SOS mutator activity: Unequal mutagenesis on leading and lagging strands

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A major pathway of mutagenesis in *Escherichia coli* is mediated by the inducible SOS response. Current models of SOS mutagenesis invoke the interaction of RecA and UmuD′$_2$C proteins with a stalled DNA replication complex at sites of DNA lesions or poorly extendable terminal mismatches, resulting in an (error-prone) continuation of DNA synthesis. The precise mechanisms of SOS-mediated lesion bypass or mismatch extension are not known. Here, we have studied mutagenesis on the *E. coli* chromosome in *recA730* strains. In *recA730* strains, the SOS system is expressed constitutively, resulting in a spontaneous mutator effect (SOS mutator) because of reduced replication fidelity. We investigated whether during SOS mutator activity replication fidelity might be altered differentially in the leading and lagging strand of replication. Pairs of *recA730* strains were constructed differing in the orientation of the *lac* operon relative to the origin of replication. The strains were also mismatch-repair defective (*mutL*) to facilitate scoring of replication errors. Within each pair, a given *lac* sequence is replicated by the leading-strand machinery in one orientation and by the lagging-strand machinery in the other orientation. Measurements of defined *lac* mutant frequencies in such pairs revealed large differences between the two orientations. Furthermore, in all cases, the frequency bias was the opposite of that seen in normal cells. We suggest that, for the *lacZ* target used in this study, SOS mutator activity operates with very different efficiency in the two strands. Specifically, the lagging strand of replication appears most susceptible to the SOS mutator effect.

In the bacterium *Escherichia coli*, mutagenesis by UV light and many chemical agents is not a passive process but requires the intervention of an active cellular system (the SOS system) that processes the damaged DNA in an error-prone fashion (for review, see refs. 1 and 2). SOS induction occurs when RecA protein binds to regions of single-stranded DNA that are produced as a consequence of DNA replication blockage, for example when DNA polymerase III, which replicates the bacterial chromosome, stalls at the sites of DNA lesions. RecA then undergoes a conformation change that leads to activation of its latent coprotease activity. The RecA coprotease then promotes cleavage of LexA, the repressor of the SOS regulon. On LexA inactivation, some 20–30 proteins are induced, including the genes of the *umuDC* operon, which are essential for SOS mutagenesis (1, 2). RecA also facilitates cleavage of UmuD to produce the mutationally active form, UmuD′. The UmuD′$_2$C complex then acts jointly with RecA protein, which also has a direct role in the process, to create mutations at the sites of DNA lesions (targeted mutagenesis). The UmuD′$_2$C complex has recently been demonstrated to possess a polymerase activity (pol V) (3–6), which likely plays a critical role in producing the mutation. UmuC is an example of a specialized polymerase, a number of which have been reported from a variety of organisms and which are generally thought to function in mutagenesis and DNA damage processing (for reviews, see refs. 7 and 8). Among these is also *E. coli* pol IV (9), the product of the *dinB* gene (9–12), which is also under SOS control (1, 10). The role of this polymerase in SOS mutagenesis is less clear at this time, as most targeted SOS mutagenesis depends on *umuDC* (1, 2) and not *dinB* (10).

One particularly intriguing aspect of SOS mutagenesis is the SOS mutator effect. This spontaneous mutator effect is observed in certain *recA* strains (*recA441*, *recA730*, and others) in which there is a constitutive expression of the SOS system because of the "spontaneous" activation of RecA (13–15). In such strains, increased mutagenesis occurs in the absence of any DNA damaging treatment (untargeted mutagenesis). This mutagenesis, when occurring on the bacterial chromosome or F′ episome, depends, like targeted mutagenesis, on the action of pol V, as the *recA730*- or *recA441*-induced mutator activities are not observed in *umuDC* mutants (16–19). Interestingly, another type of untargeted mutagenesis does not depend on pol V and RecA, but instead on pol IV, the *dinB* gene product (9–12). This *dinB*-mediated form of untargeted mutagenesis has been demonstrated to occur on undamaged bacteriophage λ when infecting UV-irradiated *E. coli* (10). However, Pol IV can also operate on the *E. coli* chromosome, as its overproduction from a plasmid in otherwise normal cells also leads to a mutator phenotype (11, 12).

