Translesion DNA Polymerases

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Living cells are continually exposed to DNA-damaging agents that threaten their genomic integrity. Although DNA repair processes rapidly target the damaged DNA for repair, some lesions nevertheless persist and block genome duplication by the cell's replicase. To avoid the deleterious consequence of a stalled replication fork, cells use specialized polymerases to traverse the damage. This process, termed "translesion DNA synthesis" (TLS), affords the cell additional time to repair the damage before the replicase returns to complete genome duplication. In many cases, this damage-tolerance mechanism is error-prone, and cell survival is often associated with an increased risk of mutagenesis and carcinogenesis. Despite being tightly regulated by a variety of transcriptional and posttranslational controls, the low-fidelity TLS polymerases also gain access to undamaged DNA where their inaccurate synthesis may actually be beneficial for genetic diversity and evolutionary fitness.

The Watson–Crick (WC) DNA structure was published in 1953 (Watson and Crick 1953). Coincidentally, it was in 1953 when Weigle discovered that λ bacteriophage that had been killed by exposure to UV radiation could, in essence, be brought back to life by irradiation of its *Escherichia coli* host (Weigle 1953). The reactivation of λ, however, was accompanied by a sizable increase in phage mutagenesis (Weigle 1953). With the benefit of nearly five decades of hindsight, the increase in phage survival and mutagenesis observed by Weigle turns out to have been caused by the action of translesion DNA synthesis (TLS) polymerases. Currently, there are three TLS polymerases (Pols) in *E. coli*, and many of the 15 known polymerases in eukaryotes have the capacity to promote some degree of TLS (Table 1) (Goodman 2002; Bebenek and Kunkel 2004; Waters et al. 2009; Lange et al. 2011; Sale et al. 2012).

In this review, we revisit the concepts and experiments in *E. coli* centered on the induction and regulation of mutagenesis that began in the mid 1970s, continuing throughout the 1980s, and culminating with the identification of a new family (Y-family) of error-prone DNA polymerases (Ohmori et al. 2001). We review early TLS models based principally on genetic data from *E. coli* that were generated in the 1980s and discuss recent biochemical data leading to current models of TLS in *E. coli* and humans. The typically poor fidelity of TLS polymerases

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Table 1. Properties of key TLS polymerases across all domains of life

Organism	Gene name	Protein name	Characteristics[a]
E. coli	*polB*	Pol II	B-family polymerase Regulated by LexA; damage inducible Creates −2 frameshift mutations during TLS of N^2-dG-AAF adducts
E. coli	*dinB*	Pol IV	Regulated by LexA; damage inducible Prone to making −1 frameshifts when overexpressed in vivo Bypasses N^2-dG adducts efficiently and accurately Involved in TLS of alkylation damage in vivo Major polymerase involved in stress-induced mutagenesis
E. coli	*umuDC*	Pol V	Regulated by LexA; damage inducible Major TLS polymerase in *E. coli* Composed of a heterotrimer of $UmuD'_2$ (∼24 kDa) and UmuC (∼48 kDa) to form an ∼72-kDa $UmuD'_2C$ complex Interacts with RecA and ATP to form Pol V Mut
Sulfolobus solfataricus	*dpo4*	Dpo4	Archaeal ortholog of *E. coli* Pol IV Numerous crystal structures of Dpo4 in the process of TLS have been solved
Sulfolobus acidocaldarius	*dbh*	Dbh	First archaeal DinB homology identified Less processive than Dpo4
Saccharomyces cerevisiae	*REV3*	Rev3p	B-family polymerase Together with Rev7, Pol31 and Pol32 forms Pol ζ_4 Can bypass lesions unassisted Works with other TLS polymerases to facilitate the extension step of TLS Stimulated by an interaction with Rev1
S. cerevisiae	*REV1*	Rev1p	Specifically incorporates dCMP opposite abasic sites and undamaged template dG Interacts with Pol ζ to stimulate Pol ζ–dependent TLS in vivo
S. cerevisiae	*RAD30*	Pol η	Bypasses a thymine–thymine CPD relatively accurately and efficiently
Homo sapiens	*REV3L*	REV3	B-family polymerase Catalytic subunit of human Pol ζ Very large protein consisting of 3130 amino acids Murine homozygous knockout is embryonic lethal
H. sapiens	*REV1*	REV1	Similar to *S. cerevisiae* Rev1 protein, it specifically incorporates dCMP opposite dG and abasic sites Acts as scaffold protein that interacts with TLS polymerases ζ, η, ι, and κ Generates mutations at G-C base pairs during immunoglobulin gene somatic hypermutation
H. sapiens	*POLH/ XPV/ RAD30A*	Pol η	Bypasses a thymine–thymine CPD relatively efficiently and accurately Defects lead to the sunlight-sensitive and cancer-prone xeroderma pigmentosum variant (XP-V) phenotype Regulated by ubiquitination and phosphorylation Generates mutations at A-T base pairs during immunoglobulin gene somatic hypermutation

Continued

Table 1. *Continued*

Organism	Gene name	Protein name	Characteristics[a]
H. sapiens	*POLI/RAD30B*	Pol ι	Has unique replication fidelity; incorporates opposite template dA reasonably accurately, but opposite template dT in a highly error-prone manner In vivo functions remain unknown
H. sapiens	*POLK/DINB1*	Pol κ	Prone to making −1 frameshift mutations, but can accurately and efficiently bypass a number of N^2-dG lesions Plays additional roles in repair synthesis steps of nucleotide excision repair

[a]Unless specified, the TLS polymerase belongs to the Y-family of DNA polymerases (Ohmori et al. 2001).

can now be understood thanks to high-resolution X-ray structures that indicate a more spacious active cleft that can accommodate a variety of non-WC base pairs. Owing to their potential to cause excessive numbers of deleterious mutations compared with replication polymerases, we describe how TLS Pol expression is tightly regulated, at both the transcriptional and post-translational levels. However, owing to the need for generating beneficial mutations, we also review recent data showing that TLS Pols are involved in providing evolutionary fitness in bacteria and in generating immunological diversity in higher vertebrates. Recognizing that the cellular function(s) of many of the human TLS Pols remain unknown, it may turn out that lesion bypass is but one important property of TLS Pols, and perhaps not even their most salient one.

DNA DAMAGE-INDUCED REGULATION OF GENE EXPRESSION IN *E. coli*

Weigle's 1953 phage reactivation experiment suggested the possibility of an induced cellular DNA repair process. The presence of a bacterial repressor system that could be inactivated by excessive DNA damage was proposed by Witkin in 1967 (Witkin 1967) as a molecular model to explain UV-induced phage reactivation. The pivotal connection to mutagenesis was made by Radman, who proposed the SOS model for an inducible error-prone mechanism in *E. coli* for the repair of DNA (Radman 1974). The conceptual framework provided by Witkin and Radman paved the way for several decades of genetic and biochemical studies aimed at elucidating the molecular basis of "SOS error-prone repair" (Witkin 1976; Walker 1984; Friedberg et al. 2006; Schlacher and Goodman 2007).

