

REVIEW ARTICLE

A new model for SOS-induced mutagenesis: how RecA protein activates DNA polymerase V

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Abstract

In *Escherichia coli*, cell survival and genomic stability after UV radiation depends on repair mechanisms induced as part of the SOS response to DNA damage. The early phase of the SOS response is mostly dominated by accurate DNA repair, while the later phase is characterized with elevated mutation levels caused by error-prone DNA replication. SOS mutagenesis is largely the result of the action of DNA polymerase V (pol V), which has the ability to insert nucleotides opposite various DNA lesions in a process termed translesion DNA synthesis (TLS). Pol V is a low-fidelity polymerase that is composed of UmuD'₂C and is encoded by the *umuDC* operon. Pol V is strictly regulated in the cell so as to avoid genomic mutation overload. RecA nucleoprotein filaments (RecA*), formed by RecA binding to single-stranded DNA with ATP, are essential for pol V-catalyzed TLS both *in vivo* and *in vitro*. This review focuses on recent studies addressing the protein composition of active DNA polymerase V, and the role of RecA protein in activating this enzyme. Based on unforeseen properties of RecA*, we describe a new model for pol V-catalyzed SOS-induced mutagenesis.

Keywords: Translesion synthesis; RecA nucleoprotein filament; polymerase transactivation; mutational mechanisms

Introduction

More than 50 years ago, Weigle made the pivotal discovery that UV-irradiated λ phage could be rescued by infection into UV-irradiated *Escherichia coli* (Weigle, 1953). Fourteen years later, Witkin proposed that this phenomenon was evidence of a damage-induced DNA repair system in bacterial cells (Witkin, 1967). An increase in the number of point mutations in the phage was also characteristic of this rescue. Radman then suggested the presence of a “mutation-prone” replication mechanism involving the *lexA* and *recA* gene functions that can account for UV-induced mutations of λ phage and *E. coli*, which he named “SOS repair” (Radman, 1974), and the accompanying mutations after DNA damage came to be known as “SOS mutagenesis”.

The SOS response is triggered by DNA damage caused by exposure to UV irradiation or to chemicals (Witkin, 1976; Walker, 1984). The DNA damage leads to a halt in DNA replication as replication forks encounter unrepaired lesions or lesions undergoing repair (Setlow *et al.*, 1963; Friedberg *et al.*, 2006). The primary task of the SOS response is to restart replication before the cell dies. This system is regulated by both the LexA transcriptional repressor and the RecA recombinase (Figure 1).

There are more than 40 genes known to be involved in SOS repair (Fernandez de Henestrosa *et al.*, 2000; Courcelle *et al.*, 2001). Most of these genes are induced rapidly and are involved in error-free DNA repair, including base excision repair (BER), nucleotide excision repair (NER), and recombinational DNA repair (Friedberg *et al.*, 2006). If the DNA damage levels are so great that error-free

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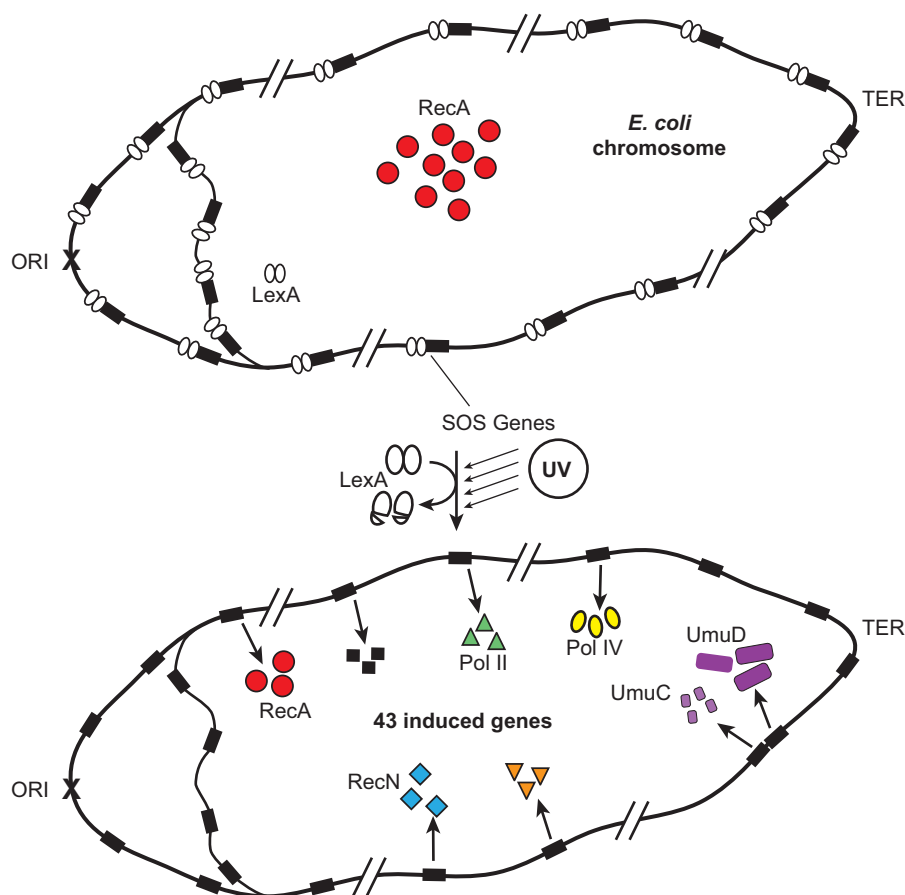


Figure 1. The SOS response in *Escherichia coli*. The system includes at least 43 known genes. Only a subset of these are shown. Under normal growth conditions, the SOS genes are repressed by the dimeric transcriptional repressor, LexA. Upon exposure to DNA damage, such as ultraviolet radiation, the LexA repressor is autocatalytically cleaved in a reaction involving the RecA protein, and the SOS genes are induced.

pathways are insufficient to complete repair and restart replication, the mutagenic phase of SOS is triggered (Walker, 1984; Echols and Goodman, 1990). This phase of the SOS response is mediated by DNA polymerases that replicate past template lesions, in a process called translesion DNA synthesis, or TLS (Goodman, 2002). TLS is inherently inaccurate, and is responsible for the large increase in mutations originally observed by Weigle.

There are three SOS-induced DNA polymerases in *E. coli*, pols II, IV and V, and all are engaged in various aspects of TLS (Goodman, 2002). It is pol V, encoded by the *umuDC* operon, that is largely responsible for the ~100-fold increase in DNA damage-induced chromosomal mutations (Kato and Shinoura, 1977; Steinborn, 1978; Woodgate, 1992). SOS mutagenesis can be considered a kind of desperation response. The mutations that occur may kill many cells. However, replication is successfully restarted, and the “lucky” cells survive.

Certain mutations in the LexA repressor, the RecA recombinase, and the UmuDC proteins abolish SOS mutagenesis. Since LexA and RecA are regulators of the SOS response, it is understandable that mutations in

these genes might render the cell non-mutable through their inability to induce the SOS response. The *umu* genes prove to be more interesting in that they do not affect other aspects of the SOS response and are thus uniquely involved only in SOS mutagenesis. Another intricacy in the system is that the RecA protein has at least three genetically separable roles in SOS mutagenesis. These include mediating self-cleavage of the LexA repressor leading to the subsequent derepression of all LexA-regulated genes, mediating self cleavage of UmuD to a shorter and mutagenically active form called UmuD' (Burckhardt *et al.*, 1988; Shinagawa *et al.*, 1988), and a direct role in SOS mutagenesis (Nohmi *et al.*, 1988; Dutreix *et al.*, 1989; Sweasy *et al.*, 1990).

