HHS Public Access

Author manuscript

DNA Repair (Amst). Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

DNA Repair (Amst). 2016 August ; 44: 42–50. doi:10.1016/j.dnarep.2016.05.005.

Insights into the complex levels of regulation imposed on *Escherichia coli* DNA polymerase V

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Abstract

It is now close to 40 years since the isolation of non-mutable *umu/uvm* strains of *Escherichia coli* and the realization that damage induced mutagenesis in *E.coli* is not a passive process. Early models of mutagenesis envisioned the Umu proteins as accessory factors to the cell's replicase that not only reduced its normally high fidelity, but also allowed the enzyme to traverse otherwise replication-blocking lesions in the genome. However, these models underwent a radical revision approximately 15 years ago, with the discovery that the Umu proteins actually encode for a DNA polymerase, *E.coli* pol V. The polymerase lacks 3′→5′ exonucleolytic proofreading activity and is inherently error-prone when replicating both undamaged and damage DNA. So as to limit any "gratuitous" mutagenesis, the activity of pol V is strictly regulated in the cell at multiple levels. This review will summarize our current understanding of the myriad levels of regulation imposed on pol V including transcriptional control, posttranslational modification, targeted proteolysis, activation of the catalytic activity of pol V through protein-protein interactions and the very recently described intracellular spatial regulation of pol V. Remarkably, despite the multiple levels at which pol V is regulated, the enzyme is nevertheless able to contribute to the genetic diversity and evolutionary fitness of *E.coli*.

Keywords

Translesion DNA synthesis; Posttranslational regulation; Proteolysis; Y-family DNA polymerase

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Conflict of interest: The authors declare no conflict of interest

1. Introduction

The pioneering studies of Weigle in 1953 [1] demonstrated that survival and mutagenesis of UV-irradiated bacteriophage λ is enhanced if the phage infected a host that had been previously subject to chromosomal DNA damage and provided one of the first hints that *E.coli* possesses a damage inducible (and potentially error-prone) repair system. These observations were followed up by Witkin who proposed a repressor system which was inactivated by DNA damage as a model to explain Weigle's phage induced reactivation [2]. The pivotal connection to mutagenesis was provided by Radman in a 1974 publication where he outlined an “SOS repair hypothesis” for inducible error-prone repair in *E.coli* [3]. Although occurring as part of a damage inducible response, it remained unclear at that time if the associated cellular mutagenesis was a passive byproduct of the SOS response, or required the active participation of cellular factors induced as part of the SOS response to facilitate mutagenesis.

The identification of the *umuC* locus by Kato and Shinoura in 1977 [4] and the allelic *uvm* locus by Steinborn in 1978 [5] lead to the realization that damage-induced mutagenesis in *E.coli* is not a passive process. Early mutagenesis models envisaged the Umu proteins as accessory factors to the cell's main replicase, pol III, which would allow it to bypass otherwise replication blocking lesions with a concomitant reduction in replication fidelity [6]. However, we now know that the Umu proteins actually encode for a low-fidelity DNA polymerase, pol V that can catalyze translesion DNA synthesis (TLS) of a variety of DNA lesions in the *E.coli* genome [7, 8]. Research over the past four decades has revealed numerous and complex levels of regulation that have been imposed on pol V so as to apparently limit its highly mutagenic functions within the cell. However, a low level of mutagenesis is actually beneficial, since it provides genetic diversity and may contribute to overall evolutionary fitness [9, 10]. Indeed, *E.coli* appears to utilize the various pol V regulatory pathways to provide “just the right amount” of pol V in times of stress, so as to help the organism overcome environmentally challenging adversity.

2. Transcriptional control

The damage-inducible SOS response is regulated through the interplay of two proteins; LexA and RecA [11]. LexA is a transcriptional repressor that binds specific nucleotide sequences located in the promoter region of genes under its control, thereby keeping the target gene's expression to a minimum. RecA is a recombinase that upon DNA damage forms nucleoprotein filaments (commonly called RecA*). Molecules of LexA that are not bound to DNA have a high affinity for the deep helical groove of RecA* [12], where the LexA protein undergoes a self-mediated cleavage reaction [13]. Cleavage of LexA inactivates its ability to serve as a transcriptional repressor. After DNA damage, intracellular levels of intact LexA protein drop and genes that are normally repressed by LexA are subsequently expressed at elevated, derepressed levels.

The LexA binding site is palindromic and has a consensus of TACTG-$(TA)_5$-CAGTA [14, 15]. Binding sites with a close match to the consensus sequence are said to have a low heterology index (H.I.), while those that deviate from the consensus have a high H.I. [16].

Genes that have a high H.I. binding-site are induced early in the SOS response, while those with a low H.I. are expressed much later in the response. The *umu* locus was shown to be under the transcriptional control of LexA in 1981 [17] and DNA sequence analysis of the *umu* loci revealed an operon consisting of two genes, *umuD* and *umuC* that have a good LexA binding site immediately upstream of the *umuD* gene [18, 19]. Analysis of the LexA binding site upstream of *umuDC* indicates that it has an H.I. of 2.77, making it one of the lowest values determined for the binding sites of 40 or so genes that are known to be transcriptionally-regulated by LexA [20]. As a consequence, it is not surprising that induction of the Umu operon occurs late in the SOS-response, and is not fully derepressed until ∼15 minutes after cells have been exposed to DNA damage [21] and significant levels of the Umu proteins do not accumulate until ∼45 minutes after DNA damage [22] (Fig. 1).