The damage-inducible SOS regulon requires interplay of the LexA and RecA proteins. The *lexA* gene encodes the LexA transcriptional repressor, which binds to sequences in the operator region of the >40 genes under its control (Fernández de Henestrosa et al. 2000; Courcelle et al. 2001). There is a minimal 6-bp operator consensus sequence, but it is the nonconsensus surrounding bases that determine repressor-operator binding constants, so that the earliest expressed genes are those whose operators are bound weakly by LexA (Lewis et al. 1994; Fernández de Henestrosa et al. 2000). It is estimated that there are roughly 8000 RecA molecules in an undamaged cell (Boudsocq et al. 1997). When the cellular DNA is damaged, RecA assembles as a nucleoprotein filament on single-stranded DNA (ssDNA), requiring the presence of ATP (Kuzminov 1999). This nucleoprotein filament, commonly referred to as RecA*, acts as a coprotease to mediate self-cleavage of LexA molecules in solution (Little et al. 1980). As the overall cellular concentration of intact LexA diminishes, only those genes that bind LexA tightly remain repressed.

The earliest genes to be induced after DNA damage are those involved in error-free repair, such as nucleotide excision repair (*uvrA*, *uvrB*, *uvrD*, and *cho*), as well as recombinational repair (*recA*, *recN*). *E. coli*'s three TLS polymerases

are also regulated by LexA. The operators for Pol II (*polB*) and Pol IV (*dinB*) bind LexA weakly (Fernández de Henestrosa et al. 2000) and are induced early in the SOS response. In contrast, the *umuDC* operon (encoding Pol V) has one of the tightest LexA-binding sites and is induced late in the response, 30–40 min after DNA damage (Sommer et al. 1993). Given their early induction and relatively high basal expression levels in the absence of DNA damage, it is thought that Pol II and Pol IV are likely to participate in mostly error-free TLS of specific DNA lesions. In contrast, Pol V can traverse a wide range of DNA lesions and does so in a much more error-prone manner. Its induction late in the SOS response therefore suggests that *E. coli* only uses Pol V as a last resort, once all other error-free repair pathways have been exhausted (Sommer et al. 1993).

E. coli AS A MODEL SYSTEM FOR TLS AND MUTAGENESIS

By the mid-1970s, early 1980s, genetic experiments had led to the identification of the principal players involved in damage-induced mutagenesis, *lexA*, *recA*, along with *umuD* and *umuC*. The same mechanism used by RecA* to mediate LexA cleavage (Little et al. 1980) is also used to cleave UmuD to form UmuD′ (Burckhardt et al. 1988; Shinagawa et al. 1988) and is an absolute requirement for SOS mutagenesis (Nohmi et al. 1988). By isolating RecA mutants that were UV non-mutable, despite retaining the ability to cleave LexA and UmuD, Devoret and colleagues showed that RecA* also had a separate *direct* role in SOS mutagenesis (Dutreix et al. 1989). This mutagenic role for RecA* remained a mystery until 2009 (Jiang et al. 2009); its biochemical mechanism is discussed below.

Early TLS Models

The first model to address the function of the Umu proteins during UV-induced TLS was proposed by Bridges and Woodgate in 1985 (Bridges and Woodgate 1985a,b). According to this model, TLS occurred in a two-step process in which Pol III incorporates a nucleotide opposite the first (3′) T of a T-T CPD, requiring the presence of RecA protein bound to the template proximal to the lesion. Pol III would subsequently interact with the UmuDC proteins to incorporate another nucleotide at the second (5′) T of the CPD (Fig. 1A). At least one of the two incorporations would be non-WC, thereby causing a mutation targeted at the site of the CPD.

A model proposed by Echols and Goodman in 1990 (Echols and Goodman 1990) envisioned complete blockage of the Pol III holoenzyme (Pol III core, β-sliding clamp, γ-clamp-loading complex) when encountering a template lesion, followed by the assembly of a damage-localized nucleoprotein complex involving RecA, UmuC, UmuD′, SSB, and Pol III holoenzyme—a "mutasome"—to copy past a template lesion (Woodgate et al. 1989; Echols and Goodman 1991). This model took into account the finding that RecA* facilitates cleavage of UmuD to a mutagenically active form, UmuD′ (Burckhardt et al. 1988; Nohmi et al. 1988; Shinagawa et al. 1988). Subsequently, it was shown that it was actually a dimeric $UmuD_2$ that is cleaved to $UmuD'_2$ and that subsequently interacts with UmuC to form a stable complex of $UmuD'_2C$ (Woodgate et al. 1989; Bruck et al. 1996), identified in 1999 as Pol V (Tang et al. 1999). Whereas a Pol III replisome performs rapid and processive "error-free" genome replication on undamaged DNA (Johnson and O'Donnell 2005; McHenry 2011), a mutasome would perform slow, poorly processive, "error-prone" TLS.

Biochemical Characterization of the *E. coli* Umu Proteins and Evolution of TLS Models

At the time of the mutasome model, UmuD′ could be obtained in high purity and yield; not so for UmuC, which formed insoluble inclusion bodies in *E. coli* cell lysates. Woodgate, working with Echols, denatured and then renatured UmuC to obtain soluble UmuC that was included in a biochemical reconstitution assay along with UmuD′, SSB, β-sliding clamp, γ-clamp loader, and Pol III core. Using this combination of proteins to copy a template DNA containing an abasic moiety, a small