The UmuC protein and dimeric UmuD'₂ interact (Woodgate *et al.*, 1989; Bruck *et al.*, 1996) to form error prone DNA polymerase V (pol V) (Reuven *et al.*, 1999; Tang *et al.*, 1999). DNA polymerase V inserts nucleotides opposite a variety of DNA lesions to facilitate TLS (Tang *et al.*, 2000). Since pol V is a low-fidelity polymerase it quite often promiscuously inserts incorrect bases opposite of the lesion, thereby explaining the observed

increase in damage-induced SOS mutagenesis *in vivo* (Tang *et al.*, 2000).

An active RecA nucleoprotein filament, RecA* is required for activation of pol V-catalyzed TLS both *in vivo* (Dutreix *et al.*, 1989; Sweasy *et al.*, 1990) and *in vitro* (Tang *et al.*, 1998; 1999; Reuven *et al.*, 1999; Fujii *et al.*, 2004; Schlacher *et al.*, 2006a; Jiang *et al.*, 2009). RecA* is formed in the presence of ATP by RecA binding to single-stranded DNA gaps. Such gaps are formed at stalled replication forks, at sites where lesions are simply bypassed (Heller and Marians, 2006; Marians, 2008), and at sites of intensive DNA repair throughout the genome. As detailed below, the filaments need not be in the same genomic location as the site where pol V action is required (Schlacher *et al.*, 2006a; Jiang *et al.*, 2009).

Irrespective of their location in the cell, these active RecA* filaments are essential for pol V-catalyzed TLS. The exact role for this requirement has only recently been discovered (Jiang *et al.*, 2009). The term RecA* has historically been associated with RecA filaments that were active in the promotion of SOS induction and SOS mutagenesis. In this article, we associate the term with active RecA filaments bound to DNA in the presence of ATP or an ATP analog, filaments that are active for the complete range of activities normally associated with RecA.

This review centers on our understanding of three proteins: RecA, UmuC, and UmuD', and their actions and roles in SOS mutagenesis. We address two longstanding, enigmatic aspects of SOS mutagenesis: the molecular composition of mutagenically active pol V and the precise role of RecA* in activating pol V for DNA synthesis. Based on the properties of RecA* during pol V activation, we describe a new model to account for the biochemical basis of SOS-induced mutagenesis (Jiang *et al.*, 2009).

The RecA protein

Proteins homologous to RecA in structure and function are ubiquitous in all classes of life. The archaea homolog is called Rada (Seitz *et al.*, 1998; Yang *et al.*, 2001; Wu *et al.*, 2004). In eukaryotes, there are two RecA homologs, the Rad51 and Dmcl proteins (Bishop *et al.*, 1992; Shinohara *et al.*, 1992; Sung, 1994). The *recA* gene was initially identified and characterized genetically in a screen for recombination deficient mutants of *E. coli* (Clark and Margulies, 1965). It was quickly found to have multiple roles in recombination and repair; for a review see Clark and Sandler (1994).

The RecA protein is a 352 amino acid polypeptide with a molecular weight of 38 kDa. In uninduced cells, it is present at less than 10,000 monomers per cell. Upon SOS induction the level of RecA can increase to over 70,000 monomers per cell (Sommer *et al.*, 1998). The bacterial RecA protein is a DNA-dependent ATPase. The

RecA of *Escherichia coli* hydrolyzes ATP at a rate of 20–30 per minute, depending on the nature of the bound DNA (Weinstock *et al.*, 1981; Pugh and Cox, 1987). RecA binds to DNA as a nucleoprotein (np) filament that forms most rapidly onto single-stranded DNA.

Filaments are formed in two steps, with a slow nucleation followed by a more rapid, cooperative extension of the filament in the 5' to 3' direction (Register and Griffith, 1985; Shan *et al.*, 1997; Kuzminov, 1999) (Figure 2); however, there is recent evidence for slower filament growth in the opposite direction on DNA at a single molecule level (Galletto *et al.*, 2006; Joo *et al.*, 2006). Five monomers of RecA are sufficient for nucleation (Joo *et al.*, 2006). Filament formation requires ATP binding, but not ATP hydrolysis. When ATP is hydrolyzed, it causes filament disassembly, also in the 5' to 3' direction (Shan *et al.*, 1997; Arenson *et al.*, 1999; Bork *et al.*, 2001). The footprint of RecA on DNA is one monomer of RecA per three nucleotides of DNA. The bound single stranded DNA is extended by 50% and underwound so that there are 18 base pairs, or six RecA monomers, per helical turn (Egelman and Stasiak, 1986; Yu and Egelman, 1992; Stasiak and Egelman, 1994). There are three binding sites for DNA strands within the np-filament and these sites facilitate the strand exchange reaction (Takahashi *et al.*, 1991; Takahashi

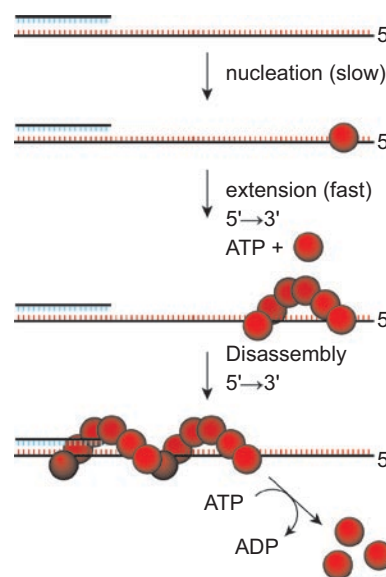


Figure 2. Assembly and disassembly of RecA protein filaments. RecA filament nucleation is the slow step, normally occurs on single-stranded DNA, requires ATP, and involves an oligomer with 4–5 RecA subunits in it. Extension of the filament occurs rapidly in the 5' → 3' direction by addition of RecA subunits. Extension will continue into adjacent regions of double stranded DNA. Disassembly also occurs in the 5' → 3' direction, and from the opposite end of the filament. Disassembly is coupled to ATP hydrolysis. RecA filaments formed on any DNA in the presence of ATP are active in activating DNA polymerase V, and have been designated RecA*.

and Norden, 1995; Kurumizaka and Shibata, 1996; Kubista *et al.*, 1996).

RecA has four known functions in the *E. coli* cell: catalyzing the DNA strand exchange reaction in the context of recombinational DNA repair, induction of the SOS response by promotion of the autocatalytic cleavage of the LexA repressor, activation of UmuD' by mediating autocatalytic cleavage of UmuD, and direct participation in SOS mutagenesis by activation of DNA polymerase V (Schlachter *et al.*, 2006b; Jiang *et al.*, 2009) (Figure 3).

All RecA-class recombinases promote a DNA strand exchange reaction that is central to their function in recombinational DNA repair. Once formed, the active nucleoprotein filament (RecA*) aligns the bound single

strand with homologous sequences in a different duplex DNA. It then promotes a strand exchange reaction in which one strand of the duplex is transferred to the bound single strand to create a new duplex (Figure 3A). The second strand from the original duplex is displaced. There are many standard assays to investigate this phenomenon *in vitro* (Cox, 2003).

The SOS response is normally suppressed by the LexA repressor protein. LexA binds to the operator regions of the genes that are involved in SOS and thus hinders their expression. RecA is present at constitutive levels in the cell in order to function during homologous recombination. In cells exposed to DNA damage, RecA is induced to attain much higher levels. RecA* filaments formed on free ssDNA or, perhaps much more likely, at single strand gaps, serve to turn on the SOS response. RecA* promotes this second cellular function – SOS induction – by facilitating the autocatalytic cleavage of the LexA repressor (Little, 1984) (Figure 3B). In principle, the RecA* filaments are located at any site where a single strand gap appears in the genome. Such gaps are typically associated with DNA lesions, occurring where lesions are being repaired (e.g., small gaps created during DNA excision repair or mismatch repair), where lesions are bypassed during replication (Heller and Mariani, 2006; Mariani, 2008), or where replication forks are stalled.