In a previous study of the *recA730*-induced SOS mutator (20), we presented evidence that this activity does not reflect mutagenesis at endogenous DNA lesions, but rather an increase in base misincorporation errors during ongoing DNA replication. The effect is observed for both transition and transversion errors but is most pronounced for transversions. As transversion mismatches are more difficult to extend and thus more likely to result in DNA polymerase stalling, we suggested that the SOS mutator effect results from the transient stalling of polymerases at those terminal mismatches (20). This stalling provides an entry point for the constitutively present SOS proteins, leading to increased probability of mismatch extension and, hence, mutation. In view of the recent discovery of a polymerase activity for UmuC or UmuD$_2$C (pol V) (3–6), it is likely that this polymerase is responsible for the mismatch extension. However, the exact chain of events leading to mismatch extension, including the precise exchange and interplay between pol III and pol V, remains to be elucidated.

In the present study, we have investigated whether mutations arising through the SOS mutator activity occur with equal efficiency on the leading and the lagging strand of DNA replication. The question of differential replication fidelity within the two strands is a subject of current interest (21–27). A strand preference of the SOS process, if observed, would be of mechanistic importance. Because of the antiparallel nature of the two DNA strands, DNA synthesis at a replication fork is functionally asymmetric (for review, see ref. 28). The leading strand is

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Table 1. Mutant frequencies in SOS-induced pairs of strains containing the *lac* operon in opposite (L and R) orientations on the *E. coli* chromosome

Experiment	*lac* allele (mutation)	Genotype	*lac* orientation	Lac$^+$/10^8	L/R ratio*	Rif^r/10^8
1	CC104	*mutL*	L	3.0	1.9 (0.005)	110
	(G·C→T·A)	*mutL*	R	1.6		90
		recA730, mutL	L	5.0	0.45 (0.0002)	260
		recA730, mutL	R	11		270
2	CC105	*mutL*	L	1.2	4.0 (0.007)	49
	(A·T→T·A)	*mutL*	R	0.3		54
		recA730, mutL	L	6.7	0.08 (<0.0001)	280
		recA730, mutL	R	79		280
3	CC106	*mutL*	L	1.2	0.25 (0.002)	130
	(A·T→G·C)	*mutL*	R	4.8		140
		recA730, mutL	L	18	1.3 (0.90)	590
		recA730, mutL	R	14		610

These experiments were performed with strains of the NR11531 background (see *Materials and Methods*). Although between individual experiments *lac* and rif$^+$ mutant frequencies sometimes vary significantly, the relative mutant frequencies within each experiment are generally very reproducible (see also ref. 27).
*In parentheses, the *P* value indicates significance of L- vs. R-frequency differences, calculated as described in *Materials and Methods.*

synthesized continuously but the lagging strand discontinuously in short Okazaki fragments, 1–2 kb in length. Lagging-strand synthesis is a particularly complicated process that requires the cyclical repetition of several different reactions in a defined temporal sequence. The different enzymology within the two strands may provide a basis for differential fidelity.

To address the question of differential mutability of the two strands, we used an assay system that is based on measurements of mutagenesis of the *lacZ* gene in pairs of near-identical strains. Within a pair, the strains differ only in the orientation of the *lacZ* gene with respect to the origin of DNA replication. Depending on its orientation, a given *lacZ* sequence will be replicated as a leading strand in one orientation and as a lagging strand in the other. Differences in replication fidelity may then be observed through different *lacZ* mutant frequencies for the two orientations. Previously, we used this system to analyze normal replication fidelity by studying several defined *lacZ* reversions in mismatch-repair-defective strains (in which spontaneous mutations reflect uncorrected replication errors) (27). Modest but consistent differences (2- to 6-fold, depending on the mutational marker) were observed between the two orientations, indicating that normal replication fidelity may indeed be different in the two strands (27). In the present study, by using mismatch-repair-defective *recA730* cells, we observe again differences between the two opposing orientations. The differences are larger than observed for normal replication errors and, remarkably, of the opposite bias. We discuss these data with regard to the possible strandedness of the SOS mutator activity.