3. Lon-dependent Proteolysis of UmuD and UmuC

While the *umuDC* operon is one of the tightest regulated by LexA, transcriptional control is nevertheless insufficient to completely eliminate expression of UmuD and UmuC in an undamaged cell [23]. Levels of UmuD and UmuC are, however, kept to a minimum through their targeted proteolysis by the ATP-dependent protease, Lon [24, 25] (Fig. 2). The rapid degradation of the UmuD and UmuC proteins aides the illusion of much tighter transcriptional control than it is in reality since accumulation of the Umu proteins some 45 mins after DNA damage only occurs when their expression outweighs their degradation (Fig. 1). This is probably helped by the fact that other substrates of Lon, such as the cell division inhibitor, SulA [26], are also induced upon DNA damage. The primary Lon recognition site in UmuD has been identified as being located between residues 15 -18 (FPLF), with an auxiliary site between residues 26 - 29 (FPSP) in the amino terminus of UmuD [25]. The Lon recognition site in UmuC has yet to be identified, but is likely to reside in the last 51 carboxyl-terminal amino acid residues of UmuC, since a mutant lacking these residues is extremely stable compared to the normally labile wild-type UmuC protein [27].

4. Posttranslational conversion of UmuD to UmuD′

RecA plays a central role in the induction of the LexA-regulated SOS response. However mutants of *recA* were characterized in the early 1980s that were proficient for LexA cleavage, yet were phenotypically non-mutable after DNA damage [28], indicating that RecA played a direct, 2nd role in induced mutagenesis. Clues as to what this role might be emerged from the DNA sequence analysis of the cloned *umuD* gene [18, 19], which was shown to have limited homology to the carboxyl-terminus of LexA protein, including a potential cleavage site between residues 24-25 in the N-terminus of UmuD, as well as conserved Serine and Lysine active site residues.

UmuD was shown to undergo RecA-mediated cleavage *in vivo* [29] and *in vitro* [30] in 1988. Unlike LexA which is inactivated upon self-cleavage, the cleaved form of UmuD, called UmuD′, was shown to be active for inducible mutagenesis [31]. Indeed, all three of the original *umuD* mutants isolated by Kato and Shinoura [4] and independently by Steinborn [5] were subsequently shown to encode non-cleavable forms of UmuD [32]. Conversion of mutagenically inactive *E.coli* UmuD to mutagenically active UmuD′ is

extremely slow compared to LexA cleavage *in vitro* [30]. UmuD exists as a dimer in solution and studies on the nature of the cleavage reaction revealed that it can occur via an intermolecular reaction [33], whereby the cleavage site of one protomer is positioned in the active site of the partner protomer. The slow posttranslational processing of UmuD compared to LexA makes teleological sense, since the cell would want to rapidly induce DNA repair proteins under LexA control, yet also provide time for the repair proteins to work before converting inactive UmuD into a mutagenically active UmuD′ protein.

5. UmuD-UmuD′ heterodimerization and ClpXP proteolysis

It seems unlikely that cleavage of the N-terminal tail of the two UmuD protomers occurs simultaneously, meaning that the proteins would exist as a heterodimer of intact UmuD and processed UmuD′, until such time that the tail of the intact UmuD protomer is eventually processed to form homodimeric UmuD′ [34] (Fig. 2). Unlike homodimeric $UmuD_2$, which is labile and rapidly degraded by the Lon protease, homodimeric $UmuD'_2$ is largely insensitive to Lon-mediated proteolysis and is much more stable than $UmuD_2$ [24].

However UmuD′ in the context of the UmuD/D′ heterodimer is extremely labile and rapidly degraded by the ClpXP protease both *in vivo* and *in vitro* [24, 35, 36]. $UmuD_2$ has also been reported to be substrate of ClpXP *in vitro* [36], but the biological significance of this finding remains unclear, since $UmuD_2$ is stable in a *lon*$^-$ *clpXP*$^+$ strain *in vivo* [24]. Rapid ClpXP-dependent proteolysis of UmuD′ in the UmuD/D′ heterodimer therefore provides an additional way to delay the accumulation of mutagenically active $UmuD'_2$ molecules (Fig. 2). Indeed, rapid proteolysis of UmuD′ in the context of UmuD/D′ gives the impression that posttranslational conversion of UmuD to UmuD′ *in viv*o is slower than it is in reality. It is tacitly assumed that processing of UmuD to UmuD′ only occurs when the cell is severely damaged, yet considerable conversion of UmuD to UmuD′ was observed in an undamaged *recA*$^+$ *lexA*(Def) *clpX*$^-$ strain, indicating that UmuD cleavage does occur in an undamaged cell, but UmuD′ does not accumulate due to rapid proteolysis by ClpXP (Fig. 2 and [37]). The fact that homodimeric $UmuD'_2$ is not degraded by ClpXP indicated that the signal for UmuD′ degradation in the context of the UmuD/D′ heterodimer must reside in the N-terminal tail of intact UmuD. Alanine scanning mutagenesis revealed that the ClpXP recognition site that targets UmuD′ for degradation is located between residues 9-12 of UmuD (LREI) [35].