There are differential degrees of repression based on the binding affinity of LexA to the operator regions of the genes, which exhibit considerable variability outside a shared consensus sequence (Little and Mount, 1982; Walker, 1984; Lewis *et al.*, 1994; Fernandez de Henestrosa *et al.*, 2000). LexA binds weakly to the *recA* operator; therefore, RecA is induced “immediately,” <1 min after exposure to high UV doses (Figure 3B). *In vitro*, under specific conditions, LexA is able to autocleave in the absence of RecA* (Little, 1984; 1991). However, this is unlikely to occur under physiological conditions and LexA is only cleaved upon contact with RecA* *in vivo*. LexA binds deep within the helical groove of the nucleoprotein filament and is cleaved into two inactive fragments rapidly upon contact with the filament (Little *et al.*, 1980; Yu and Egelman, 1993). As the amount of LexA protein is reduced, dissociation of LexA from the operator regions leads to induction of the LexA (SOS)-regulated genes (Figure 1). Therefore, the genes with the highest affinity LexA binding sites lose the LexA repressor last and are induced later in the response. Among these genes are *umuC* and *umuD* that act in SOS mutagenesis, which are turned on ~30 min after exposure to UV (Sommer *et al.*, 1998).

However, despite being expressed, UmuD is inactive for SOS mutagenesis (Nohmi *et al.*, 1988). The protein is activated for its mutagenesis functions after its N-terminal 24 amino acid tail is removed in a RecA-mediated self cleavage reaction that is mechanistically similar to LexA

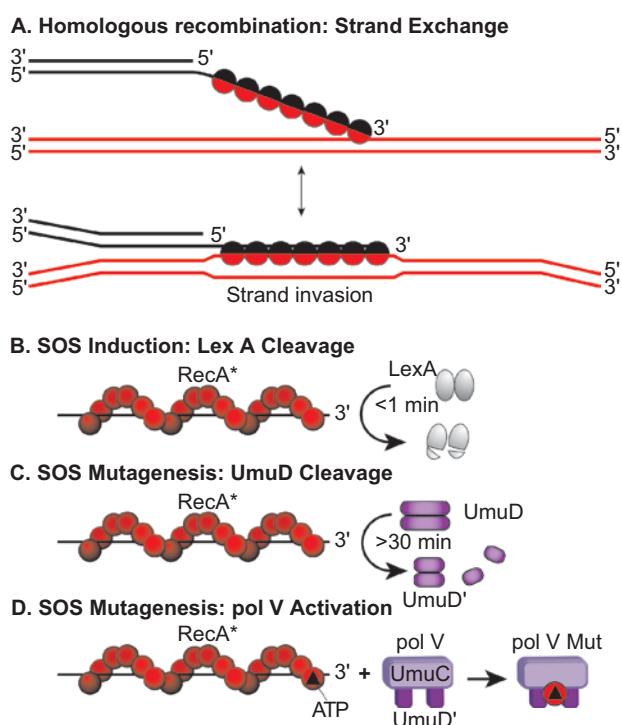


Figure 3. The four cellular functions of the RecA protein in *Escherichia coli*. (A) RecA protein is involved in many aspects of recombinational DNA repair, and promotes a variety of DNA strand exchange reactions in the context of this function. One key type of reaction is shown – DNA strand invasion. In this process, RecA forms a filament (RecA*) on the 3' end of a single-stranded DNA, aligns that DNA with its complement in a duplex DNA, and pairs the bound DNA with the complementary strand of the duplex (displacing the other duplex strand). (B) The autocatalytic cleavage of LexA repressor occurs rapidly in the presence of RecA* filaments. It is the number and extent of filament formation that governs the efficiency of LexA auto cleavage. As a result of excessive DNA damage, the number of single strand DNA gaps is increased. LexA cleavage and the accompanying SOS system induction generally occur only in response to such damage. (C) RecA* is required for the autocatalytic cleavage of the UmuD protein to form active UmuD'. This reaction is much slower than LexA cleavage and occurs >30 min after DNA damage. (D) A RecA monomer and a molecule of ATP are transferred to pol V from the 3'-proximal end of a RecA* filament to form the active pol V Mut.

cleavage (Burckhardt *et al.*, 1988; Nohmi *et al.*, 1988; Shinagawa *et al.*, 1988; Paetzel and Woodgate, 2004). UmuD cleavage is much less efficient than LexA cleavage (Burckhardt *et al.*, 1988) and as a consequence, UmuD' does not accumulate in the cell until some 45 minutes post UV-irradiation (Sommer *et al.*, 1998). Removal of the N-terminal tail from UmuD presumably leads to a conformational change in the structure of the protein that allows dimeric UmuD' to form a tight complex with UmuC (UmuD'₂C) (Woodgate *et al.*, 1989; Bruck *et al.*, 1996) (Figure 3C).

Even when recombinant UmuD'₂C was fully derepressed in a $\Delta recA$ background, no UV-induced mutagenesis was observed, implying that RecA plays a direct role in the mutagenic process (Nohmi *et al.*, 1988). However, $\Delta recA$ strains are extremely UV-sensitive and the lack of any detectable UV-induced mutagenesis might have simply arisen through the lack of bacteria surviving irradiation. To circumvent these problems, Devoret and colleagues isolated a missense *recA* mutant, *recAS117F* (*recA1730*), that was defective in UV-induced SOS mutagenesis (Dutreix *et al.*, 1989). When expressed in a *lexA*⁺ background, the *recAS117F* allele was defective in most RecA functions. However, overexpression of the mutant protein in a *lexA*(Def) background helped it regain its recombination activity, yet the strain remained non-mutable upon UV-treatment, even in the presence of recombinant *umuD'*C genes on a plasmid. These observations confirmed the notion that there is a direct requirement for RecA* in SOS mutagenesis, independent of its role in LexA and UmuD cleavage. The theory was further cemented by strains that overproduced UmuD'₂C, in which LexA and UmuD processing was not required, but mutagenesis was still distinctly dependent on the specific *recA* allele present (Sweasy *et al.*, 1990).

This "third role" of RecA in SOS mutagenesis remained enigmatic for many years. However, it has recently been shown that RecA is required to directly activate the UmuD'₂C polymerase, by transferring a molecule of RecA and ATP to form an activated pol V mutasome, UmuD'₂C-RecA-ATP (Jiang *et al.*, 2009) (Figure 3D). The elucidation of the direct role of RecA in SOS mutagenesis will comprise the take-home message from this review.

The Umu proteins

The *umuDC* genes were identified as encoding key components in SOS mutagenesis in the late 1970s (Kato and Shinoura, 1977; Steinborn, 1978; 1979). In a direct genetic screen for SOS mutagenesis, they were the only genes identified that solely affected mutagenesis and not other aspects of SOS repair (Kato and Shinoura, 1977).

The *umuDC* genes are regulated at the transcriptional level by LexA and at the post-translational level

via RecA-mediated cleavage of UmuD to mutagenically active UmuD'. In addition, intracellular levels of the Umu proteins are kept to a minimum through regulated proteolysis. Both UmuC and UmuD are rapidly degraded by the Lon protease (Frank *et al.*, 1996; Gonzalez *et al.*, 1998). UmuD' is largely resistant to the actions of Lon, but is, instead, rapidly degraded by the ClpXP protease when in a heterodimeric complex with UmuD (Frank *et al.*, 1996; Gonzalez *et al.*, 2000). In an uninduced cell, it is estimated that there are approximately 200 molecules of UmuD and only 15 molecules of UmuC per cell (Woodgate and Ennis, 1991). Upon DNA damage, steady-state levels of the two proteins increase and UmuD is converted to UmuD'. However, even when maximally expressed some 45 min after DNA damage, there are at most 60 molecules of UmuD'₂C in a cell (Sommer *et al.*, 1998). Presumably such regulation is designed to keep the mutagenically active UmuD'₂C (pol V) complex to an absolute minimum, such that it is only utilized under dire circumstances.

