, *gapA*, and *gnd*, in natural strains of *E. coli* and *Salmonella enterica*. Substitutions in these genes were reconstructed along their phylogenies obtained by both neighbor-joining (*10–13*) and parsimony methods (*14, 15*). Frequencies of complementary substitutions along each phylogenetic tree were compared both within each species and for *S. enterica* and *E. coli* together. Figure 1 illustrates the most salient feature of the patterns obtained, which appeared in all genes over both species, and along either type of phylogenetic reconstruction. The only consistent asymmetry detected is between the complementary transitions C→T and G→A, whereas no asymmetries between complementary transversions (Y→R and R→Y) were observed in any gene.

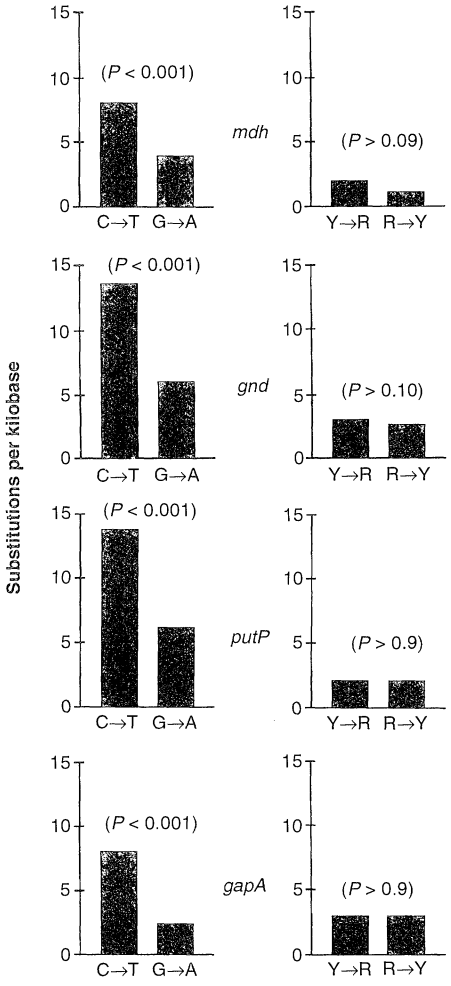

Fig. 1. Comparisons of complementary substitution frequencies (C→T versus G→A and Y→R versus R→Y) in *E. coli* and *S. enterica*. Sequences were obtained from the following sources: *mdh* (*10*), *gnd* (*13*), *putP* (*11*), and *gapA* (*12*). Because mean sequence divergence does not exceed 5% within, and 16% between, species (*30*), sequence alignments were performed manually, and no gaps were introduced. For each gene, only substitutions that could be unambiguously reconstructed were scored on the coding strand, along the most parsimonious phylogenetic reconstructions (*14, 15*). Substitution frequencies were computed as the number of a particular substitution divided by the total number of the original nucleotide in the sequences considered (×1000) and compared by means of χ^2 tests (*8*).

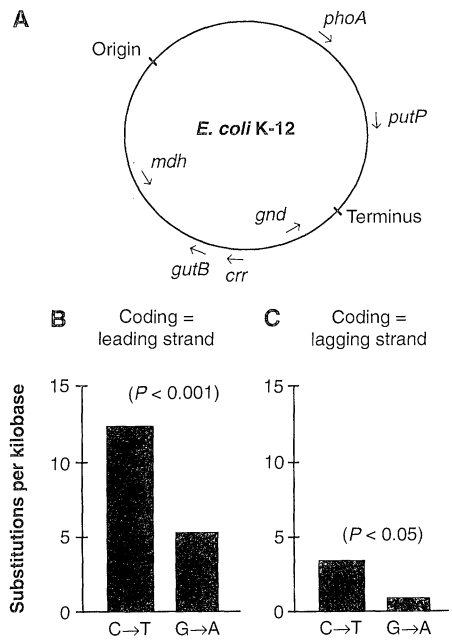

Fig. 2. (**A**) Map position and complementary transition frequencies of genes coded on the (**B**) leading and (**C**) lagging strands of the *E. coli* chromosome. Because replication proceeds bidirectionally from a single origin, the coding strands of *phoA*, *putP*, *gnd*, and *mdh* are replicated as leading strands, whereas those of *crr* and *gutB* are replicated as lagging. Sequences were obtained from the following sources: *phoA* (*31*), *mdh* (*10*), *gnd* (*13*), *putP* (*11*), and *crr* and *gutB* (*27*). For each gene, the mean sequence divergence was <5% and the substitutions were scored as for Fig. 1 (*32*). Total substitution frequencies for each class of genes [those coded on the leading strand (B) and those on the lagging strand (C)] were obtained by summing the numbers of substitutions for each gene within each class and dividing by the total number of the original nucleotide across genes in that class (×1000). Because of sample sizes, complementary transition frequencies were compared by means of χ^2 tests (*8*) for genes on the leading strand and by means of a one-tailed binomial test for those on the lagging strand.

To address the origins of this asymmetry, we analyzed the patterns of substitutions in three additional genes, *phoA*, *crr*, and *gutB*, each of which has been sequenced in numerous strains of *E. coli*. The location and orientation of these genes on the *E. coli* K12 chromosome, as well as those of *gnd*, *mdh*, and *putP*, are shown in Fig. 2. From the map position of these genes, it can be deduced whether their coding strands are synthesized as leading or as lagging strands: *gnd*, *mdh*, *putP*, and *phoA* are coded by the leading strand, and *crr* and *gutB* are coded on the lagging. For all genes, C→T transitions are more frequent than G→A when substitutions are scored on the coding strand (Fig. 2). Therefore, asymmetry is generated by a process that distinguishes between the coding and the noncoding strands of a gene, not on the mode of replication.

