Replication slippage is a particular type of error caused by DNA polymerases believed to occur both in bacterial and eukaryotic cells. Previous studies have shown that deletion events can occur in *Escherichia coli* by replication slippage between short duplications and that the main *E. coli* polymerase, DNA polymerase III holoenzyme is prone to such slippage. In this work, we present evidence that the two other DNA polymerases of *E. coli*, DNA polymerase I and DNA polymerase II, as well as polymerases of two phages, T4 (T4 pol) and T7 (T7 pol), undergo slippage *in vitro*, whereas DNA polymerase from another phage, φ29, does not. Furthermore, we have measured the strand displacement activity of the different polymerases tested for slippage in the absence and in the presence of the *E. coli* single-stranded DNA-binding protein (SSB), and we show that: (i) polymerases having a strong strand displacement activity cannot slip (DNA polymerase from φ29); (ii) polymerases devoid of any strand displacement activity slip very efficiently (DNA polymerase II and T4 pol); and (iii) stimulation of the strand displacement activity by *E. coli* SSB (DNA polymerase I and T7 pol), by phagic SSB (T4 pol), or by a mutation that affects the 3′→5′ exonuclease domain (DNA polymerase II exo− and T7 pol exo−) is correlated with the inhibition of slippage. We propose that these observations can be interpreted in terms of a model, for which we have shown that high strand displacement activity of a polymerase diminishes its propensity to slip.

Replication slippage has been widely proposed as a probable mechanism of genome rearrangements, such as deletions between short duplications in bacteria (1–3), yeast (4), and mammalian mitochondria (5) or deletions between long tandem repeats in *Escherichia coli* (6–8), as well as microsatellite instability (for reviews see Refs. 9–12). Direct evidence for the slippage has been obtained *in vivo*, in *E. coli* (13), and *in vitro* (14). In the latter study, it was shown that *E. coli* DNA polymerase III holoenzyme (pol III HE),† the enzyme that replicates the cell chromosome (for review see Ref. 15), was able to slip, which is of particular significance in view of the very high replication accuracy required to maintain the integrity of the genome. In the present work we tested the slippage ability of the two other DNA polymerases from *E. coli*, pol I and pol II, and three phage DNA polymerases from T4, T7, and φ29 to provide insight in the generality of this kind of replication mistakes and in its mechanism.

Pol I is involved in DNA repair and completion of Okazaki fragments (16); it does not usually replicate long stretches of DNA and has been shown previously *in vitro* to cause frameshifts and strand switching (16, 17). Pol I contains several enzymatic activities in a single polypeptide chain; proteolytic cleavage can separate this chain into two active fragments: a large C-terminal fragment (Klenow fragment or pol I KF) carrying polymerase and 3′→5′ exonuclease (proofreading) activities, and a small N-terminal fragment containing 5′→3′ exonuclease activity (16).

For a long time, the role of pol II was not clearly assigned, but recent evidence suggests that it functions during adaptive mutagenesis and translesion DNA synthesis (18, 19). It has also been proposed that pol II might replace pol III HE during replication of the chromosome (20). Pol II is a monomeric enzyme, with polymerase and proofreading activities; unlike pol I, it is able to use the accessory subunits of pol III (the γ complex and the β subunit), which strongly stimulate synthesis by increasing pol II processivity (21, 22).

DNA polymerases from phages T4 and T7 are well characterized enzymes (for reviews see Refs. 23 and 24). In the case of T4 pol, we studied the action of the catalytic polymerase subunit alone (the gene 43 product, or gp43), which has proofreading activity, and refer to it in this study as T4 pol. The complete holoenzyme contains in addition three accessory proteins and a specific single-stranded DNA-binding protein (the gene 32