Early models proposed that these genes were accessory factors required by the replicative polymerase, pol III, to bypass lesion sites in the DNA (Figure 4). These models were based on the observation that temperature sensitive strains carrying a mutation in the gene that encodes the α subunit of pol III had fixed UV-induced mutations when grown at permissive, but not restrictive, temperatures (Bridges and Mottershead, 1976).

Direct biochemical analysis of the Umu proteins was made difficult owing to the insoluble nature of UmuC. For many years, only a minimal amount of UmuC protein was recoverable by traditional means of purification, hampering efforts in several laboratories. The protein formed inclusion bodies and the earliest protocols involved refolding the protein in the presence of chaperones, after application of harsh denaturing conditions (Woodgate *et al.*, 1989; Petit *et al.*, 1994). *In vitro* reconstitution of TLS was made feasible when a more efficient method of purification was formulated. UmuC was expressed in a soluble form by purifying it as a complex with a dimer of UmuD' (Bruck *et al.*, 1996). This yielded much larger amounts of the UmuD'₂C complex. The new protocol also held the advantage of not requiring treatment by strong denaturants, which can negatively affect the enzymatic activities of any protein. Shortly after reconstitution of the system, it was discovered that UmuD'₂C was a bona fide polymerase (Reuven *et al.*, 1999; Tang *et al.*, 1999). DNA synthesis was observed in the presence of UmuD'₂C and RecA protein, but in the absence of pol III (Tang *et al.*, 1998; 1999). This new polymerase, termed pol V (Reuven *et al.*, 1999; Tang *et al.*, 1999), was able to synthesize past many different lesions in DNA (Tang *et al.*, 2000).

E. coli pol V is one of the founding members of a new class of polymerases: the Y-family polymerases (Ohmori *et al.*, 2001). The Y-family polymerases can be classified

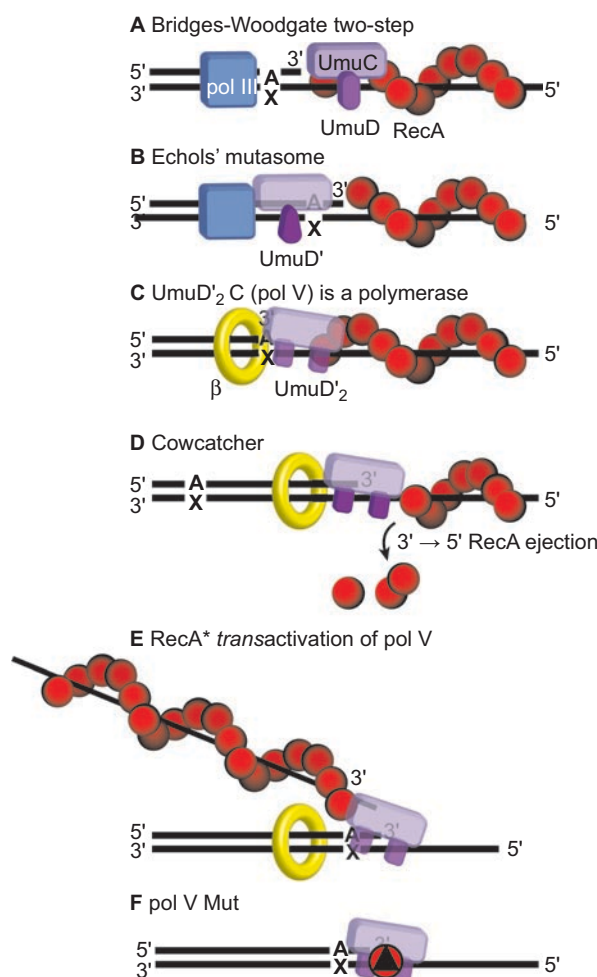


Figure 4. Translesion synthesis (TLS) models. (A) The Bridges-Woodgate two-step model. In this model, translesion synthesis (TLS) is catalyzed by DNA polymerase (pol) III. DNA pol III (which requires RecA in a first step) inserts a nucleotide opposite a template lesion (for example, A opposite X) and, in a second step, also copies past the lesion, which requires the UV mutagenesis gene products UmuDC. (B) The Echols' mutasome model. A multiprotein complex that includes UmuC, UmuD' and DNA pol III holoenzyme is recruited to a DNA lesion by a RecA nucleoprotein filament. The mutasome complex enables replication to take place across the lesion, which results in mutations. (C) UmuD'₂C (pol V) is a DNA polymerase. An *in vitro* system composed of UmuD'₂C, RecA and the β -sliding clamp carries out TLS in the absence of DNA pol III. The UmuD'₂C complex (designated DNA pol V) was shown to have intrinsic DNA polymerase activity in its UmuC subunit. (D) The cowcatcher model. The presence of a RecA nucleoprotein filament proximal to a lesion blocks TLS that is mediated by DNA pol V. Analogous to a cowcatcher on the front of a locomotive, DNA pol V in the presence of single-strand binding protein (SSB, not shown) removes RecA in a 3' \rightarrow 5' direction ahead of the advancing DNA pol V to allow TLS to occur. (E) RecA* transactivation of DNA pol V model. TLS requires DNA pol V to be activated by interacting with the 3'-proximal tip of RecA bound to a separate single-stranded (ss) DNA molecule in trans. A proficient transactivating RecA nucleoprotein filament is formed on gapped DNA. (F) Pol V Mut model. RecA* is required to directly activate pol V by transferring a molecule of RecA ("red circle" and ATP "black triangle" from the 3'-proximal tip of RecA* to form an activated pol V mutasome, UmuD'₂C-RecA-ATP, which catalyzes TLS in the absence of RecA*.

based upon phylogenetic relationships. In addition to *E. coli* UmuC, the Y family includes polymerases related to *E. coli* DinB (pol IV/Dpo4/pol κ), and eukaryotic Rev1 and Rad30/pol η and pol ι (Goodman and Tippin, 2000; Ohmori *et al.*, 2001; Goodman, 2002). These polymerases all lack a 3' \rightarrow 5' proofreading exonuclease activity, and also lack the 5' \rightarrow 3' nick translation activity of *E. coli* DNA polymerase I (Goodman and Tippin, 2000; Goodman, 2002). They are usually weakly processive and have low fidelity. Structurally, they have stubby "finger" and "thumb" domains and their active sites provide fewer constraints for base pairing (Ling *et al.*, 2001; Silvian *et al.*, 2001; Yang and Woodgate, 2007).

These polymerases have a variety of roles in the cell including the extension of mismatched primer ends in the case of pol IV and involvement in immunoglobulin hypermutation in the case of human pol η (Goodman, 2002). Owing to their low fidelity, when left unchecked, these polymerase activities can have deleterious consequences for the cell. DNA pol V makes base substitution errors with a frequency of 10^{-2} – 10^{-3} on undamaged DNA (Tang *et al.*, 2000). *In vitro*, it preferentially misincorporates dG opposite the 3' T of a TT-(6-4) photoproduct (Tang *et al.*, 2000), consistent with the profile of SOS mutations observed *in vivo* (LeClerc *et al.*, 1991; Szekeres *et al.*, 1996).

Models for pol V/RecA interactions

Our understanding of the role of RecA* in TLS has gone through many iterations over a period of 25 years. Even in the pre-pol V era, there were several different proposals for the role of RecA* in TLS. Shortly after the discovery of the *umuDC* genes, a two-step model for TLS was proposed by Bridges and Woodgate, which incorporated pol III, UmuDC, and RecA (Figure 4A). In the first step, pol III would incorporate a nucleotide opposite of the lesion with the help of RecA. This would be followed by lesion bypass by pol III with the help of UmuDC (Bridges and Woodgate, 1985). In 1990, Echols proposed that a multi-protein "mutasome", including UmuD'C, pol III, and RecA is assembled to bypass a DNA template lesion (Echols and Goodman, 1990) (Figure 4B). Once a small amount of purified UmuC was available, detectable levels of TLS were generated in a reconstituted system *in vitro* with UmuD'C, pol III, and RecA (Rajagopalan *et al.*, 1992).

