Chapter 773

UmuD and UmuD′ Proteins

Databanks

MEROPS name: UmuD protein
MEROPS classification: clan SF, family S24, peptidase S24.003
Tertiary structure: Available
Species distribution: superkingdoms Eukaryota, Bacteria
Reference sequence from: *Escherichia coli* (UniProt: P0AG11)

Name and History

The *Escherichia coli umuC* locus was identified in the late 1970s in genetic screens for strains that were **U**V-non**mu**table [1]. In their manuscript, Kato and Shinoura reported the identification of three discrete loci that they called *umuA*, *umuB* and *umuC*. Mapping studies indicated that *umuA* and *umuB* mutants were likely to reside in *lexA* and *recA* respectively, while *umuC* appeared to be a novel locus involved in damage-induced mutagenesis [1]. Shortly thereafter, in an independent study, Steinborn similarly isolated mutants of *E. coli* that were also non-mutable after exposure to UV-light, which he called *uvm* (for **UV-m**utagenesis) [2]. The use of the *uvm* name was, however, dropped in the early 1980s when it was realized that the locus is allelic with *umuC*. Cloning and sequencing of the *umu* locus revealed that instead of encoding a single gene, it in fact consists of a two-gene operon that is regulated by the transcriptional repressor LexA [3–7]. The larger of the two genes was called *umuC*, while the smaller gene was called *umuD*. The *umuD* gene is located upstream of *umuC* with the TGA stop codon of *umuD* and the ATG start codon of *umuC* overlapping by one base-pair [6,7]. Perry *et al.* also noticed that the smaller *umuD* gene encodes a protein with similarity to the C-terminal domain of LexA, including conserved cleavage site and active site residues, suggesting that UmuD may undergo post-translational processing [7]. Indeed, in

1988, Shinagawa *et al.* and Burckhardt *et al.* demonstrated that the 15 kDa UmuD protein undergoes both RecA-mediated cleavage and autocatalysis at high pH to generate peptides of ~3 kDa and ~12 kDa [8,9]. At the same time, Nohmi *et al.* reported that unlike LexA, which is inactivated for its repressor functions upon proteolysis, the larger of the two UmuD cleavage products, called UmuD′, is active and actually required for damage-induced mutagenesis [10]. In the years since their initial discovery, several orthologs of *umuDC* have been identified, cloned and characterized. Many share the same general operon organization with a *umuD*-like gene located immediately upstream of a *umuC*-like gene. Various names have been given to these orthologs including *mucAB* (**m**utagenesis, **U**V and **c**hemical) [7,11]; *samAB* (***Sa****lmonella* **m**utagenesis) [12]; *impAB* (**I**-group **m**utagenesis and **p**rotection) [13,14]; *rumAB* (**R**-plasmid ***umu***-homolog) [15,16]; and *rulAB* (**r**esistance to **U**V-**l**ight) [17].

Activity and Specificity

The ability of *E. coli* UmuD [8,9] and several of its orthologs, including *Salmonella typhimurium* UmuD [18], the R-plasmid-encoded MucA [19,20], or RumA [16] proteins to undergo post-translational cleavage *in vitro* and *in vivo* has been reported. In all cases, processing is greatly stimulated by RecA protein. *In vivo*, moderately efficient cleavage occurs in cells that have been exposed to cellular DNA damage and in which RecA is believed to be in a so-called 'activated' filamentous state bound to single-stranded DNA. Constitutive cleavage occurs in *E. coli* strains expressing mutant RecAs that are in the activated state in the absence of exogenous DNA damage [8,21–23]. *In vitro*, the RecA-mediated reaction requires single-stranded DNA and magnesium for efficient nucleoprotein filament formation. In the absence of RecA, *E. coli* UmuD, MucA and RumA all undergo autoproteolysis at alkaline pH. The rate of autoproteolysis varies considerably between the orthologs with the fastest being MucA ($t_{1/2}$ at pH 10 ~ 75 min) [20] and the slowest being *E. coli* UmuD ($t_{1/2}$ at pH 10 > 10 h) [9,20]. Both of which are considerably slower than that of *E. coli* LexA ($t_{1/2}$ at pH 10 ~ 8 min) [20,24].

