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Escherichia coli Y family DNA polymerases

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TABLE OF CONTENTS

1. ABSTRACT

DNA damage is ubiquitous, arising from both environmental and endogenous sources. All organisms have evolved multiple pathways to respond to DNA damage and maintain genomic integrity. *Escherichia coli* possesses two DNA polymerases, pol IV and pol V, that are members of the Y family. These polymerases are characterized by their specialized ability to copy damaged DNA as well as their relatively low fidelity on undamaged DNA. Pol IV and pol V are regulated by the SOS response to DNA damage and by their multiple interactions with other proteins. These two Y family DNA polymerases copy DNA damaged by distinct agents. Pol IV is capable of replicating DNA containing N^2-dG adducts, while pol V bypasses abasic sites and thymine-thymine dimers, which result from exposure to UV radiation. In addition to their roles in copying damaged DNA, the two Y family DNA polymerases in *E. coli* act in regulation of DNA replication and contribute to bacterial mutagenesis in response to cellular stress.

2. INTRODUCTION

The process by which DNA polymerases replicate damaged DNA is known as translesion synthesis (TLS) and was first described 35 years ago (1). It was observed that DNA damage induced the *E. coli* SOS response, which is accompanied by mutagenesis of the DNA (1). Originally DNA damage-induced mutagenesis was thought to result from the modification of replicative DNA polymerases, which allowed them to bypass DNA damage, albeit sometimes mutagenically (2). However it was later discovered that the UmuC/UmuD′ complex (UmuD′$_2$C, pol V) and DinB (pol IV) are Y family DNA polymerases that have the specialized ability to carry out potentially mutagenic TLS (3).

Y family DNA polymerases (4), found throughout all domains of life, have five conserved sequence motifs but the overall size of the proteins can vary considerably (Figure 1) (3, 5-9). In addition to bacterial pol IV and pol V, the eukaryotic members of the family include

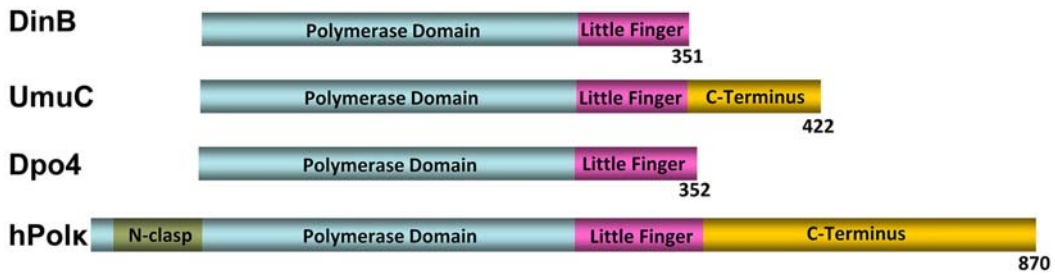


Figure 1. The domains and relative sizes of some Y family polymerases (159). The DinB ortholog human DNA polymerase kappa is represented as hPolk. Dpo4 represents DNA polymerase IV from *Sulfolobus solfataricus.*

Rev1, pol eta, pol kappa, and pol iota (4). The domains of both replicative and Y family DNA polymerases are named for their general resemblance to the parts of a right hand, including thumb, palm, and finger domains. Y family polymerases also possess a domain known as the 'little finger' domain found only in the Y family (10). The Y family polymerases are characterized by small finger and thumb domains relative to replicative DNA polymerases, which result in an open, solvent-accessible active site in the palm domain of Y family members (3, 11). The active site of replicative polymerases contains an 'O-helix', the role of which is to act as a steric check on fidelity and allow only a correct base pair to be formed (12-14). Y family polymerases lack the O-helix, contributing to their more open and flexible active sites and allowing them to accommodate lesions on the DNA template (10, 15). The available crystal structures of Y family DNA polymerases (6) tend to support the model of an open active site, as seen in the structure of *Sulfolobus solfataricus* Dpo4 in complex with DNA containing a thymine-thymine (T-T) cyclobutane pyrimidine dimer (16). This structure demonstrates that both thymines are accommodated in the active site simultaneously (16). Structures of other Y family DNA polymerases with or without DNA also generally show that these proteins have small finger domains and open, solvent-accessible active sites, suggesting a structural basis both for their ability to accommodate DNA lesions and for their low fidelity when copying undamaged DNA (3, 6, 10-11, 17-18).

3. TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION

In *E. coli*, the expression of Y family polymerases along with other genes is induced via the SOS response to damaged DNA. This cellular response was named the SOS response by Miroslav Radman because there is a "danger signal which induces SOS repair" (19). That "danger signal" is usually considered to be a DNA lesion that disrupts normal DNA replication (5, 19). Evelyn Witkin suggested that there was a pathway in *E. coli* that is controlled by a repressor whose function is inactivated when DNA damage occurs and that again becomes activated as a repressor once the repair of DNA damage is complete (20). This repressor was discovered to be the LexA protein, the repressor of the SOS genes. The SOS response is initiated when a lesion in the DNA template prevents replicative polymerases from continuing with efficient replication, causing a region of single-stranded DNA (ssDNA) to develop (Figure 2). RecA is activated upon binding to ssDNA, forming a RecA/ssDNA nucleoprotein filament (5). LexA then binds the RecA/ssDNA nucleoprotein filament, inducing LexA to cleave itself at its Ala^{84}-Gly^{85} bond, approximately in the middle of the protein (21). Once LexA is cleaved it no longer represses the SOS genes, allowing at least 57 genes, including *umuC*, *umuD*, and *dinB*, to be expressed during the SOS response (5, 22). In addition to its role in initiating the SOS response, RecA also plays more direct roles in the ability of Pol V to bypass lesions (see Section 5.5).

The *umuD* gene products contribute an additional level of regulation of Y family DNA polymerases in *E. coli* (5). Upon expression, $UmuD_2$ binds to the RecA/ssDNA nucleoprotein filament, stimulating the ability of UmuD to cleave itself at its Cys^{24}-Gly^{25} bond and removing its N-terminal 24-amino acids to form $UmuD'_2$ by a mechanism similar to that of LexA (23-25). The full-length $UmuD_2$ protein persists for approximately 20-40 min after expression is induced, after which time the cleaved form $UmuD'_2$ becomes the predominant form (Figure 2) (26). Full-length $UmuD_2$ and cleaved $UmuD'_2$ play distinct roles in the cellular response to DNA damage; $UmuD_2$ contributes to accurate DNA replication and repair while $UmuD'_2$ facilitates mutagenesis (26-32). Therefore, this lag in the appearance of $UmuD'_2$ delays the use of a potentially mutagenic pathway, in part via direct interactions between the *umuD* gene products and Y family DNA polymerases.

4. DNA POLYMERASE IV: DinB

DinB is one of two Y family polymerases found in *E. coli* (29, 31, 33). The *dinB* (*d*amage-*in*ducible) gene was identified as being induced upon treatment with DNA damaging agents (9, 34). Subsequently, the *dinB* gene product was demonstrated to be a DNA-dependent DNA polymerase (33). DinB was also shown to possess the ability to accommodate misaligned or bulged primer-template structures into its active site and to lack intrinsic 3′-5′ exonuclease proofreading activity (33).

4.1. DinB in general stress responses

DinB is expressed at a level of approximately 250 molecules per cell under non-SOS induced conditions (35).