Another interesting observation related to the regulation of the UmuD′ protein by ClpXP is that when homodimers of $UmuD_2$ and $UmuD'_2$ are mixed *in vitro*, the proteins rapidly rearrange to form heterodimeric UmuD/D′ [38, 39]. A similar rearrangement was recently demonstrated under equilibrium conditions *in vitro*, confirming that formation of the heterodimeric UmuD/D′ complex is preferred over homodimeric $UmuD_2$ or $UmuD'_2$ [34]. The fact that formation of a UmuD/D′ heterodimer is preferred over formation of homodimers and that UmuD′ becomes susceptible to degradation by ClpXP only when it is part of the heterodimeric complex with UmuD provides a mechanism whereby *E.coli* can reduce the intracellular levels of the Umu proteins *after* they have facilitated survival of the damaged cell. As the damage is repaired, the inducing signal that activates RecA* dissipates and the conversion of $UmuD_2$ to $UmuD'_2$ slows. Any homodimeric $UmuD'_2$ already formed

in the cell will readily rearrange into a heterodimer with intact UmuD and will be rapidly degraded by ClpXP. Once UmuD′ is degraded, both $UmuD_2$ and UmuC will be degraded by the Lon protease (Fig. 3).

6. Activation of pol V via protein-protein interactions

Why is $UmuD_2$ unable to promote mutagenesis, whereas $UmuD'_2$ is considered as being mutagenically active? We believe that the answer lies in their respective ability to interact with UmuC. Homodimeric $UmuD'_2$ was shown in 1989 to interact with UmuC forming a soluble and stable ~70 kDa $UmuD'_2C$ complex [40] which can be purified from *E.coli* when both subunits are co-expressed *in vivo* [41]. This is in contrast to UmuC, which when expressed alone, is essentially insoluble [40]. We have taken advantage of the tight association between $UmuD'_2$ and UmuC to develop a simple and efficient method to purify milligram quantities of the soluble $UmuD'_2C$ complex from just a few liters of culture [42].

Briefly, N-terminal histidine tagged UmuC was expressed at very low levels along with high levels of untagged $UmuD'_2$. The high levels of $UmuD'_2$ help to “solubilize” UmuC and the $UmuD'_2$-His-UmuC complex was readily purified to homogeneity using a handful of chromatographic steps [42]. By comparison, when we substituted intact $UmuD_2$ for $UmuD'_2$ in the expression system, no soluble UmuC or $UmuD_2C$ complex was obtained (unpublished observations), indicating that the interaction between UmuC and $UmuD_2$ and $UmuD'_2$ is very different. Furthermore, when $UmuD_2$ was added to the purified $UmuD'_2C$ complex *in vitro*, heterodimerization between UmuD and UmuD′ occurred and UmuC precipitated out of solution [39] (Fig. 3). In essence, UmuC is only truly “soluble” when bound in a complex with $UmuD'_2$. Presumably binding of $UmuD'_2$ to UmuC masks hydrophobic regions of UmuC that would otherwise be solvent exposed in the absence of $UmuD'_2$.

Based upon structural modeling studies, it has been suggested that one protomer of the UmuD′ dimer interacts with UmuC residues 82, 90 and 126-132, while the other protomer interacts with UmuC residues 89, 93, 94 and 239 [43]. In contrast, at least two independent studies have indicated that UmuD′ interacts with the N- and C- terminus of UmuC. By using deletions of UmuC in a yeast two hybrid assay, Jonczyk and Nowicka determined that residues 1-13 and 397-422 are required for an interaction between UmuD′ and UmuC [44]. Sutton *et al.*, also concluded that the 26 C-terminal residues of UmuC are required for the interaction with UmuD′ [45]. By taking advantage of the fact that UmuC is labile and degraded by the Lon protease *in vivo* unless the protein is in a complex with $UmuD'_2$, we have found that deletion of just one C-terminal residue renders UmuC unstable such that it is barely detectable in the lon^+ strain and reduces Umu-dependent mutagenesis to about 20% of that seen with wild-type $UmuD'_2C$, most likely because the mutant has a reduced ability to interact with UmuD′ (Fig .4). Deletion of two or more amino acids essentially renders the strain non-mutable, presumably as a result of the complete loss of the $UmuD'_2$-UmuC interaction. In support of this notion, the Δ2 or Δ3 mutant is unable to promote cellular mutagenesis in a lon^- strain, even though the cell contains close to wild-type levels of UmuC (Fig. 4). With the ability to purify milligram quantities of the $UmuD'_2C$ complex [42], it surely must only be a matter of time before the crystal structure of pol V is finally determined and the regions of interaction identified.