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† To whom correspondence should be addressed. Tel.: 33-1-34-65-25-12; Fax: 33-1-34-65-25-21; E-mail: cancelle@biotec.jouy.inra.fr.
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† The abbreviations used are: pol III HE, DNA polymerase III holoenzyme from *E. coli*; pol II, DNA polymerase II from *E. coli*; pol I, DNA polymerase I from *E. coli*; pol I KF, the Klenow fragment of pol I; T4 pol, DNA polymerase from phage T4; T7 pol, DNA polymerase from phage T7; φ29 pol, DNA polymerase from phage φ29; exo−, exonuclease-deficient; SSB, single-stranded DNA-binding protein; ssDNA, single-stranded DNA; gp32, gene 32 protein from phage T4; gp43, gene 43 protein from phage T4; gp5, gene 5 protein from phage T7; gp2.5, gene 2.5 protein from phage T7; p5, gene 5 protein from phage φ29; DTT, dithiothreitol; BSA, bovine serum albumin; bp, base pairs.

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product, or gp32). T7 pol is a highly processive enzyme constituted of 2 subunits: the gene 5 product (gp5), which contains both polymerase and proofreading activities, and a host-encoded protein, called thioredoxin, which acts as a processivity factor (25). We refer here to the gp5-thioredoxin complex as T7 pol. The Bacillus subtilis phage Φ29 polymerase (Φ29 pol) is also very well characterized (for reviews see Refs. 26 and 27). It is a protein-primed DNA polymerase that contains polymerase and proofreading activities in a single polypeptide, does not have a separate processivity subunit, and replicates very long stretches of duplex DNA in the absence of any helicase, because it possesses a strong strand displacement activity (28). We show here that all polymerases except that of phage Φ29 can slip during replication, albeit with different efficiencies, and demonstrate that the propensity of a polymerase to slip is decreased by its strand displacement activity.

We have previously observed that single-stranded DNA-binding protein of E. coli (SSB) stimulates slippage of pol III HE (14) and therefore decided to test its effect on the other polymerases. SSB is essential for cell viability and is involved in various DNA transactions, such as replication, recombination, and repair (for reviews see Refs. 29 and 30). It suppresses secondary structures in DNA but has no unwinding activity. It may also interact directly with DNA polymerases, as reported for pol II (62) and for the χ subunit of pol III (63, 64). Here we present evidence that the efficiency of the replication slippage of different polymerases is affected in a different way by the E. coli SSB. The protein inhibits slippage of pol I and T7 pol, does not affect that of pol II and T4 pol, and is able to stimulate that of pol III HE. We show in this work that SSB affects the capacity of different polymerases to slip by modulating their strand displacement activity rather than by a suppression of secondary structures in DNA.

**EXPERIMENTAL PROCEDURES**

**Proteins**—Pol II and pol II exo were kind gifts of Dr. M. Goodman (University of Southern California, Los Angeles). Φ29 pol (wild type and exo) were kind gifts of Dr. M. Salas and Dr. L. Blanco (Centro de Biología Molecular, Madrid, Spain). Pol III was purified as described (14). Pol I, pol I KF, T4 pol, and gp32 were purchased from Roche Molecular Biochemicals. T7 pol was purchased from New England Biolabs. T7 pol exo (Sequenase™, version 2) was from U. S. Biochemical Corp, and sequencing on single- or double-stranded DNA templates was carried out according to the protocol of the Sequenase version 2 sequencing kit (U. S. Biochemical Corp.). E. coli SSB was purchased from U. S. Biochemical Corp. Proteinase K was from Roche Molecular Biochemicals. For all polymerases, we have used the units ion 2 sequencing kit (U. S. Biochemical Corp.), and sequencing on single- or double-stranded DNA template.

10% glycerol; (ii) for Sequenase: 40 mM Tris-HCl, pH 7.5, 20 mM MgCl2, 5 mM dNTP (each), and 100 μM 32P-labeled primer was used, or 250 μM dNTP (each) if a separate processivity subunit, and replicates very long stretches of duplex DNA in the absence of any helicase, because it possesses a strong strand displacement activity (28). We show here that all polymerases except that of phage Φ29 can slip during replication, albeit with different efficiencies, and demonstrate that the propensity of a polymerase to slip is decreased by its strand displacement activity.