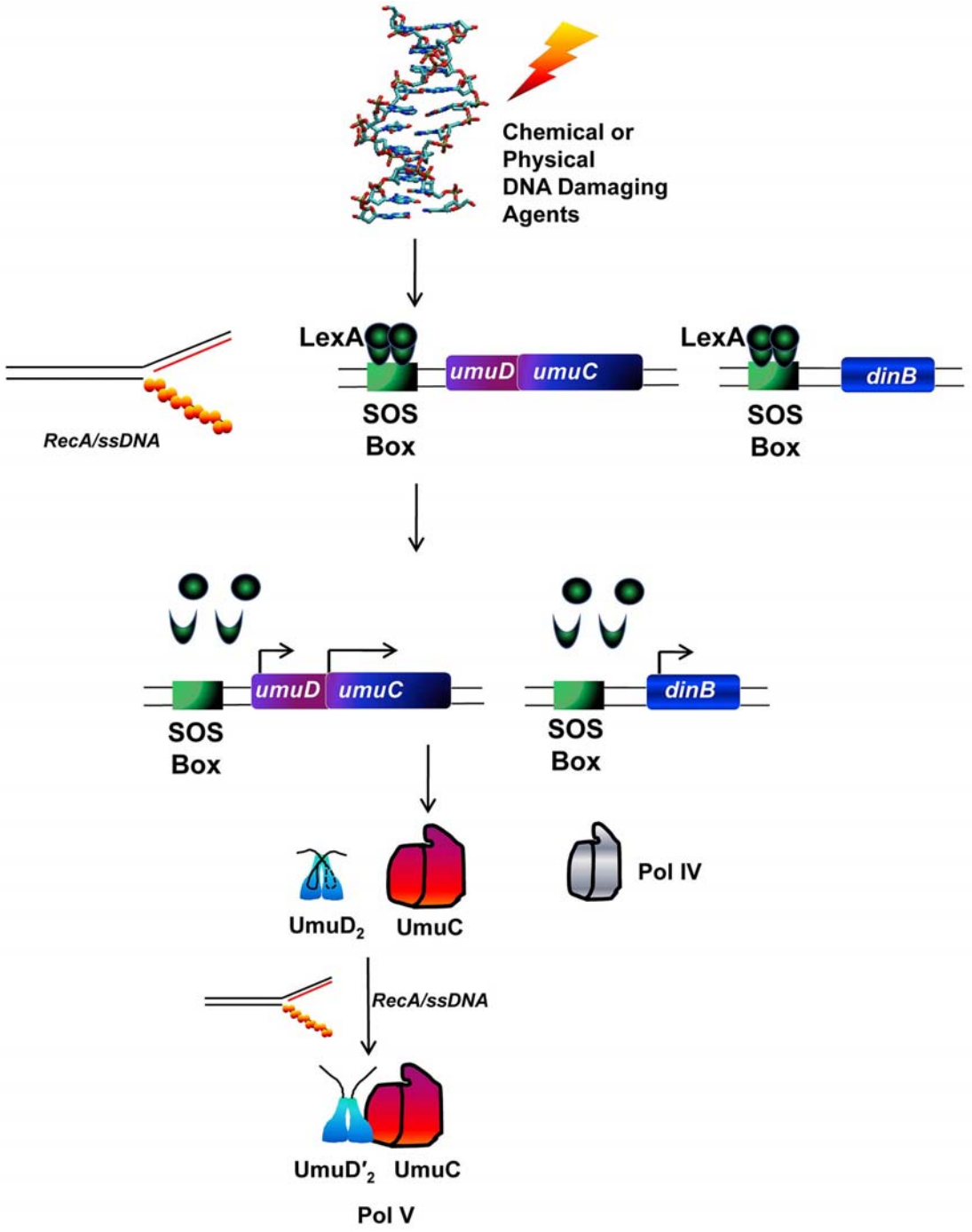
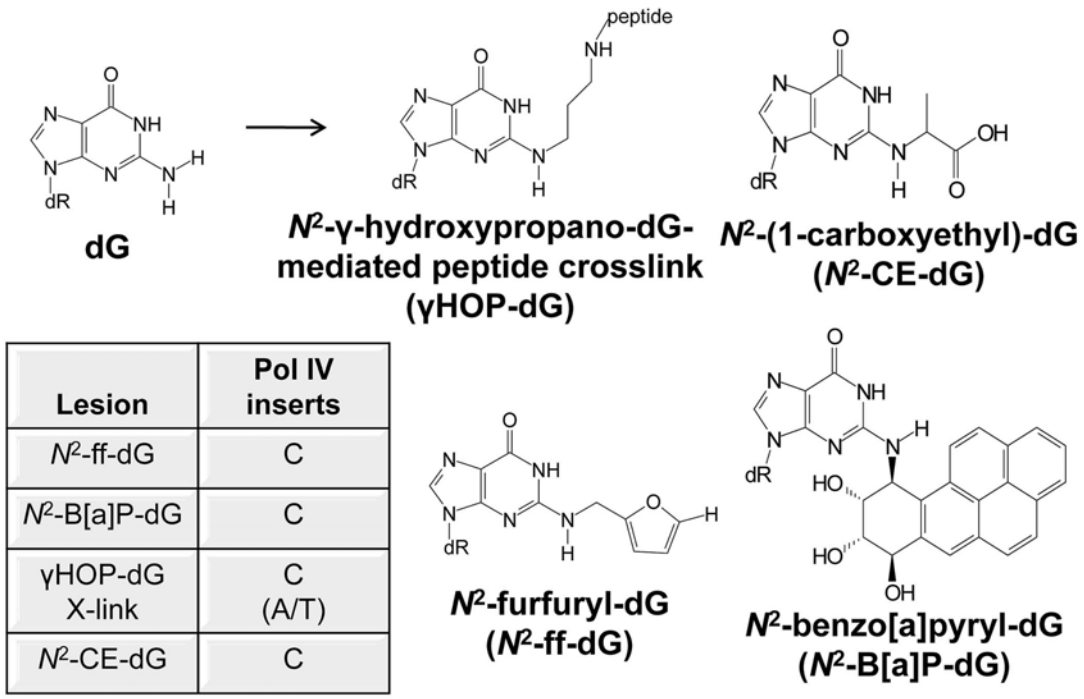


Figure 2. DNA damaging agents lead to the formation of lesions in DNA that disrupt replication and induce the SOS response. Single-stranded DNA (ssDNA) develops and becomes coated with RecA creating a RecA/ssDNA nucleoprotein filament, which signals the initiation of the SOS response. At least 57 genes are regulated by the LexA repressor, which represses the SOS genes by binding to consensus sequences ("SOS boxes") in the promoter regions. LexA cleaves itself upon its interaction with the RecA/ssDNA nucleoprotein filament (5, 22). The cleavage of LexA ablates its repressor function and allows for the expression of the *umuDC* and *dinB* genes, among others. $UmuD_2$ also undergoes a cleavage reaction facilitated by the RecA/ssDNA nucleoprotein filament to form $UmuD'_2$, the active form in SOS mutagenesis (23-25). The dashed line in the $UmuD_2$ cartoon signifies that the arm is behind the globular domain, as the monomers are related to each other by a C_2 axis of symmetry. Both $UmuD'_2C$ (pol V) and DinB (pol IV) perform translesion synthesis (TLS) to bypass DNA adducts. It should be noted that it is not yet known exactly how UmuC and $UmuD'_2$ interact and the cartoon merely indicates that they form a complex.



Lesion	Pol IV inserts
N^2-ff-dG	C
N^2-B[a]P-dG	C
γHOP-dG X-link	C (A/T)
N^2-CE-dG	C

Figure 3. Adducts of deoxyguanine bypassed by *E. coli* pol IV: N^2-dG-gamma-hydroxypropano-dG (64), N^2-(1-carboxylethyl)-2′-dG (N^2-CEdG) (59), N^2-furfuryl-dG (56) N^2-[+*trans, anti*]-benzo[a]pyryl-dG (60-62). Unmodified dG is shown for comparison. The table shows the identity of the nucleotide inserted by pol IV opposite each lesion.

