Functions of Eukaryotic DNA Polymerases

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A major function of DNA polymerases is to accurately replicate the six billion nucleotides that constitute the human genome. This task is complicated by the fact that the genome is constantly challenged by a variety of endogenous and exogenous DNA-damaging agents. DNA damage can block DNA replication or alter base coding potential, resulting in mutations. In addition, the accumulation of damage in nonreplicating DNA can affect gene expression, which leads to the malfunction of many cellular processes. A number of DNA repair systems operate in cells to remove DNA lesions, and several DNA polymerases are known to be the key components of these repair systems. In the past few years, a number of novel DNA polymerases have been discovered that likely function in replicative bypass of DNA damage missed by DNA repair enzymes or in specialized forms of repair. Furthermore, DNA polymerases can act as sensors in cell cycle checkpoint pathways that prevent entry into mitosis until damaged DNA is repaired and replication is completed. The list of DNA template-dependent eukaryotic DNA polymerases now consists of 14 enzymes with amazingly different properties. In this review, we discuss the possible functions of these polymerases in DNA damage repair, the replication of intact and damaged chromosomes, and cell cycle checkpoints.

The Growing Number of DNA Polymerases

The past several years have seen dramatic progress in our understanding of the world of eukaryotic DNA polymerases. Human cells are now known to contain at least 14 different DNA template-dependent DNA polymerases. They are listed in Fig. 1 using currently accepted nomenclature (*1*), along with the human and yeast genes that encode their catalytic subunits. The 14 enzymes have been categorized into four distinct families on the basis of their primary structures. Family A includes DNA polymerases homologous to *Escherichia coli* DNA polymerase I, the product of the *polA* gene. Two members of this family are present in human cells: Pol θ (*2*) and the mitochondrial replicative enzyme Pol γ (*3*). Family B is defined by homology with *E. coli* DNA polymerase II, the product of the *polB* gene, and includes human DNA polymerases α, δ, ε, and ζ. Human Pol β is regarded as a prototype of the X family, which also includes Pol λ (*4-6*), Pol μ (*4*, *7*), Pol σ (*8*), and terminal transferase, a template-independent DNA polymerase. Most recently discovered is the Y family of DNA polymerases [reviewed in (*9*)], which includes human Pol η, Pol ι, and Pol κ, as well as REV1, which was initially described as a DNA template-dependent deoxycytidyl transferase and is now referred to as a G template-specific DNA polymerase (*10*).

These enzymes differ in their polymerization properties, such as catalytic efficiency, fidelity, processivity (the number of nucleotides polymerized continuously without dissociation from the template), and preferred DNA substrates. Some have additional enzymatic activities, such as an intrinsic 3′→5′ exonuclease or a 5′-deoxyribose phosphate (dRP) lyase (Fig. 1). dRP lyase enzymes remove a dRP group through a lyase (β-elimination) mechanism (that is, they cleave the phosphodiester bond 3′ to the dRP group). Pol α has a DNA primase activity in a separate but strongly associated subunit. In addition to protein domains required for enzymatic activity, some of these polymerases also contain other motifs (for example, a BRCT domain, which was originally found in the C-terminal region of the breast cancer susceptibility gene *BRCA1*, or a DNA binding zinc finger motif) that are likely to be functionally important. The role that each enzyme plays in the cell is presumably defined by its enzymatic properties, by its ability to interact with accessory proteins, and by other regulatory mechanisms, such as transcriptional regulation or posttranslational modification. For example, acetylation of Pol β has recently been observed in vivo and shown to regulate the activity of this polymerase (*11*). Despite some 40 years of outstanding work on polymerase functions, the pace of recent discovery has led to many more questions than answers. One of the most challenging tasks is to decipher when and how different polymerases come into play to deal with different substrates during the various DNA transactions required to accurately replicate and stably maintain eukaryotic genomes. In this review, we summarize our current understanding of the possible functions of the multiple DNA polymerases as implied by their distinctive features.

Providing Good Templates for the Copying Machines

Cellular DNA is continuously damaged by endogenous genotoxicants, such as reactive oxygen species, and exogenous agents, such as ultraviolet (UV) irradiation or the environmental contaminant benzo[*a*]pyrene. This results in the loss or modification of bases, single- and double-strand breaks in the DNA, and intra- and interstrand cross-links. Such damage presents a threat to genome stability by creating obstacles for DNA replication and transcription complexes and by altering base coding potential, which leads to replication and transcription errors. Fortunately, cells possess specialized repair pathways that remove various types of damage. A variety of helix-distorting lesions, including UV radiation-induced damage and bulky chemical adducts, are removed by nucleotide excision repair (NER) [reviewed in (*12-15*)]. Damaged bases, such as those produced by reactive oxygen species or methylating agents, or uracil, which results from cytosine deamination, are removed primarily by base excision repair (BER) [for review, see (*16-18*)]. Both repair processes require a DNA synthesis step to replace the damaged nucleotides that are excised. However, the two pathways generate distinct substrates for DNA synthesis and have somewhat different DNA polymerase requirements (see Fig. 2 for a summary of lesions and the polymerases involved in their repair).