Materials and Methods

Media. Solid and liquid media were as described (29). Minimal plates were supplemented with 0.4% glucose, 0.4% D-sorbitol, or 0.4% lactose as a carbon source, 5 μg/ml thiamine, and 50 μg/ml of amino acids, if required. Antibiotics were added as follows: tetracycline, 12.5 μg/ml; kanamycin 25 μg/ml; ampicillin, 25 μg/ml; and rifampicin, 100 μg/ml.

Strains. Strains containing the *lac* operon inserted in the chromosomal phage λ attachment site (*attL*) in the two possible orientations, created by the method of Diederich *et al.* (30), have been described (27). These strains have the *lac* operon deleted from its normal location near 8 min on the *E. coli* map (31). Strains EC3126, EC3132, EC3138, EC3144, EC3150, and EC3156 contain the *lac* operon derived from strains CC104, CC105, and CC106 (32) in the L and R orientation, respectively (27).

For the present work, two series of strains were constructed. One, used in the experiments of Table 1, was derived from strain NR11531 (*ara*, *thi*, Δ*prolac*, *sulA366*, *recA730*, *srl*::Tn*10*) (20). This strain was converted to its *rec*$^+$, *srl*$^+$ counterpart by P1 transduction, selecting for growth on minimal medium containing D-sorbitol as carbon source. Then, the *attB*::*lacZ* (Ap) insertions from EC3126, EC3132, EC3138, EC3144, EC3150, and EC3156 (27) were introduced into both the *recA730* and *rec*$^+$ strains by P1 transduction by using the ampicillin resistance conferred by the *attB*::*lacZ* insertion as selective marker. Finally, the *mutL*::Tn*5* mismatch-repair deficiency derived from NR9559 (29) was introduced in each strain by P1 transduction by using selection for kanamycin resistance.

The second series of strains, used in the experiments of Table 2, was derived from the original EC3126, EC3132, EC3138, EC3144, EC3150, and EC3156 series (MC4100 derivatives) (27). These strains made *sulA366* by the two-step procedure described by Fijalkowska *et al.* (20). The *sulA* derivatives were then made *recA730* in another two-step procedure by first making the strains *srl*::Tn*10* and then *srl*$^+$ *recA730* by using NR11239 as P1 donor (20) by selecting on minimal-sorbitol medium. As a last step, the *mutL*::Tn*10* mismatch-repair-deficiency was introduced from strain NR9163 (33) by using tetracycline selection.

Plasmid pRW134, carrying the umuD′C genes, was obtained from R. Woodgate (National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD) (34).

Mutant Frequency Measurements. Mutant frequencies for each indicated strain were determined by starting 20 to 30 cultures (1 ml of LB or minimal medium) from single colonies and growing them to saturation at 37°C with agitation. For each genotype, the colonies were taken from three independent *lac* integrants for each orientation and usually from several independent transductants (*mutL*, *recA730*, etc.) derived from each integrant. Appropriate dilutions of the cultures were plated on minimal-Lac and LB-Rif plates to determine the number of *lac*$^+$ and Rif^r mutants, respectively, and on LB or minimal plates to determine the total cell count. To calculate mutant frequencies, the median number of mutants per plate was determined and divided by median (or average) number of total cells. The nonparametric Mann–Whitney criterion (35) was applied to the

Table 2. Mutant frequencies (*lac*$^+$ mutants per 10^8 cells) in SOS-induced pairs of strains containing the *lac* operon in opposite (L and R) orientations and carrying plasmid pRW134 (*umuD′C*)

Experiment	*lac* allele (mutation)	Relevant genotype	*lac* orientation	Lac$^+$/10^8	L/R ratio	(*P* value)*
1	CC104 (G·C→T·A)	*mutL*	L	0.9	1.2	(0.53)
			R	0.75		
		recA730, mutL	L	4.7	0.36	(0.003)
			R	13		
		mutL [pRW134]	L	1.0	1.7	(0.09)
			R	0.6		
		recA730, mutL [pRW134]	L	7.6	0.18	(<0.0001)
			R	43		
2	CC105 (A·T→T·A)	*mutL*	L	0.5	2.5	(0.02)
			R	0.2		
		recA730, mutL	L	5.3	0.095	(<0.0001)
			R	56		
		mutL [pRW134]	L	0.9	2.3	(0.50)
			R	0.4		
		recA730, mutL [pRW134]	L	17	0.11	(<0.0001)
			R	151		