However, this number increases 10-fold after SOS induction; therefore DinB is the most abundant DNA polymerase in *E. coli* during times of cellular stress (35). This level of upregulation of DinB leads to inhibition of pol III, decreasing the ability of pol III to access DNA and ultimately leading to cell death (36-37).

A phenomenon known as adaptive mutagenesis involves *dinB*-dependent increased mutability in starving, non-dividing cells (38-39). It has been suggested that adaptive mutagenesis provides mutations that confer a selective advantage in times of cellular stress (40). DinB induction occurs late in stationary phase and the higher levels may be maintained for at least several days with maximum expression and mutagenesis occurring in cells that have active RNA polymerase sigma factor (RpoS) (41-42). Adaptive mutagenesis is a cellular starvation stress response system, which is partially distinct from the SOS response. Notably, of the SOS genes, only DinB at elevated levels is required for stress-induced mutagenesis (43), although the exact molecular mechanism of adaptive mutagenesis may not be entirely clear (44-46). A variety of aspects of adaptive mutagenesis have been reviewed recently (40, 47-49). The GroE heat shock response chaperone system has also been shown to influence DinB protein levels as well as adaptive mutagenesis, although no direct interaction between GroE and DinB has been detected (50).

Classically, expression of the *dinB* gene is repressed by LexA and induced as part of the SOS response (22, 34). However, the *dinB* gene can also be expressed under other conditions of cellular stress. For example, *dinB* expression is induced by beta-lactam antibiotic-mediated inhibition of the synthesis of the bacterial cell wall independent of LexA (51). This suggests that transcription of the *dinB* gene can be considered a general stress response mechanism. Increased mutagenesis by DinB, and possibly pol V, may be a contributing factor to antigenic variation or antibiotic resistance (52-55).

4.2. Specificity

DinB displays a preference for certain damaged DNA substrates. For example, DinB possesses a 15-fold preference to insert C opposite N^2-furfuryl-dG and a 25-fold preference to extend from a base pair with N^2-furfuryl-dG in comparison to undamaged dG in a DNA template (Figure 3) (56-57). While the endogenous source of N^2-furfuryl-dG has not yet been determined, by analogy to the formation of kinetin (N^6-furfuryl-dA), it may be a product of ribose oxidation (58). Strains in which *dinB* has been deleted show a striking sensitivity to nitrofurazone and 4-nitroquinoline-1-oxide (4NQO), both of which are thought to form N^2-dG adducts, as well as possibly other adducts (5, 56). DinB also efficiently and accurately bypasses N^2-(1-carboxyethyl)-2′-deoxyguanine (N^2-CEdG), which was detected in 1 in 10^7 bases in melanoma cells and is formed as an adduct of methylglyoxal, a common byproduct of glycolysis (Figure 3) (59). The presence of DinB significantly increases bypass of the N^2-dG adduct of benzo[a]pyrene (B[a]P), a potent carcinogen consisting of a large, bulky polycyclic hydrocarbon (Figure 3) (60-63).

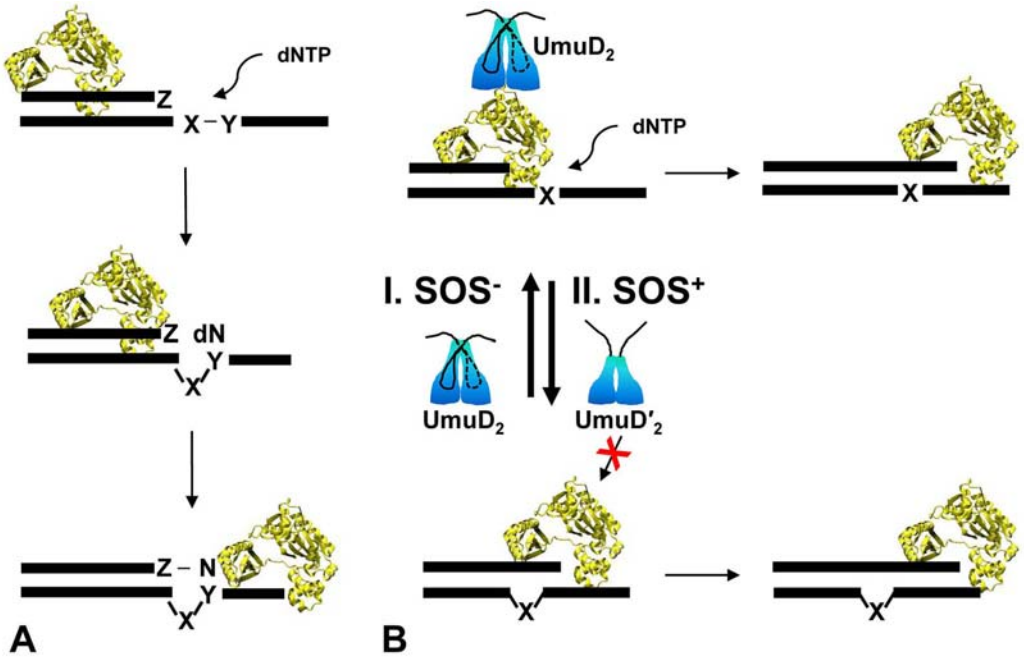


Figure 4. Mechanisms for the generation of -1 frameshift mutations by *E. coli* pol IV. Pol IV is represented by the crystal structure of Dpo4 (yellow) (10), while $UmuD_2$ and $UmuD'_2$ are shown in blue. A. dNTP-stabilized misalignment mechanism (69, 72) B. Template slippage mechanism I. Prior to SOS induction, full-length $UmuD_2$ regulates pol IV to enhance error-free synthesis (top). II. Upon SOS induction, cleaved $UmuD'_2$ does not bind pol IV and so pol IV is free to generate -1 frameshift mutations via the template slippage mechanism (bottom) (71, 73)

However, efficient bypass of some isomers of B[a]P also requires pol V, suggesting that even subtle changes in the conformation of adducts can alter how they are processed by DinB (60). Acrolein is a potent toxin and has tumor initiating properties, but it is also an endogenous byproduct of fatty acid metabolism (64). DinB inserts dCTP across from gamma-hydroxypropano-deoxyguanosine (HOPdG), the N^2-dG adduct of acrolein, as well as peptide cross-links to gamma-HOPdG (Figure 3) (64). In addition to bypass of DNA-peptide cross-links, DinB is also proficient in bypassing N^2-N^2-dG interstrand cross-links (65). DinB may have a functional duality as a bypass polymerase for certain metabolism-induced DNA lesions such as HOPdG, N^2-CEdG, and N^2-furfuryl-dG, and as a general bypass polymerase capable of negotiating larger N^2-dG adducts such as benzo[a]pyrene.

Pol kappa is the eukaryotic ortholog of DinB. Although mammalian pol kappa, like DinB, exhibits a preference for N^2-furfuryl-dG (56), there are differences in the abilities of the two enzymes to replicate DNA containing other adducts. Notably, the C^8-dG adducts of N-2-acetylaminofluorene (2-AAF) and aminofluorine (2-AF), which are prototypical aromatic amides and carcinogens, are readily bypassed by human pol kappa, but these same adducts block insertion and extension by *E. coli* DinB (66).

DinB exhibits varying efficiency for bypass of a number of lesions resulting from reactive oxygen species, including 8-oxoG, 2-oxoA, thymine glycol, 5-formyluracil, and 5-hydroxymethyluracil (67) and is able to incorporate into DNA the oxidized nucleotides 2-oxo-dATP and 8-oxo-dGTP (68). DinB has been shown to bypass abasic (AP) sites *in vitro* generating either -1 or -2 frameshift mutations (69-70). The presence of accessory proteins, specifically the beta processivity clamp and the gamma clamp loader, greatly increase the efficiency of bypass (70) and decrease frameshift mutagenesis (69).

