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 exonuclease and T7 pol exonuclease) 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.

Misalignment of two DNA strands during replication can lead to DNA rearrangements such as deletions or duplications of varying lengths ranging from several nucleotides to entire genes. This process, designated replication slippage (as well as strand slippage) of Okazaki fragments (16); it does not usually replicate long stretches of DNA and has been shown previously in vitro to cause fr or 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 mutation 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 product of the gene 32 gene, designated gp32).

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; 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 F29 polymerase (F29 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 F29 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). F29 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. Protease K was from Roche Molecular Biochemicals. For all polymerases, we have used the units defined for pol I: one unit of enzyme catalyzes the incorporation of 10 nmol of total nucleotide into acid insoluble material in 30 min at 37 °C.

**ssDNA Template and Primer Extension Reactions**—Plasmids pH727FXb and pH727FXc (see Fig. 1), the preparation of the ssDNA template was describe previously (14). Pol I, pol I KF, T4 pol, and gp32 were purchased from Roche Molecular Biochemicals.

**RESULTS**

**Experimental System**—To test the ability of different polymerases to undergo slippage, we carried out primer extension reactions with radiolabeled nucleotides on a single-stranded circular plasmid DNA. This template carries two 27-bp direct repeats that flank a pair of 300-bp inverted repeats (Fig. 1A, left). E. coli pol III HE mainly generates one intermediate and two final products on this template (14). The intermediate, a partially replicated template, is due to the arrest of the polymerase at the hairpin formed by annealing of the two inverted repeats (Fig. 1A, center, S). One final product is a fully double-stranded molecule, termed parental (Fig. 1A, right, P), which results from synthesis through the hairpin and therefore involves separation of duplex DNA strands. The other is a heteroduplex molecule (Fig. 1A, right, H), composed of one recombinant and one parental DNA strand, resulting from a polymerase slippage. The recombinant strand lacks the segment flanked by the direct repeats (2 kilobases) and one of the direct repeats. The ability of the polymerase to carry out either reaction can be estimated from the proportion of these products. Two templates used in this work, pH727FXb and pH727FXc, differ slightly by the sequence at the base of the palindrome (Fig. 1B, FXb and FXc, 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 Supporting Information (see “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.
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 at low enzyme concentrations (Fig. 2, lanes 17–20). In contrast, Φ29 pol did not form heteroduplex molecules under any condition; instead, it synthesized mainly slowly migrating high molecular weight molecules (Fig. 2, lanes 21–23). These are presumably due to rolling circle replication, which implies that after completion of one round of replication, the newly synthesized strand is displaced and the synthesis continued. Φ29 pol is known to possess a very potent strand displacement activity (28). The absence of recombinant heteroduplex was verified by analyzing the restricted DNA samples on a sequencing gel, a method of detection that is more sensitive than the agarose gel electrophoresis (not shown). We conclude from these results that all polymerases but one, endowed with an exceptionally high strand displacement activity, can slip.

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 palindrome 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 which 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. 10J).² In addition, we tested two exo⁻ mutants for which no data concerning their strand displacement activity were previously reported: (i) pol I KP 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 produces 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 KP exo⁻ mutant enzyme was essentially indistinguishable from pol I and pol I KP 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...

**Fig. 4.** Effect of E. coli SSB on synthesis and slippage promoted by pol I. The primer extension reactions were carried out as described in the legend to Fig. 2 on 25 ng of FXc template but with three different amounts of pol I, as indicated above the lanes and increasing amounts of SSB ranging from 0, 0.1, 1, 3, to 10 times the saturating amount. Lanes 1, 6, and 11, no SSB. Lanes 2, 7, and 12, 7.5 ng of SSB. Lanes 3, 8, and 13, 75 ng of SSB. Lanes 4, 9, and 14, 225 ng of SSB. Lanes 5, 10, and 15, 750 ng of SSB.

**Fig. 5.** Effect of E. coli SSB on synthesis and slippage promoted by T7 pol. The primer extension reactions were carried out as described in the legend to Fig. 2 on 25 ng of FXc template but with three different amounts of T7 pol, as indicated above the lanes and increasing amounts of SSB ranging from 0, 0.1, 1, to 10 times the saturating amount. Lanes 1, 5, and 9, no SSB. Lanes 2, 6, and 10, 7.5 ng of SSB. Lanes 3, 7, and 11, 75 ng of SSB. Lanes 4, 8, and 12, 750 ng of SSB.

