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Translesion synthesis DNA polymerases and control of genome stability

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1. ABSTRACT

Eukaryotic and prokaryotic genomes are replicated with amazingly high fidelity to assure faithful transmission of genetic information from one generation to the next. The accuracy of replication relies heavily on the ability of replicative DNA polymerases to efficiently select correct nucleotides for the polymerization reaction and excise mistakenly incorporated nucleotides using their intrinsic exonucleases. Cell also possess a variety of specialized DNA polymerases that help to overcome replication blocks when occasional unrepaired DNA lesions stall the replication machinery. The translesion synthesis (TLS) polymerases have an extremely low fidelity during copying undamaged DNA substrates, such that uncontrolled participation of these polymerases in DNA replication could present a threat to the genome stability. In this article, we discuss the properties of prokaryotic and eukaryotic TLS polymerases and their roles in modulating the rate of spontaneous and genotoxicant-induced mutations. We also review recent insights into the molecular mechanisms regulating the participation of error-prone TLS polymerases in the genome replication. Finally, we discuss the relationship between the functions of TLS polymerases and human disease.

2. INTRODUCTION

Human cells are now known to contain at least 14 template-dependent DNA polymerases, and five DNA polymerases have been recognized in *E. coli*. All DNA polymerases are classified into several distinct families based on the primary structure of their catalytic subunits. Families A, B and C are defined by homology to *Escherichia coli* DNA polymerases I, II and III encoded by the *polA*, *polB* and *polC* (*dnaE*) genes, respectively. In addition to the prototype enzymes, family A includes mitochondrial replicase Pol gamma and two recently discovered eukaryotic enzymes, Pol theta and Pol nu. Family B includes the eukaryotic replicative enzymes Pol alpha, Pol delta and Pol epsilon, as well as eukaryotic Pol zeta. No representatives of family C other than the DnaE, the *E. coli* replicase, and homologous proteins from other bacteria are known. Family X includes eukaryotic Pol beta, Pol lambda and Pol mu. Family Y (1) includes *E. coli* Pol IV, Pol V, and eukaryotic Pol eta, Pol iota, Pol kappa and a DNA template-dependent deoxycytidyl transferase REV1.

Among the most recently identified members of this group are enzymes that function in replicative bypass of DNA damage that obstruct the progression of replication

Table 1. Replicative and TLS DNA polymerases from *E. coli*, *S. cerevisiae* and humans

Organism	Polymerase	Family	Catalytic subunit		Associated activities
			Mass (kDa)	Gene (alias)	
Replicative DNA polymerases					
E. coli	Pol III	C	130	*polC (dnaE)*	3′ exonuclease
	Pol I	A	103	*polA*	3′ exonuclease; 5’
	Pol II	B	89	*polB*	3’ exonuclease
Eukaryotes[1] (*S. cerevisiae*)	Pol alpha	B	167	*POL1 (CDC17, HPR3, CRT5)*	Primase
	Pol delta	B	125	*CDC2 (POL3, HPR6, TEX1)*	3′ exonuclease
	Pol epsilon	B	256	*POL2 (DUN2)*	3′ exonuclease
Eukaryotes[1] (human)	Pol alpha	B	165	*POLA*	Primase
	Pol delta	B	124	*POLD1 (POLD)*	3′ exonuclease
	Pol epsilon	B	262	*POLE (POLE1)*	3′ exonuclease
TLS polymerases					
E. coli	Pol II	B	89	*polB*	3′ exonuclease
	Pol IV	Y	40	*dinB*	AP lyase; dRP lyase
	Pol V	Y	46	*umuC*	AP lyase; dRP lyase
Eukaryotes (*S. cerevisiae*)	Pol zeta	B	173	*REV3 (PSO1)*	
	Pol eta	Y	72	*RAD30 (DBH1)*	
	REV1	Y	112	*REV1*	TdT (for dC)
Eukaryotes (human)	Pol zeta	B	353	*POLZ (REV3)*	
	Pol eta	Y	78	*POLH (RAD30, RAD30A, XPV)*	
	Pol iota	Y	80	*POLI (RAD30B)*	dRP lyase
	Pol kappa	Y	76	*POLK (DINB1)*	
	REV1	Y	138	*REV1*	TdT (for dC)

[1] Not listed is Pol gamma that replicates mitochondrial genomes in both lower and high eukaryotes.

forks. In *E. coli*, the term “translesion synthesis (TLS) polymerases” is applied to three enzymes, Pol II, Pol IV and Pol V, that are upregulated, along with ~40 other genes, during DNA damage-induced SOS response. In eukaryotes, the B-family enzyme Pol zeta and the four Y-family members are traditionally regarded as TLS polymerases. We will focus on these eight enzymes in our review, although other polymerases, for example, eukaryotic Pol theta and Pol delta, may be able to bypass certain types of DNA lesions as well (e.g., see Refs. 2 and 3).

The properties of Y-family polymerases are strikingly different from those of main replicative enzymes that are responsible for copying the bulk of genomic DNA during the S-phase of the cell cycle. In particular, the error rate during replication of undamaged DNA substrates by the Y-family polymerases is several orders of magnitude higher than typically observed with replicative enzymes. The existence of several DNA polymerases with very low fidelity in the cells suggests the need of careful regulation of their access to the primer terminus to prevent their potential adverse effects on the genome stability. We begin our review by describing the mechanisms that provide the basis for the high fidelity DNA synthesis by replicative DNA polymerases. We then describe the properties and biological roles of TLS polymerases mainly focusing on their contribution to mutagenesis *in vivo* during normal DNA replication and after exposure to exogenous DNA damaging agents. We then review our current understanding of the mechanisms that control the participation of TLS polymerases in replication. We end by briefly describing the connection between TLS polymerases and human disease. Additional information on the properties and function of TLS polymerases can be found in several recent reviews (4-9). Beyond the scope of our review is the role of TLS polymerases in somatic hypermutation, a specialized DNA transaction that occurs in B lymphocytes and is responsible for diversification of immunoglobulin genes. The extensive studies on the relationship between TLS polymerases and somatic hypermutation are discussed elsewere (10, 11).

3. REPLICATIVE DNA POLYMERASES AND CONTROL OF GENOME STABILITY

3.1. Fidelity of replicative DNA polymerases

As mentioned prevously, chromosomal DNA replication is a highly accurate process. The fidelity of replication relies heavily on faithful DNA synthesis by replicative DNA polymerases. Replication of the *E. coli* chromosome is performed by DNA polymerase III holoenzyme (Pol III HE) (Table 1), an asymmetric dimer that simultaneously replicates the leading and lagging strand of replication fork (12). The catalytic subunit of Pol III HE belongs to the C family of DNA polymerases, representatives of which are found only in bacteria. DNA polymerase I, an A-family polymerase performs gap-filling during maturation of Okazaki fragments. In addition, the 3’ exonuclease activity of DNA polymerase II, a B-family enzyme, is implicated in correction of errors during chromosomal DNA replication (47).

In eukaryotes, three B-family DNA polymerases, Pol alpha, Pol delta and Pol epsilon, are implicated in replication of chromosomal DNA in the S-phase of the cell cycle (13). Pol alpha has a tightly associated primase activity that is used to synthesize RNA primers at replication origins and on the lagging DNA strand. Pol alpha extends these RNA primers by synthesizing short stretches of DNA, and then a switch occurs to processive synthesis by Pol delta and/or Pol epsilon. The precise roles of these two polymerases in genome replication are not yet completely understood. In yeast *Saccharomyces cerevisiae*, the most carefully studied organism in this respect, genetic and biochemical evidence is consistent

Table 2. Fidelity of replicative and TLS DNA polymerases during copying of undamaged DNA

DNA polymerase	Family	Error rate (x 10^{-5})		Reference
		Base substitution	Frameshift	
Replicative DNA polymerases				
E.c. Pol III	C	≤0.6	≤0.4	27
yPol epsilon	B	≤2	≤0.05	19
yPol delta	B	≤ 2	1.3	18
yPol alpha	B	9.6	3.1	263
Exonuclease-deficient variants of replicative DNA polymerases				
E.c. Pol III exo$^-$	C	1.7	2.2	27
yPol epsilon exo$^-$	B	24	5.6	19
yPol delta exo$^-$	B	13	7.0	18
TLS DNA polymerases				
E.c. Pol II	B	≤ 0.1	≤ 0.1	40
E.c. Pol IV	Y	20	5.0	144
E.c. Pol V	Y	19	2.3	145
hPol kappa	Y	580	180	143
hPol eta	Y	3500	240	142
hPol iota	Y	72,000[1]		126

[1] The Pol iota value is for the T-dGTP mismatch only. Pol iota error rates and or misinsertion rates for other mismatches vary widely.

with the idea that Pol epsilon performs the bulk of the DNA synthesis on the leading DNA strand and Pol delta takes over Pol alpha to complete Okazaki fragments on the lagging strand (14-16; reviewed in 13).

