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Translesion DNA synthesis

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Abstract

All living organisms are continually exposed to agents that damage their DNA, which threatens the integrity of their genome. As a consequence, cells are equipped with a plethora of DNA repair enzymes to remove the damaged DNA. Unfortunately, situations nevertheless arise where lesions persist, and these lesions block the progression of the cell's replicase. Under these situations, cells are forced to choose between recombination-mediated "damage avoidance" pathways, or use a specialized DNA polymerase (pol) to traverse the blocking lesion. The latter process is referred to as Translesion DNA Synthesis (TLS). As inferred by its name, TLS not only results in bases being (mis)incorporated opposite DNA lesions, but also downstream of the replicase-blocking lesion, so as to ensure continued genome duplication and cell survival. Escherichia coli and Salmonella typhimurium possess five DNA polymerases, and while all have been shown to facilitate TLS under certain experimental conditions, it is clear that the LexA-regulated and damage-inducible pols II, IV and V perform the vast majority of TLS under physiological conditions. Pol V can traverse a wide range of DNA lesions and performs the bulk of mutagenic TLS, whereas pol II and pol IV appear to be more specialized TLS polymerases.

Keywords

DNA polymerase II; DNA polymerase IV; DNA polymerase V; RecA, LexA, β-clamp; Y-family DNA polymerase; Mutagenesis; Replication Restart; Recombination; SOS response

HISTORICAL OVERVIEW

We now know that TLS is largely facilitated by specialized DNA polymerases that can accommodate bulky adducts in their active sites. Such properties are usually associated with a dramatic decrease in replication fidelity and as a consequence, TLS is often error-prone, leading to an increase in cellular mutagenesis. Significant progress in understanding the

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FURTHER READING

For additional recent reviews on TLS-related topics see references (6, 76, 101, 122, 167, 173, 179, 180, 203, 214).

molecular mechanisms of TLS have been achieved in the past decade, following the successful purification and biochemical characterization of a number of TLS polymerases. However, prior to the characterization of the highly purified proteins, the pioneering studies on TLS were largely focused on its genetic endpoints, namely its mutagenic consequences. Indeed, the first hint that damage-induced mutagenesis is not a passive process came in the early ‘50s when Jean Weigle reported that mutagenesis of irradiated bacteriophage λ was significantly enhanced, if the host bacterium had also been irradiated (247). However, it was not until the pioneering studies of Evelyn Witkin working with bacteria that could be killed by UV irradiation, but not mutated, that the concept of damage inducible error-prone translesion DNA synthesis was born (249–251, 253). These ideas were further expanded in 1970 by Miroslav Radman in a privately circulated letter in which he suggested that “SOS replication” was the result of the induction of an error-prone DNA polymerase under the control of the *recA* and *lexA* genes (reproduced in (30)). (This idea subsequently evolved into the “SOS repair hypothesis”(194, 252) (See Eco-Sal III: Module 5.4.3. The SOS Regulatory Network, and Module 7.2.8. The SOS Response).

Interestingly, damage-induced mutagenesis was not only dependent upon chromosomally encoded bacterial genes, but could be dramatically increased if the host bacterium harbored certain self-transmissible R-plasmids, such as ColIb (96), or R-Utrecht (R205) (143). Indeed, Donald MacPhee even presented evidence in the early ‘70s that R-Utrecht encoded for an error-prone DNA polymerase (142). The ability of R-plasmids to increase cellular mutagenesis prompted Ames and colleagues to introduce pKM101 into *Salmonella* strains they had developed to detect carcinogens, so as to increase the sensitivity of their assays (147). Further support for the notion of so-called “mutagenesis proteins” was provided shortly thereafter, when Kato and Shinoura (105) and independently Steinborn (219), isolated *umu/uvm* strains of *E. coli* that were specifically defective for damage-induced mutagenesis. Interestingly, DNA sequence analysis of the mutagenesis-promoting genes from pKM101 (called *mucAB*) revealed that they were closely related to the *E. coli umuDC* genes (57, 109, 185, 186, 212). During the late ‘70s and early ‘80s, many other R-plasmids from various incompatibility groups were also shown to possess mutagenesis-promoting potential (7, 159, 190, 237, 239). Many of these plasmids have now been sequenced in their entirety and similar to pKM101, shown to harbor orthologs of the *E. coli umuDC* genes (see more detailed information below).

The precise mechanism of Umu-dependent mutagenesis remained elusive for many years, largely because the UmuC protein is insoluble when overexpressed in *E. coli* (260). Based upon genetic experiments, it was hypothesized that the UmuDC proteins acted to coerce the cells main replicase, pol III, to perform TLS in a two-step process that involved a RecA-dependent (mis)insertion event opposite the lesion, followed by a second UmuDC-dependent extension step (Fig. 1A) (31, 32, 257). However, with the advent of novel expression and purification protocols, the Umu proteins were finally purified in sufficient quantities for detailed biochemical analysis (36). These studies revealed that rather than being accessory factors of pol III, the Umu proteins assemble into a complex that possesses intrinsic polymerase activity and is capable of carrying out unassisted lesion bypass (Fig.

1B). As the fifth *E. coli* polymerase reported in the literature, the Umu complex subsequently became known as pol V (200, 232).

We now know that pol V belongs to the much larger Y-family of DNA polymerases that are found in all domains of life (171). Interestingly, many organisms possess multiple Y-family polymerases. *E. coli* and *S.typhimurium* are no exception, since in addition to multiple pol V homologs (see below), they also contain the *dinB*-encoded pol IV (243) (Fig. 2), another Y-family DNA polymerase. The primary cellular role of Y-family polymerases appears to be TLS. While pol V is predominantly error-prone when promoting TLS, other Y-family TLS polymerases, such as *E. coli* pol IV, are remarkably accurate when bypassing certain DNA lesions, such as N^2-dG adducts (85, 103, 158, 265), which perhaps explains why its TLS properties went largely undetected for close to 20 years after the *dinB* gene was first identified (106). In a similar vein, a role for *E. coli*'s sole B-family polymerase, pol II (Fig. 2), in TLS also went unnoticed for nearly three decades after its initial discovery in the early 1970s (84, 115). However, by using strains specifically lacking pol II and plasmids harboring site-directed DNA lesions, it has been shown that, like pol IV and pol V, pol II can promote both error-free and error-prone TLS by itself, or in combination with other TLS polymerases (6, 17, 76, 79, 116, 117). While pols II, IV and V perform the vast majority of TLS under physiological conditions, pol III, the cell's main replicase, nevertheless also has the capacity to facilitate TLS, especially when its 3'–5' exonucleolytic proofreading function is inactivated (178). Indeed, *E. coli* pol III holoenzyme can bypass a synthetic abasic site *in vitro* with high efficiency (234) and in the absence of pol I, pol II, pol IV and pol V, an exonuclease-deficient pol III can actually facilitate efficient TLS of a cyclobutane thymine dimer *in vivo*, which is quite remarkable, given the constraints of its small active site (26).

REGULATION OF TLS POLYMERASES

LexA Regulation

All three *E. coli* TLS polymerases are regulated at the transcriptional level by the LexA repressor (8, 49, 106) (and see Eco-Sal III: Module 5.4.3. The SOS Regulatory Network, and Module 7.2.8. The SOS Response (Fig.3). LexA binds to specific sequences in the operators of genes it regulates, thereby diminishing transcription of the downstream gene. The consensus LexA-binding site is 5'-TACTG$(TA)_5$CAGTA-3' and genes with a close match to the consensus sequence are said to have a low Heterology Index (H.I.) (131), while those with a more divergent LexA-binding site have a high H.I. Upon DNA damage, LexA undergoes a RecA-mediated cleavage reaction leading to its inactivation as a transcriptional repressor (137, 214). As the intracellular levels of intact LexA drops, LexA-regulated genes with binding-sites with a high H.I. are the first to be derepressed, while those with a lower H.I. are the last to become fully derepressed. Interestingly, the LexA-binding sites in the promoter region of both, *polB*, encoding pol II, and *dinB*, encoding pol IV, have relatively high H.I. values of 12.09 and 12.84, respectively (65), and as a consequence, both enzymes are expressed at relatively high basal levels. It is estimated that there are ~50 molecules of pol II per undamaged cell (193) and these levels increase approximately 7-fold upon DNA damage (24) (Table 1). Similarly, pol IV is expressed at high basal levels, with an estimated intracellular concentration of ~250 molecules per undamaged cell and these levels increase a

further 10-fold in a *lexA*(Def) strain (108) (Table 1). In contrast, pol V is much more tightly regulated by LexA. Indeed, the LexA binding site in the *umuDC* operator has a close match to the consensus binding-site with an H.I. value of just 2.77, meaning that it is one of the last LexA-regulated genes/operons to be derepressed after DNA damage (49, 65). Despite tight regulation by LexA, it is estimated that there are nevertheless ~200 molecules of dimeric UmuD in an undamaged cell. However, levels of UmuC are much lower and are essentially undetectable (258). Undamaged cells harboring a *lexA*(Def) mutation encoding a defective LexA repressor, express ~2400 molecules of $UmuD_2$ and about 200 molecules of UmuC, with the latter being roughly equivalent to basal levels of pol IV (108, 258) (Table 1).

RecA-mediated cleavage of UmuD

Although the Umu proteins are constitutively expressed in *lexA*(Def) strains, the cells do not exhibit high levels of pol V-dependent mutagenesis (58), suggesting that TLS may be subject to additional levels of control, over and above simple transcriptional regulation by LexA. Clues to the mechanism of regulation came with the DNA sequence analysis of the *E. coli umuDC* and pKM101 *mucAB* operons (109, 185). Quite remarkably, both UmuD and MucA exhibited sequence similarity to the C-terminus of the LexA repressor, including conserved active site and cleavage site residues, suggesting that like LexA, UmuD and MucA might also be subject to RecA-mediated cleavage (185). Indeed, RecA-mediated cleavage of UmuD was subsequently shown to occur *in vivo* (211) and *in vitro* (39). However, unlike LexA cleavage, which inactivates repressor functions, UmuD cleavage generates a product, $UmuD'_2$, that is activated for mutagenic TLS (168) (Fig.3). The molecular basis for this activation appears to reside in the respective abilities of $UmuD_2$ and $UmuD'_2$ to interact with UmuC. While it is thought that intact UmuD is capable of an interaction with UmuC, such interactions have yet to be shown definitively (209). In contrast, $UmuD'_2$ interacts with UmuC (260) (to form $UmuD'_2C$ = pol V) that can be isolated and purified as a complex (36, 80, 230).

RecA does not directly act as a protease to cleave LexA and UmuD, rather it appears to act like a co-protease that helps modify the respective structure of the two proteins, such that they undergo a self-cleavage reaction that is mechanistically similar to signal peptidases (39, 136, 175). While cleavage of LexA is thought to occur via an intramolecular reaction (215), cleavage of dimeric UmuD occurs via an intermolecular reaction (150). That is, cleavage of one UmuD protomer in a UmuD dimer, occurs in the active site of the other protomer of the dimer (Fig. 4). While RecA-mediated cleavage of *E. coli* UmuD to UmuD' is quite slow, cleavage of *S. typhimurium* UmuD to UmuD' is very efficient (259) and the rapid cleavage can be attributed to a single amino acid change (Q23P) juxtaposed to the active site of the *S.typhimurium* UmuD protein (151). Interestingly, despite the efficient cleavage of UmuD to UmuD' that is required for mutagenesis, *S.typhimurium* is poorly mutable when compared to *E. coli* (204, 205, 216). Such differences may be attributed to amino acid substitutions in *S. typhimurium* UmuC that lead to an attenuation of UmuC activity (112).

Regulation of pol V-mediated mutagenesis via proteolysis

While both UmuD and UmuD' proteins can form homodimers in solution (39), when mixed *in vitro*, they preferentially form UmuD/UmuD' heterodimers (12). Interestingly, when non-

cleavable variants of UmuD were expressed *in vivo* in excess of cleavable UmuD, there was a dramatic reduction in the amount of damage-inducible mutagenesis (12), suggesting that the UmuD/D' heterodimer may, in some way, attenuate mutagenesis. Possible clues to the mechanisms underlying the attenuation came from studies investigating how the Umu proteins might be regulated via proteolysis (72). Both UmuD and UmuC are rapidly degraded *in vivo* (72) and *in vitro* (86) by the Lon serine protease (Fig. 3). However, heterodimeric UmuD/D' is largely resistant to Lon, but is, instead, degraded by another serine protease, ClpXP (72, 87, 165). Interestingly, only the UmuD' protomer of the UmuD/D' heterodimer is degraded (165). Furthermore, UmuD' is only degraded by ClpXP when associated with UmuD, and is largely resistant to ClpXP-mediated proteolysis in its homodimeric form. Thus, by forming heterodimers, UmuD' is specifically targeted for degradation, thereby dramatically reducing the intracellular concentration of homodimeric UmuD' available in the cell to interact with UmuC and form mutagenic pol V. Such regulation therefore serves two purposes. First, it helps postpone mutagenic pol V-dependent TLS until the damage-inducing signal is sufficiently elevated to promote more rapid RecA-mediated conversion of UmuD to UmuD', and second, it provides a mechanism whereby intracellular levels of pol V are returned to basal levels once the cellular damage has been repaired and the inducing signal mitigated. (72).

Multiple roles for RecA in TLS

It was known for many years that RecA protein plays multiple roles in pol V-dependent TLS. The first two roles have been described above and are related to RecA's ability to mediate cleavage of LexA, so as to derepress the *umu* operon, and to mediate cleavage of UmuD, so as to generate the mutagenically active UmuD' protein. However, Δ*recA* strains of *E. coli* expressing recombinant UmuD'C are rendered non-mutable (168), suggesting that RecA is required for a third, possibly direct role, in TLS. Indeed, a direct role for RecA in TLS was confirmed when Devoret and colleagues isolated the *recA1730* (F117S) missense allele that was functional for all of RecA's known activities, yet was specifically unable to promote pol V-dependent TLS (55). Furthermore, studies by Sweasy and Witkin demonstrated that even in the presence of overproduced UmuD'C, the level of spontaneous mutagenesis observed in *lexA*(Def) cells was dependent upon the particular allele of *recA* in the cell (227). In particular, mutant *recA* alleles encoding proteins that spontaneously form nucleoprotein filaments (often referred to as RecA*) in the absence of DNA damage, showed the highest levels of spontaneous mutagenesis. Clearly, RecA* actively participates in TLS, yet its “third role” in TLS remained enigmatic for close to two decades. Recent biochemical studies have, however, revealed that the third role of RecA* is to physically interact with $UmuD'_2C$ and stimulate the catalytic activity of the polymerase (see below) (104).

Additional levels of regulation imposed on pol IV (DinB)

Like pol V, pol IV is also subject to multiple levels of regulation. In addition to transcriptional control by LexA, the intracellular level of pol IV is modulated by the stress-induced sigma factor, RpoS (Fig. 3), since the expression of pol IV (DinB) is reduced 2-fold in *rpoS* defective cells (125). Furthermore, Pol IV expression also appears to be induced in a LexA- and RpoS-independent manner by the inhibition of cell wall synthesis (184). In

addition, it is likely that pol IV, similar to UmuC (54), is stabilized through an interaction with the *groE* gene products (126) (Fig. 3). Although there is no direct evidence of posttranslational modification of DinB, there is an indication that the activity and/or fidelity of pol IV might be subject to control beyond transcriptional regulation. For example, it has been proposed that the properties of pol IV are modulated by the *ppk* gene, encoding polyphosphate kinase (222), which catalyzes the polymerization of inorganic phosphate into long chains of polyphosphate. Indeed, both pol IV dependent stationary-phase adaptive mutagenesis and growth-dependent pol IV mutagenesis decrease in *ppk*-deficient cells (222). Very recently, DinB has been reported to interact with NusA, which modulates RNA polymerase and it has been hypothesized that such interactions help couple TLS to transcription at sites of DNA damage by targeting pol IV to stalled transcription complexes (46). It has also been shown that uncleaved UmuD protein can physically interact with DinB and in doing so, increases the fidelity of the normally error-prone pol IV (71, 85), possibly by suppressing pol IV-dependent primer-template slippage. Indeed, it may be no mere coincidence that the two proteins (UmuD and DinB) are expressed at similar intracellular levels in both undamaged and damaged cells (Table 1).

