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Review Article

The Roles of UmuD in Regulating Mutagenesis

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All organisms are subject to DNA damage from both endogenous and environmental sources. DNA damage that is not fully repaired can lead to mutations. Mutagenesis is now understood to be an active process, in part facilitated by lower-fidelity DNA polymerases that replicate DNA in an error-prone manner. Y-family DNA polymerases, found throughout all domains of life, are characterized by their lower fidelity on undamaged DNA and their specialized ability to copy damaged DNA. Two *E. coli* Y-family DNA polymerases are responsible for copying damaged DNA as well as for mutagenesis. These DNA polymerases interact with different forms of UmuD, a dynamic protein that regulates mutagenesis. The UmuD gene products, regulated by the SOS response, exist in two principal forms: $UmuD_2$, which prevents mutagenesis, and $UmuD'_2$, which facilitates UV-induced mutagenesis. This paper focuses on the multiple conformations of the UmuD gene products and how their protein interactions regulate mutagenesis.

1. Mutagenesis Due to Y-Family DNA Polymerases

The observation of nonmutable phenotypes of *E. coli umu* (UV-nonmutable) mutants led to the discovery that mutagenesis in *E. coli* is an active process [1–4]. The mutagenesis process utilizes specialized DNA polymerases belonging to the Y family [5]. Y-family DNA polymerases are found in all domains of life and have the specialized ability to replicate damaged DNA, a process known as translesion synthesis (TLS) [5–8]. This specialized ability comes at the cost of lower fidelity in replicating undamaged DNA compared to replicative DNA polymerases. Indeed, Y-family polymerases are from one to several orders of magnitude less accurate than replicative DNA polymerases [9–11]. Moreover, Y-family polymerases lack intrinsic 3′-5′-exonuclease activity and have inherent low processivity [6, 8, 12–14]. Because the cellular functions of Y-family DNA polymerases are potentially mutagenic, their activities are tightly regulated. *E. coli* has two members of the Y family, DNA pol IV (DinB, encoded by the *dinB* gene) [15] and pol V ($UmuD'_2C$, encoded by the UmuD and UmuC genes) [16, 17], whose functions are regulated on multiple levels. A key feature of their regulation is their interactions with products of the UmuD gene.

2. SOS Regulation

The UmuD gene is found in an operon with UmuC [18, 19]. The expression of these genes, as well as the *dinB* gene, is negatively regulated by the LexA repressor as part of the SOS response [7, 20]. LexA binds to a sequence in the operator region of regulated genes called the "SOS box," with a consensus sequence of 5′taCTGtatatatataCAGta, where the most conserved residues are in capital letters [21]. Upon DNA damage, a region of single-stranded DNA (ssDNA) forms due to the inability to continue replication of the damaged DNA. RecA polymerizes on the ssDNA, forming a RecA/ssDNA nucleoprotein filament, which is the inducing signal for the SOS response (Figure 1) [22]. Upon binding to the RecA/ssDNA filament, LexA undergoes a conformational change that stimulates its latent ability to cleave itself [23]. LexA cleavage inactivates it as a repressor and exposes a