The UmuD-like proteins usually exist as dimers in solution [25,26] and it was originally believed that cleavage occurs via an intermolecular reaction in which the substrate tail of one protomer is cleaved in the active site of a dimer mate [18,27]. However, Ollivierre *et al.* [28] recently reported that a *umuD* mutant (N41D) which is unable to dimerize, nevertheless undergoes cleavage, indicating that the UmuD cleavage reaction can occur via both intermolecular and intramolecular pathways. While both proteins form homodimers in solution, when $UmuD_2$ and $UmuD'_2$ are mixed together *in vitro*, they preferentially associate to form UmuD/UmuD′ heterodimers [26].

Structural Chemistry

The *E. coli* UmuD protein is 139 amino acids in length and has a calculated molecular mass of 15 063 Da and a pI of 4.5. The 115 residue UmuD′ protein has a molecular mass of 12 285 Da and also has a pI of 4.5 [6,7].

The crystal structure of the $UmuD'_2$ protein was solved at 2.5 Å resolution revealing an extended N-terminal tail and a globular C-terminal catalytic and dimerization domain containing a mostly beta protein fold [29,30] (Figure 773.1A). NMR spectroscopy has been used to map the UmuD′ dimerization interface [31]; to solve the UmuD′ solution structure [32] (Figure 773.1B); and to propose a structure for the UmuD/UmuD′ heterodimer [32,33].

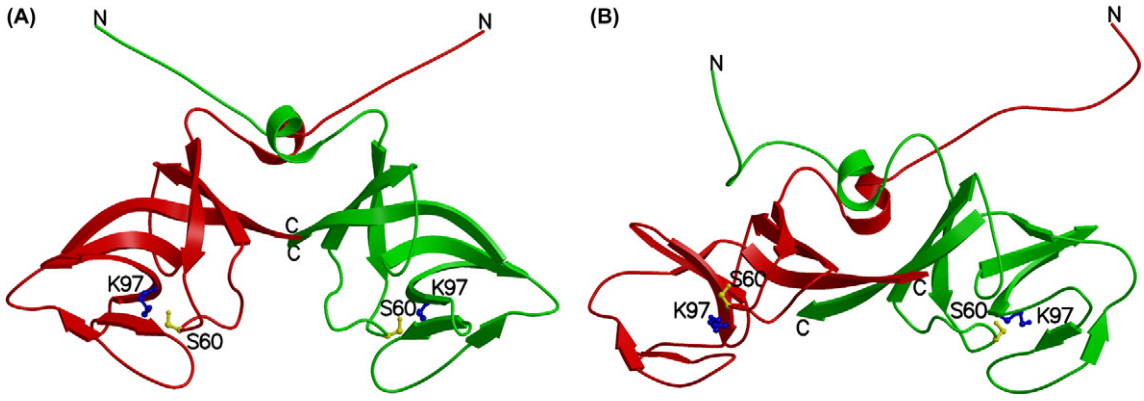

FIGURE 773.1 (A) The crystallographic structure of the $UmuD'_2$ dimer. The protein fold is shown as a ribbon with the side chains of the nucleophilic Ser 60 and general base Lys 97 shown in ball-and-stick. The atomic coordinates 1UMU (pdb code) were used to produce this figure after generating the symmetry-related molecules; (B) The NMR-determined structure of the $UmuD'_2$ dimer. Molecules A and B from the atomic coordinates 1I4V (pdb code) were used to produce this figure.