Although formation of UmuD′$_2$C is a pre-requisite for mutagenesis, genetic and biochemical studies have revealed that pol V alone has very weak catalytic activity [42] which is strongly enhanced through interactions with protein partners. One of these partners is RecA. Clues to a direct role of RecA in the pol V-dependent mutagenic process came from genetic studies of Devoret and colleagues who isolated the *recA1730* (F117S) allele that is proficient for both LexA and UmuD cleavage, but is unable to promote UmuD′$_2$C (pol V)-dependent mutagenesis *in vivo* [46-48]. The so-called "3rd role" of RecA in damage induced mutagenesis remained elusive for over 25 years. However, reconstitution of pol V-dependent translesion DNA synthesis *in vitro* revealed that for efficient TLS to occur, a single molecule of RecA, along with an ATP molecule must be transferred from the 3′ tip of a RecA* filament to UmuD′$_2$C to generate a higher order structure termed pol V Mut [49, 50]. Subsequent biochemical studies have revealed that the ATP molecule is required for pol V Mut to bind a primer-terminus and that an intrinsic ATPase activity of pol V leads to ATP hydrolysis, which in turn, causes pol V Mut to dissociate from DNA [51]. The autoregulatory ATPase activity of pol V therefore helps limit the extent of error-prone DNA synthesis facilitated by pol V Mut (Fig. 3).

Greatest stimulation of TLS is observed when RecA* is *trans*-activating. *Cis*-activation could in principle also stimulate pol V activity, but such a mechanism would generate downstream problems, since the RecA* filament formed on the DNA strand being replicated would need to be displaced for pol V Mut to copy DNA [42, 52]. A study showing repetitive "on – off" deactivation – reactivation of pol V Mut, implied that RecA, which remains bound to pol V Mut in both states, is likely to bind in different locations [50]. Very recently, cross-linking studies have indicated that the 3′ tip of RecA* (residues 112-117) interacts with two separate regions of UmuC that include residues 257-277 and 362-377 that are over 40Å apart, suggesting at least two distinct binding modes of the RecA-UmuC interaction [53].

Like all of *E.coli*'s DNA polymerases, pol V interacts with the β–sliding clamp through a conserved canonical binding site [54]. This interaction is essential for pol V-dependent mutagenesis *in vivo* [55]. As expected, the interaction with β–clamp increases the processivity of pol V [56]. Pol V replicates DNA very slowly and is estimated to only incorporate one nucleotide every 2 seconds [42]. In the absence of the β-clamp, pol V can only replicate ∼30 nucleotides (in 2 mins) before dissociating from the primer-template. In contrast, in the presence of the β–sliding clamp, pol V can replicate several hundred nucleotides, if assayed under extended reaction times in the presence of ATPγS [42]. Interestingly, even in the presence of the β-clamp pol V exhibited very limited synthesis in the absence of single stranded binding (SSB) protein [42], indicating that SSB is another key co-factor required to stimulate pol V synthesis (Fig. 3).

7. Spatial regulation of pol V

One could easily imagine that the levels of regulation imposed on pol V and described above would be sufficient to keep the enzyme "in check". However, recent studies have revealed yet another level of regulation, namely spatial regulation of pol V [57]. Through the use of single molecule fluorescent microscopy, electron microscopy and western blotting of soluble

E.coli extracts, it was discovered that when first induced after DNA damage, UmuC is sequestered on the inner membrane. Over time, it re-localizes to the cytosol. The timing of the re-localization is concurrent with the conversion of UmuD to UmuD′ and the accumulation of soluble UmuD′$_2$C [57] (Fig. 5). At the present time, it is unknown how UmuC is initially sequestered on the inner membrane, but it is interesting to speculate that an exposed hydrophobic surface on monomeric UmuC is occluded when UmuD′$_2$ binds to UmuC to generate soluble UmuD′$_2$C (Fig. 5).

8. Alternative mechanisms of regulation imposed on pol V orthologs

It is now known that many gram-negative bacteria and their plasmids harbor pol V orthologs. Very few of these orthologs have been as well characterized as *E.coli* pol V, but where they have, it is interesting to determine how the activity of the pol V ortholog is regulated. Compared to *E.coli*, *Salmonella typhimurium* is poorly mutable. Like *E. coli* UmuD, *S. typhimurium* UmuD is subject to rapid proteolysis by the Lon protease [37]. Yet Lon regulation is largely circumvented because *S. typhimurium* UmuD is very efficiently converted to homodimeric UmuD′$_2$ [58], thereby avoiding both Lon-mediated degradation of homodimeric UmuD$_2$ and ClpXP-degradation of heterodimeric UmuD/D′.

It appears that *S. typhimurim* has no need to utilize targeted proteolysis as a form of regulation to limit the mutagenic activity of pol V since the *S. typhiumurim* UmuC protein is unable to promote significant levels of cellular mutagenesis [59]. Based upon analysis of chimera with regions of *E. coli* UmuC interchanged with *S. typhimurium* UmuC, the 8 residues that differ between amino acids 26-60 that comprise the substrate lid of the fingers domain of the polymerase [60] appear to be responsible for the poor mutability [61]. At the present time, *S. typhimurium* pol V has not been purified and characterized biochemically and it is unknown if the poor mutability is due to a loss of catalytic activity, or an increase in the fidelity of the *S. typhimurium* pol V compared to *E.coli* pol V.