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Polymerase[a]	Family	Catalytic subunit				Associated activities	Proposed functions
		Molecular mass (kDa)[b]	Human gene (alias)	Chromosomal location[c]	Yeast gene[d] (alias)		
α (alpha)	B	165	*POLA*	Xp22.1-p 21.3	*POL1* (*CDC17*)	Primase	chromosomal replication, S-phase checkpoint, DSB repair
β (beta)	X	39	*POLB*	8p11.2	-	dRP & AP lyase	BER, single strand break repair
γ (gamma)	A	140	*POLG*	15q25	*MIP1*	3'→5' exonuclease, dRP lyase	mitochondrial replication, mitochondrial BER
δ (delta)	B	125	*POLD1*	19q13.3	*POL3 (CDC2)*	3'→5' exonuclease	chromosomal replication, NER, BER, MMR, DSB repair
ε (epsilon)	B	255	*POLE*	12q24.3	*POL2*	3'→5' exonuclease	chromosomal replication, NER, BER, MMR, DSB repair, S-phase checkpoint
ζ (zeta)	B	353	*POLZ* (*REV3*)	6q21	*REV3*		TLS, DSB repair, ICL repair?, SHM
η (eta)	Y	78	*POLH* (*RAD30, RAD30A, XPV*)	6p21.1	*RAD30*		TLS, SHM
θ (theta)	A	198	*POLQ*	3q13.33	-		ICL repair?
ι (iota)	Y	80	*POLI* (*RAD30B*)	18q21.1	-	dRP lyase	TLS?, BER?, SHM
κ (kappa)	Y	76	*POLK* (*DINB1*)	5q13	-		TLS
λ (lambda)	X	66	*POLL*	10q23	*POL4* (*POLX*)	dRP lyase	DSB repair, BER?
μ (mu)	X	55	*POLM*	7p13	-	TdT	DSB repair
σ (sigma)	X	60	*POLS* (*TRF4-1*)	5p15	*TRF4*		sister chromatid cohesion
REV1	Y	138	*REV1*	2q11.1-q11.2	*REV1*	TdT (for dC)	TLS

Fig. 1. Human template-dependent DNA polymerases. [a]Rapid identification of several of these DNA polymerases in a short time resulted in some confusion in nomenclature that is worth mentioning briefly. The product of the human *DINB1* gene was first named Pol θ (*138*), but because that designation had already been used (*2*), the human *DINB1* gene product is now designated as Pol κ. When first identified, DNA polymerase λ was found to be homologous to Pol β, so that in one instance it was designated as Pol β2 (*6*), but now it has its own Greek letter designation. The *S. cerevisiae* X-family enzyme encoded by the *POL4* gene was first considered to be the yeast equivalent of mammalian Pol β, but is now known to be more closely related to mammalian Pol λ (*5*). Finally, the products of the yeast *TRF4* and human *TRF4-1* genes were originally designated Pol κ (*8*). Because that name was already assigned to a different gene product (*92*), the *TRF4* gene products are now called Pol σ. [b]Deduced from protein primary structure. [c]From the Human Genome database (http://www.ncbi.nlm.nih.gov/genome/guide/human). [d]*S. cerevisiae*. DSB, double-strand break repair.

Nucleotide Excision Repair

NER removes DNA lesions by excising an oligonucleotide containing the lesion, leaving a single-stranded DNA gap of ~30 nucleotides. There is substantial evidence that these gaps are filled by one or both of the B-family polymerases Pol δ or Pol ε. Either of these two polymerases can fill the DNA gap during in vitro reconstituted NER with purified mammalian proteins (*19*). Using either permeabilized human fibroblasts or HeLa cell nuclear extracts, both Pol δ and Pol ε were found to be necessary for NER of UV-irradiated DNA (*20*, *21*) [see also references in (*14*)]. Studies in yeast have indicated that either Pol δ or Pol ε is sufficient to complete repair of UV-induced damage (*22*). And in a later study, both Pol δ and Pol ε were reported to be required for efficient NER synthesis in yeast extracts (*23*). Processive DNA synthesis in vitro by Pol δ, and under certain conditions by Pol ε, requires an accessory factor, proliferating cell nuclear antigen (PCNA), which is loaded onto DNA by a five-subunit complex, replication factor C (RFC). Repair synthesis by either polymerase also requires PCNA and RFC.

Base Excision Repair

During BER, damaged bases are first removed by a DNA glycosylase. Monofunctional DNA glycosylases, such as uracil DNA glycosylase, cleave the glycosydic bond, generating an abasic (AP) site, and subsequent action of an AP endonuclease creates a nick with 3′-OH and 5′-dRP termini. This intermediate can be further processed by more than one BER pathway involving different DNA polymerases. In the major mammalian BER pathway, Pol β inserts a single nucleotide using the 3′-OH as a primer and removes the 5′-dRP using its dRP lyase activity. This generates a nick that is sealed by DNA ligase, with the entire reaction accomplishing the replacement of a single damaged nucleotide (single-nucleotide BER). Alternatively, in some cases, such as when the dRP is modified and cannot be removed by the dRP lyase activity of Pol β, a DNA polymerase may instead perform limited displacement synthesis to incorporate 2 to 13 nucleotides (long patch BER). This process generates a single-stranded DNA flap that is subsequently removed by the *FEN1* flap endonuclease. Pol β, Pol δ, and Pol ε can per-

form DNA resynthesis during long patch BER in vitro (*24*-*27*). The identity of the polymerase involved in the long patch BER in vivo is not certain, but it has been reported that Pol β always initiates DNA synthesis (*28*).

A distinct BER pathway is initiated by bifunctional DNA glycosylases that have an AP lyase activity in addition to their glycosylase activity [reviewed in (*29*)]. Combined action of a DNA glycosylase/AP lyase and an AP endonuclease generates a single-nucleotide gap with 3′-OH and 5′-phosphate termini that are filled predominantly by Pol β (*30*).