On undamaged DNA, DinB has a relatively high error frequency of 2.1×10^{-4} for frameshift mutations and 5.1×10^{-5} for base substitution mutations (69). The majority of the frameshift mutations are single base deletions (~81%), whereas a substantial number of frameshift mutations are two base deletions (~15%) (69). The mechanisms by which the -1 frameshift mutations occur in TLS by DinB is most likely a combination of Streisinger slippage (71) and dNTP-stabilized misalignment in which a nucleotide downstream from the primer terminus is flipped out of the DNA helix and is skipped by DinB generating a -1 frameshift mutation (Figure 4) (69, 72-73). The dNTP-mediated mechanism may be at odds however with the recently published mechanism of template slippage on homopolymeric runs by DinB (73). This type of slippage, which generates -1 frameshift mutations, is inhibited by $UmuD_2$ (Figure 4) (73). Base skipping occurs as the template strand opposite the 5′ terminus folds into the extrahelical space and the next nucleotide on the template strand becomes the base opposite the primer terminus (74-75). This mechanism was also observed in similar studies with the DinB ortholog *Sulfolobus acidocaldaricus* Dbh (76).

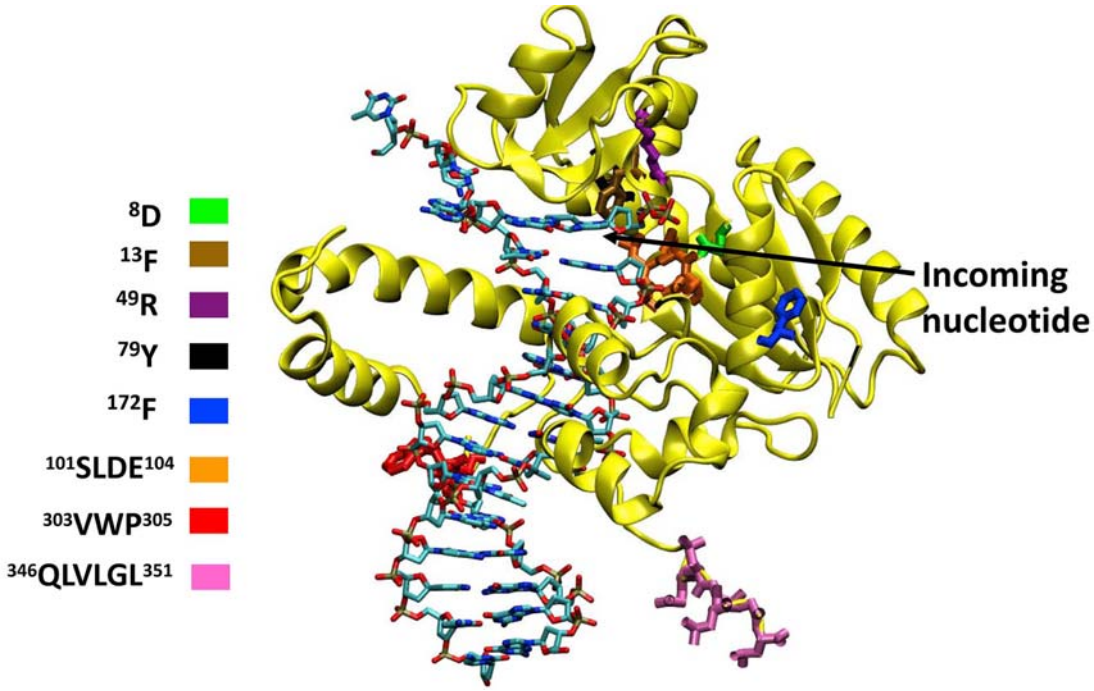


Figure 5. Model of DinB with some important residues highlighted (56). F13 (brown) is involved in accomodation of the lesion in the active site (56). D8 (green), D103 and E104 (orange), R49 (purple) each when mutated confer a lower mutation frequency than wild-type DinB (33). D103 and E104 are involved in coordinating the divalent magnesium ion necessary for catalysis (33). Y79 (black) is important in regulating the steric gate residue (57). Residues ^{346}QLVLGL^{351} (pink) comprise one of the interaction sites with the beta clamp (83), while ^{303}VWP^{305} (red) comprise the site of interaction with the dimer interface of the beta clamp (85).

4.3. DinB variants

Currently, there is no high-resolution structure of DinB but homology models (56, 77) have been constructed based on the crystal structures of other homologous proteins: *Sulfolobus solfataricus* Dpo4 (10) and *Sulfolobus acidocaldaricus* Dbh (17). Therefore, interpretation of experiments in which DinB variants have been constructed relies on the use of homology models. Cells containing overexpressed wild-type DinB had a mutation frequency of 68,466.5 x 10^7 (33), which is approximately 3600-fold greater than cells without overexpressed DinB (33). DinB mutations D8A, D8H, R49A, R49F, D103A, D103N (*dinB003*), and E104A led to between 850- and 3700-fold lower mutation frequencies (Figure 5) (33). D103 and E104 reside in the S[LI]DE box whose negatively charged residues in the active site coordinate the divalent magnesium ions needed for adding the incoming nucleotide to the DNA primer (10, 33). Along with D103 and E104, D8 is strictly conserved (33). R49 is predicted to lie in a loop region that is near the incoming nucleotide.

The steric gate is a single residue in DNA polymerases that prevents the incorporation of ribonucleotides into DNA by sterically occluding nucleotides with a 2′ hydroxyl group (78). The steric gate residue of Y family DNA polymerases is most frequently tyrosine or phenylalanine (56, 79-80). Mutation of the steric gate residue F13 in DinB increases the frequency of ribonucleotide misincorporation from $<10^{-5}$ to 10^{-3} (56). It was hypothesized that the pocket in which F13 resides is involved in the accommodation of a lesion in the active site (Figure 5) (56). The substitution F13V inhibits the ability of DinB to bypass N^2-furfuryl-dG and also slightly enhances the ability of DinB to replicate undamaged DNA (56).

Near the steric gate residue and conserved among all orthologous DinBs, Y79 is hypothesized to regulate the function of the steric gate residue (57). Mutations at this position have a modest effect on primer extension on undamaged DNA but prevent DinB from extending more than a few nucleotides beyond a lesion and result in extreme cellular sensitivity to nitrofurazone (57). As demonstrated in these studies, single mutations can have a large effect on both the DNA replication and TLS activities of DinB.

4.4. Cellular interactions of DinB

DinB is regulated through protein-protein interactions with $UmuD_2$, RecA, and NusA, as well as with the beta clamp (27, 81). Addition of RecA and $UmuD_2$ to a primer extension assay in which there are correctly paired bases at the primer terminus increases the polymerization proficiency of DinB (27). It appears that the deleterious -1 frameshift mutator activity of DinB is a result of the elevated number of molecules of DinB present in a cell relative to the amount of $UmuD_2$ present (27). Indeed, co-upregulation of both $UmuD_2$ and DinB suppresses the -1

frameshift mutation activity, while -1 frameshift mutations are elevated in the absence of *umuD* (Figure 4) (27). Modeling studies suggest that RecA and $UmuD_2$ may suppress the -1 frameshift activity innate to DinB by decreasing the openness of the DinB active site (27). This regulation may explain the dual nature of the polymerase activity of DinB, which accurately bypasses bulky N^2-dG adducts but is also responsible for highly mutagenic -1 frameshift mutations (56, 69, 82). Deletion of *umuD* did not affect DinB-dependent resistance to nitrofurazone, suggesting that the -1 frameshift mutator activity and TLS functions are to some extent distinct (27). DinB residue F172 is highly conserved in DinB sequences from organisms that also harbor *umuD* and has been shown to mediate the interaction between DinB and UmuD (Figure 5) (27).