Similarly, the most error-prone pol V ortholog characterized to date is pol V$_{R391}$ encoded by *rumAB* from the integrating conjugating element (ICE) 391 (formerly known as IncJ R391) [62-64]). When located in its native environment (an 88.5kb ICE), pol V$_{R391}$ promotes very low levels of cellular mutagenesis in *E.coli* [65]. The observed level of mutagenesis increased dramatically when the *rumAB* operon was sub-cloned [62]. RumA is cleaved to a RumA′ at a similar rate to *E.coli* UmuD, indicating that inefficient cleavage may help attenuate pol V$_{R391}$ activity [63], but it does not explain why the cellular mutagenesis increased when the *rumAB* operon was sub-cloned.

Analysis of the DNA sequence of the ICE391 (Genbank AY090559) reveals that it encodes a Lon protease homolog (orf31) that may degrade RumA and/or RumB; a putative 3′→5′ exonuclease (orf13) immediately upstream of the *rumAB* operon that may proofread any errors made by pol V$_{R391}$; and a lambda *cI*-like repressor, called SetR (orf 96) [66] that may also down-regulate *rumAB* expression. We assume that these proteins combine to substantially reduce pol V$_{R391}$–dependent mutagenesis when expressed from the ICE391 and as such, the catalytic activity of pol V$_{R391}$ has not been subjected to any evolutionary pressure to curtail its mutagenesis promoting ability, thereby explaining why the enzyme is

so potent when cloned away from its *cis*-acting regulating proteins. The fact that pol V_{R391} retains the potential to be highly mutagenic when unshackled from its normal regulation is worrisome. Antibiotics are known to induce the SOS response in bacteria [67, 68] and the mobilization of ICE391 [69]. The ICE391 elements are widely distributed in pathogenic strains of *Vibrio cholera* and prolonged exposure to SOS-inducing antibiotics may lead to the attenuation of regulatory mechanisms that normally keep pol V_{R391} activity to a minimum and may allow the pathogenic bacteria to adapt and ultimately acquire antibiotic resistance.

9. Concluding remarks and future perspectives

It is clear that bacteria use a variety of regulatory mechanisms to keep the mutagenesis-promoting activity of pol V enzymes at a level they can tolerate. In the case of *E.coli*, it is at multiple levels. *S. typhimurium* appears, on the surface, to do so with one fail swoop through a few amino acid substitutions in its UmuC protein. In contrast, pol V_{R391} has not been subjected to evolutionary pressures to curtail its very efficient mutagenesis-promoting activity because the enzyme is usually "kept in check" by a multitude of *cis*-acting regulatory factors. The result is that the TLS and mutagenesis promoting activity of pol V enzymes are minimalized until such time that they are needed for cell survival and mutagenesis, so as to maintain the evolutionary fitness of the host organism [9, 10, 70].

Such complex levels of regulation could hardly be imagined some 40 years ago when the *umu* operon was first discovered. Indeed, one gets the sense that we may have only revealed the "tip of the iceberg" on understanding how "simple" bacteria regulate the mutagenic process. To date, much of the studies on pol V have centered on *E.coli*, yet as noted above, there are hints that other bacteria utilize alternate mechanism to keep the error-prone polymerase in check. The advent of new technologies, such as single cell fluorescent microscopy, for example, opens up endless avenues of research on understanding (in three dimensions), how and when, any given bacterium (not just *E.coli*) responds to environmental damage (such as chronic exposure to antibiotics), and the subsequent evolution of antibiotic resistance that ensures cell survival. Based upon previous studies, it likely that a variety of novel and ingenious mechanisms that regulate the mutagenic process still remain to be discovered. One can only imagine where our understanding of such regulation might be in five years time, let alone another forty.

Acknowledgments

This study was made possible by funding from the NIH/NICHD Intramural Research Program to R.W. and ES012259, GM21422 to M.F.G. We thank Alexandra Vaisman for generating the figures shown in this review article.

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Abbreviations

nt	nucleotide
dNTP	deoxyribonucleoside triphosphate
rNTP	ribonucleoside triphosphate
pol	DNA polymerase
TLS	Translesion DNA synthesis
CPD	cyclobutane pyrimidine dimer
RecA*	RecA bound to single-stranded DNA forming a nucleoprotein filament