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ssDNA Template and Primer Extension Reactions—Plasmids pH727FXb and pH727FXc (see Fig. 1), the preparation of the ssDNA template and primer extension reactions. Two templates used in this work, pH727FXb and pH727FXc, differ slightly by the sequence at the base of the priming (Fig. 1B, F1 and F2, respectively). Essentially identical results were obtained with both, and for simplicity, only those obtained with the second was shown throughout the manuscript. The differences observed with pol II and T4 pol are presented in the last section under “Results.”

To determine the slippage efficiency the reaction products were analyzed by electrophoresis on agarose gel and revealed by autoradiography. An example of such analysis, carried out with pol III HE, is shown on Fig. 2 (lanes 9–12). The two products, parental (P) and heteroduplex (H) display slow and intermediate migration on agarose gels, whereas the incompletely replicated molecules (S) migrate fast. Detailed characterization of the molecules obtained with pol III HE by digestion with restriction enzymes, followed by electrophoresis on sequencing gel, autoradiography, and determination of the size of specific restriction fragments were reported previously (14).
It was carried out for the polymerases studied here in many experiments but is not presented for simplicity.

The Three E. coli DNA Polymerases Can Slip—Slippage ability of two E. coli DNA polymerases, pol I and pol II, was compared with that of the previously studied pol III HE. Pol I generated both parental and heteroduplex molecules (Fig. 2, lanes 1–4). We deduce that this polymerase can slip under the conditions used. The proportion of the heteroduplex varied inversely with pol I concentration. A similar phenomenon was previously observed with pol III HE (14) and was confirmed here (Fig. 2, lanes 9–12).Somewhat unexpectedly, pol I generated three heteroduplex products (Fig. 2, lanes 3 and 4), formed by slippage at different short repeats, present either at the bottom of the hairpin (Fig. 1B) or within it, as deduced from the restriction analysis and electrophoresis on sequencing gels (not shown). Pol I KF was also tested and found to be indistinguishable from wild-type in this experiment.

FIG. 1. Experimental system. A, schematic structure of the recombination units and of the primer extension reaction used in this work. Recombination unit of plasmids pH727FXb and pH727FXc is constituted of two 27-bp direct repeats (DR, open boxed arrows), flanking a pair of 300-bp inverted repeats (IR, thick arrows) and a central 1370-bp region (insert). The template strand is represented by a thick line, and the neosynthesized strand is represented by a thin line. B, detailed sequences of the two recombination units used in this work: pH727FXb and pH727FXc. Direct repeats (boxed arrows) and the beginning of the inverted repeat are represented. The GC-rich region at the bottom of the inverted repeat is boxed.

FIG. 2. Different DNA polymerases can promote slippage. The primer extension reactions were carried out as described under "Experimental Procedures." Decreasing amounts of each polymerase were used as indicated above the lanes, in the presence of labeled dNTPs and primer 1233 annealed to the FXc template (75 ng), followed by electrophoresis on agarose gel. E. coli SSB was present (at a saturating amount, i.e. 225 ng) in pol II, pol III, T4 pol, and T7 pol reactions. No SSB was added in pol I and F29 pol reactions. To the left of the figure, P, H, and S refer to the parental, heteroduplex, and stop (because of polymerase pausing at the hairpin) molecules, respectively. Lanes 1–4, 2, 1, 0.5, and 0.25 units of pol I, respectively. Lanes 5–8, 1.5, 0.75, 0.37, and 0.18 units of pol II, respectively. Lanes 9–12, 0.28, 0.14, 0.1, and 0.06 units of pol III HE, respectively. Lanes 13–16, 2, 1, 0.5, and 0.25 units of T4 pol, respectively. Lanes 17–20, 0.3, 0.1, 0.03, and 0.01 units of T7 pol, respectively. Lanes 21–23, 40, 20, and 10 ng of F29 pol, respectively.
able from pol I (not shown). Pol II slipped more efficiently than the other two polymerases (Fig. 2, lanes 5–8), forming predominantly heteroduplex molecules under all tested conditions.