Table 2 shows error rates for purified replicative and TLS DNA polymerases measured in gap-filling reactions containing all four dNTPs. These rates reflect the ability of a DNA polymerase to incorrectly insert nucleotides and then extend these mismatched primer termini to generate stable misincorporations. In accordance with their important roles in genome replication, *E. coli* Pol III HE and eukaryotic Pol delta and Pol epsilon are among the most accurate DNA polymerases (Table 2; reviewed in Refs. 4, 17). They incorporate incorrect nucleotides at rates of 10^{-5} or less during replication of undamaged DNA templates *in vitro*. A similar fidelity is observed with *E. coli* Pol I. All four polymerases have an intrinsic 3′ exonucleolytic proofreading activity that is used to remove non-complementary nucleotides mistakenly inserted by the polymerases from the primer terminus. The proofreading activity makes a substantial contribution to the fidelity of DNA synthesis. Inactivation of the exonuclease activity of Pol delta and Pol epsilon reduces polymerization fidelity by factors of about 10- to 100- fold, depending on the type of error and the DNA sequence context (Table 2; Refs. 18, 19). The fidelity of Pol alpha that is naturally devoid of proofreading activity is similar to the fidelity of the exonuclease-deficient forms of Pol delta and Pol epsilon, with error rates being in the range of 10^{-4} to 10^{-5} (Table 2). In the subsection below, we will discuss the molecular basis for the fidelity of replicative DNA polymerases, as suggested by studies of crystal structures of DNA polymerases and extensive kinetic analysis.

3.2. Molecular mechanisms determining nucleotide selectivity of replicative DNA polymerases

Most of our current understanding of the mechanisms underlying the high fidelity of replicative DNA polymerases stems from kinetic studies, wherein nucleotide insertion and extension of matched and mismatched primer termini have been analyzed individually in reactions containing correct or incorrect dNTPs, as well as from X-ray structural studies of DNA polymerases bound to template DNA and an incoming dNTP (reviewed recently in Refs. 20-24). The key step in the polymerization reaction that provides the basis for accurate DNA synthesis is a conformational change in the DNA polymerase and template-primer induced upon binding of an incoming dNTP. In the absence of a dNTP, DNA polymerases adopt a so-called "open" conformation. Binding of a dNTP induces conformational changes in the polymerase-DNA-dNTP complex ("closing" of the complex) that result in the assembly of a solvent-inaccessible binding pocket for the nascent base pair. The four correct base pairs are geometrically equivalent and fit snugly in the binding pocket of accurate replicative DNA polymerases. The proper fit is a prerequisite for the formation of the "closed" complex, wherein the alpha-phosphate of the incoming dNTP and the 3′-OH of the primer are positioned in a way required for efficient catalysis. Incorrect base pairs can not easily be accommodated in the active sites due to their improper geometry, and thus make catalysis unfavorable. Protein-DNA contacts that are important for checking the geometry of the newly forming base pair include the polymerase interactions with the DNA minor groove at the active site and for several base pairs upstream of the primer terminus in the duplex DNA.

The key concepts described above emerged mainly from the studies of enzymes belonging to family A, such as T7 DNA Pol and the Klenow fragment of Pol I of *E. coli*, as well as family X (Pol beta) and HIV-1 reverse transcriptase. The structural studies of a B family DNA polymerase, the replicative enzyme of bacteriophage RB69, complexed with DNA and dNTP (25) suggest that these concepts hold true for RB69 Pol, and, therefore, can likely be extended to eukaryotic replicative DNA polymerases as well. No structural information is currently available on C family DNA polymerases that include the replicase of *E. coli* and related bacteria. It remains to be determined whether the mechanism of error avoidance involving formation of the nucleotide binding pocket is shared by the C family enzymes.

3.3. Sequential correction of DNA replication errors by exonucleolytic proofreading and DNA mismatch repair

Although the geometric selection mechanism provides the basis for efficient discrimination against mispairs, incorrect nucleotides are sometimes inserted by replicative DNA polymerases. At least some of the misinsertions may result from the incorrect base pair adopting an unusual conformation with geometry suitable for catalysis. Such misinsertions create mismatched primer termini that are extended at least 10,000-fold less efficiently than matched primers by replicative DNA polymerases (reviewed in Ref. 26). For polymerases with intrinsic proofreading exonuclease activity, slow mismatch extension allows the primer terminus to fray and move to the exonuclease active site, and the incorrect nucleotide can be thus removed by the exonuclease (reviewed in Ref. 23). For polymerases with no intrinsic exonuclease activity, slow mismatch extension could allow dissociation of the enzyme from the template, which would make it possible for an extrinsic exonuclease to correct the mismatch.

As mentioned above, the *E. coli* replicase Pol III HE and two of the three eukaryotic replicative DNA polymerases, Pol delta and Pol epsilon, have proofreading exonucleases that correct over 90% of errors made by these polymerases during DNA synthesis *in vitro* (Table 2; Refs. 18, 19, 27). Contribution of proofreading by replicative DNA polymerases in the overall fidelity of DNA replication *in vivo* can be estimated by analyzing the mutation rate in cells that have proofreading-deficient versions of these polymerases. Mutations eliminating the 3′ exonuclease activity of Pol delta result in up to a several hundred-fold increase in the mutation rate in yeast (28-30) and at least in a 15-fold increase in mice (31). Inactivating the proofreading function of Pol epsilon results in up to a 40-fold elevation of the mutation rate in yeast (32). This represents only minimal estimates of the contribution of proofreading by Pol delta and Pol epsilon to replication fidelity *in vivo*, since exonucleases of these two polymerases can compensate for the absence of each other, presumably by correcting each other's errors (33). Recent evidence suggests that the exonuclease of Pol delta can also correct errors made by proofreading-deficient Pol alpha, thus reducing the contribution of this less accurate polymerase to genome instability (15).

The amazingly high fidelity with which replicative DNA polymerases with the proofreading activity synthesize DNA is still not sufficient to accurately replicate large eukaryotic genomes, such as the three billion base pairs human genome. The rare nucleotide misinsertions that escaped both the geometry check at the polymerase active and proofreading give rise to mismatches in the newly formed double-stranded DNA. If left uncorrected, these mismatches will result in mutations after subsequent rounds of replication. *In vivo*, most of these mismatches are corrected by the DNA mismatch repair system (MMR) (reviewed in Ref. 34). MMR reduces the rate of mutation by 10 to 10,000-fold depending on the reporter gene in bacteria, yeast and human cell lines. The critical role of correcting DNA polymerase errors for the stability of the eukaryotic genome is demonstrated by the fact that defects in MMR in humans are associated with hereditary non-polyposis colorectal cancer characterized by a mutator phenotype, as well as several types of sporadic cancers.

4. TRANSLESION SYNTHESIS DNA POLYMERASES

The fidelity mechanisms described above have evolved to ensure accurate and efficient DNA synthesis on undamaged template DNA. However, cellular DNA is continuously damaged by genotoxic products of normal cellular metabolism, for example, reactive oxygen species, as well as by exogenous agents, for example, ultraviolet (UV) irradiation or environmental contaminant benzo[*a*]pyrene. Although cells possess specialized repair pathways that remove various types of DNA lesions, DNA damage occurs throughout the cell cycle, including the S-phase. The replication machinery, therefore, occasionally encounters damaged DNA templates. Many types of lesions present a block for replicative DNA polymerases that can snugly accommodate only the four correct Watson-Crick base pairs in their active sites. The molecular details of the replication block were recently revealed by crystallographic studies of the A family T7 DNA polymerase and B family RB69 DNA polymerase with DNA containing lesions (Refs. 35-38; reviewed in Ref. 39). The presence of a lesion at the active site disrupts the proper contacts between the protein and DNA and makes these DNA polymerases remain in an open, catalytically inactive conformation. In the past decade, several new DNA polymerases were discovered that have the ability to bypass lesions in template DNA that block replicative DNA polymerases. In the following subsections, we will review the properties of these polymerases, including their structural features and fidelity during DNA synthesis on undamaged and damaged DNA templates, the roles of these enzymes in maintaining the genome stability, and the recent insights into the mechanisms regulating their function during DNA replication.