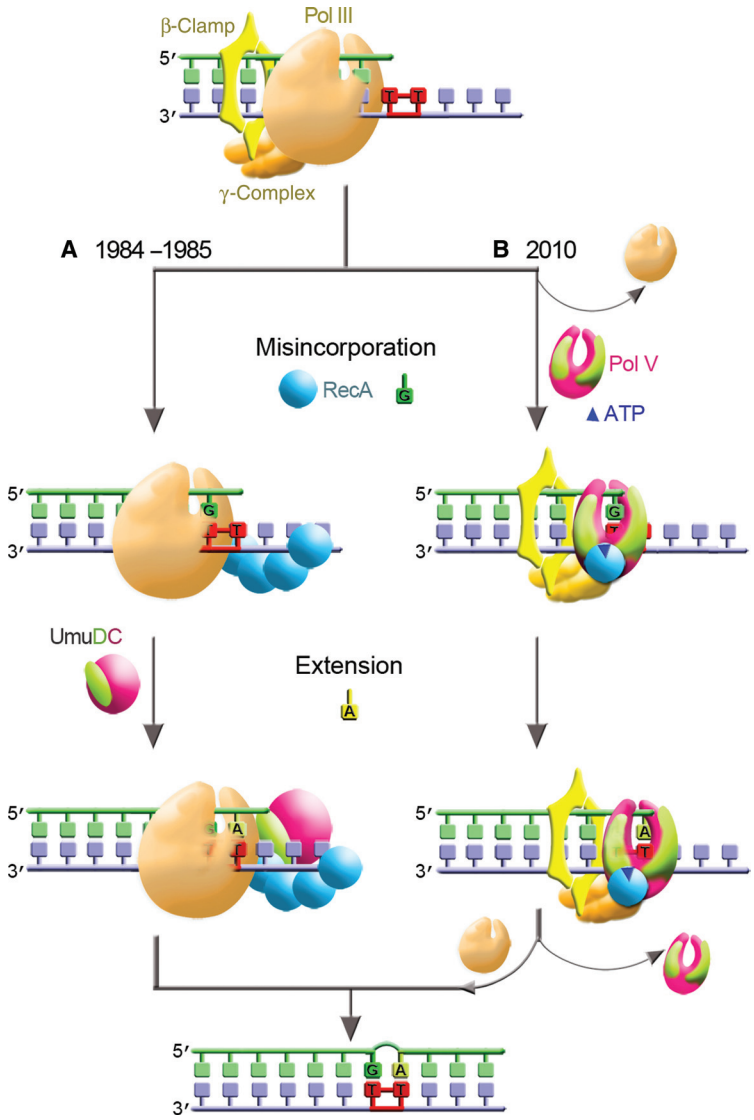

Figure 1. Two-step model for UV mutagenesis in *E. coli.* (*A*) The two-step model for UV mutagenesis proposed in 1984/1985 assumed that TLS in vivo is performed by the replicative Pol III. The first step, nucleotide misincorporation opposite a 3′ T of a CPD, was hypothesized to be mediated by the RecA protein (represented as blue sphere). The misincorporated base was subsequently fixed as a mutation in a second extension/bypass step that was dependent on UmuC (purple) and UmuD (lime) (Bridges and Woodgate 1984, 1985a,b). (*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 as purple UmuC and two lime UmuD′ subunits assembled in the shape of a right hand) (Reuven et al. 1999; Tang et al. 1999), which interacts with RecA* in *trans* (not shown) from which molecules of RecA (blue sphere) and ATP (dark blue triangle) are transferred from the 3′-filament tip to generate Pol V Mut (Jiang et al. 2009). Pol V Mut can perform both the (mis)insertion and extension steps of TLS. After traversing the damaged DNA, Pol V Mut is replaced by Pol III holenzyme, which resumes high-fidelity chromosomal duplication.

amount of primer was extended past the lesion (Rajagopalan et al. 1992). The difficulties with obtaining sufficient quantities of UmuC for detailed biochemical characterization were circumvented in 1996, by the isolation and purification of the UmuD′$_2$C complex (from 200 L of *E. coli*!) (Bruck et al. 1996). When UmuD′$_2$C was used in replication assays, robust TLS was observed. Remarkably, TLS also occurred in the *absence* of Pol III core (Tang et al. 1998). It appeared, therefore, that UmuD′$_2$C by itself had the ability to copy undamaged and damaged DNA templates, and, about a year later, UmuD′$_2$C was conclusively shown to be a DNA polymerase, Pol V (Reuven et al. 1999; Tang et al. 1999).

A key feature of the mutasome model is the assembly of RecA* on ssDNA proximal to the lesion (Fig. 1A). When a replication fork encounters a lesion, an uncoupling of leading- and lagging-strand synthesis may ensue. Then, one of the TLS Pols can replace Pol III on the β-clamp and copy the damaged DNA. If the lesion were present on the leading strand, then DNA unwinding by the DnaB helicase downstream from the lesion would leave a region of ssDNA on which a RecA* filament could form. In the case of a lagging-strand lesion, ssDNA is likely to be present as a consequence of Okazaki fragment synthesis and processing. Thus, for both leading- and lagging-strand lesions, one can easily envision how RecA* could assemble in *cis*, that is, on the template strand being copied, proximal to the lesion. However, the presence of RecA bound in *cis* to the template blocks DNA synthesis and would assuredly have to be removed to permit synthesis to occur beyond the lesion (Pham et al. 2001). Because both RecA* assembly and disassembly normally occur in a 5′→3′ direction (Kuzminov 1999), removal of a blocking RecA* ahead of an advancing Pol V would entail filament disassembly in an "abnormal" 3′→5′ direction (Pham et al. 2001).

The problematic issue of RecA* formation and dissolution on the template strand would be irrelevant if RecA* were absent from the DNA template, but rather acted in *trans* by forming on a non-template ssDNA strand (Schlacher and Goodman 2007; Patel et al. 2010). And that appears to be what is happening. Transactivation of Pol V by RecA* to perform TLS has been shown to take place in vitro (Schlacher et al. 2006; Jiang et al. 2009), giving rise to a Pol V mutasome model for TLS (Fig. 1B). The unanticipated role for RecA* in the transactivation of Pol V provided the key to establishing its biochemical function. By incubating Pol V with RecA* bound to resin and then spinning down the resin-bound RecA*, what remained in solution along with "unreacted" Pol V was a new form of Pol V, named Pol V Mut=UmuD′$_2$C-RecA-ATP (Jiang et al. 2009). Once formed, Pol V Mut copies undamaged and damaged DNA (i.e., performs TLS) in the *absence* of RecA*.

Thus, the direct role of RecA* in SOS-mediated TLS, described by Devoret and colleagues in 1989 (Dutreix et al. 1989), is to transfer a molecule of RecA from its 3′-filament tip along with a molecule of ATP to convert UmuD′$_2$C into Pol V Mut, which can traverse a variety of DNA lesions unassisted (Fig. 1B). Pol V Mut has the unique property that it exists in two conformational states, either activated (able to copy DNA) or deactivated (unable to copy DNA), perhaps depending on where RecA-ATP is bound in relation to the polymerase subunits UmuD′$_2$ and UmuC (Jiang et al. 2009). A deactivated Pol V Mut is completely reactivated when re-exposed to RecA*, which is used to replace the old RecA-ATP with new RecA-ATP from the 3′-filament tip (Jiang et al. 2009). This type of repeating on–off switching has not been observed for any other DNA polymerase, and perhaps provides a way for *E. coli* to protect undamaged DNA from gratuitous mutation, enabling the cell to activate Pol V Mut whenever replication forks have stalled at DNA template damage sites.

POL V ORTHOLOGS

E. coli Pol V has been the subject of intense study, but many orthologs are now known to exist in a wide range of prokaryotes and self-transmissible plasmids that they harbor (Vaisman et al. 2012). Of particular interest are Pol V orthologs MucA′B and RumA′B found on the IncN R-plasmid R46/pKM101 (Perry and Walker 1982) and integrating conjugative element

(ICE) R391 (Kulaeva et al. 1995), respectively. The ability of MucA′B to increase mutagenesis in strains of *Salmonella typhimurium* exposed to a variety of DNA-damaging agents plays an important role in increasing the efficacy of the "Ames test" that was proposed as a possible way to identify human carcinogens (McCann et al. 1975). Although the MucA′B proteins appear to be significantly more mutagenic than their *E. coli* counterparts, the RumA′B proteins are the most potent at promoting cellular mutagenesis than any other Pol V ortholog identified so far (Mead et al. 2007). This is especially worrisome, because the *rum* genes are found on natural isolates of the STX/R391 ICE family of mobile elements that are involved in antibiotic resistance spread among γ-proteobacteria, including virulent strains of *Vibrio cholera* and *Proteus mirabilis*, and their ability to provide genetic diversification to the host bacterium may pose a significant risk to human health (Beaber et al. 2004).

