Roles of DNA Polymerase V and RecA Protein in SOS Damage-Induced Mutation

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Contents

1. Brief Historical Perspective of SOS Regulation and DNA Damage-Induced Mutation in Escherichia coli

Damage to the DNA of bacteriophage λ results in a loss of phage viability upon infection of *Escherichia coli*. However, phage viability is restored if the bacterial DNA is damaged prior to infection by exposure to UV radiation. This process, referred to as Weigle or W-reactivation, is accompanied by an increase in the number of base substitution mutations in λ.[1] The rescue and mutagenesis of the damaged lambda phage is a byproduct of the action of DNA damage-inducible genes in *E. coli*, expressed as part of the SOS regulon.[2–4] More generally, the large increase ($\sim$100-fold) observed in both chromosomal and episomal mutagenesis associated with SOS induction is referred to as "SOS error-prone repair".[5]

There are more than 40 SOS genes,[6] and many of the gene products are used to repair damaged DNA with base excision repair (BER), nucleotide excision repair (NER), or recombination repair. SOS genes are also involved in triggering cell division, which occurs only after the genome has been fully replicated and it is safe for the cell to divide. Among the SOS genes are those encoding the specialized DNA polymerases, Pols II, IV, and V.[7] The SOS polymerases catalyze translesion synthesis (TLS) by replacing a replicative Pol III that stalls when encountering a damaged template base. Once past the damage site, Pol III takes over to restart normal DNA replication. TLS, which results in mutations targeted to the sites of DNA damage, appears to be the biological basis of SOS mutagenesis. The regulation of SOS is governed by the action of two key proteins, the LexA repressor and RecA.

RecA is engaged in three distinct roles in the cell. Its principal role is to catalyze DNA strand pairing, leading to homologous recombination.[8] There are, however, two additional roles for RecA. One involves induction of the SOS response, and another is necessary for triggering SOS mutation.[3,9] RecA is absolutely essential for all three processes. A tacit assumption has been that for RecA to function in each it must first assemble as a nucleoprotein filament on single-stranded DNA (ssDNA). Indeed, along with death and taxes, there appears to be an inevitability of RecA nucleoprotein filaments, at least with regard to RecA's intracellular interactions. These activated RecA* filaments have clearly been shown to be involved in the processes of homologous recombination[8] and turning on SOS by acting as a coprotease during the cleavage of LexA repressor protein.[10]

We begin this review by presenting a general overview of RecA to establish its import as an essential element in the coupled "3R" processes of replication, recombination, and repair. We will then review past genetic evidence, especially the work of Devoret[11,12] and Witkin,[13,14] that demonstrated a role for RecA in SOS mutagenesis, a role that was found to be distinct from those during recombination and SOS induction.

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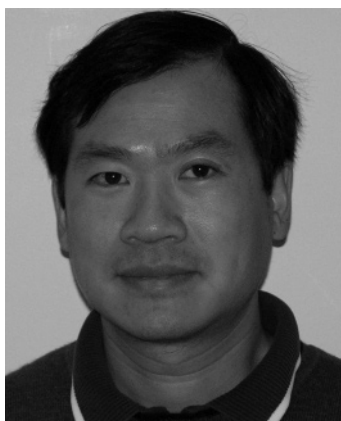
Phuong Pham was born in Hai-phong, Vietnam, in 1967. He earned his M. Sc. (Biology, in 1989) and Ph.D. (Genetics, in 1993) at St. Petersburg State Univesity, St. Petersburg, Russia. After pursuing postdoctoral work with Roel Schaaper at the National Institute of Environmental Health Services, North Carolina, he moved to the University of Southern California in 1999, where he is now a Research Assistance Professor. His research interests have included the genetic and biochemical basis of sponta- neous and SOS mutagenesis, and currently focuses on ssDNA cytosine deaminase enzymes, which play major roles in mammalian immuno- globuline antibody diversification and innate resistance against HIV and retroviral infection.

Michael Cox is professor and assistant chair in the Department of Biochemistry at the University of Wisconsin−Madison. Prior to his appointment at Madison in 1982, he obtained his Ph.D. under William P. Jencks at Brandeis University and was a postdoctoral fellow in the laboratory of I. Robert Lehman at Stanford University. His research centers on recombinational DNA repair of replication forks, the enzymology and regulation of RecA protein and its homologues, and mechanisms of radiation resistance in the bacterium *Deinococcus radiodurans*. His awards include the 1989 Eli Lily Award sponsored by the ACS Divsion of Biological Chemistry. In addition to teaching and research, Cox is a coauthor of *Lehninger Principles of Biochemistry*.

Myron F. Goodman performed graduate studies at Johns Hopkins University and received a Ph.D. in Electrical Engineering in 1968. His thesis investigated a theory of laser interactions with complex polyatomic molecules with an eye toward achieving nonthermal laser-induced bond- selective chemistry. Instead of starting a career at Bell Laboratories, he made the somewhat peculiar choice of switching gears to study the biochemistry of DNA polymerases as a postdoctoral associate with Maurice J. Bessman at John Hopkins. In 1973, he joined the faculty at the University of Southern California, in the Department of Biological Sciences, and divided efforts between developing a theory of laser-stimulated decomposition of molecules, which led to infrared laser-driven separation and enrichment of isotopes of uranium, and studies on the fidelity of DNA polymerases and SOS mutagenesis. A study to model SOS mutagenesis in vitro, which involved an exceptionally pleasant and fruitful collaboration with Hatch Echols at Berkeley, resulted in the discovery of the "sloppier copier" *E. coli* DNA polymerase V. His current research is focused on the role of activation-induced cytidine deaminase in somatic hypermutation of immunoglobulin genes, the biochemical basis of SOS mutagenesis involving Pol V and RecA, which is the subject of this review, and the "inner workings" of DNA polymerases that determine their fidelity.

This third essential role of RecA in SOS mutation serves as the focal point of this review. We will discuss recent evidence showing that in contrast to recombination and SOS induction Pol-V-catalyzed TLS requires the absence of a RecA filament proximal to a lesion.[15,16] Biochemical data using a RecA mutant, Devoret's so-called "third role" RecA mutant, will clarify how and why RecA1730 fails to elicit mutations above spontaneous background mutations in cells induced for SOS. Here, a new role for RecA is described, one that appears to involve two separate RecA monomeric binding interactions with Pol V. Although one of the RecA− Pol V binding modes requires the presence of DNA and ATP, the other does not. We have recently shown that the specialized role of RecA in SOS mutagenesis is to activate Pol V for TLS, by serving as an obligate subunit of a Pol V holoenzyme complex.[16] Since Pol V is almost "dead" in the absence of RecA,[16] its coupling to RecA monomers is essential for mutagenic TLS.

2. Interdependent 3R System: Replication, Recombination, and Repair

The SOS response provides a vivid illustration of the integration of cellular DNA metabolism. The fabled three Rs, replication, repair, and recombination, work in concert to restore the genome to normal order after it has absorbed

substantial abuse. These are coordinated to the cell cycle and to each other by an elaborate regulatory matrix.

When bacterial cells are subjected to sufficient DNA damage to induce the SOS response, a key event is the collapse of replication forks as they encounter the multitude of newly introduced DNA lesions in the template DNA. The primary goal of the SOS system is to productively restart replication. SOS has at least two phases.[8,17−21] SOS mutagenesis occurs in the second phase, preceded by a largely accurate phase dominated by accurate repair processes such as excision repair and recombinational DNA repair. Mutagenesis is thus not a required outcome of the SOS response. Instead, mutagenic translesion replication comes into play late in the response under circumstances in which the nonmutagenic processes have proven insufficient to restart replication on their own.

The first phase of SOS is a complex dance of multiple enzymatic systems. At the sites of stalled or collapsed replication forks, recombinational repair systems serve to reconstruct or alter the fork structure to allow repair. A dedicated origin-independent replication restart system then puts the replication cycle back on track. The fork will collapse again if there is additional damage downstream, and classical repair systems (nucleotide excision repair,[22−24] base excision repair,[25−27] and direct repair[28−30]) operate throughout the damaged genome to remove these potential impediments. If damage is sufficiently extensive that forks are caused to stall repeatedly, then the mutagenic phase of SOS ensues. This involves activation of the translesion DNA polymerase V.[16,31−36]

DNA polymerase V functions in concert with RecA protein and possibly other recombination functions. To appreciate the role of RecA in DNA polymerase V activity, it is necessary to review its function in other contexts.

2.1. RecA Protein

The recA gene was identified in *E. coli* by Clark and Margulies in 1965[37] and soon shown to have multiple roles in recombination and repair.[38] RecA is a 38-kDa polypeptide and is central to recombination functions in bacteria. Structural and functional homologues of RecA exist in all classes of organisms, including the RadA protein in archaeans[39] and the Rad51 and Dmc1 proteins in eukaryotes.[40] RecA proteins are nearly ubiquitous in bacterial species, with the only known exceptions occurring in bacterial species undergoing genome degeneration as part of an adaptation to an endosymbiotic lifestyle.[41−43] RecA is a DNA-dependent ATPase, and the RecA of *E. coli* (RecA$_{Ec}$) hydrolyzes ATP with a k_{cat} of 20−30 min^{-1}, depending on the nature (single strand or duplex) of the bound DNA.[44−46]

The recombination functions of RecA are a reflection of the protein's well-studied DNA strand exchange activities. RecA typically binds to single-stranded DNA, aligns that strand with homologous sequences in a duplex DNA, and then promotes a strand exchange in which one strand of the duplex is transferred to the single strand to create a new duplex, and the other strand from the original duplex is displaced. There are several classical assays for this activity, as shown in Figure 1. The exchange can readily encompass thousands of DNA base pairs.

The active form of RecA in DNA strand exchange reactions is a nucleoprotein filament, formed in several steps. A nucleation step is generally rate-limiting, followed by an extension of the filament in a 5′ to 3′ direction along single-
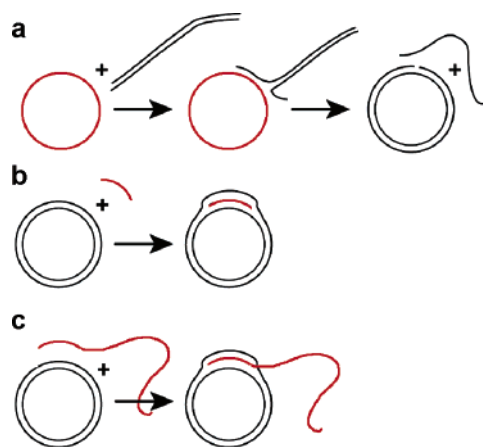

Figure 1. DNA pairing reactions promoted by the RecA protein. DNA strand exchange (top) occurs when a single-stranded DNA coated with RecA protein (red) invades a homologous duplex DNA. The exchange is completed as a strand is transferred progressively from the duplex to the single strand, creating a new duplex and a displaced strand originating from the original duplex. The latter stages of this reaction are facilitated by ATP hydrolysis. D-loop formation (middle) involves the incorporation of a short oligonucleotide into a duplex DNA, with one strand of the duplex displaced over the region of the pairing to form a structure similar to a "D". The duplex DNA must be supercoiled for this reaction to proceed efficiently. DNA strand invasion (bottom) is similar to D-loop formation but involves single-stranded DNAs too long to overcome topological constraints for full incorporation into the duplex. Thus, a single-strand extension remains. This reaction mimics a key step in certain pathways for replication fork repair (Figure 3).
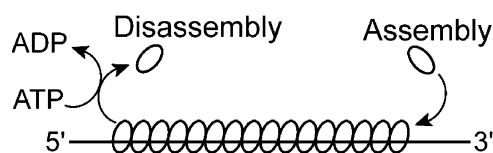

Figure 2. Assembly and disassembly of RecA filaments. Both processes are unidirectional, proceeding 5′ to 3′ along a single-stranded DNA. Thus, RecA monomers are added at the 3′-proximal end and subtracted at the 5′-proximal end. The disassembly process requires ATP hydrolysis. ATP is actually hydrolyzed throughout the filament uniformly but can result in dissociation when it occurs in a monomer at the disassembly end.

stranded DNA.[47,48] Filament assembly requires bound ATP but not ATP hydrolysis. RecA protein can bind directly to duplex DNA, but nucleation is much slower than it is when ssDNA is used. However, when a filament is assembled in a single-strand gap such that nucleation has occurred in a single-strand region, filament extension readily proceeds to encompass the adjacent duplex DNA. Filaments can also disassemble. The disassembly process requires ATP hydrolysis, and it also proceeds 5′ to 3′ such that RecA monomers are added to one end of a filament and subtracted from the other (Figure 2).[48−50] ATP hydrolysis occurs uniformly in all RecA monomers across the filament, with dissociation of RecA generally occurring only at the disassembling (5′-proximal) end. Filament assembly is faster than disassembly, as must be the case if a filament is to form.