4.1. Overview of *E. coli* DNA polymerases induced during the SOS response

DNA polymerase II (Pol II) encoded by the *polB* (*dinA*) gene belongs to the B-family of polymerases. Like eukaryotic replicative B family polymerases, Pol delta and Pol epsilon, Pol II is a high fidelity enzyme possessing 3→5' exonuclease activity (40). The constitutive intracellular concentration of Pol II (30-50 molecules/cell) is comparable to the estimated concentration of Pol III HE (30 molecules/cell) (41, 42). During the UV-induced SOS response, Pol II is induced seven-fold (42-45). Available data suggests that Pol II plays a key role in "error-free" replication restart after UV-irradiation (46). It can also substitute for Pol III HE when the latter has difficulties extending a mismatched primer terminus (47). In addition, Pol II was implicated in episomal DNA replication *in vivo* (48), as well as in DNA synthesis in non-dividing cells (49-51). Pol II also contributes to the repair of DNA damaged by UV irradiation (52), oxidation (49) and to the repair of inter-strand cross-links (53).

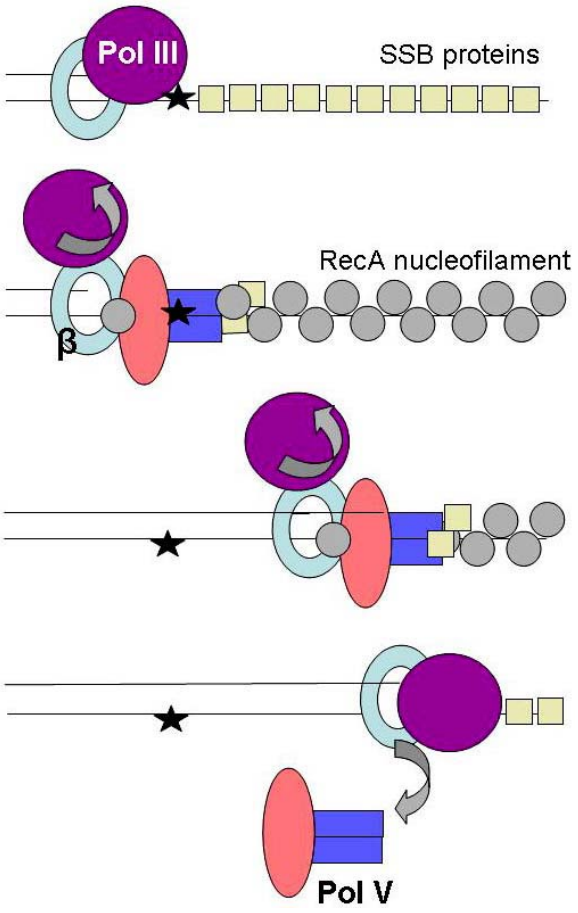

Figure 1. Schematic model of TLS in *E. coli*. Pol III bound to the processivity beta clamp performs DNA synthesis up to the blocking lesion. The lesion impedes the Pol III-mediated replication, and a switch to Pol V occurs. Pol V, in the presence of RecA nucleofilament, continues DNA synthesis through the lesion. A fragment of DNA is synthesized, then Pol V dissociates allowing Pol III to rebind the primer terminus and continue processive replication. SSB protein is not required for minimal TLS (264). Goodman and coworkers suggested that SSB molecules act together with Pol V as a "locomotive cowcatcher" that removes RecA monomers from the 3' end of the filament (64). Experiments by Livneh laboratory (63) also suggest that SSB is necessary for TLS. In contrast, it was shown by Fujii et al (62) that TLS reaction does not require SSB.

DNA polymerase IV (Pol IV), a member of the Y family of DNA polymerases, is encoded by the *dinB* gene. The basal level of Pol IV in uninduced cells is fairly high (250 molecules per cell) (54). The *dinB* gene is induced 10-fold during the SOS response, producing 2500 molecules of Pol IV per cell (54). The physiological role of DNA Pol IV is not well defined. Pol IV is able to carry on error-free or error-prone TLS, depending upon the nature of DNA damage and sequence context (55). It was proposed that an important function of this polymerase may be to restart stalled replication forks (6). Pol IV is involved in the generation of spontaneous mutations in a variety of circumstances including adaptive mutation (Refs. 50, 56, 57; see also Sections 4.6 and 4.7). Recently, apurinic/apyrimidinic 5'-deoxyribose phosphate (AP/5'-dRP) lyase activities intrinsic to Pol IV polymerase were observed *in vitro* (58), suggesting that Pol IV may be also involved in base excision repair.

DNA polymerase V (Pol V) is a heterotrimer built of the catalytic subunit encoded by the *umuC* gene and two identical subunits that are modified products of the *umuD* gene (UmuD'). The products of the *umuC* and *umuD* genes were known for many years to play a critical role in DNA damage-induced mutagenesis, but were only recently recognized as subunits of a DNA polymerase (59, 60). In uninduced cells, the number of Pol V molecules is below a detectable level (<15 per cell) (61). During SOS induction, the levels of UmuD and UmuC increase to approximately 2400 and 200 molecules per cell, respectively. How many molecules of active Pol V are assembled in fully SOS induced cells is an open question. Pol V requires the presence of RecA protein, β-clamp (a processivity factor of Pol III HE), and single-stranded DNA binding protein (SSB) for efficient TLS (Figure 1), although the influence of these accessory proteins varied depending on experimental conditions, TLS assay, different Pol V preparations, and different DNA substrates (60, 62-65). Pol V catalyzes TLS through several types of lesions performing both nucleotide insertion opposite the lesion and extension of the resulting primer terminus. These include TT *cis-syn* cyclobutane dimers (62, 66), AP sites (59, 60), TT (6-4) photoproducts (62, 66), and covalent adducts like N-2-acetylaminofluorene-dG (62). Like Pol IV, Pol V was reported to have an associated AP lyase and 5′-dRP lyase activities (58).

4.2. Overview of eukaryotic DNA polymerases capable of lesion bypass

Pol eta, a member of the Y family of DNA polymerases identified in 1999 (67, 68), is the product of the *POLH*/*RAD30*/*XPV* gene in humans and the *RAD30* gene is yeast. Discovery of Pol eta caused a particular interest, because mutations in the *XPV* gene in humans lead to a rare disease, a variant form of xeroderma pigmentosum (XPV) (68, 69). This disorder is characterized by high susceptibility to sunlight-induced skin cancer (70), and cells of XPV patients are defective in replication of UV-damaged DNA (71, 72). A distinctive feature of Pol eta is its ability to efficiently bypass a *cis-syn* thymine-thymine (TT) dimer, a major lesion generated by UV irradiation, incorporating predominantly two As opposite the two Ts of the dimer. Consistent with this, both the phenotypes of human XPV patients and genetic studies in yeast suggest a role for Pol eta in reducing the frequency of UV-induced mutations (see below). Although the bypass of TT dimers appears to be a key biologically significant function of Pol eta, this polymerase can perform DNA synthesis on templates containing a number of other adducts *in vitro* with varying efficiencies. These include 8-oxoguanine, a common product of oxidative damage, ^{6}O-methyl guanine and others (reviewed in Refs. 6, 8). It remains to be

established whether Pol eta plays a role in the bypass of these lesions *in vivo*. Pol eta is also involved in somatic hypermutation of immunoglobulin genes (see Refs. 11 and 73 for review). In addition, it has recently been reported that Pol eta is required for homologous recombination-dependent gene conversion in immunoglobulin genes, another process occurring during antibody diversification (74). Consistent with this, Pol eta, unlike other DNA polymerases, is able to use D-loop DNA structures that are generated during recombination as a substrate for DNA synthesis, suggesting an important role for Pol eta in homologous recombination (75).

Pol kappa is a homolog of *E. coli* Pol IV. Multiple studies suggest a role for this enzyme in the bypass of polycyclic aromatic hydrocarbon (PAH) DNA adducts, such as lesions generated by BPDE that are not efficiently bypassed by other polymerases. BPDE is a mutagenic metabolite of benzo[*a*]pyrene, a common environmental contaminant (76). BPDE reacts preferentially with the N^2 position of guanine in DNA to form covalent BPDE-N^2-dG adducts, mostly (+)-*trans*-*anti*-BPDE-N^2-dG adduct (77-80). This lesion can be removed by DNA repair enzymes, but if left unrepaired, the adduct blocks DNA replication by replicative DNA polymerases (see, for example, Ref. 81). Pol kappa bypasses the BPDE-N^2-dG adduct *in vitro*, inserting primarily a C opposite the damaged G (82, 83), which would reduce the mutagenic potential of this lesion. Indeed, Pol kappa-deficient mouse cells are hypersensitive to benzo[*a*]pyrene, show increased benzo[*a*]pyrene-induced mutagenesis (84) and are specifically defective in the bypass of a site-specific BPDE adduct introduced on a plasmid (85). Expression of the gene encoding Pol kappa is under the control of the arylhydrocarbon receptor (AhR) that mediates conversion of benzo[*a*]pyrene to BPDE (86). Treatment of human cells with BPDE induces localization of Pol kappa in nuclear foci coinciding with sites of active DNA synthesis (87). Finally, Pol kappa is specifically required for recovery from the S-phase checkpoint arrest that mammalian cells undergo after exposure to BPDE (87). In addition to its involvement in the bypass of PAH adducts, Pol kappa has a remarkable ability to extend aberrant primer termini that have their 3' terminal nucleotide paired with different damaged nucleotides (88, 89). It remains to be determined whether the extension capacity of Pol kappa is utilized during the bypass of lesions other than PAH adducts *in vivo*.