Interactions of the TLS polymerases with the β-clamp

All five *E. coli* DNA polymerases bind the cells homodimeric replicative sliding-clamp β and this interaction is required for both processive synthesis *in vitro* and the respective enzyme's cellular functions (16, 25, 38, 51, 130, 140, 188, 241). An interaction of the TLS polymerases with the β-clamp is absolutely required for their biological functions *in vivo*, since mutations in the β-binding motif, or deletion of the motif in either *umuC* or *dinB* leads to a complete loss of TLS (16). Several genetic studies have indicated the existence of a usage hierarchy among *E. coli*'s five DNA polymerases that appears to depend on the relative affinity of the polymerases for the β-clamp in the following order: pol III > pol I > pol II > pol IV > pol V (53, 94, 223, 224). Furthermore, the TLS polymerases and replicative polymerases compete with each other for a common docking site in a hydrophobic cleft on the surface near the C-terminal tail of the β-clamp. Within this region, the main interactions occur at overlapping, although distinct sites of each β-protomer (139). The primary β-clamp interacting site of different DNA polymerases is described by the eubacterial clamp-binding motif with a consensus sequence of QL(S/D)LF (51). In pol II, the motif spans residues 778–783 (QLGLF) at the very carboxyl terminus of the polymerase. Similarly, the β-binding motif in pol IV is also located at the C-terminus of DinB and spans residues 346–350 (QLVLG). In contrast, in UmuC, the motif is located approximately 60 amino acids from the C-terminus and spans residues 357–361 (QLNLF) (51). Non-canonical regions of interaction with the β-clamp have also been described for *E. coli* pol IV. This secondary protein-protein interaction interface involves the Glu93 and Leu98 residues on the rim of the clamp and the C-terminal part of the polymerase that is substantially larger than the main clamp-binding motif (240).

Since the β-clamp is a homodimeric structure, it can likely bind two polymerases at the same time in a manner analogous to a “toolbelt” (176) (Fig. 5). In agreement with this hypothesis, concurrent binding of catalytic subunit of pol III and pol IV to the β-clamp has been demonstrated (98). Furthermore, since at least one TLS polymerase, pol IV, has been shown

to possess two binding interfaces with the β-clamp (38, 240), it is likely that two different polymerases can concurrently interact with the same β-clamp chain (93). Thus, during processive DNA replication pol III is bound to the β-clamp at a hydrophobic cleft, while pol IV is kept in close proximity by interacting with the rim of the same molecule (Fig. 5). Different modes of interaction with the β-clamp have also been identified in UmuC (20), suggesting that the proposed "toolbelt" model for the recruitment of TLS polymerases to the progressing replication fork could be universal. Based upon all the supporting data, the current paradigm assumes that β-clamp plays an essential role in coordinating the switch between replicative and TLS polymerases. In addition, by increasing the processivity and catalytic activity of TLS polymerases (19, 80, 231, 241), the β-clamp facilitates synthesis of "TLS patches" which are long enough to prevent premature switching to the replicative pol III and subsequent proofreading/re-synthesis that results in futile cycling (78).

PHENOTYPES OF E. COLI STRAINS WITH DELETIONS OF POL II, IV OR V, OR OVERPRODUCTION OF POL II, IV OR V

Improved evolutionary fitness

Deletion alleles of all three TLS polymerases have been generated in both *E. coli* and *S.typhimurium*, indicating that they are not absolutely required for cell viability. Indeed, long-term growth and death rates of *E. coli* strains with individual deletions of either *polB*, *dinB* or *umuDC* are virtually identical to that of the TLS-proficient wild-type strains, when each strain is incubated separately in rich medium (263). Interestingly, however, when co-cultured under the same conditions in competition with a wild-type strain, the TLS-defective strains exhibit a reduction in fitness and are rapidly replaced by the wild-type strain. However, none of the strains lacking individual SOS-inducible polymerase displayed any clear advantage or disadvantage versus other mutant strains (263).

Similar to the wild-type cells, *E. coli* strains with individual deletions of *polB, dinB* or *umuDC* express the GASP (Growth Advantage in Stationary Phase) phenotype when they undergo long-term stationary-phase starvation. Indeed in all three mutant strains, aged cells out-competed the co-cultured non-aged parents. However, the GASP phenotype in these strains is disrupted at least to some extent when they compete with the wild type non-aged cells (263). Pol V mutants show the greatest reduction in fitness, suggesting that it plays a more significant role in the acquisition of the GASP phenotype. Thus, while not essential, the TLS polymerases are nevertheless critical for long-term cell survival during stationary phase and overall evolutionary fitness of the bacterium.

Replication Restart

The cellular role of pol II remained enigmatic for many years after its initial discovery in the early '70s. However, by pulse-labeling DNA with ^{3}H thymidine and comparing the rates of post-UV DNA synthesis in various strains, Rangarajan *et al*., concluded that pol II plays a critical role in restarting replication after cells have incurred DNA damage (195, 196). In wild-type cells, there is usually a transient inhibition of DNA replication for ~5–10 mins after DNA damage. In contrast, in *E. coli* strains lacking pol II, the period of inhibition is significantly prolonged to ~50 mins and the resumption of DNA synthesis coincides with the

appearance of high levels of pol V. Based upon these observations, Rangarajan *et al*., hypothesized that pol II plays a major role in the early stages of "Replication Restart", while pol V appears to act in a back-up process, presumably by promoting TLS of the replication-blocking lesions and continued cell survival. Using a similar approach, but slightly different assay conditions, Courcelle *et al*., observed no effect of pol II or pol IV on the kinetics of replication restart, but instead, found that replication restart was dependent upon nucleotide excision repair (*uvrA*) and pol V-dependent TLS (48). In addition, in an independent study Rudolph *et al*., found that post-UV replication does not change in the absence of pol II, thereby questioning pol II's role, if any, in replication restart (201).

Effects of overproducing TLS polymerases on potential damage-induced replication checkpoints and recombination

As noted earlier, the intracellular concentrations of the TLS polymerases are quite low and at a maximum, increase 7- to 10-fold upon DNA damage (Table 1). In addition, the TLS polymerase activity is regulated by a plethora of protein-protein interactions (e.g. pol II with the β– clamp; pol IV with β– clamp, UmuD and chaperones; and pol V with $UmuD_2$, UmuD/D', $UmuD'_2$, β–clamp, RecA and various proteases). It is not surprising, therefore, that there are often pleotropic effects when the TLS polymerases are significantly overproduced compared to their normally low intracellular levels. Given the fact the levels of the TLS polymerases are clearly strictly regulated, even a small increase in intracellular concentration may lead to phenotypic differences. Indeed, overproduction of pol II and pol IV have been shown to slow pol III-dependent fork progression (81, 97, 236) and overproduction of pol V leads to a cell-cycle checkpoint (161, 174) (See Eco-Sal III: Module 5.4.3. "The SOS Regulatory Network"), as well as inhibiting RecA-dependent recombination (27, 217). Such phenotypes can be attributed to the overproduced polymerases sequestering essential components normally needed for replication and recombination (such as the β–clamp and RecA). However, the physiological relevance of these phenotypes remains in question. In the case of pol V-dependent inhibition of recombination, inhibition was only observed when RecA levels were kept to a minimum, while pol V was artificially elevated. At high cellular concentrations of RecA, no inhibition of recombination was observed (27). In a normal SOS response, just a few hundred molecules of pol V accumulate some 45 mins after DNA damage, when there is an estimated 70,000 molecules of RecA per cell (218). As a consequence, pol V is unlikely to have any significant effect on RecA-dependent recombination under normal SOS-inducing conditions. (See Eco-Sal III: Module 7.2.8. "The SOS Response", for more discussion on interpreting the phenotypes observed when TLS polymerases are overproduced).

Contributions of TLS polymerases to mutagenesis *in vivo*

Spontaneous mutagenesis in actively dividing cells—Neither pol IV, nor pol V, possess intrinsic 3'–5' proofreading activity and are therefore considered to be low-fidelity DNA polymerases when they duplicate undamaged DNA. Indeed, in SOS-induced cells overexpressing DinB, there is a dramatic increase in untargeted mutations in bacteriophage λ (34), as well as –1 frameshift mutations in exponentially growing cells (1, 107, 119, 244). As noted above, pol IV is expressed at moderately high basal levels even in an undamaged cell, and could, potentially, contribute to significant number of spontaneous mutations on the

E. coli chromosome. However, it appears that basal levels of pol IV are unable to compete with the cell's replicase, pol III so as to gain access to a primer terminus, since the rates and spectra of spontaneous mutations arising in actively dividing cells does not change in *dinB*$^+$ or Δ*dinB* cells (119, 153, 154, 229, 254). Nevertheless, these data do not exclude the possibility that pol IV is involved in processing of rare spontaneous DNA lesions. Indeed, while the mutation frequency remained unchanged in Δ*dinB* strains defective for repair of abasic sites, bulky DNA adducts, and oxidative DNA damage, inactivation of the glycosylases responsible for excision of 3-methyladenine (3-meA) and 3-methylguanine (3-meG) in cells lacking *dinB* resulted in a significant increase in spontaneous mutagenesis. Thus, is appears that not only does pol IV replicate DNA with spontaneous alkylation base damage, but actually does it in error-free manner (22). The role of pol IV in the bypass of spontaneously arising alkylation damage could not be detected in cells with functional alkylation repair because the number of alkyl lesions in the genome is probably too low to influence the overall rate of spontaneous mutagenesis. However, the study by Bjedov *et al*., clearly implies that even at basal steady-state levels, pol IV can successfully compete with pol III for access to the replication fork (22).

The role of pol V in promoting spontaneous mutagenesis is more obvious. While pol V is tightly regulated so as to keep its activity on undamaged DNA to a minimum, the enzyme can nevertheless compete with pol III and gain access to genomic DNA, as the number of spontaneously arising *his*$^+$ revertants drops 5 to10-fold in a Δ*umuDC* strain compared to the isogenic *umuDC*$^+$ parent (256). Pol V-dependent spontaneous mutagenesis increases dramatically in strains with missense mutations in *recA* that lead to constitutive co-protease functions, such a *recA730* (66, 227) and is further enhanced in strains with an impaired replicase (66, 238). For many years, it was thought that the so-called "SOS mutator" activity was entirely dependent upon the *umuC* gene product (43). However, it now appears that roughly 40–50% of the spontaneously arising mutations are also dependent upon pol IV (50, 120). The simplest interpretation of this observation, is that the mutations arise through a two-step process (33, 257), (Fig. 1B) in which pol V makes the first error-prone misinsertion and this is subsequently extended either by pol V itself, or by pol IV.

Of the three bacterial TLS polymerases, only pol II has intrinsic 3'–5' proofreading (Fig. 2) and therefore exhibits much higher fidelity than pol IV or pol V. Indeed, rather than promoting spontaneous mutagenesis, it is believed that pol II helps maintain *E. coli*'s genomic integrity by removing replication errors generated by pols III, IV and V (9, 50, 83).

Mutagenesis in stationary phase cells—During conditions of starvation, *E. coli* cells employ the survival strategy of "stress-induced" adaptive mutagenesis, which requires expression of SOS-induced DNA polymerases, even in the absence of exogenous DNA damage (68, 82). The mutagenesis is clearly dependent upon error-prone pol IV, since it is abolished in cells with a deletion of *dinB* (153, 235) and is also reduced in *rpoS* cells where the cellular level of pol IV is diminished (125, 138, 191, 221). Historically, stress induced mutagenesis has been followed through the reversion of a +1 frameshift allele *lacZ*. However, stress induced mutagenesis that is independent of *lac* reversion can also be observed in *ampD* leading to increased antibiotic resistance (187), as well as reversion of a *tet* gene (37, 47, 67, 191). Stress induced mutagenesis appears to be initiated when double

strand breaks are repaired (70, 90, 91). Although pol IV plays a major role in stress-induced mutagenesis, other polymerase can also participate in the process. Indeed, it has recently been shown that pol II is responsible for roughly 15% of frameshift reversions that are pol IV-independent (75). In addition, pols I, III and V may reduce pol IV-dependent stress-induced mutagenesis by simply competing with pol IV at a nascent primer terminus generated during double strand break repair (69, 92, 191).

Damage-induced mutagenesis

Since the TLS polymerases are not essential for viability, it has been possible to generate strains of *E. coli* and *S.typhimurium*, which carry deletions of each polymerase, either individually, or in combination. Conversely, cloning of the genes encoding the TLS polymerases into low-copy vectors allows for over-expression of respective polymerases. By using such strains, one can analyze the ability of each respective polymerase to promote error-free, or error-prone TLS events after exposing cells to different kinds of environmental stress (117), or to different DNA damaging agents. Using such an approach, Takehiko Nohmi and colleagues reported the effect of chemically induced frameshift mutagenesis in *S. typhimurium* strains either lacking (113), or overexpressing (146) one or more TLS polymerase. These studies revealed that for many lesions a combination of polymerases is required in order to successfully complete TLS and many lesions could be bypassed by more than one TLS polymerase. For example, it was shown that pol IV and pol V have distinct, but partly overlapping substrate specificities, yet they can cooperate with pol II in the two-polymerase mechanism of TLS, and when overexpressed, both polymerases are able to assume TLS functions normally performed by pol III holoenzyme. Interestingly, overexpression of *E. coli* pol I reduced Y-family polymerase-dependent chemically induced mutagenesis to the levels observed in the strains lacking all TLS polymerases possibly by suppressing the access of these polymerases to the replication fork (146). Given the fact that each chemical compound is likely to generate a range of DNA lesions *in vivo*, it is hardly surprising that more than one polymerase is involved in a cellular response to a single DNA damaging agent.

While examination of TLS in randomly damaged cells provides valuable knowledge about polymerase selectivity, more precise information regarding the exact role for each polymerase in TLS has been gained through the use of vectors containing a single defined lesion. The first such studies of this type were undertaken by Chris Lawrence and colleagues, who assayed TLS of a single *cis-syn* cyclobutane pyrimidine dimer (CPD) *in vivo* (11). These were quickly followed by studies on other DNA lesions in the single-stranded substrate, including an abasic site (apurinic/apyrimidinic site generated spontaneously, or as a byproduct of chemical or radiation DNA damage) (123) and a UV-induced *trans-syn* CPD (10) and T-T and T-C 6-4 photoproducts (6-4PP) (95, 127). In each instance, the defined lesion gave rise to a unique mutagenic signature. In all cases, mutagenesis increased in SOS-induced cells and was largely dependent upon pol V since bypass was greatly reduced in a Δ*umuDC* strain (124, 228). In agreement with the *in vivo* data, *in vitro* reconstitution of TLS has shown that in the presence of accessory proteins, pol V is the most efficient among SOS-inducible polymerases in the TLS of CPDs, 6-4PP, and abasic site (24, 144, 232). Furthermore, the nucleotide incorporation specificity of pol V

opposite these lesions is in excellent agreement with mutations observed *in vivo*. For example, pol III and pol IV favor the non-mutagenic incorporation of dA opposite a 3'T of the 6-4PP *in vitro*, while pol V catalyzes the preferential incorporation of dG, which would result in the T to C transitions often observed *in vivo* (127, 188, 189). Compared with the 6-4PP, the CPD is a much less mutagenic lesion when replicated by pol V. Interestingly, when *E. coli* pol V was substituted by R-plasmid encoded orthologs (see below) MucAB (pol RI), or RumAB (pol $V_{(R391)}$), the efficiency of T-T CPDs bypass and mutagenic spectrum changed considerably, even though the overall mutagenic frequency remained low (124, 170).