E. coli TLS POL II AND POL IV

Special mention should be made regarding Pol II in relation to SOS and TLS. The discovery in 1970 of Pol II (Knippers 1970) in the De Lucia and Cairns *polA* mutant (De Lucia and Cairns 1969) that had a severely compromised Pol I protein was followed shortly thereafter by the discovery of Pol III (Gefter et al. 1971). The isolation of a conditionally lethal temperature-sensitive Pol III mutant showed that Pol III was absolutely required for *E. coli* survival (Gefter et al. 1971), acting as the principal replicative polymerase responsible for copying almost the entire bacterial genome (Johnson and O'Donnell 2005; McHenry 2011).

Mutations in Pol II, however, proved to be non-informative, at least not until relatively recently (Foster et al. 1995; Rangarajan et al. 1997; Frisch et al. 2010). Pol II was "rediscovered" in 1988, as a polymerase activity isolated from UV-irradiated cells that could replicate past abasic template lesions (Bonner et al. 1988). This activity was induced sevenfold in response to UV radiation that depended on expression of the *lexA* gene. It became evident upon purification that the induced lesion-copying protein was Pol II (Bonner et al. 1988) and that the DNA damage-inducible *dinA* gene identified in 1980 by Kenyon and Walker (Kenyon and Walker 1980) encodes Pol II (Bonner et al. 1990; Iwasaki et al. 1990) and is allelic with the *polB* gene that had been identified in 1970 (Qiu and Goodman 1997). In addition to bypassing abasic sites, Pol II appears adept at bypassing N^2-deoxyguanosine-acetyl aminofluorene (AAF) adducts in an error-prone manner that produces −2 frameshift mutations (Table 1) (Fuchs and Fujii 2007).

The *dinB* gene was identified by Kenyon and Walker in 1980 as being induced as part of the cellular SOS response to DNA damage (Kenyon and Walker 1980). However, its function remained largely unknown for many years aside from a paper in 1986 implicating a role in producing "untargeted" mutagenesis of bacteriophage λ (Brotcorne-Lannoye and Maenhaut-Michel 1986). Some 15 years after the *dinB* locus was first identified, Ohmori et al. (1995) discovered a damage-inducible gene, *dinP*, in *E. coli* that mapped to the same general region of the *E. coli* chromosome as *dinB*. A short time thereafter, the *dinB*/*P* genes were also shown to be allelic (Kim et al. 1997) and encode the Y-family DNA Pol IV (Wagner et al. 1999). DinB orthologs are the most ubiquitous of Y-family polymerases, because they are found in all domains of life (Ohmori et al. 2001). However, like many other TLS polymerases, they are not absolutely essential for life, and some organisms (such as *Saccharomyces cerevisiae*) lack any obvious *dinB* ortholog. DinB's role in TLS was largely overlooked because it is not highly mutagenic (unlike Pol V). Indeed, Pol IV appears to bypass certain N^2-dG adducts (such as N^2-furfuryl-dG) in an error-free manner (Table 1) (Jarosz et al. 2006) and can even copy past N^2-N^2-guanine interstrand cross-links with high fidelity (Kumari et al. 2008).

TLS POLYMERASES IN ARCHAEA AND EUKARYOTES

Ever since the discovery of "reversionless" (*REV*) strains of *S. cerevisiae* (Lemontt 1971) and non-mutable (*umu*/*uvm*) strains of *E. coli* (Kato

and Shinoura 1977; Steinborn 1978) in the 1970s, it was known that cellular mutagenesis is not a passive process but instead required the active participation of certain so-called mutagenesis proteins, which we now know are bona fide DNA polymerases. Over the years, the number of mutagenesis proteins discovered increased steadily, and with the advent of whole-genome sequencing projects, orthologs were identified at an ever-increasing pace. By the mid 1990s, orthologs of the Rev1, Rev3, UmuC, and DinB proteins had been identified in bacteria, archaea, and eukaryotes.

The first archaeal ortholog was reported in the literature in 1996, as a DinB homolog (Dbh) from what was originally believed at the time to be *Sulfolobus solfataricus* (Table 1) (Kulaeva et al. 1996). However, analysis of the sequenced *S. solfataricus* genome (She et al. 2001) identified a different DinB ortholog (Dpo4), which is only 53% identical to the Dbh protein (Boudsocq et al. 2001). Subsequent studies suggest that the original *dbh* gene appears to be from the closely related organism *Sulfolobus acidocaldarius* instead of *S. solfataricus* (Chen et al. 2005; McDonald et al. 2006). Both archaeal Dbh and Dpo4 proteins have proven extremely useful for subsequent structure–function studies into TLS (Table 1) (Ling et al. 2001, 2003, 2004a,b; Silvian et al. 2001; Zhou et al. 2001; Boudsocq et al. 2004; Yang and Woodgate 2007; Wilson and Pata 2008; Pata 2010) (see below).

S. cerevisiae RAD30 was identified in 1997, as an ortholog of *E. coli* UmuC and DinB proteins that is involved in error-free repair of UV damage (McDonald et al. 1997; Roush et al. 1998). In 1999, the *RAD30* gene was subsequently shown to encode Pol η, which can bypass a T-T CPD efficiently and with the same accuracy as undamaged DNA (Johnson et al. 1999b). A human ortholog of *S. cerevisiae* Pol η was also identified in 1999 and shown to possess similar properties to the yeast enzyme (Masutani et al. 1999a; Johnson et al. 2000b; Matsuda et al. 2001), but perhaps more importantly, was found to be defective in humans with the sunlight-sensitive and cancer-prone xeroderma pigmentosum variant (XP-V) syndrome (Johnson et al. 1999a; Masutani et al. 1999a,b). A second Rad30 ortholog (McDonald et al. 1999) and *E. coli* DinB ortholog (Gerlach et al. 1999; Ogi et al. 1999) were identified in humans and mice and were subsequently shown to encode DNA polymerases ι and κ, respectively (Johnson et al. 2000a; Ohashi et al. 2000; Tissier et al. 2000; Gerlach et al. 2001; Zhang et al. 2001). Thus, within a period of a roughly 18 mo, a significant number of the phylogenetically related "mutagenesis proteins," which were once thought to be merely accessory factors to replicative polymerases, evolved into the "Y-family" of DNA polymerases (Ohmori et al. 2001) that can perform unassisted TLS.