Pol zeta is the only eukaryotic TLS polymerase that belongs to B family of DNA polymerases. The B family includes the three eukaryotic replicative enzymes, Pol alpha, delta and epsilon, as well as replicases of a number of bacteriophages and viruses. The B family enzymes are typically accurate when replicating undamaged DNA templates and are blocked by lesions that distort helix geometry. Apparently, Pol zeta has evolved to possess structural features that differ from the other polymerases in the family and that provide the basis for the TLS capacity. Pol zeta from yeast *Saccharomyces cerevisiae* is a two-subunit enzyme encoded by the *REV3* and *REV7* genes. Homologs of both genes are present in high eukaryotes, and there is good evidence that the products of human and mouse *REV3* gene encoding the catalytic subunit of Pol zeta are involved in TLS (90-92). The two-subunit Pol zeta has not been purified from mammalian cells, however, and our understanding of the biochemical properties of this enzyme is based on the studies of yeast Pol zeta. The yeast enzyme is a template-directed DNA polymerase that can synthesize, with limited efficiency, past several types of DNA lesions that block DNA synthesis by normal replicative DNA polymerases (93, 94). The main function of Pol zeta, however, appears to be the extension from nucleotides incorporated opposite DNA lesions by other DNA polymerases. Like Pol kappa, Pol zeta is renowned for its ability to efficiently extend mismatched primer termini or those containing a terminal nucleotide opposite a non-coding or distorting lesion (reviewed in Refs. 8 and 95). Bypass of a variety of lesions can be accomplished *in vitro* through insertion of a nucleotide opposite the lesion by one of the Y family DNA polymerases or by replicative Pol delta followed by extension of the resulting primer terminus by Pol zeta (96-99). *In vivo*, Pol zeta is responsible for nearly all mutagenesis induced by various DNA damaging agents, indicating that Pol zeta-mediated TLS is mostly error-prone (see Sections 4.5 and 4.6 for a detailed discussion of the role of Pol zeta in genome instability). Consistent with the importance of Pol zeta-mediated TLS in providing DNA damage tolerance, mutations in the yeast *REV3* gene confer sensitivity to many mutagens. The list of these mutagens includes, among others, intra-strand cross-link (ICL)-inducing agents (100-102). This suggests that Pol zeta may play a role in ICL repair, although it is not known whether the TLS ability of Pol zeta or other features of this polymerase are critical for this function. In mammalian cells, Pol zeta may have another important function, in addition to TLS. In addition to the DNA polymerase domain, the mammalian REV3 protein contains a large N-terminal part which is not present in its yeast homolog and the function of which is not known. While inactivation of the *REV3* gene has no effect on viability of yeast cells, *Rev3*$^{-/-}$ mouse embryos die around mid-gestation, suggesting the importance of Pol zeta for normal development (103-107).

REV1 is a template-dependent deoxycytidyl transferase. Although it belongs to the Y family of DNA polymerases, REV1 is not a *bona fide* DNA polymerase, since the only reaction that it efficiently performs is incorporation of C opposite G or opposite a non-coding lesion, such as an abasic site, in the template DNA (108-110). Recent structural studies revealed a unique mechanism of nucleotide incorporation by REV1 that is not shared by any of the known DNA polymerases (111). In the crystal structure of yeast Rev1 with template DNA and an incoming dCTP, the nucleotide pairs with an arginine residue in the protein rather than with the template G, which explains the high specificity of the enzyme for insertion of a C. The template G is flipped out of the DNA helix and makes hydrogen bonds with a part of the protein. The interactions are such that neither one of the other three nucleotides, A, T or C, could be stably bound at this position. This, in turn, explains the strong preference of REV1 for a template G. REV1 functions in TLS in concert with Pol zeta. Human, mouse and yeast REV1 are known

to interact with REV7 (112-115). Genetic studies in yeast indicate that all three proteins, REV3, REV7 and REV1 are required for the mutagenic bypass of a number of lesions, including those generated by UV light, ionizing radiation and alkylating agents (reviewed in Ref. 95). However, the essential role of REV1 in TLS likely does not reflects its deoxycytidyl transferase activity, but another, yet undefined function of this protein. In the case of UV-induced lesions, the incorporation of C opposite the lesion is rarely seen. The C is frequently inserted opposite an abasic site in yeast (116-119), and the incorporation of C is specifically lost in mutants defective in the deoxycytidyl transferase activity of Rev1. However, the overall efficiency of TLS and the frequency of DNA damage-induced mutation is not affected in these mutants (96, 118), indicating that another polymerase can efficiently substitute for Rev1 when it is inactive. On the other hand, mutations affecting the BRCT domain of REV1 cause a defect in induced mutagenesis (117, 118, 120). This implies that the role of REV1 in the Pol zeta-dependent TLS is largely structural. In addition, REV1 has recently been shown to interact with Pol eta, Pol iota and Pol kappa, with the interaction requiring the same ~100 C-terminal amino acid residues of REV1 (113, 121, 122), suggesting that REV1 may act as a scaffold to regulate the assembly of various DNA polymerase complexes at the replication fork.

Pol iota is the only Y-family member, whose involvement in TLS *in vivo* has not been demonstrated. *In vitro*, Pol iota can incorporate nucleotides opposite several types of lesions, but is not able to extend the resulting primer termini (reviewed in Ref. 123), such that efficient bypass requires extension by a second DNA polymerase (see, for example, Refs. 98, 124, 125). In addition to the ability to participate in TLS, Pol iota has a unique nucleotide insertion specificity that is strikingly different from all other known DNA polymerases. First, it inserts dTMP opposite an A in the DNA template much more efficiently than it forms the other three Watson-Crick base pairs (126-128). Second, it misinserts dGMP opposite a template T at a rate that exceeds that of correct dAMP insertion opposite T, thus being the only known DNA polymerase that roughly violates Watson-Crick base-pairing rules (98, 126-128). Third, on templates that contain two or more consecutive T's, Pol iota preferentially incorporates dGMP only opposite the first template T residue, which is followed by preferential correct dAMP incorporation opposite the second template T (126, 129). The discovery of these unusual properties of Pol iota has brought about several ideas regarding its possible *in vivo* functions. The efficient insertion of T opposite A could be utilized during base excision repair (BER) of DNA containing uracil residues resulting from dUMP incorporation during DNA replication. On the other hand, the preferential insertion of G opposite T could help replace dG if it is removed by a glycosylase from a G-T or G-U mismatch that resulted from deamination of a 5-methyl-cytosine or cytosine in DNA (126). The presence of a dRP lyase activity in Pol iota (126) is consistent with its possible role in BER. Finally, an attractive possibility is that Pol iota functions during TLS of uracil-containing cyclobutane pyrimidine dimers that result from deamination of cytosine in UV-induced TC photoproducts (130). Such deamination occurs at a high rate *in vivo*, and incorporation of G opposite the 5'U of the deaminated photoproduct followed by correct incorporation of A opposite the 3'T of the photoproduct could help reduce the mutagenic potential of this lesion. To date, all of these ideas remain speculative. More clues to the *in vivo* role of Pol iota are expected to come from analysis of phenotypes of Pol iota-deficient cells.