DinB also interacts physically with the beta processivity clamp. In the presence of the beta clamp, DinB is recruited to the primer terminus to form a stable complex with DNA, which substantially stimulates its processivity (81). The specific residues involved in this protein-protein interaction have been identified as ^{346}QLVLGL^{351} (83-84), which is at the C-terminus of DinB (Figure 5). The co-crystal structure of the little finger domain of DinB with the beta clamp reveals a second interaction site between the two proteins at DinB residues ^{303}VWP^{305} and near the dimer interface of beta (85). When the structure of full-length Dpo4 was superimposed on the structure of the DinB little finger, the DNA polymerase did not seem to be in the proper position to access the primer terminus (85). This suggests that this conformation, while likely not catalytically relevant, may be one way in which the beta processivity clamp facilitates recruitment of DinB to replication forks (85-86). DinB can remove pol III from the beta clamp when pol III is stalled at a primer terminus *in vitro* thus inhibiting the continuation of DNA synthesis by the pol III holoenzyme (37).

Recently, a role for the NusA protein in stress-induced mutagenesis has been found involving an interaction with DinB. NusA plays an important role in the elongation, termination, and anti-termination phases of transcription (87-89). It was shown that DinB and NusA physically interact with one another, so it was proposed that NusA recruits DinB to gaps that stall RNA polymerase during transcription (90). Though the exact location of the interaction has yet to be identified, the C-terminal domain of NusA and surface residues near the *nusA11*^{Ts} mutation are likely sites (90-91). Genetic interactions have been observed between *nusA* and both *dinB* and *umuDC*, which may indicate a genetic link between TLS and transcription (90). Moreover, NusA plays a role in transcription-coupled nucleotide excision repair as well as in recruiting DinB for transcription-coupled TLS of lesions that result in gaps in the DNA template that disrupt RNA polymerase (92). Additionally, NusA has been found to be required for DinB-dependent stress-induced, or adaptive, mutagenesis (93).

5. DNA POLYMERASE V: $UmuD'_2C$

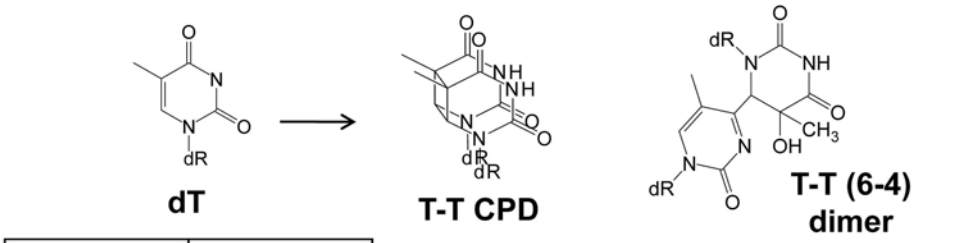
Y family DNA polymerase pol V, discovered in an experiment to identify nonmutable mutants of *E. coli*, is encoded by the *umuDC* genes and induced by the SOS response (94-95). This specialized polymerase consists of one molecule of UmuC which possesses the polymerase activity, and one $UmuD'_2$ dimer, therefore pol V is also referred to as $UmuD'_2C$. Pol V is responsible for the majority of UV-induced mutagenesis in *E. coli* and it has been shown to bypass common lesions from UV radiation.

5.1. Discovery of pol V and SOS mutagenesis

The *umuC* gene was discovered by characterizing mutants of *E. coli* that were deficient for UV-induced mutagenesis but were still viable (95). Kato and Shinoura screened for UV-nonmutable (Umu) mutants by using the mutagen 4-nitroquinoline-1-oxide (4NQO) and then using UV irradiation in a second screen (95). The *umuC* gene appeared to encode a protein that participates in "mutagenic repair" as well as reactivation by UV irradiation (95). Steinborn independently discovered *uvm* mutants that are deficient in UV mutability using a similar method to Kato and Shinoura (96). It was thought that perhaps the *uvm* gene was related to *umuC* in that they displayed similar nonmutable characteristics when *uvm* mutants were exposed to UV light, a phenotype of UmuC that is now well known (96). Characterization of the *umuC* gene and its role in SOS mutagenesis was carried out long before the biochemical function of its gene product in translesion synthesis was determined.

Polymerase V is an error-prone DNA polymerase that is responsible for most SOS mutagenesis. It was first suggested that the UmuC and UmuD proteins were mediators that enabled DNA polymerase III holoenzyme (pol III HE), which typically stalls at sites of damage, to bypass DNA lesions (97-98). This stalling may occur for one of two reasons: either the polymerase cannot recognize the lesion as an instructional base, or the exonucleolytic proofreading subunit of pol III HE recognizes any base insertion as incorrect and hydrolyzes the newly incorporated nucleotide (2, 5, 99-101). It was thought that UmuC-UmuD′ allows pol III HE to successfully replicate past a DNA lesion but with low fidelity (2, 98). A two-step model for UV mutagenesis was proposed. In the model, the first step involves a RecA-mediated misincorporation event opposite the lesion. In the second step, *umuC* extends the primer from the misincorporated nucleotide, allowing replication to continue beyond this point (97, 100). At the same time, it was discovered that the *umuD* gene product participates along with UmuC in this two-step process (97).

Tang *et al.* showed that $UmuD'_2C$ in the presence of RecA, beta clamp, gamma clamp loader, SSB, and either polymerase III or II facilitates the bypass of an abasic lesion, at which time it was speculated that $UmuD'_2C$ had polymerase activity (102). By 1999, two separate groups reported purifying different forms of the UmuC protein. The Livneh group purified a soluble form of UmuC, an N-terminal fusion with maltose binding protein (MBP) (29). The Woodgate and Goodman groups collaborated to purify a soluble complex $UmuD'_2C$ (31, 102-103). Both groups determined that $UmuD'_2C$ is in fact a DNA polymerase (29, 31). UmuC had weak DNA polymerase activity, but with the addition of cofactors such as UmuD′, RecA, and SSB,



Lesion	Pol V inserts
T-T CPD	A-A
T-T (6-4)	A-G
Abasic site	A
C^8-AAF-dG	C
N^2-B[a]P-dG	A/C

Figure 6. *E. coli* DNA polymerase V is known to bypass common lesions that occur from UV radiation, such as thymine-thymine (T-T) *cis-syn* cyclobutane pyrimidine dimers (CPD) and T-T (6-4) photoproducts, as well as abasic sites and the C^8-dG adduct formed from *N*-2-acetylaminofluorine (C^8-AAF) (101, 116-118). The table shows the identity of the nucleotide inserted by pol V opposite each lesion.

this activity increased despite the absence of pol III HE (29). The UmuD′$_2$C complex also inhibits homologous recombination mediated by RecA, suggesting that the appearance of pol V actively prevents the relatively accurate recombination repair pathway while enabling SOS mutagenesis (104-106).

5.2. Roles of Pol V in responding to replication stress

Upon DNA damage, replication forks undergo regression; these regressed forks are stabilized by RecA and RecF (107-108). RecJ and RecQ partially degrade nascent DNA at stalled replication forks, while preventing TLS from occurring (109). Recovery of DNA synthesis typically occurs once the generally accurate nucleotide excision repair process removes the lesion (110). If the capacity of nucleotide excision repair is exhausted, pol V specifically allows DNA replication to recover (111). In the absence of *umuDC*, recovery of DNA synthesis is modestly delayed (110). However, in the absence of *recJ*, replication restart is significantly delayed and in the absence of both *recJ* and *umuDC*, replication essentially does not recover. Without RecJ present to process the stalled replication fork, TLS by pol V is required for survival (110, 112).