The experiments were performed with strains of the MC4100 background (see *Materials and Methods*).
*In parentheses, the *P* value indicating the significance of L vs. R frequency differences, calculated as described in *Materials and Methods*).

mutant frequency distributions of the individual cultures for the two compared (L and R) orientations by using the statistical analysis program PRISM (GraphPad, San Diego) to determine the significance of any L and R orientation differences.

Results

We have described an experimental system that permits investigation of the potentially different replication fidelity during leading and lagging DNA synthesis on the *E. coli* chromosome (27). In this system, we determine the reversion frequency of certain defined *lacZ* missense alleles (32) as an indicator of DNA replication fidelity. Pairs of strains are constructed containing the *lacZ* sequence of interest in the two possible orientations in the *E. coli* chromosome with respect to the origin of DNA replication (Fig. 1*A*). In any given pair, a certain *lacZ* sequence is replicated as a leading strand in one orientation but as a lagging strand in the other orientation. Under conditions in which the observed mutant frequency represents the frequency of DNA replication errors, such as in mismatch-repair defective *mutH*, *mutL*, or *mutS* strains, a difference in the mutant frequency between the two orientations indicates differential replication fidelity.

The example in Fig. 1*B* can be used to illustrate the basic logic behind these experiments. The *lacZ* allele of strain CC105 (32), which reverts to *lac*$^+$ specifically by A·T → T·A transversion (32), is presented. A·T → T·A transversions can result from both A·A and T·T mismatches occurring in opposite strands. In the L orientation (defined, arbitrarily, as the case whereby the *lac* operon is transcribed leftward as drawn in Fig. 1), the A·A mismatch will be occurring in the lagging strand and the T·T mismatch in the leading strand, whereas the reverse is true in the R orientation. Although the observed mutant frequency will reflect the sum of the A·A and T·T mispairings, it is generally unlikely that these two events occur at the same, or even similar, rate, and the observed frequency will reflect therefore only the most frequent of the two. If the rate of this most frequent mispair is different in the leading vs. the lagging strand—because of intrinsic fidelity differences between the two strands—the *lac* reversion frequencies for L- and R-oriented strains will be different. Such differences, in the range of 2- to 6-fold, depending on the *lacZ* marker used, were indeed discovered in our previous study of normal replication errors in mismatch-repair-defective strains (27). We also concluded (based on certain assumptions regarding the most frequent mispair) that the majority of mutations resulted from leading strand replication (and that the lagging strand was therefore the more accurate) (27).

To study possible orientation biases in *recA730* strains, we chose three well-defined *lacZ* alleles that are part of a set of six that have been used widely for studies of mutational specificity (32). We used one transition allele (derived from strain CC106, which reverts specifically by A·T → G·C transition) and two transversion alleles (derived from strains CC104 and CC105, which revert specifically by G·C → T·A and A·T → T·A transversion, respectively). These three alleles (when residing on F′*prolac*) were shown previously to be particularly responsive to SOS induction, their corresponding mutant frequencies being increased 6-, 18-, and 55-fold, respectively (20). As SOS-induced replication errors are subject to DNA mismatch repair like normal errors (20, 36), we again conducted the experiments in a mismatch-repair-defective (*mutL*) background, permitting a ready analysis of the observed mutant frequencies in terms of replication error frequencies.

The results of the experiments of Table 1 reiterate those obtained previously with regard to the mismatch-repair-deficient *mutL* control strains. In the case of the G·C → T·A and A·T → T·A alleles, the *lac* mutant frequency is 2- to 4-fold higher for the strain containing the L-oriented *lac* gene, whereas for the A·T → G·C allele, it is about 4-fold higher for the strain with the R-oriented gene. [In contrast, the frequency of rifampicin-resistant mutants is not significantly different within each pair of L- and R-oriented strains, as also shown before (27). This lack of change is as expected, as the target gene for these mutations (*rpoB*) is not subject to inversion.] The bias in favor of the L orientation for the CC104 and CC105 alleles and the R orientation for the CC106 allele was previously interpreted in terms of a lower replication fidelity in the leading strand (27).