To date, only defects in Pol η have been associated with a human disease (XP-V), and this phenotype has been recapitulated in mouse models (Dumstorf et al. 2006; Ohkumo et al. 2006). A loss of Pol η function is also responsible for the absence of mutations at A-T sites in immunoglobulin variable regions during somatic hypermutation (Rogozin et al. 2001; Zeng et al. 2001). Thus, Pol η is responsible for copying damaged DNA (i.e., pyrimidine dimers) accurately, in the avoidance of skin cancer, and for copying undamaged DNA (i.e., Ig V-gene repair gaps) inaccurately, to generate mutations that contribute to antibody diversity (Peled et al. 2008). Mice with defects in Pol η, Pol ι, or Rev1 are viable but do not show any gross phenotypic changes (Ogi et al. 2002; Schenten et al. 2002; McDonald et al. 2003; Jansen et al. 2006). In contrast, homozygous inactivation of murine Rev3 (catalytic subunit of Pol ζ) is embryonic lethal, suggesting that Pol ζ plays additional cellular roles besides TLS (Bemark et al. 2000; Esposito et al. 2000; Wittschieben et al. 2000, 2006). Distinct roles for Pol ζ is supported by the recent discovery of a four-subunit complex, Pol ζ_4 (Rev3-Rev7-Pol31-Pol32) (Baranovskiy et al. 2012; Johnson et al. 2012; Makarova et al. 2012), where the binding of the Pol31 and Pol32 accessory subunits of Pol δ are required for damaged-induced mutagenesis in yeast (Johnson et al. 2012; Makarova et al. 2012).

The molecular mechanism of TLS appears to be conserved from *E. coli* to humans (Fig. 2). Similar to the original TLS model proposed by

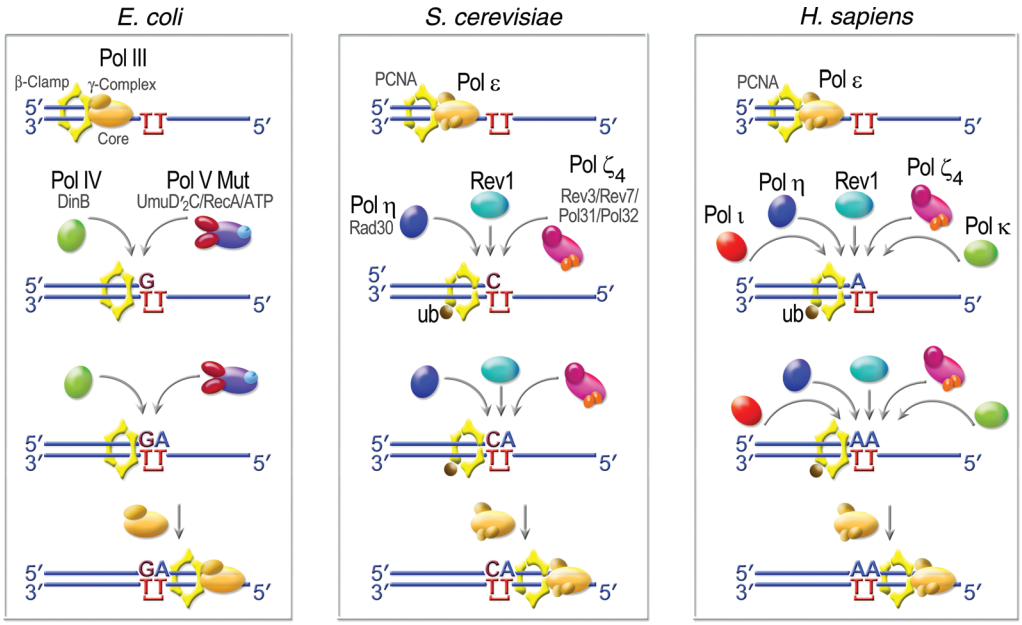

Figure 2. Evolutionarily conserved roles of TLS polymerases. Replicative polymerases such as *E. coli* Pol III or eukaryotic Pol ε stall at the site of a DNA lesion. TLS polymerases are recruited to the site via interactions with the replicative sliding processivity clamp (β-subunit in *E. coli* and PCNA in eukaryotes). *E. coli* has a choice of three TLS polymerases: Pol II, Pol IV, and Pol V Mut (composed of UmuD′$_2$C-RecA-ATP) (Jiang et al. 2009). *S. cerevisiae* also has a choice of three TLS polymerases: Pol ζ_4, Rev1, and Pol η. In humans, at least five TLS polymerases can be recruited to sites of arrested replication, including Pol η, Pol ι, Pol κ, Rev1, and Pol ζ_4. Additional human DNA polymerases, such as Pol β, Pol λ, Pol θ, and/or Pol ν (Maga et al. 2007; Seki and Wood 2008; Shtygasheva et al. 2008; Yamanaka et al. 2010; Hogg et al. 2011; Villani et al. 2011), may also facilitate TLS under certain conditions, but these have been omitted for clarity. The likelihood that this insertion step is error-prone, as shown for *E. coli* and *S. cerevisiae*, or error-free, as shown for humans, will depend on the DNA lesion encountered and the polymerase used for TLS. The extension step may be facilitated by the same enzyme that performed the (mis)insertion or by a different polymerase. Once the nascent DNA chain has been extended beyond the lesion, the TLS polymerase is replaced by the cell's replicative DNA polymerase so as to complete genome duplication.

Bridges and Woodgate in 1985, it is generally thought to occur in two kinetically separable steps: misincorporation followed by extension. In principle, a single TLS enzyme (such as *E. coli* Pol V or human Pol η) can facilitate both steps. However, it is also evident that some TLS polymerases are more proficient at extension (such as Pol η and Pol ζ) than incorporation; thus, TLS not only occurs in two steps but may also require two polymerases for complete lesion bypass to occur (Fig. 2). Support for the proposed polymerase switching model comes from biochemical studies that reveal a dynamic exchange process in which *E. coli* Pol IV (Indiani et al. 2005, 2009; Furukohri et al. 2008) and Pol II (Indiani et al. 2009) can replace Pol III on the β-sliding clamp at a stalled replication fork, with a subsequent resumption of rapid DNA synthesis following the displacement of Pol IV and Pol II by Pol III.

STRUCTURAL INSIGHTS INTO TLS AND MUTAGENIC SPECIFICITY

Probably the biggest surprise to researchers studying TLS was the fact that the mutagenesis proteins turned out to be bona fide (Y-family) DNA polymerases, because the primary amino acid sequence of the proteins show virtually no sequence homology to DNA polymerases from

other families. However, quite remarkably, crystallographical studies of archaeal Dbh and Dpo4 revealed a topology similar to replicases, including domains likened to a right-hand with palm, thumb, and fingers subdomains (Ling et al. 2001; Silvian et al. 2001; Zhou et al. 2001). The TLS polymerases also have an additional domain that has been called the little finger (to coincide with the analogy to a hand) (Ling et al. 2001) or polymerase-associated domain (Fig. 3A,B) (Trincao et al. 2001). As with high-fidelity replicases, residues required for catalysis are located in the palm domain of the Y-family polymerases. In contrast to the replicases, the remaining domains are much smaller and are stubby.