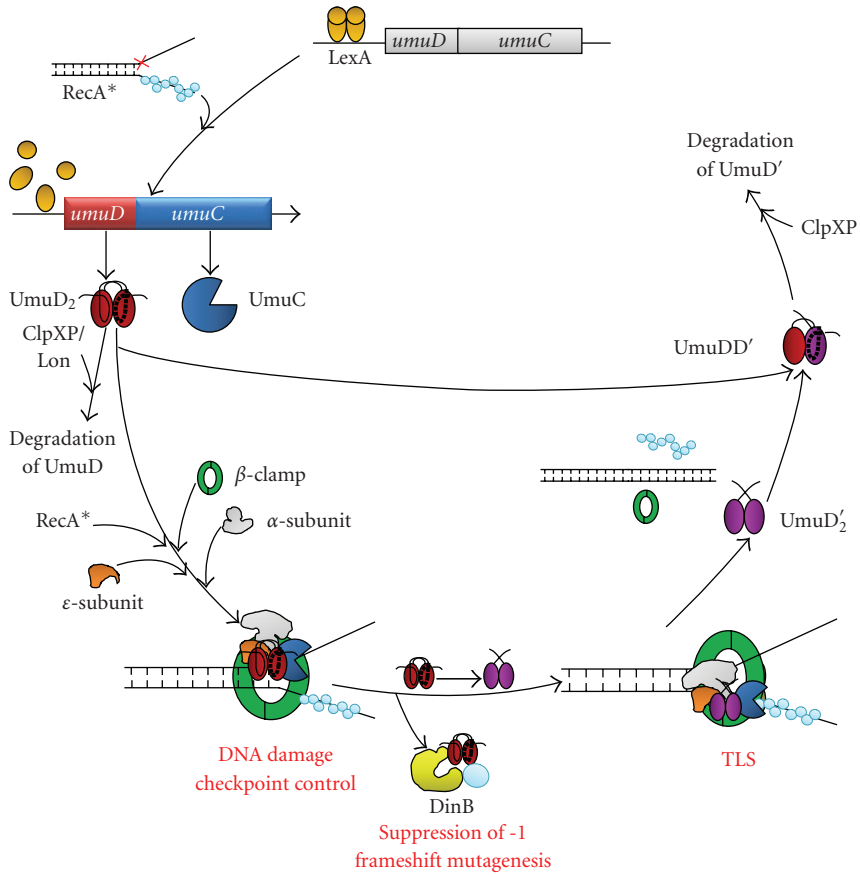

Figure 1: Life cycle and interactions of UmuD gene products. Details are described in the text.

proteolysis signal sequence, leading to degradation of LexA [24] and to increased expression of at least 57 SOS-regulated genes, including UmuD [20]. The cellular levels of UmuD, UmuC, and DinB all increase approximately 10-fold upon SOS induction, with UmuD increasing from ~180 to ~2400 molecules, UmuC increasing from ~15 to ~200 molecules, and DinB increasing from ~250 to ~2500 molecules per cell [25, 26]. The products of SOS-regulated genes are involved in DNA repair, DNA damage tolerance, and regulation of cell division. As the cell recovers from genotoxic stresses, it is presumed that the concentration of ssDNA is reduced, resulting in a decrease of RecA/ssDNA filament in the cell. This occurrence allows intact LexA to accumulate, thereby diminishing the SOS response [4].

3. UmuD is a Molecular Adaptor That Regulates Mutagenesis

Following initiation of the SOS response, $UmuD_2$ is the predominant form of the protein for 20–30 minutes [27]. The presence of UmuD and UmuC protects the cell from the potential deleterious effects of the error-prone DNA damage response pathway, a function which is genetically distinct from their role in SOS mutagenesis [27, 28]. $UmuD_2$, together with UmuC, may act in a primitive DNA damage checkpoint, as they specifically inhibit DNA replication without an effect on transcription or translation when present at elevated levels in cells grown at 30°C [27, 29]. UmuD and UmuC also slow the resumption of DNA replication after UV irradiation [27]. Therefore, UmuDC acts in a noncatalytic fashion by delaying SOS mutagenesis and thereby allowing accurate pathways such as nucleotide excision repair time to proceed [27, 28]. Moreover, UmuD interacts with DinB and inhibits its mutagenic −1 frameshift activity [30].

$UmuD_2$ interacts with the RecA/ssDNA filament, which stimulates the ability of UmuD to cleave itself, removing its N-terminal 24 amino acids [31–33]. UmuD is homologous to the C-terminal domain of LexA, and their cleavage reactions are remarkably similar: both proteins utilize a Ser-Lys (S60-K97 in UmuD) catalytic dyad, which is also similar to the reaction carried out by signal peptidases [34]. By analogy to signal peptidases, K97 is proposed to deprotonate S60, which is then capable of nucleophilic attack on the peptide backbone [34]. Therefore, $UmuD_2$ and LexA also undergo autodigestion at elevated pH [23, 33]. The kinetics of cleavage are remarkably different for $UmuD_2$ and LexA, with cleavage of LexA much more efficient than that of $UmuD_2$ in both RecA- and alkaline-mediated cleavage [33]. Moreover, LexA undergoes intramolecular cleavage [35] while $UmuD_2$ is capable of intermolecular cleavage [36–38].