These early models incorporated pol III for copying of DNA past the lesion. RecA* was assembled *cis* in these models, as a filament on the template ahead of the lesion. This expectation was built on the known properties of the RecA protein, which nucleates the formation of a RecA* filament most readily in single-stranded DNA gaps and was not known to have any function when not part of a nucleoprotein filament. The UmuD'C proteins

were predicted to be mutagenic factors to help pol III synthesize past the lesion site.

The discovery that UmuD'₂C was an error-prone polymerase (Reuven *et al.*, 1999; Tang *et al.*, 1999), removed pol III from the translesion synthesis models (Figure 4C). The first model for pol V function proposed that pol V carried out TLS in the presence of the β -sliding clamp, but in the absence of pol III (Tang *et al.*, 1998). It was clear that RecA* was still required, and the most plausible location for RecA* was in the single strand gap downstream of the advancing pol V. Thus, this model still relied upon RecA* in *cis*, on the template containing the lesion as proposed in the earlier efforts with pol III (Echols and Goodman, 1990).

The idea of positioning RecA* in *cis*, downstream of pol V, requires a pol V capacity not only to interact with the RecA*, but to displace it as it advanced. The proposed activity would require the ejection of RecA* subunits from the end of the RecA filament where disassembly does not normally occur. The capacity of pol V to displace a RecA* filament was called into question when substantial inhibition of pol V movement was demonstrated in the presence of the filaments (Pham *et al.*, 2001). However, some DNA synthesis did occur when the single stranded DNA binding (SSB) protein was included in the reaction. A model was presented in which pol V, in the presence of SSB, acting similar to a cowcatcher on the front of a locomotive, pushed the impeding RecA molecules off the DNA template in front of the oncoming pol V (Pham *et al.*, 2001) (Figure 4D).

The effects of RecA* remained enigmatic, and the activity of pol V was difficult to decipher. In particular, primer utilization by pol V was generally poor, rarely above 20 to 30% when filaments were formed with ATP γ S, wherein the filaments could assemble but filament disassembly, which requires ATP hydrolysis, was hindered. Although the best reactions were observed with RecA levels that approximated optimal filament formation, 1 RecA protein per 3–5 DNA nucleotides (nt) (Pham *et al.*, 2002; Schlacher *et al.*, 2006b), a significant TLS efficiency was observed even at ~1 RecA/50 DNA nt, where it was unlikely that significant filament assembly would occur (Pham *et al.*, 2002; Schlacher *et al.*, 2006b) (Figure 5). Higher concentrations of RecA were highly inhibitory (Pham *et al.*, 2002). We viewed these data as a potential “fly in the *cis* RecA* filament ointment”.

Since high levels of RecA protein clearly impeded pol V-mediated DNA synthesis, and extremely low levels of RecA protein were able to support TLS, the models were developed further. It was proposed that short RecA protomers could take part in forming a minimal mutasome by binding to DNA pol V (Schlacher *et al.*, 2005). The model was supported by the observation that RecA binds directly to DNA pol V in the absence of DNA, and

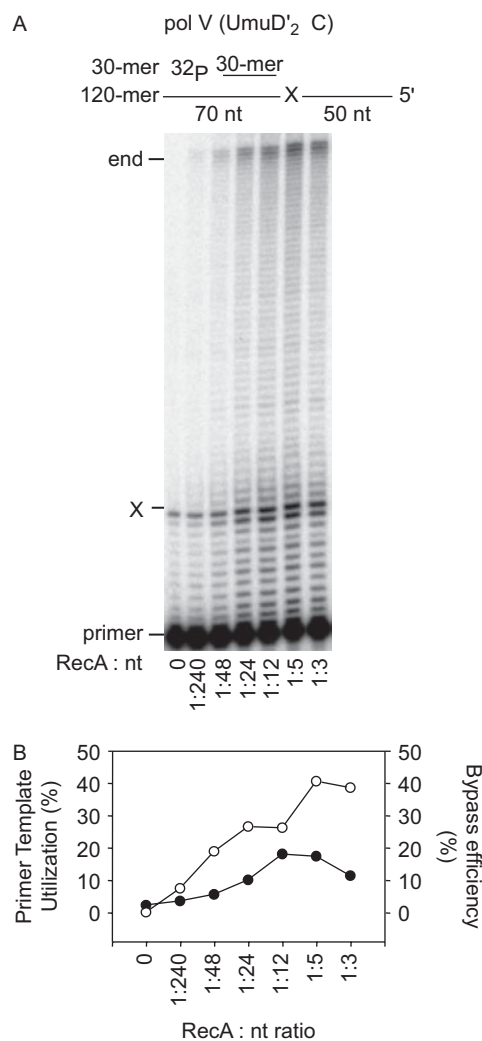


Figure 5. “Fly in the *cis* RecA* filament ointment”. (A) Pol V (UmuD'₂C) synthesis and TLS measured on a 64 nt ssDNA template overhang containing an abasic lesion, X, located 50 nt from the 5'-template end, in the presence of ATP γ S, SSB, and different RecA/DNA nt ratios, as indicated. (B) Primer utilization (●) and lesion bypass efficiencies (○) calculated from the data in Figure 5A. Although a much more robust lesion bypass reaction occurs when conditions are optimal for filament assembly with ~1 RecA molecule per three nucleotides, Pol-V-catalyzed TLS is clearly observed at a stoichiometry of ~1 RecA molecule per 50 nucleotides, conditions under which RecA* formation is absent.

binds by a separate mechanism in the presence of DNA (Schlacher *et al.*, 2005). In all of these efforts, full primer utilization was never achieved by pol V, either in the absence of β , γ complex (Figure 5), or when polymerase accessory proteins were present in the reaction (Pham *et al.*, 2001; Schlacher *et al.*, 2006b). Pol V was instead thought to either be poorly functional or to contain a relatively high level of inactive protein in the pure preparations of UmuD'₂C.

The pol V was not inactive. It was simply being impeded by the RecA* that was bound to many (but not all) of the added primer/templates. The experiments

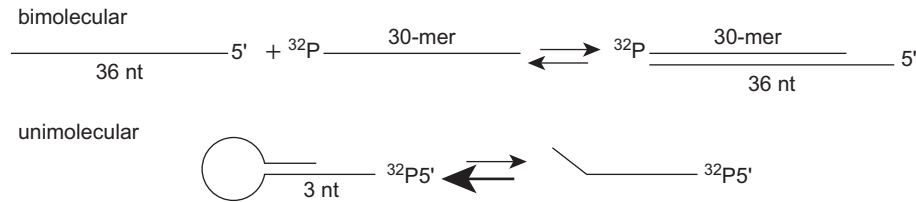


Figure 6. Kinetics of primer/template annealing. When forming primer/template DNA molecules by mixing single-stranded DNA primers and templates and then allowing them to anneal in a bimolecular reaction, the presence of ssDNA on which a RecA filament can assemble cannot be avoided. There will always be un-annealed template and primer DNA, and therefore an indeterminate amount of single stranded DNA in *all* experiments utilizing annealed primer/template substrates. A simple way to eliminate excess ssDNA is to form a stable hairpin structure so that almost all of the DNA anneals in the form of a hairpin, in a unimolecular reaction, with a very small proportion of non-hairpin ssDNA remaining. By having a short template overhang (3 nt), RecA filament assembly on the hairpin primer/template DNA is eliminated, because the footprint of a single RecA monomer is 3 nt.

above were all carried out in the context of an unrecognized and unappreciated ambiguity. If RecA protein is added to any kind of artificial primer/template along with UmuD'₂C, it is impossible to know if RecA* is acting in *cis* or in *trans*. Optimization of the polymerase reaction required RecA protein concentrations that were sufficient to bind only some of the template/primers. RecA* assembly occurs as a strongly cooperative binding of RecA to ssDNA (Kuzminov, 1999). At the RecA concentrations that were optimal for pol V activation, filament assembly is heterogeneous. Consequently, some DNA molecules will form RecA*, others not. Were the RecA* filaments promoting pol V-mediated DNA synthesis on the same DNA substrates to which they were bound (in *cis*), or were they promoting DNA synthesis in *trans* on other DNA substrates that were not bound by RecA*?