Within the nucleoprotein filament, bound DNA is extended by about 50% and underwound such that there are about 18 base pairs per helical turn.[51−53] Each RecA monomer binds to three nucleotides of DNA or DNA base pairs, so that there are six RecA monomers per turn in the helical nucleoprotein filament. There are binding sites in the filament for as many as three strands of DNA.[54−58] DNA strand exchange is

facilitated via the interchange between these DNA binding sites. The extent of the DNA strand exchange reactions reflects the length of the RecA nucleoprotein filaments, which can encompass many thousands of DNA base pairs.

2.2. RecA Protein in the Induction of the SOS Response

RecA nucleoprotein filaments have quite a different role in the induction of the SOS response. SOS is regulated by a repressor protein called LexA. When it is bound to its specific binding sites in the *E. coli* genome, the 40+ genes of the SOS system are repressed. The extent of repression is dependent on the extent of LexA affinity to the binding site, which differs greatly between the genes. The result is a timely hierarchy of SOS gene expression. LexA undergoes an inactivating autocatalytic cleavage under certain conditions in vitro.[59,60] Under physiological conditions, this same autocatalytic cleavage does not occur to any significant extent except when LexA comes into contact with a RecA nucleoprotein filament.[59,60] Such filaments form at DNA gaps created when replication forks stall or collapse at sites where DNA damage is introduced to the cell. As the supply of active LexA is depleted by cleavage, the genes of the SOS response undergo transcriptional induction. RecA protein levels rise, along with the concentrations of many other repair functions. The LexA is cleaved into two inactive fragments.

Since RecA does not function as a classical protease but instead facilitates an autocatalytic cleavage of LexA, the RecA activity is generally referred to as a coprotease function. The coprotease is not limited to LexA cleavage. The bacteriophage λ repressor also undergoes an autocatalytic cleavage facilitated by RecA, as does the UmuD protein.[61−63] The UmuD′ protein fragment generated by UmuD cleavage is an active subunit of DNA polymerase V.

2.3. RecA Protein in the Repair of Stalled Replication Forks

A replication fork can stall or collapse at a variety of DNA lesions. If the collapse occurs at a template strand break, one arm of the replication fork is detached (Figure 3). Repair requires the RecBCD helicase/nuclease as well as the RecA protein, single-strand binding (SSB) protein, and additional recombination functions. In brief, the RecBCD enzyme binds to the broken DNA end and moves along the DNA. The 3′-ending strand is preferentially degraded as the DNA is unwound.[64−67] When the enzyme encounters the sequence 5′-GCTGGTGG-3′ (a Chi site), its activity is altered. The 5′-ending strand is now preferentially degraded, creating a 3′-ending single-strand extension.[64−67] RecBCD then loads the RecA protein onto this single-stranded DNA.[68,69] RecA then promotes a DNA strand invasion as the first step in the repair of the collapsed replication fork (Figure 3).

At a stalled replication fork with no template strand break, RecA can promote several different reactions to effect repair. First, if there is a gap on the leading strand template, RecA can readily promote the regression of the fork structure (Figure 4). This reaction requires ATP hydrolysis and is in effect a motor function of RecA.[70,71] If a gap is left on the lagging strand DNA template, then the loaded RecA filament (oriented in the direction opposite to what it would be on the leading strand) can unwind the fork for up to 400 bp.[72]

2.4. Regulation of RecA Protein

Unchecked recombination between repeated sequences in the genome could result in the loss of the intervening DNA.
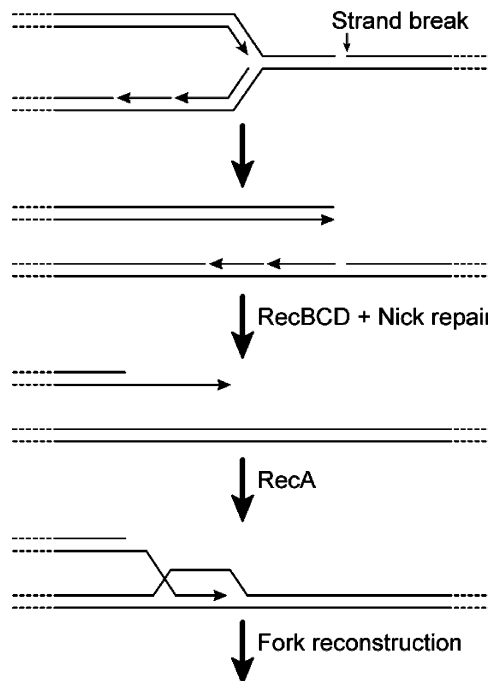

Figure 3. Recombinational repair of replication forks. The double strand break repair pathway is shown, one of several that vary depending on the nature of the DNA damage encountered by the fork. In this instance, a fork has encountered a strand break on one of the template strands, leading to the separation of one arm of the fork. The replication apparatus must disassemble, and the end of the broken arm is processed by the RecBCD enzyme. RecBCD unwinds the broken end and degrades the DNA so as to produce a DNA end with a 3′ single-strand extension. RecBCD then loads RecA protein onto the extension, leading to strand invasion (Figure 1). Further processing occurs to restore a viable replication fork, employing a series of helicases, nucleases, and ligase.
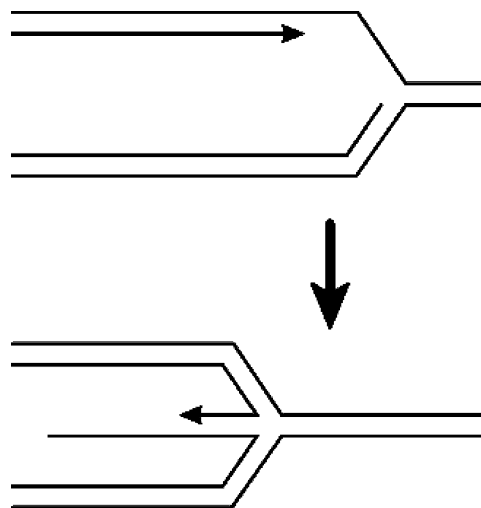
Figure 4. Replication fork regression. This reaction is often a key part of the replication fork repair process when forks encounter DNA lesions that halt fork progression but leave the fork arms attached. The original template strands are re-paired, displacing the newly synthesized strands and moving the fork backward. The newly synthesized strands themselves pair, forming a four-armed junction or Holliday junction. This reaction is promoted by the RecA protein and by the RecG helicase. In both cases, ATP hydrolysis is required.

Not surprisingly, RecA function is regulated on multiple levels so that it is focused on the sites requiring it.

The first level of regulation involves the RecA C-terminus. Removal of 17 amino acid residues results in a RecA variant

that is more robust in virtually every RecA function.[73−75] Thus, these 17 amino acids are part of an autoregulatory suppression of RecA function. The wild-type RecA protein does not function in DNA strand exchange in vitro unless an unphysiological level of free Mg^{++} ion (generally 6−8 mM) is included in the reaction in addition to the Mg^{2+} needed to chelate the added ATP. The need for added Mg^{2+} is eliminated when the wild-type protein is replaced by the deletion mutant, RecAΔC17.[73] The deletion mutant also exhibits a more robust coprotease activity as well as enhanced functions described below.

The second level of regulation involves modulation of RecA function by other proteins. Most of this modulation is directed at the RecA filament assembly and disassembly processes. The *E. coli* single-stranded DNA binding protein, SSB, is a passive participant. SSB impedes the nucleation step of RecA nucleoprotein filament formation, resulting in a lag in binding that can be measured in hours. However, once RecA nucleation has occurred, SSB has a positive role in the extension phase of nucleoprotein filament formation. RecA does not bind well to regions of DNA secondary structure. SSB melts these duplex regions and is then displaced by RecA in the extension phase to create a contiguous filament on the DNA.[48,76] RecAΔC17 readily displaces SSB protein even in the nucleation phase. Thus, the barrier to nucleation on SSB-coated ssDNA is ensconced in the RecA C-terminus.[75]

Additional proteins modulate almost every phase of RecA filament assembly and disassembly. The RecO and RecR proteins form a complex that facilitates the nucleation of wild-type RecA protein onto SSB-coated ssDNA.[77−80] The RecF protein is also involved in RecA filament assembly. RecF facilitates RecOR function under at least one set of conditions,[81] although there is no evidence for the formation of a RecFOR complex. RecF will separately form a complex with RecR protein, competing with RecO for RecR interaction.[79] The RecFR complex binds tightly to dsDNA and can limit the extension of RecA nucleoprotein filaments into duplex regions adjacent to ssDNA gaps.[82]

The RecX protein inhibits RecA function by interfering with RecA filament formation.[83−85] It does so by blocking filament extension, most likely by capping the filament.[84] The DinI protein has a generally positive effect on RecA function. DinI binds stoichiometrically to RecA filaments, exerting a substantial stabilizing effect on them.[86,87] In the presence of DinI, filament disassembly is blocked, but assembly can proceed. In the presence of RecX, assembly is blocked, but disassembly can proceed. Each of these two proteins thus antagonizes the function of the other.[86] Notably, one RecA function is suppressed by DinI. When DinI is bound to RecA, the LexA coprotease function is intact, but the UmuD cleavage reaction is blocked.[86,88] The DinI protein is expressed early in the SOS response,[89] reaching maximum levels in 20 min or less. DinI may be part of a regulatory system that modulates the temporal course of SOS. As long as DinI−RecA filaments are present, UmuD should not be cleaved, and the activation of DNA polymerase V to initiate the mutagenic phase of SOS is thus postponed.

2.5. Predominance of RecA Filaments in RecA Function

The RecA nucleoprotein filament is the active species in all of the processes considered to this point. DNA strand exchange occurs within a filament. The coprotease function requires the establishment of a filament on DNA. All of the activities of RecA in the recombinational repair of replication forks require an active RecA filament. Thus, the paradigm has developed that RecA functions exclusively as a nucleoprotein filament. This paradigm is challenged in the case of RecA function in the context of SOS mutagenesis.

