Lesion Bypass by the *Escherichia coli* DNA Polymerase V Requires Assembly of a RecA Nucleoprotein Filament*  

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Translesion replication is carried out in *Escherichia coli* by the SOS-inducible DNA polymerase V (UmuC), an error-prone polymerase, which is specialized for replicating through lesions in DNA, leading to the formation of mutations. Lesion bypass by pol V requires the SOS-regulated proteins UmuD and RecA and the single-strand DNA-binding protein (SSB). Using an *in vitro* assay system for translesion replication based on a gapped plasmid carrying a site-specific synthetic abasic site, we show that the assembly of a RecA nucleoprotein filament is required for lesion bypass by pol V. This is based on the reaction requirements for stoichiometric amounts of RecA and for single-stranded gaps longer than 100 nucleotides and on direct visualization of RecA-DNA filaments by electron microscopy. SSB is likely to facilitate the assembly of the RecA nucleoprotein filament; however, it has at least one additional role in lesion bypass. ATPγS, which is known to strongly increase binding of RecA to DNA, caused a drastic inhibition of pol V activity. Lesion bypass does not require stoichiometric binding of UmuD along RecA filaments. In summary, the RecA nucleoprotein filament, previously known to be required for SOS induction and homologous recombination, is also a critical intermediate in translesion replication.

Genomic DNA is afflicted by numerous lesions that might interfere with its propagation and with gene expression (1). Most of these lesions, which are usually base modifications, are repaired by cellular DNA repair mechanisms (1). When the replication fork encounters a blocking DNA lesion that has escaped repair, replication stops forming a ssDNA region in DNA (2). In *Escherichia coli* at least two mechanisms, which are regulated by the SOS response (3), act to repair the gap by converting the ssDNA region into a dsDNA region without actually removing the damaged nucleotide. Recombination repair patches the gap with a complementary DNA segment from the fully replicated sister chromatid (4, 5), whereas translesion replication fills in the gap by DNA synthesis. This pathway, also termed lesion bypass or error-prone repair, is mutagenic, because DNA lesions often cause misincorporation by DNA polymerases, leading to the formation of mutations (2, 6).

The *in vitro* reconstitution of SOS translesion replication with purified components (7–9) established that SOS-targeted mutagenesis occurs by replication through DNA lesions by DNA polymerase V (UmuC) in the presence of UmuD, RecA, and SSB (10, 11). Pol V effectively bypasses a synthetic abasic site (10, 11), a cyclobutyl TT dimer and a 6-4 TT adduct (12), leading to targeted mutations. When replicating an undamaged DNA template pol V is highly mutagenic and forms preferentially purine-purine and pyrimidine-pyrimidine mismatches, resulting in transversion mutations (13). These activities of pol V are responsible for SOS mutagenesis targeted to DNA lesions and for untargeted mutagenesis, which occurs in undamaged DNA regions.

Proteins similar to UmuC are widespread from *E. coli* to humans. Several of them were shown to encode DNA polymerases in bacteria (14), in *Saccharomyces cerevisiae* (15, 16), and in humans (reviewed in Refs. 17–19). Unlike pol V, these polymerases do not require any additional proteins for their polymerase and/or lesion bypass activities, at least *in vitro*. In this sense pol V is more similar to the recently discovered DNA polymerase RI, product of the plasmidic *mucB* gene, whose activity also requires RecA and SSB (20). Most intriguing is the requirement for RecA, a protein known to be required also for gap filling by recombinational repair. Here we show that the assembly of a RecA nucleoprotein filament is required for translesion synthesis by pol V.

**MATERIALS AND METHODS**

**Proteins**—UmuDand MBP-UmuC (a fusion of UmuC to maltose-binding protein) were overexpressed and purified as previously described (8, 11). The degree of purity was estimated to be >95% for UmuD and 90–95% for MBP-UmuC. SSB and RecA were purified as described (21, 22), except that a phosphocellulose purification step was added for RecA. Their degree of purity was estimated to be >95%. Restriction nuclease T4 DNA ligase, and T4 polynucleotide kinase were from New England BioLabs; T7 gp6 exonuclease was from Pharmacia Amersham Biotech, and ATPγS was from Sigma.

**DNA Substrates**—The preparation of the gapped plasmid GP21 carrying a site-specific lesion was recently described (23, 24). GP21 contains a site-specific synthetic (tetrahydrofuran) abasic site and a ssDNA region of ~350 nucleotides (see Fig. 1).  