4.3. Structural basis for TLS capacity

As described above, accurate replicative DNA polymerases undergo a conformational change upon binding of a correct dNTP, which results in the assembly of a tight binding pocket for the nascent base pair. A strikingly different way of accommodating nucleotides at the active site is revealed in structural studies of the Y-family DNA polymerases. To date, crystal structures have been described for two Y family members from archea, Dpo4 from *Sulfolobus solfataricus* and Dbh from *Sulfolobus acidocaldarius*, as well as for human Pol eta, Pol kappa and Pol iota (131-140). Among those are structures of complexes of Dpo4 DNA polymerase with several DNA lesions, including a *cis-syn* thymine dimer (131), an abasic site (133), and a BPDE adduct (134). The Y family TLS DNA polymerases have the same overall domain organization as the replicative DNA polymerases, with thumb, palm and fingers subdomains and the catalytic amino acid residues in the palm subdomain. However, Y family DNA polymerases have an extra DNA binding domain and a preformed large and solvent-accessible active site that can accommodate a variety of bulky lesions and aberrant base pairs. For example, studies of the crystal structure of Dpo4 complexed with DNA containing a *cis-syn* thymine dimer have shown that both thymines of the dimer are accepted in the active site at the same time (131). The 3'T forms a Watson-Crick base pair with an A at the 3' end of the primer, while the 5'T of the dimer forms a Hoogsteen base pair with an incoming nucleotide. In the complex of Dpo4 with DNA containing an abasic site, a frameshift intermediate is observed at the active center with an extra nucleotide in the minor groove (133). The binding pockets of these polymerases are formed by smaller side chains, and much less interaction is seen between the protein and the DNA minor groove. Apparently, these polymerases do not closely check for correct geometry of the newly forming base pair, which explains their remarkable TLS abilities. More comprehensive discussions of the structural features of TLS polymerases can be found in several recent reviews (8, 39, 141).

4.4. Fidelity of TLS polymerases during replication of undamaged DNA

The features of the TLS DNA polymerases that provide the basis for efficient lesion bypass, namely the large solvent-accessible active sites, have dramatic consequences for the fidelity with which they copy undamaged DNA templates. The Y family enzymes are the least accurate DNA polymerases studied to date. This is consistent with the results of structural studies suggesting that the principle of geometric selection, which is the key fidelity mechanism for replicative DNA polymerases, is not

used by the TLS polymerases (see Section 4.3 and Refs. 21 and 73 for review). The Y-family DNA polymerases are also devoid of proofreading 3'→5 exonuclease activity. Table 2 lists the average rates at which purified *E. coli* Pol IV and human Pol eta, Pol kappa and Pol iota generate single base substitutions and single base deletions during gap-filling DNA synthesis on undamaged DNA templates *in vitro*. The base substitution error rates for Y-family polymerases typically range from 10^{-1} to 10^{-3} (Refs. 126, 142, 143; reviewed in Ref. 73). Rates for individual errors can vary more than 1,000-fold depending on the type of the error and the DNA sequence context. A peculiar error signature is observed for Pol iota that generates template T-dGMP mispairs at a rate approaching 1 (see Section 4.2 and Table 2). At the same time, Pol iota discriminates against other types of mismatches substantially better. For example, misinsertions opposite a template A occur at a rate of ~10^{-4}, which is about 100-fold less frequently than during DNA synthesis by Pol eta or Pol kappa.

Surprisingly, *E. coli* Pol IV appeared to be significantly more accurate than other Y-family polymerases, with an average base substitution error rate of 0.5 x 10^{-4} (144). Another striking feature of Pol IV is the predominant generation of frameshifts (80%) compared to the base substitutions (17%). Although human Pol kappa and Pol eta also generate frameshift mutations at a high rate, their base substitution fidelity is extremely low, such that base substitutions predominate in the spectrum of errors that they make. Kobayashi *et al*. (144) have shown that a "dNTP-stabilized" misalignment mechanism is responsible for the creation of -1 frameshift mutations by Pol IV. Incoming dNTP is incorporated "correctly" opposite a complementary downstream template base on a misaligned ('looped out") primer/template DNA. Such a mechanism has been postulated previously when the Y-family DNA polymerase Dpo4 was crystallized as a ternary complex with DNA and an incoming nucleotide (132). In this complex, Dpo4 accommodates two undamaged template bases in its active site, and the incoming nucleotide pairs with the second (5') template base. Pol V exhibits a 5- to 10-fold lower fidelity for base substitutions than Pol IV, with the error rates being around 10^{-4} to 10^{-3}, as measured by steady-state kinetics (66) and a gap-filling assay (145). However, Pol V mostly generates base substitutions, such that the rate of frameshift mutations is about 10-fold lower than that seen with Pol IV (145).

The base substitution fidelity of a DNA polymerase is determined by the ability of this polymerase both to misinsert a nucleotide and to extend the resulting mismatched primer terminus. In the same way, the frameshift fidelity is determined by the rate at which a misalignment is generated in the primer-template and the rate at which the misaligned primer is extended. The kinetic studies have shown that, among the Y-family polymerases, Pol kappa has an extraordinary ability to efficiently extend primers with 3'-terminal mismatches (89) that are poor substrates for most of the other DNA polymerases. Interestingly, Pol zeta, a B-family polymerase whose fidelity during copying of long stretches of undamaged DNA has not been described yet, is also known to possess an extraordinary mismatch extension capacity (96, 98). This property of Pol zeta and Pol kappa has important implications for the roles of these polymerases in regulating the genome stability, since it suggests that these two polymerases can contribute to mutagenesis by extending mismatches created by other polymerases (see Sections 4.6 and 4.7). *E. coli* Pol IV extends mismatched primer termini 10- to 1000-fold less efficiently than its human counterpart, Pol kappa (89, 144), which is consistent with the higher fidelity of Pol IV during gap-filling DNA synthesis (Table 2).

E. coli Pol II is distinct from all known specialized TLS polymerases from prokaryotes and eukaryotes. Similar to replicative DNA polymerases, it is highly accurate, with base substitution and frameshift error rates being $\leq 10^{-6}$ and with proofreading 3'→5 exonuclease activity making an important contribution to the fidelity (40).

4.5. Role of TLS polymerases in DNA damage-induced mutagenesis

DNA lesions can be bypassed accurately, *i.e.* in a way that restores the original DNA sequence, or inaccurately, *i.e.* in a way that generates mutations. The fidelity of TLS depends on the type of lesion and on the particular DNA polymerase that accomplishes the bypass. In eukaryotes, there is extensive evidence that TLS dependent on Pol zeta and Rev1 is mutagenic. In yeast *Saccharomyces cerevisiae*, deletion of the gene encoding either one of the two subunits of Pol zeta or Rev1 protein eliminates nearly all mutagenesis associated with exposure to almost every exogenous DNA damaging agent that has been tested (reviewed in Ref. 95). In mammalian cells, the product of the *REV3* gene has been shown to be required for the vast majority of mutagenesis induced by UV light and BPDE (90-92), and the product of the *REV1* gene is required for the UV-induced mutagenesis (146). As mentioned in Section 4.2, Rev1 contributes to induced mutagenesis not through its deoxycytidyl transferase activity, but through another, possibly structural, function. At the same time, DNA polymerase activity of Pol zeta is apparently required for the mutagenic lesion bypass, since point mutations in the polymerase active site of Pol zeta abolish UV-induced mutagenesis (Ref. 184; M. Diaz, personal communication). This is consistent with the *in vitro* data that the efficient bypass of most DNA lesions requires extention of aberrant primer termini by Pol zeta.

Quite contrary to Pol zeta, a major function of Pol eta is thought to be the prevention of UV-induced mutagenesis by performing mostly accurate replicative bypass of thymine-thymine dimers. Pol eta-deficient human cells show increased frequency of UV-induced mutations (147-150). The fidelity of Pol eta during DNA synthesis on CPD-containing DNA *in vitro* is low. The most frequent error, dGMP incorporation opposite 3'T of the dimer, occurs at a rate of 3.9×10^{-2} (one incorrect dGMP incorporation for every 26 bypass events), which is similar to the fidelity of Pol eta with undamaged DNA templates (151). However, even with this high error rate, Pol eta helps avoid mutations, since in the absence of Pol

eta bypass of CPDs apparently occurs through a pathway that is more error-prone. Genetic data in yeast suggest that Pol eta may reduce the incidence of UV-induced C->T transitions mediated by the formation of CPD and (6-4) photoproducts at CC and TC sequences (152), as well as reduce the mutagenic potential of other lesions produced by sunlight, including 8-oxoguanine (153, 154). Pol eta also reduces the frequency of mutations induced by methylmethane sulfonate (155). Studies of the replicative bypass of individual site-specific lesions *in vivo* have suggested that Pol η can contribute to mutagenic bypass of certain lesions. For example, Pol η may be involved in generation of 3'-T->C transitions during the bypass of a T(6-4)T photoproduct (156), and, to some extent, in mutagenic bypass of *N*-2-acetylaminofluorene (AAF) (156) and acrolein-derived deoxyguanosine adducts (157, 158).

Available evidence suggests that Pol kappa contributes to genome stability by reducing mutagenicity of benzo[*a*]pyrene-dG adducts (84, 85). It remains to be determined whether Pol kappa helps avoid, or, *vice versa*, generate mutations induced by other DNA lesions. It also remains an open question whether Pol iota plays any role in modulating DNA damage-induced mutagenesis.