Additional complexity is indicated by the observations that two of the newly discovered DNA polymerases, Pol ι and Pol λ, also have dRP lyase activity (*31*, *32*). Thus, like Pol β, it is possible that they too participate in damage repair that requires removal of a dRP residue. Pol λ is a close homolog of Pol β (*4*-*6*), with certain enzymatic properties in common (*33*, *34*). Like Pol β, it is devoid of 3′→5′ exonuclease activity and has low processivity during primer extension on single-stranded DNA templates (*4*, *33*). Also like Pol β, Pol λ is processive when filling small gaps containing a 5′ phosphate group (*33*). Thus, Pol λ is a suitable candidate for BER synthesis. In fact, Pol λ can substitute for Pol β in reconstituted BER of uracil-containing DNA in vitro (*32*). However, *Polλ*$^{-/-}$ mouse cells are not sensitive to methylmethane sulfonate (MMS) or hydrogen peroxide, the agents that produce, among other types of damage, AP sites and oxidized bases (*35*). This suggests that Pol λ is not essential for BER in cells that contain Pol β, Pol δ, and Pol ε. Interestingly, Pol λ can efficiently polymerize DNA using a very low (less than 1 μM) concentration of deoxynucleoside triphosphates (dNTPs) (*33*), which might allow it to participate in BER or other repair processes in quiescent cells that have a small dNTP pool [reviewed in (*36*)]. In support of this hypothesis, Pol λ expression was found to be cell cycle-dependent, with higher expression observed in cells undergoing the S- to M-phase transition and in quiescent cells (*6*).

Pol ι belongs to a different family of DNA polymerases, family Y. A major function suggested for Y family enzymes is the bypassing of DNA lesions that block replicative DNA polymerases. However, several properties of Pol ι suggest that it may also participate in specialized BER processes [reviewed in (*37*)]. Pol ι has low processivity, lacks a proofreading 3′→5′ exonuclease, and can fill gaps of one to five nucleotides in vitro (*31*, *38*, *39*). Using its dRP lyase and DNA polymerase activities, Pol ι can substitute for Pol β in BER of uracil-containing DNA in vitro (*31*). Studies of the kinetics of nucleotide incorporation have led to two suggestions for repair involving Pol ι. Pol ι incorporates dTMP opposite an A in the DNA template much more efficiently than it forms the other three Watson-Crick base pairs (*31*, *40*, *41*), and the fidelity of DNA synthesis opposite A's in the template is comparable to that of Pol β. These findings led to the idea that Pol ι may participate in BER of uracil residues in DNA that result from dUTP incorporation opposite a template A during DNA replication (*31*). Pol ι also has the unprecedented ability to misinsert dGMP opposite a template T at a rate that exceeds that of correct dAMP incorporation (*31*, *40*-*42*). Moreover, on templates that contain two or more consecutive T's, preferential dGMP incorporation opposite T residues was followed by preferential (and correct) A incorporation opposite a second template T (*31*, *43*). This unique specificity led to the speculation that Pol ι may participate in a specialized BER reaction that replaces dG if it is inadvertently removed by a glycosylase from a G-T or G-U mismatch that arose by deamination of a 5-methyl-cytosine or cytosine in DNA (*31*). Possible roles for Pol ι in translesion DNA synthesis and somatic hypermutation are considered below.

The potential for the participation of five nuclear DNA polymerases, three with dRP lyase activity, in BER will likely require future studies in cells and animal models lacking more than one polymerase, as well as a better understanding of the substrate preferences and polymerase-accessory protein interactions used for different BER pathways. In addition to BER, Pol β is thought to function in the repair of single-strand DNA breaks, a process that has recently been found to be defective in patients with hereditary spinocerebellar ataxia [reviewed in (*44*)]. Single-strand breaks generated by endogenous or environmental agents often have one-nucleotide gaps between the DNA ends and modified 3′ and/or 5′ termini, substrates that are similar to those that arise during BER. It would be interesting to investigate whether other DNA polymerases with dRP lyase activity and properties consistent with their possible function in BER, such as Pol ι and Pol λ, could also participate in single-strand break repair.

The fourth DNA polymerase in human cells that has a dRP lyase activity is mitochondrial Pol γ (*45*). Pol γ is the only DNA polymerase found in mitochondria, and it is therefore responsible for all DNA synthesis transactions in this organelle, including DNA replication and repair of DNA damage. Mitochondria are subject to extensive DNA damage by reactive oxygen species generated during oxidative phosphorylation. This type of damage can be efficiently repaired by a set of mitochondrial proteins that includes AP endonuclease, Pol γ, and mitochondrial DNA (mtDNA) ligase, and the process is similar to single-nucleotide BER in nuclear DNA [reviewed in (*46*)].

Repair of Interstrand Cross-Links

Interstrand cross-links (ICLs) are induced by a variety of agents, including psoralen, diepoxybutane, and nitrogen mustard. ICLs present a special challenge to DNA repair systems, because both strands are damaged and thus neither strand retains the correct genetic information. Little is known about the mechanism by which ICLs are repaired in mammalian cells. Mutations in a number of genes confer sensitivity to ICL-inducing agents in model organisms. At least two of these genes are now known to encode DNA polymerases. An allele of the *Saccharomyces cerevisiae REV3* gene, which encodes the catalytic subunit of Pol ζ, was isolated in a screen for mutants hypersensitive to photoactivated psoralens (*47*). Pol ζ is one of the main players in translesion DNA synthesis in human cells. Further studies are needed to determine whether the lesion bypass ability of Pol ζ or other features of this polymerase are critical for its function in ICL repair. Another DNA polymerase, human Pol θ, was identified by homology to the *Drosophila mus308* gene product, a putative DNA polymerase-helicase (*2*). The *mus308* mutants are hypersensitive to and display chromosomal aberrations upon exposure to DNA cross-linking agents (*48*, *49*), suggesting involvement in ICL repair. The *mus308* mutants are also hypermutable after exposure to a monofunctional *O*-alkylating agent *N*-ethyl-*N*-nitrosourea (*50*), suggesting that Pol θ may also participate in repair of other types of DNA damage.