3. Genetics of SOS Mutations

3.1. SOS Mutations Targeted to DNA Damage

The first indications of increased mutagenesis in *E. coli* and bacteriophage after exposure to DNA damage were observed in experiments with λ phage. UV irradiation of *E. coli* host prior to infection of UV-irradiated phage resulted in increased survival (W-reactivation) and mutagenesis (W-mutagenesis) of λ phage.[1] Studies of bacterial chromosome mutagenesis showed that *E. coli* strains with either an inactive RecA protein (recA(Def)) or a mutant LexA protein that could not support induction of the SOS response (lexA(Ind−)) were not mutable by UV irradiation.[90−92] Similarly, experiments with λ phage demonstrated that both W-reactivation and W-mutagenesis were not observed in preirradiated cells with either recA(Def) or lexA(Ind−) mutation.[93] These data were instrumental in formulating a model referred to as "SOS error-prone repair",[4] in which it was proposed that the activation of an SOS system is required for repair and bypass of UV-induced DNA lesions and that UV-induced mutations arise as a consequence of an error-prone bypass of the DNA lesions.[2,3] Subsequent genetic and biochemical studies have confirmed that SOS error-prone translesion synthesis is essential to the organism to cope with DNA damage by UV light or chemicals by repairing and bypassing replication-blocking lesions.

More than 40 genes are upregulated upon exposure to DNA-damaging agents.[6] SOS induction is regulated by a two-component repressor/activator system of the LexA and RecA proteins (Figure 5). The LexA repressor binds to a 20 base pair consensus sequence in the operator region of the SOS genes, suppressing their expression.[94,95] A direct screen for genes affecting SOS mutagenesis of cells in response to UV light or 4-nitroquinoline 1-oxide revealed a requirement of two other genes besides lexA and recA, namely, umuC and umuD, which constitute the umuDC operon.[96,97] *E. coli* strains with certain mutations in umuD or umuC are largely nonmutable by UV irradiation and other SOS-inducing agents.[4]

Early genetic studies showed the umuDC genes to be involved in error-prone bypass of UV lesions. For example, an umuC mutation in an excision repair defective strain abolished W-reactivation of UV-irradiated lambda phage.[98] Twenty years later it was shown that the umuDC gene products, in the form of a heterotrimeric UmuD′₂C complex,[99] is an error-prone DNA polymerase, *E. coli* Pol V.[31,100,101] Reconstitution of translesion synthesis reactions in vitro with purified proteins, including RecA, showed that Pol V bypasses major UV lesions, TT cis−syn cyclobutane dimers and TT (6−4) photoproducts, with mutation specificities similar to those observed in vivo for UV-induced mutagenesis.[102] These data provide a biochemical basis supporting a direct role for Pol V in generating the types of mutations occurring during TLS in vivo.

The key component of the SOS system is the RecA protein. RecA was one of the first genes to be identified as essential for SOS induction. Acting almost immediately in
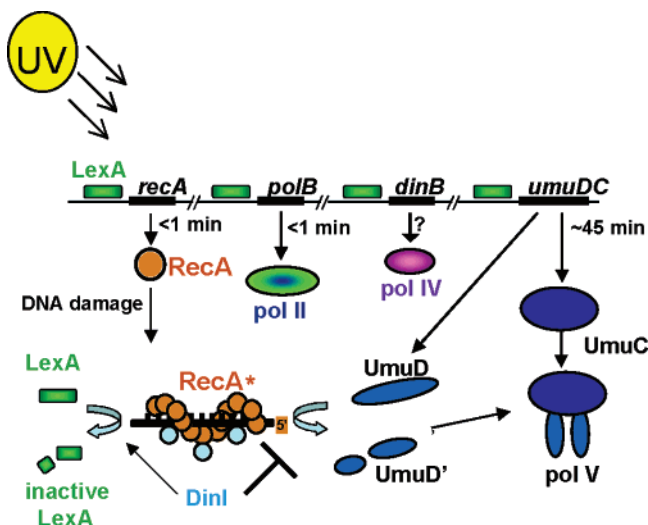
Figure 5. Regulation of the SOS response. LexA protein (green) represses protein expression by binding to the SOS box in the consensus operator sequence. Upon UV irradiation, RecA forms an activated filament on single-stranded DNA, which assists the autocleavage of LexA. Differing LexA binding affinities to distinctive operon sequences of the various proteins regulate the protein induction in a time-dependent manner. Proteins with operators bound weakly to LexA are induced soon after DNA damage whereas proteins with strongly bound operators are induced later. RecA (orange) is one of the first proteins induced in response to DNA damage, whereas UmuDC (blue) undergoes induction much later. DinI regulates late Pol V expression by inhibiting RecA-mediated cleavage of UmuD to UmuD′ during the early phase of the SOS response.

response to DNA damage, RecA forms a nucleoprotein filament on ssDNA and functions as a coprotease to cleave and thereby inactivate the LexA repressor.[10,103] Its direct involvement in mutagenesis was revealed by its ability to act in a manner similar to cleave UmuD to mutagenically active UmuD′,[62,63] required to make Pol V (UmuD′₂C). However, in 1989 Dutreix and co-workers identified a mutant RecA, RecA1730 that was proficient in homologous recombination and LexA/UmuD cleavage upon overexpression but failed to exhibit an increase in mutagenesis.[12] Further characterization of this mutant RecA led to the conclusion that there must be an independent third role for RecA responsible SOS mutator activity. This newly revealed mutagenic role for RecA is separate from LexA and UmuD processing and is dependent on interactions with the UmuD′ and UmuC proteins.[14] As discussed in section 4, the mutagenic role of RecA involves a direct stimulation of Pol V activity, which in contrast to its roles in homologous recombination and induction of SOS, occurs in the absence of a RecA nucleoprotein filament.[16,104]

Pols II and IV are also induced in response to DNA damage as part of the SOS regulon,[7] and while neither polymerase seems to copy UV-induced chromosomal damage, they do cause mutations in damaged plasmid DNA and may also cause chromosomal mutations targeted at non-UV-induced lesions.[105−108] However, in contrast to Pol V, RecA is not involved in either Pol-II- or Pol-IV-catalyzed TLS.

3.2. SOS Untargeted Mutagenesis

The induction of SOS is accompanied by a large increase in untargeted mutations at DNA sites that appear to remain undamaged. For example, infection of a pre-UV-irradiated *E. coli* host with undamaged λ phage creates increased phage

mutation rates.[109] Similarly, SOS mutator effects on undamaged DNA were found to occur on the *E. coli* chromosome and on the F′ episome DNA in SOS-constitutive strains containing mutant recA alleles, recA441 or recA730.[13,110−112] Subsequent studies showed that untargeted mutagenesis on the *E. coli* chromosome as opposed to an infecting λ phage requires different genetic factors. SOS-induced untargeted chromosomal and episomal (F′) mutations appear to be strictly dependent on RecA and Pol V,[3,113] whereas mutations on undamaged λ phage require UvrABC and the genes encoding Pols I (polA) and IV (dinB).[114,115] Untargeted SOS mutagenesis on chromosomal DNA is characterized by large numbers of transversions that can be corrected by mismatch repair (MutHLS).[112,116,117] The genetic data viewed alongside recent biochemical data measuring error-prone polymerase mutation specificity[102,108,118,119] suggest that SOS untargeted mutations are most likely generated by spontaneous replication errors caused by inappropriate copying of undamaged chromosomal DNA by Pol V and episomal DNA by Pol IV.

3.2.1. SOS Polymerases and Adaptive Mutations

When nondividing *E. coli* are placed under nonlethal selective pressure, mutations accumulate seemingly in response to the selective environment.[120] This phenomenon is known as adaptive mutation. Adaptive mutations are typically characterized by measuring the reversion of a lacZ frameshift mutation carried on an F′ episome,[121] in a lacZ⁻ cell, and are also accompanied by nonselected mutations.[122] While Pol V does not appear to participate in causing adaptive mutations,[121,123] the other two SOS polymerases are clearly involved. Pol IV is upregulated during stationary phase[124] and is required for most (∼80%) adaptive mutations, which are typically −1 frameshift deletions.[125,126] Small deletions are characteristic of Pol IV's mutational spectrum in vitro.[119,127] In contrast, Pol II, through its 3′-exonuclease proofreading function, serves to regulate the level of adaptive mutations by causing an approximate 5-fold reduction in magnitude.[128]

3.2.2. SOS Polymerases during Long-Term Survival and Fitness

In the absence of SOS induction Pol V has not been detected (<15 molecules/cell), whereas Pols II and IV are present at significant constitutive levels.[129] Pol II is maintained at about 50 molecules/cell, and its number is increased by 7-fold following SOS induction.[130,131] Pol IV levels are about 250 molecules/cell and increase about 10-fold when SOS is turned on.[132] All three SOS polymerases appear to be upregulated during stationary phase in the absence of exogenous DNA damage.[124,133,134] Aside from their ability to perform TLS, the error-prone enzymes play an important role in promoting genetic diversity under normal physiological conditions in *E. coli* grown deep into stationary phase.

Single and multiple Pol II, Pol IV, and Pol V mutants, when cultured individually, can survive for months on end in stationary phase without addition of supplementary nutrients. However, when cocultured in the presence of wild-type *E. coli*, the mutant cells deficient in any one or any combination of SOS polymerases fail to survive for more than about 10−14 days.[133] Clearly, even a single dysfunctional SOS polymerase confers a significant disadvantage for the bacteria when they are competing with the wild-type for limited energy resources. Double and triple error-prone polymerase mutants suffer even a greater loss in fitness when

competing with wild-type cells.[133] Studies of long-term survival of bacterial cells during stationary phase have established that the cell populations undergo a highly dynamic process known as the "growth advantage in stationary phase" (GASP) phenotype. GASP is defined as the ability of aged cells, experiencing a long stationary period, to out compete a young cell population in survival and is thought to result from the appearance of and selection for advantageous mutations.[135,136] All mutants in SOS polymerases are shown to be defective in expressing a strong GASP phenotype.[133] The reduced fitness of SOS polymerase mutants demonstrates their microevolutionary importance as reflected in their ability to help generate sufficient genetic diversity, enabling a cell to cope with long-term nutrient deprivation. However, since the GASP phenotype is influenced by a variety of mutations, it does not result solely from error-prone synthesis performed by the three SOS-regulated polymerases.

4. Biochemistry of SOS Mutation

4.1. Polymerase Trafficking at Blocked Replication Forks

In the course of the past decade, it has became clear that DNA replication and repair no longer can be considered to be separate processes but that the two intercept and complement each other on numerous levels. The most prominent example for this connection is RecA as discussed above, with its various roles for homologous recombination, replication fork maintenance, SOS induction, UmuD processing, and Pol V stimulation. Another protein, the β-clamp, which originally was thought to be only a prominent factor in replication, also turned out to be a major player in bridging the two processes.