In subsequent years, a variety of DNA lesions have been introduced into single-stranded vectors. This includes (but is not limited to) N^2-acetylaminofluorene (AAF) and N^2-aminofluorene (AF) modified guanines (80, 162, 177, 226), benzo[*a*]pyrene diol epoxide (BaP) guanine and adenine adducts (129, 162, 208), 8-oxoguanine (8-oxoG) and other guanine oxidation products (141, 163, 164), oxidized abasic lesions (118), N^2-dG and other acrolein-induced and related adducts (102, 103, 157, 265), butadiene-induced guanine intrastrand and N^2-N^2-guanine interstrand crosslinks (41, 121), and alkylated bases (52). By using the single-lesion containing vectors in *in vitro* reconstituted reactions and by introducing them into strains lacking one or more TLS polymerase, it is straightforward to determine the contribution of each polymerase to the efficiency and accuracy of *in vivo* lesion bypass. Although pol V is responsible for the bulk of mutagenic TLS, it is not surprising that some lesions require the presence of other SOS-inducible TLS polymerases. For example, only pol IV is capable of the efficient nucleotide incorporation opposite N^2-guanine linked DNA–peptide crosslinks and this incorporation proceeds with high fidelity (Fig. 6) (157). Consistent with *in vitro* data, pol IV-deficient cells lack the ability to replicate DNA vectors with N^2-dG–peptide crosslinks. Similar results were obtained with modified DNA containing other bulky N^2-dG adducts and N^2-N^2-guanine interstrand crosslinks (103, 121, 265). Interestingly, pol IV is particularly efficient at extension after dC incorporation opposite the damaged guanine catalyzing the first nucleotide addition after the lesion with up to 25-fold greater catalytic efficiency than after undamaged guanine (102). The exceptional ability of pol IV to accurately copy DNA containing N^2-dG damaged bases suggests that this type of lesions is the cognate substrate for pol IV. Pol IV is also involved in the TLS of BaP-induced N^2-dG adducts, however in this case pol V and, to a lesser extent, pol II are also likely to play additional roles in TLS (129, 162, 208) (Fig. 7). Moreover, even the cell's replicase, pol III, is able to carry out limited synthesis past the dG-BaP in a particular specific sequence context (208).

Interestingly, despite pol IV's preference for N^2-dG adducts, *in vivo* replication past N^2-dG-AAF does not require pol IV. Instead, pol II, and pol V appear to compete for the bypass of the lesion (Fig. 8) (15, 162). When pol V catalyzes bypass of the AAF adduct, it proceeds in an error-free manner. In contrast, pol II-dependent TLS is error-prone and leads to −2bp frameshift events *in vivo*. It appears that the pathway is initiated by the insertion of the correct nucleotide opposite the AAF-dG lesion by either pol III or pol V. This is often followed by primer-template slippage that generates a −2 frameshift intermediate that is subsequently extended and fixed as a mutation by pol II (Fig. 8). In contrast, when pol II

facilitates TLS of 5-guanidino-4-nitroimidazole (NI), lesion bypass is error-free, while pol V-catalyzed misinsertion at the lesion site causes G to T transversions (164). However, both pol V-dependent error-prone and pol II-dependent error-free TLS pathways require pol V for extension of the nascent strand after nucleotide incorporation opposite the NI lesion. Evidence for the concerted action of SOS-inducible polymerases is also found when assessing TLS of an oxidized abasic site. When the lesion is replicated in *E. coli*, pol V incorporates dA opposite this lesion producing full-length replication products, while pol II and pol IV utilize a dNTP-stabilized misalignment mechanism to create single-nucleotide deletion products (118).

Taken together, studies of TLS *in vivo* and *in vitro* indicate that participation of each polymerase in lesion bypass not only depends on the chemical nature of the lesion, but its local sequence context. In each particular case, cells can recruit a single, or a combination of TLS polymerases to try and accomplish TLS with maximal accuracy and efficiency. The driving goal is to ensure survival of the cell in the face of otherwise certain death and with a minimal amount of mutagenesis. Unfortunately, it's not a perfect world and a certain amount of mutagenesis is an unavoidable consequence of TLS. Most of these mutations are likely to be deleterious, with just a small fraction ultimately improving the cellular fitness of the bacterium (6).

BIOCHEMICAL PROPERTIES OF E. COLI TLS POLYMERASES

In vitro fidelity of TLS polymerases

Pol II consists of a single 89.9-kDa polypeptide that possesses both polymerase and 3'→5' exonuclease activities (Fig. 2) (23, 40). As a consequence, pol II-dependent replication on undamaged DNA is reasonably accurate with misincorporations occurring *in vitro* in the range of ~1×10^{-6} (40). On damaged templates, pol II is also quite accurate, with the notable exception of AAF adducts where it promotes −2 frameshifts. Pol IV is also encoded by a single ~40 kDa polypeptide, but the protein lacks intrinsic 3'→ 5' exonuclease activity (Fig. 2) and is a low-fidelity enzyme with a misincorporation frequency in the range of 10^{-3} to 10^{-5} depending on the nature of the mispair formed (231). When replicating its cognate substrates, such as the N^2-dG adducts, pol IV replicates the lesion containing DNA with the same, or perhaps even higher accuracy than the undamaged template (103). Pol V is an ~72 kDa complex comprised of dimeric UmuD' (~24 kDa) and UmuC (~48 kDa) proteins (Fig. 2). Like pol IV, the pol V complex lacks proofreading activity and is error-prone with misincorporations occurring in the range of 10^{-3}-10^{-4} (231). However, when replicating various damaged substrates, pol V is generally less accurate, which leads to a clear mutagenic signature *in vivo*.

In addition to low-fidelity incorporation of deoxynucleoside triphosphates during TLS, both pol IV and pol V have been shown to readily incorporate oxidized deoxynucleoside triphosphates opposite undamaged template bases (262). Such properties may contribute to a significant amount of cellular mutagenesis when bacteria are exposed to environmental oxidative stress (192)

Last, but not least, both pol IV and pol V exhibit apurinic/apyrimidinic lyase activity (210), although relevance for this function *in vivo* has yet to be established.

Cofactor requirements that modify the fidelity and processivity of TLS polymerases *in vitro*

All three TLS polymerases physically interact with the cell's replicative sliding β-clamp (51) and this helps stimulate the processivity and catalytic activity of each enzyme *in vitro* (19, 80, 231, 241). In the case of pol II, processivity increases from just five nucleotides in the absence of the β-clamp to greater than 1600 nucleotides per template binding event in its presence (25). The processivity of purified pol IV *in vitro* is very low, as it can only incorporate just a few nucleotides before dissociating (243). However, with the assistance of the β-clamp, the processivity of pol IV increases by 2- to 4-orders of magnitude enabling it to synthesize over 1 kb DNA (241). Recently, it has been suggested that UmuD physically interacts with Pol IV (85) and in doing so, helps cap the polymerase's active site, so as to increase pol IVs fidelity (71, 85).

Pol V ($UmuD'_2C$) is an extremely weak polymerase (200, 232). However, the catalytic activity of pol V can be greatly stimulated upon the addition of co-factors. Interestingly, pol V's cofactor requirements *in vitro* are quite varied and appear to depend upon the template being replicated (80). On long single-stranded templates, the greatest stimulation of pol V's catalytic efficiency and processivity is observed in the presence of the β-clamp, single stranded binding (SSB) protein and RecA (80). On shorter, hairpin templates, SSB is dispensable, but a requirement for RecA remains. Even though the β-clamp is not necessary for replication of short DNA templates by RecA-activated pol V, the processivity of the polymerase increases significantly when it is present in the reaction mixture (202).

Velocity of DNA synthesis

Compared with the cell's replicase, pol III, which catalyzes elongation of DNA chains at a rate of about 1 kb per second, the velocity of nucleotide incorporation by the TLS polymerases is incredibly slow. In the presence of accessory proteins the rate of pol II-dependent DNA synthesis has been estimated to be ~20–30 nucleotides per second (25). By comparison, the velocity of pol IV-dependent synthesis is between 2–8 nucleotides per second (98, 130, 231, 242). Pol V is by far the slowest TLS polymerase, with a rate of DNA synthesis estimated to be less than 1 nucleotide per second (77) (Vaisman and Woodgate, unpublished data).

Nature of RecA filaments

Stimulation of Pol V's activity *in vitro* by RecA fits nicely with the genetic requirement for RecA in pol V-dependent TLS *in vivo* (55, 168, 227). For many years, it was tacitly assumed that RecA stimulated pol V in the form of a nucleoprotein filament that formed *in cis* (Fig. 9A). That is, the RecA filament forms on the single-stranded DNA immediately 3' of the blocked replication complex. However, by using a short hairpin substrate with a 3' nucleotide overhang that is incapable of forming a RecA filament, Goodman and colleagues demonstrated that RecA activation of pol V can also occur *in trans*; i.e., on another DNA template with no relation to the blocked lesion-containing template (Fig.9B) (202). The fact

that RecA can activate pol V *in trans* does not mutually exclude the possibility that RecA filaments formed *in cis* do activate pol V *in vitro*, but as yet, it has proven impossible to test this hypothesis directly, since in any *in vitro* reaction involving an oligonucleotide primer and a separate template, there will always be some *trans* activating DNA in the reaction (either from unannealed primer, if the primer is in excess, or template, if it's in excess). Furthermore, *cis*-activation is also likely to be problematic for TLS, since the *cis*-activating RecA nucleoprotein filament would need to be displaced for DNA synthesis to occur. It is even possible that a *cis*-acting RecA filament could even inhibit TLS, as it was shown to interfere with pol III holoenzyme-catalyzed DNA replication (213).

Cis- or *trans*-activation aside; what is the molecular basis of RecA's ability to stimulate pol V *in vitro*? Recent studies by Goodman and colleagues have shown that the sole purpose of the RecA nucleoprotein filament is to transfer a single RecA protomer and ATP molecule from the 3' tip of the filament to pol V to generate a stable and highly active complex consisting of UmuD'$_2$C•RecA•ATP that they called pol V Mut (Fig. 9C) (104). The term "Mut" is short for "Mutasome", a multi-protein complex that the late Hatch Echols hypothesized some 20 years ago would facilitate TLS (56).

STRUCTURAL INSIGHTS INTO TLS

Crystal structure of pol II reveals its capacity to promote frameshift TLS events

The apo structure of pol II was deposited in the Protein Data Base (PDB) some 16 years ago (PDB Accession number: 1Q8I) (5). However, the structure of pol II in a ternary complex with DNA and incoming dNTP has only recently been solved (245). Indeed, Wang and Yang solved eight different crystal structures of pol II, including apo, binary, and ternary complexes with normal and abasic-lesion containing DNAs (Fig. 10A) (PDB accession numbers: 3K57, 3K58, 3K59, 3K5A, 3K5L, 3K5M, 3K5N, and 3K5O). Given that pol II is a B-family polymerase, it was not too surprising that the ternary structure of pol II with undamaged DNA and incoming nucleotide was virtually superimposable with structures of the related B-family ϕ29 replicative polymerase and RB69 polymerase, gp43 (18, 73). Unfortunately, these structures did not provide any clear insights into how pol II might facilitate TLS. Like all known polymerases, pol II has palm, finger and thumb domains (220), as well as an additional N-domain and Exo domain (Fig. 2). However, the crystal structure of pol II in a ternary complex with abasic lesion-containing DNA revealed that there were two major differences between TLS pol II and replicative gp43 polymerase. First, significant changes were observed in the DNA duplex upstream of the abasic site and in the thumb domain of pol II. These resulted in small cavities 1 and 2 bp upstream from the pol II active site that accommodated the looping out of not only the abasic site, but also the two adjacent downstream template bases. The second major difference between pol II and gp43, is that pol II has a 20 amino acid insertion in its N-domain that alters the position of the Exo-domain relative to the polymerase active site in the palm domain, so as to reduce exonucleolytic proofreading activity relative to polymerization. Thus, it appears that a combination of the small pockets in pol II that accommodate multiple template strand "acrobatics" and a weakened proofreading capacity allows pol II to facilitate TLS (245).

Archaeal Dpo4 as a model for *E. coli* pol IV

Although pol IV can be purified in high yield and great purity, the structure of the full-length polymerase has yet to be determined. However, archaeal orthologs Dpo4 from *Sulfolobus solfataricus* (Fig. 10B) (PDB accession number: 1JXL)(133) and Dbh from *Sulfolobus acidocladarius* (PDB accession numbers: 3BQ1) (248) have been solved in a ternary complex with undamaged DNA and incoming dNTPs and have served as a model for *E. coli* pol IV. Despite virtually no obvious amino acid homology to DNA polymerases from other families, the crystal structures of Dpo4 and Dbh reveal a topology resembling a right hand similar to that of high fidelity polymerases with palm, thumb and finger domains (133). In addition, these proteins have another domain called the “little finger” (LF) that wraps around DNA and helps secure the polymerase to the primer-template (28, 29, 133). When compared to high fidelity polymerases, the domains are rather “stubby” and instead of having a constricted active site, the active site of Dpo4 is spacious and solvent exposed, helping to explain how the enzyme can accommodate a variety of DNA lesions in its active site (Fig. 10B). In the 10 years since the first ternary structure of Dpo4 was reported some 56 separate lesion-containing structures of Dpo4 have now been solved and deposited in the PDB (Table 2).

Dpo4 (pol IV) interaction with the replicative sliding clamp

As noted above, a prerequisite for TLS is the ability of the respective TLS polymerase to interact with the β-clamp. The first structural insights into how this interaction might promote TLS came when Bunting and colleague solved the crystal structure of the *E. coli* pol IV LF-domain bound to the β-clamp (PDB: 1UNN) (38). Interestingly, by superimposing the full-length structure of Dpo4 onto the *E. coli* DinB LF- β-clamp structure, the model revealed that when the interaction occurs through the rim of the clamp, the active site of Dpo4 would not be engaged at the primer-terminus of the nascent DNA chain, but is rather “hanging off to the side” in what would be a catalytically inactive configuration (Fig. 11A). In contrast, when pol IV is flexibly attached to the clamp via the C-terminal peptide, it can simultaneously contact the DNA template, thus assuming an “active” orientation (Fig. 11B). The structure therefore provided the first evidence of a potential “toolbelt” model for TLS polymerases in which multiple enzymes could be bound to the replicative clamp simultaneously and engaged at the primer terminus only when, and if, needed (98, 176). Very recently, the crystallographic studies of Dpo4 in a complex with heterodimeric PCNA1–PCNA2 (PDB: 3FDS)(261) has shown that a flexible structure allows the polymerase to adopt one of the several different conformational states in order to optimize its contact with PCNA and/or DNA (Figs. 11C and D). In addition to the expected specific binding of Dpo4 to PCNA1 via its PCNA-interacting peptide (PIP)-box, the structure revealed additional protein-protein interactions with the clamp via the finger, thumb and little finger domains (261). Both structural studies of *E. coli* DinB LF with β-clamp and Dpo4 with PCNA1–PCNA2 corroborate the “toolbelt” model for polymerase switching (Fig. 5).

