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Y-family DNA polymerases in *Escherichia coli*

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The observation that mutations in the *Escherichia coli* genes *umuC*⁺ and *umuD*⁺ abolish mutagenesis induced by UV light strongly supported the counterintuitive notion that such mutagenesis is an active rather than passive process. Genetic and biochemical studies have revealed that *umuC*⁺ and its homolog *dinB*⁺ encode novel DNA polymerases with the ability to catalyze synthesis past DNA lesions that otherwise stall replication – a process termed translesion synthesis (TLS). Similar polymerases have been identified in nearly all organisms, constituting a new enzyme superfamily. Although typically viewed as unfaithful copiers of DNA, recent studies suggest that certain TLS polymerases can perform proficient and moderately accurate bypass of particular types of DNA damage. Moreover, various cellular factors can modulate their activity and mutagenic potential.

SOS transcriptional regulation

The SOS response to DNA damage was the first inducible response to genotoxic stress to be characterized. Many molecular details of this response are now well understood (Figure 1) [1]. Transcription of genes induced as part of the SOS response is typically repressed by the product of the *lexA*⁺ gene. When replication is stalled by DNA damage or another mechanism, the *recA*⁺ gene product binds to single-stranded DNA (ssDNA) produced at the replication fork, forming a nucleoprotein filament in the presence of nucleoside triphosphates. This filament stimulates a latent autoproteolytic activity of LexA, thereby enabling transcription of >40 genes. Both *lexA*⁺ and *recA*⁺ are also SOS-regulated [1]. However, recent results have indicated that this simple view of the SOS response is far from complete. Agents that do not damage DNA, such as β -lactam antibiotics, can induce the SOS response [2] through the two-component signal transduction system *dpiBA*, presumably in an attempt to mitigate antimicrobial lethality by inhibiting cell division, and induce the expression of the *dinB* gene in particular through a *lexA*-independent mechanism [3]. This observation raises the possibility that crosstalk between the SOS response and other cellular signaling pathways could be more extensive than previously realized. Maximal transcription of *dinB* in stationary phase requires a functional *rpoS* gene, an effect

that is also *lexA*-independent [4]. This might have particularly important implications for bacteria living under conditions of nutrient starvation. The SOS response also seems to be oscillatory at the single-cell level, and this oscillation is dependent on the *umuDC* genes [5]. Finally, the SOS response is one component of a broader cellular response to DNA damage. Exposure of *Escherichia coli* to the DNA-damaging agent mitomycin C (MMC) results in expression changes of >1000 genes [6].

Several of the genes regulated by the SOS response were initially identified using randomly generated Mu d1-generated transcriptional fusions to the *lac* operon. Mu d1 is a derivative of bacteriophage Mu that has been engineered to create such transcriptional fusions when it inserts into the chromosome. A collection of *E. coli* strains bearing these fusions was treated with MMC and examined for expression of β -galactosidase. Some of these fusions exhibited inducible expression of β -galactosidase, which was dependent on *recA*⁺ and *lexA*⁺; thus, they were named *din* (for damage-inducible) [7]. Many of these genes and their gene products have still not been characterized in detail. Although *dinB* [which encodes the translesion synthesis (TLS) polymerase (pol), DNA pol IV] was identified in this experiment, deletion of the gene did not initially show any marked phenotypes – this was in striking contrast to *umuD* and *umuC* (see later). Both *umuD* and *umuC* were subsequently shown to be transcriptionally induced as part of the SOS response using Mu d1-*lac* operon fusions [1]. This review will focus on the two SOS-regulated Y-family DNA polymerases found in *E. coli*, DinB (DNA pol IV) and UmuD₂C (DNA pol V), and their effects on the fidelity of replication.

Mutagenic function of *umuD*⁺-C⁺ and *dinB*⁺

Early studies of mutagenesis induced by UV irradiation indicated that mutation of either the *recA*⁺ or *lexA*⁺ genes could result in a nonmutable phenotype [1]. A screen for additional nonmutable mutants identified the *umuD*⁺ and *umuC*⁺ genes [8]. Loss-of-function mutants of each of these *umu* genes also show modest sensitivity to UV irradiation [1]. UmuD and LexA are structurally related to the lambda repressor, which undergoes RecA-nucleoprotein activated autocleavage, and to peptide hydrolases that employ a Ser-Lys catalytic diad in their mechanism [1]. Both LexA and UmuD form homodimers in solution and, similarly to LexA, interaction of UmuD₂ with the RecA nucleoprotein filament induces a latent autoproteolytic activity causing