The β-clamp is a ring-shaped dimer that tethers Pol III to a DNA template primer $3'$ end, thereby increasing polymerase processivity from about 10 nucleotides (nt's) to several thousand nt's per template binding event.[137−139] β also stimulates the SOS induced polymerases, Pol II, IV, and V.[15,31,118,140,141] All of the polymerases, in addition to MutS and DNA ligase, possess a pentapeptide motif having a consensus sequence QL(SD)LF used for binding to the hydrophobic pocket located on each β monomer surface.[142,143] In vitro and in vivo studies suggest that this interaction is essential to ensure strong polymerase−DNA binding and enhanced processivity.[144−147] Pol IV, which by itself binds poorly to both DNA and dNTP substrates, also exhibits a large increase in dNTP binding affinity in the presence of β.[148]

The β-clamp presumably acts as a platform, on which translesion synthesis polymerases switch with Pol III, when the replicative polymerase becomes stalled at a damaged template base. Since each protein appears to interact with the clamp at the same site and since the Pol III core does not simply dissociate from β-clamp when loaded on a primed template for at least 30 min,[149,150] a competitive model was proposed in which various polymerases compete for the hydrophobic binding site on the clamp.[144,147] In an in vitro model system used to copy primed circular DNA containing a site-directed lesion, Fujii and Fuchs[151] observed sequential usage of a β-clamp by Pol III and Pol V. Alternatively, since the dimeric nature of the β-clamp provides two hydrophobic pockets available for proteins to bind, a tool-belt model could allow two polymerases to be present simultaneously on a

single β dimer.[145,152] Recently, just such a ternary complex has been identified with Pol III, Pol IV, and β.[153]

Interactions with the conserved consensus sequence alone might not be sufficient for polymerase activity in vivo.[154] In addition to the conserved binding pocket common to all polymerases, each polymerase contains a unique binding site for the β[152,155,156] subunit. The crystal structure revealed interactions between Pol IV and β on the interface between the two β subunits in addition to the main binding occurring between the C-terminal tail of Pol IV's little finger domain and the hydrophobic channel of the β-clamp. This secondary interface may maintain the polymerase in an inactive orientation and could regulate a switch between polymerases,[152] perhaps by a tool-belt mechanism in which a stalled Pol III is replaced by an error-prone polymerase just long enough for TLS to occur, whereupon Pol III takes over to complete replication.

Competitive versus tool-belt switching may not be mutually exclusive mechanisms, and each might operate under different sets of circumstances, such as whether replication is taking place on chromosomal or extrachromosomal DNA and whether cells are dividing in log or stationary phase or perhaps not dividing. It is known, for example, that TLS bypass efficiencies in vivo depend on the identity of the lesion and the polymerase used to copy it; for example, Pol V abasic sites, TT dimers, and 6−4 photoproducts much more efficiently than either Pol II or Pol IV,[102] whereas bulky adducts such as acetyl aminofluorenes (AAFs) and benzopyrenes are better substrates for Pols II and IV.[106,108] Making matters even more complex are genetic data showing that Pol V is almost entirely responsible for SOS mutations occurring on chromosomal DNA in exponentially dividing cells,[96,97] whereas Pol IV appears not to mutate the chromosome during exponential growth.[114,157,158] A consequence of a competitive switching mechanism is that the "best" repair enzyme may not always be chosen to copy a specific lesion. Evidence favoring competitive polymerase selection in vivo has been obtained with plasmid DNA, where the relative numbers of Pol II and Pol V in the cells were found to determine whether AAF guanine adducts are copied in an error-prone or error-free manner.[108] A large increase in −2 frameshift mutations occurred when Pol II was expressed at higher levels than Pol V, whereas frameshift mutations were absent when Pol V/Pol II ratios were reversed.[108] However, a more stringent selection among polymerases could occur for chromosomal replication where access to β might be restricted, in which case a tool-belt mechanism might be in force.

4.2. "Cowcatcher" Model for Pol-V-Catalyzed Translesion Synthesis

The familiar trombone model for DNA replication reflects coupled leading and lagging strand DNA synthesis occurring at the replication fork (Figure 6a). Collapse of the replication fork and uncoupling of DNA synthesis is believed to occur when a damaged DNA base is encountered on either strand. About 15 years prior to the initial identification of UmuD$'_2$C as a DNA polymerase,[31,100,101] a model by Bridges and Woodgate[159] proposed that the Umu proteins, acting in conjunction with a RecA filament, helped shepherd a blocked Pol III past the site of a lesion by reducing Pol III fidelity, perhaps by inhibiting proofreading. Woodgate and Lawrence have shown that proofreading-deficient Pol III is in fact able to fully replicate a plasmid containing a single TT dimer.[160]
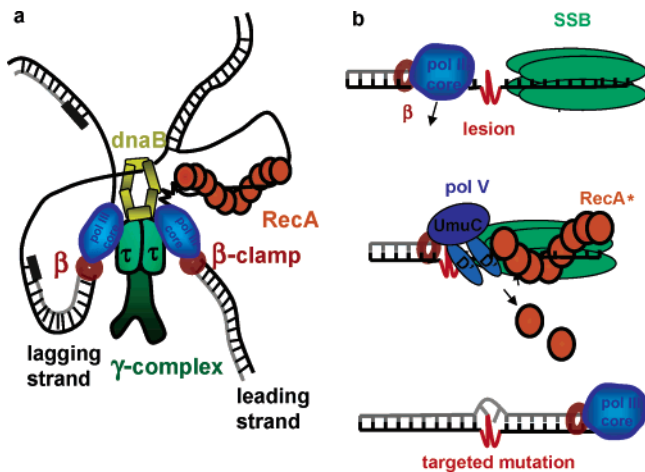
Figure 6. Replication fork encountering a DNA template lesion. (a) "Trombone model" of *E. coli* Pol III replisome, consisting of Pol III core (blue), the processivity β-clamp (dark red), and γ loading complex (green), together with DnaB helicase (yellow) and RecA filament (orange), shown encountering a lesion (ziz zag line) on the leading strand. (b) Schematic diagram of lesion bypass during late SOS response. When replicative Pol III stalls at a damaged base, SSB (green) will coat the single-stranded regions arising presumably by ongoing helicase activity. Pol V replaces Pol III on the processivity clamp (β-clamp), copies past the damaged site, with an absolute requirement for RecA, and is in turn replaced by Pol III to resume normal DNA replication.

But proofreading deficient Pol III catalyzed TLS does not involve Pol V. Under normal conditions during late SOS phase in the cell, Pol V presumably replaces Pol III on the β-clamp and copies past the lesion. Once past the lesion, Pol V is in turn replaced by Pol III to resume normal DNA replication (Figure 6b).

Devoret's genetic data showed that SOS mutagenesis requires the presence of RecA protein.[11,12] It was therefore natural to assume that assembly of a nucleoprotein filament had to occur. RecA was known to work as a filament during homologous strand exchange, cleavage of LexA to turn on the global SOS response, and cleavage of UmuD to turn on SOS mutagenesis. Continued unwinding of the DNA by the DnaB helicase immediately ahead of a stalled replication fork could provide a stretch of ssDNA downstream from the lesion on which a RecA filament could readily assemble (Figure 6a).

There is, however, an obvious topological difficulty copying an intact nucleoprotein filament by Pol V attached to β-clamp. How, for example, could a filament of ~100 Å in diameter[8] be threaded through a β-clamp with an opening of only 38 Å?[138] Indeed, formation of a stable RecA filament with non-hydrolysable ATPγS effectively blocked Pol V DNA synthesis in vitro (Figure 7, lane 1).

The "cowcatcher" model[15] addressed this issue, using the *E. coli* SSB protein as a reagent in the in vitro assay to destabilize the RecA filament allowing atypical disassembly in the 3′ to 5′ direction immediately ahead of an advancing Pol V (Figure 7, lane 2 and 4), which therefore allows Pol-V-mediated TLS to occur.[15] We have characterized the concerted action of Pol V and SSB by analogy with a "locomotive cowcatcher",[15] a large triangular piece of metal affixed to front of a locomotive, designed to clear cows from the track of on an advancing train. As each RecA is ejected in turn from the filament, contact is presumably maintained between the advancing Pol V and the RecA monomer at the receding 3′ filament tip. Whether or not SSB has a role in
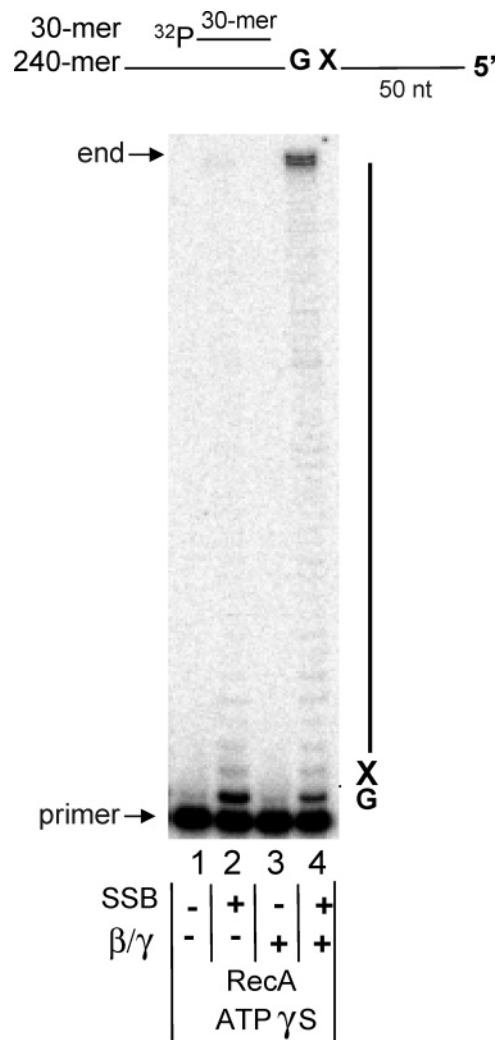

Figure 7. Pol-V-catalyzed TLS in the presence of a ATPγS-stabilized RecA nucleoprotein filament. Pol-V-catalyzed translesion synthesis was measured on a primer template containing an abasic site (a terahydrofuran moiety) on a 50 nucleotide template overhang in the presence of RecA protein, slowly hydrolyzable ATPγS, SSB, and β/γ. The TLS process requires that Pol V acting in conjunction with SSB facilitates disassembly of a stabilized RecA nucleoprotein filament, in a 3′ to 5′ direction, in a reaction that does not involve ATP hydrolysis, as described by the "locomotive cowcatcher" model depicted in Figure 6b, middle sketch. Reprinted with permission from *Nature* (http://www.nature.com), ref 15. Copyright 2001 Nature Publishing Group.

SOS mutagenesis in vivo remains an open question. What seems clear from the data, however, is that a stabilized RecA nucleoprotein filament if present must be disassembled to permit TLS.