**Translesion Replication Assay**—The translesion replication reaction was performed as previously described (8, 11) with minor changes. The

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‡ The abbreviations used are: ssDNA, single-stranded DNA; SSB, single-strand DNA-binding protein; MBP, maltose-binding protein; ATPγS, adenosine 5′-O-thiotriphosphate; PAGE, polyacrylamide gel electrophoresis.

§ We define UmuC as pol V, whereas Goodman and co-workers (10, 12) define the UmuD′C as pol V. Until the function of UmuD′ is elucidated, we prefer to avoid including UmuD′ as part of the polymerase. Notice that UmuD′ has no effect on the intrinsic polymerase activity of UmuC (in the absence of RecA and SSB, Ref. 11).
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RESULTS

RecA Is Needed in Stochiometric Amounts for Lesion Bypass—The experimental system for assaying lesion bypass was previously described (8, 23). It is based on a gapped plasmid carrying a site-specific synthetic abasic site in the ssDNA region and an internal radiolabeled phosphate in the primer strand (Fig. 1). Upon addition of pol V the 3′-primer terminus is extended up to the abasic site, where synthesis may terminate or proceed through the abasic site (lesion bypass). At the end of the reaction the DNA is isolated and restricted with MspAII, which cleaves 4 nucleotides upstream of the radiolabel, and with Asp700, which cleaves downstream of the lesion. This yields radiolabeled DNA fragments of 19, 29, and 47 nucleotides long, for the unextended primer, the block at the lesion, and the bypass product, respectively (Fig. 1). Bands in the length range of 20–46 nucleotides represent pol V pause or dissociation sites (see for example Figs. 2 and 6). In some cases the DNA products were restricted with Asp700 and BstXI, yielding products which were 4 nucleotides shorter than with the Asp700/MspAII cleavage. The DNA were fractionated by urea-PAGE and visualized and quantified by phosphorimaging.

The activity of the RecA protein in recombination or SOS induction requires its binding to DNA. This binding is stoichiometric and cooperative, leading to the formation of a helical RecA filament along ssDNA, composed of multiple RecA monomers (reviewed in Refs. 26, 27). We examined whether the formation of a RecA nucleoprotein filament is required also for lesion bypass. First, a titration experiment was performed, to determine the amount of RecA needed to saturate the bypass reaction. As can be seen in Fig. 2, although lesion bypass could be observed at 0.5 μM RecA, 2 μM RecA was required to reach a saturation level of bypass. Further increases up to 16 μM RecA had little effect on lesion bypass (Fig. 2). For comparison, a titration of pol V was performed. As can be seen in Fig. 3, bypass activity reached saturation at 50–100 nM pol V, 20-40 fold lower than the saturating concentration of RecA. The con-
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Lesion Bypass Requires Single-stranded Gaps That Are Longer than 100 Nucleotides—The formation of a stable RecA nucleoprotein filament requires a ssDNA region longer than 50 nucleotides (26). Thus, if a stable RecA nucleoprotein filament is required for lesion bypass, this should be reflected in a requirement for a long ssDNA region in the substrate DNA. This prediction was examined by using DNA substrates with single-stranded regions 12, 100, 350, or 850 nucleotides long. Fig. 4 shows the results of lesion bypass with DNA gaps 12 and 350 nucleotides long. In these experiments, the primer terminus was located opposite the nucleotide preceding the lesion in the template strand. This configuration of the DNA substrate gave lesion bypass results similar to those obtained with the standard substrate where the primer terminus was located 11 nucleotides upstream of the lesion (data not shown). As can be seen in Fig. 4, when the DNA substrate contained a small gap of 12 nucleotides, no bypass was observed by pol V, UmuD', RecA, and SSB (Fig. 4, lane 7). Furthermore, in a control reaction, bypass by pol I was higher than pol V on this substrate (Fig. 4, lane 2 versus lane 7). Attempts to examine whether omission of one of the protein components will allow lesion bypass on this DNA substrate showed no bypass under all the conditions examined (Fig. 4, lanes 3–6). No bypass was observed even with a 100-nucleotide-long gap (data not shown). In contrast, effective bypass was observed with gaps of 350 (Fig. 4, lane 14; see also Figs. 2 and 3) or 850 nucleotides long (see Fig. 8 below).