In *E. coli*, Pol V is the major polymerase involved in damage-induced mutagenesis. Mutants lacking Pol V are completely deficient in UV-induced mutagenesis (159, 160), suggesting the involvement of Pol V in error-prone bypass of major UV photoproducts. Indeed, the nucleotide insertion specificity of Pol V during bypass of TT *cis-syn* cyclobutane dimers and TT (6-4) photoproducts *in vitro* corresponds to the specificity of mutations induced by UV light *in vivo* (66). Analysis of TLS through site-specific lesions *in vivo* have also indicated that Pol V is involved in mutagenic bypass of TT (6-4) photoproducts (161). Pol V can also contribute to an accurate bypass of AAF adducts, and to both accurate and inaccurate bypass of benzo[*a*]pyrene and BPDE adducts (55, 162).

E. coli Pol IV can also participate in accurate or inaccurate TLS, either alone or in combination with Pol V, depending upon the nature of the lesion and its local sequence context (55, 163). Pol IV, as well as Pol V, is needed for the generation of both error-free TLS products and -1 frameshifts during bypass of BPDE adducts *in vivo* (55, 164). Pol IV is also involved in mutagenesis induced by 4-nitroquinoline *N*-oxide (4-NQO), benzo[*a*]pyrene adducts and oxidative damage (54, 55, 162-164).

Pol II plays an essential role in the replication restart in UV irradiated *E. coli* cells (46). After inhibition of replication caused by DNA damage, the resumption of DNA synthesis occurs rapidly in wild-type cells, within about ten minutes after exposure to UV light (165). In mutant cells lacking Pol II, a 50-minute delay in the replication restart was observed (46). In the absence of Pol II, post-UV DNA synthesis depended on Pol V. In accordance with its high fidelity observed *in vitro*, Pol II synthesizes chromosomal DNA in an error-free manner during replication restart. Since Pol II is induced almost immediately after SOS induction (46), prior to the appearance of Pol V (166), the mutation-free replication restart process can take place ahead of the error-prone translesion synthesis (46, 167). However, even the "high-fidelity" Pol II enzyme can promote error-prone bypass of certain lesions. For example, Pol II is responsible for generating −2 frameshifts during TLS through *N*-2-acetylaminofluorene (AAF) guanine adducts, while the "low-fidelity" Pol V is performing error-free bypass of the same adduct (163, 168, 169). Pol II is also responsible for mutagenic bypass of some intrastrand DNA crosslinks (170).

4.6. Role of TLS polymerases in spontaneous mutagenesis

There is extensive data suggesting that yeast Pol zeta is involved in the generation of mutations not only when cells are exposed to exogenous DNA damaging agents, but also in a variety of other circumstances. Pol zeta is needed for 50 to 70% of spontaneous mutations in wild-type yeast strains (171-173). It is also responsible for the increase in mutation rate caused by defects in nucleotide-excision repair (173, 174), base excision repair (175), post-replicative DNA repair (176-178), homologous recombination (174, 177), overproduction of 3-methyladenine DNA glycosylase (179), and expression of altered uracil-DNA glycosylases that remove undamaged cytosines and thymines in a base excision repair-defective strain (180). In these cases, the Pol zeta-dependent mutagenesis likely reflects the function of this polymerase in the error-prone bypass of endogenous DNA damage. In addition, DNA synthesis associated with repair of a double-strand break by homologous recombination in yeast was found to be highly mutagenic (181), with mutagenesis depending largely on Pol zeta (182). This suggests that Pol zeta can also perform error-prone DNA synthesis on substrates that are generated during recombination. Moreover, increased Pol zeta-dependent mutagenesis is observed in strains with defects in normal replicative DNA polymerases (Refs. 183, 184; M. R. Northam and P. V. Shcherbakova, unpublished data), suggesting that Pol zeta may be recruited to perform DNA synthesis at replication forks stalled or slowed down for reasons other than DNA damage. A Pol zeta-dependent increase in spontaneous mutation rate has been reported to be associated with high levels of transcription (185) and with mitochondrial disfunction (186). The important roles of Pol zeta in spontaneous mutagenesis are likely to be relevant to its remarkable ability to extend terminally mismatched primers and other aberrant substrates created by other DNA polymerases. Although Pol zeta contributes substantially to the generation of point mutations, its function is apparently required to maintain the proper chromosome stability. Mammalian cells defective in Pol zeta display aneuploidy and chromosomal rearrangements (187).

It has been suggested that Pol kappa can compete with Pol zeta in mammalian cells, since it also efficiently extends aberrant primer termini (89). Although it is not known whether the wild-type levels of Pol kappa could substantially affect spontaneous mutagenesis in eukaryotes, the *E. coli* homolog of Pol kappa, Pol IV, is clearly implicated in the generation of spontaneous mutation in

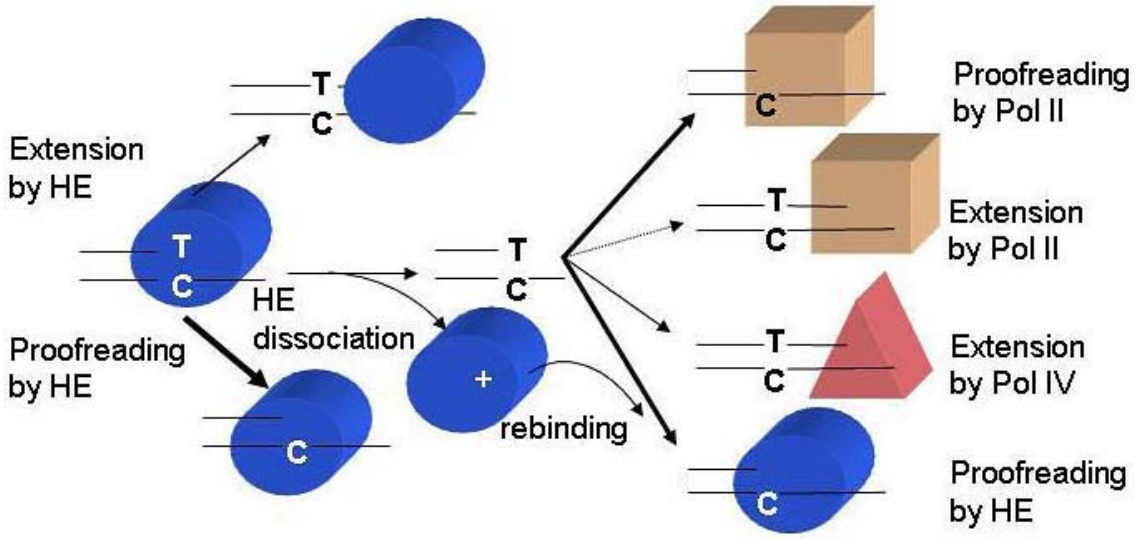

Figure 2. Competition of multiple *E. coli* DNA polymerases for the 3'-terminal mismatch. A mismatch created by Pol III HE may be extended to yield a mutation, proofread, or Pol III HE may dissociate allowing the other polymerases to compete for the primer terminus. Pol II possessing proofreading activity may remove the incorrect nucleotide from the 3' terminus. The low-fidelity Pol IV may extend the mismatched primer terminus causing a mutation. Rebinding of Pol III to the mismatch most likely will cause removal of the terminal nucleotide by the exonuclease and error-free continuation of replication. Based on Refs. 191 and 47.

some circumstances. Loss of Pol IV does not significantly affect the level of spontaneous mutations in the chromosome in growing cells (188-190). Since the basal level of Pol IV in uninduced cells is approximately ten-fold higher than that of Pol III (41, 54), this suggests that either Pol IV has limited access to the chromosomal growing point, or its involvement in DNA synthesis is mostly error-free. However, loss of Pol IV activity significantly reduces the mutator phenotype of the strains with impaired DNA polymerase activity of Pol III (47, 191, 192). About 50% of the mutations in these strains are considered to result from Pol IV action. It was proposed that Pol IV generates mutations by extending mismatched primer termini that are created by the defective Pol III HE and become available to error-prone Pol IV due to increased dissociation of Pol III. Pol IV acts unequally on the two strands and is preferentially responsible for mutations originating in the lagging strand (191). Presumably, the mechanism of lagging-strand replication which favors dissociation of major replicase after generating a mispair, creates a greater opportunity for Pol IV to participate in DNA synthesis. At the same time, there is no evidence that Pol V participates in spontaneous mutagenesis when SOS in uninduced and RecA is not activated.