Structure of *E. coli* UmuD' and modeling of *E. coli* UmuD

The structure of pol V ($UmuD'_2C$) or pol V Mut ($UmuD'_2C$•RecA•ATP) has yet to be solved, at least in part because it has been historically difficult to obtain large quantities of the enzyme for crystallographic studies. However, recombinant UmuD' is readily overexpressed in *E. coli* and both the crystal structure (182), and the NMR-solution structure (64) of $UmuD'_2$ have been solved. Two dimer interfaces were observed in the crystal structure. One termed the “Molecular” dimer in which the N-terminal tail of each protomer is in opposite directions and the Ser-Lys dyad of the peptidase active site is occluded (PDB: 1AY9) (Fig. 12A). The second dimer observed in the crystal was termed the “Filament”, and in this interface, the N-terminal tails of the two protomers cross and are likely to be in close proximity to the active site Ser60-Lys97 residues of the adjacent protomer (PDB: 1UMU) (Fig. 12B). The latter structure is consistent with the observation that UmuD cleavage occurs via an intermolecular reaction (150) (Fig. 4).

While two dimeric interfaces were observed in the crystal structure, only one, corresponding to the so-called “Filament” dimer, was observed in the NMR solution structure (PDB: accession code 1I4V) (64), and it is clearly the filament dimer that is active for mutagenesis in *E. coli* (155, 172). In the crystal structure, the catalytically active Ser-Lys residues are close to each other and are poised for catalysis, whereas in the NMR-solution structure they are 7Å apart (Fig. 12C). Presumably, the functional role of RecA in mediating UmuD cleavage is to induce a conformational change in UmuD such that the Ser-Lys active site residues are juxtaposed, so as to induce its self-cleavage.

Although UmuD only differs from UmuD' by the presence of a 24 amino acid N-terminal tail, the structure of UmuD has yet to be determined. Molecular models of UmuD have, however, been generated. Early models were based upon the ease with which mono-cysteine mutants of UmuD could be chemically cross-linked (89, 128) and subsequently mapped on to the NMR structure of UmuD' (64). The model was subsequently refined based upon additional data including solvent accessibility and electron paramagnetic spin resonance (EPR) studies (225). Based upon these studies, Sutton *et al*. proposed that the N-terminal tails of UmuD (residues 1–39) form an extended interface in the $UmuD_2$ homodimer by folding down over the globular domains of their respective intra-dimer partners (225). While this clearly has to be the case to ensure the appropriate orientation of the cleavage site of one protomer in the active site of the adjacent protomer, recent UmuD models that are based upon the crystal structures of UmuD' and LexA (21) and hydrogen–deuterium exchange mass spectrometry (HXMS), reveal that the N-terminal tail of UmuD is actually very dynamic and highly flexible with the N-terminus solvent exposed in solution (63). One can easily imagine that it is the interaction with RecA that helps to trap these flexible tails in a “bear-hug” manner, so as to facilitate autodigestion of UmuD to UmuD'.

SIMILARITIES AND DIFFERENCES BETWEEN *E. COLI* AND *S.TYPHIMURIUM* TLS POLYMERASES

Genomic locations of TLS polymerases in *E. coli* and *S.typhimurium*

Overall, the *E. coli* and *S.typhimurium* genomes are nearly 90% identical at the nucleotide level. In particular, the pol II and DinB proteins from *E. coli* and *S.typhimurium* are 89% and 91% identical, respectively. Pol II is located at 1.4 mins of the *E. coli* genome and is flanked by the *hepA* and *araD* genes (xBase). *S.typhimurium polB* is located at a roughly similar position of the genome and is flanked by *hepA* and several intervening putative genes and then followed by the *araD* gene (Fig. 13).

E. coli dinB gene is located at 5.4 mins on the chromosome. *yafM* gene and two pseudogenes are located upstream of *dinB*, while *yafN*, *yafO* and *yafP* are located immediately downstream of *dinB* with all four genes expressed as a single operon (154). *S.typhimurium dinB* is located at 7.2 mins and lacks the downstream *yafN*, *yafO* and *yafP* genes but is, instead, flanked by the *yafK* and *prfH* genes. The lack of a downstream *yafP* gene is intriguing as the operon arrangement is apparently conserved in a number of naturally occurring *E.coli* and YafP seems to work with DinB to protect cells from cytotoxic effects of exposure to nitroaromatic compounds (88). Despite the lack of an obvious *dinB-yafN-yafO-yafP* operon in *S. typhimurium*, its *dinB* gene is nevertheless located in a similar genomic region to *E.coli dinB*, since four genes upstream of the *E. coli yafM* gene is the *yafK* gene and 1.4 kb downstream of the *yafN* gene is a *prfH*-like pseudogene (Fig. 13). Thus, both the *polB* genes and the *dinB* genes from both *E. coli* and *S.typhimurium* are positioned within analogous genomic locations and architectures.

Interestingly, the UmuD and UmuC proteins from these two organisms are considerably more diverged than the rest of the genome with only 73% and 83% identity, respectively. Furthermore, their chromosomal locations are substantially different. Whereas the *E. coli umu* operon is located at 26.5 mins on the chromosome and flanked by *hlyE* and *dsbB*, the *S.typhimurium umuDC* operon is located at 42.9 mins on the map and is flanked by STM1999 and *cspB* and is within the large 588 kbp inversion (148), that is a major distinguishing factor between the *E. coli* and *S.typhimurium* genomes (Fig. 13). Closer analysis of the two loci reveals that the region of the *S.typhimurium* chromosome, which is analogous to the *E. coli* 26.5 minute interval is located at 41.3 minutes of the *S.typhimurium* genome and is identical to the *E. coli* interval, except that it lacks the *umuDC* operon (Fig. 13). Similarly, the region of the 42.9 minute interval of the *S. typhimurium* chromosome encompassing the *umuDC* operon corresponds to *E. coli* 44 minutes and has the same gene arrangement, except that it lacks the *umuDC* operon (Fig. 13). These observations, together with fact that the *umuDC* operon is more diverged than the rest of the *E. coli* and *S.typhimurium* genomes, suggest that the *umuDC* operon was possibly acquired by independent horizontal gene transfer from a plasmid-encoded transposable element instead of evolving from a single common ancestor.

An additional UmuD' ortholog in the *S.typhimurium* LT2 genome

BLAST analysis (2) of the *S.typhimurium* genome reveals another protein with similarity to *E. coli* UmuD. The protein annotated as STM2230 in GENBANK NC_003197.1 is 156 amino acids long and its C-terminal 128 residues are 40 and 45% identical to *E. coli* and *S.typhimurium* UmuD', respectively. Further analysis of the nucleotide sequence of SMT2230 suggests that the protein may, however, be incorrectly annotated and we suggest that the gene may actually encode a 129 amino acid protein since a consensus ribosome binding site can be identified upstream of the Methionine located at position 28 of SMT2230. This shorter protein shares highest homology with the HumD protein found on bacteriophages P1, P7, N15 and φKO2 (Fig. 14) and SMT2230 therefore appears to originate from one of the many cryptic prophages that inhabit *S.typhimurium* (149). Bacteriophage P1 HumD protein has previously been shown to function with *E. coli* UmuC (expressed from a low-copy number plasmid), so as to restore damage-induced mutagenesis to Δ*umuDC* strains of *E. coli* (156) indicating that the P1 HumD protein is a functional ortholog of UmuD'. Why *S.typhimurium* would retain another UmuD' ortholog in its genome remains a mystery, especially since *S.typhimurium* UmuD is cleaved to UmuD' very efficiently (259).

Many strains of *S.typhimurium* carry R-plasmids expressing pol V orthologs

Interestingly, in addition to possessing a genomic copy of HumD, many natural isolates of *S.typhimurium* harbor R-plasmids carrying orthologs of pol V. Indeed, *S.typhimurium* LT2 harbors the 60 MDa virulence plasmid (pSLT) (35) encoding the pol V ortholog SamAB (169). Similarly, *S.typhimurium* LT7 harbors the Col11a plasmid encoding pol V ortholog ImpAB (111). Quite remarkably, despite carrying multiple orthologs of low-fidelity pol V, *S. typhimurium* is poorly mutable compared to *E. coli* (205, 216, 233). It was partly for this reason that Ames and colleagues introduced yet another R-plasmid (pKM101), encoding pol V orthologs *mucAB* into *S. typhimurium* tester strains that they developed to identify potential mutagens and carcinogens (3, 4, 147).

It is now known that pol V orthologs are found on many broad-host range plasmids, including those from the “pre-antibiotic” era (206) that are capable of existing in either *E. coli* or *S.typhimurium* (Table 3). Interestingly, the genes encoding pol V orthologs are often located very close to the origin of transfer (oriT) of the R-plasmid (181) or ICE (42), meaning that they are among the first genes transferred to the host bacterium during conjugation. In this process, “rolling-circle” replication introduces a single-strand DNA into the recipient cell. The single-stranded nature of the transferred DNA prevents nucleotide excision repair of any lesion in the plasmid DNA, while the absence of an intact homolog blocks any subsequent recombinational repair of the daughter-strand gaps left after synthesis by the host bacterial replicase. While the host TLS polymerases may be able to provide assistance, it may be insufficient for the plasmid to survive. However, the damaged plasmid has a much higher chance of survival, if it quickly expresses its own pol V in the host bacterium. Plasmid survival is clearly the evolutionary driving force for the retention of pol V orthologs on the mosaic R-plasmids. This is most evident from Chris Lawrence’s TLS studies with single-stranded M13 phage (228). In the absence of pol V, survival of an M13 genome with a single *cis-syn* cyclobutane dimer is ~0.5%. When the same damaged DNA is

introduced into a host expressing recombinant pol V, survival dramatically increased to ~70%, with only 2–5% of the survivors acquiring mutations (228). To phrase this differently, 95–98% of the survivors (that would have died in the absence of pol V) did so without any mutational consequences. Of course, once the plasmid is established in its new host, the low-fidelity plasmid encoded pol V ortholog may nevertheless contribute to antibiotic resistance and pathogenicity, particularly when the host cells are stressed (14, 44).

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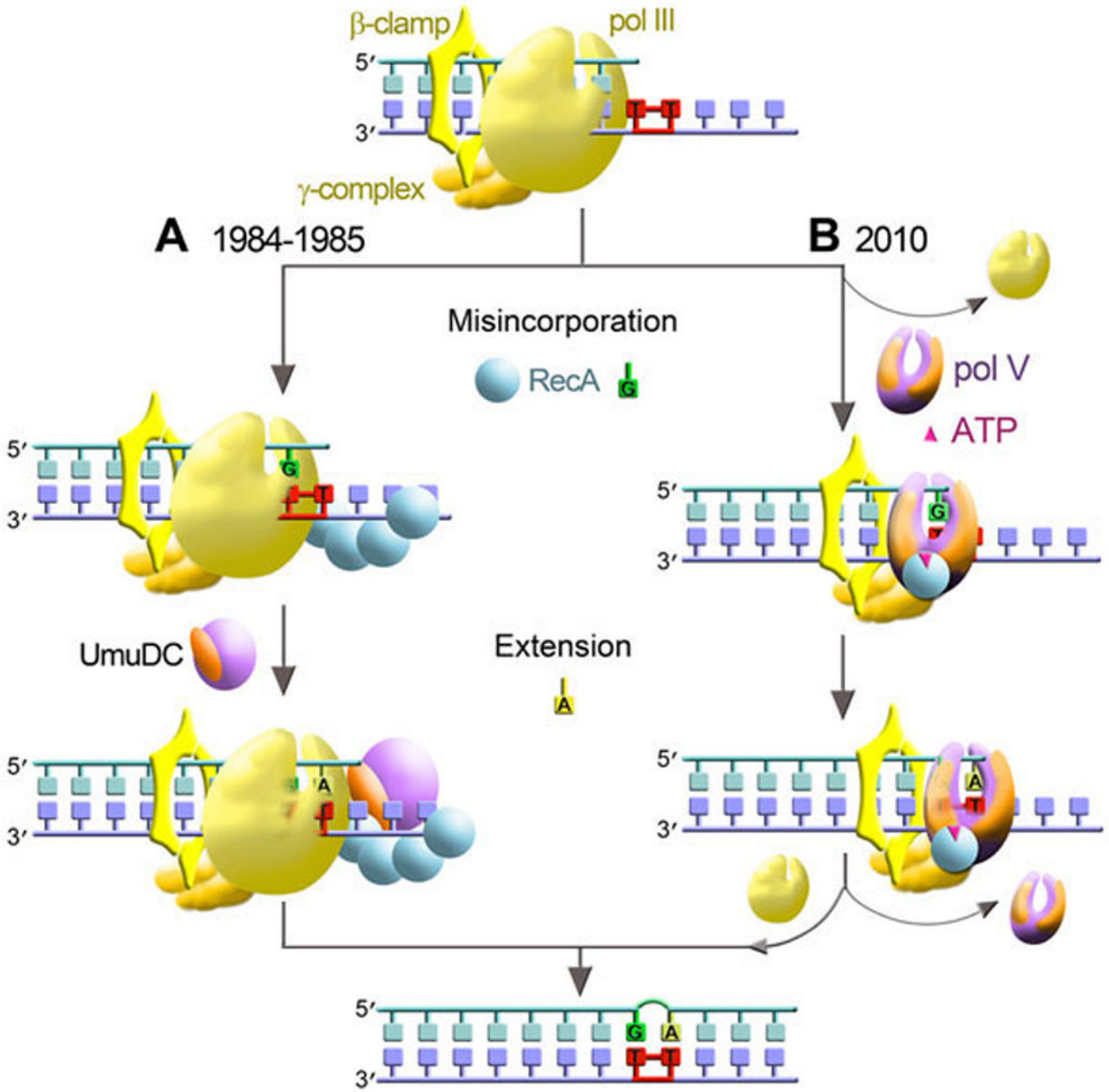

Figure 1. Two-step model For UV-mutagenesis; then and now

A. The original “two-step” model for UV-mutagenesis reported in 1984/1985 assumed that TLS *in vivo* is performed by the replicative pol III holoenzyme [consisting of catalytic core (α, θ, ε, shown here and in figures 5–7 as a yellow shape resembling a “right hand”), β-clamp (shown in figures 1, 3, 5 and 9 as yellow hexagonal shape) and γ-clamp loading complex (shown as light orange five-subunit shape)]. The first step, nucleotide misincorporation opposite a 3' T of a CPD, was hypothesized to be mediated by the RecA protein (represented as blue sphere in figures 1, 2, and 9). The misincorporated base was subsequently fixed as a mutation in a second elongation/bypass step that depended upon the

UmuC (shown in purple) and UmuD (orange shape) proteins (31–33). **B**. Subsequent studies revealed that rather than being accessory factors of pol III, the products of *umuDC* genes encode a *bona fide* DNA polymerase, pol V (shown here and in figures 3, 7, 8, and 9 as a purple UmuC and two orange UmuD subunits assembled in a shape of a “right hand”), that executes TLS *in vivo* (200, 232). Thus, at a replication blocking lesion, such as a CPD, pol III is replaced by pol VMut (UmuD'$_2$C-RecA-ATP complex) (104), which can carry out both the (mis)insertion and extension steps of TLS past the CPD. After traversing the damaged DNA, pol VMut is replaced by pol III holenzyme, which resumes high-fidelity chromosomal duplication (178).