Involvement of DNA Polymerases in Double-Strand Break Repair

Double-strand breaks can be repaired by recombination with a

homologous DNA sequence or by nonhomologous DNA end joining (NHEJ) in vertebrate cells (*51*) [reviewed in (*52*, *53*)]. At least two DNA polymerases are implicated in the microhomology-mediated pathway of NHEJ in eukaryotic cells. Several studies suggest that the *S. cerevisiae* homolog of human Pol λ, the product of the *POL4* gene, is involved in repair of double-strand breaks. The *pol4* mutants display increased frequency of illegitimate mating and meiotic recombination (*54*). Genetic data implicated yeast Pol4 in microhomology-mediated joining of incompatible DNA ends that requires fill-in synthesis by a DNA polymerase (*55*). Small gaps formed by the alignment of linear duplex DNA molecules were shown to be a preferential substrate for yeast Pol4, and the protein was shown to interact physically and functionally with the Dnl4/Lif1 complex, an essential component of the NHEJ pathway (*56*). This interaction is mediated by the BRCT domain of Pol4. A BRCT domain is also found in Pol μ and terminal deoxynucleotidyl transferase (TdT), and may help recruit these polymerases to their appropriate in vivo substrates (*56*).

Pol μ and TdT are Pol X family enzymes that share 41% sequence identity (*7*). TdT is expressed in lymphoid tissue and is involved in the template-independent additions of nucleotides during V(D)J recombination in antigen receptor genes [reviewed in (*57*)]. Pol μ is unusual in that it can polymerize DNA in both a DNA template-dependent and template-independent manner (*7*), and it can efficiently extend template primers containing up to four mismatches (*58*). These properties make Pol μ suitable for function in the NHEJ pathway of double-strand break repair. Indeed, Pol μ interacts with Ku, one of the central players in the NHEJ pathway, and stably associates with DNA only in the presence of Ku and another end-joining factor, the XRCC4-ligase IV complex (*59*). This complex can efficiently perform an end-joining reaction that requires the annealing of partially overlapping DNA ends and the filling of a one-nucleotide gap. Also, exposure of human cells to ionizing radiation results in increased Pol μ levels and localization of Pol μ in discrete nuclear foci that coincide with sites of double-strand breaks (*59*).

Repair of double-strand breaks by homologous recombination requires DNA synthesis that is initiated by invasion of the 3′ end of the broken DNA into a donor sequence. In *S. cerevisiae*, all three DNA polymerases required for S-phase replication of chromosomes (Pol α, Pol δ, and Pol ε) were found to be necessary for DNA synthesis associated with double-strand break-induced recombination (*60*). The requirement of both leading and lagging strand replicative polymerases led to a model wherein a strand invasion created a replication fork involving leading and lagging strand DNA synthesis (*60*). Replicative DNA polymerases are characterized by high fidelity, which is consistent with the notion that recombinational repair of DNA damage does not frequently generate mutations (hence it is often referred to as "error-free" repair). However, DNA synthesis associated with double-strand break repair in yeast can be highly mutagenic (*61*), with mutagenesis depending largely on Pol ζ (*62*), a DNA polymerase implicated in DNA lesion bypass. This suggests that Pol ζ can also be recruited to perform DNA synthesis on substrates that are generated during homologous recombination. Although Pol ζ is responsible for most of the mutagenesis during double-strand break repair, the repair process itself is not defective in the absence of Pol ζ (*62*), indicating some degree of functional redundancy among polymerases for this type of DNA repair.

Replication of Normal and Damaged DNA

In the following sections, we discuss the various polymerases that are involved in the replication of normal and damaged DNA and in cell cycle checkpoint control (see Fig. 3 for a summary of these polymerases and their functions).

Synthesis of Leading and Lagging DNA Strands During Chromosomal DNA Replication

Chromosomal DNA replication in eukaryotic cells requires three DNA polymerases: Pol α, Pol δ, and Pol ε [reviewed in (*63*)]. Pol α is not highly processive and lacks a 3′→5′ proofreading exonuclease activity; therefore, it is not well suited for fast and accurate replication of the large nuclear genome. However, Pol α is the only polymerase that has a tightly associated activity for synthesis of RNA primers at replication origins and on the lagging DNA strand. Pol α extends these RNA primers by synthesizing short stretches of DNA, and then a switch occurs to processive synthesis by Pol δ and/or Pol ε. The precise roles of these two polymerases in genome replication are not yet completely understood. It has been proposed that one of the two polymerases performs the bulk of the DNA synthesis on the leading DNA strand and the other is responsible for synthesis on the lagging strand (*64*, *65*). Indeed, genetic data in yeast suggest that 3′→5′ exonucleases associated with the two polymerases proofread replication errors on opposite DNA strands during chromosomal (*66*) or plasmid (*67*) DNA replication. In the in vitro replication of