Two of the Three Phage DNA Polymerases Can Slip—We tested the slippage ability of three different phage DNA polymerases: T7 pol (which is phylogenetically related to pol I) and T4 pol and Φ29 pol (both related to pol II). T4 pol generated large amounts of heteroduplex molecules and no parental molecules, even at the highest concentrations (Fig. 2, lanes 13–16) and thus resembled pol II. T7 pol resembled pol I and pol III, forming a higher proportion of heteroduplex molecules and no parental molecules under all conditions tested.

Mutations in DNA Polymerases Can Affect Their Slippage Efficiency—Synthesis of parental molecules requires opening of the duplex DNA formed by the annealing of the palindromic present on the single-stranded templates (Fig. 1). Such opening should be promoted by the strand displacement activity that a polymerase may have. As a consequence, high strand displacement activity should interfere with slippage, as observed for Φ29 pol, above. It is known that the strand displacement activity of certain exo⁻ mutants DNA polymerases is modified, probably because the same structural domain of the polymerase is required for both activities. Such exo⁻ mutants affected in strand displacement activity were described for T7 pol (32, 33), Φ29 pol (34, 35), and T4 pol (36). To test the putative negative correlation between strand displacement activity and slippage efficiency, we used two such mutants: (i) T7 pol exo⁻ (Sequenase™, version 2, a genetically engineered protein that misses 28 amino acids; Ref. 37) that has lost the proofreading activity and has acquired a strand displacement activity (~10% of the wild type enzyme; Refs. 34 and 35). This activity is, however, still higher than that of pol I (see Fig. 3).

In addition, we tested two exo⁻ mutants for which no data concerning their strand displacement activity were previously reported: (i) pol I KF exo⁻ that has lost both the 5' → 3' (nick translation) and the 3' → 5' (proofreading) exonuclease activities (38) and (ii) pol II exo⁻ that has lost the proofreading activity (39).

The acquisition of a strand displacement activity by T7 pol exo⁻ rendered the polymerase unable to slip (Fig. 3A, lanes 4–6). This supports the hypothesis that the opening of the duplex interferes with slippage. In contrast, partial loss of the strand displacement activity of Φ29 pol exo⁻ did not promote slippage (Fig. 3B, lanes 4–6). This activity is clearly lower in the mutant than in the wild type Φ29 pol, because it mainly produced parental molecules rather than high molecular weight material (Fig. 3B, lanes 1–3). However, it appears strong enough to efficiently open the duplex formed by the palindrome. It was reported that the strand displacement activity can be reduced further by decreasing the reaction temperature (to 10 °C) or by increasing the salt concentration (40). However, even under such conditions no slippage was detected (not shown).

The pol I KF exo⁻ mutant enzyme was essentially indistinguishable from pol I and pol I KF under all conditions tested (not shown). In contrast, pol II exo⁻ has become able to synthesize parental molecules to the detriment of the recombinant heteroduplex (Fig. 3C, compare lanes 1 and 2 to lanes 5 and 6). It is possible that the mutation in pol I does not affect the strand displacement activity, whereas the mutation in pol II endows the enzyme with some strand displacement activity and thus enables it to open the duplex portion of the template.

SSB Can Modulate the Slippage—Study of pol III HE revealed that E. coli SSB could stimulate replication slippage (14). We therefore investigated the effect of SSB on slippage of other DNA polymerases. The amounts of SSB ranged from one-tenth to 10 times that required to cover all the single-stranded DNA present in the assay.

Two effects of E. coli SSB were observed, one on slippage and another on overall DNA synthesis. The slippage of pol I was inhibited by SSB, because almost no heteroduplex molecules were detected at high SSB concentrations (Fig. 4). In parallel, the amount of parental size molecules decreased, and the molecules migrating more slowly appeared, suggesting that the strand displacement activity of pol I was stimulated by SSB. The overall synthesis was not affected greatly, irrespective of

2 M. Salas and L. Blanco, personal communication.
the pol I concentration. Analogous results were obtained with pol I KF (not shown). A similar inhibitory effect of SSB on slippage of T7 pol was observed (Fig. 5, lanes 1–4, 7, and 8). In contrast to pol I, SSB stimulated overall synthesis by T7 pol, particularly at low polymerase concentrations (Fig. 5, lanes 5–8 and 9–12).