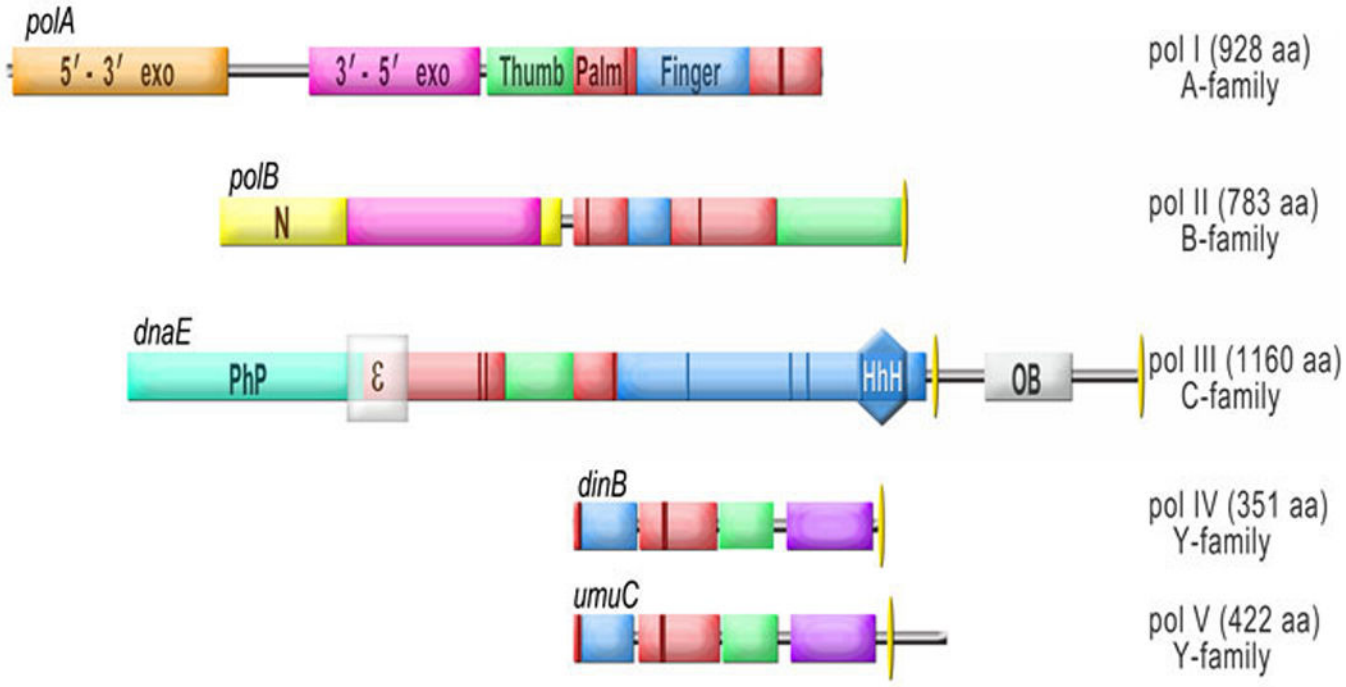

Figure 2. Domain organization of *E. coli* polymerases

The name and phylogenetic family relationship of each of *E. coli*'s five DNA polymerases along with number of amino acid residues in each polymerase is indicated on the right, while the name of the gene encoding the polymerase is noted on the left above the domain structure of each respective polymerase. The structural domains present in all polymerases are color coded as shown for pol I (palm - red, thumb - green, finger - blue). The palm domain consists of two segments separated by the insertion of the finger domain for all polymerases except pol III (α-catalytic subunit), in which thumb domain separates these two segments. The five DNA polymerases have been aligned to the smaller segment of the palm domain. Other domains are: 5'-3' exonuclease of pol I– orange; 3'-5' exonuclease of pol I, and pol II– violet; N-terminal domain of pol II – yellow, little finger of pol IV and pol V – purple; PhP (Polymerases and Histidinol Phosphatase) domain- light blue. The region of the DnaE that binds to the ε-(3'-5' proofreading) subunit of pol III is indicated by a semitransparent grey box labeled "ε". Other regions of DnaE that mediate protein-protein and protein-DNA interactions are the "helix-hairpin-helix" (HhH, shown as a dark blue hexagon) and Oligonucleotide/oligosaccharide Binding (OB, shown as a grey box) domains. The HhH domain provides binding to dsDNA, while the OB domain is involved in preferential interaction with ssDNA. The finger domain of DnaE has four sub-domains (marked by dark blue vertical bars). The acidic catalytic residues of each DNA polymerase are indicated in the diagram as dark red vertical sticks. The narrow yellow ovals mark the region of each polymerase that mediates their respective binding to the β-clamp.

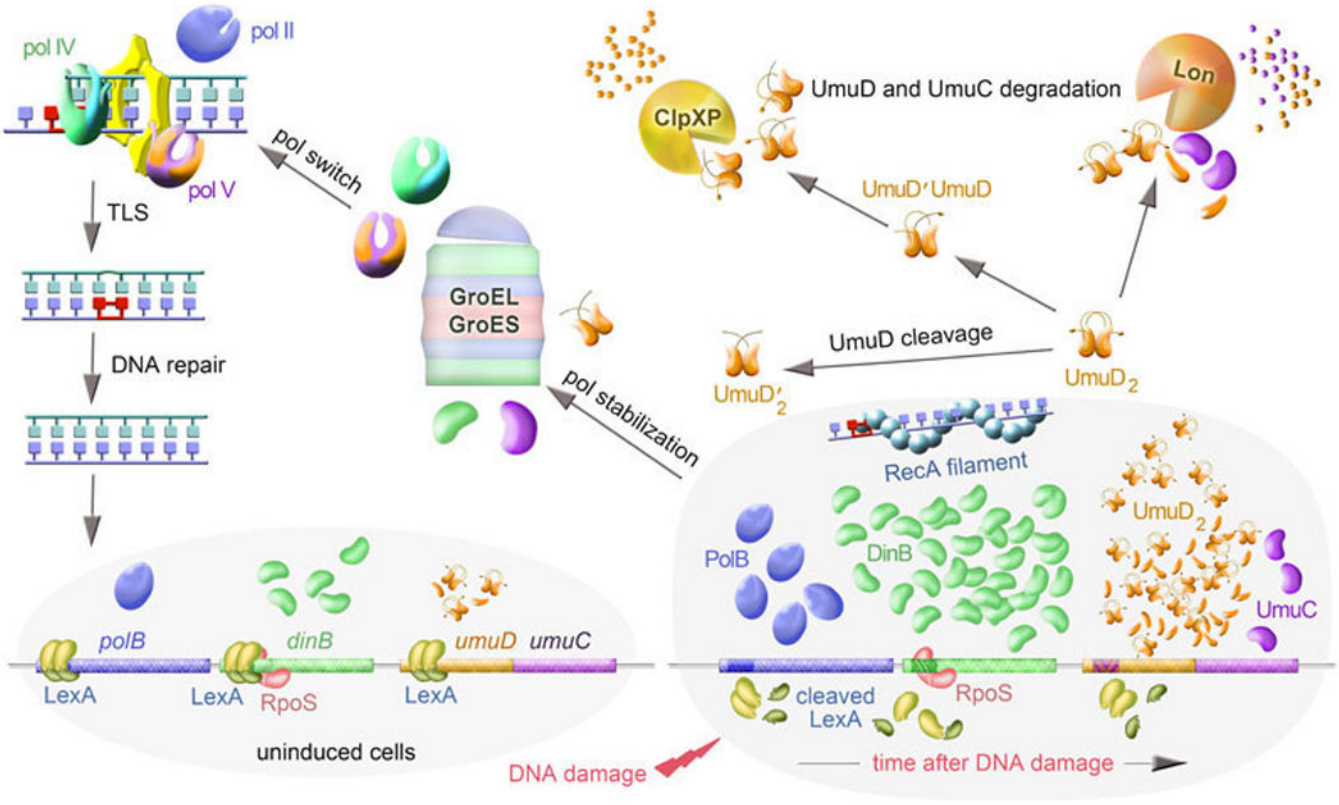

Figure 3. Transcriptional and posttranslational regulation of TLS polymerases

In uninduced cells, LexA (shown as yellow homodimer in which each monomer consists of an N-terminal DNA-binding domain and a C-terminal dimerization domain) represses expression of TLS polymerases (pol II represented in figures 3, 7, and 8 as a dark blue "right hand" shape and pol IV shown here and in figures 5–7 as a green "right hand" shape with a blue ""little finger" domain, as well as both, UmuC and UmuD subunits of pol V) to a different extend depending on Heterology Index (H.I.) of the LexA-binding site in the respective operator/promoter region of each polymerase. The H.I. also determines how soon after LexA cleavage expression of each gene is derepressed. DNA damage-induced formation of a RecA nucleoprotein filament on ssDNA mediates autocleavage of LexA and the derepression of genes in the LexA-regulon. It also mediates autocleavage of UmuD to UmuD'. Uncleaved and cleaved UmuD together with UmuC form different complexes of which $UmuD'_2C$ is the most stable. UmuD' is targeted for degradation by ClpXP through preferential formation of UmuD/UmuD' heterodimers. Both UmuC and UmuD are unstable and rapidly degraded by the Lon serine protease. However, UmuC is stabilized by the GroEL and GroES molecular chaperones. The *groE* gene products also stabilize pol IV. In addition to SOS induction, pol IV expression is also stimulated by RpoS (represented by a pink arched shape) in stressed cells. Upon repair of DNA damage, the disappearance of the RecA filament allows the newly synthesized LexA molecules to block transcription of all LexA-regulated genes and as a consequence, the intracellular concentrations of TLS polymerases return to their basal uninduced levels.

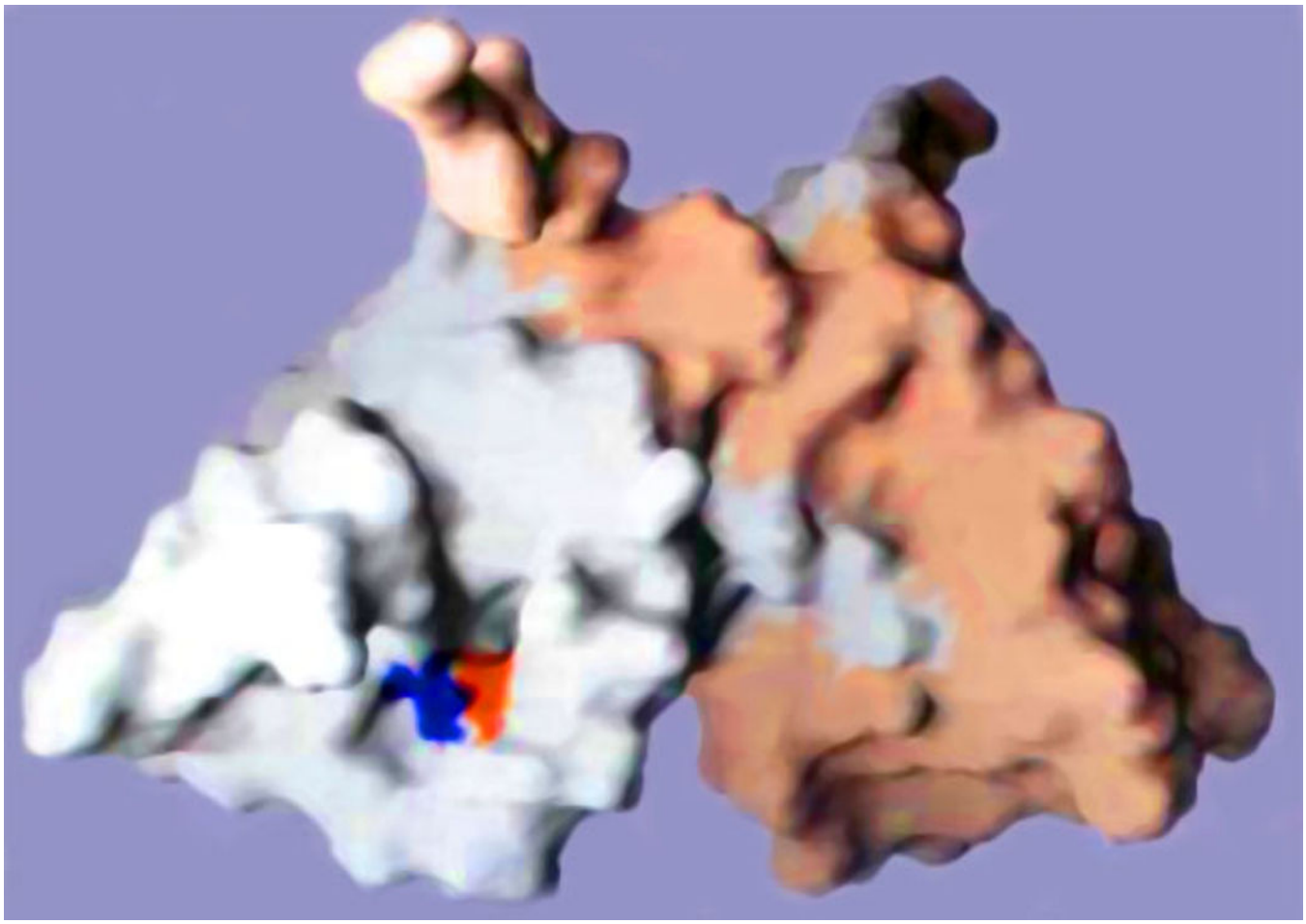

Figure 4. Structural basis for intermolecular UmuD cleavage

Surface representation of a UmuD' dimer (PDB: 1UMU). One protomer is colored pale gray and another one is colored pale orange. In this orientation, the active site (Ser60 is shown in red and Lys97 is in blue) of the gray protomer is clearly visible at the end of a cleft in the UmuD' protein. By forming such a dimer, the N-terminal tail of the pale orange UmuD' protomer is brought into relatively close proximity with the active site of its gray dimer mate (64, 183). It is thought that a similar configuration is assumed during RecA-mediated cleavage of UmuD and that such protein-protein interactions promote intermolecular UmuD cleavage (21, 152). The figure was generated using the program GRASP (166) and is reproduced with permission from McDonald et al (152).