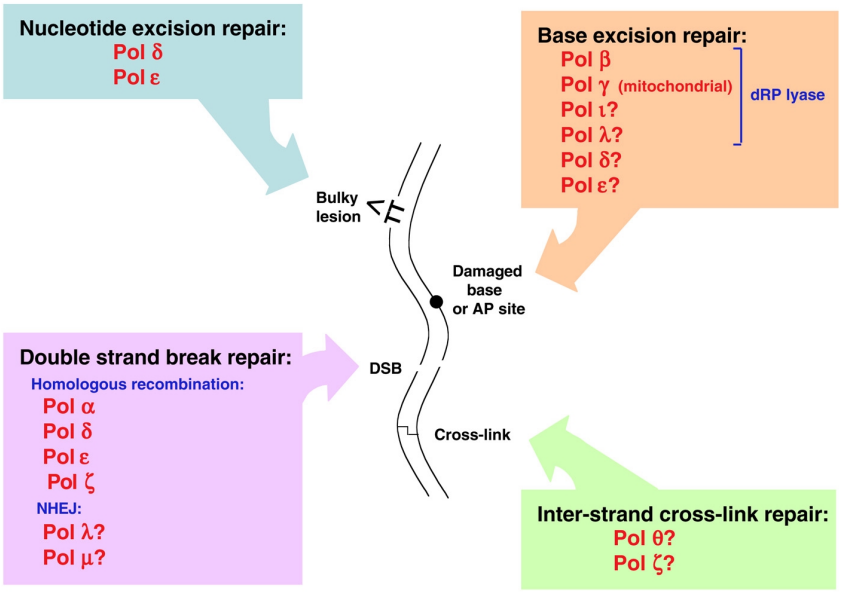

Fig. 2. DNA polymerases involved in DNA damage repair.

plasmid DNA containing the SV40 replication origin, Pol α initiates DNA synthesis at the origin and on the lagging DNA strand, and Pol δ is responsible for the rest of DNA synthesis on both strands (*68*). Because there is substantial evidence for the involvement of Pol ε in chromosomal replication [see (*69*) and references therein], it is possible that in vivo replication that initiates at chromosomal origins and is strongly cell cycle-regulated has different polymerase requirements than does SV40 origin-dependent plasmid replication. Alternatively, it was suggested that the role of Pol ε in chromosomal replication is nonessential, because yeast mutants with a deletion of the catalytic domain of Pol ε are viable (*70*). However, these mutants display severe growth and replication defects (*69*), and mutants with single amino acid substitutions in the active site of Pol ε are inviable (*71*, *72*). These observations suggest that Pol ε is normally a component of the replication machinery, but in the absense of Pol ε another polymerase can partially substitute for it. Another study suggests that Pol ε participates in chromosomal replication in late, but not early, S phase in human cells (*73*).

Accurate chromosome segregation in mitosis requires that sister chromatids remain attached to each other by cohesin complexes until they separate in anaphase. It was recently discovered that one of the proteins required for establishing sister chromatid cohesion in S phase, the product of the *TRF4* gene, belongs to the X family of DNA polymerases (*8*), and this polymerase was designated Pol σ (*74*). These observations led to the suggestion that Pol σ might be a specialized DNA polymerase necessary for replication through the cohesion sites, which could present an obstacle for the major replicative polymerases Pol δ and Pol ε (*74*).

Both Pol δ and Pol ε replicate undamaged DNA with high fidelity (*75*, *76*). This property reflects the high nucleotide selectivity of the polymerase active site, followed by proofreading of mismatches by the intrinsic 3′→5′ exonuclease activities of these polymerases. DNA polymerase errors missed by the proofreading activities can be corrected by postreplicative DNA mismatch repair [MMR, reviewed in (*77*)]. This repair system recognizes base-base and insertion/deletion mismatches in double-stranded DNA and corrects them through a process involving mismatch excision followed by correct DNA synthesis. The DNA synthesis step requires an aphidicolin-sensitive and PCNA-dependent DNA polymerase, most likely Pol δ (*78*, *79*). It has also been proposed that the 3′→5′ exonuclease activities of Pol δ and Pol ε might function in the excision step of MMR (*80*).

Replication of the Mitochondrial Genome

The sole mitochondrial DNA polymerase, Pol γ, is responsible for replication of both strands of the mitochondrial genome [reviewed in (*81*)]. Human Pol γ is highly accurate, because of efficient discrimination against the insertion of incorrect nucleotides and proofreading of DNA synthesis errors via an intrinsic 3′→5′ exonuclease activity [see (*82*) and references therein]. As mentioned below, mutations in the gene encoding the catalytic subunit of Pol γ are associated with human disease.

When DNA Replication Machinery Encounters an Aberrant Substrate

The repair processes that provide the replication machinery with undamaged substrates are not perfect, and DNA damage occurs throughout the cell cycle, including during S phase. The replication machinery, therefore, occasionally encounters damaged DNA templates, with consequences that depend on the nature of the damage. Unrepaired uracil arising from cytosine deamination codes like thymine and is therefore highly mutagenic. Modified bases, such as 8-oxyguanine, which arises from oxidative stress, or O^6-methyl guanine, which arises from alkylation, have ambiguous base coding potential during replication, resulting in mismatches that yield point mutations. Abasic sites that have no base coding potential and bulky adducts that distort DNA helix geometry impede synthesis by replicative DNA polymerases. Structural studies show that family A and B polymerases have active sites that snugly accommodate the four correct and geometrically equivalent Watson-Crick base pairs to allow efficient and accurate synthesis [reviewed in (*31*, *83*, *84*)]. Bulky lesions cannot be readily accommodated in the active sites of these polymerases, which probably accounts for the replication block.