Results with pol II were markedly different. The slippage was affected little by SSB, because the heteroduplex molecules were the major product whenever the synthesis was efficient enough (bands migrating faster than the heteroduplex, detected when the synthesis was inefficient, are presumably due to polymerase pausing before completion of replication; Fig. 6, lanes 6, 11, and 12). Overall DNA synthesis was stimulated by SSB, in particular at low pol II concentrations (Fig. 6, lanes 6, 7, and 11–13). T4 pol was affected in a similar way (no effect on slippage, stimulation of overall DNA synthesis; Fig. 7). F29 pol was not affected by SSB (not shown).

The contrasting effects of E. coli SSB on different polymerases are difficult to reconcile with the notion that the protein affects the DNA structure only, modifying its capacity to serve as a template for regular synthesis or for slippage. They may indicate the existence of interactions between the SSB and the different polymerases that can alter the properties of the enzyme. We therefore considered the possibility that interaction of phage polymerases with their cognate SSB could also alter the slippage propensity of the enzymes. The effects of two phage SSB proteins, gp32 from T4 and p5 from F29, were tested. Low gp32 levels (below or at saturation) stimulated both the synthesis and slippage mediated by T4 pol at low polymerase concentration (Fig. 8, lanes 1–3) and had no effect at high polymerase concentration (Fig. 8, lanes 7–10). At higher gp32 levels (2 or 5 times over saturation), slippage was inhibited, and parental molecules were synthesized (Fig. 8, lanes 11 and 12). Partially replicated molecules were also detected (Fig. 8, lanes 5–6, 11, and 12), their accumulation could be due to limited opening of the base of the hairpin by gp32. To test whether the formation of parental molecules was due to the opening of the entire hairpin by gp32, this protein was used in conjunction with pol I KF. Under conditions where this polymerase produced essentially the heteroduplex molecules, gp32 did not mediate the appearance of parental molecules and, at high concentration, inhibited the overall DNA synthesis (Fig. 8, lanes 13–18). This argues against the hypothesis that gp32 was able to open the entire hairpin on the single-stranded template and suggests the existence of specific interaction between gp32 and T4 pol. No effect of p5 on phage F29 pol was detected (not shown).

Taken together, our results show that SSB from E. coli and phage T4 affect slippage of three different polymerases (pol I, T7 pol, and T4 pol) but not two other polymerases (pol II and F29 pol). They suggest that the effect, when observed, may be due to the modulation of the strand displacement ability of a polymerase.

SSB Can Modulate Strand Displacement Activity of DNA Polymerases—To test the hypothesis that the strand displacement activity affects slippage efficiency of different polymerases, we have set up a system allowing us to independently estimate this activity under conditions used for studying the slippage. The system is represented schematically at the top of Fig. 10. It consists of a circular ssDNA template carrying two primers. One of 17 bases is fully homologous to the template and is labeled at its 5’ end. The other, placed 74 nucleotides...
downstream, is not homologous to the template over the 5’-terminal 10 bases and is annealed over the remaining 20 bases. Upon initiation of DNA synthesis both primers are elongated, and a double-stranded DNA molecule is generated. Replication from the labeled primer is monitored by withdrawing aliquots at different times and analyzing them on denaturing sequencing gel. A polymerase devoid of any strand displacement activity should be arrested upon encountering the annealed portion of the second primer, thus generating a labeled fragment of precisely 91 nucleotides, easily detectable on a sequencing gel. In contrast, a polymerase endowed with strand displacement ability should progress through the double-stranded region and generate ssDNA fragments of increasing length. The system was used to test strand displacement activity of different polymerases in the absence of SSB or in the presence of different SSB concentrations: 10-fold below saturation, at saturation, and 10-fold above saturation. The results are presented in Fig. 10.