When UmuC and UmuD are overexpressed in *E. coli*, strains are cold sensitive, meaning the cells exhibit extremely slow growth at 30 °C without a growth defect at 42 °C. The cold sensitivity phenotype correlates with a specific decrease in the rate of DNA replication (28). This decrease in the rate of replication likely delays restart of replication in response to DNA damage to allow time for accurate methods of DNA repair such as nucleotide excision repair to operate and therefore may serve as a primitive DNA damage checkpoint (26). In this model, cleavage of UmuD to form UmuD′ releases the checkpoint, in part because UmuD′ has lower affinity for the beta clamp than does UmuD (113-114), and allows TLS to occur (26, 30, 115). The cold sensitivity phenotype conferred by UmuD and UmuC is independent of their roles in TLS (30). Taken together, these observations suggest that inappropriate levels of the *umuDC* gene products, whether because they are deleted or overexpressed, disrupt the cellular responses to DNA damage or replication stress.

5.3. Specificity of pol V

Polymerase V bypasses common lesions that result from UV radiation, such as thymine-thymine (T-T) *cis-syn* cyclobutane pyrimidine dimers (CPD) and T-T (6-4) photoproducts, as well as abasic sites and the C^8-dG adduct formed from *N*-2-acetylaminofluorine (C^8-AAF) (Figure 6) (101, 116-119). Pol V efficiently bypasses UV photoproducts as well as abasic sites when in the presence of the beta clamp, RecA/ssDNA, and SSB (118). Pol V inserts G six-fold more frequently than it inserts A opposite the 3′-T of T-T (6-4) photoproducts, consistent with its mutagenic signature *in vivo* (116, 118). The CPD UV photoproduct is bypassed in an error-free manner (116, 118). *N*-2-acetylaminofluorine forms adducts at the C^8 position of the guanine base (117), giving C^8-AAF-dG, which is bypassed in an error-free manner by pol V when the lesion occurs in the context of (5′-GGCG^{AAF}CC-) (117). These lesions also commonly cause -2 frameshift mutations in continuous G sequences, including the *Nar*I sequence, as well as -1 frameshift mutations in sequences containing three or four continuous Gs (5). Pol V efficiently bypasses N^6-benzo[a]pyrene-dA and is implicated in bypass of some N^2-benzo[a]pyrene-dG adducts (Figure 3), as well as some oxidized bases (60-61, 63, 120). Pol V displays error rates of 10^{-3} – 10^{-4} when copying undamaged DNA, therefore it is even less accurate than DinB (118).

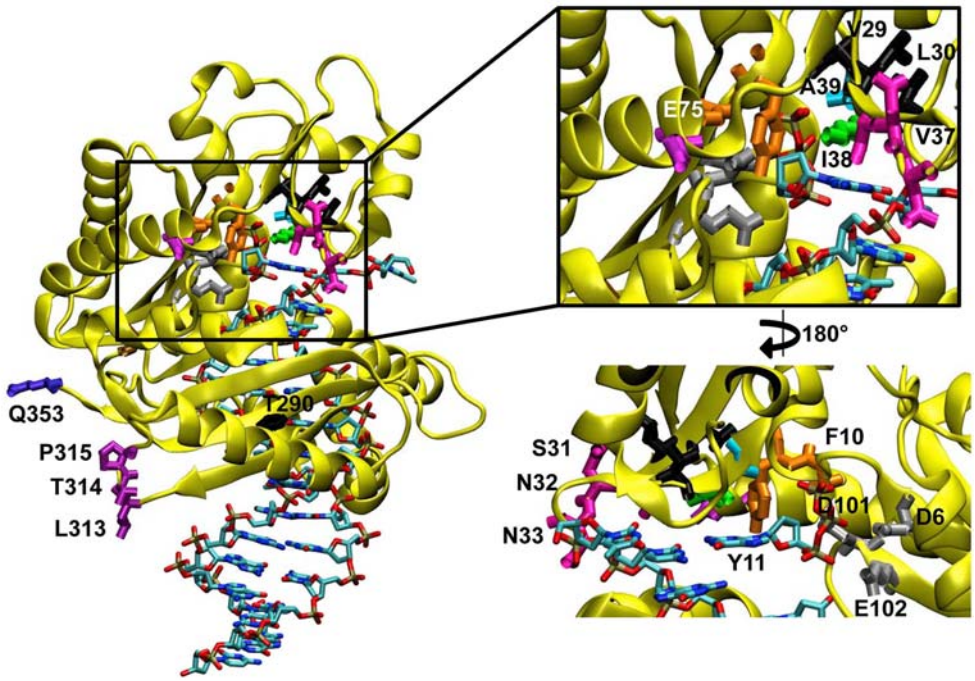


Figure 7. Model of UmuC, the DNA polymerase subunit of UmuD′$_2$C (121). Residues are highlighted as follows: F10 and Y11 steric gate (orange) (80); ^{31}SNN^{33} (pink) are proposed to control the size of the active site (124); V29, L30, and V37 (black) are thought to anchor an active site loop; I38 (green) is likely to be adjacent to the incoming nucleotide (124); D6, D101, and E102 (silver) are conserved catalytic residues (8, 10, 96); E75 (pink) is potentially a site of interaction between UmuC and UmuD′$_2$ (126-127); T290 (black) is in the hydrophobic core of the little finger and may contribute to its stability (126); A39 (teal) is important for mutagenesis, UV resistance, and the cold sensitive phenotype (123); ^{313}LTP^{315} (violet) is the site of interaction with the dimer interface of the beta clamp (121); Q353 (purple) is the C-terminal residue of the model. This model of UmuC is a truncation, as the C-terminal domain of UmuC lacks homology to proteins of known structure. The UmuC beta binding motif ^{357}QLNLF^{361} would be located just after Q353 (purple) (121, 128).

5.4. UmuC variants

There is currently no crystal structure of UmuC, so interpretation of experiments with UmuC variants must rely on models based on homology to Dpo4 and other Y family DNA polymerases (121-122). UmuC variants with mutations at several residues have been characterized (Figure 7). Due to the lack of an efficient purification scheme for pol V, most characterization of variants has been carried out *in vivo* using complementation assays. Residues D101, E102, and D6 are strictly conserved catalytic residues for metal ion binding, with D101 and E102 part of the conserved S[LI]DE motif (10, 96). The UmuC D101N (*umuC104*) variant made by Steinborn cannot carry out UV-induced mutagenesis and polymerase activity is severely diminished (10, 96).

The steric gate residue of UmuC has been identified as Y11 (Figure 7) (80). F10 is the residue N-terminal to the steric gate residue and was chosen for analysis based on analogy to the F13 steric gate residue of DinB (80). Mutating either UmuC F10 or Y11 to alanine caused cells harboring these variants to be hypersensitive to UV light, which was alleviated by combining either of these mutations with ablation of the beta-binding motif in UmuC (80). This observation suggests that the toxic effect of the F10 or Y11 variants is conferred via their access to replication forks.

Cells harboring the UmuC variant A39V were very sensitive to UV radiation and had decreased UV-induced mutagenesis (Figure 7). Also, the A39V mutation fails to complement the *umuDC*-dependent cold sensitivity phenotype (123). Even though pol V contributes substantially to UV-induced mutagenesis, it contributes only modestly to survival after exposure to UV (5). However, mutations at F10, Y11, and A39 are examples of point mutations in UmuC that confer dramatically increased sensitivity to UV on cells that harbor them.