Intriguingly, in the corresponding *recA730* strains, the exact opposite strand bias is observed for each of the three cases. This inversion is the apparent result of an unequal mutator effect for the two gene orientations. For example, for the G·C → T·A marker, the strain with the L-oriented *lac* operon is subject to only a modest 1.7-fold SOS mutator effect (5.0/3.0), whereas the strain with the R-oriented operon shows a 6.9-fold (11/1.6)

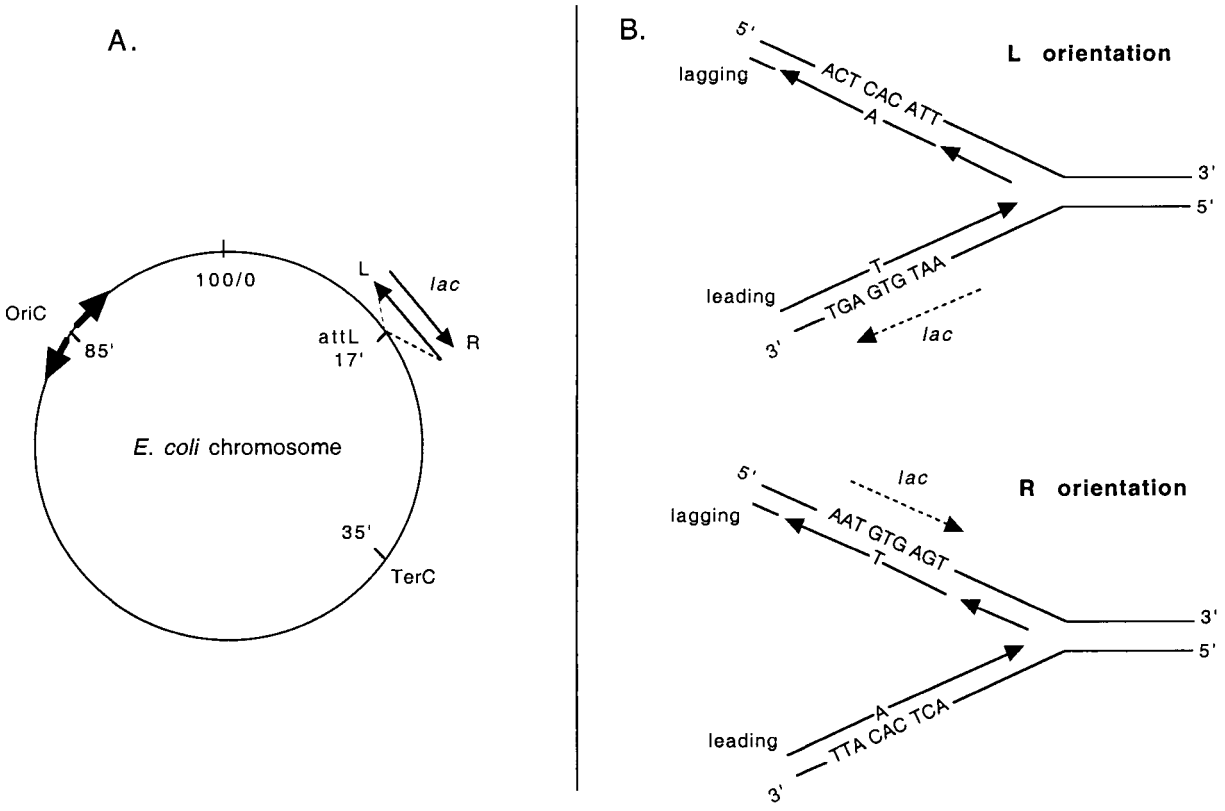

Fig. 1. (*A*) Insertion of the *lac* operon into the *attL* site of the *E. coli* chromosome in two orientations with regard to the chromosomal replication origin *oriC*. The orientation in which the *lac* operon is transcribed in the same direction as the movement of the replication fork through the target is designated as the right (R) orientation, whereas the left (L) orientation indicates *lac* transcription in a direction opposite to the movement of the replication fork. The thick arrows at *oriC* represent the two forks initiated at this site. (*B*) Presented is a more detailed drawing of the replication fork advancing (*Left to Right*) through the *lacZ* target of the CC105 allele that reverts by A·T → T·A transversion (GTG → GAG codon change), along with the potential A·A and T·T mispairs that can cause this transversion in L or R orientations. The dashed arrow indicates the direction of *lac* transcription. The assignment of A·A and T·T mispairs to either leading or lagging strand replication can be deduced as follows. The sequence 5′-AAT-GTG-AGT-3′ (underline, base to be mutated) represents the (+) strand *lacZ* coding sequence for this allele (32). The 5′ → 3′ direction of this sequence is, by necessity, also the direction of transcription. As defined above, in the R orientation (*B*, lower diagram) the direction of transcription has the same direction as the advancing replication fork. As a consequence, the 5′-AAT-GTG-AGT-3′ sequence is copied by the lagging-strand replication machinery. This places the T·T mispair in the lagging strand and the A·A mispair in the leading strand. In the L orientation (*B*, upper diagram) the situation is reversed. Table 4 of reference 27 delineates the corresponding mispairs for the other *lacZ* alleles used. Thus, for the R orientation, the CC104 (G·C→T·A) allele is characterized by $(C{\cdot}T)_{lagging}$ and $(G{\cdot}A)_{leading}$, whereas the CC106 (A·T→G·C) allele is characterized by $(A{\cdot}C)_{lagging}$ and $(T{\cdot}G)_{leading}$ (for each mispair, the template base is stated first).