As a consequence, the active site of the Y-family TLS polymerases is much more spacious

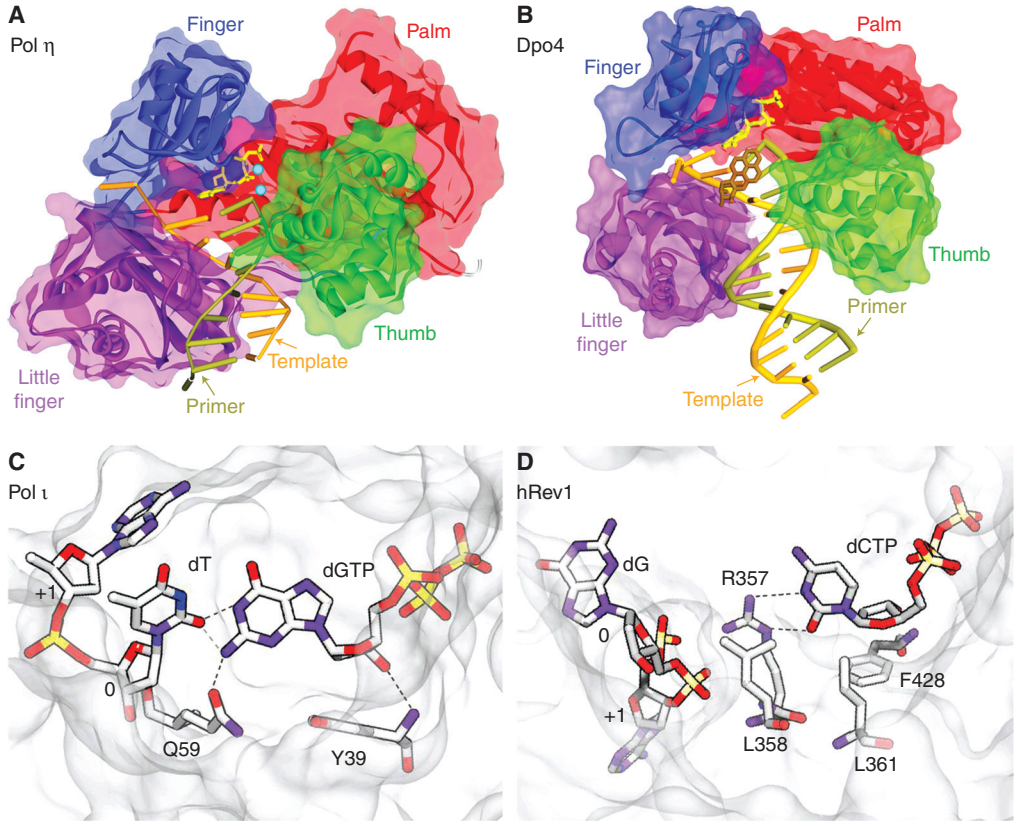

Figure 3. Structural insights into TLS polymerases and their mutagenic specificity. In panels *A* and *B*, the main domains are color-coded: (red) palm; (green) thumb; (blue) fingers; (purple) little finger. (*A*) Crystal structure of human Pol η in a ternary complex with a CPD. In this view, the 3′T of the CPD is in the active site and is correctly paired with incoming dATP (PDB: 3MR3) (Biertümpfel et al. 2010). (Rust) The template strand; (olive green) the primer; (yellow) the incoming dNTP. (Burgundy stick) The position of the CPD; (small blue spheres) the metal ions. The protein backbone is represented by the ribbon surrounded by the semitransparent solvent-accessible surface. (*B*) Crystal structure of the *S. solfataricus* Dpo4 in a ternary complex with DNA containing a benzo[*a*]pyrene lesion (brown) and incoming nucleotide (yellow) (PDB: 1S0M_BP-2) (Ling et al. 2004b). As can be seen, the benzo[*a*]pyrene lesion is flipped into the major groove, so as to accommodate base pairing. (*C*) Human Pol ι making a G:T mispair (PDB:3GV8); note that the template dT and incoming dG are both in an *anti* conformation (Kirouac and Ling 2009) and the mispair is stabilized through hydrogen bonds with Gln59. (*D*) Arg357-directed dC incorporation by human Rev1 (PDB: 3GQC) (Swan et al. 2009). (*C*,*D*) (White/gray) The protein surface; (dotted lines) hydrogen bonds.

and solvent exposed (Ling et al. 2001), and it can often accommodate large bulky DNA lesions that are unable to fit into the tighter constraints of the high-fidelity replicases. Based on a thermodynamic model, it has been proposed that high-fidelity Pols enhance WC base-pairing specificities by partially excluding bulk water from the Pol active cleft (Petruska et al. 1986; Petruska and Goodman 1995). By having a more open architecture that enables water to compete with nucleobases in forming H-bonds, the error-prone Pols generally show less ability to discriminate between forming WC and non-WC base pairs. Having said that, "not all Y-family TLS polymerases are created equal." Pol η appears to have evolved to bypass CPDs efficiently and accurately. It does so by acting as a "molecular splint" straightening the normally kinked damaged DNA backbone, so that the covalently linked pyrimidines of the CPD can be "read" and accurately copied (Fig. 3A) (Biertümpfel et al. 2010).

Structural studies have also revealed that key residues within the active sites of Pol ι and Rev1 contribute to their unique mutagenic "signature." For example, Pol ι is best characterized in vitro for its ability to misincorporate dG opposite template T threefold to 10-fold better than the normal base dA (Table 1) (Johnson et al. 2000a; Tissier et al. 2000; Zhang et al. 2000). This is in contrast to efficient and accurate (for a Y-family polymerase) incorporation of T opposite dA (Johnson et al. 2000a; Tissier et al. 2000; Zhang et al. 2000).

Such diverse properties can be explained by the presence of amino acids (most notably Gln59) of the finger domain that protrude into the active-site cavity and direct the orientation of the templating base. In the case of the G:T mispair, the template base, dT, is always held in an *anti* conformation irrespective of the incoming dNTP. The mispaired dG also adopts an *anti* conformation and is further stabilized by hydrogen bonds with Gln59 (Fig. 3C) (Kirouac and Ling 2009). In contrast to a dT, a template dA is driven into a *syn* conformation by the incoming dTTP, which remains in an *anti* conformation. As a consequence, base pairing is limited so that dA makes a Hoogsteen, rather than WC base pair with the incoming dTTP (Nair et al. 2004, 2006). Similarly, Rev1 is characterized as a dCMP transferase (Nelson et al. 1996a), but this specificity is not dependent on bonding of the incoming dCMP with a template base, but rather is determined by a particular arginine residue (K324 in *S. cerevisiae* and K357 in humans) of the little finger domain of the Rev1 protein (Fig. 3D) (Nair et al. 2005; Swan et al. 2009).