4.3. A "Fly in the RecA Filament Ointment"

The requirement for RecA filament disassembly ahead of the polymerase raised another challenging question: How can the tip of a RecA *filament* be necessary if filament destruction is needed for synthesis to occur? In Figure 8, the paradigm of a necessity for a RecA filament in lesion bypass is challenged. Pol-V-catalyzed TLS is clearly observed at a stoichiometry of ~1 RecA molecule per 50 nt's (Figure 8a). Filamentation is absent under these conditions as shown by the absence of a conversion from UmuD to UmuD′ (Figure 8c). The coproteolytic cleavage of UmuD to form UmuD′ is a definitive property of RecA nucleopro-
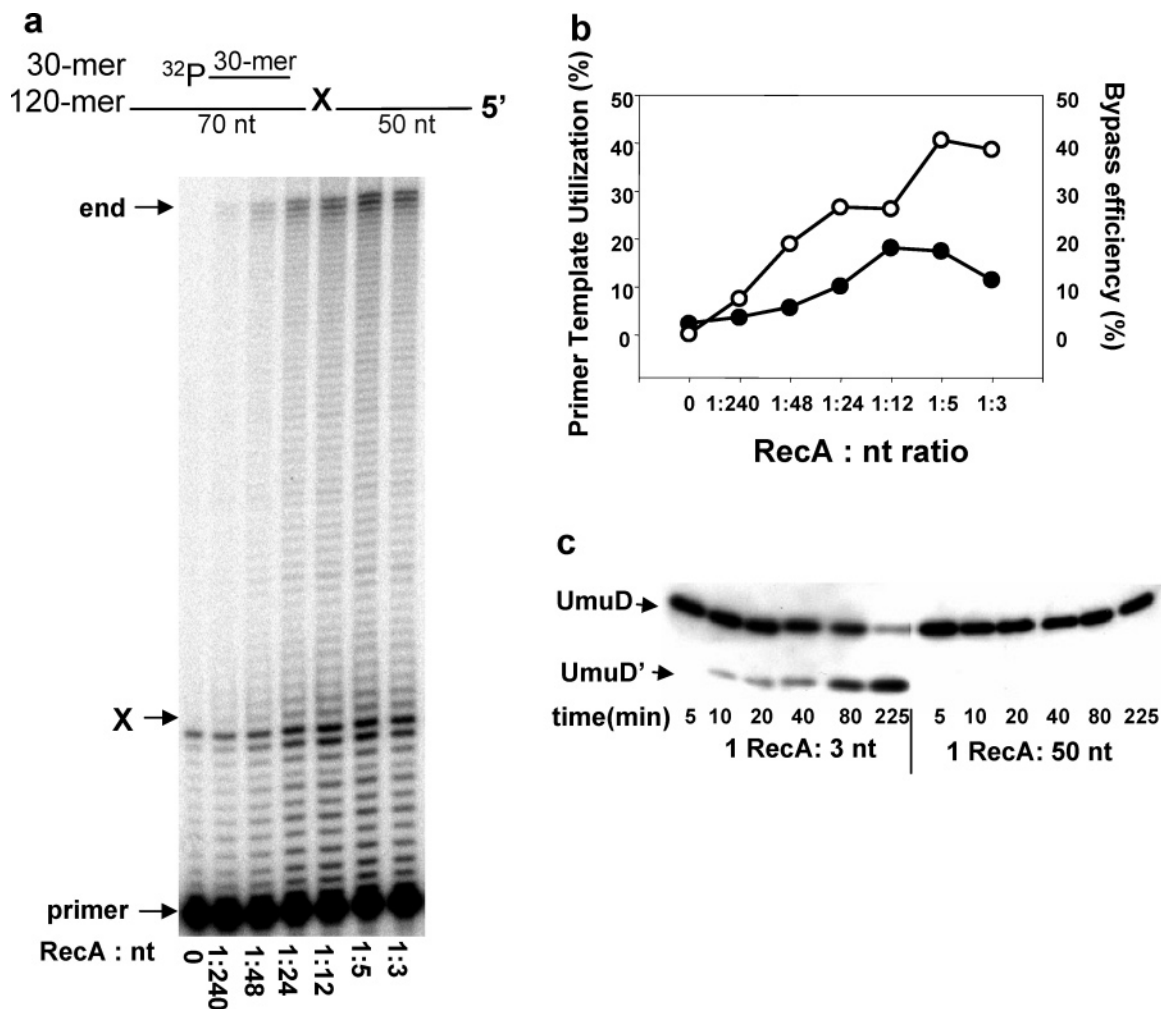
Figure 8. "Fly in the RecA filament ointment". (a) Pol V synthesis and TLS measured on a 64 nt ssDNA template overhang containing an abasic lesion X, located 50 nt from the 5′-template end, in the presence of ATPγS, SSB, and different RecA/DNA nt ratios, as indicated. (b) Primer utilization (●) and lesion bypass efficiencies (○) calculated from the data in Figure 8a. (c) Conversion of UmuD to UmuD′ by the coproteolytic action of a RecA nucleoprotein formed at a ratio of 1 RecA molecule per 3 nt's ssDNA (left gel). At a RecA-to-nucleotide ratio of 1:50, no conversion of UmuD to UmuD′ is detected (right gel), while Pol-V-catalyzed TLS occurs (Figures 8a and 8b).

tein filaments.[62,63,161] Although a much more robust lesion bypass reaction occurs when conditions are optimal for filament assembly with ~1 RecA molecule per 3 nt's (Figures 8a and 8b) where conversion of UmuD to UmuD′ happens (Figure 8c), it suggested that the presence of a nucleoprotein filament is not essential for Pol-V-catalyzed TLS.

The reactions in Figure 8 were performed in the presence of slowly hydrolyzable ATPγS and therefore required (SSB). Obviously, ATPγS does not exist in vivo. Therefore, the key question is whether RecA in the presence of hydrolyzable ATP disassembles rapidly enough for Pol-V-mediated TLS to occur. In the case of wild-type RecA, Pol-V-catalyzed TLS occurs in the absence of a RecA nucleoprotein filament (Figure 9a) but does not occur when a RecA filament is present (Figure 9b).[16] Notably, the short (9 nt) template overhang on which TLS occurs cannot support RecA filament formation, as shown by the absence of conversion of UmuD to UmuD′, whereas the longer (21 nt) template overhang on which TLS fails to occur does support filament assembly, as shown by the avid UmuD to UmuD′ conversion. Thus, a RecA filament, even under more physiological conditions where RecA can dynamically assemble and disassemble, is counterproductive to lesion bypass.[16]

Supposedly, the inability of Devoret's mutant RecA1730 to support SOS mutagenesis could be caused by a defect in the interaction of the mutant RecA at the 3′ tip of the filament with Pol V. However, since Pol-V-catalyzed TLS occurs in the absence of a RecA filament (Figure 9a), the time was ripe to dispense with preconceived notions about RecA filaments and to characterize the biochemical interactions of RecA with Pol V.[16]

4.4. Enigmatic Third Role for RecA in TLS: Stimulating Pol V Activity

The activity of Pol V exhibits strong dependence on RecA concentration (Figure 10, left gel). Of course, Pol V is a DNA polymerase in the absence of RecA protein,[31,100,101] but it is a fairly feeble one. A comparison of Pol V stimulation by wild-type (WT) RecA and RecA1730 (S117F) reveals the biochemical basis for the absence of SOS mutagenesis with the mutant RecA, namely, that Pol V is virtually "dead" in the presence of the nonmutable RecA1730 (S117F) (Figure 10, right gel).[16]

These data allow us to propose that the enigmatic third role of RecA in SOS mutagenesis is principally to activate Pol V. Wild-type RecA protein stimulates the specific activity of Pol V by about 350-fold.[15] The activation of Pol V by
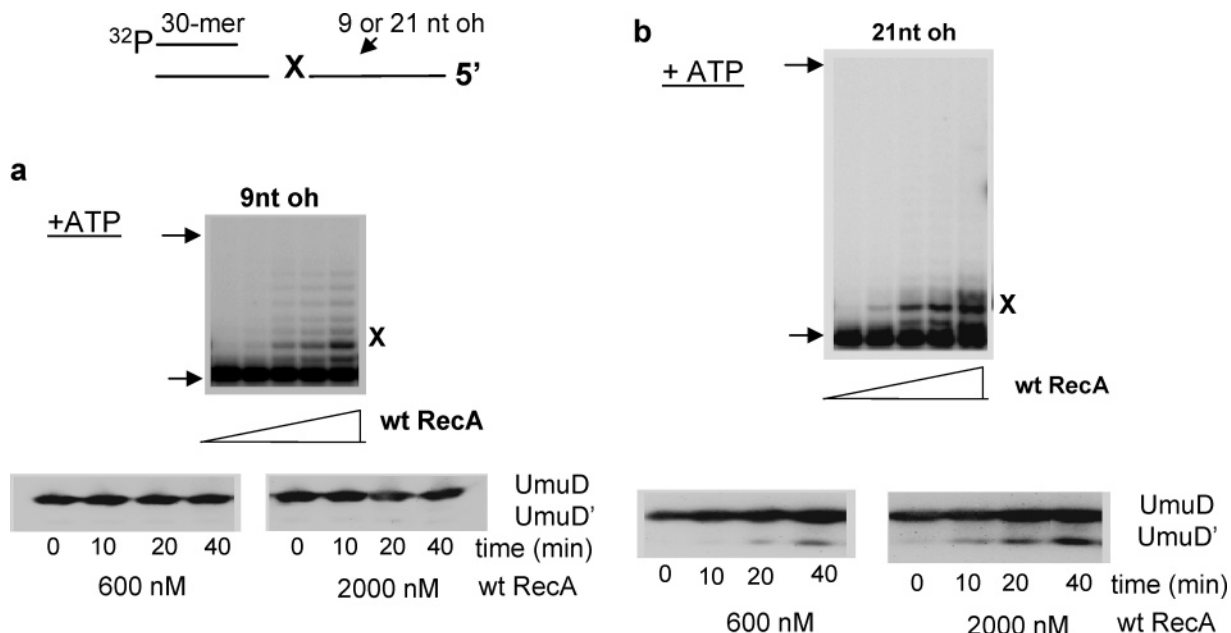
Figure 9. Pol-V-catalyzed lesion bypass requiring RecA but not a RecA filament. (a) TLS measured using primer template DNA containing a 9 nt template overhang, in the presence of continuously regenerating ATP and different RecA concentrations (0−2000 nM RecA protein). When identical reaction conditions were used, no conversion of UmuD to UmuD′ was observed at low (600 nM) or high (2000 nM) RecA concentrations, confirming the absence of a RecA filament. (b) Pol-V-catalyzed TLS failing to occur on primer template DNA having a 21 nt ssDNA overhang. Under identical reaction conditions, conversion of UmuD to UmuD′ was observed at low and high RecA concentrations, demonstrating that in the absence of any other accessory proteins the presence of a RecA nucleoprotein filament inhibits Pol-V-catalyzed TLS. Reprinted with permission from ref 16. Copyright 2005 Elsevier.
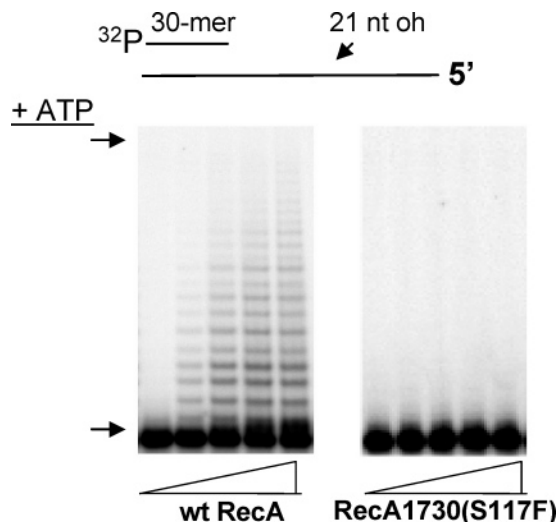

Figure 10. Third role for RecA during translesion synthesis: the direct stimulation of Pol V activity. Pol-V-catalyzed synthesis requires RecA and ATP (left gel). In contrast, mutant RecA1730 (S117F) fails to stimulate Pol V under any conditions. The concentrations of RecA were varied between 0 and 2000 nM. Reprinted with permission from ref 16. Copyright 2005 Elsevier.

RecA is both necessary and sufficient for TLS when RecA filamentation is precluded (Figure 9).[16] An examination of the interactions between RecA and Pol V sheds further light on this process.