**Fig. 6.** Effect of E. coli SSB on synthesis and slippage promoted by pol II. The primer extension reactions were carried out as described in the legend to Fig. 2 on 25 ng of FXc template but with three different amounts of pol II, as indicated above the lanes and increasing amounts of SSB ranging from 0, 0.1, 1, 3, to 10 times the saturating amount. Lanes 1, 6, and 11, no SSB. Lanes 2, 7, and 12, 7.5 ng of SSB. Lanes 3, 8, and 13, 75 ng of SSB. Lanes 4, 9, and 14, 225 ng of SSB. Lanes 5, 10, and 15, 750 ng of SSB.
Fig. 7. Effect of E. coli SSB on synthesis and slippage promoted by T4 pol. The primer extension reactions were carried out as described in the legend to Fig. 2 on 25 ng of FXc template but with two different amounts of T4 pol and with pol I KF as a control, as indicated above the lanes and increasing amounts of SSB ranging from 0, 0.1, 1, to 10 times the saturating amount. Lanes 1 and 3, no SSB. Lanes 2 and 6, 7, 5 ng of SSB. Lanes 3 and 7, 75 ng of SSB. Lanes 4 and 8, 750 ng of SSB.

Fig. 8. Effect of gp32 on synthesis and slippage promoted by T4 pol. The primer extension reactions were carried out as described in the legend to Fig. 2 on 75 ng of FXc template but with two different amounts of T4 pol and with pol I KF as a control, as indicated above the lanes and increasing amounts of gp32 ranging from 0, 0.5, 1, 2, to 5 times the saturating amount. Lanes 1, 7, and 13, no gp32. Lanes 2, 8, and 14, 100 ng of gp32. Lanes 3, 9, and 15, 500 ng of gp32. Lanes 4, 10, and 16, 1000 ng of gp32. Lanes 1, 11, and 17, 2000 ng of gp32. Lanes 6, 12, and 18, 5000 ng of gp32.

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 activity 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 II and T4 pol slippage (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 exo- 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 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 I) 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

| Polymerase | SSB | Slippage | Strand displacement |
|------------|-----|----------|---------------------|
| T4 pol     |     |          |                     |
| pol II     |     |          |                     |
| pol II exo-|     | Only     |                     |
| T7 pol     |     |          |                     |
| pol I KF   |     |          |                     |
| pol I KF exo-| | Some    |                     |
| T7 pol exo-|     |          |                     |
| Φ29 pol exo-| | None    |                     |
| Φ29 pol    |     |          |                     |

The different DNA polymerases have been tested in the absence or presence of SSB, except pol III HE which has not been tested without SSB, because it requires SSB for synthesis, and furthermore, our preparation contains some SSB.
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 subsaturating 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 Φ29 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. Φ29 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 Φ29 pol mutant, having a reduced strand displacement activity and used under suboptimal 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 Φ29 pol (protein p5), which have been shown to stimulate DNA synthesis by Φ29 pol, probably indirectly by preventing the unproductive binding of Φ29 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 exo− 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-
Replication Slippage in Vitro

Fig. 10. Effect of SSB on strand displacement activity of the different DNA polymerases. Top, schematic representation of the experimental system. M13mp18 ssDNA is represented as a circle, and the two primers are represented as arrows. The labeled primer 1212 is indicated with an asterisk, and the downstream primer 37, which is only partially homologous to the template, is represented with a flapping tail. The number within the circle (91 nt) refers to the length of ssDNA that separates the two primers (including the 17 bases of the labeled primer). The first product is expected from DNA synthesis on single-stranded template only, the second is expected from the synthesis by displacement of the partially annealed primer, and the third is expected from the synthesis by displacement of both primers. Reaction times are indicated below the straight arrow. Bottom, the primer extension reactions. These were carried out in 50 μl as described under “Experimental Procedures.” Reactions were preincubated 5 min at 37 °C in the absence or in the presence of SSB, before DNA polymerase addition, as indicated above each panel. Aliquots were withdrawn at times indicated at the top, measured from DNA polymerase addition. The amounts of added SSB correspond to saturating amount (noted 1), ten times less (noted 0.1) and ten times more (noted 10), that is 37.5, 375, and 3750 ng, respectively, for 125 ng of ssDNA. However, in the case of gp32, the amounts were 0, 0.5, 1, and 5 times the saturating amount, that is 330, 1650, and 8250 ng, respectively. A, 5 units of pol I KF; B, 2.5 units of pol II; C, 15 units of T4 pol; D, 0.5 units of T7 pol; E, 100 ng of Φ29 pol; F, 5 units of pol I KF exo-. G, 2.5 units of pol II exo-. H, 15 units of T4 pol; I, 0.5 units of T7 pol exo-. J, 100 ng Φ29 pol exo-. A sequencing ladder of M13mp18 obtained with the primer 1212 was loaded on each side of each panel. The arrow labeled 91 nt indicates the position of migration of the labeled fragment that results from replication of ssDNA from the labeled primer 1212 up to the downstream primer.