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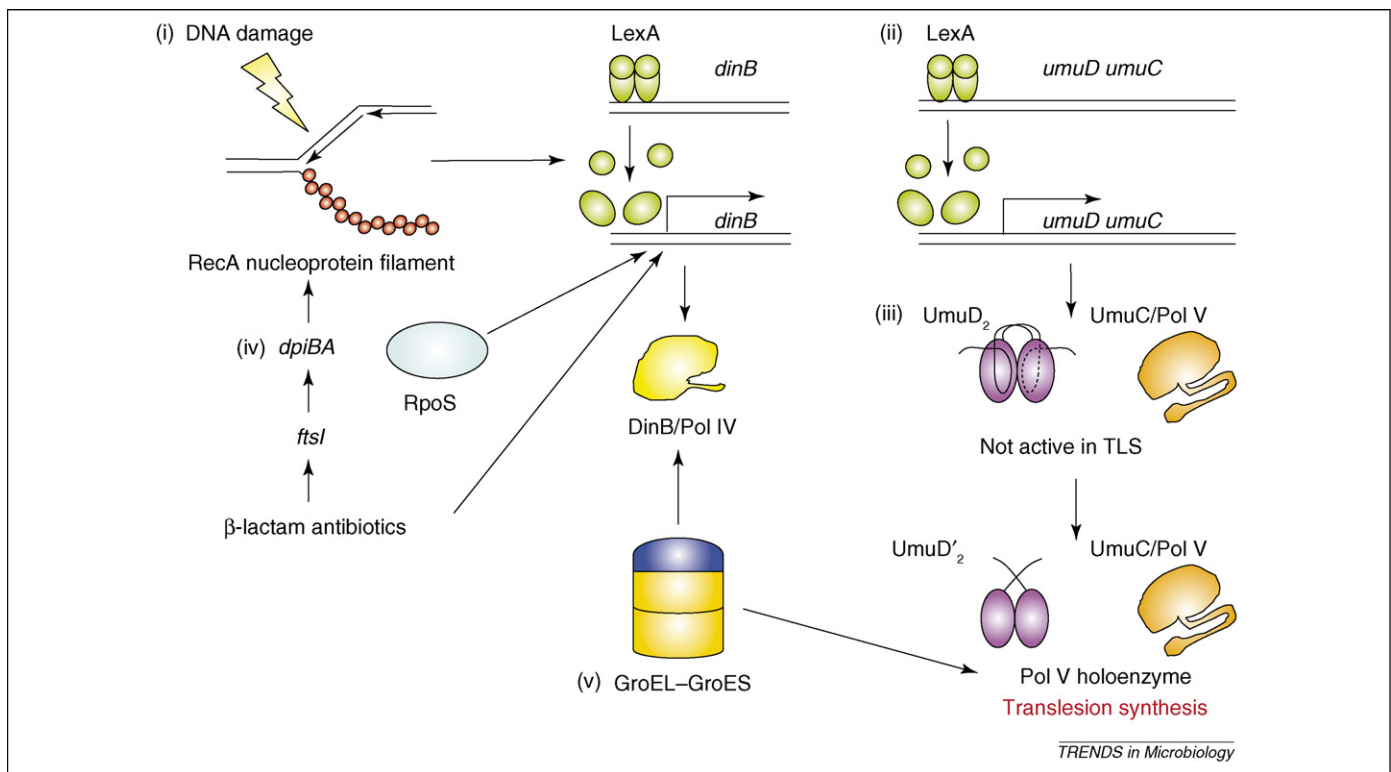


Figure 1. Multifaceted regulation of Y-family DNA polymerases in *Escherichia coli*. (i) The inducing signal for the SOS response forms when RecA polymerizes on a region of ssDNA, which is formed as a result of the failure to replicate damaged DNA. The RecA-ssDNA nucleoprotein filament is referred to as RecA*. (ii) Binding to RecA* induces LexA to undergo autoproteolytic cleavage, which inactivates it as a transcriptional repressor and leads to the induction of at least 40 genes, among which are those that encode the Y family DNA polymerases UmuD'₂C and DinB. (iii) The cleavage of UmuD to UmuD' is also facilitated by the binding of UmuD₂ to RecA*, which provides temporal regulation of the potentially mutagenic translesion synthesis activity of UmuC. (iv) Transcription of *dinB* is also regulated by *rpoS*, *dpiBA* and β-lactam antibiotics. (v) The chaperone GroEL-GroES is required for both DinB and UmuD'₂C function. Extensions on UmuD₂ and UmuD'₂ represent the N-terminal arms; extensions from DinB and UmuC represent their C termini including their β-binding motifs.

UmuD₂ to remove its N-terminal 24 amino acids to form UmuD'₂ [1]. It is UmuD'₂ that is active in UV-induced mutagenesis and associates with UmuC to form DNA pol V (UmuD'₂C) [1,9].