Several residues were proposed to be important for UmuC activity based on analysis of the active site region thought to accommodate bulky lesions (77, 122, 124). Residues ^{31}SNN^{33} are metaphorically referred to as the "flue" of a "chimney," meaning these residues perhaps control the size of the active site opening of UmuC, which in turn would control the size of the adducts that can be bypassed (Figure 7) (124). S31 was shown experimentally to be important for UV-induced mutagenesis, while I38 and V39 contribute to bypass of benzo[a]pyrene (77, 125). N32 theoretically plugs the chimney hole, controlling the size of the opening (Figure 7) (124). V29, L30, V37, and I38 are thought to anchor an active site loop, referred to as loop 1, of UmuC. Furthermore, I38 is identified as the "roof" amino acid which influences dNTP insertion due to its location directly adjacent to the incoming

nucleotide. L30 is a "flue handle" that controls the flue opening of the chimney (77, 124).

Strains harboring the *umuC36* allele (E75K) were rendered nonmutable (126). E75 is potentially a site of interaction between UmuC and UmuD′$_2$, because in strains with UmuD′$_2$ present at elevated levels, the non-mutable phenotype was suppressed (126-127). The T290K mutation (*umuC25*) causes strains harboring it to be non-mutable (126). By analogy to Dpo4, T290 is predicted to be in the hydrophobic core of the little finger and may contribute to protein stability (Figure 7) (8).

The model of UmuC shown in Figure 7 is a truncation, with the last residue in the model indicated at Q353, as the C-terminal domain of UmuC lacks homology to proteins of known structure. The UmuC C-terminal domain is important for UV-induced mutagenesis as well as for interactions with UmuD and UmuD′ (30). The UmuC beta-binding motif ^{357}QLNLF^{361} would be located just C-terminal to the end of the model, Q353 (83, 121, 128). Mutations in this motif cause almost a complete loss in UmuC-dependent UV-induced mutagenesis (83, 121). The second area of interaction between UmuC and the beta clamp, ^{313}LTP^{315}, did not cause a loss in UV-induced mutagenesis when mutated. However, the UmuC-dependent cold-sensitivity phenotype was suppressed when either of these sites was mutated (121).

5.5. Cellular interactions of UmuC

Initial characterization through a series of co-immunoprecipitation experiments showed that UmuC interacts with UmuD$_2$ and interacts more strongly with UmuD′$_2$ (2). UmuD′$_2$ is the form of the *umuD* gene products that is active in mutagenesis, with the presence of UmuD′$_2$ specifically required to facilitate mutagenesis (24). Moreover, induction of *umuDC* is all that is required for SOS mutagenesis (129). It was not until approximately twenty years after the discovery of the *umuDC* genes and their roles in SOS mutagenesis that UmuD′$_2$C was found to be a DNA polymerase capable of copying damaged DNA (29, 31).

Several additional proteins facilitate TLS by UmuD′$_2$C. These proteins include RecA, the beta clamp and gamma clamp loader, and SSB. Activated RecA is strictly required for pol V-dependent TLS, although the exact mechanism by which RecA must be activated in still in question (see below). The use of a variety of experimental systems by three separate groups to study the biochemical properties of pol V has contributed to different conclusions about the roles and requirements of these accessory proteins (summarized in Figure 8). First, different forms of UmuC have been used, with one group using a maltose binding protein tag (MBP) to purify UmuC (29) while others purified a native UmuC-UmuD′ complex (31, 117). Another major experimental difference among the three groups is the DNA substrate used (Figure 8). The Livneh group used a gapped plasmid with an ssDNA region of approximately 339 nucleotides (29, 130-132). The Goodman group originally used linear ssDNA with the lesion located 50 nucleotides from the 5′ end (31, 118-119); in their more recent work the length of the DNA varies (133-136). Lastly, the Fuchs group used circular ssDNA that is about 2700 nucleotides in length (101, 117, 137). These differences in experimental design possibly set the stage for the discrepancies in biochemical requirements for pol V as summarized in Figure 8 and discussed below.

RecA is a 38-kDa protein that is the product of the *recA* gene (138). In addition to the significant roles RecA is known to play in responses to DNA damage, including homologous recombination, induction of the SOS response by serving as a coprotease in the autoproteolytic cleavage of LexA, and the regulation of SOS mutagenesis by cleavage of UmuD$_2$ to UmuD′$_2$, RecA also has a direct role in TLS (139-140). Furthermore, it was proposed that RecA has two distinct roles in pol V-mediated TLS (134). First, RecA at the 3′ end of a primer stimulates pol V for TLS activity. Second, RecA bound to the template strand mediates extension past the lesion (134). The RecA nucleoprotein filament may provide an "activated" RecA monomer for TLS, but the filament itself may or may not participate in the actual TLS reaction. A six-nucleotide overhang can only bind two RecA monomers, and TLS still occurs, an observation that argues against the need for a RecA filament (134). Bypass of an abasic site in a three-nucleotide gap in the DNA to which RecA can still bind was successful as well; however, bypass of a lesion in a two-nucleotide gap was not (134).

One viewpoint is that a "minimal mutasome," which includes pol V, ATP, and two RecA molecules, one bound to UmuC and one bound to UmuD′$_2$, is the active form in TLS (136, 141). Furthermore, in this model, RecA acts in *trans*, such that the RecA/ssDNA filament is formed on a DNA strand not actively being copied by pol V (135). In other words, the trans RecA/ssDNA transfers a RecA monomer as well as a molecule of ATP from its 3′ end to a molecule of pol V, thereby activating pol V for TLS (133). The newly activated pol V then performs a single round of TLS, and upon dissociation from the DNA is inactivated and must once again be activated by RecA and ATP (133). On the other hand, there is also evidence that the RecA/ssDNA nucleoprotein filament primarily acts in cis for TLS by forming a filament on the single-stranded DNA downstream from the lesion on the primer strand, thus facilitating TLS by pol V (137).

The beta clamp significantly increases the processivity of the replicative DNA polymerase pol III (142). This enhancement of processivity extends to other polymerases as well, including pol V. The beta clamp and gamma clamp loader provide additional stability to the complex and may help pol V remain tethered to DNA (119). However, the beta clamp only increases processivity of pol V modestly, with determinations ranging from three- to five-fold to ~100-fold (117, 131). It has been consistently observed that the beta clamp stimulates Pol V, but to varying extents and with different requirements for co-factors (Figure 8) (29, 31, 101-102, 117-119, 131). The Livneh and Fuchs groups determined that with native ATP, the presence of the beta clamp increased processivity of Pol V (117, 131), whereas the Goodman group observed a three- to five-fold increase in processivity with the beta clamp present but only with ATP-gamma-S and SSB also present (119). The Fuchs group suggested that SSB must be