Structural studies with archaeal Dpo4 have been particularly enlightening because the protein readily crystallizes in a ternary complex with an incoming nucleotide and lesion-containing template DNA. There are more than 60 different structures in the PDB database with individual "snapshots" of Dpo4 in the process of facilitating TLS (Vaisman et al. 2012). What is evident is that the enzyme can perform a wide range of molecular gymnastics with either the template base or incoming nucleotide so as to allow TLS to occur. An example is shown in Figure 3B, in which a large bulky benzo[*a*]pyrene adduct linked to dA is flipped into the major groove of the template DNA, so as to allow base pairing of the adducted dA with the incoming T.

Last, we mention *E. coli* Pol II. The apo structure of Pol II was first reported in 1994 (Anderson et al. 1994), but only recently has the ternary complex with template DNA and incoming nucleotide been solved. It appears that Pol II contains many molecular "nooks and crannies" near the active site of the polymerase that allow for manipulation of the lesion-containing template DNA (Wang and Yang 2009). In the case of an N^2-dG-AAF adduct, the template loops out in such a manner that two bases are skipped, leading to the −2 frameshift mutagenesis that is a characteristic of Pol II bypass of AAF adducts (Fuchs and Fujii 2007).

REGULATION OF TLS POLYMERASES

The ability to traverse a wide array of DNA lesions often comes at the price of reduced fidelity when replicating undamaged DNA. With the exception of *E. coli* Pol II, which has intrinsic 3′–5′ exonucleolytic proofreading, none of the

other TLS polymerases has the capacity to excise errors made when duplicating an undamaged DNA template. It is therefore not surprising that cells use several mechanisms to keep the error-prone TLS polymerases in check, so that they are only used as, and when required. Perhaps the best-studied example is in *E. coli*, where the three TLS polymerases are first negatively regulated at the transcriptional level by the LexA repressor. Although no additional regulation of Pol II is required because of its high fidelity ensured by intrinsic proofreading, the Y-family polymerases, Pol IV and Pol V, are exonuclease deficient and are subject to a variety of posttranslational controls.

Most notably, the UmuD′ subunit of Pol V has to be activated via damage-inducible cleavage of UmuD (Nohmi et al. 1988), and the UmuD, UmuD′, and UmuC proteins are all subject to rapid proteolytic degradation by the Lon and ClpXP proteases (Frank et al. 1996; Gonzalez et al. 1998, 2000; Neher et al. 2003). Any $UmuD'_2C$ complex that escapes degradation is essentially catalytically inactive until activated through protein interactions with $RecA^*$ to form Pol V Mut, whose activity is enhanced by binding to the β-clamp (Schlacher et al. 2006; Karata et al. 2012). Pol IV activity is enhanced through protein interactions with UmuD, RecA, and the β-clamp (Wagner et al. 2001; Becherel et al. 2002; Godoy et al. 2007).

Although the *S. cerevisiae RAD30* (Pol η) transcript appears to be induced roughly threefold after DNA damage (McDonald et al. 1997), much of the regulation of TLS polymerases in eukaryotes appears to rely on their posttranslational modification and/or specific protein–protein interactions that target them to the site of DNA damage. A key player in this process is ubiquitin. In *S. cerevisiae*, ubiquitination of Rad30 (Pol η) leads to its rapid degradation by the proteasome (Skoneczna et al. 2007). Similarly, ubiquitination of human Pol η by the E3 ubiquitin ligase Pirh2 results in its degraded by the 20S proteasome (Jung et al. 2010). In addition to ubiquitination via conventional E3 ligases, the human Y-family polymerases can undergo self-ubiquitination that is mediated by their UBMs (found in Pol ι or Rev1) or UBZ motifs (found in Pol η and Pol κ) (Fig. 4) (Bienko et al. 2005, 2010; Guo et al. 2006; Plosky et al. 2006; Jung et al. 2011). These motifs bind ubiquitin in a noncovalent manner and induce a conformational change in the TLS polymerase that prevents any subsequent interaction with other ubiquitinated proteins, such as PCNA. As a consequence, the enzymes are no longer correctly targeted to sites of DNA damage, which precludes their ability to facilitate TLS (Bienko et al. 2005, 2010; Plosky et al. 2006).

The eukaryotic Y-family TLS polymerases are also regulated through key protein–protein interactions. One such interaction is with the replicative clamp, PCNA. The Y-family polymerases possess specific PCNA-binding motifs (PIP-box) that allow for a direct interaction with the clamp in a manner similar to β-clamp binding by the bacterial polymerases (Fig. 4) (Warbrick 1998, 2000; Dalrymple et al. 2001). Upon DNA damage, PCNA is monoubquitinated (Hoege et al. 2002), and this further strengthens the interaction with the TLS polymerases because they bind to the monoubiquitinated PCNA via the PIP box *and* the UBM/UBZ motifs (Bienko et al. 2005, 2010; Guo et al. 2006; Plosky et al. 2006). Another important protein interaction regulating the eukaryotic TLS polymerases is with the Rev1 protein. In addition to its catalytic dCMP transferase activity, Rev1 plays a crucial structural function by interacting with Pol ζ (Lawrence and Hinkle 1996; Nelson et al. 1996b) and with Pol η, Pol ι, and Pol κ (Fig. 4) (Guo et al. 2003; Ohashi et al. 2004; Tissier et al. 2004) and targeting them to sites of DNA damage.

CONCLUDING REMARKS: IS TLS PERHAPS THE TIP OF THE ICEBERG?

Although the TLS polymerases are best characterized by their ability to traverse lesions that would otherwise block replicases, it is becoming increasingly obvious that they also participate in other cellular pathways. In some cases, such as Pol IV–dependent stress-induced mutagenesis in *E. coli* (Bull et al. 2001; Tompkins et al. 2003) or Pol η/Rev1-dependent somatic hypermutation of immunoglobulin genes in humans