In contrast to the marked phenotypes displayed by mutants of *umuD* and *umuC*, mutants of *dinB* show more enigmatic phenotypes [10]. Although deletion of the *dinB*⁺ gene has almost no discernable effect on spontaneous mutagenesis [11], the *dinB*⁺ gene is required for untargeted mutagenesis of λ phage, in which *E. coli* are UV-irradiated and transfected with unirradiated λ but UV-induced mutagenesis is seen in the λ DNA [12]. The mutation spectrum observed is distributed between base substitution mutations and -1 frameshift events with a strong preference for mutation at G:C base pairs [1]. The *dinB*⁺ gene is also important for the phenomenon of adaptive mutagenesis in *E. coli* [13]. In this form of mutagenesis, stationary-phase *E. coli* bearing a +1 frameshift mutation in an episomal copy of a *lacI-lacZ* fusion are plated under conditions of nonlethal selection, namely on minimal medium with lactose as the sole carbon source, and mutants appear on the plate over many days. Although some mechanistic details of this phenomenon remain controversial, it is clear that deletion of *dinB* results in a 5–10-fold reduction in the number of adaptive mutants that appear [13]. Adaptive mutagenesis is also regulated by several genes including *rpoS* [4,14], the chaperones *groES* and *groEL* [15], and *ppk* [16]. Both *groES*

and *groEL* mutants are also impaired for *umuDC*-dependent UV-induced mutagenesis [17].

Overproduction of *dinB* leads to an increase in spontaneous and 4-nitroquinoline 1-oxide (4-NQO)-induced base -1 frameshift and, to a lesser extent, spontaneous base substitution mutagenesis [18,19]. Curiously, a preference is observed for spontaneous mutagenesis on the lagging strand and this seems to result from extension of terminal mismatches [20]. Moreover, a considerable fraction of the lagging-strand-directed mutator phenotype of a constitutively SOS-induced *recA730* strain requires *dinB*⁺ [21]. It has recently been shown that Δ*dinB* strains of *E. coli* display increased sensitivity to the DNA damaging agents nitrofurazone (NFZ) and 4-NQO [22]. Despite this marked sensitivity to both NFZ and 4-NQO, deletion of the *dinB*⁺ gene does not reduce mutagenesis induced by either agent [22]. These data suggest that the *dinB*⁺ gene product is able to contend with DNA damage produced by at least some DNA damaging agents with comparable fidelity to other repair processes available to the *E. coli* cell.

Biochemical activities of DinB and UmuD'₂C

Although decades of genetic characterization clearly established their roles in spontaneous and induced mutagenesis, the biochemical function of the *umuDC*⁺-C⁺ and *dinB*⁺ gene products remained elusive for many years. Early clues came when UmuD'₂C was shown to bind to DNA [23] and the eukaryotic Y-family member *REVI* was

Box 1. DNA polymerases in TLS and DNA repair in *E. coli* and eukaryotes

DNA polymerases are divided among six families based on sequence homology. Y-family polymerases usually participate in lesion bypass but X- and B-family polymerases can also be involved. *Escherichia coli* has five DNA polymerases [1]: DNA pol I (A family) and DNA pol III (C family) are high-fidelity polymerases that replicate the majority of the genome; DNA pol II is a relatively accurate X-family polymerase that is involved in translesion synthesis; DNA pol IV/DinB (Y family) and DNA pol V/UmuD₂C (Y family) are translesion polymerases that lack exonuclease activity and are, therefore, relatively error-prone [1]. Important eukaryotic Y-family polymerases that participate in translesion synthesis include pol κ (DinB ortholog), pol ι, pol η/XP-V (UmuC functional ortholog) and Rev1. Y-family DNA polymerases are found in all domains of life and are characterized by their ability to replicate damaged DNA, that is, to perform translesion synthesis [9]. The family was named in 2001 [27], although the catalytic activity of at least some members had been known since 1996 [24]. They typically exhibit 10–1000-fold lower fidelity than replicative DNA polymerases when replicating undamaged DNA [1]. Thus, translesion synthesis by Y-family polymerases comes at a considerable mutagenic potential.

Crystal structures of Y-family DNA polymerases show that these enzymes adopt a similar right-hand fold to that of replicative DNA polymerases [1,37,38], which is striking considering that they bear relatively little sequence homology to replicative polymerases. Y-family polymerases also have an additional domain, referred to as the 'little-finger' domain [previously also called the 'wrist' or 'polymerase associated domain' (PAD)] that provides additional DNA-binding contacts [1,39]. These structures reveal an accommodating active site and short, stubby finger domains relative to replicative DNA polymerases. To date, the only crystal structure of a Y-family polymerase from *E. coli* is of the little finger domain of DinB bound to the β processivity clamp [60]. Future structural studies will be required to understand the specific structural features of Y-family polymerases from *E. coli*.