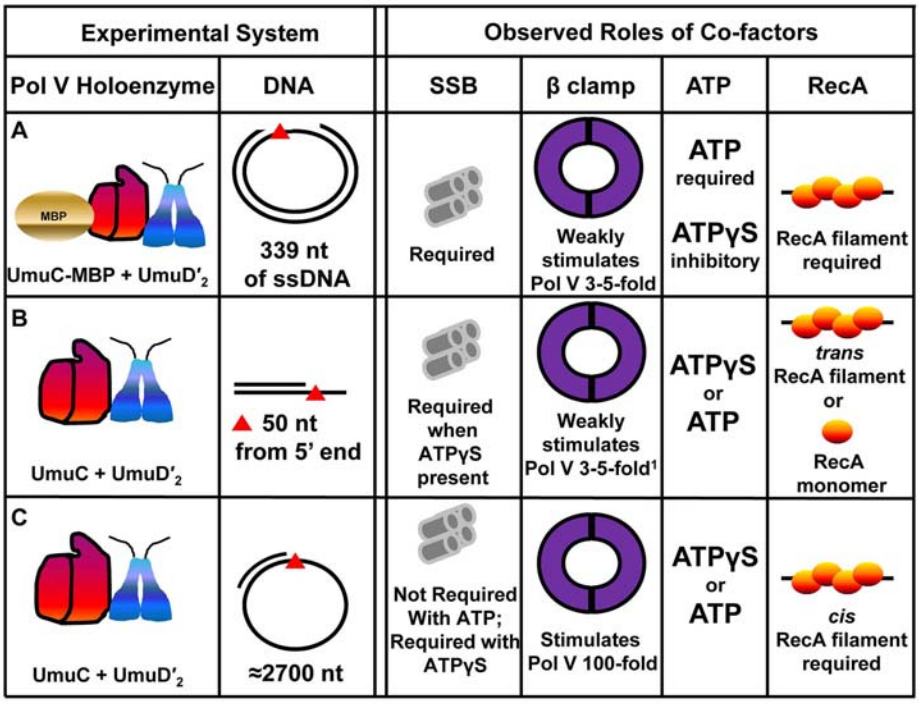


Figure 8. A comparison of biochemical requirements for TLS by pol V. (A) UmuC was purified by using a maltose binding protein tag (29). The DNA substrate used was a circular plasmid with approximately 339 nt of ssDNA. It was found that with SSB, ATP, and a RecA/ssDNA nucleoprotein filament, the beta clamp stimulates nucleotide incorporation by three- to five-fold (29, 130-132). (B) The DNA substrate used was a single strand of linear DNA with the lesion 50-nt away from the 5′ end of the DNA. SSB and the beta clamp were required when ATP-gamma-S was present. A RecA nucleoprotein filament in *trans* or only a single RecA monomer is needed for pol V activation (31, 118-119, 133-136, 139, 160). (C) The circular DNA substrate used was approximately 2700-nt long. SSB was not required when ATP was present; the beta clamp stimulates TLS by 100-fold. The RecA nucleoprotein filament is in *cis* (101, 117, 137). [1]The processivity of Pol V is increased three- to five-fold when ATP is replaced with ATP-gamma-S (119).

present when ATP-gamma-S is used, perhaps to destabilize the highly stabilized RecA filament that is formed in the presence of ATP-gamma-S (62).

A direct physical interaction has been detected between UmuC and single-stranded DNA binding protein (SSB) (130). SSB coats single-stranded DNA and helps to prevent dissociation of RecA from the ssDNA formed after a replicative polymerase stalls at a lesion (143). SSB also stimulates the formation of the RecA filament by over 50-fold (144). Lesion bypass using MBP-UmuC (29) was at its most efficient when the concentration of SSB in the reaction was 50 nM and ATP was present (132). Using a purified UmuD′$_2$C complex, it was determined that lesion bypass was optimal at 60 nM SSB and that SSB stimulated TLS by 1,040-fold when ATP-gamma-S was present (119). Similarly, SSB likely helps to form the RecA nucleoprotein filament in the presence of ATP (132). In a lesion bypass experiment in the absence of SSB in which a functional RecA/ssDNA nucleoprotein filament was formed, no bypass was observed, leading to the conclusion that SSB may have a second function in TLS besides stimulation of RecA nucleoprotein filament formation (132). On the other hand, it has been suggested that SSB is not absolutely required for Pol V TLS in the presence of ATP, but SSB may be needed to disrupt the more stable RecA filament formed in the presence of ATP-gamma-S (117). Furthermore, it is thought that SSB may play a direct role in recruiting pol V to the 3′ end of the primer coated with RecA via a direct interaction with UmuC (130), as SSB interacts with pol V at the C-terminus of SSB (130). It is important again to consider the differences in the form of pol V as well as the DNA substrate used for these experiments, which is summarized in Figure 8.

6. POLYMERASE SWITCHING

There are a variety of models used to describe how the multiple DNA polymerases in *E. coli* are utilized appropriately. The beta clamp facilitates polymerase switching that must take place in order for pol V to replace pol III on the DNA template (101, 117, 137, 145). In *E. coli*, it was observed that the beta clamp is donated by pol III to recruit pol V to the DNA template near the lesion (101). The "dynamic processivity" model was suggested from experiments using a catalytically inactive variant of the T4 DNA polymerase gp43 that was exchanged in less than a minute to replace the wild-type replicative polymerase (146). Thus, this model suggests that DNA polymerases may exchange in a stochastic and rapid

manner. The observation that elevated levels of DinB inhibit pol III suggests a model of rapid replacement of polymerases at the replication fork in *E. coli* as well (36-37). Another model of polymerase switching is the "tool belt" model, which holds that multiple DNA polymerases are tethered to the beta clamp at the same time, allowing several DNA polymerases to be present at the replication fork and used when needed (5, 147). However, the observation that only one binding site on the beta clamp is used to facilitate a switch between pol III and pol IV is inconsistent with the tool-belt model (148). Finally, the gap filling model suggests that gaps are left at lesion sites when a replicative polymerase cannot bypass the lesion. Replication is initiated downstream of the lesion and the resulting gap is subsequently filled in by Y family polymerases performing TLS (149-152). These models are not mutually exclusive.

Although pol V is very poorly processive, the processivity offered by the beta clamp to pol V allows the polymerase to synthesize an appropriate-length "TLS patch." This patch of at least six nucleotides allows pol V to bypass the lesion and extend past it far enough for pol III to resume DNA synthesis without triggering proofreading at the newly bypassed lesion (101). Pol III can detect distortions in DNA caused by inserting a nucleotide opposite a lesion even when pol III is recruited back to the replication fork four to five nucleotides after the lesion so a TLS polymerase must extend at least beyond that point.

Mutations in the beta clamp, notably a poly-A sequence substituted at residues 148-152 (replacing ^{148}HQDVR^{152}) severely inhibited DNA replication by pol IV, but did not affect the polymerase functionality of pol III (153). Even though pol IV has been observed to inhibit replication by pol III (36-37), pol IV only displaces an impaired pol III that is not actively replicating and does not seem to displace an actively replicating pol III (148). Pol IV is proposed to accomplish this by two separate interactions with the beta clamp, at what is termed the 'cleft' and the 'rim' dimer interface regions of the beta clamp (85-86, 148). First, DinB binds the rim adjacent to the cleft bound by a replicating pol III alpha subunit and then gains control of the cleft once the replicative polymerase stalls at a lesion site (148). The DinB little finger-rim interaction is dispensable for TLS but necessary for recruitment of pol IV to replication forks (86).

Other work suggests that the situation is more complicated than a simple exchange between two DNA polymerases, as apparently several polymerases can compete or cooperate to bypass specific lesions (111, 120, 154-157). The presence of pol III, pol II, pol IV and pol V influence one another's ability to access the primer terminus of the replication fork (158). The two Y family DNA polymerases contribute to bypass of benzo[a]pyrene, for example (60, 63). Additionally, by using the characteristic mutagenic signature of each DNA polymerase, it was found that the polymerases in *E. coli* compete for access to DNA and that both pol IV and pol V contribute to spontaneous mutagenesis (154).

7. SUMMARY

E. coli Y family DNA polymerases play important roles in conferring resistance to DNA damaging agents. The two Y family polymerases present in *E. coli* are proficient for bypassing distinct sets of DNA lesions, which suggests that they play roles complementary to each other in cells faced with DNA damage. The Y family polymerases also regulate DNA replication in response to DNA damage and other replication stress. Because of their role in mutagenesis, the Y family polymerases may be involved in antigenic variation or antibiotic resistance. Thus, understanding both the regulation and the inherent basis of specificity of Y family DNA polymerases is critical.

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