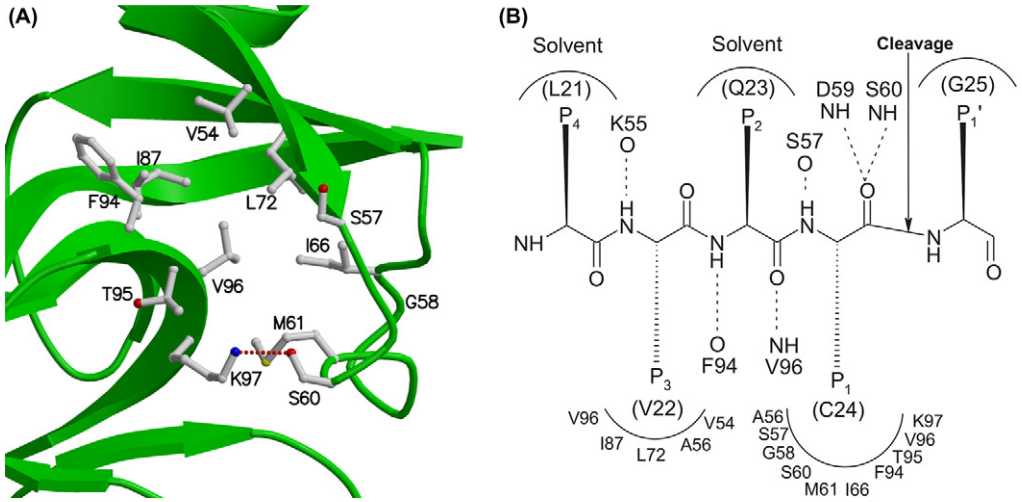

FIGURE 773.2 (A) The UmuD active site. The side chains of residues in the S1 and S3 binding pockets are shown in ball-and-stick. The crystal structure of UmuD′ shows that Ser 60 Oγ and Lys 97 Nζ are within hydrogen bonding distance [30]. The atomic coordinates 1UMU (pdb code) were used to produce this figure; (B) A schematic of the possible interactions between the UmuD cleavage site region in the binding site of its dimer mate. The residues involved in forming the S1 and S3 binding sites are indicated. The cleavage site residues are in parenthesis. Potential hydrogen bonding interactions between the extended cleavage site region and the β-strands that line each side of the binding sites are shown. The main chain amide hydrogens of Ser60 and Asp59 would make up the oxyanion hole.

UmuD utilizes a serine-lysine dyad mechanism. Site-directed mutagenesis [10] and structural studies [30] are consistent with Ser60 serving as the nucleophile and Lys97 the general base. A structural alignment of UmuD′ with the acyl-enzyme of signal peptidase (another member of the clan SF) suggested an orientation for the cleavage site in the UmuD binding site. These studies also revealed that the nucleophilic Ser60 hydroxyl of UmuD attacks the scissile bond (located between Cys24↓Gly25 of UmuD) from the *si*-face rather than the *re*-face as seen in most serine proteases [34] (Figure 773.2).

NMR analysis of the UmuD/UmuD′ dimer [32] and crystallographic analysis of the analogous protein LexA [35] are consistent with the proposed substrate orientation. The crystal structure of LexA with its bound cleavage site suggests that the main chain amide hydrogens from Ser60 and Asp59 in UmuD could serve as the oxyanion hole [35] (Figure 773.2B). Crystal structures are now available for members of the clan SF: UmuD′, λ CI repressor, LexA repressor, and signal peptidase [30,35–38]. A superposition of their active sites reveals that the Nζ of the lysine general base is coordinated by three hydrogen bonds. In the case of UmuD′, the neutral ε-amino group of Lys97 (the deprotonated state is a requirement for it serving as the general base) would have two hydrogen bond acceptors (Val96 O and Thr95 Oγ) and one hydrogen bond donor (Ser60 OγH). It is proposed that the pK_a of the ε-amino group of Lys97 is depressed by its burial upon binding of the cleavage site in an energetically unfavorable position [35] (Figure 773.2).

The NMR solution structure suggests that the UmuD′$_2$ dimer is structurally dynamic and that Ser60 and Lys97 are not within hydrogen bonding distance in solution [32] (Figire 773.1B). In contrast, all crystal structures of the clan SF proteases so far have shown the nucleophilic serine and general-base lysine to be within hydrogen bonding distance. Ferentz and collegues [32] suggest that the crystal packing forces may, therefore, result in the stabilization of the catalytically competent conformation and that an interaction with a RecA nucleoprotein filament *in vivo* facilitates the cleavable conformation.

Other proteases utilizing the serine-lysine catalytic dyad mechanism whose structure have recently been solved include the clan SJ proteases: Lon-A peptidase (family S16, PDB: 1RRE) [39] and birnavirus VP4 protease from blotched snakehead virus (family S50, PDB: 2GEF) [40], infectious pancreatic necrosis virus (family S50, PDB: 2PNL, 2PNM) [41] and *tellina* virus 1 (family S69, PDB: 3P06) [42]. Clan SK proteases that utilize the serine-lysine dyad mechanism include: C-terminal processing peptidase-1 (family S41, PDB: 1FC6) [43], and bacterial signal peptide peptidase A (family S49, PDB: 3BF0, 3BEZ) [44].