The past several years have seen the discovery of DNA polymerases that have the remarkable ability to bypass lesions in template DNA that block replicative DNA polymerases. Among these is one B-family enzyme, Pol ζ, and a whole new family of polymerases, the Y family (Pol η, Pol ι, Pol κ, and REV1) (Fig. 1) (*9*). Clues to their remarkable translesion synthesis (TLS) abilities come from initial structural studies revealing that Y-family polymerases have an extra DNA binding domain and a

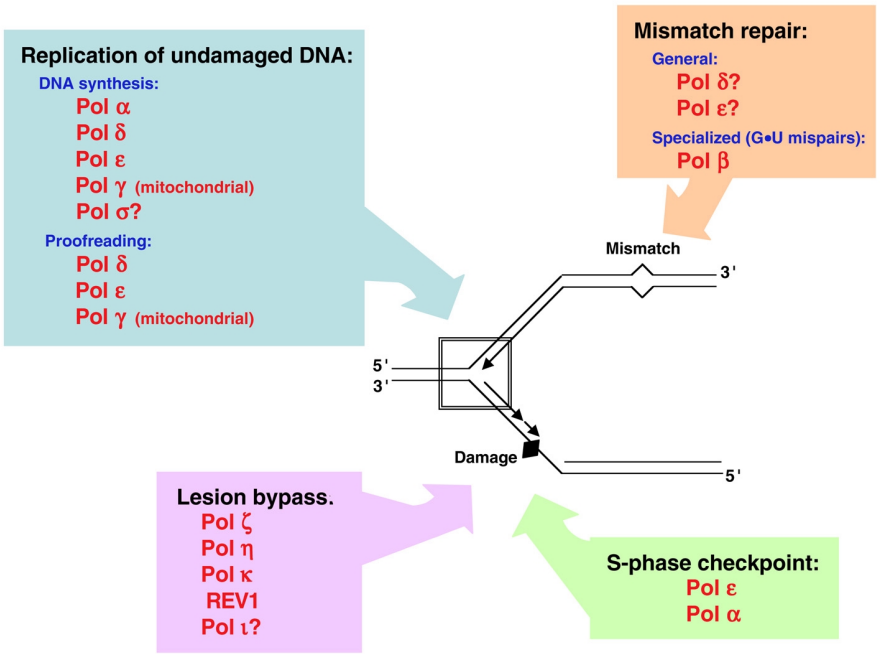

Fig. 3. DNA polymerases involved in replication of normal and damaged DNA.

generally more open active site than do polymerases in other families, thus allowing Y-family polymerases to accommodate and copy bulky DNA lesions [reviewed in (*85*, *86*)]. These DNA polymerases have remarkably low fidelity when copying undamaged DNA, which is thought to reflect the lower selectivity of their active sites [reviewed in (*37*, *84*)].

We are just beginning to understand the cellular roles of these polymerases in damage bypass. Data accumulated thus far suggest that properties and lesion bypass specificities of the TLS polymerases are different and perhaps adapted for specific functions in the replication of damaged DNA. For example, Pol η is particularly efficient in bypassing a *cis-syn* thymine-thymine (TT) dimer, a major lesion generated by UV irradiation [reviewed in (*87*)], whereas Pol κ readily bypasses polycyclic aromatic hydrocarbon adducts, such as benzo[*a*]pyrene-N^2-dG lesions (*88-91*) and acetylaminofluorene adducts (*88*, *92*, *93*). TLS polymerases also show functional redundancy in the bypass of certain lesions. For example, studies in yeast have demonstrated that (6-4) TT photoproducts can be bypassed both accurately and inaccurately, with inaccurate bypass depending on Pol η (*94*).

Among the DNA polymerases implicated in TLS, Pol ζ is renowned for its ability to efficiently extend mismatched primer termini or those containing a terminal nucleotide opposite a noncoding or distorting lesion. At the same time, Pol ζ relatively inefficiently incorporates nucleotides opposite DNA lesions by itself. This provided the basis for a "two-polymerase" lesion bypass model, wherein nucleotide incorporation opposite a lesion by one polymerase is followed by extension by another polymerase (*87*, *94*, *95*). Several TLS polymerases could function in the first incorporation step. For example, Pol ι is able to efficiently incorporate nucleotides opposite certain lesions, such as (6-4) TT photoproducts, but is not able to extend the resulting primer terminus (*40*, *42*, *96*, *97*). REV1 interacts with a subunit of Pol ζ in vitro (*98*, *99*) and can cooperate with Pol ζ in the bypass of abasic sites (*95*). Such a bypass could involve the incorporation of dCMP opposite the lesion by REV1 and subsequent extension by Pol ζ (*100*), although studies in yeast have demonstrated that the deoxycytidyl transferase activity of Rev1 is not essential for lesion bypass (*101*). It was proposed that Pol κ could compete with Pol ζ for the extension of aberrant primer termini (*102*), as it is also able to efficiently extend primers that have their 3′ terminal nucleotide paired with different damaged nucleotides (*102*, *103*).

Pol ζ , Pol κ, and Pol η also efficiently extend terminal mispairs formed by undamaged nucleotides (*102*, *104*, *105*). Genetic studies in yeast suggest that Pol ζ can take part in DNA replication not only when DNA is damaged, but also when the replicative machinery experiences difficulties because the machinery itself is defective. Mutations that affect replicative DNA polymerases ε or δ lead to a mutator phenotype that is dependent on functional Pol ζ (*72*, *106*). These observations resemble the Pol ζ-dependent mutagenesis associated with double-strand break repair, which also presumably involves DNA synthesis on undamaged templates (*62*). It is possible that the remarkable extension capacity of Pol ζ allows it to synthesize on damaged DNA and on undamaged substrates that are poorly used by other polymerases.