increase in mutant frequency. As a consequence, the initial 1.9-fold bias in favor of the L orientation becomes a 2.3-fold bias in favor of the R orientation. Even more dramatic results are obtained with the A·T → T·A transversion. SOS induction enhances mutagenesis of the strain carrying the L-oriented *lac* gene by 5.6-fold (6.7/1.2) but enhances mutagenesis of the R-oriented gene by 263-fold (79/0.3). As a consequence, the initial 4-fold bias in favor of the L orientation becomes a 12.5-fold bias in favor of the R orientation. For the A·T → G·C marker (a transition event sensitive to the SOS mutator effect) (20), unequal enhancement of mutagenesis for the two orientations is also observed, again leading to a switch in the L vs. R bias. For the strain with the L-oriented operon, the SOS mutator effect is 15-fold (18/1.2), whereas it is only 2.9-fold (14/4.8) for the strain with the R-oriented operon. The combined data clearly show that the effect of the SOS mutator depends on the orientation of the target gene. Remarkably, for each of the three tested *lac* alleles, the SOS-imposed bias is the opposite of that observed under noninduced conditions. As in the case of normal replication errors, the most straightforward interpretation is likely to be found in the differential operation of the SOS mutator in the leading vs. the lagging strand of replication (see *Discussion*).

Next, we investigated the orientation dependence of the SOS mutator effect after introduction into the strains of plasmid pRW134, a (low-copy) plasmid carrying the *umuD′* and *umuC* genes (34). The UmuD2′C complex is rate limiting for SOS mutagenesis (37), and therefore the magnitude of the SOS mutator effect may be significantly enhanced by the presence of this plasmid. These experiments (see Table 2), performed with the G·C → T·A and A·T → T·A transversion alleles in a slightly different strain background (see *Materials and Methods*), clearly showed that pRW134 enhances the level of SOS-dependent mutations. pRW134-mediated enhancement of *recA730*-induced mutagenesis occurs for both L- and R-oriented strains, with retention of the bias in favor of the R orientation. For the G·C → T·A allele, the bias in favor of the R orientation becomes significantly more pronounced in the presence of the plasmid. Although *recA730* enhances mutagenesis in the L- and R-oriented strains by 5- and 17-fold, respectively, in the presence of pRW134 these factors are 7- and 72-fold, respectively, enhancing the bias in favor of the R orientation to about 6 (43/7.6). For the more readily mutable A·T → T·A allele, *recA730*

enhances mutagenesis in the L- and R-oriented strains by 10- and 280-fold, respectively, increasing to 19- and 380-fold in the pRW134-containing strains. Thus, for this allele, the plasmid enhances the mutability of both orientations approximately equally. In both cases, the bias in favor of the R orientation is about 10-fold.