First, in the absence of SSB, pol II, T4 pol, and T7 pol were devoid of strand displacement activity (see Fig. 10, B–D). In contrast, pol I KF and Φ29 pol were able to progress through the double-stranded region (see Fig. 10, A and E). However, progression was slower and less efficient for pol I KF compared with Φ29 pol, for which no replication pauses are detected. The correlation between strand displacement activity and the ability to slip appears to be rather good, because pol II and T4 pol, the two polymerases that slipped most efficiently, lack the strand displacement activity, and Φ29 pol, the polymerase that did not slip, had the strongest strand displacement activity, whereas pol I was intermediate by both criteria (Fig. 2). Slippage of T7 pol, although somewhat less efficient than that of pol II and T4 pol, was more efficient than that of pol I.

Second, SSB clearly stimulated the strand displacement activity of pol I KF and T7 pol (see Fig. 10, A and D) but had no effect on T4 pol and pol II (see Fig. 10, B and C; only trace amount of long fragments were detected with pol II at high SSB amounts). This parallels perfectly the inhibition of pol I and T7 pol slippage by SSB (Figs. 4 and 5) and lack of effect of SSB on pol slippage by T4 pol (Figs. 6 and 7). At high concentration SSB inhibited DNA synthesis by Φ29 pol (see Fig. 10E).

Third, a mutation affecting the exonuclease domain of pol II or T7 pol conferred certain strand displacement activity to these polymerases (see Fig. 10, G and I) and interfered with their slippage (Fig. 3, A and C). This was not the case for pol I KF exonuclease mutation (see Fig. 10F). Interestingly, the strand displacement activities of pol II exo+ and T7 pol exo+ were stimulated even further by SSB. Concerning Φ29 pol exo−, this test did not reveal a significant slight decrease of its strand displacement activity, presumably because of the low resolution of the gel for larger DNA molecules.

Finally, gp32 but not E. coli SSB conferred certain strand displacement activity to T4 pol when present above saturating amount (see Fig. 10H). The former but not the latter protein interfered with the slippage of T4 pol (Figs. 7 and 8). Taken together, the results (summarized in Table 1) indicate the existence of a negative correlation between the strand displacement ability of a polymerase and its capacity to undergo replication slippage. 

**Effects of the Template Structure**—The two templates used throughout this work differ in only one aspect. In FXb the direct repeats flank the palindrome, whereas in FXc the last 15 bp of the repeat proximal to the primer are part of the palindrome (Fig. 1). Therefore, the hairpin formed by annealing of the palindrome arms encompasses none of the direct repeat in FXb but does encompass about a half of it in FXc. Because

![Fig. 7. Effect of E. coli SSB on synthesis and slippage promoted by T4 pol.](image)

![Fig. 8. Effect of gp32 on synthesis and slippage promoted by T4 pol.](image)
slippage requires arrest of the polymerase within the repeat (14), it should occur on FXb only when a polymerase pauses at the bottom of the hairpin but could occur on FXc also when it pauses within the first 15 bases of the hairpin. Most of the polymerases we studied generated very similar products on both templates but two gave different results.

Pol II and T4 pol generated mainly the heteroduplex molecules on the FXc template, irrespective of the concentration of SSB. On FXb template, pol II still generated mainly the heteroduplex but only at sub saturating SSB concentration (Fig. 9, lane 2). At higher SSB concentration little heteroduplex was observed, and partially replicated molecules accumulated instead, together with some parental molecules (Fig. 9, lanes 3 and 4). This indicates that pol II could enter the hairpin under these conditions but remained mostly blocked there. Possibly, SSB could open the hairpin slightly, thus allowing penetration of pol II into the hairpin. Only partially replicated molecules were observed with T4 pol on FXb template (Fig. 9, lanes 11 and 12), indicating that the enzyme entered the hairpin and was blocked there.