discovered to encode an enzyme with dCMP transferase activity [24]. Shortly thereafter, UmuD₂C and DinB were purified and shown to have bona fide DNA polymerase activity, thereby contributing to the recognition of a new superfamily of DNA polymerases known as the Y family [25–28] (Box 1). Unlike DNA pol III (the replicative DNA polymerase of *E. coli*), DinB and UmuD₂C catalyze relatively distributive DNA synthesis that is modestly stimulated by the addition of the β processivity clamp subunit of DNA pol III [29–31]. (The β processivity clamp is a ring shaped protein that encircles the DNA helix.) AP lyase activity has been demonstrated for both DinB and UmuD₂C, although genetic studies have not established a relevance for this function *in vivo* [32].

The *in vitro* DNA polymerase activity of UmuD₂C and DinB on both damaged and undamaged DNA has been examined in some detail. Their specialized function comes with a mutagenic risk because Y-family polymerases replicate DNA with lower fidelity than their replicative relatives. Although UmuD₂C and DinB display poor activity and fidelity on undamaged DNA relative to replicative DNA polymerases, they compare far more favourably on certain types of damaged templates. UmuD₂C replicates undamaged templates with an error frequency of 10⁻³–10⁻⁴ and has an error frequency of 10⁻² for T^AT cyclobutane dimers [33,34], a common photoproduct resulting from UV irradiation that covalently links two adjacent thymines. DinB replicates both undamaged templates and

an apparent cognate substrate, an adduct at the N² position of guanine (N²-dG), with an error frequency of 10⁻³–10⁻⁵ [22,33]. The difference between the fidelity of these polymerases when replicating damaged substrates might correlate with the clear UV-induced mutagenic signature of *umuDC in vivo* and the comparative lack of *dinB*⁺-dependent mutagenesis induced by NFZ or 4-NQO [8,22]. Furthermore, DinB shows an increased catalytic proficiency on an N²-dG damaged substrate relative to an undamaged control, which is dependent on a single active-site residue [22]. Conceptually similar behavior is also observed for human DNA polymerase η with respect to UV irradiation and T^AT-damaged substrates [35,36]. Additional work will be required to determine whether a hallmark of a cognate substrate for Y-family polymerases is comparable efficiency and/or fidelity on damaged and undamaged templates and, if so, what the endogenous sources of such cognate substrates might be.

Loose grips and open active sites

Although structures of the Y-family polymerases from *E. coli* have not yet been solved, structural analysis of *Sulfolobus solfataricus* (Dpo4) and *Sulfolobus acidocaldarius* (Dbh) homologs have yielded profound insights into function [37,38]. Whereas these enzymes share no clear sequence homology with replicative polymerases, their structures reveal a similar right-hand fold consisting of a thumb, palm and fingers domain. However, Y-family polymerases have an additional little-finger domain that seems to play an important part in both substrate specificity and processivity [39]. Unlike the tight grip seen in active sites of canonical DNA polymerases [40], Y-family polymerases have open active sites that are relatively solvent-accessible (Figure 2). Moreover, an α-helix responsible for several orders of magnitude of fidelity in canonical DNA polymerases (the O-helix) is entirely absent in Y-family polymerases, providing a structural rationale for their comparatively low fidelity when replicating undamaged DNA.

Structural insight into Y-family polymerases encountering their cognate substrates is considerably more limited. A study of Dpo4 encountering a cyclobutane pyrimidine dimer is the most definitive to date [41]. Such UV-induced damage presents a particular problem for replicative polymerases because their active sites can only accommodate one base at a time. The relative openness of the Dpo4 active site enables the enzyme to fit a covalently linked T^AT cyclobutane pyrimidine dimer within its active site [41]. Whereas Dpo4 replicates past the 3' T of the T^AT with appreciable efficiency, it replicates past the second base with considerably higher activity and fidelity [41]. This is particularly interesting given that structural analysis of the second addition reveals that the incipient base pair adopts a Hoogsteen conformation rather than the canonical *anti* conformation. Named after Karst Hoogsteen, who first modeled these pairings in 1963, Hoogsteen basepairing occurs in the major groove and involves the N7 atom of purines in contrast to canonical Watson–Crick basepairing, which occurs in the minor groove [42]. In the case of Dpo4, the conformation seems to be induced by the enzyme because both bases in a T^AT in duplex DNA adopt a Watson–Crick conformation [41].