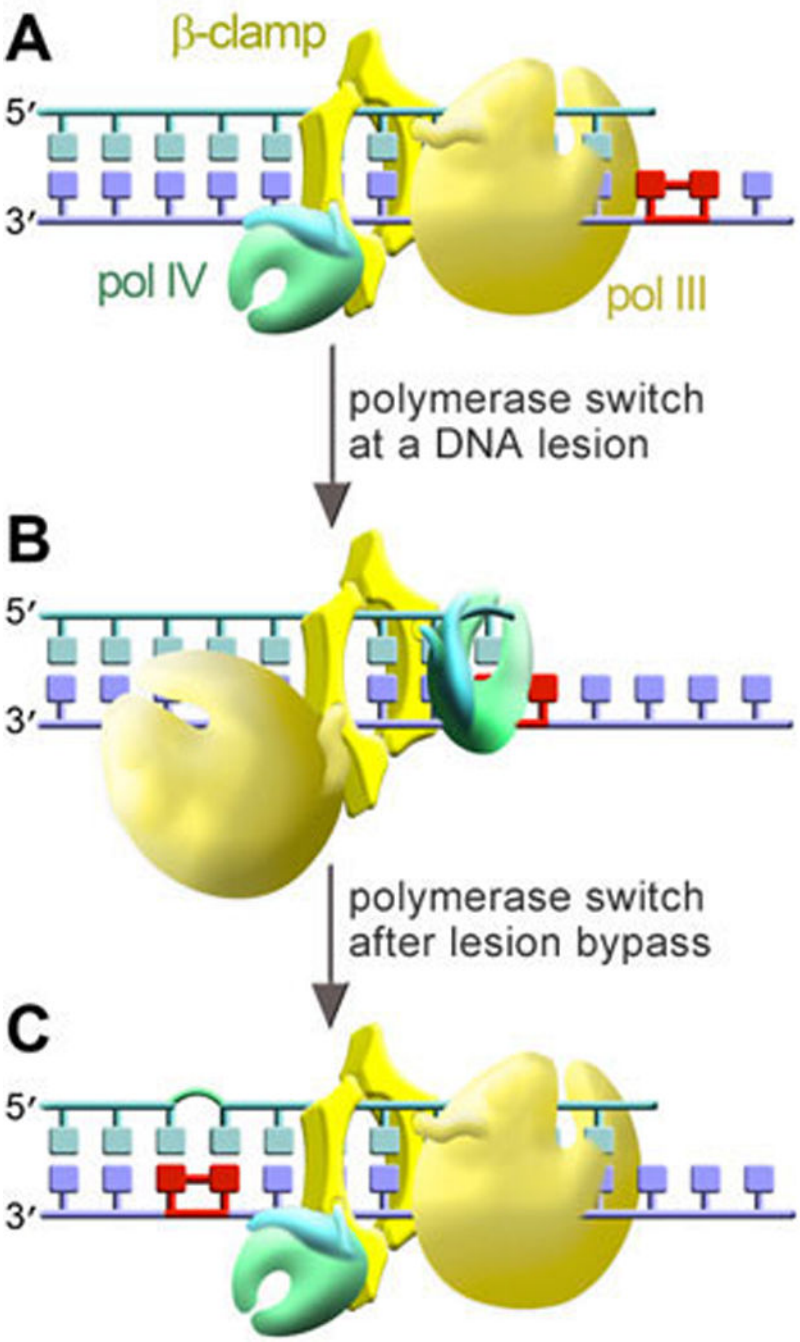

Figure 5. Tool-belt model for polymerase switching

The cartoon depicts pol III (yellow) and pol IV (green with a blue little finger) simultaneously bound to the dimeric β-clamp. In the top panel, the main replicase, pol III, is engaged at the primer terminus. At a DNA lesion (middle panel), pol III disengages from the primer terminus and pol IV rotates into position so as to promote TLS. After TLS has occurred (bottom panel), pol IV is replaced by pol III, which resumes genome duplication. Cartoon modified from Pages and Fuchs (176).

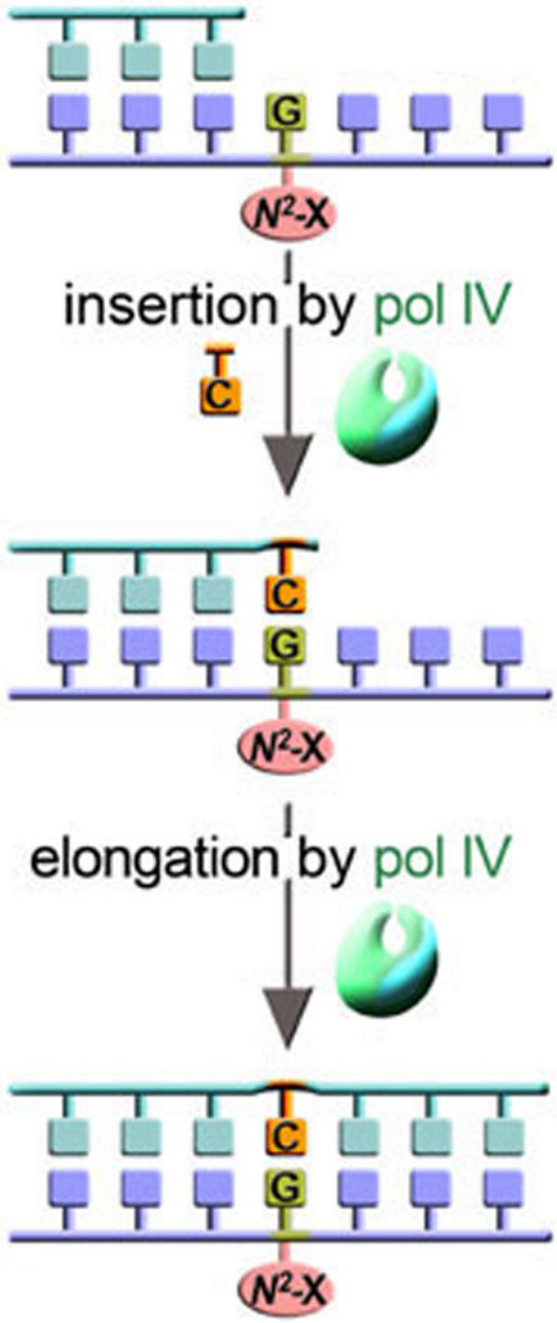

Figure 6. Pol IV can promote unassisted error-free TLS of certain N2 dG lesions

Recent data suggests that pol IV can efficiently and accurately insert nucleotides opposite 1, N^2-dG adducts, such as those generated by exposure to nitrofurazone, as well as to extend from the resulting primer termini. As a consequence, cells devoid of *dinB* are much more sensitive to the killing effects of nitrofurazone-induced adducts (102).

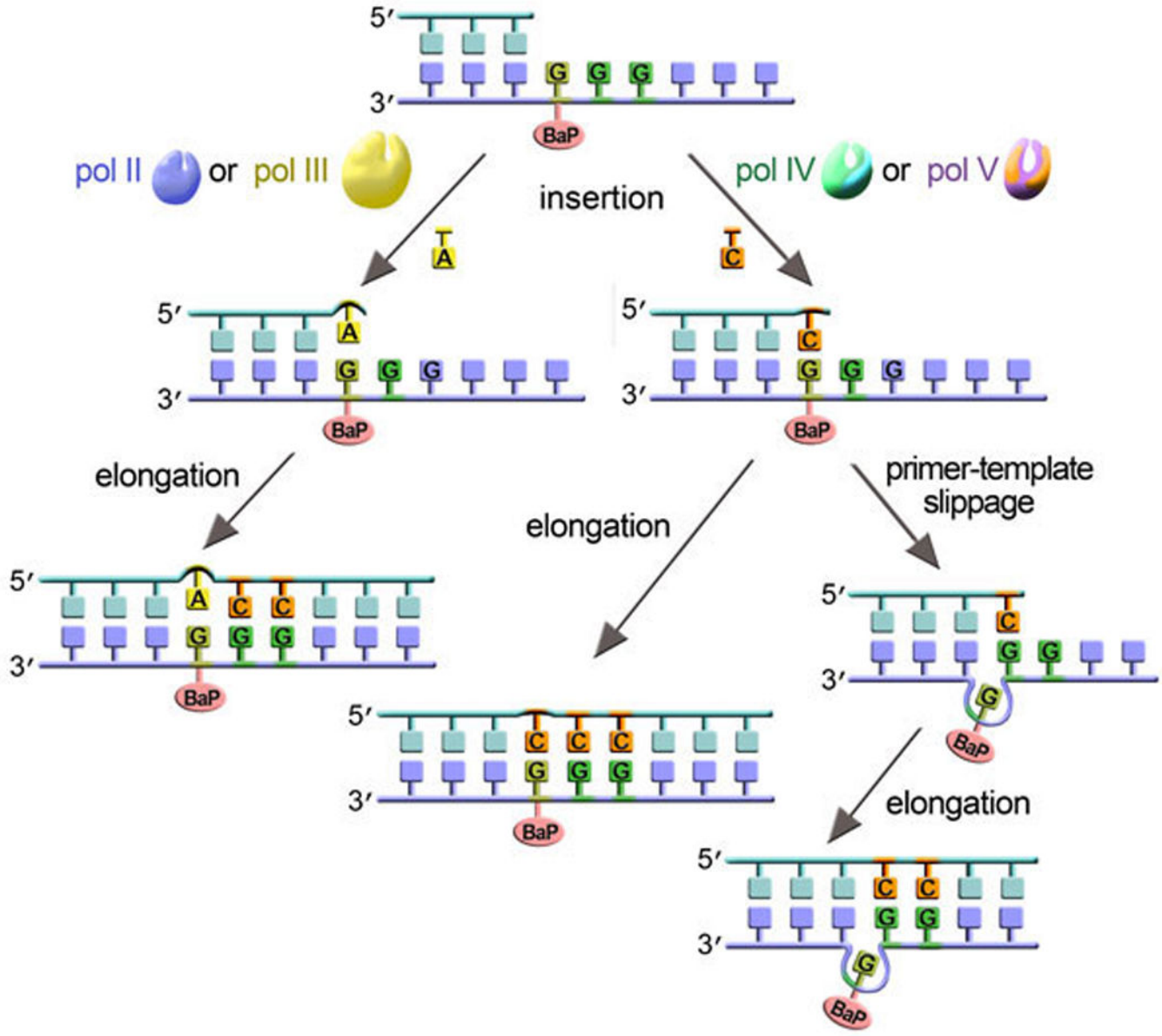

Figure 7. Mutliple TLS polymerases work together to promote BaP lesion bypass

Different DNA polymerases may be involved in TLS of benzo[*a*]pyrene (BaP) adducts depending on the sequence context surrounding the damaged site, conformation of the adduct and identity of the damaged template base (A vs G) (129, 162, 207, 208, 264). For example, in SOS-induced cells both error-free and −1 frameshift TLS of BaP located within a short run of Gs has been shown to depend largely on pol V and pol IV (108, 134). In this case, bypass is initiated from the correct incorporation of C opposite the dG-BaP. This intermediate product could either be elongated in an error-free manner, or adopt a slipped conformation, elongation of which would result in −1 frameshift mutation. The SOS independent G to T base substitution pathway consists of misincorporation of a dA opposite the dG-BaP and elongation of an A-lesion terminus with both steps likely to be carried out by pol III and/or pol II (162, 208).

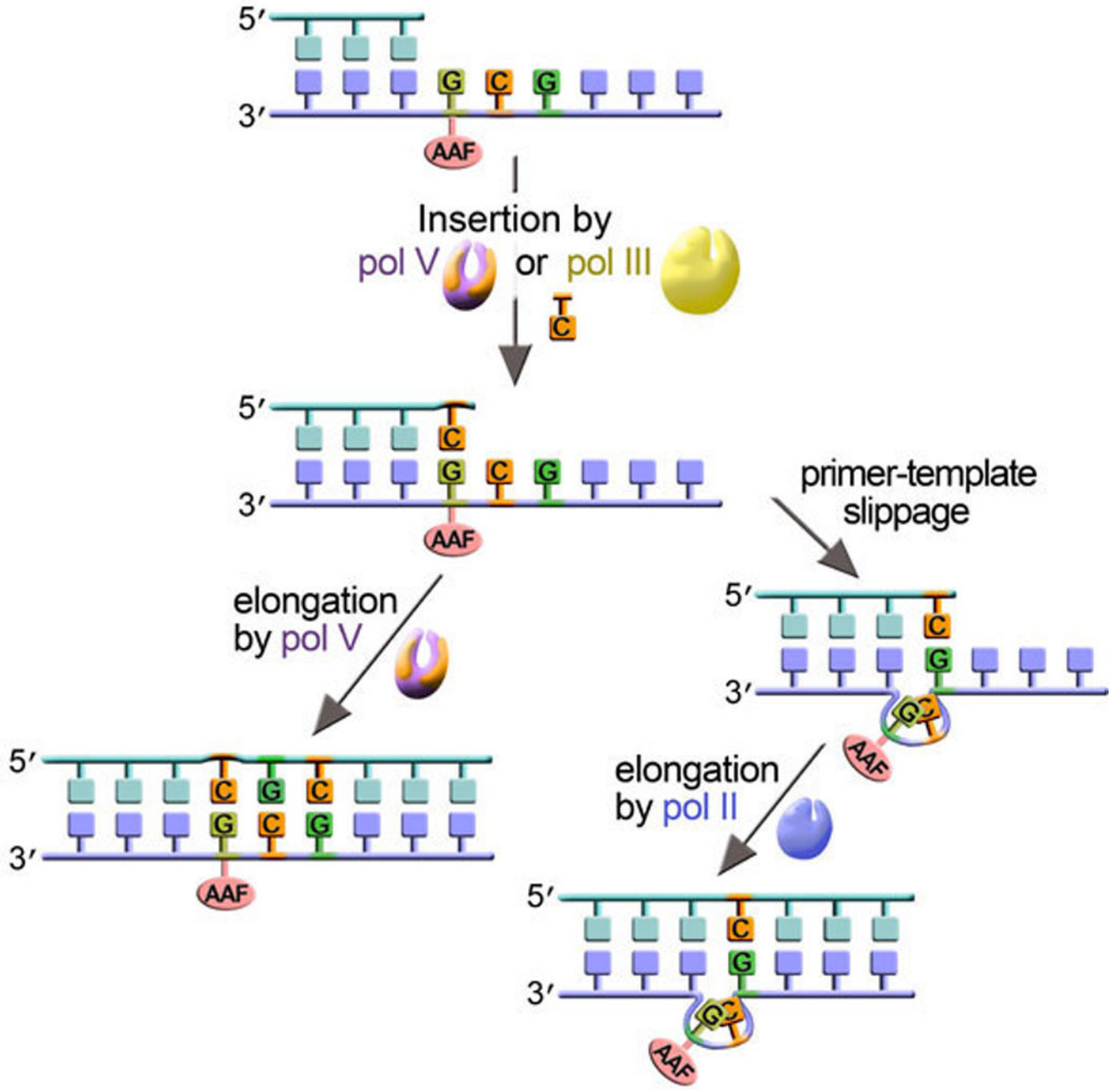

Figure 8. Pol V and pol II can promote both error-free and error-prone TLS of AAF-dG

Either pol V or pol III can insert a base opposite an N-Acetyl-2-Aminofluorene (AAF)-dG lesion and both do so in a relatively error-free manner (shown on the left hand side of the figure). Pol V can extend the primer-terminus leading to error free TLS of the AAF-dG adduct. Alternatively, when the AAF-dG lesion is positioned in the 3'-GCG sequence context, and pol II performs the extension step (right-hand side), −2 base-pair frameshifts occur (76, 162).