Where could the *trans* RecA* molecules come from? When forming primer/template molecules by mixing single-stranded DNA primers and templates and then allowing them to anneal in a bimolecular reaction, there will always be un-annealed template and primer DNA (Figure 6). It is thermodynamically unavoidable. Therefore, there were indeterminate amounts of single stranded DNA in *all* experiments utilizing annealed primer/template substrates. RecA* can, and doubtless does, form on the un-annealed single strands as well as on the annealed primer/templates. There is, however, a simple way to eliminate excess ssDNA, and that is to form a stable hairpin structure so that almost all of the DNA anneals in the form of a hairpin, in a unimolecular reaction, with a very small proportion of non-hairpin ssDNA remaining (Figure 6). By having a short template overhang (3 nt), one also hinders RecA filament assembly on the hairpin primer/template DNA.

A simple alteration of the experimental protocol soon demonstrated that the RecA* was functioning in *trans* (Schlacher *et al.*, 2006a) (Figure 4E). Full primer utilization was observed in a pol V reaction for the first time when pol V was incubated with hairpin template primer, RecA* filaments were formed on single stranded oligomeric DNA (36-mers) in a separate test tube, and the two

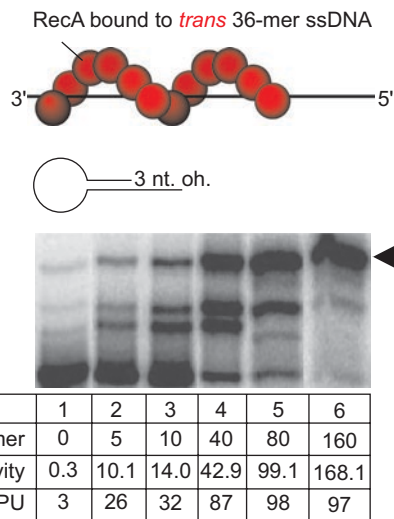


Figure 7. Pol V transactivation by RecA*. Pol V-catalyzed primer utilization when copying a hairpin containing a 3-nt-long template overhang was measured in the presence of ATPγS and RecA (2 μM) at varying *trans* ssDNA (36-mer) concentrations (in nM). The specific activity of pol V is measured in pmol DNA per μg pol V per min. The arrowhead indicates the position of the full-length product. “%PU” represents the fraction of primer utilized, i.e. extended by at least 1 nt.

were subsequently mixed (Figure 7). The improvements in reaction efficiency were dramatic; a RecA* filament was indeed necessary for TLS by pol V, but the reaction worked best when the RecA* was on a different molecule of DNA than the one pol V was acting on (Schlacher *et al.*, 2006a) (Figures 4E and 7). The reaction exhibited second order kinetics, the velocity increasing linearly with the addition of *trans* RecA* and extrapolating to zero in the absence of *trans* 36 mer DNA. In addition, pol V is activated by interacting with the 3'-proximal tip of RecA bound to that single-stranded (ss) DNA molecule in *trans* (Schlacher *et al.*, 2006a). This mechanism began to resolve the paradox that *cis* RecA filaments obstruct TLS despite the absolute requirement of RecA* for SOS mutagenesis.

What remained un-contemplated is that the role of RecA* might actually be indirect; that it was not interaction with a RecA* filament, be it located either in *trans* or in *cis* that activated pol V, but the incorporation of a RecA monomer that was transferred to the UmuD'₂C from the RecA* filament. This brings us to the current understanding of the role of RecA* in TLS (Jiang *et al.*, 2009). RecA* transfers one ATP-bound RecA subunit from its 3'-proximal end to UmuD'₂C to form an active mutasome with the composition UmuD'₂C-RecA-ATP, i.e. pol V Mut (Jiang *et al.*, 2009) (Figure 4F). The active pol V Mut complex synthesizes DNA in the absence of RecA* (Jiang *et al.*, 2009). Echols' mutasome is now re-envisioned with the minimal necessary components being UmuD'₂C, ATP, and RecA. Thus, the previously indeterminate 3rd role of RecA* in SOS mutagenesis is to transfer a RecA monomer and ATP from its 3'-proximal tip to pol V and activate pol V for DNA synthesis (Jiang *et al.*, 2009) (Figure 4F).

Discovery of pol V Mut

Since the late 1980s the most important but unmanageable questions in SOS mutagenesis were "what is the molecular composition of the pol V mutasome?" and "what is RecA* doing?" The answers to the above questions have always been obscured by the ambiguities inherent in standard experimental protocols as described above, namely the location of RecA* on a primer/template being copied, or on one not being copied.

Further refinement of the experimental protocol allowed a definitive test of RecA* function in TLS. RecA* filaments were formed on biotinylated single strand oligonucleotides that were linked to streptavidin-coated agarose resin to aid in the removal of RecA* when necessary. Stable RecA* filaments were formed on the linked oligonucleotides in the presence of ATP γ S. UmuD'₂C was incubated with the RecA* resin in the absence of any primer/template. The RecA* was then removed by centrifugation, leaving pol V in the supernatant. When primer/template was subsequently added to the experiment, efficient primer extension mediated by pol V was observed (Jiang *et al.*, 2009). When RecA* bound to resin was preincubated and centrifuged prior to the addition of pol V and primer/template, there was very little reaction by pol V, demonstrating that the centrifugation was effective in removing the active RecA* from the reaction (Jiang *et al.*, 2009).

In the supernatant present after centrifugation of RecA* and prior to the addition of primer/template, UmuD'₂, UmuC, and RecA were found in a 1:1:1 ratio. ATP was also in the complex, stoichiometric with the three protein components. The amounts of RecA present were linearly related to the amount of UmuD'₂C added

to the pre-incubation with the beads, consistent with a stoichiometric transfer of RecA subunits from the RecA* to pol V (Jiang *et al.*, 2009).

The multi-protein pol V Mut complex was resolved and quantitated using laser multi-angle light scattering (MALS) coupled with size exclusion chromatography (Figure 8). MALS allows an independent measurement of the absolute molar mass of a complex (Wyatt, 1993). The size exclusion peak that included RecA, as well as UmuD'₂C, had a molar mass of 113 kDa, in good accord with the value predicted for UmuD'₂C•RecA, 110 kDa (Figure 8). A second peak that did not include RecA had a molar mass of 73 kDa, consistent with the predicted molecular weight of UmuD'₂C alone, 72 kDa (Jiang *et al.*, 2009). The pol V mutasome foreseen by Hatch Echols and colleagues is a UmuD'₂C-RecA-ATP complex that is active even when RecA* is not to be found near the DNA that is being synthesized.