RecA Nucleoprotein Filaments Are Formed under Bypass Reaction Conditions—The formation of RecA nucleoprotein filaments under the conditions of the bypass reaction was examined using electron microscopy. The components of the reaction were mixed and incubated at 37 °C. Then the mixture was treated with glutaraldehyde to form protein-protein and protein-DNA cross-links and visualized by electron microscopy. As can be seen in Fig. 5C, RecA formed a filament along the ssDNA region in the DNA substrate. The length of the filament corresponds to the size of the gap in the plasmid. A similar picture was obtained when both RecA and SSB were present (Fig. 5D) or when the complete bypass reaction mixture was analyzed (Fig. 5F), although the size of the protein filament is somewhat larger. This may reflect coverage of the dsDNA adjacent to the gap; however, binding of RecA was largely restricted to the ssDNA region. This is not because of a shortage in RecA, because upon addition of ATPγS, all DNA molecules were entirely covered with RecA (Fig. 5E).

ATPγS Inhibits Lesion Bypass—ATPγS is a nonhydrolyzable ATP analog, which is known to strongly stabilize RecA nucleoprotein filaments (26). When lesion bypass was examined with ATPγS instead of ATP, there was a strong reduction in lesion bypass (Fig. 6, lanes 5–7). This effect of ATPγS was also observed in the presence of ATP (Fig. 6, lanes 11–13). Lesion bypass was calculated in this case by two methods. 1) The usual measure of lesion bypass was calculated as the percentage of bypass products of the primers that were extended. This measures lesion bypass directly, correcting for the efficiency of initiation of synthesis at the primer terminus. Molecules bypassed of total is the percentage of molecules on which bypass has occurred. For a substrate in which the primer terminus is located several nucleotides upstream to the lesion, this will be lower than lesion bypass, because usually not all primers are extended. As can be see in Fig. 6, the effect of ATPγS is primarily on the initiation of DNA synthesis, as indicated by the decrease in the fraction of primers that were extended, and by the molecules bypassed of total (Fig. 6, lanes 2–4 versus

**Fig. 4.** Pol V is unable to perform translesion synthesis on a substrate with a 12-nucleotide gap. The gapped plasmid substrates contain a primer ending one nucleotide before the lesion and gaps of 12 or 350 nucleotides long. Reactions were performed with the indicated combinations of proteins for 8 min. The concentrations of the proteins were: MBP-UmuC, 220 nM; UmuD', 1.25 μM; RecA, 4 μM; SSB, 600 nM (as tetramers); and pol I, 90 nM. Restriction of the DNA products prior to PAGE analysis was done with Asp700 and BstXI. The details are described under "Materials and Methods." The phosphorimage of the gel is presented.
lanes 5–7). Additionally, it seems that the bypass step itself is also inhibited, but the effect is considerably smaller (Fig. 6; lesion bypassed, %). In the presence of ATPγS, the RecA filaments extend to cover the entire DNA molecule under our reaction conditions (see Fig. 5, below). Therefore, the inhibition of pol V by ATPγS can be because of reduced accessibility to the primer terminus caused by the tight RecA-DNA complex. Alternatively, the altered conformation of the RecA nucleoprotein in the presence of ATPγS may be inhibitory. Lesion bypass was also observed in the absence of either ATP or ATPγS (Fig. 6, lanes 8–10), presumably because of the presence of dATP. This is consistent with previous results showing that dATP can replace ATP as a cofactor for RecA activities (26).

**Requirement for SSB in Lesion Bypass**—SSB was previously shown to stimulate RecA-catalyzed reactions by facilitating the formation of a RecA nucleoprotein filament (31–33). This occurs at low SSB concentrations, which favor a limited cooperativity mode of binding to DNA, leading to the appearance of beads (octamers of SSB) on a string (ssDNA Ref. 33). To examine whether the SSB requirement in lesion bypass is related to the loading of RecA on DNA, a titration of SSB was performed. As can be seen in Fig. 7, the highest bypass activity was observed at 50 nM SSB. Increasing SSB concentration above 50 nM caused a slight decrease in bypass activity, followed by a plateau. Electron microscopy analysis of SSB binding to the gapped plasmid under our reaction conditions (50 nM SSB)
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Lesion bypass, %
- 0 10 20 30 40 50 60 70 80 90 100