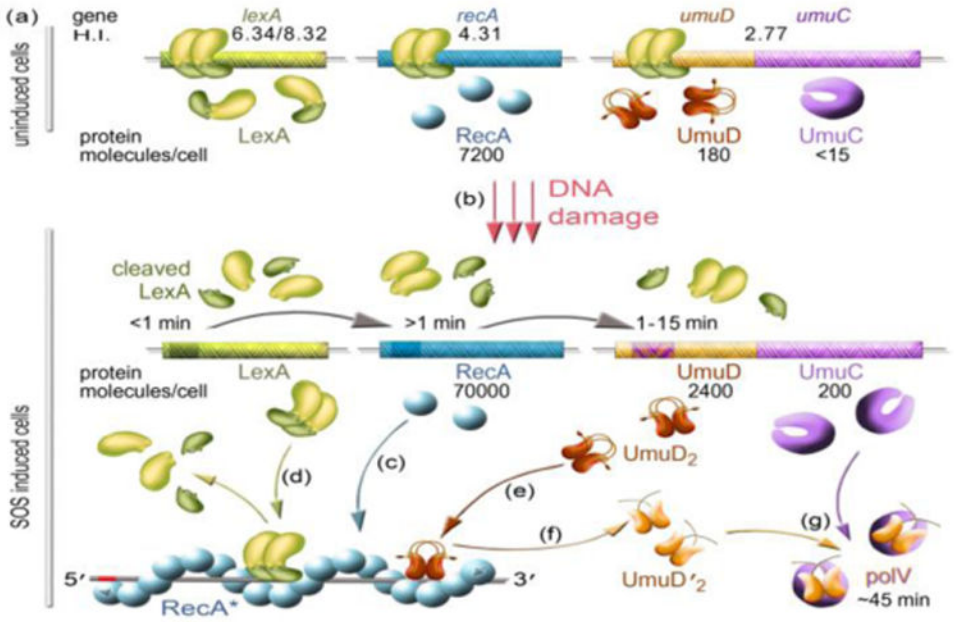

Figure 1. Transcriptional regulation of pol V-catalyzed TLS *in E. coli*

In undamaged cells, LexA repressor binds to specific sequences in the promoter region of SOS genes (including *recA*, *lexA*, *umuDC*) **(a)**. The relative affinity of LexA for these sites is determined by the extent the binding-site differs from the consensus site and is determined by the "heterology index" (H.I.). Genes with a high H.I., are expressed at higher basal levels than those with a low H.I. RecA has an H.I., of 4.31 and it is estimated that there are ∼7000 molecules of RecA in an undamaged cell. The *umu* operon is tightly regulated with an H.I., of 2.77. It is estimated that there are ∼180 molecules of UmuD and ∼15 molecules of UmuC in an undamaged cell. Cellular DNA damage results in the derepression of the SOS regulon **(b)**. RecA protein forms a nucleoprotein filament (RecA*) on single stranded DNA after DNA damage **(c)**. LexA protein has a high affinity for the deep helical groove of RecA* where it undergoes a self-cleavage reaction that inactivates its ability to serve as a transcriptional repressor **(d)**. SOS regulated genes with a high H.I., index such as LexA and RecA are induced early in the SOS response, while those with a low H.I., such as the UmuD and UmuC proteins are induced late in the SOS response. It is estimated that after DNA damage the concentration of RecA increases 10-fold to ∼70,000 molecules per cell. The Umu proteins are induced ∼13-fold, with ∼2400 molecules of UmuD and 200 molecules of UmuC per cell. Like LexA, UmuD has affinity for the deep helical groove of RecA* **(e)**. UmuD undergoes a self-cleavage reaction to generate $UmuD'_2$ **(f)**. Unlike LexA, which is inactivated after cleavage, cleavage of UmuD to UmuD′ activates the protein for its mutagenesis functions. $UmuD'_2$ can subsequently interact with UmuC to generate pol V with peak levels of pol V occurring ∼45 mins after DNA damage **(g)**.