4.5 Pol V−RecA Interactions

Since RecA is required for Pol-V-catalyzed TLS whereas RecA filaments are not needed, then how do RecA and Pol V interact? It is commonly believed that RecA-mediated interactions involve at least one step in which RecA binds cooperatively to ssDNA with ATP present as a cofactor.[8]

However, RecA protein and RecA mutants exhibit optimal stimulation of Pol V at concentrations too low and with DNA templates too short to support RecA filament formation. In addition, concentrations and conditions that tend to support RecA filament formation tend to inhibit Pol V function (Figure 9).[15,16,104] These observations led us to explore the interaction between RecA and UmuD′₂C.

We have recently found that RecA and Pol V are able to interact in the absence of DNA.[16] This interaction, referred to as mode 1 binding, takes place in the absence of ATP. Mode 1 binding is measured as an increase in rotational anisotropy (fluorescence depolarization) of a fluorescent-tagged RecA molecule when bound to Pol V (Figure 11a). An increase in steady-state rotational anisotropy reflects the reduction in rotational diffusion when Pol V forms a complex with RecA. In other words, a bound RecA−Pol V complex rotates more slowly in solution than free RecA.

A second mode of binding between Pol V and RecA occurs in the presence of primer/template (p/t) DNA, detected as an increase in the rotational anisotropy of a fluorescent-tagged DNA molecule interacting with both Pol V and RecA proteins (Figure 11b). Although Pol V is able to bind to DNA on its own (Figure 11b, inset), the presence of RecA and a nucleotide cofactor enhances the binding affinity 2-fold, represented by a 2-fold drop in K_d. Mode 2 binding requires the presence of ATP but not ATP hydrolysis (Figure 11b).[16] Notably, the DNA-dependent binding is the same for Pol V as that for the UmuD′ subunit of Pol V (Figure 11b, inset) in the absence of UmuC, which suggests that mode 2 binding involves a ternary complex with RecA, DNA, and the UmuD′ subunit of Pol V. Mode 1 binding is likely to involve an interaction between RecA and UmuC on the basis of the inability of RecA to bind to UmuD′ in the absence of DNA and ATP (Figure 11a). However, it is difficult to measure this interaction directly owing to the insolubility of UmuC in aqueous solution.[99,162,163]
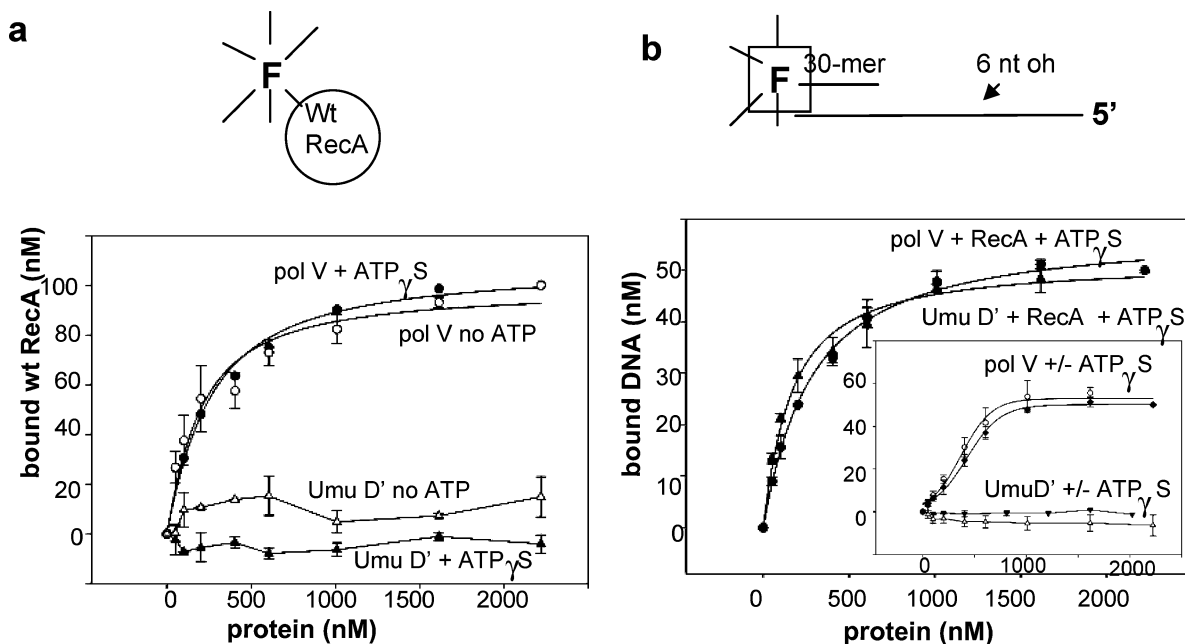
Figure 11. Pol V–RecA interactions. (a) RecA–Pol V mode 1 interaction. Fluorescent-labeled RecA binding to Pol V (● in the presence of ATPγS and ○ without ATP) or to UmuD' (▲ in the presence of ATPγS and △ without ATP) was measured as a change in steady-state rotational anisotropy. While UmuD' does not interact with RecA in the absence of DNA, Pol V (UmuD'$_2$C) binds to RecA with an apparent dissociation constant $K_d \approx 200$nM, suggesting the likelihood that UmuC interacts with RecA in solution. (b) RecA–Pol V mode 2 interaction. In the presence of ATPγS and 2000 nM WT RecA, Pol V (●) binds to a fluorescent-tagged primer template DNA with roughly the same affinity ($K_d \approx 250$ nM) as does UmuD' (▲, $K_d \approx 300$ nM). In the inset, Pol V in the absence of RecA (○) or in the absence of RecA and ATPγS (●) binds with a 2-fold lower affinity to the DNA ($K_d \approx 500$ nM), while UmuD' fails to interact with DNA completely in the absence of RecA (△) and ATPγS (▼). Reprinted with permission from ref 16. Copyright 2005 Elsevier.

4.6. Pol V–RecA, a Minimal Mutasome

By analogy with a replisome (Figure 6a), which refers to the protein complex assembled at the replication fork that copies DNA with high fidelity, Echols coined the term "mutasome"[5] to specify the proteins involved in low-fidelity TLS. The composition of the mutasome, postulated 12 twelve years prior to the discovery of Pol V, included UmuC, UmuD', Pol III holoenzyme (including the sliding β-clamp), and RecA in the form of a nucleoprotein filament and ATP.[5] There can be no doubt that β-clamp is required for TLS in vivo,[146,154] but it is not an essential biochemical element for copying damaged DNA in vitro.[15,16,104] Instead, β-clamp is used as a docking site for Pol III–Pol V exchange prior to and after TLS. Thus, we can see that, even at this very early stage of biochemical analysis, Echols' view of the mutasome was essentially correct, although not surprisingly subsequent revisions had to be made to the original model.[15,16,100] These revisions are embodied in a "minimal mutasome" model[16] (Figure 12), containing just those elements needed to copy past a DNA template lesion: Pol V (UmuD'$_2$C), RecA, and ATP. In the model, there is likely to be two RecA monomers present, but a RecA nucleoprotein filament does not need to be assembled (Figure 12).[16,104] One RecA molecule is bound to Pol V through the UmuD' subunit (mode 2 binding), requiring p/t DNA and ATP but not ATP hydrolysis. A second RecA molecule is bound to Pol V, probably via UmuC (mode 1 binding), that does not involve either DNA or ATP. Despite having characterized DNA- and ATP-independent binding of RecA to Pol V using rotational anisotropy (Figure 11a) along with scanning force field microscopy,[16] we cannot presently say whether mode 1 binding is actually part of the TLS process. Mode 2 binding is clearly required for TLS; there is no detectable DNA
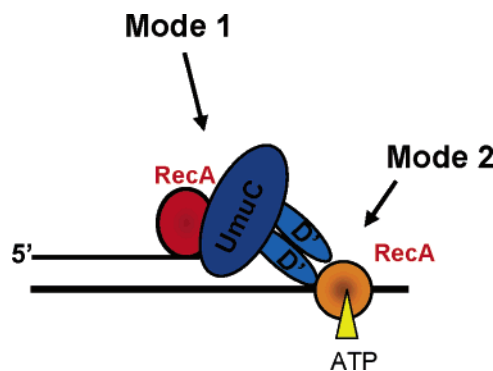

Figure 12. Pol V–RecA minimal mutasome model. Pol V (UmuD'$_2$C) and RecA, are the only proteins necessary for TLS. RecA interacts with Pol V in a DNA- and ATP-independent fashion (mode 1), and another RecA binds UmuD'$_2$, requiring ATP and DNA (mode 2). Reprinted with permission from ref 16. Copyright 2005 Elsevier.

synthesis in the absence of either ATP or non-hydrolyzable ATPγS.[16]

5. Future Perspectives

The genetic integrity of simple and complex organisms is closely tied to the action of the recombination proteins RecA in *E. coli* and Rad51 in eukaryotes. This review describes, as a work in progress, the even more intimate emerging role of RecA in translesion synthesis in *E. coli*, as alluded to in a recent review by Bryn Bridges.[164] Recently, new properties of RecA have been discovered, properties for which RecA need not assemble on single-stranded DNA in the form of a nucleoprotein filament on a template strand undergoing replication.

The nonfilamentous properties of RecA, which are required for activating Pol V, allowing it to copy past DNA

template lesions, have served as the focal point of this review. Strictly speaking, our data show that the assembly of a RecA nucleoprotein filament acts to block Pol V from copying undamaged and damaged DNA. Thus, if a filament were to assemble on the template strand proximal to a lesion, then it would have to be disassembled to allow TLS. The data further suggest that RecA nucleoprotein filament assembly per se on the template strand is not a prerequisite for TLS and thus differs from previous models. Recent studies suggesting that a RecA filament is required for Pol-V-catalyzed TLS[165,166] were performed in the presence of sliding β-clamp, SSB, and ATP, thus facilitating filament assembly and disassembly. We posit that lesion bypass in these experiments is likely taking place on templates lacking a RecA filament, i.e., on templates on which a RecA filament has been disassembled.

Perhaps our most salient result is that RecA is a required cofactor for the stimulation of Pol V activity. Pol V fails to perform TLS in the absence of RecA, and its activity is severely compromised on undamaged DNA. However, the precise mechanism for activation of Pol V is not known. RecA and Pol V interact in the absence of DNA, we think via UmuC, and further interact in the presence of DNA, via UmuD′. We suppose, as depicted in Figure 12, that there could be two distinct modes of interaction, each possibly involving a separate RecA monomer. Alternatively, the two modes might reflect different sides of the same coin, in which mode 1 is converted to mode 2, so that just one molecule of RecA is needed to activate Pol V. One might imagine that a molecule of RecA binds initially to the UmuC subunit of Pol V and that this same RecA binds subsequently to UmuD′ when Pol V binds to a primer template DNA molecule. Further experiments are required to define the precise nature of events leading to RecA activation of Pol V.

Beyond addressing the highly specialized intimate roles of RecA in activation of Pol V and TLS, a more general question can be raised: How in fact is SOS induced? Despite over 30 years of investigation, the biological SOS induction mechanism remains elusive. We know of no evidence showing that LexA repressor proteins dissociate from each of their more than 40 separate operators and then migrate to the vicinity of a stable RecA nucleoprotein filament to become cleaved. Perhaps, instead, one or just a few RecA molecules become activated when forming a transient complex with short DNA fragments generated during BER or NER. Migration of a short activated form of RecA to the vicinity of the LexA-SOS operator complex might be responsible for trapping and cleaving transiently dissociated LexA molecules. We believe that the involvement of extended "large scale" RecA nucleoprotein filaments in processes other than homologous recombination remains an open issue.