The ongoing *cis* versus *trans* issue

A recent report continues to attribute *cis* RecA* stimulation of pol V (Fujii and Fuchs, 2009). Instead, we suggest that the data presented can be interpreted as *trans* stimulation by virtue of the ambiguities already

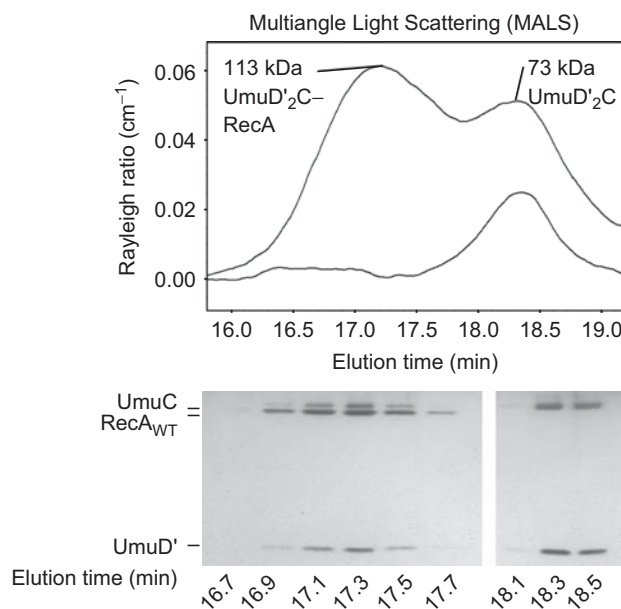


Figure 8. Determination of the molecular mass of pol V Mut by MALS. In the upper panel, after RecA*-mediated transactivation of UmuD'₂C and removal of RecA*, the mixture of pol V Mut and non-activated pol V was resolved by size-exclusion chromatography (upper trace), and the molecular mass corresponding to each peak was measured by multiangle light scattering (MALS). Non-activated pol V run separately on the silica gel elutes at 18.4 min (lower trace). In the lower panel, silver-stained SDS-polyacrylamide gel shows the protein composition from the two peaks contained in the upper panel (upper trace).

noted. The presence of ssDNA cannot be avoided when primer/template DNA is formed by annealing primer and template strands (Schlachter *et al.*, 2006a) (Figure 6). Moreover, RecA* that assembles on the template strand of primer/template DNA can transactivate pol V located on a different template/primer, as we have shown previously (Schlachter *et al.*, 2006a). Within this model, *cis* activation may indeed still occur. If RecA* assembles on primer/template DNA downstream from a lesion with bound pol V, as was assumed to be happening in earlier models of TLS (Figures 4A–4D), then a molecule of RecA and ATP could be transferred to UmuD'₂C to form pol V Mut. Once the downstream RecA filament had dissociated, TLS could proceed. Removal of RecA could, in principle, occur in accord with the “cowcatcher” model (Figure 4D), where pol V Mut, possibly including SSB, could facilitate RecA dissociation in a 3' → 5' direction immediately ahead of an advancing replication fork (Pham *et al.*, 2001). However, the inhibition of pol V observed when RecA protein concentration is increased (Pham *et al.*, 2002) suggests that pol V-mediated RecA filament displacement is limited – if it occurs at all.

The historical positioning of RecA* downstream of pol V on the template being copied has always been based on an Occam's razor-like rationale. As the 1920s bank robber Willie Sutton famously replied when asked why he robbed banks, “because that's where the money is”. The analogy is apt in the sense that it was clear that RecA* was required for TLS, and therefore it needed to interact with pol V directly. The most plausible place for that to occur would seemingly be at a blocked replication fork.

The capacity of RecA* to activate pol V from a location remote from the replication fork has important implications for its *in vivo* function. First, there are several places in the cell where RecA* could interact with pol V. One example might be where UmuD undergoes RecA-mediated cleavage. This reaction could take place at any RecA* filament, either proximal or distal to a stalled replication fork. Pol V can function in TLS at a stalled fork without impediment by a downstream RecA* filament. Second, since pol V (UmuD'₂C) is essentially inactive until it is converted to pol V Mut (UmuD'₂C-RecA-ATP), it would perhaps seem unlikely that it could displace pol III on the β-clamp and then undergo activation. We speculate that an already active pol V Mut may be better suited for this role.

Pol V Mut properties and kinetics

Pol V Mut is avidly active for DNA synthesis on a hairpin substrate with a 3-nt overhang in the absence of RecA* (Jiang *et al.*, 2009). Pol V Mut is also able to copy past a *cis-syn* TT dimer and an abasic moiety on primer/template (p/t) DNA and is virtually inactive

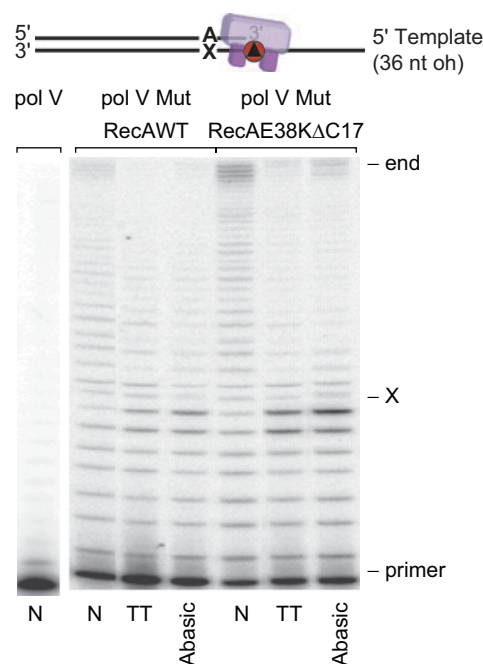


Figure 9. TLS performed by pol V Mut in the absence of RecA*. DNA template lesions, a *cis-syn* TT dimer and an abasic moiety, are copied by pol V Mut-RecA WT or pol V Mut-RecA E38K/ΔC17 in the absence of preformed *trans* RecA* filaments. Without preactivation, pol V is virtually inactive. “N” refers to a normal undamaged DNA template strand. “X” denotes a lesion. The primer strand is 12 nucleotides long and the template overhang is 36 nucleotides long.

in the absence of pre-activation by RecA* (Figure 9). There is approximately a two-fold higher efficiency with pol V Mut made using the constitutively active double mutant of RecA, RecAE38K/ΔC17, compared to pol V Mut made with wild-type RecA. This observation holds true for DNA synthesis on undamaged and damaged DNA (Jiang *et al.*, 2009). There is, however, a clear mechanistic distinction in the properties of pol V Mut formed with wild type RecA* versus RecAE38K/ΔC17*; additional “free” ATP or ATPγS was required for pol V function after activation with wild type RecA* but not for RecAE38K/ΔC17* (Jiang *et al.*, 2009).

Another important observation in the system is the requirement for RecA subunit transfer from the 3'-tip of RecA* (Schlachter *et al.*, 2006a; Jiang *et al.*, 2009). Pol V can be incubated alternatively with resin in which the 5' tip or the 3' tip of RecA* is made available. In both cases, pol V is able to strip a RecA and ATP from the filament, but only pol V Mut made with the 3'-proximal tip of RecA* exposed is active for DNA synthesis (Jiang *et al.*, 2009). With RecA* formed using the RecA mutant, RecA S117F (RecA1730, deficient for SOS mutagenesis), pol V is able to efficiently strip RecA and ATP from the filament, but this complex is inactive for DNA synthesis (Jiang *et al.*, 2009). The S117F mutation is located at the surface facing 3' on a RecA filament end (Boudsocq *et al.*, 1997). These

data strongly point to a conformational requirement for the active complex.

Deactivation of pol V Mut in the absence of RecA* occurs through two independent pathways: either in the presence or absence of DNA substrate (Jiang *et al.*, 2009). In the absence of primer/template, deactivation occurs slowly. The decrease in activity is approximately exponential, and is similar for pol V Mut activated with either RecA* using RecAE38K/ Δ C17 or wild-type RecA. The loss in activity is reversible; addition of RecA* *in trans* at any time point fully restores pol V Mut activity. In the presence of primer/template, deactivation is observed after every round of primer extension (Jiang *et al.*, 2009). Each active pol V Mut complex can promote only one round of DNA synthesis (Figure 10), after which it cannot reinitiate synthesis on a different p/t DNA. Deactivation is thus much faster in the presence of DNA substrate. Again, the deactivated complex can be fully resurrected by addition of RecA* (Figure 10).