**DISCUSSION**

We have reported previously that the main *E. coli* polymerase, pol III HE, which replicates the chromosome, can slip in vitro between directly repeated sequences (14). The model proposed to account for this process is shown in Fig. 11. A critical step is polymerase pausing at the base of the hairpin, within a direct repeat. The arrested polymerase is believed to dissociate from the template, which allows pairing of the newly synthesized strand with the second repeat. Polymerase can then load at the tip of this strand and restart the replication. A heteroduplex molecule is thus synthesized, composed of one parental strand and one strand lacking one of the repeats and all the sequences between the repeats. An alternative process that generates parental duplex molecule also takes place. It may involve duplex opening, possibly without dissociation of the polymerase from its template, followed by the replication restart (Fig. 11). It was suggested that pol III HE, not known to possess an intrinsic helicase activity (44), may take advantage of transient opening of the duplex ends to add the nucleotides at the tip of the newly synthesized strand and thus progress in a step-by-step manner inside the duplex (14). Here we have studied five additional polymerases, two from *E. coli* (pol I and pol II) and three from bacteriophages (T4 and T7 from *E. coli* and F29 from *B. subtilis*). All but the last polymerases were able to slip, which generalizes the phenomenon described for pol III HE. The efficiency of the process was, however, not the same for all enzymes. We propose that the differences may be due essentially to one polymerase property studied here: its capacity to open the duplex molecule by a strand displacement activity. We have shown in this work that the process can be oriented either toward the replication of the transiently opened duplex (synthesis of parental molecules) in case of high strand displacement activity, or toward the annealing of the newly synthesized strand with the second repeat (slippage event) in case of low strand displacement activity. The results showing the negative correlation between slippage and strand displacement activity are summarized in Table I.

The polymerases we studied may be classified in three groups, as regards their slippage efficiency. F29 pol, representative of the first group, never slipped in any conditions. It is endowed with very high strand displacement activity and processivity and is able to replicate very long duplex DNA in the absence of any helicase, both in vitro and in vivo (28). A F29 pol mutant, having a reduced strand displacement activity and used under sub optimal conditions, still did not slip, indicating that the remaining strand displacement activity of the enzyme were high enough to replicate the duplex efficiently. SSB from *E. coli* or from F29 pol, probably indirectly by preventing the unproductive binding of F29 pol to ssDNA (40, 56, 66), did not allow slippage.

The second group encompasses the catalytic subunits of pol II and T4 pol, which slipped very efficiently and generated almost no parental molecules. They are both devoid of any strand displacement activity (Refs. 21, 53, and 59 and Fig. 10, B and C). The two can form a complex holoenzyme, because pol II may associate with the auxiliary subunits of pol III (β clamp and γ complex; Refs. 21 and 22), whereas T4 pol (gp43) is associated with gp45 (the processivity factor) and gp44-gp62 (the clamp loader; Ref. 23). The two polymerase subunits are far less processive than their holoenzyme counterparts (22, 23). *E. coli* SSB stimulated DNA synthesis catalyzed by pol II, as expected from previous reports (22, 49, 60, 61), maybe by direct protein-protein interactions (62), but did not affect its capacity to slip or its strand displacement activity (Ref. 21 and Fig. 10B). Interestingly, a pol II mutant enzyme devoid of exonuclease activity has simultaneously acquired strand displacement activity, which is stimulated by SSB, and lost, at least in part, the ability to slip. However, it is puzzling to note that in a gap-filling forward mutagenesis assay, pol II exonuclease appeared to generate deletions between short direct repeats at a higher rate than its wild type counterpart (39).

We show here that *E. coli* SSB can stimulate DNA synthesis catalyzed by T4 pol, which is contradictory to previous reports (60, 65). As with pol II, SSB does not affect either the strand displacement activity of T4 pol or its capacity to slip (Fig. 10C). In contrast, gp32, which stimulates specifically DNA synthesis catalyzed by T4 pol (31, 51, 60), endows the polymerase with a strand displacement activity, in agreement with previous reports (41, 53), probably by direct protein-protein interactions (23, 51, 52) and simultaneously interferes with its capacity to slip.