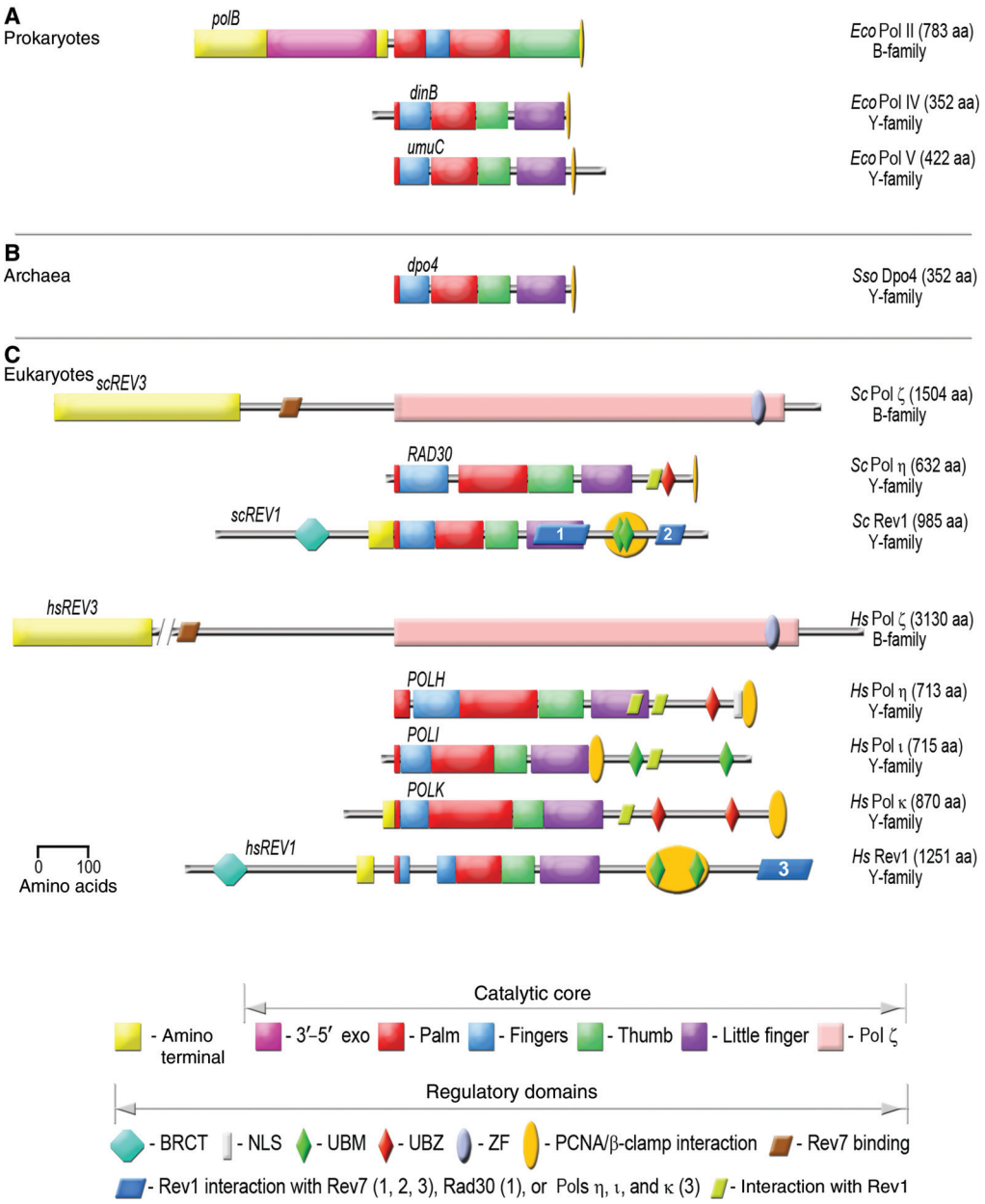

Figure 4. Domain organization of selected TLS polymerases. (*A*) Prokaryotes; (*B*) archaea; (*C*) eukaryotes. The name and phylogenetic family relationship of each of the TLS polymerases along with number of amino acid residues in each polymerase are indicated on the right-hand side of the figure. The structural catalytic domains present in all of the polymerases are color-coded as follows: (red) palm; (green) thumb; (blue) finger; (purple) little finger; (violet) 3′–5′ exonuclease of Pol II; (yellow) amino-terminal domain of Pol II and Rev3; (pink) uncharacterized catalytic domain of Rev3. Additional domains involved in localization and regulation of the TLS polymerases are as follows: (teal octagon) breast cancer-associated protein-1 carboxy-terminal domain (BRCT); (gray rectangle) nuclear localization signal (NLS); (green diamond) ubiquitin binding motif (UBM); (red diamond) ubiquitin binding zinc-finger motif (UBZ); (grayish oval) zinc finger (ZF); (gold oval) PCNA/β-clamp motif; (brown or blue rhomboid) Rev7 binding region; (olive rhomboid) Rev1-interacting region. Species: *Ec*, *E. coli*; *Ss*, *S. solfataricus*; *Sc*, *S. cerevisiae*; *Hs*, *H. sapiens*.

(Rogozin et al. 2001; Zeng et al. 2001), this occurs during error-prone replication of undamaged DNA and generates various mutations, the ultimate source of genetic diversity (Peled et al. 2008). However, there is also evidence that human Pol κ participates in nucleotide excision repair (Ogi et al. 2010) and human Pol η in recombinational repair (Kawamoto et al. 2005; McIlwraith et al. 2005), both of which are thought to be essentially error-free processes.

The heavy emphasis that has been accorded as principal roles for SOS *E. coli* Pols, and more generally for eukaryotic TLS Pols, namely, copying damaged DNA templates, has to a large extent ignored what could well turn out to be an even more important biological function in long-term survival and evolutionary fitness in the absence of exogenous DNA damage. In *E. coli*, eliminating any one of the *E. coli* SOS Pols (Pol II, IV, or V) causes a severe loss of cell fitness, so that when wild-type cells are grown in the presence of cells lacking either Pol II, Pol IV, or Pol V, the wild-type cells take over as 100% of the population of surviving cells, thus driving each of the strains lacking one of the SOS Pols to extinction within ~6–7 days of continuous growth in rich medium (Yeiser et al. 2002). For this relative fitness determination, wild-type and mutant cells compete for nutrient resources when both are inoculated at low but equal population densities ($\sim 10^2$–10^3 cells/mL) and undergo co-outgrowth while growing exponentially. Thus, when competing for nutrient resources that are diminishing with time, the elimination of any one of the SOS Pols is sufficient to ensure the mutant's eventual demise when competing against wild-type *E. coli*.

Our understanding of the molecular mechanisms of TLS has increased dramatically in the past few years, but there is still much to be learned. Although we now know of several regulatory pathways that target the TLS polymerase sites of DNA damage, how the physical switch between the replicative and TLS polymerases occurs in a living cell is still largely unknown, and insights into this process should be forthcoming with the advent of single-molecule visualization of DNA polymerases at replication forks in vivo (Reyes-Lamothe et al. 2010; Lia et al. 2012). Crystal structures of the catalytic domains of the eukaryotic TLS polymerase have been reported along with isolated PIP-box and UBM domains (Bomar et al. 2007, 2010; Hishiki et al. 2009), but to date, there have been no reports of a full-length eukaryotic TLS polymerase. Indeed, such a structure would provide a major advance by allowing us to visualize how the various regulatory domains interact with the catalytic core of the TLS polymerase. It is evident that the TLS polymerases are regulated through a multitude of direct protein–protein interactions and structures of such multiprotein complexes as Pol V Mut ($UmuD'_2C$-RecA-ATP) (Jiang et al. 2009) and Pol ζ_4 (Rev3-Rev7-Pol31-Pol32) (Baranovskiy et al. 2012; Nelson et al. 1996b; Makarova et al. 2012) are critical to further our understanding of TLS.

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