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EUKARYOTIC DNA POLYMERASES

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■ **Abstract** Any living cell is faced with the fundamental task of keeping the genome intact in order to develop in an organized manner, to function in a complex environment, to divide at the right time, and to die when it is appropriate. To achieve this goal, an efficient machinery is required to maintain the genetic information encoded in DNA during cell division, DNA repair, DNA recombination, and the bypassing of damage in DNA. DNA polymerases (pols) α, β, γ, δ, and ϵ are the key enzymes required to maintain the integrity of the genome under all these circumstances. In the last few years the number of known pols, including terminal transferase and telomerase, has increased to at least 19. A particular pol might have more than one functional task in a cell and a particular DNA synthetic event may require more than one pol, which suggests that nature has provided various safety mechanisms. This multi-functional feature is especially valid for the variety of novel pols identified in the last three years. These are the lesion-replicating enzymes pol ζ, pol η, pol ι, pol κ, and Rev1, and a group of pols called pol θ, pol λ, pol μ, pol σ, and pol ϕ that fulfill a variety of other tasks.

CONTENTS

INTRODUCTION

Complex cellular functions are performed by networks of protein machines. These protein assemblies contain highly coordinated moving parts, whose functions are in general temporally and spatially regulated by a series of ordered conformational changes that and are powered by chemical energy derived from hydrolysis of nucleoside triphosphates (1). The eukaryotic replisome is an example of how protein components interact and communicate with one another, acting in a coordinated fashion in order to duplicate the genetic information of the cell (2). At the heart of the replisome are template-directed machines for phosphoryl transfer (3): the DNA polymerases (pols). Since the discovery of pol α in 1957, the number of eukaryotic pols identified has grown (reviewed in 4). In the early 1970s pols β and γ were discovered, leading to the simple concept that pol α was the enzyme responsible for nuclear DNA replication, pol β for DNA repair, and pol γ for mitochondrial DNA replication (Table 1). The discovery of pol δ and pol ε and the intensive work done on them during the 1980s suggested that a particular pol might have more than one functional task and that a particular DNA synthetic event may require more than one pol (reviewed in 5). Moreover since 1999, at least 10 novel pols have been discovered (for details see below).

Since both DNA replication and repair are of primary importance for cells, it appears that nature created safety mechanisms by employing different pols for similar functional tasks. For example, DNA replication requires pol α, pol δ, and pol ε, while translesion DNA synthesis depends at least on pol ζ, pol η, pol ι, pol κ, and Rev1 (6). In many cases pols are multipolypeptide complexes that contain other functional subunits in addition to the polymerizing subunit, which often displays a proofreading $3' \rightarrow 5'$ exonuclease. The other functional subunits are responsible for other enzymatic activities (e.g. DNA primase to synthesize RNA primers) or allow the pol to interact with other proteins. An impressive example of these multiple functions is the finding that the replicative pols δ and ε are chaperoned by two accessory proteins, replication factor C and proliferating cell nuclear antigen (reviewed in 7 and 8, respectively), which allow accurate and fast DNA synthesis. At

TABLE 1 The classical DNA polymerases and telomerase[a]

Pol	Functional tasks
α	Initiator pol
POLA[b]	Lagging strand pol
B[c]	
β	Base excision repair pol
POLB	Recombination pol
X	Meiosis pol
	Translesion pol
	Role in neurogenesis
γ	Mitochondrial replication pol
POLG	
A	
δ	Main pol at the leading and lagging strand
POLD	Base excision repair pol
B	Nucleotide excision repair pol
	Mismatch repair pol
	Double-strand break repair pol
	Recombination pol
ϵ	Leading and lagging strand pol
POLE	Base excision repair pol
B	Recombination pol
	Checkpoint control pol
Telomerase	Telomere maintenance pol
	Homologous to reverse transcriptase

[a]For details see text and references therein.

[b]Human Genome Organization (HUGO) nomenclature.

[c]DNA polymerase family A, B, X, or Y (see References 10 and 11).

the structural level, all classical pols share a similar active site (9). Polymerization occurs through a mechanism catalyzed by two metal ions; this mechanism guarantees the incorporation of the correctly base paired deoxyribonucleoside monophosphate onto a growing primer/template duplex, with the exception of some of the translesion pols (e.g. pol ι) (6). This review first summarizes the assembled knowledge of the five "classical" and accurate pols α, β, γ, δ, and ϵ and the two important accessory proteins replication factor C and proliferating cell nuclear antigen. A second part addresses the recently discovered and mostly inaccurate pol ζ, pol η, pol θ, pol ι, pol κ, pol λ, pol μ, pol σ, pol ϕ, and Rev1.