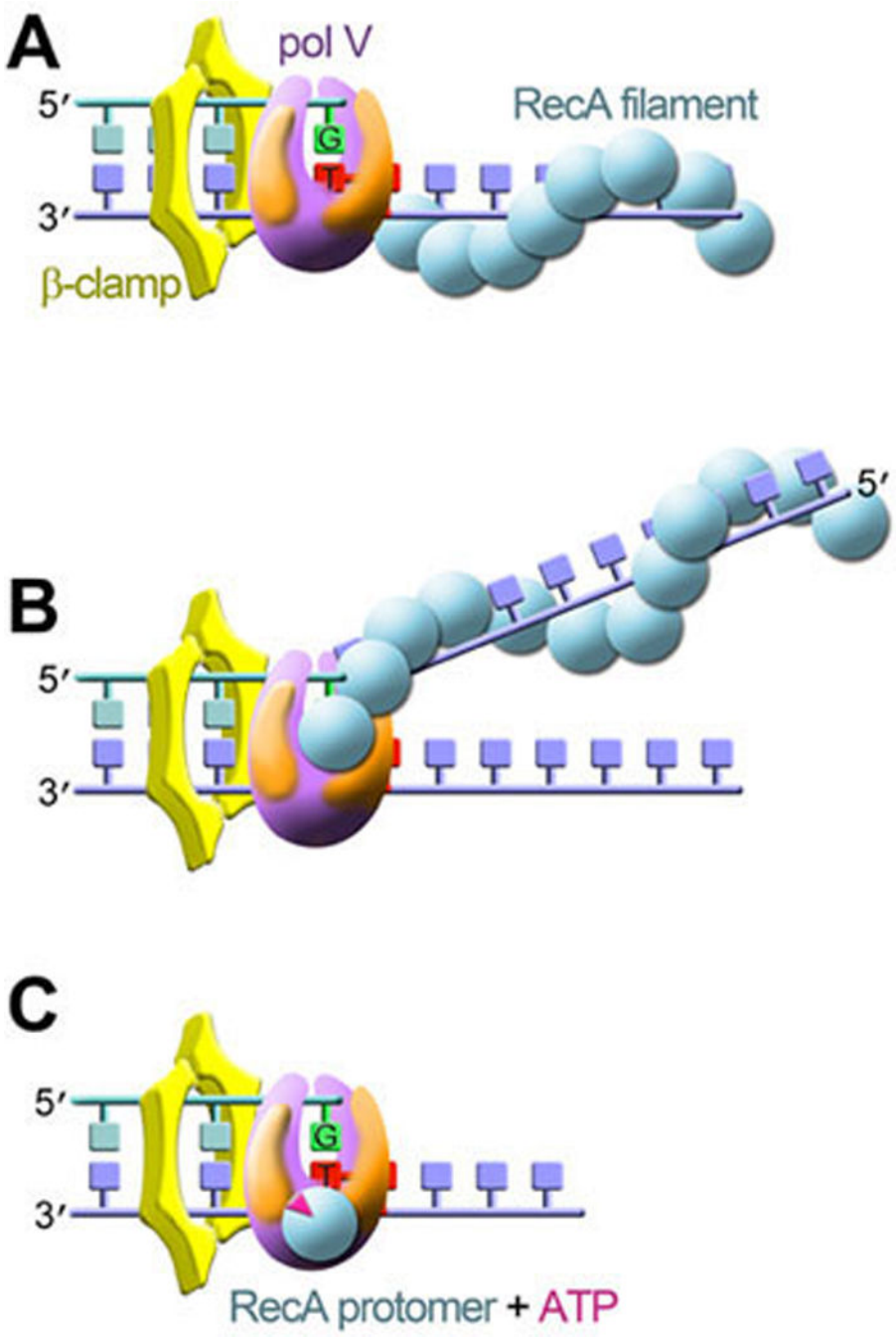

Figure 9. Evolution of RecA's "direct role" in TLS

A: In 1999, TLS was reconstituted *in vitro* in the presence of UmuD'$_2$C (pol V), β-sliding clamp and RecA protein (200, 232). Based upon genetic experiments *in vivo* (217), it was tacitly assumed that RecA stimulated pol V by forming a nucleoprotein filament on the single-stranded DNA immediately 3' of the stalled replication complex. **B**: However, in 2006 it was demonstrated that pol V can also be activated *in trans* by interacting with the 3'-tip of RecA filament formed on a separate ss DNA molecule (202). **C**: In 2009, it was subsequently determined that the highly active complex responsible for *in vivo* TLS is pol

VMut, which consists of UmuD'$_2$C•RecA•ATP and is formed by the transfer a single RecA protomer along with ATP from the 3' tip of a RecA nucleoprotein filament to pol V (104).

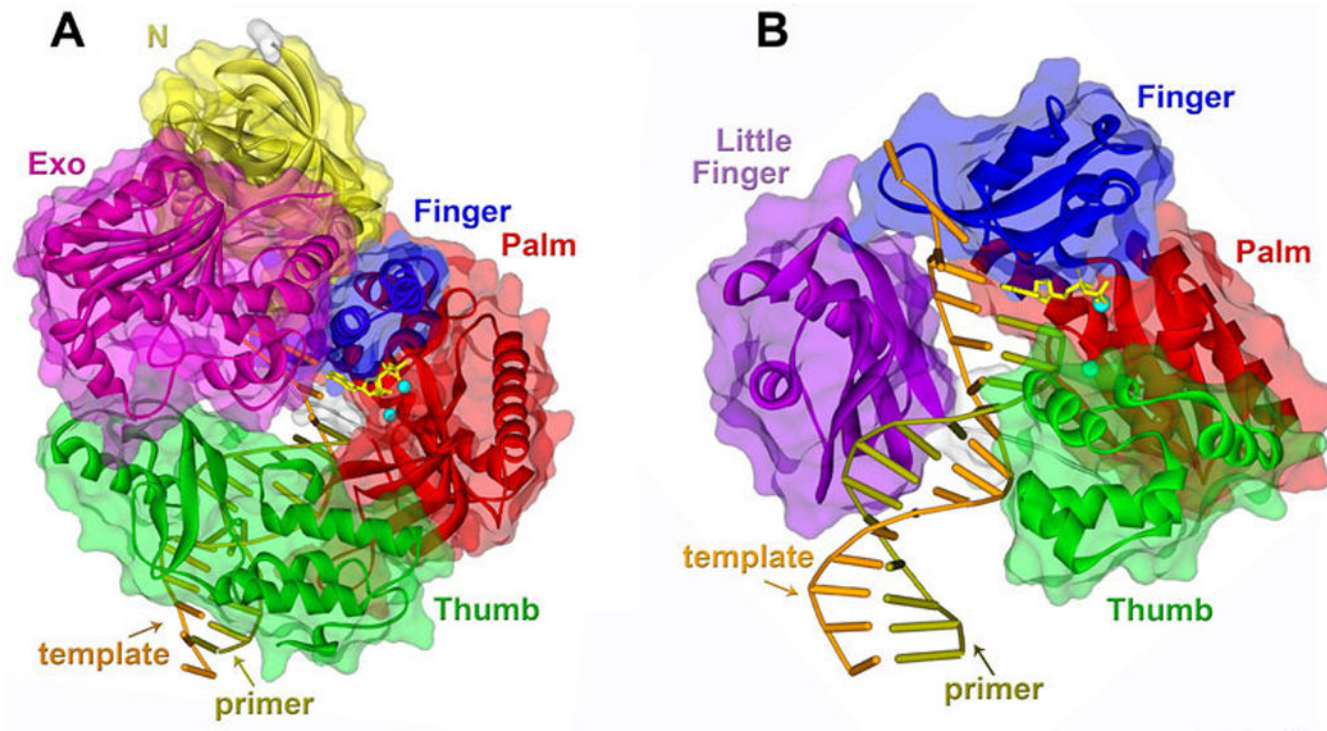

Figure 10. Crystal structure of TLS polymerases in complex with DNA and an incoming dNTP

A. Structure of pol II with DNA containing abasic site and dATP (PDB: 3K5L) (245). **B**. Crystal structure of the Dpo4 ternary complex (PDB: 1JX4) (133). The domains of both polymerases are color-coded such as: palm - red, thumb - green, finger - blue, exonuclease of pol II– violet, N-terminal domain of pol II – yellow, little finger of Dpo4 - purple. The template strand is shown in rust color, while the primer is olive green. The incoming dATP is shown in yellow. The small blue spheres represent the two metal ions. The protein backbone is represented by ribbon surrounded by semi-transparent solvent accessible surface. The structures were created using Discovery Studio Visualizer. Comparison of these structures reveals that the active site of Dpo4 is significantly more spacious than that of pol II allowing for the accommodation of bulky DNA lesions. The active site of pol II is very similar to that of high fidelity replicative polymerases, but relaxed interactions with the upstream DNA template and an altered partitioning between the polymerase and exonuclease active sites ensures the participation of pol II in TLS.

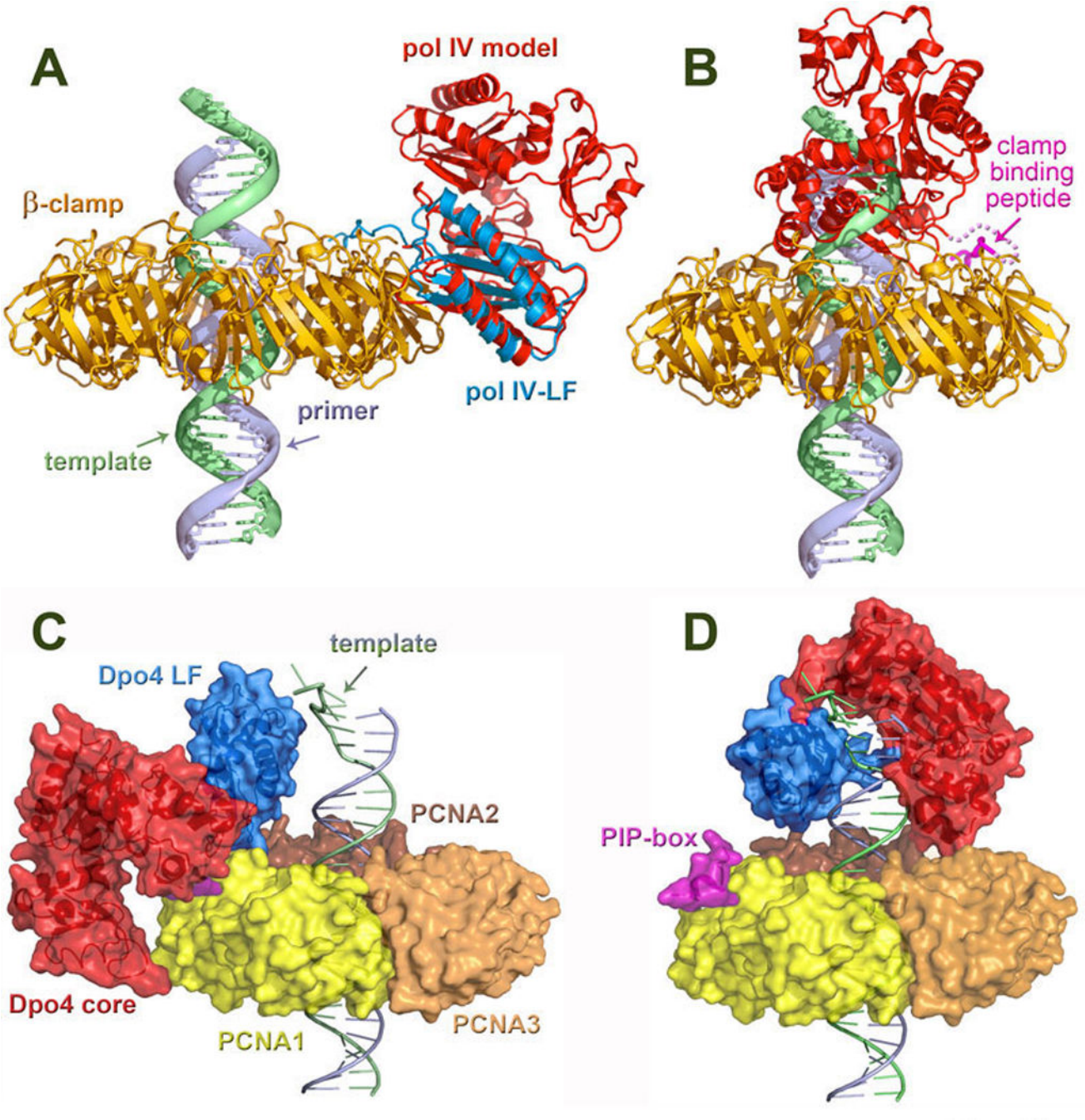

Figure 11. Structural/modeling studies of TLS polymerases bound to processivity clamps

A. Model of a pol IV (shown in red) bound to the β-clamp (gold) in an inactive position. The position of the polymerase was modeled by superimposing the little finger domain from the ternary complex of Dpo4 with DNA and an incoming nucleotide (PDB code: 1JXL) (133) onto the LF of *E. coli* pol IV (blue) in a complex with the β-clamp (PDB: 1UNN) (38). In this position, pol IV is unable to access the primer/template terminus. **B**. Model of pol IV bound to the β-clamp and DNA at the primer/template junction following a polymerase switch. The position of the polymerase was modeled by superimposing the DNA from the

ternary complex of Dpo4 onto the end of a DNA molecule running perpendicularly through the β-clamp. Contact with the clamp is maintained by the C-terminal clamp-binding peptide (pink), which tethers the polymerase to the replication complex. **C**. Dpo4 in an inactive extended form bound to PCNA (PDB: 2NTI) (261) with dsDNA passing through its central aperture. In this conformation, the core (red) and little finger (blue) of Dpo4 contact PCNA (the trimer is shown in yellow, orange, and brown) to facilitate the PIP (pink)–PCNA1 (yellow) binding. D. Model of Dpo4 in an active form bound to a DNA template and a PCNA ring. Similar to the structure shown in the panel C, in this conformation the PIP-box anchors Dpo4 to PCNA1. The DNA-bound Dpo4 is modeled onto PCNA from the type I structure (PDB: 1JX4). Panels A and B reproduced with permission from Bunting et al., (38). Panels C and D were provided through the courtesy of Dr. Hong Ling, University of Western Ontario, London, Canada. All four structures provide structural support for the proposed “tool-belt” model for polymerase switching (176).

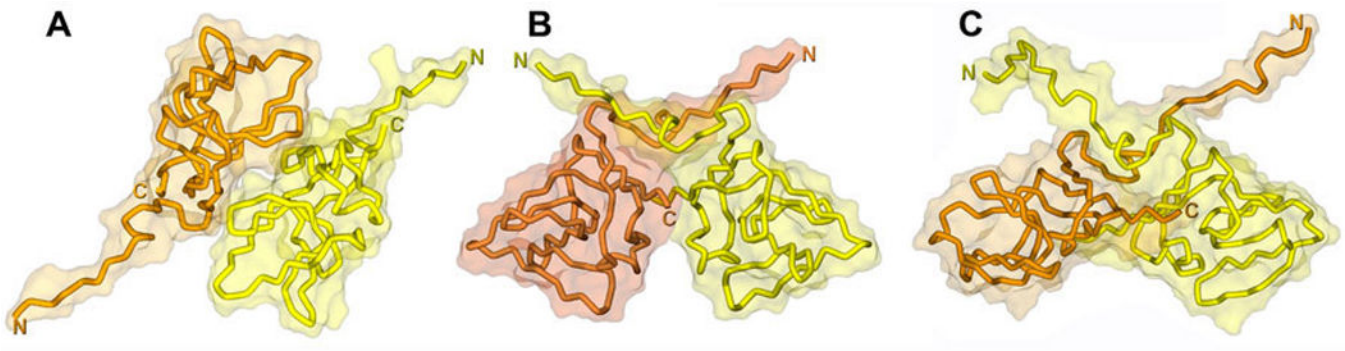

Figure 12. Comparison of the crystal and NMR structure of UmuD'$_2$

A. Crystal structure of a UmuD' "Molecular" dimer (PDB: 1AY9) in which the N-termini of each protomer are extended in diametrically opposite directions (182). **B**. Crystal structure of UmuD' "Filament" dimer (PDB: 1UMU) in which the N-terminal tail of each protomer cross over the globular body of its partner in a configuration that is likely to mimic the one in which the RecA-mediated cleavage of UmuD to UmuD' occurs (183). **C**. NMR solution structure of UmuD'$_2$ (PDB: 1I4V) revealing a structural fold similar to the crystallographic "Filament" dimer, but in which the globular domain is somewhat "flattened" and the N-terminal tails are free in solution (64). The protein backbone is depicted as a tube that is surrounded by the semi-transparent solvent accessible surface of the dimer. One UmuD' protomer is colored yellow, while the other one is in orange. The structures were created using Discovery Studio Visualizer.