It is to be noted that the strains used in Tables 1 and 2 differ with regard to the absence or presence of the *dinB* gene. Those of Table 1 carry the (*pro–lac*)X111 deletion, which includes the *dinB* gene (R.M.S., unpublished data), thus making the strains *dinB* defective, whereas the strains of Table 2 are presumed *dinB*$^+$. Thus, although *dinB* is involved in at least one form of untargeted mutagenesis (9–12), its absence in one of our strain series clearly establishes that both the *recA730*-mediated SOS mutator activity and the associated orientation bias are independent from pol IV activity.

Discussion

The mechanisms underlying the *recA730* mutator effect are of interest for both the origin of untargeted mutations and the broader mechanisms of SOS mutagenesis in general. Although SOS mutagenesis in general reflects the error-prone synthesis across a blocking lesion, the *recA730*-mediated SOS mutator effect is thought to be initiated when the polymerase makes a misinsertion error (20). The DNA polymerase complex hesitating or stalling at this lesion becomes the signal for SOS involvement. We have suggested that the SOS mutator reflects the forced extension of such terminal mismatches (20). We initially argued that this forced extension was accomplished by pol III HE under the influence of the SOS proteins, but the discovery of an intrinsic polymerase activity for UmuC or UmuD′$_2$C (pol V) makes it most likely that this enzyme is responsible for the mutagenic extension reaction (3–6). However, it remains likely that misinsertion errors by pol III HE constitute the initiating event in the SOS mutator activity, producing the mismatches that are to be extended by pol V.

Our current observations provide an intriguing new insight into the SOS mutator effect, namely that its mutagenic potential is very unequal between the two chromosomal orientations of the *lacZ* target gene. As argued previously, such inequality between the two orientations is most readily interpreted in terms of differential replication fidelity of the leading and lagging strand (27). Thus, the SOS mutator activity may have defined strand specificity, as further discussed below.

Two aspects of the data are particularly notable. One is that the mutability differences between the L and R orientations for the SOS-induced errors are generally larger than for the normal DNA replication errors. For example, greater than 10-fold L vs. R differences are observed for the SOS-induced A·T → T·A errors (Tables 1 and 2). Secondly, and most importantly, for each of the three *lacZ* alleles tested (CC104, CC105, and CC106), the bias is the opposite of that observed in noninduced cells. The fact that this switch occurs for all three alleles suggests that under SOS conditions a significant change has occurred in the factors that control replication fidelity. There are two possible scenarios that can account for the reversal of the orientation bias in SOS-induced cells. They differ with regard to the precise misinsertion errors that are responsible for the observed mutations and the strand in which these errors occur in the two gene orientations, as discussed in detail below. The arguments relate specific mispairs to the strand in which they occur and do not require an explicit assumption about which enzyme (pol III or pol V) makes the insertion error.

First, it is possible that the mispairing errors that ultimately lead to the observed mutations in normal and SOS-induced cells are not the same and, in fact, are each other's opposites. Thus, for the example of Fig. 1*B*, A·T → T·A transversion mutations might result from T·T errors under normal conditions (27), but from A·A errors under SOS-induced conditions. (Such a switch could occur because pol V instead of pol III might be responsible for the critical misinsertions or because pol III generated A·A mispairs, although less frequent than T·T, would be preferred substrates for extension by pol V.) Within this model, the intrinsic strand bias that operates in normal cells remains in effect under SOS-induced conditions. Mutations arise from the same strand in the two cases (the leading strand in the example of Fig. 1*B*), but the switch from T·T to A·A misinsertions as the basis for the observed mutations converts the L > R bias into a R > L bias. In this model, the SOS mutator effect is a mispair-specific phenomenon that retains (and even amplifies) the strand-specific fidelity bias of normal DNA replication.