Primers initiated, %
- 0 10 20 30 40 50 60 70 80 90 100

Molecules bypassed out of total, %
- 0 10 20 30 40 50 60 70 80 90 100

Fig. 6. Translesion replication by pol V in the presence of ATPγS. Reactions were performed with the gap-lesion GP21, 90 nM pol V, 50 nM SSB (as tetramers), 2 μM RecA, and 1 mM ATP or ATPγS for 2, 4, and 6 min. In lanes 11–13, ATPγS was added from the beginning of the RecA-DNA preincubation, and ATP was added with the addition of pol V. Restriction of the DNA products prior to PAGE analysis was done with Asp700 and MspAI. The details are described under “Materials and Methods.” Bypass activity was calculated in two ways: 1) as a percentage of the extended primers (lesion bypass) or 2) as a percentage of total substrate molecules (molecules bypassed out of total). The value for percentage of primers initiated (of total substrate molecules) is also presented.

Fig. 7. Effect of SSB concentration on lesion bypass by pol V. Reactions were performed with the gap-lesion plasmid GP21, 100 nM pol V, 1.25 μM UmuD2, 4 μM RecA, and the indicated concentrations of SSB for 4 min. The graph shows the results of the translesion replication assay.

Reactions were performed with the gap-lesion plasmid GP21, 100 nM pol V, or ATPγS molecules is also presented. The value for percentage of primers initiated (of total substrate molecules bypassed out of a percentage of total substrate molecules) is also presented. 3 This indicates that a functional RecA nucleoprotein filament was formed in the absence of SSB. Therefore, it seems that facilitating the formation of a RecA nucleoprotein filament is not the only role for SSB in lesion bypass.

Lesion Bypass Does Not Require Stochiometric Coverage of the RecA Nucleoprotein Filament by UmuD2 or UmuD2C—Plasmon surface resonance analysis has shown that complexes of UmuD2C bind along a RecA nucleoprotein filament (35). This binding is mediated most likely through UmuD2, which was previously shown to interact with RecA (36). So far, there was no demonstration of a direct binding between UmuC and RecA. A report that UmuC was retained on a column of immobilized activated RecA was based on a cell extract as a source of UmuC, and therefore binding could have been mediated via a third protein (37). The binding of UmuD2C along the RecA nucleoprotein filament may be related to the tendency of multiple UmuD2 molecules to form long filaments in crystalline state (38). This property of UmuD2 might be also reflected in its structure in solution (39). To examine whether such filaments might be required for lesion bypass, the following experiments were performed. In addition to the standard substrate with a gap of 350 nucleotides, a DNA substrate with an extended gap of 850 nucleotides was also constructed. A stoichiometric binding of UmuD2 or UmuD2C along the RecA-DNA complex would be reflected in a requirement for higher concentrations of these proteins in the case of the extra large gap. We performed a titration of UmuD2 under a constant concentration of 100 nM UmuC. As can be seen in Fig. 8, maximal bypass on the 350 nucleotides gap was obtained with 100 nM UmuD2. This 3 A. Berdichevsky and Z. Livneh, unpublished data.

Fig. 8. Titration of UmuD2 required to saturate lesion bypass on DNA with large gaps. Reactions were performed on gap-lesion plasmids with a 350-nucleotide gap (GP21) or a 850-nucleotide gap (GP21XL) with 100 nM pol V and the indicated concentrations of UmuD2 for 8 min. For GP21, 2 μM RecA and 50 nM SSB were added, whereas for GP21XL 6 μM RecA and 150 nM SSB were added. ○, GP21XL; □, GP21.

revealed the appearance of beads on a string (see Fig. 5B, above). This is consistent with the notion that under lesion bypass reaction conditions, SSB binds ssDNA in the limited cooperativity mode and may facilitate the formation of a RecA nucleoprotein filament.