E. coli strains deficient in Pol II activity do not exhibit any change in the genomic mutation rate (47, 48), suggesting that Pol II contributes minimally to the replication fidelity in wild-type strains. However, in *E. coli* cells carrying a mutant form of Pol III HE (*dnaE915*), Pol II is able to access the primer terminus (48) and act as a proofreader for Pol III HE-produced misinsertion errors (47). A substantial increase in mutability is observed in both episomal and chromosomal genes in these strains if the 3'->5' exonuclease acivity of Pol II is inactivated. The ability of Pol II to proofread Pol III HE errors establishes Pol II as an important back-up fidelity factor for replication in *E. coli*, with this function becoming most pronounced under conditions of increased mispair production. Moreover, Pol II not only corrects mismatches made by Pol III, but also appeares to protect the 3' terminus against the error-prone Pol IV (47). Similarly to the Pol IV-dependent mutagenesis, the contribution of Pol II to error avoidance is greater during lagging strand synthesis (47). Pol II has been also reported to prevent stationary phase adaptive mutations that depend largely on Pol IV (see Section 4.7), indicating that Pol II counteracts the activity of Pol IV in non-dividing cells (49).

Figure 2 shows possible scenarios of DNA polymerase interplay at the *E. coli* replication fork that regulates the fidelity of DNA replication. Upon creating a mismatch, Pol III may extend the mispair, creating a potential mutation. However, *in vitro* studies have indicated that Pol III HE is very inefficient in extending from terminal mismatches (27, 193). The most likely pathway at this stage is expected to be proofreading by Pol III HE itself. A third competing step is dissociation of Pol III HE, which, if it occurs, sets up a potential competition between various polymerases. Upon Pol III HE dissociation, the primer terminus may be subsequently bound by a proofreading-capable enzyme, such as Pol II, or Pol III itself, leading to excision of the mismatch. However, if Pol IV obtains access to the mismatch, it is likely to be fixed as a potential mutation. Increasing the relative amount of Pol IV will increase the chances of the error-prone fixing of the mismatch (47, 191).

4.7. Overproduction of TLS polymerases and genome stability

The discovery of a plethora of low-fidelity TLS polymerases raised an issue of regulating the access of these polymerases to the primer terminus during replication. It is obvious that uncontrolled participation of error-prone DNA polymerases in replicative DNA synthesis would present a threat to the genome stability. The access to the primer terminus is likely to be controlled by many mechanisms that may differ for different DNA polymerases. As discussed below, some TLS polymerases contribute significantly to genome instability when overproduced in cells, while others do not get access to the primer terminus during normal replication even when present at a high concentration.

In *E. coli*, Pol V is naturally overproduced in certain *recA* strains (*recA441*, *recA730*), in which there is a constitutive expression of the SOS system because of the "spontaneous" activation of RecA (194-196). In such strains, a Pol V-dependent increase in mutagenesis is observed in the absence of any DNA damaging treatment (untargeted mutagenesis) (197-200). This phenomenon is known as SOS mutator phenotype. Transversions are predominantly induced during untargeted mutagenesis (201, 202), which are also characteristic of the spectrum of mutations generated by purified Pol V *in vitro* (145). Studies of the *recA730*-induced SOS mutator phenotype (203, 204) revealed that this activity does not reflect mutagenesis at endogenous DNA lesions, but rather an increase in base misincorporation during ongoing DNA replication. The effect was observed for both transition and transversion errors, but it was most pronounced for transversions. Since transversion mismatches are more difficult to extend, they are more likely to result in DNA polymerase stalling. Therefore, it was suggested that the SOS mutator effect results from the transient stalling of Pol III HE at terminal mismatches that may result in the dissociation of Pol III. This, in turn, would provide an entry point for the constitutively present SOS proteins, leading to the increased probability of mismatch extension by Pol V (204). Additional support for this hypothesis is provided by the observation that the mutator effect of a temperature-sensitive *dnaE* mutation affecting the catalytic subunit of Pol III HE is largely dependent upon Pol V in constitutively SOS-induced cells (205, 206). This suggests that Pol V can compete effectively with the impaired Pol III HE for the 3´ primer terminus and extend mismatches made by Pol III. As in the case of Pol IV-dependent mutator phenotype, the lagging strand of replication appears more susceptible to the SOS mutator effect than the leading strand (207). The presence of additional copies of *umuDC* on a plasmid only slightly increases the level of mutagenesis during SOS response (208). However, the introduction of a low copy plasmid carrying *umuD'umuC* genes into strains with constitutive SOS mutator activity (*recA730*) caused a two-fold and 30-fold increase of mutagenesis for GC → TA and AT →TA, respectively (207).

The overproduction of Pol IV from a multicopy plasmid containing the *dinB* gene results in a mutator phenotype (191, 209, 210), with the strongest mutator effect (800-fold) being observed for -1 frameshifts (209). The mutational specificity in the Pol IV-overproducing strains (210) is in rather good agreement with the specificity of errors generated by Pol IV *in vitro*. The expression of *dinB* from a low-copy-number plasmid also increases the untargeted frameshift (209) and base-substitution (191) mutagenesis. Again, the mutations resulted predominantly from errors made during lagging strand synthesis (191). The mutator effect of Pol IV overexpression can be interpreted in one of two ways. The increased number of mutations could result from errors made by Pol IV itself, however, this would require a large amount of DNA to be synthesized by Pol IV. For example, assuming that Pol IV is 100-fold less accurate than Pol III, a 10-fold mutator effect would require 10% of the chromosome to be replicated by Pol IV. Given the biochemical properties of Pol IV, this is dubious. Alternatively, the Pol IV mutator activity could result from an error-prone extension of mismatches made by Pol III HE (see Figure 2). In support of the second hypothesis, the mutator effect of Pol IV overproduction is higher in strains carrying mutator alleles impairing the Pol III HE (191). Presumably, the frequency of terminal mispairs available for Pol IV to act on is greater in these strains. Although introduction of the *dinB* gene on a low copy plasmid does not confer any obvious negative effects on the cells growth and survival, the presence of the *dinB* gene on a high copy plasmid significantly decreased the cell count (191, 209). This suggests that an increased cellular concentration of Pol IV is not tolerated well by *E. coli* cells.

It has been shown that Pol IV plays a role in generating about 80% of the frameshift mutations in non-dividing cells on F' episomes (stationary phase or adaptive mutagenesis) (50, 56). Adaptive mutations are observed when populations of microorganisms are placed under certain nonlethal selective conditions (211, 212). Recently, it has been shown that Pol IV is induced late in the stationary phase under the positive control of RpoS, the stationary-phase sigma factor, and that high levels of Pol IV are maintained in the starving cells for at least three days due to the activity of GroE (213, 214). In the current model (see Ref. 215 for details), DNA synthesis occurs in the stationary phase on primer-template substrates generated through a RecA-dependent recombination process. The starvation causes a partial derepression of several SOS genes including *polB* or *dinB*. In addition, Pol IV is further induced in the late stationary phase under the control of RpoS. It was also suggested that amplification of the *dinB* gene in the starving cells could contribute to the induction of adaptive mutations (216). The resulting high levels of Pol IV allow it to outcompete other more accurate polymerases for access to the primer termini. The mutator activity of Pol IV in stationary phase cells is apparently counteracted by Pol II, since the frequency of adaptive mutations is increased 10-fold in *polB* mutants lacking Pol II (49).

In eukaryotes, Pol eta apparently can not freely access the primer terminus during normal DNA replication. Under conditions of Pol eta overproduction, no increase in

spontaneous mutagenesis that could be attributed to DNA synthesis by Pol eta was seen in yeast and human cells (217, 218). Although the yeast strain containing Pol eta-overproducing plasmid showed an elevation of mutagenesis, the same mutator effect was seen when a catalytically inactive variant of Pol eta was overproduced (218). This indicates that although an excess of Pol eta can interfere with the normal functioning of the replisome, the error-prone DNA polymerase itself does not contribute to replicative DNA synthesis. The overproduction of Pol kappa resulted in a ~10-fold elevation of spontaneous mutations rate (219) in mouse cells, and increased the incidence of DNA breaks, DNA exchanges and aneuploidy in human cells (220). The overproduction of Pol zeta in yeast resulted in up to a three-fold elevation in the frequency of UV-induced mutations (221), in accordance with the major role of this polymerase in DNA damage-induced mutagenesis.

4.8. Mechanisms of DNA polymerase switch at the replication fork

The mechanisms regulating participation of the multiple DNA polymerases in DNA replication are currently a subject of active investigation. Although many details of the polymerase switch process remain obscure, several components of the switch mechanism have been suggested by recent studies.