Specific 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-) inhibits its capacity to slip.

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 allowing for the unpairing of the first repeat and formation of recombinant molecules. We have shown that SSB does not stimulate DNA

3 Strand displacement activity of pol I has been demonstrated in vitro but only at a preformed replication fork, because nick translation activity will prevent any strand displacement synthesis at 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. 1A) 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

1. Trinh, T. Q., and Sinden, R. R. (1991) Nature 352, 544–547
2. Trinh, T. Q., and Sinden, R. R. (1995) Genetics 134, 409–422
3. Rosche, W. A., Trinh, T. Q., and Sinden, R. R. (1995) J. Bacteriol. 177, 4385–4391
4. Tran, H. T., Degtariareva, N. P., Koletova, N. N., Sugino, A., Masumoto, H., Gordenin, D. A., and Resnick, M. A. (1995) Mol. Biol. Cell. 15, 5607–5617
5. Madsen, C. S., Ghivizzani, S. C., and Hauswirth, W. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7671–7675
6. Lockett, S. T., and Peschenko, V. V. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7120–7124
7. Bierne, H., Vilette, D., Ehrlich, S. D., and Michel, B. (1997) Mol. Microbiol. 24, 1225–1234
8. Peschenko, V. V., and Lockett, S. T. (1998) J. Mol. Biol. 276, 559–569
9. Wells, R. D. (1996) J. Biol. Chem. 271, 2875–2878
10. Debruunere, H., Gendrel, C. G., Lechat, S., and Dutrex, M. (1997) Biochimie (Paris) 79, 577–586
11. Dijan, P. (1998) Cell 94, 155–160
12. Pearson, C. E., and Sinden, R. R. (1998) Curr. Opin. Struct. Biol. 8, 321–330
13. d’Alenc¸on, E., Petranovic, M., Michel, B., Neust, P., Auenmurier, A., Uze, M., and Ehrlich, S. D. (1994) EMBO J. 13, 2725–2734
14. Canceill, D., and Ehrlich, S. D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6647–6652
15. Kelman, Z., and O’Donnell, M. (1995) Annu. Rev. Biochem. 64, 171–200
16. Kornberg, A., and Baker, T. A. (1992) DNA Replication, 2nd Ed., W. H. Freeman & Co., New York, N.Y. p. 165–196
17. Papanicolaou, C., and Ripley, L. S. (1989) J. Mol. Biol. 207, 335–353
18. Escarelller, M., Hicks, J., Gudmundsson, G., Trump, G., Touati, D., Lovett, S., Foster, P. L., McEntee, K., and Goodman, M. F. (1994) J. Bact. 166, 6221–6228
19. Paz-Elisur, T., Takeshita, M., Goodman, M., O’Donnell, M., and Livneh, Z. (1996) J. Biol. Chem. 271, 24662–24669
20. Rangarajan, S., Gudmundsson, G., Qu, Z., Foster, P. L., and Goodman, M. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 946–951
21. Hughes, A. J., Jr., Bryan, S. K., Chen, H., Moses, R. E., and McHenry, C. S. (1991) J. Biol. Chem. 266, 4568–4573
22. Bonner, C. A., Stukenberg, P. T., Rajasopan, M., Eritja, R., O’Donnell, M., McEntee, K., Echols, H., and Goodman, M. F. (1992) J. Biol. Chem. 267, 11431–11438
23. Young, M. C., Reddy, M. K., and von Hippel, P. H. (1992) Biochemistry 31, 8675–8690
24. Richardson, C. C. (1983) Cell 33, 315–317
25. Debryzer, Z., Tabor, S., and Richardson, C. C. (1994) Cell 77, 157–166
26. Salas, M. (1991) Annu. Rev. Biochem. 60, 39–71
27. Blanco, L., and Salas, M. (1996) J. Biol. Chem. 271, 8509–8512
28. Blanco, L., Bernard, A., Lazaro, J. M., Martin, G., Garnczardz, C., and Salas, M. (1989) J. Biol. Chem. 264, 8935–8940
29. Meyer, R. R., and Laine, P. S. (1990) Microbiol. Rev. 54, 342–380
30. Lohman, T. M., and Ferrari, M. E. (1994) J. Biol. Chem. 269, 2531–2534