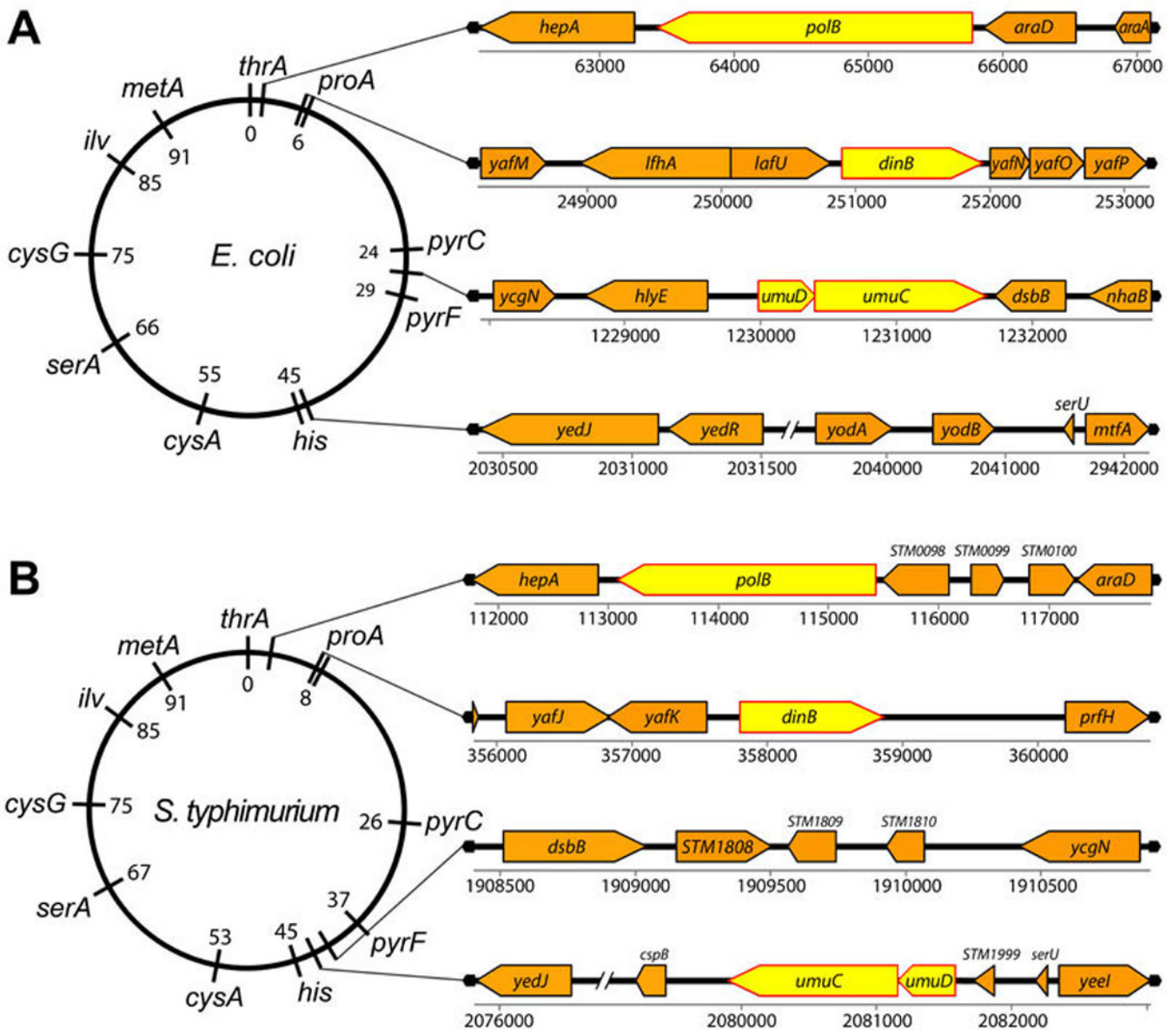

Figure 13. Comparison of the genetic map locations and genomic context of translesion DNA polymerases in *E. coli* and *S. typhimurium*

A. The genetic map locations and genomic architecture of the *E. coli polB dinB* and *umuDC* genes are depicted. **B**. The genetic map locations and genomic architecture of the *S. typhimurium polB dinB* and *umuDC* genes are depicted. The location and context of the *E. coli* and *S. typhimurium polB* and *dinB* genes are quite conserved. In contrast, the *E. coli* and *S. typhimurium umuDC* operons are located in different positions on the genetic maps, bordered by different genes and are in opposite orientations.

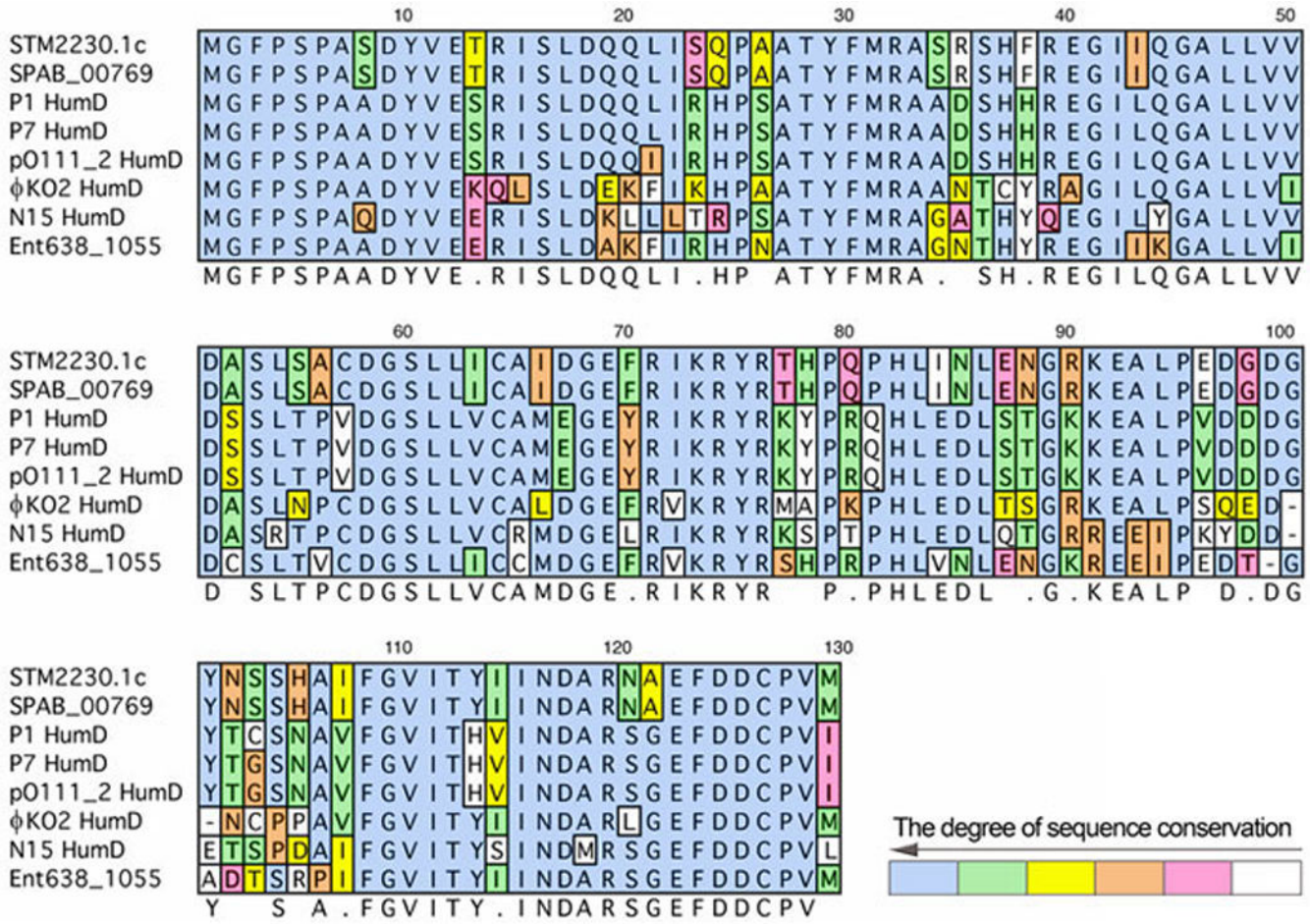

Figure 14. Homology comparison of the *S. typhimurium* STM2230 protein with various HumD-like proteins

The alignment of various HumD-like proteins was performed using MacVector 11.0.4. The HumD-like proteins include: STM2230.1c (*S. typhimurium*; NP_461173), SPAB_00769 (*S. paratyphi*; YP_001587027), P1 HumD (YP_006569), P7 HumD (ZP_07505015), pO111_2 HumD (YP_003238012), fKO2 HumD (YP_006603), N15 HumD (NP_046921) and Ent638_1055 (Enterobacter sp. 638; YP_001175788). Coloration indicates the degree of similarity to the consensus sequence. Blue indicates identity to the consensus, while green indicates an amino acid residue that is highly similar to the consensus. The degree of similarity to the consensus further decreases in this order of coloration: yellow, orange, pink and white.

Table 1

Intracellular concentration of DNA polymerases in *E. coli*

	−SOS	+SOS
pol I (PolA)	400[1]	400
pol II (PolB)	50	350[2]
pol III (holoenzyme)	~15	45[3]
pol IV (DinB)	250	2500[4,8]
pol V (UmuD'C complex)	ND[5]	60[6]
UmuD	200	2400[4]
UmuD'	ND	2500[7,8]
UmuC	ND	200[4,8]/ 700[7,8]

[1] From reference 114.

[2] Based upon the catalytic activity of pol II purified from SOS-induced cells (24).

[3] Based upon the catalytic activity of pol III purified from SOS-induced cells (24)

[4] Estimate based upon steady-state levels in strains harboring a *lexA*(Def) allele that leads to constitutive and full derepression of all LexA-regulated genes (108, 258).

[5] Not detectable

[6] Peak concentration observed in wild-type *E. coli* strains 45 mins after exposure to ultraviolet light (217).

[7] Estimate based upon steady-state levels in strains harboring a *lexA*(Def) allele that leads to constitutive and full derepression of all LexA-regulated genes and a *recA730* allele that leads to efficient and constitutive conversion of UmuD to UmuD' (258).

[8] These values are almost certainly overestimates because the levels of expression of SOS genes in a *lexA*(Def) cell are far higher than that occurring during an SOS response. Indeed, Friedman et al., (74) demonstrated that the “graded” increased expression of SOS genes with greater DNA damage is caused by more transcriptional bursts of the same small magnitude over time, and that SOS genes are never fully derepressed as in *lexA*(Def) cells.

Table 2

Crystal structures of various DNA lesions in the active site of Dpo4

DNA lesion co-crystallized with Dpo4	Number of structures	PDB accession numbers	References
1,N^2-etheno-guanine	5	2BQ3, 2BQR, 2BQU, 2BR0, 2XC9	(100, 266)
1,N^2-propanodeoxyguanosine	3	2R8G, 2R8H, 2R8I	(246)
2,4-difluorotoluene	3	2V9W, 2VA2, 2VA3	(99)
2-aminofluorene-guanine	4	3KHG, 3KHH, 3KHL, 3KHR	(197)
7,8-dihydro-8-oxoguanine	16	2ASD, 2ASJ, 2ASL, 2C22, 2C2R, 3GII, 3GIJ, 3GIK, 3GIL, 3GIM, 2UVR, 2UVU, 2UVV, 2UVW, 2XCA, 2XCP	(60, 100, 198, 199, 267)
Abasic site	5	1N48, 1N56, 1S0N, 1S0O, 1S10	(134)
Benzo[*a*]pyrene diol epoxide	1	1S0M	(135)
Cisplatin-1,2 guanine-guanine intrastrand cross-link	3	3M9M, 3M9N, 3M9O	(255)
cis-syn thymine dimer	2	1RYR, 1RYS	(132)
Malondialdehyde-deoxyguanosine	2	2V4Q, 2V4R	(62)
N^2N^2-dimethyl-deoxyguanosine	3	2W9A, 2W9B, 2W9C	(269)
N^2-naphthyl-guanine	2	2W8K, 2W8L	(268)
O^6-benzylguanine	4	2JEF, 2JEG, 2JEI, 2JEJ	(59)
O^6-methylguanine	3	2J6S, 2J6T, 2J6U	(61)

Table 3

Pol V orthologs found on R-plasmids/ICEs

Incompatibilty Group	Plasmid	Original Host	Pol V Family
IncB1	pO111_1	*E. coli*	Muc
IncFI	pSE11-2	*E. coli*	Imp[1]
IncFII[2]	pSLT	*S. typhimurium*	Sam
IncFII	pSCV50	*S. choleraesuis*	Sam
IncH	R27	*S. typhimurium*	Muc
IncH	pAPEC-O1-R	*E. coli*	Muc
IncH	pAPEC-O2-R	*E. coli*	Imp
IncIa	ColIb-P9	*E. coli*	Imp
IncIa	R64	*S. typhimurium*	Imp
IncIa	TP110	*S. typhimurium*	Imp
IncJ/ICE[3]	R391[4]	*P. rettgeri*	Rum
IncL	R471a	*S. marcescens*	Muc
IncM	R446b	*P. morganii*	Muc
IncN	R46	*S. typhimurium*	Muc
IncN	pKM101[5]	*S. typhimurium*	Muc
IncT	Rts1	*P. vulgaris*	Muc/Umu[6]
IncT	R394	*P. rettgeri*	Muc
ICE	SXT[7]	*V. cholerae*	Rum[8]
ICE	ICEPmiJpn1[7]	*P. mirabilis*	Rum
ICE	ICEPdaSpa1[7]	*P. damselae*	Rum[9]
ICE	ICEVflInd1[7]	*V. fluvialis*	Rum[10]
ICE	ICEVchmex1[4]	*V. cholerae*	Rum
ICE	ICEVchind5[4]	*V. cholerae*	Rum[11]
ICE	ICEVchind4[7]	*V. cholerae*	Rum[11]
ICE	ICEVchban5[4]	*V. cholerae*	Rum[11]
ICE	ICEPalban1[4]	*P. alcalifaciens*	Rum[12]

[1] pSE11-2 (NC_011413); the *imp*-like genes are not arranged within an operon.

[2] pSLT is believed to belong to the IncFII incompatability group (149).

[3] Originally identified as an IncJ plasmid (45), but now considered an Integrating Conjugative Element (ICE) (13).

[4] Belongs to the R391 ICE exclusion group (145)

[5] pKM101 is a naturally occurring deletion derivative of R46 (NC_003292) generated during P22 transduction of R46 into *S.typhimurium* (160)

[6] Rts1 (NC_003905); this plasmid harbors two polV-like operons. The *mucB*-like gene has a C-terminal truncation (110).

[7] Belongs to the SXT ICE exclusion group (145)

[8] The *rumB* gene of SXT is disrupted by a large 17kb insertion containing multiple antibiotic resistance genes (13).

[9] *Photobacterium damselae* ICEPdaSpa1 (AJ870986); the *rum* operon is disrupted by a transposon.

[10] *Vibrio fluvialis* ICEVflInd1 (GQ463144); the *rum*-like genes are not arranged within an operon.

[11] *Vibrio cholerae* ICEVchind5 (GQ463142), ICEVchind4 (GQ463141) and ICEVchban5 (GQ463140); the *rum*-like genes are not arranged within an operon.

[12] *P. alcalifaciens* ICEPalban1 (GQ463139); the *rum*-like genes are not arranged within an operon.