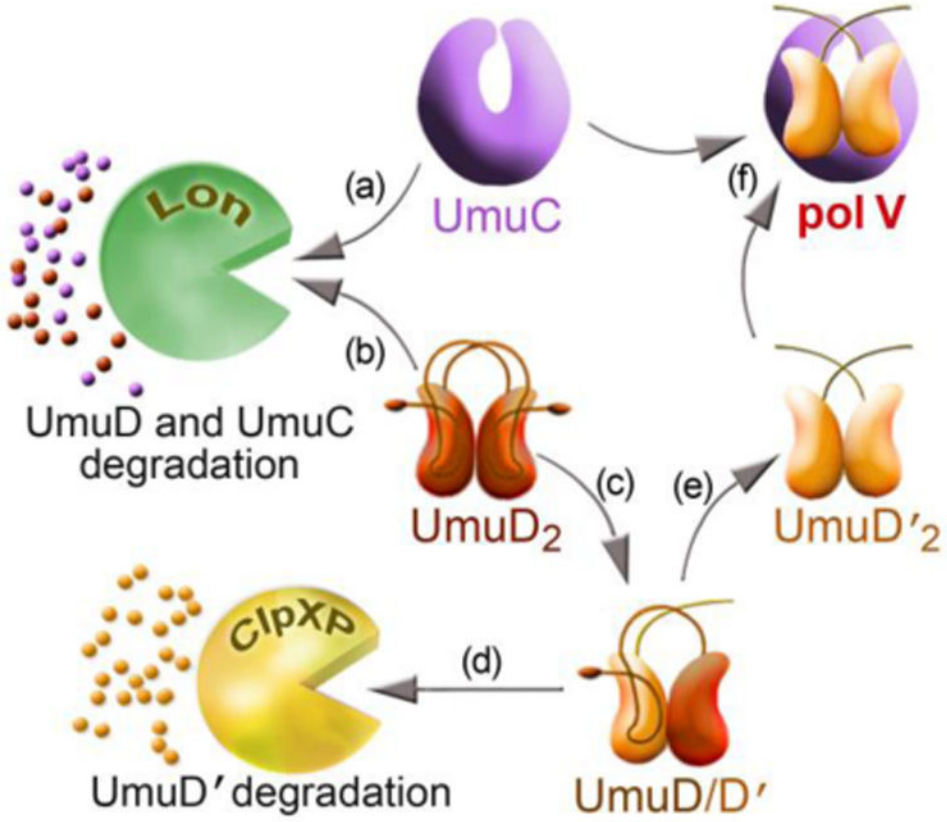

Figure 2. Proteolytic regulation of the Umu proteins helps delay their accumulation until late in the SOS response

The basal levels of the UmuC **(a)** and UmuD **(b)** proteins are kept to a minimum by the ATP-dependent protease, Lon. Late in the SOS response, $UmuD_2$ undergoes an inefficient intermolecular self-cleavage reaction **(c)**. This generates a UmuD/D′ heterodimer and in this context, UmuD′ is rapidly degraded by the ClpXP protease and postpones the accumulation of mutagenically active $UmuD'_2$ **(d)**. In the presence of persistent DNA damage and elevated levels of RecA*, UmuD/D′ is eventually converted to $UmuD'_2$, which is resistant to proteolytic degradation **(e)**. $UmuD'_2$ subsequently interacts with UmuC to generate pol V and in doing so, protects UmuC from Lon-mediated proteolysis **(f)**. This allows for the accumulation of pol V late in the SOS response, when the TLS polymerase is most needed to help facilitate cell-survival.

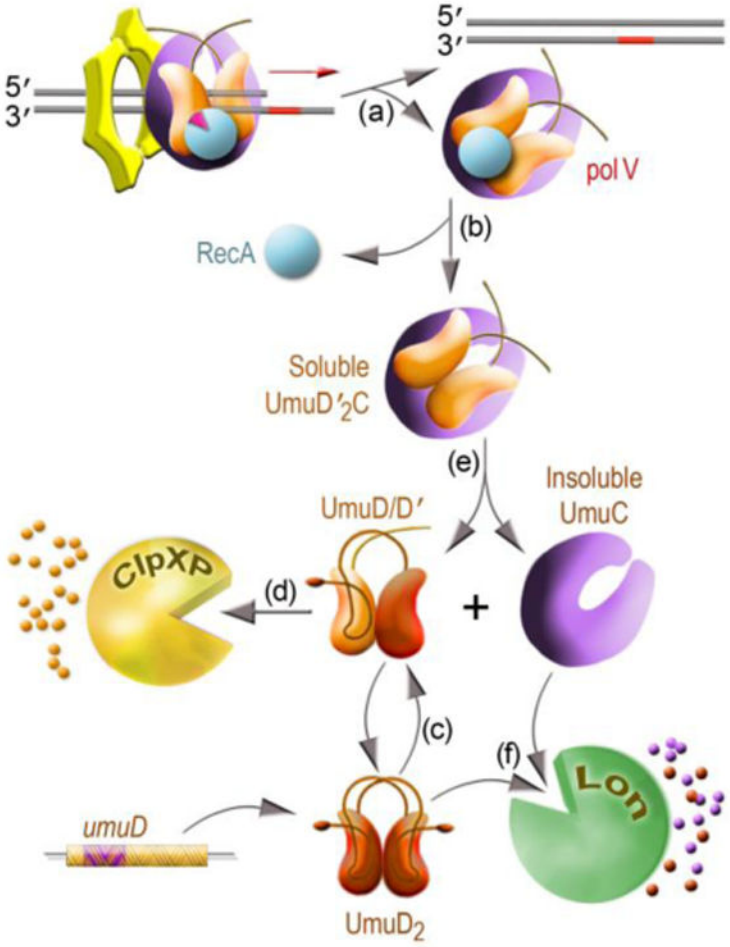

Figure 3. Returning cells to a resting state after pol V-dependent TLS has occurred

During TLS, the intrinsic ATPase of pol V hydrolyzes the ATP molecule (red triangle) associated with pol V Mut and causes the polymerase to dissociate from DNA after a short TLS track has been synthesized **(a)**. RecA also dissociates from UmuD′$_2$C to leave a soluble UmuD′$_2$C (pol V) complex, but with very weak catalytic activity **(b)**. As the SOS inducing signal wanes, the conversion of UmuD to UmuD′ slows. Intact molecules of UmuD preferentially heterodimerize with UmuD′ **(c)**, which renders UmuD′ susceptible to ClpXP-mediated degradation **(d)**. In the absence of UmuD′$_2$, UmuC is unable to remain in aqueous solution **(e)**. The levels of UmuD$_2$ and UmuC are finally returned to a basal state by their Lon-mediate degradation **(f)**.