Although natural selection could potentially produce asymmetries by favoring certain substitutions over their complementary changes, it is not likely to have generated the observed differences in the rates of C→T and G→A transitions. In that 85 to 90% of the substitutions in each gene were at third-codon positions (where transitional changes will almost invariably result in synonymous substitutions), only selection acting on codon choice could potentially create the detected asymmetry. However, neither the codon preferences in *E. coli* (*16*) nor the rules that predict such preferences (*17*) would result in consistent C→T versus G→A asymmetries. Therefore, these asymmetries must be generated by differences in the occurrence or repair (or both) of C→T or G→A changes on the coding and noncoding strands.

One possibility is that the single-stranded nature of the coding strand while RNA is being synthesized on the noncoding strand makes it prone to DNA damage (*18*). The deamination of C to U (or T if methylated) is over 100 times as fast in single-stranded as in double-stranded DNA (*19, 20*), and our results could rep-

resent an excess of C→U/T deamination events on the coding strand resulting from transcription, paralleling recent experiments in yeast showing increased mutation rates with higher levels of transcription (*21*). However, the exposure of short single-stranded regions during RNA synthesis is probably too transient to account for the level of asymmetry observed between the strands (*18*).

In contrast, the alternative process, transcription-coupled repair, is highly strand-specific and also predicts the observed differences between C→T and G→A substitution frequencies. In *E. coli*, this repair is known to act on ultraviolet-induced pyrimidine dimers and is targeted to the transcribed (that is, noncoding) strand (*22*). Because C→T transitions are the primary mutations induced by pyrimidine dimers (*23*), transcription-coupled repair will result in a deficit of C→T changes on the transcribed strand, which translates into an excess of C→T over G→A changes on the coding strand (*24*), as detected in the genes that we analyzed. If this process is responsible for the observed transitional asymmetry, the fraction of C→T changes at dipyrimidine sites would exceed 70%, the expectation based on the trinucleotide composition of the *E. coli* genome (*25*). Of the loci in Fig. 2, this fraction is above 80% in *putP* and *gutB*, supporting transcription-coupled repair at dipyrimidine sites as the cause of asymmetry; but at *mdh*, *phoA*, and *crr*, this fraction is below 65%. Because transcription-mediated repair systems have been hypothesized to operate on other types of DNA damage, including deamination of C, any C→U/T change on the transcribed strand could be preferentially corrected (*18*, *26*).

If the C→T versus G→A asymmetry is introduced during transcription, we would expect that cryptic genes, which are expressed only occasionally on an evolutionary timescale, would not display such bias. We did not detect any difference between complementary transition rates for the cryptic gene *celC* in *E. coli* (*27*), although the sample size is admittedly small (five C→T changes compared with four G→A changes; $P > 0.5$). A role for transcription-coupled repair in the evolution of enterobacterial genes has two implications regarding the process of mutation: (i) The rates of certain mutations will decline with increasing levels of gene expression, as recently suggested (*28*, *29*), because frequent transcription increases the opportunity for transcription-coupled repair; and (ii) DNA damage, rather than spontaneous replication errors, causes a substantial fraction of naturally occurring mutations. The excess of C→T over G→A substitutions represents nearly 20% of all changes in the genes analyzed, making this a minimum estimate of all naturally occurring mutations attributable to unrepaired DNA damage.

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32. Sequences for *gutB* yielded several equally parsimonious trees with different numbers of C→T substitutions: The frequency of C→T used in Fig. 2 is based on the lowest value, which would serve to underestimate the actual degree of asymmetry.

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Similarity Among the *Drosophila* (6-4)Photolyase, a Human Photolyase Homolog, and the DNA Photolyase–Blue-Light Photoreceptor Family

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Ultraviolet light (UV)–induced DNA damage can be repaired by DNA photolyase in a light-dependent manner. Two types of photolyase are known, one specific for cyclobutane pyrimidine dimers (CPD photolyase) and another specific for pyrimidine (6-4) pyrimidone photoproducts [(6-4)photolyase]. In contrast to the CPD photolyase, which has been detected in a wide variety of organisms, the (6-4)photolyase has been found only in *Drosophila melanogaster*. In the present study a gene encoding the *Drosophila* (6-4)photolyase was cloned, and the deduced amino acid sequence of the product was found to be similar to the CPD photolyase and to the blue-light photoreceptor of plants. A homolog of the *Drosophila* (6-4)photolyase gene was also cloned from human cells.

Cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts [(6-4)photoproducts] are the two major classes of cytotoxic, mutagenic, and carcinogenic photoproducts produced in DNA when cells are irradiated with UV light (*1*–*3*). The phenomenon of photoreactivation—the reduction of lethal and mutagenic effects of UV radiation by simultaneous or subsequent irradiation with near UV or visible light—has been identified in a variety of organisms (*1*, *4*). The enzyme responsible, called DNA photoreactivating enzyme (CPD photolyase), repairs CPDs by reverting them to normal bases using light energy (*4*, *5*). We discovered another type of photolyase in *Drosophila melanogaster* (*6*, *7*) that catalyzes the light-dependent repair of (6-4)photoproducts rather than CPDs. To investigate the mechanism by which the (6-4)photoproducts are repaired, we cloned the *Drosophila* (6-4)photolyase gene.

Escherichia coli normally do not photoreactivate (6-4)photoproducts (*7*) and thus would be expected to show an increased resistance to UV light when engineered to