Finally, pol I, pol III HE, and T7 pol form a third group of polymerases, with intermediate slippage properties, catalyzing formation of both parental and recombinant molecules, whose respective amounts depend on polymerase and SSB concentrations. We propose that the intermediate slippage efficiencies may result from a combination of several properties of the polymerases: intermediate strand displacement activities, spe-
cific protein-protein interactions, and differences in their processivity. A low processivity would favor heteroduplex formation by promoting the dissociation of the polymerase during replication of the first repeat, whereas high processivity would favor synthesis of parental molecules by preventing the dissociation of the polymerase.

T7 pol has no strand displacement activity in the absence of SSB (Refs. 32 and 57 and Fig. 10D), and it slips very efficiently. However, in the presence of SSB it acquires some strand displacement activity (Refs. 48 and 50 and Fig. 10D), which correlates perfectly with the (partial) inhibition of its slippage by SSB. Not surprisingly, a mutation that confers the strand displacement activity on T7 pol (T7 pol exo

It has been proposed that E. coli SSB stimulates T7 pol by suppressing secondary structures on the DNA (46) but also by increasing the affinity of the polymerase for the primer-template complex (47, 48) and by strongly increasing its processivity (43). For instance, E. coli SSB might prevent thioredoxin dissociation from gp5 and thus increase the processivity of the polymerase. In that case, this would inhibit the slippage, because the process may require dissociation of the arrested polymerase from its template (Fig. 11). These results are in agreement with the strand slippage model proposed to explain the error-prone replication of repeated DNA sequences by T7 pol in the absence of thioredoxin (45). Some authors have reported direct protein-protein interactions between SSB and T7 pol (62), but others could not reproduce this result (47). Direct protein-protein interactions were also described between the T7 SSB (gp2.5) and the polymerase (54, 55).

Pol I possesses some strand displacement activity even in the absence of SSB (Fig. 10A), which would allow for the formation of parental molecules. We propose that slippage is still possible, first because the strand displacement activity is not very high, and second, because pol I has a low processivity (16) that would favor the dissociation of the polymerase, thus favoring the dissociation of the polymerase during replication of a nick (57). As expected, pol I KF (which is devoid of 5’→3’ exonuclease) can catalyze strand displacement synthesis both at a nick and at a preformed replication fork (16, 57). In vitro, replication of double-stranded DNA by pol I KF occurs approximately 10 times more slowly than synthesis on ssDNA (58).
synthesis by pol I but stimulates its strand displacement activity (Fig. 10A) and concomitantly inhibits slippage. Interestingly, strand switching and frameshift mutations caused by pol I in vitro were observed previously but in the absence of E. coli SSB (16, 17).

In contrast to pol I, pol III HE is highly processive (42) and is believed to have little or no strand displacement activity, because it is unable to displace the 5′ end of a primer encountered during replication even in the presence of SSB (44). However, pol III HE is able to replicate to some extent through double-stranded regions, because it is able to produce parental molecules (Ref. 14 and Fig. 1). The ability of pol III HE to promote slippage despite having some strand displacement activity and high processivity might be due to specific protein-protein interactions with SSB. Another common property to pol I, pol III HE, and T7 pol is that the proportion of parental molecules synthesized by these three polymerases could be increased by increasing the polymerase concentration, possibly by promoting the step by step progression inside the palindrome, as suggested previously for pol III HE (14).

In conclusion, we show in this work that different polymerases can undergo slippage and that their strand displacement activity interferes with the slippage. We propose that the processivity of the polymerase and direct protein-protein interactions may also affect the slippage efficiency. The role of SSB is complex, because all polymerases are not affected in a similar way. These contrasting effects suggest that its action is not only via binding to single-stranded DNA, which could conceivably alter the capacity of the template to support polymerase slippage. We therefore consider the alternative explanation: SSB may act either directly or indirectly on the different polymerases by increasing their strand displacement activity, increasing their affinity for the primer-template, or increasing their processivity and thus modulates their capacity to slip. These observations may provide some guidance toward better understanding of genome rearrangements that result from replication slippage.

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References

Replication Slippage in Vitro