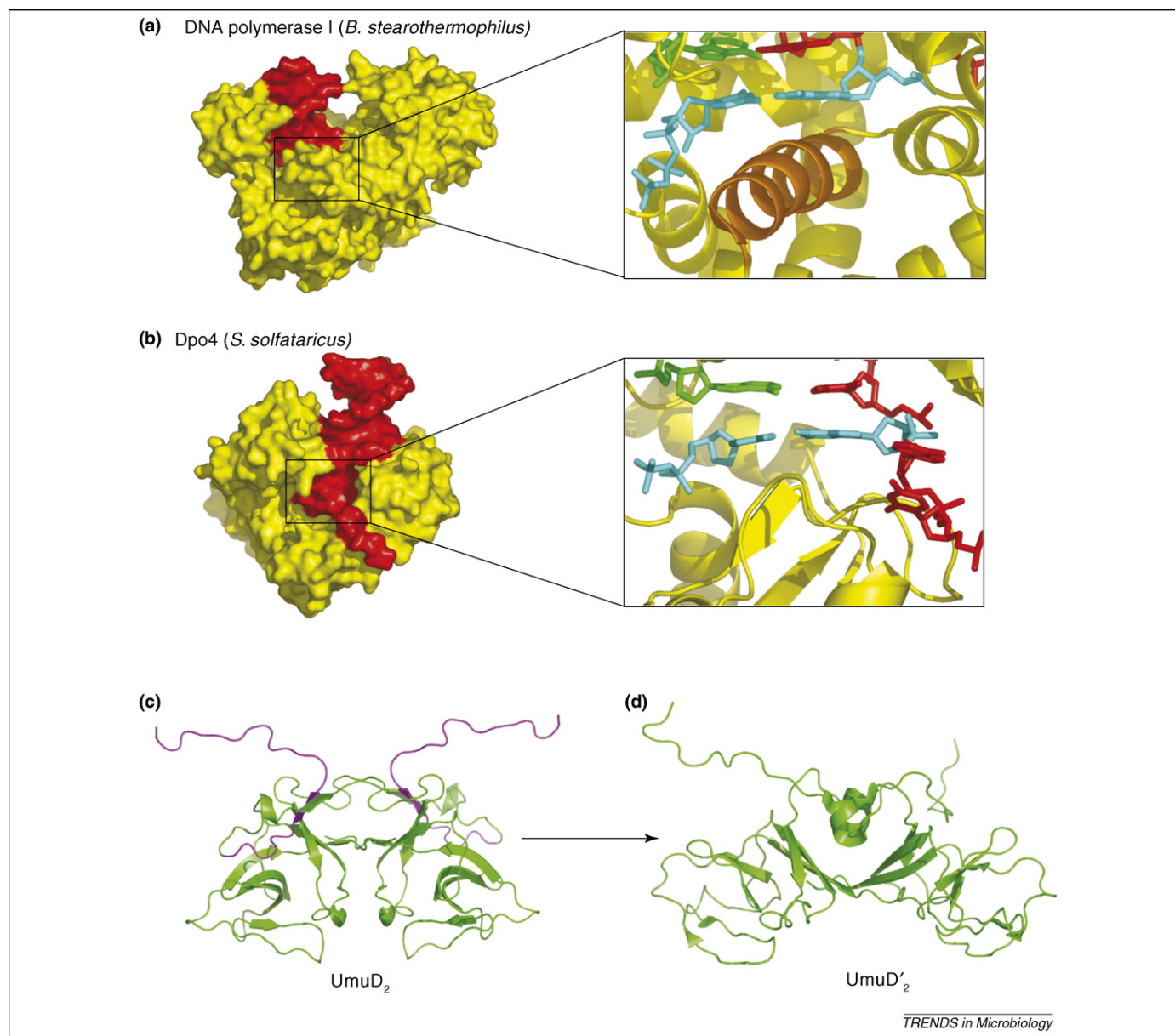


Figure 2. X-ray and NMR structures reveal key mechanistic details of TLS. (a) The structure of *Bacillus stearothermophilus* replicative DNA polymerase I in a closed conformation [28] shows numerous close protein (yellow) contacts with DNA (red). An α -helix (orange) performs a geometric check to ensure the fidelity of the incipient base pair (blue). (b) By contrast, the Y-family polymerase Dpo4 from *Sulfolobus solfataricus* [26] shows a loose grip on the DNA, a relatively open active site, and has no α -helix to check the geometry of the incipient base pair. (c) A model of UmuD₂ [37] and (d) an NMR structure of UmuD'₂ [36] indicate the structural rearrangements that occur upon RecA-mediated autocleavage. The structural plasticity of these molecules is likely to be important for their ability to interact with various cellular factors.

An induced conformational change between an open and substrate-bound closed form is a hallmark of A and B family DNA polymerases [43]. Indeed, this conformational change is believed to contribute substantially to the exquisite fidelity of replicative polymerases [44,45]. Although such a change has not been observed crystallographically for a Y-family polymerase, several studies have indicated that such a conformational change might have a crucial role in translesion synthesis [22,46,47]. These observations provide further evidence that Y-family polymerases catalyze translesion synthesis in an orchestrated fashion rather than exclusively by virtue of an open active site.