THE CLASSICAL DNA POLYMERASES α, β, γ, δ, AND ϵ

An Evolutionary Perspective: DNA Polymerases Have a Very Conserved Active Site

Based on sequence homology and structural similarities, pols have been grouped in five different families: A, B, C, X, and Y (10, 11). The eukaryotic replicative pols (pol α, pol δ, and pol ϵ) belong to family B, and the mitochondrial pol γ to family A. Albeit pols from different families are structurally quite dissimilar, several common features have emerged (9, 12–14). They fold into a conformation resembling a human right hand composed of three distinct domains designated as palm, thumb, and fingers. Figure 1*A* shows the structure of pol from bacteriophage RB69 (a prototype of the family B pol and consequently related to pol α, pol δ, and pol ϵ).

On the basis of the pol α sequence, six highly conserved regions termed I–VI have been identified among eukaryotic, prokaryotic, and viral pols. Their relative position along the primary sequence is also conserved; region IV is at the N terminus, followed by regions II, VI, III, I, and V. The functional roles of these regions have been extensively described elsewhere (12, 14). The highly conserved region I is located in the palm, close to the thumb domain, and contains one of the conserved aspartic residues that form the catalytic diad of all known pols in family B (-YGDTDS- motif). The other invariant aspartic acid belongs to region II and is located at the tip of a β-sheet that is part of the palm subdomain (-DxxSLYPS- II region). Included in this region is the highly conserved SLYPS-II region, which is important for deoxynucleoside triphosphate (dNTP) binding. Although these motifs are absolutely conserved in pol α and δ subfamilies, pol ϵ has considerably diverged from the consensus, so that the region I motif of pol ϵ is -ELDTDG- and region II has become -DxxAMYPN-. Other residues important for dNTP binding are in region III, and they fold into an α-helix located in the fingers subdomain. Region IV is located at the N terminus, in extending into a domain that is part of the $3' \rightarrow 5'$ exonuclease active site. The other two conserved regions, V and VI, are located in the thumb and fingers subdomains, respectively.

The high degree of structural and sequence conservation of these domains between eukaryotic, prokaryotic, and viral pols suggests that these pols derive from a common ancestor gene. The palm subdomain, which contains the catalytically important residues, can be superimposed among members of the pol α subfamily, *Escherichia coli* pol I family, and HIV-1 reverse transcriptase. This subdomain consists of four- to six-stranded β-sheets flanked by two α-helices. Comparison with the structure of the RB69 pol showed that the most conserved residues are located 10 Å from the active site, into three regions that emanate from the palm, the fingers, and the thumb and converge at the catalytic site, forming a continuous conserved surface.