If the only function of SSB in bypass is to help assemble the RecA nucleoprotein filament, then bypass should be observed also in the absence of SSB. Similar situations exist for other RecA-catalyzed reactions such as strand exchange. This reaction is stimulated by SSB, but it occurs also in its absence (26). When SSB is absent, however, the concentration of Mg2+ should be kept low to prevent secondary structures from interfering with RecA polymerization on DNA (34). We have therefore performed a preincubation step of RecA and the DNA, in the absence of SSB, under 1 mM Mg2+. Then pol V and UmuD2 were added, and the concentration of Mg2+ was raised to 10 mM to enable optimal activity of the polymerase. No bypass was observed under these conditions (data not shown). Other experimental protocols that were carried out included prolonged preincubation times with RecA prior to the addition of pol V, usage of higher concentrations of RecA, and addition of ATPγS instead of ATP. Under any of these conditions no bypass was observed in the absence of SSB (data not shown). In an attempt to examine whether a RecA nucleoprotein filament was formed in the absence of SSB, we assayed strand exchange between the gapped plasmid and a homologous DNA fragment. We found that RecA promoted strand exchange in the absence of SSB under the same conditions in which no lesion bypass was observed. 3 This indicates that a functional RecA nucleoprotein filament was formed in the absence of SSB. Therefore, it seems that facilitating the formation of a RecA nucleoprotein filament is not the only role for SSB in lesion bypass.
concentration is equimolar with the concentration of UmuC on the one hand, but it is also sufficient to cover the RecA-bound ssDNA region, assuming that one UmuD’2 molecule binds two RecA monomers (Ref. 35; As discussed above, there is 200 nM RecA bound to the ssDNA region). The UmuD’ titration was repeated with a gap of 850 nucleotides, in the presence of a 3-fold higher RecA concentration (because of the larger size of the gap). It was found that the saturating amount of UmuD’2 was the same, 100 nM (Fig. 8), significantly less than the amount required to cover the RecA nucleoprotein filament (at least 240 nM). Moreover, the extent of maximal bypass was similar with both DNA substrates (Fig. 8). This suggests that under our assay conditions, binding of either UmuD’2 or UmuD’2,C along the RecA nucleoprotein filament is not required for lesion bypass.

**DISCUSSION**

Two of the most striking features of the activity of pol V are the ease with which it bypasses lesions that severely block other DNA polymerases and the absolute requirement for RecA (8–12). As discussed before, RecA is known to be required for another DNA damage tolerance mechanism, namely recombinational repair. Numerous studies have documented the binding of RecA to DNA in the form of a long helical nucleoprotein filament, with RecA wrapped around the DNA (reviewed in Refs. 27, 40). The following results argue that the assembly of a RecA nucleoprotein filament is required for lesion bypass by pol V. 1) RecA is required at stoichiometric concentrations in the lesion bypass reaction. 2) Electron microscopy analysis showed the existence of RecA nucleoprotein filaments under our assay conditions. 3) No bypass was found with DNA substrates in which the lesion was located on a ssDNA 100 nucleotides or shorter, on which a stable RecA filament is difficult to assemble.

On gapped DNA, the assembly of the RecA nucleoprotein filament starts in the ssDNA region, and proceeds in the 5’→3’ direction (41). It can then continue to assemble on the double-stranded portion of the DNA (28–30). Based on the amounts of DNA and RecA, there is enough RecA to coat the DNA entirely. This is indeed seen in the electron micrograph in the presence of ATPγS (Fig. 5E). However, under standard reaction conditions, the ssDNA region is covered with RecA whereas the dsDNA region remains largely uncoated. This is not because of the presence of UmuC and UmuD’, because the same results were obtained in their absence. Glutaraldehyde effectively cross-links proteins to proteins and to ssDNA, but not to dsDNA. However, glutaraldehyde treatment does fix the RecA filament on dsDNA, probably because of the special structure of the protein-DNA complex (42). Thus, it seems that the electron micrograph results showing the dsDNA largely uncoated, reflect the situation in solution under reaction conditions.

In the presence of ATPγS, where the entire DNA molecule was covered with RecA, there was a strong inhibition of the activity of pol V, primarily because of a 10-fold reduction in initiation of synthesis. This inhibition of initiation by pol V may have been caused by the tight and extended binding of RecA to DNA in the presence of ATPγS, leading to limited accessibility of pol V to the primer terminus. In addition, the inhibition may have been caused, at least in part, by the altered conformation of RecA filaments in the presence of ATPγS. The ATPγS experiment provides some hints on the dynamics of RecA during the action of pol V. A priori it is possible that the dissociation of RecA takes place only after the polymerization/bypass step. RecA stretches and unwinds the DNA and upon this the ssDNA in the RecA nucleoprotein filament is partially exposed (4, 5). Thus, the access of the polymerase to ssDNA might not be hindered in the presence of RecA. Nevertheless, the inhibitory effect of ATPγS on bypass is consistent with a model in which polymerization requires dissociation of RecA from DNA (Fig. 6).