Preparation

The UmuD protein was initially overexpressed in *E. coli* from a temperature-inducible λP_L promoter [9]. Homodimeric UmuD′ can be purified from the same UmuD overproducing strain if the cells are also exposed to the DNA-damaging agent, mitomycin C, so as to promote *in vivo* conversion of UmuD to UmuD′ [25]. Both UmuD and recombinant UmuD′ are now routinely expressed from an IPTG-inducible T7 promoter [31,45]. Purification is relatively simple and involves ammonium

sulfate precipitation, ion-exchange and gel-filtration chromatography. Under these conditions, up to 10 mg of highly purified UmuD or UmuD′ protein can be isolated from 1 liter of an induced *E. coli* culture.

Biological Aspects

Since their discovery, the Umu proteins have been hypothesized to participate in damage-induced mutagenesis. For many years it was believed that the Umu proteins somehow modified the cell's main replicase, so that it would traverse otherwise replication-blocking lesions. However, in the late 1990s, the *E. coli* UmuC protein was shown to possess intrinsic DNA polymerase activity [46–48] and is now considered one of the founding members of the 'Y-family of DNA polymerases' [49]. *In vitro* studies suggest that homodimeric UmuD′ binds to the UmuC protein [48], to form UmuD′$_2$C, or *E.coli* DNA polymerase V (polV) [25,47,50]. The Y-family polymerases are found in all three kingdoms of life, yet interestingly, UmuD-like orthologs have only been identified in Gram-negative bacteria, their self-transmissible R-plasmids, or bacteriophages. Even more intriguing, is the fact that the P1 and N15 bacteriophage orthologs actually encode for a preprocessed UmuD′-like protein and do not undergo post-translational cleavage to become biologically active, nor are they associated with a cognate *umuC*-like gene [51]. Together, these observations have led to the suggestion that the UmuD and UmuD′-like proteins may participate in other biochemical pathways unique to Gram-negative bacteria. One such role might be in a 'cell-cycle' DNA damage-checkpoint pathway [32,52,53]. Whatever their role(s) in addition to translesion replication, it is clear that *E. coli* has gone to great lengths to minimize the cellular concentrations of both the UmuD and UmuD′ proteins *in vivo* [21]. In addition to being tightly regulated at the transcriptional level by LexA, UmuD protein is rapidly degraded by the Lon protease [54,55]. Some molecules of UmuD that escape Lon-mediated proteolysis are nevertheless converted to UmuD′ upon cellular DNA damage. But instead of forming homodimers, which are resistant to proteolysis [54,56], the UmuD′ protomers preferentially associate with intact UmuD to form a UmuD/UmuD′ heterodimer, where the UmuD′ protomer becomes a substrate for another serine protease, ClpXP [54,57]. Significant levels of UmuD′$_2$ only form when the cell is exposed to high levels of DNA damage and as a consequence, error-prone polV-dependent translesion DNA synthesis is only utilized as a last resort to enable cell survival.

Distinguishing Features

Polyclonal rabbit antibodies have been produced against both UmuD and UmuD′ [25,54] that can detect endogenous levels of the chromosomally encoded *E. coli* proteins [21,22].

Related Peptidases

The C-terminal proteolytic/dimerization domain of the UmuD protein (residues 50–136) has sequence and structural similarity to the proteolytic/dimerization domain of the large family (family S24) of λ CI [37] and LexA-like repressors [35]. It is also structurally related to the central catalytic domain of bacterial signal peptidase (family S26; [34,36,58]). The families S24 and S26 both belong to the clan SF of serine proteases.

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Further Reading

A structural analysis and comparison of the Ser/Lys protease has been previously reported [58]. For a general discussion on serine-lysine proteases see Paetzel & Dalbey [59]. For a recent review of serine proteases utilizing unconventional catalytic mechanisms see Ekici *et al.* [60]. A recent review on UmuD and its role in the SOS response can be found in Ollivierre *et al.* [61]. There have also been several recent reviews on the cellular functions of Y-family DNA polymerases; see Jarosz *et al.* [62], Fuchs *et al.* [63], Yang & Woodgate [64], and Pata [65] (and additional references therein).