One such component is likely based on intrinsic differences in substrate preferences between different polymerases that are observed in studies of DNA synthesis *in vitro* with individual purified polymerases. For example, replicative DNA polymerases are highly capable of fast and processive DNA synthesis on undamaged templates. However, they insert nucleotides very inefficiently opposite a DNA lesion, as well as opposite nucleotides immediately following the lesion. In case of a *cis-syn* thymine-thymine dimer, two nucleotides need to be incorporated beyond the dimer to allow efficient resumption of DNA synthesis by the replicative enzymes (Refs. 222, 223; reviewed in Ref. 224). An exact complementary pattern of substrate use is observed with Pol eta: it has low processivity during copying of undamaged DNA, but switches to a processive mode of DNA synthesis when copying the T-T dimer and a few nucleotides following the lesion. The probability of termination of DNA synthesis by Pol eta rises again after the lesion bypass is completed, *i.e.* exactly when replicative DNA polymerases are able to take over Pol eta and continue processive replication (222, 223).

Multiple studies suggest that an important role in the DNA polymerase switch at sites of DNA damage is played by processivity clamps of replicative DNA polymerases, PCNA in eukaryotes and beta-clamp in *E. coli*. During processive replicative DNA synthesis, the polymerase remains bound to the clamp that encircles DNA and tethers the polymerase to the template preventing its dissociation. The beta-clamp interacts with all three SOS-inducible *E. coli* DNA polymerases (161, 225-230) and increases their processivity and TLS capacity (60, 62-64, 66, 225, 230). For Pol IV, it was shown that interaction with beta clamp also enhances the polymerase affinity for deoxyribonucleotides by an order of magnitude (231). Variants of Pol II, Pol IV and Pol V with changes in the predicted clamp-binding motif are unable to participate in TLS *in vivo* (161, 227). Also, a strain with a temperature-sensitive form of the beta clamp is defective in Pol V-dependent TLS at the permissive temperature (232). These observations suggest that the interactions of TLS polymerases with the clamp are critical for TLS. Based on these observations, it was proposed that the beta clamp plays an important role in coordinating the participation of multiple DNA polymerases in replication (163, 228, 230, 233-235). The beta clamp is a dimer built of identical protomers, therefore, it contains two peptide binding pockets and may bind different DNA polymerases simultaneously. It was suggested to function like a molecular tool-belt tethering replicative and translesion DNA polymerases (62, 236, 237), such that the polymerases replace each other at the primer terminus being bound to the same beta-dimer. Thus, the clamp may play a dual role in TLS. First, it may keep the TLS polymerase in the vicinity of primer terminus. Second, it increases the processivity of TLS polymerases, allowing them to synthesize past the lesion, in a single binding event, a fragment of DNA long enough not to be degraded by proofreading exonuclease of the major replicase, Pol III (230, 238). Indiani *et al.* (239) demonstrated that Pol IV and Pol III indeed may bind to one beta clamp at the same time *in vitro*. However, Pol IV can not access the primer/template junction as long as Pol III is synthesizing DNA. Pol IV can only bind the primer/template junction efficiently and perform DNA synthesis when Pol III stalls. When the stall is relieved, the switch from Pol IV to Pol III takes place immediately. Thus, the action of Pol IV is limited and the action of accurate Pol III is privileged (239). The precise, quick and controlled switch of polymerases prevents excessive participation of Pol IV in DNA synthesis and may explain why the error-prone Pol IV does not influence the level of spontaneous mutagenesis in growing strains (188, 190). Both Pol III and Pol IV were shown to have two different sites for binding beta clamp (228, 233, 234, 240, 241), suggesting that switching between different binding modes may be used to bring one or another polymerase to the primer terminus.

Eukaryotic Pol eta and Pol iota interact with PCNA, and this interaction increases the ability of these TLS polymerases to incorporate nucleotides opposite DNA lesions in vitro (242-244). Both polymerases accumulate in replication foci following treatment with DNA damaging agents (245). Pol iota variants that can not interact with PCNA fail to accumulate at replication foci (246), suggesting that this interaction may be used to recruit Pol iota, and, possibly, other TLS polymerases, to the replication fork. Interaction with PCNA, the PCNA-loading complex RFC, and RPA also stimulates the activity of Pol kappa (247). Although physical interaction of Pol zeta with PCNA has not been demonstrated, PCNA dramatically stimulates the TLS activity of Pol zeta (2).

In addition, in eukaryotes, posttranslational modification of PCNA appears to be important for the polymerase switch. Exposure of yeast and human cells to

DNA damaging agents induces monoubiquitination of PCNA at Lys 164 by Rad6-Rad18 complex (248-250). Elegant genetic experiments with a yeast mutant that has lysine 164 of PCNA substituted for an arginine have demonstrated that the monoubiquitination of Lys164 is required for TLS by DNA polymerases eta and zeta, and for Pol zeta-dependent DNA damage-induced mutagenesis (248, 250, 251). In human cells, the activity of RAD6 and RAD18 proteins is necessary for the accumulation of Pol eta at replication foci in response to UV irradiation (252). These observations suggested that the ubiquitin conjugation to PCNA could regulate protein-protein interactions at the replication fork or the activity of replicative and/or TLS polymerases in a way that results in the polymerase switches at the primer terminus needed for efficient lesion bypass. In a recent study, a system was developed for efficient monoubiquitination of yeast PCNA at Lys164 *in vitro*, and the effects of the ubiquitinated PCNA on DNA synthesis by of several purified polymerases was analyzed (253). The ability of PCNA to support DNA synthesis by replicative Pol delta was not affected by the modification, and lesion bypass capacity of Pol zeta was similar to that seen in the presence of unmodified PCNA. However, ubiquitinated PCNA greatly stimulated the TLS activity of Pol eta and Rev1. All Y-family polymerases possess ubiquitin-binding domains (254). These domains have been shown to be required for the accumulation of Pol eta and Pol iota in replication foci, interaction of these polymerases with monoubiquitiated PCNA and TLS *in vivo* (254). Taken together, these observations suggest that monoubiquitination of PCNA contributes to the regulation of polymerase switch by promoting lesion bypass by TLS polymerases.

In addition, an important role in polymerase switching may be played by the Rev1 protein. As discussed above, the essential function of Rev1 in TLS does not depend on its deoxycytidyl transferase activity, but the presence of the protein is absolutely required for DNA damage-induced mutagenesis. Recently, multiple studies have shown that mammalian Pol eta, Pol iota, Pol kappa, and the Rev7 subunit of Pol zeta all interact with the same ~100 C-terminal amino acid residues of REV1 (113, 121, 122), such that interaction with only one polymerase at a time is possible. In yeast, the interaction between Rev1 and Rev7 was reported to involve a different region of Rev1, suggesting that the interaction mechanisms could vary between species, or there could possibly be multiple interaction sites (112). In any case, the involvement of Rev1 in interactions with all other TLS DNA polymerases suggest a possibility that Rev1, as well as PCNA, could provide a docking site used to exchange different polymerases at the replication fork.

5. TLS POLYMERASES AND HUMAN DISEASE

As described in Section 4.5, in many cases, the activity of TLS polymerases helps prevent genotoxicant-induced mutations. Therefore, it is not surprising that dysfunction of these enzymes is associated with disease. Humans carrying mutations in the *XPV (POLH/RAD30)* gene that inactivate the function of Pol eta suffer from Xeroderma pigmentosum, a rare disease characterized by increased susceptibility to sunlight-induced skin cancer (68, 69). CPDs, which Pol eta can efficiently bypass incorporating predominantly correct nucleotides, are the most common lesions generated in DNA by the UV component of solar irradiation. The current hypothesis is that CPDs, unlike other sunlight-induced photoproducts, are slowly removed by excision repair and, therefore, are encountered by the replication machinery. CPDs block DNA synthesis by accurate replicative polymerases. In *XPV* patients that lack Pol eta, CPDs are bypassed by another polymerase in a manner that generates the mutations that lead to skin cancer. Interestingly, not only the ability of Pol eta to bypass CPDs accurately, but also its ability to generate mutations at a high rate is important for human health. Humans with mutations in the gene encoding Pol eta have altered somatic hypermutation spectrum in immunoglobulin genes, likely reflecting a role for Pol eta in promoting the somatic hypermutation, one of the major events in the development of a normal immune response. Both susceptibility to UV-induced skin tumors and defects in somatic hypermutation have been recently observed in Pol eta$^{-/-}$ mice as well (10, 11, 255).

Multiple reports suggest an association of mutations in the gene encoding Pol iota with increased susceptibility to lung cancer in mice (256-258) and humans (259). Single nucleotide polymorphisms in the human *REV1* gene were also found to be associated with increased cancer risk (259). In addition, changes in the expression of genes encoding Pol iota and Pol kappa have been found in human tumors (260-262). Given the importance of TLS polymerases in the control of genome stability during normal DNA replication and upon exposure to exogenous genotoxicants, it is likely that other disorders involving malfunction of these enzymes will be discovered in the future.

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