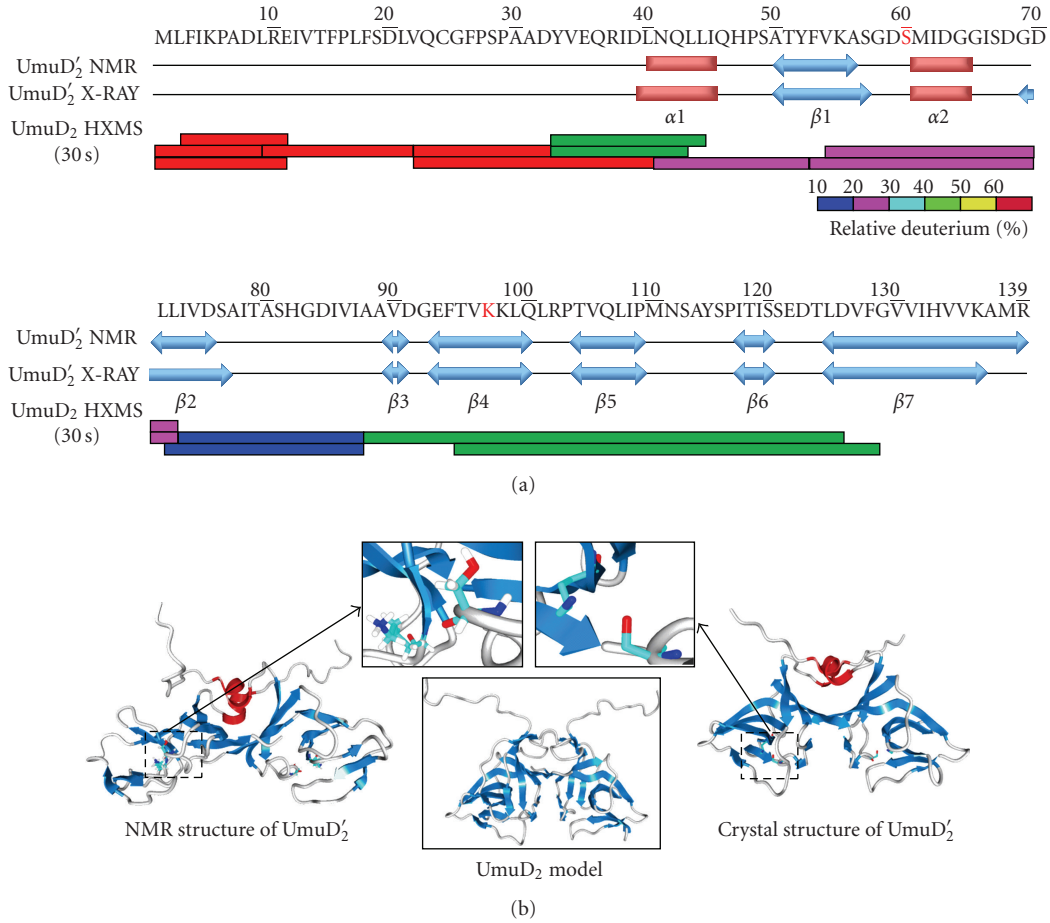

FIGURE 2: The secondary and tertiary structure of $UmuD_2$ and $UmuD'_2$. (a) Secondary structure comparison between the $UmuD'_2$ NMR [44, 45] and crystal [46] structures. The α helices are shown in red, and β sheets are shown in blue. Relative deuterium incorporation of $UmuD_2$ at 30 sec labeling in HXMS experiments is shown, and the colors are based on the relative deuterium percentage scale shown [51]. (b) Comparison of the NMR [44, 45] and crystal [46] structures of $UmuD'_2$. The color of the α helices and β sheets is consistent with (a). The active site regions are boxed and shown in the insets. A model of full-length $UmuD_2$ is shown [52].

RecA-facilitated cleavage of UmuD to UmuD′ occurs 20–40 minutes after the induction of SOS and serves to initiate TLS [4, 27]. UmuD′ together with UmuC forms the TLS polymerase Pol V that is active in the damage tolerance mechanism SOS mutagenesis [4, 16, 17, 39, 40]. Additionally, UmuD′ and UmuC inhibit RecA-dependent homologous recombination as a result of the direct interaction of UmuD′C with the RecA/ssDNA nucleoprotein filament, thereby preventing accurate recombination repair [41–43]. Taken together, these results support a model in which full-length UmuD acts to prevent mutagenesis while UmuD′ facilitates it.

4. Structure and Dynamics of UmuD

Since the UmuD gene products play crucial roles in managing the biological responses to DNA damage, the conformation and dynamics of $UmuD_2$ and $UmuD'_2$ are of great interest. To date, the structure of full-length $UmuD_2$ has not been amenable to crystallization or NMR analysis. However, the NMR [44, 45] and crystal [46] structures (Figure 2) of $UmuD'_2$ have been solved. Both structures show that $UmuD'_2$ is a homodimer with a C_2 axis of symmetry and show similar secondary structures: $UmuD'_2$ is composed primarily of β-strands with two short α-helices in each monomer. The C-terminal globular domain (residues 40–139) is mainly composed of curved antiparallel β-strands connected by tight turns with a long C-terminal strand, $\beta 7$, that spans both monomers (Figure 2). Residues between positions 132–139 in $\beta 7$ in UmuD and UmuD′ show the strongest interdimer cross-linking of their monocysteine derivatives [47]. The $\alpha 1$ helices pack against each other in the dimer interface. Both $UmuD_2$ and $UmuD'_2$ are exceptionally tight dimers with equilibrium dissociation constants $K_D{}^s <$ 10 pM [48]. The active site residue K97 is in the middle of strand $\beta 4$ while S60 is in helix $\alpha 2$ (Figure 2) [44, 46]. In both structures, the short N-terminal arms that remain after cleavage (residues 25–39) are largely unstructured [45, 46].