The fact that at least 8 of the 14 enzymes listed in Fig. 1 are required for the replication of undamaged nuclear DNA (Pol α, Pol δ, and Pol ε) and TLS (Pol ζ, Pol η, Pol ι, Pol κ, and REV1) has led to the "factory model" (107), in which many polymerases are simultaneously present in a given replication complex. TLS enzymes are called on when accurate replicative polymerases are stalled, either by a lesion or by a naturally occurring barrier to replication. After they finish performing their tasks, the TLS enzymes vacate the template primer to allow the major replicative DNA polymerases to resume synthesis.

It remains to be determined what mechanisms are used in cells to recruit one or another DNA polymerase to its particular substrate. It was reported that Pol η, Pol ι, and Pol κ are associated with replication foci during S phase and accumulate at stalled replication forks after treatment with DNA-damaging agents (*108-110*). Several protein-protein interactions were described that could probably constitute a part of the recruitment mechanisms. As mentioned above, the REV1 protein interacts with a subunit of Pol ζ in vitro (*98*, *99*), which is consistent with these two polymerases functioning together in the bypass of abasic sites (*95*). Pol η and Pol ι interact with PCNA, an accessory protein of replicative DNA polymerases δ and ε, and this interaction increases the ability of these TLS polymerases to incorporate nucleotides opposite DNA lesions (*96*, *111*). Interaction with PCNA, the PCNA-loading complex RFC, and RPA also stimulates the activity of Pol κ (*112*). It is possible that these interactions are used to recruit Pol η, Pol ι, and Pol κ to the replication fork. In addition, it was shown that localization of Pol ι in replication foci is dependent on the presence of Pol η, and that Pol ι and Pol η physically interact. This suggests that Pol η could be the protein that targets Pol ι to the replication machinery (*110*).

DNA Polymerases Act As Sensors in Cell Cycle Checkpoint Control

The dependence of cell cycle progression on completion of DNA replication and repair of DNA damage is controlled by regulatory mechanisms called checkpoints. Several observations in the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* suggest that components of the DNA replication machinery can serve as sensors of replication blocks in the S-phase checkpoint pathway and are also involved in the S-phase DNA damage checkpoint. In *S. cerevisiae*, certain mutations in Pol ε result in the S-phase checkpoint defect, so that the mutants fail to arrest the cell cycle in response to a DNA replication block and enter lethal mitosis with unreplicated chromosomes (*71*, *113*, *114*). These mutants also show a defect in the DNA damage checkpoint that is normally manifested by inhibition of cell cycle progression and transcriptional activation of genes required for DNA repair in response to DNA damage (*113-115*). A similar defect in the DNA damage checkpoint is observed in mutants with a defective primase subunit of Pol α (*116*). In fission yeast, mutations in the gene that encodes the catalytic subunit of Pol α have been shown to confer a defect in the S-phase replication checkpoint (*117*). It would be interesting to determine whether any of the newly identified damage bypass polymerases could function in the damage checkpoint pathway. It was reported that a subunit of Pol ζ shares significant homology and interacts in vitro with the MAD2 protein, a key component of the spindle assembly checkpoint pathway (*98*). The spindle assembly checkpoint ensures that cells do not enter mitosis and that chromosome segregation does not occur until all chromosomes are properly attached to the mitotic spindle [reviewed recently in (*118*)]. At present, it is not clear whether Pol

ζ has any function relevant to mitotic spindle assembly.

DNA Polymerases and Human Health

There are now several examples wherein mutations in polymerase genes that inactivate or modify enzymatic functions have consequences for human health (Fig. 4). As mentioned above, the integrity of the human mitochondrial genome depends on faithful replication by the two-subunit DNA Pol γ. Although wild-type Pol γ has a relatively high fidelity resulting from efficient base selection and exonucleolytic proofreading (*82*), the mutation rate in mtDNA is higher than that in nuclear DNA. This partly reflects increased DNA damage due to reactive oxygen species generated during oxidative phosphorylation (see "The Two Faces of Oxygen*" and Nicholls Perspective†). Point mutations and deletions in mtDNA generate mitochondrial genetic diversity within a single cell, which increases exponentially with age [reviewed recently in (*119*)], and loss of mitochondrial function due to a high mutation load causes a wide range of respiratory and tissue degeneration diseases (*119*, *120*-*122*). Recently, several mutations in the polymerase and exonuclease domains of human Pol γ have been associated with progressive external ophthalmoplegia (PEO), a rare disease characterized by the accumulation of point mutations and large deletions in mtDNA (*123*, *124*) that eventually lead to loss of mitochondrial function (see Hoopes Viewpoint‡). As an example, the Pol γ gene from one Belgian and several Italian families with PEO has a heterozygous A-to-G transition at codon 955 that changes a tyrosine to a cysteine (Y955C). The tyrosine is highly conserved among polymerases in the A family, and structural analysis of another A family member, T7 DNA polymerase, indicated that the tyrosine is in the active site binding pocket for the nascent base pair. The Y955C derivative of Pol γ has a 10- to 100-fold higher error rate for certain mismatches relative to the wild-type enzyme, as well as lower catalytic efficiency. These properties of the Y955C variant are consistent with the accumulation of mutations in mtDNA observed in patients with PEO. Additional mutations in the *POLG* gene have been found that are associated with heritable PEO (*124*). The functional consequences of these mutations, and possibly of polymorphisms in *POLG*, await future investigation.