References

[1] Kato, T., Shinoura, Y. (1977). Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol. Gen. Genet.* 156, 121–131.

[2] Steinborn, G. (1978). Uvm mutants of *Escherichia coli* K12 deficient in UV mutagenesis. I. Isolation of *uvm* mutants and their phenotypical characterization in DNA repair and mutagenesis. *Mol. Gen. Genet.* 165, 87–93.

[3] Bagg, A., Kenyon, C.J., Walker, G.C. (1981). Inducibility of a gene product required for UV and chemical mutagenesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 78, 5749–5753.

[4] Shinagawa, H., Kato, T., Ise, T., Makino, K., Nakata, A. (1983). Cloning and characterization of the *umu* operon responsible for inducible mutagenesis in *Escherichia coli*. *Gene* 23, 167–174.

[5] Elledge, S.J., Walker, G.C. (1983). Proteins required for ultraviolet light and chemical mutagenesis. Identification of the products of the *umuC* locus of *Escherichia coli*. *J. Mol. Biol.* 164, 175–192.

[6] Kitagawa, Y., Akaboshi, E., Shinagawa, H., Horii, T., Ogawa, H., Kato, T. (1985). Structural analysis of the *umu* operon required for inducible mutagenesis in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 82, 4336–4340.

[7] Perry, K.L., Elledge, S.J., Mitchell, B., Marsh, L., Walker, G.C. (1985). *umuDC* and *mucAB* operons whose products are required for UV light and chemical-induced mutagenesis: UmuD, MucA, and LexA products share homology. *Proc. Natl. Acad. Sci. USA* 82, 4331–4335.

[8] Shinagawa, H., Iwasaki, H., Kato, T., Nakata, A. (1988). RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis. *Proc. Natl. Acad. Sci. USA* 85, 1806–1810.

[9] Burckhardt, S.E., Woodgate, R., Scheuermann, R.H., Echols, H. (1988). UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification and cleavage by RecA. *Proc. Natl. Acad. Sci. USA* 85, 1811–1815.

[10] Nohmi, T., Battista, J.R., Dodson, L.A., Walker, G.C. (1988). RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. *Proc. Natl. Acad. Sci. USA* 85, 1816–1820.

[11] Perry, K.L., Walker, G.C. (1982). Identification of plasmid (pKM101) coded proteins involved in mutagenesis and UV resistance. *Nature* 300, 278–281.

[12] Nohmi, T., Hakura, A., Nakai, Y., Watanabe, M., Murayama, S.Y., Sofuni, T. (1991). *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a new *umuDC*-like operon (*samAB*) present in a 60-megadalton cryptic plasmid of *S. typhimurium*. *J. Bacteriol.* 173, 1051–1063.

[13] Glazebrook, J.A., Grewal, K.K., Strike, P. (1986). Molecular analysis of the UV protection and mutation genes carried by the I incompatibility group plasmid TP110. *J. Bacteriol.* 168, 251–256.

[14] Lodwick, D., Owen, D., Strike, P. (1990). DNA sequence analysis of the *imp* UV protection and mutation operon of the plasmid TP110: identification of a third gene. *Nucleic Acids Res.* 18, 5045–5050.

[15] Ho, C., Kulaeva, O.I., Levine, A.S., Woodgate, R. (1993). A rapid method for cloning mutagenic DNA repair genes: isolation of *umu*-complementing genes from multidrug resistance plasmids R391, R446b, and R471a. *J. Bacteriol.* 175, 5411–5419.

[16] Kulaeva, O.I., Wootton, J.C., Levine, A.S., Woodgate, R. (1995). Characterization of the *umu*-complementing operon from R391. *J. Bacteriol.* 177, 2737–2743.

[17] Sundin, G.W., Kidambi, S.P., Ullrich, M., Bender, C.L. (1996). Resistance to ultraviolet light in *Pseudomonas syringae*: sequence and functional analysis of the plasmid-encoded *rulAB* genes. *Gene* 177, 77–81.