The differences between the X-ray and NMR structures of UmuD′ are not insignificant [44]. The RMSD of the backbone atoms (residues 40–139) between the two

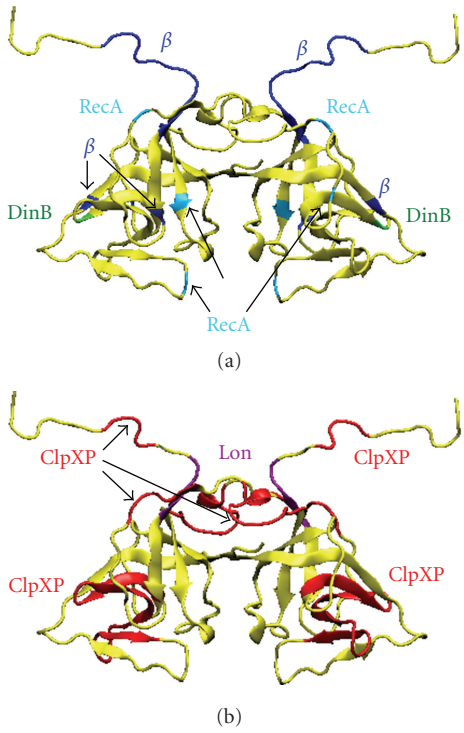

FIGURE 3: Protein interaction sites on UmuD. (a) The β clamp interacts with residues 14–19, 24, 52, and 126 (blue) [53]. RecA interacts with residues 34, 81, 57, 67, and 112 (cyan) [49]. DinB interacts with residue 91 on UmuD (green) [30]. (b) ClpXP interacts with residues 9–12, 33–37, 41–51, and 85–109 (red) [123]. Lon binds to regions close to the residues that are important for interaction with ClpXP, residues 15–19 (violet) [125].

structures of $UmuD'_2$ is 4.59 Å [44]. Moreover, the active site in the crystal structure appears correctly positioned for catalysis, while in the NMR structure, the catalytic residues Ser60 and Lys97 are over 7 Å apart and are not positioned appropriately (Figure 2) [44, 46]. It has been suggested that the conformation of $UmuD'_2$ in the crystal structure is similar to the conformation of $UmuD_2$ bound to the RecA/ssDNA nucleoprotein filament [44], which might indicate the mechanism whereby RecA/ssDNA acts as a coprotease in facilitating UmuD cleavage. Several residues on the outer loops and the surface of $UmuD_2$, specifically Val34, Ser57, Ser67, Ser81, and Ser112, each when changed to single cysteines, have been shown to cross-link to RecA and therefore are likely sites of interaction between the two proteins (Figure 3) [49]. The structure of $UmuD'_2$C-RecA/ssDNA complex determined by using cryo-electron microscopy shows $UmuD'_2$C bound deep in the groove of the RecA/ssDNA nucleoprotein filament and a second binding mode with $UmuD'_2$C at the end of the RecA/ssDNA filament [50].

The differences in the X-ray and NMR structures of UmuD′ and other findings suggested that UmuD and UmuD′ may be quite dynamic proteins. Indeed, $UmuD_2$ and $UmuD'_2$ were recently found to be intrinsically disordered proteins [48]. Despite the predominantly β-sheet character in the solved structures of UmuD′, the circular dichroism spectra of both UmuD′ and UmuD at physiological concentrations (5 μM) in solution are more characteristic of a random coil than of β sheets [48]. Higher concentrations of UmuD or UmuD′ (2 mM) or incubation with crowding agents or partner proteins including DinB or the β clamp induced CD spectra more characteristic of a predominantly β-sheet structure [48].

An analysis of the dynamics of UmuD using hydrogen-deuterium exchange mass spectrometry (HXMS) found that many regions of $UmuD_2$ were highly dynamic in solution, especially its N-terminal arms (Figure 2), consistent with previous suggestions [36, 44, 51]. In addition, the comparison of the conformations and dynamics of $UmuD_2$ and $UmuD'_2$ in solution by HXMS indicated that the N-terminal arm was a key factor governing the dynamics of $UmuD_2$ and $UmuD'_2$. In the absence of the N-terminal 24 residues in $UmuD'_2$, regions of the globular domain likely to contact the arm underwent more exchange than in $UmuD_2$ [51]. The predicted dimer interface of $UmuD_2$ was the most resistant to deuteration indicating that this region is the most stable and structured part of the protein. The results of HXMS were consistent both with the proposed model of $UmuD_2$ [52] and with the observation that $UmuD_2$ is relatively unstructured [48].