Humans carrying mutations in the *XPV* (*POLH*) gene that inactivate the function of Pol η suffer from xeroderma pigmentosum, a rare disease characterized by increased susceptibility to sunlight-induced skin cancer. The current hypothesis regarding this association (*87*, *107*, *125*) is that cyclobutane pyrimidine dimers (CPDs) resulting from sunlight exposure are removed slowly by excision repair and therefore are sometimes encountered by the replication machinery. CPDs block DNA synthesis by accurate replicative polymerases, whereas Pol η efficiently bypasses them, incorporating predominantly correct nucleotides. Because Pol η is absent in XPV patients, CPDs are bypassed by another polymerase in a manner that generates the mutations that lead to skin cancer. It remains to be determined whether other types of cancer are associated with inactivation of other polymerases that participate in translesion DNA synthesis (for example, Pol κ or Pol ζ).

The consequences of a defect in proofreading have recently been studied in mice carrying a point mutation that inactivates the 3′-to-5′ exonuclease of DNA polymerase δ (*126*, *127*). Inactivation of the exonuclease eliminates its proofreading function, resulting in a recessive mutator phenotype. The mice also have a recessive cancer phenotype characterized by reduced life-span (median survival, 10 months) and an increased incidence of several tumor types, predominantly of epithelial cell origin. This implies that DNA polymerase errors that are not proofread contribute to carcinogenesis. Thus, the consequences of the loss of proofreading during replication conform, in general, to the mutator hypothesis for the origins of cancer, which posits that an early event in tumorigenesis is the expression of a mutator phenotype resulting from mutations in genes that normally function to maintain genome stability (*128*).

Given the cancer phenotype of mice with one proofreading defect, it is also worth mentioning that many of the DNA polymerases listed in Fig. 1 are intrinsically inaccurate and do not have a proofreading activity. For example, the average base substitution error rate of exonuclease-deficient DNA

* http://sageke.sciencemag.org/cgi/content/full/sageke;2001/1/oa5
† http://sageke.sciencemag.org/cgi/content/full/sageke;2002/31/pe12
‡ http://sageke.sciencemag.org/cgi/content/full/sageke;2002/45/vp6

DNA polymerase	Alteration	Effect on protein function	Effect on health	Comments
Human Pol γ	Tyr to Cys in polymerase active site	Reduces activity and fidelity	Progressive external ophthalmoplegia	
Human Pol η	Gene mutations	Inactivate polymerase	Cancer, altered SHM specificity	
Mouse Pol δ	Asp to Ala in exonuclease active site	Inactivates proofreading	Cancer	
Human Pol β	Elevated expression		Cancer	
Human Pol ι	Gene deletion	Inactivates polymerase	Altered SHM specificity	Demonstrated using a Burkitt's lymphoma cell line (BL2)
Human Pol ζ	Inhibition of expression		Decreased SHM	Demonstrated using human B cells[a]

[a] Reduced expression of the gene that encodes Pol ζ also results in decreased SHM in mice.

Fig. 4. Consequences of altered mammalian DNA polymerase function.

polymerase β alone is sufficient to introduce more than one mismatch into the genome per day during BER of the ≥10,000 damaged bases estimated to arise every day in a human cell. There is evidence to suggest that exonucleases encoded by different genes may proofread errors made by the exonuclease-deficient polymerases. Interestingly, the apurinic endonuclease that incises the DNA backbone after DNA glycosylase removal of a damaged base has recently been shown to contain an intrinsic 3′-to-5′ exonuclease that can preferentially excise mismatched bases from primer termini (129). Thus, errors made during BER might be proofread by this exonuclease. This could be important for preventing the accumulation of mismatches in the human genome, perhaps especially for quiescent cells that are subject to stresses that result in damage repaired by BER (for example, oxidative stress).

The existence of several human polymerases with very low fidelity suggests the need for careful regulation of their functions by protein-protein interactions or by transcriptional and/or translational regulation. That the permanent or transient loss of regulation may be relevant to human cancer is suggested by the observation that ectopic expression of the gene that encodes DNA polymerase β in mice is tumorigenic [see (*109*) and references therein].

The mutator hypothesis for cancer emphasizes the negative consequences of mutations that result from DNA biosynthetic errors. However, there is a DNA transaction that occurs in human B cells in germinal centers that results in mutations beneficial to health: somatic hypermutation (SHM) of immunoglobulin genes to generate high-affinity antibodies. The DNA that encodes the variable regions of immunoglobulin genes undergoes mutagenesis at a rate estimated to be a million times greater than the rate of spontaneous mutation in the rest of the nuclear genome. This suggests that targeted, highly inaccurate DNA synthesis is occurring, with low-fidelity DNA polymerases being prime candidates for participation. Indeed, there is now evidence supporting roles for Pol ζ, Pol η, and Pol ι in SHM, as well as suggestions that other DNA polymerases might also be involved [reviewed in (*37*, *130*-*132*) and references therein]. The nature of the DNA transactions that introduce the mutations at high rates are the subject of intense investigation. Models involving replication, double-strand break repair, and BER have all been considered. The idea that BER might be involved in SHM is strongly supported by evidence indicating that SHM is initiated upon deamination of cytosine in DNA (*133*-*136*) by the activation-induced cytidine deaminase known to be required for SHM (*137*).

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Further Reading

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