[18] McDonald, J.P., Frank, E.G., Levine, A.S., Woodgate, R. (1998). Intermolecular cleavage of the UmuD-like mutagenesis proteins. *Proc. Natl. Acad. Sci. USA* 95, 1478–1483.

[19] Shiba, T., Iwasaki, H., Nakata, A., Shinagawa, H. (1990). Proteolytic processing of MucA protein in SOS mutagenesis: both processed and unprocessed MucA may be active in mutagenesis. *Mol. Gen. Genet.* 224, 169–176.

[20] Hauser, J., Levine, A.S., Ennis, D.G., Chumakov, K.M., Woodgate, R. (1992). The enhanced mutagenic potential of the MucAB proteins correlates with the highly efficient processing of the MucA protein. *J. Bacteriol.* 174, 6844–6851.

[21] Woodgate, R., Ennis, D.G. (1991). Levels of chromosomally encoded Umu proteins and requirements for in vivo UmuD cleavage. *Mol. Gen. Genet.* 229, 10–16.

[22] Ennis, D.G., Levine, A.S., Koch, W.H., Woodgate, R. (1995). Analysis of *recA* mutants with altered SOS functions. *Mutat. Res.* 336, 39–48.

[23] Konola, J.T., Guzzo, A., Gow, J.B., Walker, G.C., Knight, K.L. (1998). Differential cleavage of LexA and UmuD mediated by *recA* Pro67 mutants: implications for common LexA and UmuD binding sites on RecA. *J. Mol. Biol.* 276, 405–415.

[24] Little, J.W. (1984). Autodigestion of LexA and phage repressors. *Proc. Natl. Acad. Sci. USA* 81, 1375–1379.

[25] Woodgate, R., Rajagopalan, M., Lu, C., Echols, H. (1989). UmuC mutagenesis protein of *Escherichia coli*: purification and interaction with UmuD and UmuD′. *Proc. Natl. Acad. Sci. USA* 86, 7301–7305.

[26] Battista, J.R., Ohta, T., Nohmi, T., Sun, W., Walker, G.C. (1990). Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis. *Proc. Natl. Acad. Sci. USA* 87, 7190–7194.

[27] McDonald, J.P., Peat, T.S., Levine, A.S., Woodgate, R. (1999). Intermolecular cleavage by UmuD-like enzymes: identification of residues required for cleavage and substrate specificity. *J. Mol. Biol.* 285, 2199–2209.

[28] Ollivierre, J.N., Sikora, J.L., Beuning, P.J. (2011). The dimeric SOS mutagenesis protein UmuD is active as a monomer. *J. Biol. Chem.* 286, 3607–3617.

[29] Peat, T.S., Frank, E.G., McDonald, J.P., Levine, A.S., Woodgate, R., Hendrickson, W.A. (1996). The UmuD′ protein filament and its potential role in damage induced mutagenesis. *Structure* 4, 1401–1412.

[30] Peat, T., Frank, E.G., McDonald, J.P., Levine, A.S., Woodgate, R., Hendrickson, W.A. (1996). Structure of the UmuD′ protein and its regulation in response to DNA damage. *Nature* 380, 727–730.

[31] Ferentz, A.E., Opperman, T., Walker, G.C., Wagner, G. (1997). Dimerization of the UmuD′ protein in solution and its implications for regulation of SOS mutagenesis. *Nat. Struct. Biol.* 4, 979–983.

[32] Ferentz, A.E., Walker, G.C., Wagner, G. (2001). Converting a DNA damage checkpoint effector ($UmuD_2C$) into a lesion bypass polymerase ($UmuD'_2C$). *EMBO J.* 20, 4287–4298.

[33] Sutton, M.D., Guzzo, A., Narumi, I., Costanzo, M., Altenbach, C., Ferentz, A.E., Hubbell, W.L., Walker, G.C. (2002). A model for the structure of the *Escherichia coli* SOS-regulated $UmuD_2$ protein. *DNA Repair* 1, 77–93.

[34] Paetzel, M., Strynadka, N.C. (1999). Common protein architecture and binding sites in proteases utilizing a Ser/Lys dyad mechanism. *Protein Sci.* 8, 2533–2536.