31. Konigsberg, W. H., and Spicer, E. K. (1991) J. Biol. Chem. 266, 25873–25879
32. McEntee, K., Echols, H., and Goodman, M. F. (1981) J. Biol. Chem. 256, 4087–4094
33. Engler, M. J., Lechner, R. L., and Richardson, C. C. (1983) J. Biol. Chem. 258, 11165–11173
34. Lechner, R. L., Engler, M. J., and Richardson, C. C. (1983) J. Biol. Chem. 258, 11174–11184
35. Soengas, M. S., Esteban, J. A., Lazaro, J. M., Bernard, A., Blasco, M. A., Salas, M., and Blanco, L. (1992) EMBO J. 11, 4227–4237
36. Esteban, J. A., Soengas, M. S., Salas, M., and Blanco, L. (1994) J. Biol. Chem. 269, 31946–31954
37. Reha-Krantz, L. J., Stecki, S., Nonay, R. L., Dimayuga, E., Goodrich, L. D., Konigsberg, W. H., and Spicer, E. K. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2417–2421
38. Tabor, S., and Richardson, C. C. (1989) J. Biol. Chem. 264, 6447–6458
39. Derbyshire, V., Freemont, P. S., Sanderson, M. R., Reese, L., Friedman, J. M., Joyce, C. M., and Stitz, T. A. (1988) Science 240, 199–201
40. Cai, H., Yu, H., McEntee, K., Kunkel, T. A., and Goodman, M. F. (1995) J. Biol. Chem. 270, 15327–15335
41. Soengas, M. S., Gutierrez, C., and Salas, M. (1995) J. Mol. Biol. 253, 517–529
41. Nossal, N. G. (1974) J. Biol. Chem. 249, 5668–5676
42. Fay, P. J., Johnson, R. O., McHenry, C. S., and Bambara, R. A. (1981) J. Biol. Chem. 256, 976–983
43. Tabor, S., Huber, H. E., and Richardson, C. C. (1987) J. Biol. Chem. 262, 16212–16223
44. O’Donnell, M. E., and Kornberg, A. (1985) J. Biol. Chem. 260, 12875–12883
45. Kunkel, T. A., Patel, S. S., and Johnson, K. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6830–6834
46. Romano, L. J., and Richardson, C. C. (1979) J. Biol. Chem. 254, 10476–10482
47. Myers, T. W., and Romano, L. J. (1988) J. Biol. Chem. 263, 17006–17015
48. Rigler, M. N., and Romano, L. J. (1995) J. Biol. Chem. 270, 8910–8919
49. Molinaux, I. J., Friedman, S., and Gefter, M. L. (1974) J. Biol. Chem. 249, 6090–6098
50. Nakai, H., and Richardson, C. C. (1988) J. Biol. Chem. 263, 9831–9839
51. Huberman, J. A., Kornberg, A., and Alberts, B. M. (1971) J. Mol. Biol. 62, 39–52
52. Formosa, T., Burke, R. L., and Alberts, B. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2442–2446
53. Cha, T. A., and Alberts, B. M. (1989) J. Biol. Chem. 264, 12220–12225
54. Kim, Y. T., Tabor, S., Churchich, J. E., and Richardson, C. C. (1992) J. Biol. Chem. 267, 15032–15040
55. Kim, Y. T., and Richardson, C. C. (1994) J. Biol. Chem. 269, 5270–5278
56. Gutierrez, C., Martin, G., Sogo, J. M., and Salas, M. (1991) J. Biol. Chem. 266, 2104–2111
57. Lechner, R. L., and Richardson, C. C. (1983) J. Biol. Chem. 258, 11185–11196
58. Hillebrand, G. G., and Beattie, K. L. (1985) J. Biol. Chem. 260, 3116–3125
59. Wickner, R. B., Ginsberg, B., and Hurvitz, J. (1972) J. Biol. Chem. 247, 498–504
60. Sigal, N., Delius, H., Kornberg, T., Gefter, M. L., and Alberts, B. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 3537–3541
61. Sherman, L. A., and Gefter, M. L. (1976) J. Mol. Biol. 103, 61–76
62. Molinaux, I. J., and Gefter, M. L. (1975) J. Mol. Biol. 98, 811–825
63. Kelman, Z., Yushakov, A., Andjelkovic, J., and O'Donnell, M. (1998) EMBO J. 17, 2436–2449
64. Glover, B. P., and McHenry, C. S. (1998) J. Biol. Chem. 273, 23476–23484
65. Burke, R. L., Alberts, B. M., and Hosoda, J. (1980) J. Biol. Chem. 255, 11484–11493
66. Martin, G., Lazaro, J. M., Mendez, E., and Salas, M. (1989) Nucleic Acids Res. 17, 3663–3672