SSB is known to facilitate the formation of RecA nucleoprotein filaments by removing secondary structures from DNA (27, 40). This occurs in the beaded limited cooperativity mode of binding of SSB to DNA. In this mode, octamers of SSB bind ssDNA forming a beaded structure, representing a binding mode of 65 nucleotides per SSB octamer (43, 44). Such binding allows entry sites for RecA, which then forms filaments via cooperative binding, with the displacement of SSB. Electron micrograph analysis under our reaction conditions revealed the appearance of complexes (Fig. 5B), which were consistent with the beaded structure of SSB-DNA complexes reported by Griffith (43). This supports a role for SSB in facilitating the formation of the RecA nucleoprotein filament required for lesion bypass. We were unable to observe lesion bypass in the absence of SSB. This means one of two possibilities, which are not mutually exclusive. 1) The RecA nucleoprotein filament in the presence of SSB is different from the filament of RecA alone, or 2) SSB has an essential role other than the loading of RecA on DNA. It is possible that mixed RecA-SSB complexes with DNA are required for lesion bypass. Such mixed complexes were previously described (45).

The importance of the proper assembly of a RecA nucleoprotein filament is likely to be the reason for the difference between our results and those of Goodman and co-workers (10). Whereas in our system only UmuC, UmuD’, RecA and SSB were required for lesion bypass (11), in their system the processivity subunits of DNA polymerase III holoenzyme (the β-subunit and the five subunit γ complex) were also required (10). This is not likely to be caused by a contamination of the 6 proteins in our protein preparations, which were highly purified. The extra requirements stem, in our view, from the difficulty in assembling a stable and functional RecA nucleoprotein filament on the particular substrate used by Goodman and his co-workers (10). They used a linear DNA substrate, with the lesion located on a single-stranded region, 50 nucleotides from the 5’-end of the DNA. Because RecA filaments assemble on DNA in a polar 5’→3’ direction (41), this relatively short
stretches of ssDNA 5’ to the lesion may be incompletely covered with RecA, or else the complex may be unstable at the primer terminus. Addition of the processivity proteins presumably stabilized the protein-DNA complex, enabling bypass. This interpretation is supported by their observation that the requirement for processivity proteins was alleviated when ATPγS was used instead of ATP (12). In that case, the presence of ATPγS presumably promoted formation of a stable RecA filament, which could enable bypass to a low extent. Nevertheless, whereas not absolutely required for lesion bypass, the processivity proteins might have a stimulatory effect on pol V.

The inhibitory effect of ATPγS on bypass described above seems to contradict the report of Goodman and co-workers (12), who found that ATPγS stimulated bypass by pol V. This difference stems, most likely, from the difference in the absolute extents of bypass in the two systems. Goodman and co-workers (12) report a stimulation of bypass from a state of no bypass to low bypass, whereas we observed an inhibition from high bypass to low bypass (Fig. 6). Because no quantification was given in their paper for bypass in the presence of ATPγS, it is difficult to make a precise comparison. However, in both laboratories the bypass in the presence of ATPγS was low.

The RecA nucleoprotein filament is a tool used by the cell to protect the integrity of its DNA when replication is blocked at DNA lesions and single-stranded regions are exposed. Coating of the ssDNA with RecA protects it from degradation on the one hand (46) and provides the platform for repairing the gap by recombinational repair and translesion synthesis, on the other hand. Having all these activities require a common intermediate, i.e., the RecA nucleoprotein filament, offers an obvious effective mode of regulation and coordination of these processes. The relationship between recombinational repair and translesion synthesis is not clear. The two mechanisms might compete with each other or there might be a switching mechanism that governs the activities of the two pathways. In this context it should be mentioned that pol V itself was proposed to switch the cellular repair systems from recombination to bypass, by inhibiting recombinational repair (47, 48).

Based on current knowledge, the following model can be drawn for translesion replication (Fig. 9): When a replication fork encounters a blocking lesion, the DNA polymerase stops. At that stage the RecA protein displaces SSB from DNA, forming a RecA nucleoprotein filament. This nucleoprotein filament serves as a substrate for DNA polymerase V. The interaction between pol V and RecA is mediated most likely by UmuD\textsuperscript{2}, which interacts both with RecA and pol V. Pol V then replicates across the damaged site, incorporating a limited number of nucleotides. Because replication of undamaged regions of the DNA is both inefficient (because of the low processivity of pol V) and mutagenic, there is a switch back to error-free and efficient replication by DNA polymerase III holoenzyme. Obviously, this model provides only a preliminary scenario for translesion replication by pol V. Further studies are needed to elucidate the detailed mechanism of action of this fascinating DNA polymerase.

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