[35] Luo, Y., Pfuetzner, R.A., Mosimann, S., Paetzel, M., Frey, E.A., Cherney, M., Kim, B., Little, J.W., Strynadka, N.C. (2001). Crystal structure of LexA: a conformational switch for regulation of self-cleavage. *Cell* 106, 585–594.

[36] Paetzel, M., Dalbey, R.E., Strynadka, N.C. (1998). Crystal structure of a bacterial signal peptidase in complex with a β-lactam inhibitor. *Nature* 396, 186–190.

[37] Bell, C.E., Frescura, P., Hochschild, A., Lewis, M. (2000). Crystal structure of the λ repressor C-terminal domain provides a model for cooperative operator binding. *Cell* 101, 801–811.

[38] Luo, C., Roussel, P., Dreier, J., Page, M.G., Paetzel, M. (2009). Crystallographic analysis of bacterial signal peptidase in ternary complex with arylomycin A2 and a β-sultam inhibitor. *Biochemistry* 48(38), 8976–8984.

[39] Botos, I., Melnikov, E.E., Cherry, S., Tropea, J.E., Khalatova, A.G., Rasulova, F., Dauter, Z., Maurizi, M.R., Rotanova, T.V., Wlodawer, A., Gustchina, A. (2004). The catalytic domain of *Escherichia coli* Lon protease has a unique fold and a Ser-Lys dyad in the active site. *J. Biol. Chem.* 279(9), 8140–8148.

[40] Feldman, A.R., Lee, J., Delmas, B., Paetzel, M. (2006). Crystal structure of a novel viral protease with a serine/lysine catalytic dyad mechanism. *J. Mol. Biol.* 358(5), 1378–1389.

[41] Lee, J., Feldman, A.R., Delmas, B., Paetzel, M. (2007). Crystal structure of the VP4 protease from infectious pancreatic necrosis virus reveals the acyl-enzyme complex for an intermolecular self-cleavage reaction. *J. Biol. Chem.* 282(34), 24928–24937.

[42] Chung, I.Y., Paetzel, M. (2011). Crystal structure of a viral protease intramolecular acyl-enzyme complex: Insights into cis-cleavage at the VP3/VP4 junction of *Tellina* birnavirus. *J. Biol. Chem.* 286(14), 12475–12482.

[43] Liao, D.I., Qian, J., Chisholm, D.A., Jordan, D.B., Diner, B.A. (2000). Crystal structures of the photosystem II D1 C-terminal processing protease. *Nat. Struct. Biol.* 7(9), 749–753.

[44] Kim, A.C., Oliver, D.C., Paetzel, M. (2008). Crystal structure of a bacterial signal peptide peptidase. *J. Mol. Biol.* 376(2), 352–366.

[45] Frank, E.G., Hauser, J., Levine, A.S., Woodgate, R. (1993). Targeting of the UmuD, UmuD′ and MucA′ mutagenesis proteins to DNA by RecA protein. *Proc. Natl. Acad. Sci. USA* 90, 8169–8173.

[46] Tang, M., Shen, X., Frank, E.G., O'Donnell, M., Woodgate, R., Goodman, M.F. (1999). UmuD′$_2$C is an error-prone DNA polymerase, *Escherichia coli*, DNA pol V. *Proc. Natl. Acad. Sci. USA* 96, 8919–8924.

[47] Tang, M., Bruck, I., Eritja, R., Turner, J., Frank, E.G., Woodgate, R., O'Donnell, M., Goodman, M.F. (1998). Biochemical basis of SOS-induced mutagenesis in *Escherichia coli*: reconstitution of *in vitro* lesion bypass dependent on the UmuD′$_2$C mutagenic complex and RecA. *Proc. Natl. Acad. Sci. USA* 95, 9755–9760.

[48] Reuven, N.B., Arad, G., Maor-Shoshani, A., Livneh, Z. (1999). The mutagenesis protein UmuC is a DNA polymerase activated by UmuD′, RecA, and SSB and is specialized for translesion replication. *J. Biol. Chem.* 274, 31763–31766.