6. Acknowledgments

7. References

(1) Weigle, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1953**, *39*, 628.
(2) Radman, M. In *Molecular Mechanisms for Repair of DNA, Part A*; Hanawalt, P., Setlow, R. B., Eds.; Basic Life Sciences 5; Plenum Press: New York, 1975; pp 355−367.
(3) Witkin, E. M. *Bacteriol. Rev.* **1976**, *40*, 869.
(4) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*; ASM Press: Washington, DC, 1995.
(5) Echols, H.; Goodman, M. F. *Mutat. Res.* **1990**, *236*, 301.
(6) Courcelle, J.; Khodursky, A.; Peter, B.; Brown, P. O.; Hanawalt, P. C. *Genetics* **2001**, *158*, 41.
(7) Goodman, M. F. *Annu. Rev. Biochem.* **2002**, *71*, 17.
(8) Kuzminov, A. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 751.
(9) Walker, G. C. *Microbiol. Rev.* **1984**, *48*, 60.
(10) Little, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1375.
(11) Bailone, A.; Sommer, S.; Knezevic, J.; Dutreix, M.; Devoret, R. *Biochemie* **1991**, *73*, 479.
(12) Dutreix, M.; Moreau, P. L.; Bailone, A.; Galibert, F.; Battista, J. R.; Walker, G. C.; Devoret, R. *J. Bacteriol.* **1989**, *171*, 2415.
(13) Witkin, E. M.; McCall, J. O.; Volkert, M. R.; Wermundsen, I. E. *Mol. Gen. Genet.* **1982**, *185*, 43.
(14) Sweasy, J. B.; Witkin, E. M.; Sinha, N.; Roegner-Maniscalco, V. *J. Bacteriol.* **1990**, *172*, 3030.
(15) Pham, P.; Bertram, J. G.; O'Donnell, M.; Woodgate, R.; Goodman, M. F. *Nature* **2001**, *409*, 366.
(16) Schlacher, K.; Leslie, K.; Wyman, C.; Woodgate, R.; Cox, M. M.; Goodman, M. F. *Mol. Cell* **2005**, *17*, 561.
(17) Defais, M.; Devoret, R. SOS responses. In *Encyclopedia of Life Sciences*; John Wiley & Sons: Chichester, U.K., 2005. http://www.els.net/ (doi: 10.1038/npg.els.0003874).
(18) Shinagawa, H. *EXS* **1996**, *77*, 221.
(19) Smith, B. T.; Walker, G. C. *Genetics* **1998**, *148*, 1599.
(20) Sommer, S.; Boudsocq, F.; Devoret, R.; Bailone, A. *Mol. Microbiol.* **1998**, *28*, 281.
(21) Walker, G. C.; Smith, B. T.; Sutton, M. D. In *Bacterial Stress Responses*; Storz, G., Hengge-Aronis, R., Eds.; American Society of Microbiology: Washington, DC, 2000, pp 131−144.
(22) Sancar, A.; Reardon, J. T. *DNA Repair Replication* **2004**, *69*, 43.
(23) Prakash, S.; Prakash, L. *Mutat. Res.* **2000**, *451*, 13.
(24) de Laat, W. L.; Jaspers, N. G. J.; Hoeijmakers, J. H. J. *Genes Dev.* **1999**, *13*, 768.
(25) Fromme, J. C.; Verdine, G. L. *DNA Repair Replication* **2004**, *69*, 1.
(26) Mol, C. D.; Hosfield, D. J.; Tainer, J. A. *Mutat. Res.* **2000**, *460*, 211.
(27) McCullough, A. K.; Dodson, M. L.; Lloyd, R. S. *Annu. Rev. Biochem.* **1999**, *68*, 255.
(28) Wood, R. D. *Annu. Rev. Biochem.* **1996**, *35*, 135.
(29) Sancar, G. B. *Mutat. Res.* **2000**, *451*, 25.
(30) Pegg, A. E. *Mutat. Res.* **2000**, *462*, 83.
(31) Tang, M. J.; Shen, X.; Frank, E. G.; O'Donnell, M.; Woodgate, R.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8919.
(32) Goodman, M. F.; Tippin, B. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 101.
(33) Fuchs, R. P.; Fujii, S.; Wagner, J. *DNA Repair Replication* **2004**, *69*, 229.
(34) Rattray, A. J.; Strathern, J. N. *Annu. Rev. Genet.* **2003**, *37*, 31.
(35) Sutton, M. D.; Smith, B. T.; Godoy, V. G.; Walker, G. C. *Annu. Rev. Genet.* **2000**, *34*, 479.
(36) Gonzalez, M.; Woodgate, R. *Bioessays* **2002**, *24*, 141.
(37) Clark, A. J.; Margulies, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *53*, 451.
(38) Clark, A. J. *Annu. Rev. Genet.* **1973**, *7*, 67.
(39) Yang, S. X.; Yu, X.; Seitz, E. M.; Kowalczykowski, S. C.; Egelman, E. H. *J. Mol. Biol.* **2001**, *314*, 1077.
(40) Masson, J. Y.; West, S. C. *Trends Biochem. Sci.* **2001**, *26*, 131.
(41) Wernegreen, J. J.; Ochman, H.; Jones, I. B.; Moran, N. A. *J. Bacteriol.* **2000**, *182*, 3867.
(42) Tamas, I.; Klasson, L.; Canback, B.; Naslund, A. K.; Eriksson, A. S.; Wernegreen, J. J.; Sandstrom, J. P.; Moran, N. A.; Andersson, S. G. E. *Science* **2002**, *296*, 2376.
(43) Moran, N. A.; Baumann, P. *Curr. Opin. Microbiol.* **2000**, *3*, 270.
(44) Weinstock, G. M.; McEntee, K.; Lehman, I. R. *J. Biol. Chem.* **1981**, *256*, 8845.
(45) Pugh, B. F.; Cox, M. M. *J. Biol. Chem.* **1987**, *262*, 1326.
(46) Brenner, S. L.; Mitchell, R. S.; Morrical, S. W.; Neuendorf, S. K.; Schutte, B. C.; Cox, M. M. *J. Biol. Chem.* **1987**, *262*, 4011.
(47) Register, J. C., III; Griffith, J. *J. Biol. Chem.* **1985**, *260*, 12308.
(48) Shan, Q.; Bork, J. M.; Webb, B. L.; Inman, R. B.; Cox, M. M. *J. Mol. Biol.* **1997**, *265*, 519.
(49) Bork, J. M.; Cox, M. M.; Inman, R. B. *J. Biol. Chem.* **2001**, *276*, 45740.
(50) Arenson, T. A.; Tsodikov, O. V.; Cox, M. M. *J. Mol. Biol.* **1999**, *288*, 391.
(51) Egelman, E. H.; Stasiak, A. *J. Mol. Biol.* **1986**, *191*, 677.
(52) Yu, X.; Egelman, E. H. *J. Mol. Biol.* **1992**, *227*, 334.
(53) Stasiak, A.; Egelman, E. H. *Experientia* **1994**, *50*, 192.
(54) Takahashi, M.; Kubista, M.; Nordén, B. *Biochimie* **1991**, *73*, 219.
(55) Takahashi, M.; Norden, B. *J. Biochem.* **1995**, *117*, 947.