Gas-phase hydrogen-deuterium exchange experiments, which specifically detect side-chain hydrogen exchange at msec time scales, show that when the arm is truncated, in $UmuD'_2$, more side-chain sites can be labeled, reinforcing the idea that the arm protects part of the globular domain of the protein from interactions with solvent [51]. Therefore, the flexible N-terminal arm and the extended binding interface are potential sites for $UmuD_2$ to interact with other partner proteins. Indeed, the β processivity clamp has been shown to interact with specific amino acids in both the N-terminal arm and the globular domain of $UmuD_2$ [53].

Also in support of the dynamic nature of UmuD, it was found that Leu101 and Arg102 are important for proper positioning of the Ser/Lys active site dyad upon interaction with the RecA/ssDNA filament [54]. HXMS experiments showed that the peptides including these residues (89–125 and 95–128) are highly deuterated (Figure 2) [51]. Additionally, from molecular modeling experiments four distinct conformations of $UmuD_2$ were calculated; all four were isoenergetic, suggesting that all four conformations may be physiologically relevant [52]. Thus, the flexibility of $UmuD_2$ is likely to be a key feature governing its cleavage activity as well as interactions with its numerous protein partners.

Not only are the monomer units of UmuD and UmuD′ highly flexible, but multiple dimeric forms are also observed. Homodimers of $UmuD_2$ and $UmuD'_2$ readily exchange to form the UmuDD′ heterodimer, which has been found to be the most thermodynamically stable dimeric form [55]. Additionally, the X-ray structure of $UmuD'_2$ suggested two possible dimer interfaces [46, 56]. Much biochemical data, as well as solution NMR and HXMS experiments, are consistent with the dimer interface shown in Figure 2 [45, 47, 51]. However, some experiments suggest that the other dimer

interface (not shown) may also form [56]. Both dimer interfaces may be present in solution, as indicated by the observation of higher order cross-linked UmuD multimers of molecular weights consistent with tetramers and hexamers and larger complexes [28, 56]. UmuD and UmuD′ appear to be intrinsically highly dynamic proteins that can adopt multiple dimeric, and possibly higher order, forms.

5. The Interactions of the UmuD Gene Products with the α, β, and ε Subunits of DNA Polymerase III

DNA pol III is the 10-subunit complex responsible for most DNA replication in *E. coli* [57, 58]. Although pol III and $UmuD'_2C$ reduce the primer extension activity of each other by competing for DNA primer termini, they also appear to directly interact as $UmuD'_2C$ enhances the polymerase activity of pol III with a temperature-sensitive α protein *in vitro* [17, 59, 60]. $UmuD_2$ and $UmuD'_2$ directly interact with components of the replicative DNA polymerase III, including the α catalytic, β processivity, and ε proofreading subunits [28, 61]. The UmuD gene products display differential interactions with these components of the replisome. $UmuD_2$binds more strongly to the β processivity clamp than $UmuD'_2$ does whereas $UmuD'_2$ binds more strongly to the α polymerase subunit than $UmuD_2$ does, which is consistent with the UmuD gene products serving temporally separate roles in coordinating the replication machinery in response to DNA damage [61].

The ε subunit possesses 3′ to 5′ exonuclease activity and serves as the proofreading subunit of the replicative DNA polymerase [57]. Both $UmuD_2$ and $UmuD'_2$ interact with the C-terminal domain of ε, which is the same region of ε that contacts α [61–63]. The overexpression of the ε subunit suppresses UmuDC-mediated cold sensitivity whereas overexpression of any of the other pol III subunits does not [62].

By far, the best characterized interactions of UmuD or UmuD′ with the replisome are the interactions between UmuD or UmuD′ and the β processivity clamp. Overexpression of the β processivity clamp exacerbates UmuDC-mediated cold sensitivity, which was used as the basis of selection to identify additional sites of interaction between UmuD′, UmuC, and the β clamp [62, 64, 65]. It was suggested that the exacerbation of the cold sensitive phenotype is due to an exaggerated checkpoint response [61, 64].