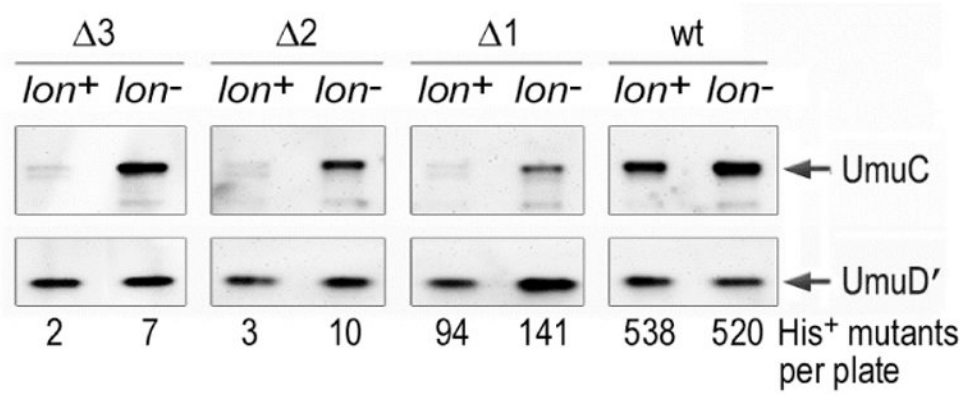

Fig 4. Removal of C-terminal residues of UmuC disrupts the interaction with UmuD′ and renders UmuC susceptible to Lon-mediated proteolysis

Western blot showing levels of UmuD′ and UmuC in isogenic *lon*$^+$ and Δ*lon recA730 lexA*(Def) strains. The UmuD′C proteins were expressed from a low copy plasmid, pRW134 [71]. UmuD′ is not degraded by the Lon protease and similar levels of UmuD′ are observed in both *lon*$^+$ and Δ*lon* strains. In contrast, UmuC is highly susceptible to Lon-mediated degradation unless it is in a complex with UmuD′$_2$. As clearly seen, UmuC mutants lacking one, two or three C-terminal amino acids are extremely unstable in a *lon*$^+$ strain, but are stabilized in a Δ*lon* strain. A UmuC mutant lacking just one C-terminal residue exhibited significantly reduced levels of *UmuD′C*-dependent spontaneous mutagenesis, while mutants lacking two, or three, C-terminal residues of UmuC were rendered essentially non-mutable, as judged by the number of His$^+$ revertants observed per plate. Together, these observations are consistent with the hypothesis that UmuD′ physically interacts with the extreme C-terminus of UmuC and in doing so, protects it from Lon-mediated degradation.

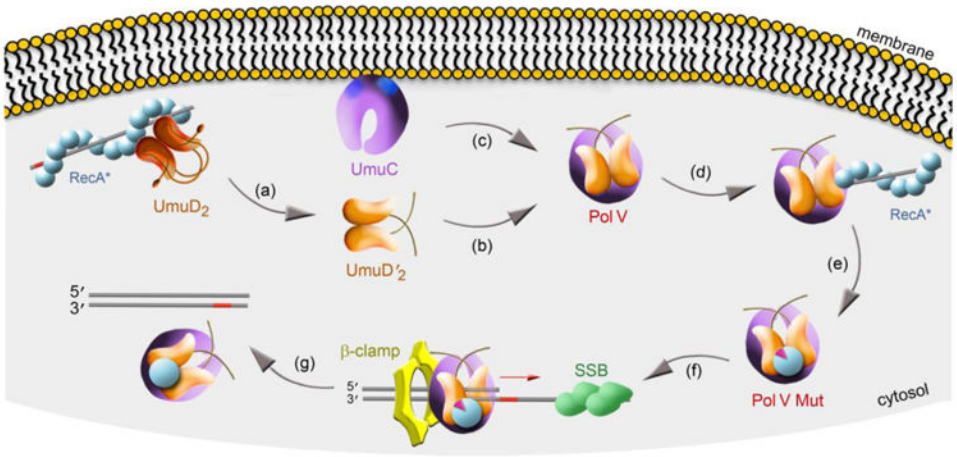

Figure 5. Spatial regulation of pol V

UmuC protein expressed >30 min after DNA damage is sequestered on the inner cellular membrane, possibly as a result of exposed hydrophobic patches on its surface (blue spots). Inefficient cleavage UmuD to UmuD′ occurs in the deep helical groove of RecA* that may also be membrane associated [72, 73] **(a)**. Once generated, $UmuD'_2$ **(b)** has high affinity for UmuC **(c)**. We hypothesize that when $UmuD'_2$ binds to UmuC, it occludes the previously exposed hydrophobic surface on monomeric UmuC and allows the soluble $UmuD'_2C$ (pol V) complex to re-localize to the cytosol. Soluble pol V transiently interacts with the 3′ tip of RecA* **(d)**, where it acquires a molecule of RecA and ATP to generate pol V Mut ∼45 min after the damage **(e)**. Pol V Mut is targeted to a lesion-containing (red bar) primer-template through an interaction with the β–sliding clamp protein and is further stimulated in the presence of single stranded binding (SSB) **(f)**. After TLS, ATP-hydrolysis catalyzed by the intrinsic DNA-dependent ATPase activity of pol V triggers dissociation of pol V Mut from DNA, thus minimizing the error-prone tracts of DNA synthesized by pol V Mut **(g)**.