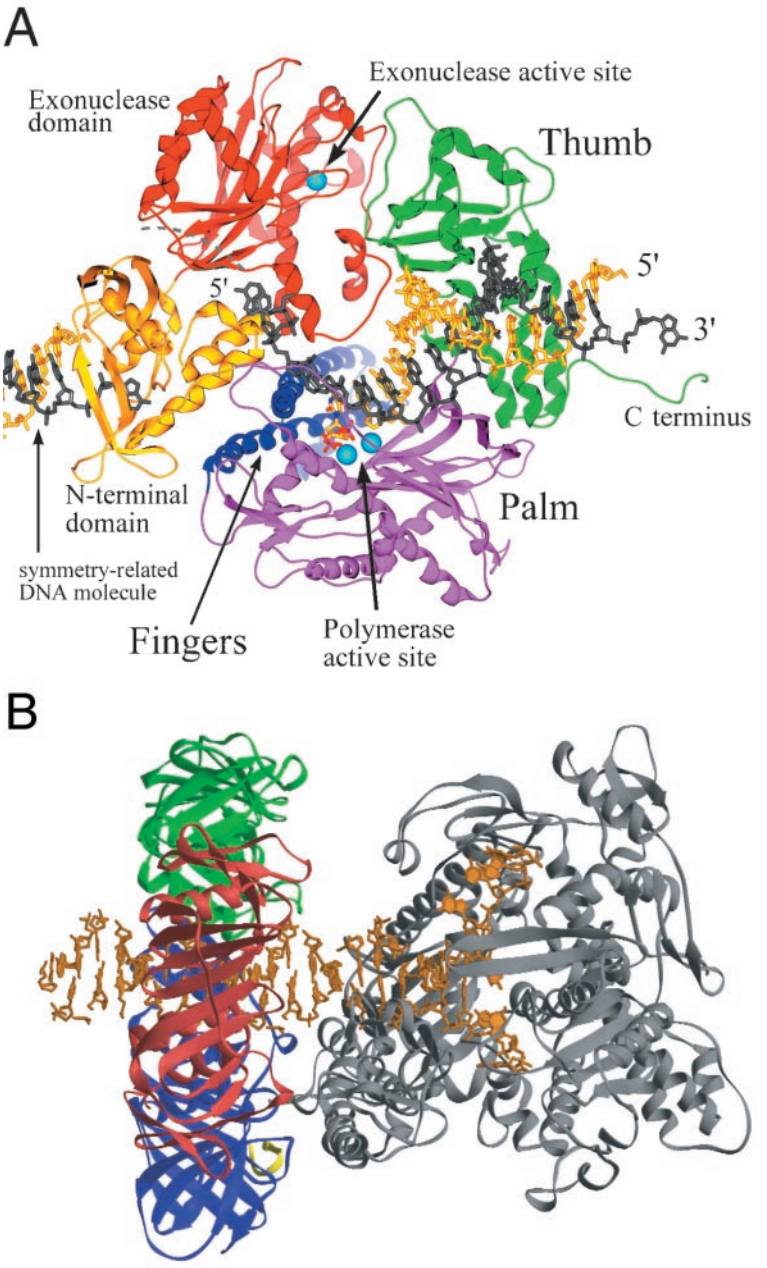

Figure 1 Structure of a replicative DNA polymerase. (*A*) Structure of ternary complex of RB69 pol with primer/template duplex DNA and dTTP. (From Reference 19.) (*B*) Structure of RB69 pol complexed with the sliding clamp gp45. (From Reference 20.) For details see text. (Reproduced with permission from T. Steitz.)

Unlike the palm, the other two subdomains, thumb and fingers, are unrelated among the four families, but they function similarly using analogous secondary structural elements. The fingers are involved in correctly positioning the template and the incoming complementary dNTP, and the thumb is important in DNA binding and processivity (13).

As discussed in the following section, eukaryotic pols are heteromultimers composed of a large subunit and a variety of smaller subunits (see also Table 2). The latter have been implicated in stabilization of the catalytic subunit, establishment of protein-protein interactions, cell-cycle regulation, and even checkpoint function.

The large subunits of pols α, δ, and ϵ can be grouped into three distinct subfamilies within the B-type pols. Pol δ is the most conserved, with identity ranging from 93% between human and mouse, to 49% between human and yeast (15). The identity between human and yeast genes for pol α is only 35% and for pol ϵ 39%. The N-terminal part of pol δ (amino acid residues 1–305) is generally poorly conserved among δ-like pols, but it contains three regions of high homology: a nuclear targeting signal (NTS) and the so-called NT-1 and NT-2 regions. The C-terminal part (amino acids 850–1105) is more conserved, with three highly identical regions termed CT-1 to 3 and a zinc-finger domain (ZnF2), which is 89% identical between human and yeast pol δ. C-terminal zinc-finger domains are also present in all α- and ϵ-like pols. Both the N- and C-terminal parts of pol ϵ show about 25% identity between human and yeast. The C-terminal region of pol ϵ contains 1000 extra amino acids, is found only in pol ϵ subfamily members, and contains a highly acidic region (residues 1918–1948) and the zinc-finger domain (residues 2125–2222). Thus, both from sequence and structural analysis, it appears that the catalytic subunits of eukaryotic pols are composed of a central domain that is evolutionarily very conserved.

A Mechanistic Perspective: DNA Polymerases Are Built by Addition of Specific Domains to a Conserved Core with Essential Catalytic Activity

STRUCTURAL AND FUNCTIONAL CONSERVATION WITHIN THE CATALYTIC CORE

Pols are template-directed enzymes that catalyze for phosphoryl transfer. Hence since they have the ability to synthesize long polymers of nucleoside monophosphates, whose linear spatial disposition is dictated by the sequence of the complementary template DNA strand (16). Their overall structure has been optimized through evolution to suit the specialized tasks each pol performs within the cell. The most conserved domains are usually responsible for essential basic catalytic functions, whereas more divergent parts have evolved independently to fulfill specific roles (14).