$UmuD_2$ binds to β in the vicinity of the same hydrophobic pocket region where other β-binding proteins interact [53, 66]. As the β clamp is a homodimer, it has two such interaction sites per functional protein. However, there is still likely to be a hierarchy or competition for binding to the clamp because at least eight proteins are likely to interact with the β clamp at the same site, some of which possess different affinities for the β clamp (Table 1) [66–74]. By using site-directed mutagenesis and cross-linking experiments, it was reported that $UmuD_2$, $UmuD'_2$, and the α catalytic subunits of Pol III share some common contacts with β, but each of these proteins possesses a different affinity for β [66]. The N-terminal region of $UmuD_2$ contains a canonical β clamp-binding motif (^{14}TLPLF^{18}) (Figures 2 and 3, Table 1); this motif is used by a number of proteins to bind to the hydrophobic pocket on the β clamp (Figure 3) [67]. By constructing truncations of UmuD, it was determined that the residues between 9 and 19 are critical for interactions with the β clamp [53]. A $UmuD_2$ variant containing mutations in the canonical β clamp interaction motif was found to bind β with the same affinity as wild-type UmuD but with a different tryptophan fluorescence emission spectrum of β [52], which indicates that the motif itself is not necessary for the interaction, but it likely indicates a conformational change in the β clamp upon UmuD binding [6]. Additionally, residues in the C-terminal globular domain of UmuD and UmuD′ are also involved in interactions with the β clamp (Figure 3) [53]. Therefore, $UmuD_2$ interacts with the β clamp by both its N-terminal arm and C-terminal globular domain.

Table 1: *E. coli* proteins that interact with the β clamp via the β-binding pentapeptide motif QL[SD]LF or similar sequence [67].

β-interacting proteins	β-binding sequence	References
UmuD	^{14}TFPLF^{18}	[52, 53](1)
DNA Pol V (UmuC)	^{357}QLNLF^{361}	[127, 128]
DNA Pol IV (DinB)	^{346}QLVLGL^{351}	[68, 127, 129]
DNA Pol II (Pol B)	^{779}QLGLF^{783}	[127]
DNA Pol III (α-subunit)	^{920}QADMF^{924}	[130]
δ-subunit Clamp Loader	^{70}AMSLF^{74}	[131]
MutS	^{812}QMSLL^{816}	[132]
Hda	^{6}QLSLPL^{11}	[133]

(1)Although these residues reside in an important region for interactions with the β clamp, their identity is not required for UmuD to interact with β (see text Section 5).

6. Molecular Interactions of UmuD with Y-Family DNA Polymerases UmuC and DinB

6.1. Molecular Interactions of umuD Gene Products with UmuC. Disruptions to the *umuDC* operon result in nonmutability by UV, 4-nitroquinoline 1-oxide (4-NQO), methyl methanesulfonate (MMS), and other agents [1–4], presumably due to the lack of TLS by pol V. Pol V has a base substitution error frequency of 10^{-3}– 10^{-5} on undamaged DNA, compared to 10^{-4}–10^{-6} for the replicative DNA polymerase pol III [75, 76]. Pol V copies DNA-containing lesions such as abasic sites, thymine-thymine cyclobutane pyrimidine dimers, (6-4) photoproducts, as well as the C^8-dG adduct of *N*-2-acetylaminofluorene, while preferentially misincorporating dG opposite the 3′ T of the thymine-thymine (6-4) photoproducts [16, 17, 59, 75, 77, 78]. This specific mutagenic bypass of the (6-4) photoproduct is a major contributor to the observed UmuC-dependent SOS mutagenesis [75, 79]. UmuC contains intrinsic DNA polymerase activity and is therefore capable of DNA synthesis on undamaged DNA, but TLS activity requires the formation of the UmuD′C complex and the presence of RecA [16, 80].

Other cofactors, including SSB and the β processivity clamp and clamp loader, also support TLS by pol V [16, 17, 59, 75, 80–86].

Whereas UmuD′ is required for TLS by UmuC, full-length UmuD does not support TLS [16, 87]. Cells expressing UmuD and UmuC at elevated levels exhibit a cold sensitive for growth phenotype that is not yet well understood [29]. Full-length UmuD also plays a role together with UmuC in inhibiting the recovery of DNA replication after UV exposure [27]. Moreover, full-length UmuD that cannot be cleaved because it harbors the S60A active site mutation causes a dramatic reduction in UV-induced mutagenesis while UmuD′-S60A shows only a modest decrease in UV-induced mutagenesis [32]. Cells expressing UmuC together with noncleavable UmuD-S60A are sensitive to UV relative to cells expressing wild-type UmuD and UmuC but are resistant to killing by UV irradiation relative to cells that are Δ*umuDC* [27, 52, 55]. Taken together, these findings suggest that full-length UmuD specifically prevents mutagenesis, presumably at least in part by preventing UmuC from engaging in mutagenic TLS.