Modulation of function by protein–protein interactions

Genetic characterization over the past 30 years has underscored the importance of the *recA* and *umuD* gene products

in regulation of *umuC*-dependent mutagenesis [1]. Recent studies have recapitulated these results with purified components and identified the pivotal role of the β processivity clamp in dictating UmuD'₂C function. Initial reports of UmuD'₂C polymerase activity invoked a requirement for UmuD'₂, RecA, ssDNA binding protein (SSB) and, in one case, various components of the polymerase III holoenzyme for UmuC activity [31]. The demonstration of polymerase activity of UmuD'₂C established UmuD'₂ as a subunit of DNA pol V.

X-ray and NMR structures of the polymerase V subunit UmuD'₂ have yielded considerable insight into its function [48,49]. Additionally, distance constraints derived from electron paramagnetic resonance spectroscopy have been used to model the structure of full-length UmuD₂ [50]. In the X-ray structure of UmuD'₂, the catalytic serine and

lysine required for autoproteolysis are located within hydrogen-bonding distance of each other and the N terminus containing the scissile bond is located >50 Å from the active site [48]. By contrast, the UmuD₂ model suggests that the N terminus of the molecule curls upon itself to bring the scissile bond in proximity to the active site [50]. Such structural plasticity might be especially important given the relatively large number of proteins with which UmuD₂ and UmuD'₂ interact [51]. A heterodimeric form of the *umuD* gene products, UmuD·D', is the most thermodynamically stable form of the protein and targets it for ClpXP-mediated proteolysis [1]. A structural model of UmuD·D' has been constructed based upon NMR analysis [36].

Aside from activating UmuD'₂C, RecA has numerous cellular roles. The *recA* gene is required not only for induction of the SOS response but also for homologous recombination [1]. Biochemical studies differ to some extent on the mode of RecA activation of UmuD'₂C and on the role of ATP in the process [31]. Recent studies have suggested that RecA binds to UmuC as a subunit of the UmuD'₂C holoenzyme and that another molecule of ATP-associated RecA binds to UmuD'₂, thereby stimulating the affinity of the holoenzyme for the primer terminus [52]. It was originally assumed that RecA is bound to the ssDNA template in this activating role, but it has now been proposed that stimulation of UmuD'₂C activity by the RecA-nucleoprotein filament occurs *in trans* [53]. This has important implications for models of UmuD'₂C action given that the most proficient transactivating RecA nucleoprotein filament is one formed on gapped DNA. These observations foreshadow what seems to be remarkably complex regulation of Y-family polymerases through protein–protein interactions. Initial studies of UmuD'₂C activity also reported an enhancement of activity provided by SSB [31]. This effect, observed at substoichiometric quantities of SSB, has now been attributed to increased formation of dynamic RecA filaments on short ssDNA templates in the presence of DNA [31].

Protein regulators of DinB function have been comparatively less well characterized. A recent report has implicated certain forms of the *umuD* gene products in regulation of a novel function of DinB [54], and the chaperone GroEL–GroES affects DinB levels, perhaps indirectly [15]. The recent identification of an additional phenotype for Δ *dinB* *E. coli* strains [22] should enable knowledge of DinB regulation to expand considerably over the coming years.

Management role of the processivity clamp

Interactions with replicative processivity clamps are crucial for regulating Y-family polymerase activity and dictating their access to DNA. Although they are characterized by low processivity on undamaged DNA, Y-family polymerases exhibit an increased processivity in the presence of the β clamp. Indeed, DinB processivity is enhanced 300-fold by the β clamp [29], whereas that of UmuC is stimulated between 5- and 100-fold [30,31]. In either case, the processivity enhancement as a result of β is far less than that of polymerase III ($\sim 10^5$ -fold) [55]. Mutation or deletion of the β interaction motif in either UmuC or DinB

causes a loss of translesion synthesis *in vivo* [56]. Most prokaryotic proteins that interact with the β processivity clamp do so through a conserved interaction motif: QL[S/D]LF [57], which bears similarity to the conserved eukaryotic proliferating cell nuclear antigen (PCNA) interaction motif, QXXLXXFF [58].

Recent structural studies have shown that proteins as diverse as the δ subunit of the clamp loader and DinB, which interact with β through the conserved interaction motif, bind to the same hydrophobic channel on β at the interface between β domains II and III [59–61]. Thus, mutations in β near this hydrophobic channel can regulate specific DNA polymerase usage [62,63]. A co-crystal structure of the C-terminal little-finger domain of DinB with the β clamp illustrates that, in addition to the conserved β -binding motif interaction, DinB also interacts with β at its dimer interface through a hydrophobic loop in the little-finger domain [60]. When the structure of full-length *S. solfataricus* Dpo4 was superimposed on the DinB little finger in this structure, the active site of Dpo4 was surprisingly far from the DNA that is expected to be running through the center of the β clamp, leading the authors to speculate that this orientation of DinB could represent a recruited-but-inactive state (Figure 3) [60].