[49] Ohmori, H., Friedberg, E.C., Fuchs, R.P.P., Goodman, M.F., Hanaoka, F., Hinkle, D., Kunkel, T.A., Lawrence, C.W., Livneh, Z., Nohmi, T., Prakash, L., Prakash, S., Todo, T., Walker, G.C., Wang, Z., Woodgate, R. (2001). The Y-family of DNA polymerases. *Mol. Cell* 8, 7–8.

[50] Bruck, I., Woodgate, R., McEntee, K., Goodman, M.F. (1996). Purification of a soluble UmuD′C complex from *Escherichia coli*: Cooperative binding of UmuD′C to single-stranded DNA. *J. Biol. Chem.* 271, 10767–10774.

[51] McLenigan, M.P., Kulaeva, O.I., Ennis, D.G., Levine, A.S., Woodgate, R. (1999). The bacteriophage P1 HumD protein is a functional homolog of the prokaryotic UmuD′-like proteins and facilitates SOS mutagenesis in *Escherichia coli*. *J. Bacteriol.* 181, 7005–7013.

[52] Opperman, T., Murli, S., Smith, B.T., Walker, G.C. (1999). A model for a *umuDC*-dependent prokaryotic DNA damage checkpoint. *Proc. Natl. Acad. Sci. USA* 96, 9218–9223.

[53] Sutton, M.D., Walker, G.C. (2001). Managing DNA polymerases: coordinating DNA replication, DNA repair, and DNA recombination. *Proc. Natl. Acad. Sci. USA* 98, 8342–8349.

[54] Frank, E.G., Ennis, D.G., Gonzalez, M., Levine, A.S., Woodgate, R. (1996). Regulation of SOS mutagenesis by proteolysis. *Proc. Natl. Acad. Sci. USA* 93, 10291–10296.

[55] Gonzalez, M., Frank, E.G., Levine, A.S., Woodgate, R. (1998). Lon-mediated proteolysis of the *Escherichia coli* UmuD mutagenesis protein: *in vitro* degradation and identification of residues required for proteolysis. *Genes Dev.* 12, 3889–3899.

[56] Frank, E.G., Gonzalez, M., Ennis, D.G., Levine, A.S., Woodgate, R. (1996). In vivo stability of the Umu mutagenesis proteins: a major role for RecA. *J. Bacteriol.* 178, 3550–3556.

[57] Gonzalez, M., Rasulova, F., Maurizi, M.R., Woodgate, R. (2000). Subunit-specific degradation of the UmuD/D′ heterodimer by the ClpXP protease: The role of *trans* recognition in UmuD′ stability. *EMBO J.* 19, 5251–5258.

[58] Paetzel, M., Dalbey, R.E., Strynadka, N.C. (2002). Crystal structure of a bacterial signal peptidase apoenzyme. Implications for signal peptide binding and the Ser-Lys dyad mechanism. *J. Biol. Chem.* 277, 9512–9519.

[59] Paetzel, M., Dalbey, R.E. (1997). Catalytic hydroxyl/amine dyads within serine proteases. *Trends Biochem. Sci.* 22, 28–31.

[60] Ekici, O.D., Paetzel, M., Dalbey, R.E. (2008). Unconventional serine proteases: variations on the catalytic Ser/His/Asp triad configuration. *Protein Sci.* 17(12), 2023–2037.

[61] Ollivierre, J.N., Fang, J., Beuning, P.J. (2010). The roles of UmuD in regulating mutagenesis. *J. Nucleic Acids*, 94768030, pii

[62] Jarosz, D.F., Beuning, P.J., Cohen, S.E., Walker, G.C. (2007). Y-family DNA polymerases in *Escherichia coli*. *Trends Microbiol.* 15, 70–77.

[63] Fuchs, R.P., Fujii, S., Wagner, J. (2004). Properties and functions of *Escherichia coli*: Pol IV and Pol V. *Adv. Protein Chem.* 69, 229–264.

[64] Yang, W., Woodgate, R. (2007). What a difference a decade makes: insights into translesion DNA synthesis. *Proc. Natl. Acad. Sci. USA* 104, 15591–15598.

[65] Pata, J.D. (2010). Structural diversity of the Y-family DNA polymerases. *Biochim. Biophys. Acta* 1804, 1124–1135.

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