Due to the difficulty in acquiring large quantities of pure, active UmuC and pol V, protein interaction studies have been somewhat limited, especially considering that the UmuC gene was identified in the 1970s. However, the physical interaction between UmuD′ and UmuC was confirmed using immunoprecipitation, yeast two-hybrid assay and glycerol gradient analysis [88, 89]. Additionally, the interaction between full-length UmuD and UmuC was verified by using affinity chromatography and velocity sedimentation in glycerol gradients, but not immunoprecipitation from cell extracts [88]. From this, it was concluded that UmuC associates strongly with UmuD′*in vivo* whereas, *in vitro*, UmuC interacts efficiently with both forms of the UmuD gene products [88]. The likely stoichiometry was determined to be one UmuC with either a dimeric UmuD or UmuD′ [88]. UmuD and UmuD′ appear to interact with the C-terminus of UmuC, as a UmuC construct lacking its C-terminal 25 residues showed dramatically reduced binding to both UmuD and UmuD′ [28]. In addition to the UmuD and UmuD′ homodimers, UmuC also interacts with the UmuDD′ heterodimer, which acts to inhibit SOS mutagenesis, possibly by titrating out the dimeric UmuD′ species that is active in TLS [88–91].

6.2. Molecular Interactions of UmuD and DinB. The *dinB* (damage-inducible) gene encoding DNA pol IV (DinB) was discovered in a screen using reporter fusions to identify DNA damage-inducible genes [92]. DinB (Pol IV) is the other Y-family lesion bypass polymerase in *E. coli* and is the only Y-family polymerase that is conserved throughout all domains of life [5, 15]. The expression level of chromosomal DinB under DNA damaging conditions is 6–12 times higher than that of UmuC or PolB (DNA pol II) with about 2500 molecules of DinB in an SOS-induced cell [25]. DinB is also found on the recombinant F′ plasmid that was constructed to determine mutation spectra of specific revertible *lac*$^-$ alleles [25, 93]. The expression level of DinB in an uninduced state from the F′ plasmid in *E. coli* strain CC108 is approximately 750 molecules, as compared to 250 molecules expressed from the chromosome in the absence of SOS induction [25]. DinB has a misincorporation error frequency of 10^{-3} –10^{-5} [94]. Unlike UmuD′C, DinB elongates templates with bulged structures causing potentially deleterious -1 frameshift mutations [95, 96]. It was also shown that DinB and its eukaryotic ortholog Pol κ can accurately and efficiently perform TLS on templates containing N^2-deoxyguanosine (N^2-dG) adducts, suggesting that these proteins are specialized for relatively accurate TLS over some N^2-dG adducts [97–99].

UmuD, UmuD′, and RecA play important roles in the regulation of DinB, and direct physical interactions between DinB and UmuD, UmuD′, and RecA have been detected [30]. Although this may have initially seemed surprising, the expression levels of UmuD (180 molecules uninduced; 2400 molecules in SOS-induced cells) and DinB (250 molecules uninduced; 2500 molecules in SOS-induced cells) before and after SOS induction align [25, 26]. The stoichiometry of the complex was found to be one DinB molecule to one $UmuD_2$ dimer [30]. DinB and $UmuD_2$ bind with a K_D of 0.62 μM [30]. It was also determined that DinB, RecA, and $UmuD_2$ form a stable ternary complex under physiological conditions *in vitro* [30]. Genetic and biochemical analysis shows that full-length UmuD as well as the noncleavable UmuD variant UmuD S60A strongly inhibits the -1 frameshift mutator effect of DinB [30]. UmuD and UmuD′ also inhibit DinB activity in adaptive mutagenesis [30]. Presteady-state kinetics experiments led to the proposal that DinB bound to DNA containing a repetitive sequence is in equilibrium between a template slipped conformation, which leads to frameshift mutagenesis and a nonslipped conformation [100]. UmuD appears to prevent DinB-dependent frameshift mutagenesis by favoring the nonslipped conformation upon binding to DinB [100]. UmuD also modulates DinB function by facilitating efficient extension of correctly paired primer termini while blocking extension of mismatched termini [30, 100].

Using peptide array mapping and structural homology models of both DinB and UmuD, it was proposed that UmuD interacts with several hydrophobic residues on the surface of DinB in the thumb and finger domains. DinB residue F172 in the thumb domain was identified as a likely site of interaction with UmuD. Indeed, DinB F172A has lower affinity for UmuD (K_D reduced ~56-fold) and exhibits less UmuD-dependent -1 frameshift suppression *in vivo* and *in vitro* than wild-type DinB [30]. The DinB interacting surface on UmuD is a discontinuous surface when mapped onto a model of *trans*-UmuD [47, 52]. Alternatively, isoenergetic models of UmuD in which the N-terminal arms are in a noncleavable conformation provide alternative interacting surfaces across the side of UmuD [52]. UmuD D91, on the outer surface of UmuD, was proposed as a likely residue to be important for interaction with DinB (Figure 3). UmuD D91A has reduced affinity for DinB (K_D reduced by over 24-fold) and dramatically reduced suppression of -1 frameshift mutagenesis compared to wild-type UmuD [30]. This suggests that there may be multiple biologically relevant