What is the role of the β clamp in managing multiple DNA polymerases? Notably, all DNA polymerases in *E. coli* interact with β at the same site [64]. The co-crystal structure of the DinB little finger and the β clamp suggests that it might be possible for β to bind two DNA polymerases simultaneously, with one polymerase in an inactive but still recruited conformation. Indeed, both DinB and the α catalytic subunit of polymerase III were found to bind to β simultaneously [65]. Thus, switching polymerase access to

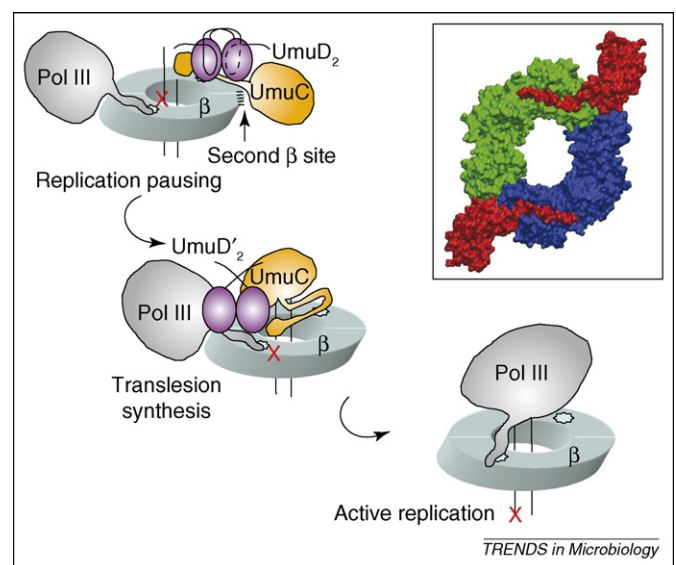


Figure 3. A model for polymerase switching that might occur in the transition from a DNA-damage checkpoint to translesion synthesis and replication. In a DNA damage checkpoint, UmuC functions in concert with UmuD₂ to slow the rate of DNA synthesis. Autocleavage of UmuD₂, which removes its N-terminal 24 amino acids to form UmuD'₂, releases the checkpoint and is required for UmuC polymerase function. After UmuC polymerizes several base pairs past the lesion [21], the replicative polymerase DnaE (polymerase III α subunit) can resume DNA synthesis. The inset shows the crystal structure of the little-finger domain of DinB (red) with β (one monomer in blue, one monomer in green) [46]. Inset structure reproduced, with permission, from Ref. [60].

the primer terminus could occur with two DNA polymerases bound to the β clamp [65]. The hierarchy of affinities of DNA polymerases in *E. coli* for the processivity clamp has been investigated genetically [62,66,67]. Upon UV irradiation, polymerase III seems to have the greatest affinity followed by pol IV, pol V and pol II [62], whereas during conjugal replication, the hierarchy seems to be pol III first, then pol II, pol IV and finally pol V [67]. Further work will be required to analyze competition among polymerases for access to the β clamp under various conditions.

The β clamp also interacts with UmuD₂ and UmuD'₂. Moreover, UmuD₂ interacts with β more strongly than UmuD'₂ does, possibly indicating a role in *umuDC*-dependent replication pausing [51]. UmuD binds to β in the vicinity of the same hydrophobic channel where other β -binding proteins interact [68]. Curiously, the N-terminal region of UmuD contains a cryptic β -binding motif (¹⁴TLPLF¹⁸) that by itself is insufficient to bind to β [57]. UmuD variants containing mutations in this motif bind to β with essentially the same affinity as wild-type UmuD [69] but with a strikingly different tryptophan fluorescence emission spectrum of β [69], indicating that although this motif itself is not responsible for the interaction, it is important for determining the nature of the complex.

In eukaryotes, polymerase management is even more complex. The processivity clamp PCNA is subject to several different post-translational modifications that dictate its roles in replication, DNA repair and DNA damage tolerance mediated by Y-family DNA polymerases [70,71]. Additionally, the alternative processivity clamp in eukaryotes (Rad9-Rad1-Hus1) is important for modulating the activity of Y-family polymerases [70].

Novel phenomena involving *dinB* and *umuDC*

In addition to the well-known function of Y-family polymerases in TLS, other functions of *umuDC* and *dinB* include UmuD₂C-dependent cold sensitivity, involvement in a primitive DNA damage 'checkpoint', enhanced survival in response to DNA-damage-independent replication stalling, and replication arrest-stimulated recombination [1,54,72–74].