In the second possibility, the same mispairing errors that underlie the mutations in normal cells are also responsible for the mutations in SOS-induced cells. Instead, the reversal in L/R ratio simply reflects a switch in the strand from which the mutations arise. Thus, in the example of Fig. 1*B*, mutations in normal cells would result mainly from T·T errors in the leading strand as proposed (27) (promoting the L orientation), but in SOS-induced cells they would result from T·T errors in the lagging strand (promoting the R orientation). In this scenario, the SOS mutator effect is a strand-specific phenomenon.

SOS Mutagenesis Likely Occurs in the Lagging Strand. At this time, we have no direct evidence for distinguishing between the two opposing scenarios described above. However, arguments can be forwarded (see below) that favor the second possibility, namely that the SOS mutator is strand specific and creates mutations preferentially in the opposite strand compared with normal replication errors. Because we have argued that normal replication errors are most frequently generated in the leading strand (27), it follows further that the SOS mutator creates mutations preferentially in the lagging strand. Preferential operation of the SOS mutator in the lagging strand would be consistent with the known enzymology of chromosomal replication and with certain features of the SOS machinery, as discussed below.

First, the clear reversal in L/R bias is observed for each of the three tested *lacZ* alleles (Tables 1 and 2). Such reversal can be explained more simply and parsimoniously by a general switch in strandedness than by a switch in the underlying misinsertion errors, as the latter would have to apply to all three individual sets of complementary mispairs, which is both qualitatively and quantitatively less likely. Second, within the hypothesis that the SOS mutator reflects polV-mediated extension of misinsertions made by pol III (20), an explanation based on the same misinsertions is more easily accommodated than an explanation based on the alternative misinsertions. Third, the requirement for the RecA nucleofilament in the SOS response is more readily satisfied by the lagging strand than by the leading strand. By its very nature, the lagging strand is characterized by the presence of single-stranded DNA, both behind and ahead of the growing Okazaki fragment. These stretches are thought to be covered by single-stranded binding protein (SSB). In contrast, in the leading strand, the polymerase is connected directly via the τ subunit to the helicase-primase complex that motors the opening of the fork, no single-strand DNA is exposed, and SSB is not required (38). The single-stranded DNA in the lagging strand, in particular the stretch that is ahead of the growing Okazaki fragment, would provide a ready entry point for generation of the RecA or RecA/UmuDC nucleofilament (39). Fourth, the lagging-strand polymerase can be assumed to dissociate relatively more readily from its primer terminus than the leading-strand polymerase, because such dissociation is a normal event in the lagging-strand replication when the enzyme reaches the end of an Okazaki fragment. It seems reasonable that the greater dissociability of the lagging-strand polymerase would also apply when synthesis is halted by a mismatch. Thus, the opportunity for pol V to

replace pol III at the 3′ terminus would be greater in the lagging strand. In our view, the above arguments suggest strongly that the enhancement of untargeted mutations in SOS-induced cells results from a disproportionately large increase in replication errors in the lagging strand.

Our conclusion that SOS mutator activity proceeds differentially in the two strands is based on our analysis of three different *lacZ* alleles in three different sequence contexts and establishes the phenomenon for this target. However, whether an identical or similar asymmetry exists for other targets around the *E. coli* chromosome remains to be established.

The SOS Mutator Effect vs. DNA Damage-Induced Mutations. In DNA damage-induced SOS mutagenesis, replication is blocked by a lesion residing in the template strand. Although the precise events following the initial blockage are not clear, whether entailing a simple persistent blockage or a collapse of the replication fork (by dissociation of the replication complex), it seems nevertheless likely that lesions in both leading and lagging strands could promote such events, ultimately leading to SOS-mediated bypass in either strand. Thus, there may be a difference between the transient blocks resulting from polymerase misinsertion errors that become substrates for the SOS mutator effect and the persistent template-strand lesions. This line of thought suggests that SOS mutagenesis occurring at DNA lesions potentially might not show the strand preference as demonstrated here. This question remains to be investigated.

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