conformations of UmuD that can interact with DinB or other polymerases [48, 51, 52, 101]. These interactions may aid in the suppression of frameshift mutagenesis by blocking the open active site that is needed to elongate bulged templates [13, 14, 30, 102]. By creating a ternary complex model of DinB, $UmuD_2$, and RecA, it was suggested that $UmuD_2$ and RecA work together in restricting the open active site of DinB thereby preventing -1 frameshift mutagenesis on bulged templates [30, 100]. Therefore, the presence of full-length UmuD actually enhances accurate TLS by DinB while suppressing extension of bulged templates that would cause frameshift mutagenesis.

7. Molecular Interactions of UmuD and UmuD′ with Lon and ClpXP Proteases

Regulation of UmuD protein levels by ClpXP and Lon proteases is an important part of the SOS response to DNA damage. Proteolytic degradation of the UmuD gene products is involved in cessation of SOS mutagenesis [4, 103, 104]. ClpXP is composed of the ATP-dependent unfoldase ClpX hexamer and the double-ringed, 14-subunit serine protease, ClpP [105–109]. The domain structure of the Lon protease is quite similar in that it contains an ATPase domain, a sensor and substrate discrimination domain (SSD), and a protease domain [110]. The mechanism of degradation begins when ClpX unfolds the substrates using repeated cycles of ATP hydrolysis and translocates the unfolded peptide into the ClpP chamber where proteolysis occurs. Substrate recognition involves the N- or C-terminal regions of the target protein binding to the substrate-processing site on ClpX [111, 112]. These signals may become apparent after cleavage, as in the case of LexA, or upon a conformational change in the target protein [113, 114]. However, the addition of an 11-amino acid (AANDENYALAA) ssrA tag to improperly translated nascent polypeptides will result in direct targeting to ClpXP for degradation [107, 108, 115–118]. This C-terminal ssrA tag is encoded by the ssrA transfer mRNA and is added cotranslationally to proteins translated without an in-frame stop codon [117, 118]. In addition, substrate recognition by ClpXP involves the interaction of tethering sites with adaptor proteins. These adaptor proteins are not degraded themselves but work to enhance the degradation of the target protein [119, 120]. One example is the SspB-mediated degradation of ssrA-tagged protein. Here, one part of the target protein binds the tethering site on ClpX while the SspB protein interacts with the ssrA tag enabling efficient delivery to ClpXP for degradation [121, 122].

Similar to SspB-facilitated degradation of ssrA-tagged target proteins, UmuD′ is a substrate for ClpXP but is only degraded when bound to full-length UmuD [123, 124]. Therefore, the preferential formation of UmuDD′ heterodimer specifically leads to a decrease in the steady-state levels of UmuD′ *in vivo* [123]. Although the residues found within the N-terminal 24 amino acids of UmuD serve as the degradation signal for ClpXP degradation of UmuD′, UmuD serves as an adaptor and is not itself degraded [124]. UmuD also serves as an adaptor in the context of $UmuD_2$ homodimers, leading to degradation of one UmuD in the dimer [123]. UmuD residues 9–12 are necessary for UmuD′ instability and therefore protease recognition (Figure 3) [124]. Amino acids 15–19 of UmuD are also implicated in the degradation of the UmuDD′ heterodimer by ClpXP (Figure 3) [124]. On the other hand, while residues 15–19 are also important for Lon-mediated degradation of UmuD, residues 9–12 are not involved in recognition by Lon [125]. ClpXP recognition sites can also be found on the surface of UmuD′, in particular, residues 33–37, 41–51, and 85–109 were found to interact robustly with ClpXP (Figure 3) [124]. The UmuD-facilitated degradation of UmuD′ can be impeded by the SspB-tethering peptide, and the SspB-tethering motif is interchangeable with the sequence in UmuD. Because the N-terminal domain of ClpX mediates interactions with both SspB and UmuD, it was determined that UmuD acts as a ClpX delivery factor that is critical in tethering itself and UmuD′ to ClpX. This seems to be a primary mechanism for bringing SOS mutagenesis to an end [126].

8. Conclusions

Although the UmuD gene was discovered over 30 years ago, new findings regarding how the UmuD gene products regulate mutagenesis have been made even within the last few years. This is despite the fact that there is still no high-resolution structure of full-length UmuD. The extremely dynamic nature of UmuD and UmuD′ has only recently come to light and provides insights into the large number of specific protein interactions of which the UmuD gene products are capable. Because of the role of UmuD in regulating mutagenesis, it could be important in bacterial evolution and is therefore potentially an important drug target.

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