Somewhat unexpectedly, deactivation of pol V Mut is neither caused nor accompanied by a concomitant loss of RecA or ATP. We suggest that deactivation of pol V Mut confers a conformational change of the enzyme that may reflect the two distinct binding modes of pol V to RecA described previously (Schlachter *et al.*, 2005). Activation/deactivation may involve alternative binding of the RecA subunit to either UmuD' or UmuC. Reactivation of inactivated pol V occurs as a result of the resident RecA-ATP being displaced from the inactive complex and replaced

with fresh RecA-ATP transferred from the 3' tip of a new RecA* (Jiang *et al.*, 2009).

Model of pol V Mut

The current model for pol V activation by RecA* is shown in Figure 11. UmuD'₂C is inactive in the absence of RecA*. The specific role of RecA* in SOS mutagenesis is to transfer a RecA•ATP from its 3'-proximal end to pol V (UmuD'₂C), thereby activating it for mutagenesis (Jiang *et al.*, 2009). This active species of pol V is named pol V mutasome, or in short, "pol V Mut", and contains UmuD'₂C-RecA-ATP. Pol V Mut can now perform TLS in the absence of RecA*.

Deactivation of the pol V Mut complex in the absence of RecA* happens through two independent pathways, either with or without DNA synthesis. In the absence of DNA substrate, the protein complex undergoes a slow deactivation over a period measured in tens of minutes at 37°C. When DNA substrate is present, rapid deactivation occurs following dissociation of pol V Mut from an extended p/t DNA substrate. Each active pol V Mut complex can promote one and only one round of DNA synthesis.

Regardless of the mode of deactivation for pol V Mut, full activity can be restored to the complex by interaction with a new RecA* (Jiang *et al.*, 2009). In this way, active pol V Mut will disappear from the cell soon after the SOS

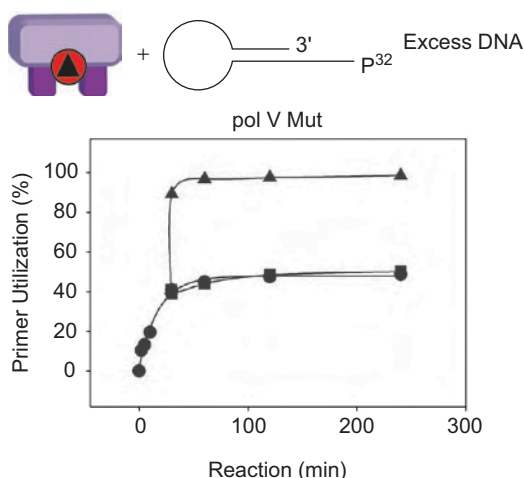


Figure 10. Pol V Mut non-cycling. Each active pol V Mut complex can promote only one round of DNA synthesis, after which it cannot reinitiate synthesis on a different p/t DNA, and is thus deactivated (●). The deactivated complex can be fully resurrected by addition of trans RecAE38K/ Δ C17*, which enables pol V Mut to reinitiate DNA synthesis on a separate p/t DNA substrate and facilitate cycling of the enzyme (▲). Addition of free RecAE38K/ Δ C17 does not reinitiate DNA synthesis (■). Trans RecA* is at 1 μ M when present. The reactions are performed under conditions of excess substrate DNA to active enzyme.

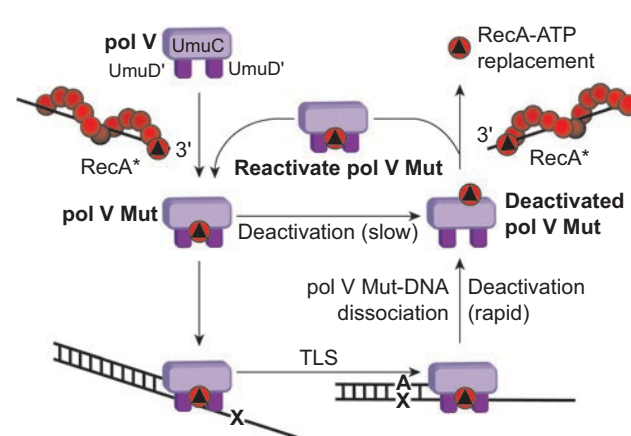


Figure 11. Model for pol V Mut function. Pol V is UmuD'₂C, and is minimally active on its own. Transfer of an ATP-bound RecA subunit from RecA* creates the active pol V Mut. Pol V Mut can migrate to a template-primer site where its activity is required. There, it will extend the primer and insert nucleotides opposite any lesion encountered (called translesion synthesis or TLS). Upon dissociation, pol V Mut is inactivated. There is also a slow inactivation of pol V Mut in solution, without carrying out TLS. Deactivated pol V Mut can be reactivated by interaction with another RecA* filament, with transfer of a new RecA-ATP subunit and displacement of the old RecA-ATP subunit.

response is switched off, and the RecA* filaments that sustain it are no longer present.

The model of Figure 11 solves a wide range of conundrums associated with the properties of pol V and RecA* filaments. Any necessity of displacing a RecA* filament that demonstrably inhibits pol V in *cis* is avoided. Obvious problems with *trans* activation are also avoided: the structural and topological problems associated with a RecA filament bound to DNA in one genomic location interacting continuously with a pol V replicating in a DNA gap somewhere else are eliminated. Pol V can be activated by an encounter with a RecA* filament anywhere, and the resulting pol V Mut can simply diffuse in an active form to a site where TLS is needed. And finally, the model provides an answer to the question of how TLS is halted once the SOS response is over. The mutational load associated with pol V function is thereby minimized, effectively restricting it to periods when SOS is induced. No RecA nucleoprotein filament needs to participate directly in TLS, either in *cis* or in *trans*, a fact obscured in previous studies because such filaments were always present when TLS was observed. Participation in TLS as a subunit of pol V Mut becomes the only filament-independent role documented for RecA.

The simple bacterium *E. coli* goes to extraordinary lengths to regulate the activity of error-prone pol V. This includes tight transcriptional control by LexA; rapid proteolysis of UmuD and UmuC by Lon; RecA-mediated post-translational activation of UmuD'; preferential UmuD/UmuD' heterodimer formation leading to ClpXP degradation of UmuD'; *trans* activation of pol V; and last, but not least, deactivation of pol V via a conformational rearrangement of RecA and ATP. The end result is to provide the cell a lifeline through the limited use of TLS pol V, when no other mechanisms of survival are available, but at the same time minimizing any "gratuitous" mutagenesis.

Pol V belongs to the Y family of DNA polymerases that are found in all domains of life. Like pol V these polymerases exhibit low fidelity DNA synthesis (Goodman, 2002). It would therefore make teleological sense that these enzymes are also tightly regulated. There is evidence in *Saccharomyces cerevisiae* that transcription of pol η (Rad30) is induced upon DNA damage (McDonald et al., 1997) and that the enzyme is subject to rapid proteolysis by the 20s proteasome (Podlaska et al., 2003; Skoneczna et al., 2007). While human pol η does not appear to be regulated at the transcriptional level, its intracellular activity is nevertheless regulated via complex mechanisms that include ubiquitination of the polymerase (Bienko et al., 2005) and interactions with ubiquitinated proteins, such as PCNA (Bienko et al., 2005). It has recently been shown in *Caenorhabditis elegans* embryos that sumoylated pol η is needed for replicating damaged chromosomes (Kim and Michael, 2008). Sumoylation

of *C. elegans* pol η protects it from proteolysis, but immediately following TLS the polymerase is presumably "deSUMOylated" and destroyed. Thus, similar to pol V Mut, pol η from *C. elegans* is degraded "on a per lesion basis" (Kim and Michael, 2008). Thus, polymerase deactivation occurs each time a lesion is copied, for *C. elegans* pol η via proteolysis and for pol V Mut via a conformational rearrangement of RecA and ATP (Jiang et al., 2009). Thus, it appears that by using different "strokes" for different species, inactivation of some of the "sloppiest copier" polymerases is a prudent course to take.

Declaration of interest

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