(56) Kubista, M.; Simonson, T.; Sjöback, R.; Widlund, H.; Johansson, A. In *Biological Structure and Function*, Proceedings of the 9th Conversation, The State University of New York; Sarma, R. H., Sarma, M. H., Eds.; Adenine Press: New York, 1996; pp 49−59.
(57) Kurumizaka, H.; Rao, B. J.; Ogawa, T.; Radding, C. M.; Shibata, T. *Nucleic Acids Res.* **1994**, *22*, 3387.
(58) Kurumizaka, H.; Shibata, T. *J. Biochem.* **1996**, *119*, 216.
(59) Little, J. W. *Biochimie* **1991**, *73*, 411.
(60) Kim, B.; Little, J. W. *Cell* **1993**, *73*, 1165.
(61) Roberts, J. W.; Roberts, C. W.; Craig, N. L. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4714.
(62) Shinagawa, H.; Iwasaki, H.; Kato, T.; Nakata, A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1806.
(63) Burckhardt, S. E.; Woodgate, R.; Scheuermann, R. H.; Echols, H. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1811.
(64) Arnold, D. A.; Kowalczykowski, S. C. RecBCD helicase/nuclease. In *Encyclopedia of Life Sciences*; John Wiley & Sons: Chichester, U.K., 1999. http://www.els.net/ (doi: 10.1038/npg./els./0000586).
(65) Dillingham, M. S.; Spies, M.; Kowalczykowski, S. C. *Nature* **2003**, *423*, 893.
(66) Singleton, M. R.; Dillingham, M. S.; Gaudier, M.; Kowalczykowski, S. C.; Wigley, D. B. *Nature* **2004**, *432*, 187.
(67) Amundsen, S. K.; Smith, G. R. *Cell* **2003**, *112*, 741.
(68) Churchill, J. J.; Anderson, D. G.; Kowalczykowski, S. C. *Genes Dev.* **1999**, *13*, 901.
(69) Arnold, D. A.; Kowalczykowski, S. C. *J. Biol. Chem.* **2000**, *275*, 12261.
(70) Robu, M. E.; Inman, R. B.; Cox, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8211.
(71) Cox, M. M. *Annu. Rev. Microbiol.* **2003**, *57*, 551.
(72) MacFarland, K. J.; Shan, Q.; Inman, R. B.; Cox, M. M. *J. Biol. Chem.* **1997**, *272*, 17675.
(73) Lusetti, S. L.; Shaw, J. J.; Cox, M. M. *J. Biol. Chem.* **2003**, *278*, 16381.
(74) Lusetti, S. L.; Wood, E. A.; Fleming, C. D.; Modica, M. J.; Korth, J.; Abbott, L.; Dwyer, D. W.; Roca, A. I.; Inman, R. B.; Cox, M. M. *J. Biol. Chem.* **2003**, *278*, 16372.
(75) Eggler, A. L.; Lusetti, S. L.; Cox, M. M. *J. Biol. Chem.* **2003**, *278*, 16389.
(76) Kowalczykowski, S. C.; Dixon, D. A.; Eggleston, A. K.; Lauder, S. D.; Rehrauer, W. M. *Microbiol. Rev.* **1994**, *58*, 401.
(77) Umezu, K.; Chi, N. W.; Kolodner, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3875.
(78) Umezu, K.; Kolodner, R. D. *J. Biol. Chem.* **1994**, *269*, 30005.
(79) Bork, J. M.; Cox, M. M.; Inman, R. B. *EMBO J.* **2001**, *20*, 7313.
(80) Lusetti, S. L.; Cox, M. M. *Annu. Rev. Biochem.* **2002**, *71*, 71.
(81) Morimatsu, K.; Kowalczykowski, S. C. *Mol. Cell* **2003**, *11*, 1337.
(82) Webb, B. L.; Cox, M. M.; Inman, R. B. *Cell* **1997**, *91*, 347.
(83) Stohl, E. A.; Brockman, J. P.; Burkle, K. L.; Morimatsu, K.; Kowalczykowski, S. C.; Siefert, H. S. *J. Biol. Chem.* **2003**, *278*, 2278.
(84) Drees, J. C.; Lusetti, S. L.; Chitteni-Pattu, S.; Inman, R. B.; Cox, M. M. *Mol. Cell* **2004**, *15*, 789.
(85) Drees, J. C.; Lusetti, S. L.; Cox, M. M. *J. Biol. Chem.* **2004**, *279*, 52991.
(86) Lusetti, S. L.; Drees, J. C.; Stohl, E. A.; Seifert, H. S.; Cox, M. M. *J. Biol. Chem.* **2004**, *279*, 55073.
(87) Lusetti, S. L.; Voloshin, O. N.; Inman, R. B.; Camerini-Otero, R. D.; Cox, M. M. *J. Biol. Chem.* **2004**, *279*, 30037.
(88) Yasuda, T.; Morimatsu, K.; Kato, R.; Usukura, J.; Takahashi, M.; Ohmori, H. *EMBO J.* **2001**, *20*, 1192.
(89) Voloshin, O. N.; Ramirez, B. E.; Bax, A.; Camerini-Otero, R. D. *Genes Dev.* **2001**, *15*, 415.
(90) Witkin, E. M. *Brookhaven Symp. Biol.* **1967**, *20*, 495.
(91) Witkin, E. M. *Annu. Rev. Microbiol.* **1969**, *23*, 487.
(92) Bridges, B. A.; Dennis, R. E.; Munson, R. J. *Genetics* **1967**, *57*, 897.
(93) Defais, M.; Fauquet, P.; Radman, M.; Errera, M. *Virology* **1971**, *43*, 495.
(94) Brent, R.; Ptashne, M. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4204.
(95) Little, J. W.; Mount, D. W.; Yanisch-Perron, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4199.
(96) Kato, T.; Shinoura, Y. *Mol. Gen. Genet.* **1977**, *156*, 121.
(97) Steinborn, G. *Mol. Gen. Genet.* **1978**, *165*, 87.
(98) Walker, G. C.; Dobson, P. P. *Mol. Gen. Genet.* **1979**, *172*, 17.
(99) Bruck, I.; Woodgate, R.; McEntee, K.; Goodman, M. F. *J. Biol. Chem.* **1996**, *271*, 10767.
(100) Tang, M.; Bruck, I.; Eritja, R.; Turner, J.; Frank, E. G.; Woodgate, R.; O'Donnell, M.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9755.
(101) Reuven, N. B.; Arad, G.; Maor-Shoshani, A.; Livneh, Z. *J. Biol. Chem.* **1999**, *274*, 31763.
(102) Tang, M.; Pham, P.; Shen, X.; Taylor, J. S.; O'Donnell, M.; Woodgate, R.; Goodman, M. F. *Nature* **2000**, *404*, 1014.
(103) Little, J. W.; Edmiston, S. H.; Pacelli, L. Z.; Mount, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3225.
(104) Pham, P.; Seitz, E. M.; Saveliev, S.; Shen, X.; Woodgate, R.; Cox, M. M.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11061.
(105) Napolitano, R.; Janel-Bintz, R.; Wagner, J.; Fuchs, R. P. *EMBO J.* **2000**, *19*, 6259.
(106) Shen, X.; Sayer, J. M.; Kroth, H.; Ponten, I.; O'Donnell, M.; Woodgate, R.; Jerina, D. M.; Goodman, M. F. *J. Biol. Chem.* **2002**, *277*, 5265.
(107) Lenne-Samuel, N.; Janel-Bintz, R.; Kolbanovskiy, A.; Geacintov, N. E.; Fuchs, R. P. *Mol. Microbiol.* **2000**, *38*, 299.
(108) Wagner, J.; Etienne, H.; Janel-Bintz, R.; Fuchs, R. P. *DNA Repair* **2002**, *1*, 159.
(109) Ichikawa-Ryo, H.; Kondo, S. *J. Mol. Biol.* **1975**, *97*, 77.
(110) Castellazzi, M.; George, J.; Buttin, G. *Mol. Gen. Genet.* **1972**, *119*, 139.
(111) Witkin, E. M. *Mol. Gen. Genet.* **1975**, *142*, 87.
(112) Fijalkowska, I. J.; Dunn, R. L.; Schaaper, R. M. *J. Bacteriol.* **1997**, *179*, 7435.
(113) Ciesla, Z. *Mol. Gen. Genet.* **1982**, *186*, 298.
(114) Brotcorne-Lannoye, A.; Maenhaut-Michel, G. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3904.
(115) Wood, R. D.; Hutchinson, F. *J. Mol. Biol.* **1984**, *173*, 293.
(116) Miller, J. H.; Low, K. B. *Cell* **1984**, *37*, 675.
(117) Yatagai, F.; Halliday, J. A.; Glickman, B. W. *Mol. Gen. Genet.* **1991**, *230*, 75.
(118) Maor-Shoshani, A.; Reuven, N. B.; Tomer, G.; Livneh, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 565.
(119) Kobayashi, S.; Valentine, M. R.; Pham, P.; O'Donnell, M.; Goodman, M. F. *J. Biol. Chem.* **2002**, *277*, 34198.
(120) Cairns, J.; Overbaugh, J.; Miller, S. *Nature* **1988**, *335*, 142.
(121) Cairns, J.; Foster, P. L. *Genetics* **1991**, *128*, 695.
(122) Rosenberg, S. M. *Nat. Rev. Genet.* **2001**, *2*, 504.
(123) McKenzie, G. J.; Harris, R. S.; Lee, P. L.; Rosenberg, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6646.
(124) Layton, J. C.; Foster, P. L. *Mol. Microbiol.* **2003**, *50*, 549.
(125) Foster, P. L. *Cold Spring Harbor Symp. Quant. Biol.* **2000**, *65*, 21.
(126) McKenzie, G. J.; Lee, P. L.; Lombardo, M. J.; Hastings, P. J.; Rosenberg, S. M. *Mol. Cell* **2001**, *7*, 571.
(127) Wagner, J.; Gruz, P.; Kim, S. R.; Yamada, M.; Matsui, K.; Fuchs, R. P.; Nohmi, T. *Mol. Cell* **1999**, *4*, 281.
(128) Foster, P. L.; Gudmundsson, G.; Trimarchi, J. M.; Cai, H.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7951.
(129) Woodgate, R.; Ennis, D. G. *Mol. Gen. Genet.* **1991**, *229*, 10.
(130) Bonner, C. A.; Randall, S. K.; Rayssiguier, C.; Radman, M.; Eritja, R.; Kaplan, B. E.; McEntee, K.; Goodman, M. F. *J. Biol. Chem.* **1988**, *263*, 18946.
(131) Qiu, Z.; Goodman, M. F. *J. Biol. Chem.* **1997**, *272*, 8611.
(132) Kim, S. R.; Matsui, K.; Yamada, M.; Gruz, P.; Nohmi, T. *Mol. Genet. Genomics* **2001**, *266*, 207.
(133) Yeiser, B.; Pepper, E. D.; Goodman, M. F.; Finkel, S. E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8737.
(134) Taddei, F.; Matic, I.; Radman, M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11736.
(135) Zambrano, M. M.; Siegele, D. A.; Almiron, M.; Tormo, A.; Kolter, R. *Science* **1993**, *259*, 1757.
(136) Finkel, S. E.; Kolter, R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4023.
(137) Kelman, Z.; O'Donnell, M. *Annu. Rev. Biochem.* **1995**, *34*, 171.
(138) Kong, X.-P.; Onrust, R.; O'Donnell, M.; Kuriyan, J. *Cell* **1992**, *69*, 425.
(139) Oakley, A. J.; Prosselkov, P.; Wijffels, G.; Beck, J. L.; Wilce, M. C.; Dixon, N. E. *Acta Crystallogr., Sect. D* **2003**, *59*, 1192.
(140) Bonner, C. A.; Stukenberg, P. T.; Rajagopalan, M.; Eritja, R.; O'Donnell, M.; McEntee, K.; Echols, H.; Goodman, M. F. *J. Biol. Chem.* **1992**, *267*, 11431.
(141) Wagner, J.; Fujii, S.; Gruz, P.; Nohmi, T.; Fuchs, R. P. *EMBO Rep.* **2000**, *1*, 484.
(142) Dalrymple, B. P.; Kongsuwan, K.; Wijffels, G.; Dixon, N. E.; Jennings, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 11627.
(143) Lopez de Saro, F. J.; O'Donnell, M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8376.
(144) Burnouf, D. Y.; Olieric, V.; Wagner, J.; Fujii, S.; Reinbolt, J.; Fuchs, R. P.; Dumas, P. *J. Mol. Biol.* **2004**, *335*, 1187.
(145) Becherel, O. J.; Fuchs, R. P.; Wagner, J. *DNA Repair* **2002**, *1*, 703.
(146) Lenne-Samuel, N.; Wagner, J.; Etienne, H.; Fuchs, R. P. *EMBO Rep.* **2002**, *3*, 45.
(147) Lopez de Saro, F. J.; Georgescu, R. E.; Goodman, M. F.; O'Donnell, M. *EMBO J.* **2003**, *22*, 6408.
(148) Bertram, J. G.; Bloom, L. B.; O'Donnell, M.; Goodman, M. F. *J. Biol. Chem.* **2004**, *279*, 33047.
(149) Johanson, K. O.; McHenry, C. S. *J. Biol. Chem.* **1982**, *257*, 12310.

(150) McHenry, C. S. *J. Biol. Chem.* **1991**, *266*, 19127.
(151) Fujii, S.; Fuchs, R. P. *EMBO J.* **2004**, *23*, 4342.
(152) Bunting, K. A.; Roe, S. M.; Pearl, L. H. *EMBO J.* **2003**, *22*, 5883.
(153) Indiani, C.; McInerney, P.; Georgescu, R.; Goodman, M. F.; O'Donnell, M. *Mol. Cell* **2005**, *19*, 805.
(154) Sutton, M. D.; Duzen, J. M.; Maul, R. W. *Mol. Microbiol.* **2005**, *55*, 1751.
(155) Sutton, M. D.; Narumi, I.; Walker, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5307.
(156) Duzen, J. M.; Walker, G. C.; Sutton, M. D. *DNA Repair* **2004**, *3*, 301.
(157) Wolff, E.; Kim, M.; Hu, K.; Yang, H.; Miller, J. H. *J. Bacteriol.* **2004**, *186*, 2900.
(158) Kuban, W.; Jonczyk, P.; Gawel, D.; Malanowska, K.; Schaaper, R. M.; Fijalkowska, I. J. *J. Bacteriol.* **2004**, *186*, 4802.

(159) Bridges, B. A.; Woodgate, R. *Mol. Gen. Genet.* **1984**, *196*, 364.
(160) Vandewiele, D.; Borden, A.; O'Grady, P. I.; Woodgate, R.; Lawrence, C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15519.
(161) Nohmi, T.; Battista, J. R.; Dodson, L. A.; Walker, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1816.
(162) Woodgate, R.; Rajagopalan, M.; Lu, C.; Echols, H. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7301.
(163) Shen, X.; Woodgate, R.; Goodman, M. F. *J. Biol. Chem.* **2003**, *278*, 52546.
(164) Bridges, B. A. *DNA Repair* **2005**, *4* (9), 1047.
(165) Reuven, N. B.; Arad, G.; Stasiak, A. Z.; Stasiak, A.; Livneh, Z. *J. Biol. Chem.* **2001**, *276*, 5511.
(166) Fujii, S.; Gasser, V.; Fuchs, R. P. *J. Mol. Biol.* **2004**, *341*, 405.