Overexpression of the *umuDC* gene products leads to inhibition of growth at 30 °C, known as *umuDC*-mediated cold sensitivity. The *umuDC* genes are the only SOS regulated genes required for the manifestation of cold sensitivity and the degree of cold sensitivity is proportional to the amount of expression. This phenomenon is associated with the rapid and reversible inhibition of DNA synthesis and *sulA*-independent filamentation [1]. Strikingly, the genetic requirements for cold sensitivity are different from those needed for TLS [1]. Namely, neither RecA nor the catalytic activity of UmuC is needed and UmuD (but not UmuD') is required. Cold sensitivity seems to result from an exaggeration of a DNA-damage-induced 'checkpoint' in which UmuD₂C delays the resumption of DNA synthesis after DNA damage, perhaps through interaction with the β clamp, to enable error-free repair processes to occur [73,75]. The response is temporally regulated by the cleavage of UmuD to UmuD'.

Both *E. coli* Y-family polymerases have been implicated in enhancing cellular survival under conditions of depleted

deoxyribonucleotide pools, such as after the addition of hydroxyurea (HU). Strains carrying a *umuC122::Tn5* allele, resulting in a truncated protein that retains an intact polymerase domain but is deficient for induced mutagenesis, are strikingly resistant to HU [54]. Although seemingly unrelated, cold sensitivity and resistance to HU share a genetic requirement for *umuD*. HU resistance requires the catalytic activity of UmuC122 and DinB and certain forms of the *umuD* gene products. Moreover, this resistance might, at least in part, be because of failed communication with the toxin–antitoxin pairs MazEF and RelBE that would normally lead to cell death [54]. The increased mutation frequency observed in a *umuC122::Tn5* strain upon HU treatment could imply that under conditions of deoxyribonucleotide limitation, DinB and UmuD'₂C take over a considerable fraction of DNA replication. Furthermore, recent studies have shown that Y-family DNA polymerases participate in oxidation-induced mutagenesis by virtue of their ability to incorporate oxidized nucleotides during replication [76,77]. Taken together, these results suggest that Y-family polymerases might have a larger role in DNA replication when the deoxyribonucleotide pool is substantially perturbed, such as under conditions of HU treatment or oxidative stress.

Interestingly, *dinB* has also been implicated in replication-arrest-stimulated recombination [74]. Deletions of *tetA* fragments that are set in tandem repeats are elevated at the permissive temperature in a strain background bearing a temperature-sensitive mutant of the replicative DNA helicase (*dnaB107*). This type of mutagenesis is reduced in a *dnaB107 dinB* strain, contributing to a model in which RadA, RecG and RuvAB can stabilize a D-loop/recombination intermediate that enables DinB to extend the invading 3' strand and promote continued replication [74].

Concluding remarks and future perspectives

Recent developments have greatly enhanced the understanding of Y-family polymerases and, particularly, their role in DNA damage tolerance and mutagenesis. Whether the paradigm for understanding their function should be that of unfaithful copiers or specialized polymerases is still a subject of some debate (Box 2). The picture is likely to be considerably more nuanced than either extreme. In the case of *E. coli*, DinB seems to be a specialized polymerase under many circumstances. However, UmuD'₂C seems to function with both lower fidelity and broader substrate specificity. The observation of novel 'checkpoint' functions associated with both *umuDC* and *dinB* have also greatly

Box 2. Major questions regarding the function and regulation of Y-family polymerases in *E. coli*

- (1) How do Y-family polymerases gain access to an appropriate primer terminus and how is their action coordinated with that of replicative polymerases?
- (2) How do protein–protein interactions regulate the activity of Y-family polymerases?
- (3) Are there families of cognate lesions for each different Y-family polymerase?
- (4) Can mutations introduced by Y-family polymerases be corrected by exonucleolytic proofreading *in trans*?

expanded our understanding of the multifaceted roles of these genes in *E. coli*. However, Y-family polymerases are not enzymes that function in isolation and considerable effort needs to be directed towards understanding their function in the context of a living cell. The protein regulators of UmuD₂C have been studied in some detail whereas those of DinB are largely unknown.

E. coli delays the mutagenic function of UmuD₂C by timing the cleavage of UmuD₂ to UmuD₂C. This temporal separation of more accurate DNA repair and error-prone DNA damage tolerance might be echoed in eukaryotes, in which the Y-family member Rev1 is not maximally expressed until the G2/M transition of the cell cycle [78]. Indeed, the exquisite regulation of Y-family polymerases could be particularly important in eukaryotes, which, according to some estimates, rely on translesion synthesis 50-fold more than prokaryotes [79].

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