The phosphoryl transfer reaction that lies at the heart of the polymerization mechanism is catalyzed by a two-metal-ion mechanism (13, 17). Two Mg^{2+} ions form a pentacoordinated transition state with the phosphate groups of the

incoming nucleotide, through interaction with conserved carboxylate residues in region I and region II. Besides the conserved chemical step catalyzed by two metal ions, another common feature of all pols is the concerted movement of the finger subdomains that rotate toward the palm to switch from an "open" to a "closed" conformation, forming the binding pocket for the incoming dNTP. Resolution of the structure of RB69 pol complexed with primer/template DNA and dTTP shows that upon formation of the ternary complex, the fingers domain rotated 60° toward the palm, resulting in a movement of the finger tips of 30 Å. The thumb domain also rotated toward the palm by 8°. The resulting closed conformation allows the interaction of conserved residues of the fingers with the dNTP binding site and the exonuclease domain. In addition the thumb is wrapped around the minor groove of the primer/template DNA duplex. The pol δ and ϵ catalytic subunits both contain a $3' \rightarrow 5'$ proofreading exonuclease domain at their N terminus. In the known crystal structures of family B members, this domain is folded around a central β-sheet that contains the active site and, together with the pol domain, creates a ring-shaped structure with a central hole, where the template/primer duplex DNA is positioned. The catalytic mechanism leading to the removal of the last incorporated nucleotide by the exonuclease activity is a phosphoryl transfer catalyzed by two metal ions, analogous to the one responsible for polymerization. The $3' \rightarrow 5'$ exonuclease activity allows the pol to remove misincorporated nucleotides, ensuring the high fidelity of DNA synthesis required for faithful genome replication.

Thus, during DNA synthesis, pol δ and ϵ repetitively shuttle between a polymerizing and an editing mode, and the balance between these two activities is regulated by a competition for the $3'$ end of the primer between the exonuclease and polymerase active sites (18). These two different functional states of exonuclease-containing pols are also reflected at the structural level. The duplex DNA occupies the same position adjacent to the thumb in either the editing or the polymerizing mode, whereas the $3'$ end is bound to the exonuclease or polymerase active sites, respectively, which can be separated by more than 30 Å. A model for the coordinated action between the polymerase and exonuclease activities of family B pols can be constructed by comparing the structure of RB69 in its polymerizing (19) and editing (20) modes. A mismatched base pair prevents the fingers from rotating toward the palm to bind the incoming dNTP. This leaves the $3'$ mismatched end available for binding to the exonuclease active site, which removes the wrong nucleotide. During the switch between polymerizing and editing modes, the DNA moves toward the exonuclease active site with a rotation in the double-helix axis. This movement is aided by the tip of the thumb subdomain, which holds contact with the DNA during the movement, guiding it on a path between the two sites.

MOLECULAR ARCHITECTURE OF POL α In all eukaryotic organisms, pol α is a heterotetrameric enzyme (Table 2). Three separate domains were identified in the catalytic p180 subunit: (*a*) an N-terminal domain (amino acids 1–329), which

appears dispensable for both the catalytic activity and the assembly of the tetrameric complex; (*b*) a central domain (amino acids 330–1279), which contains all the conserved regions responsible for DNA binding, dNTP binding, and phosphoryl transfer (see above); and (*c*) a C-terminal domain (amino acids 1235–1465), which is dispensable for catalysis but necessary for the interaction with the other subunits. The heterotetrameric pol α is unique among eukaryotic pols, since two of the three small subunits have DNA primase activity. The eukaryotic DNA primase is a heterodimeric enzyme with subunits (in human cells) of 49 and 55 kilodaltons (kDa) (21). Since DNA primases were reviewed last year in this series we do not discuss these enzymes further (22). The heterodimeric DNA primase is associated with the catalytic 180-kDa subunit and the B subunit (21). Like the corresponding polypeptides in pol δ and pol ϵ heteromultimers, the B subunit of pol α has no detectable enzymatic activity, but is essential in yeast and appears to have a role in maintaining a functional heterotetrameric complex. In addition, the finding that it is phosphorylated in a cell-cycle-dependent manner suggests regulatory functions (23).

Studies of coexpression of all four mammalian subunits led to a model for the dynamic assembly of the heterotetrameric pol α/primase. The mouse primase p55 subunit directly interacts with the catalytic p180 subunit and the second primase subunit p48. In addition, the p68 B subunit directly contacts p180 and p55/p48. These interactions are essential for tethering the heterodimeric primase to the large catalytic subunit. The p55/p48 can translocate into the nucleus independently from the other subunits, by virtue of its own nuclear localization signal located in p55, but interaction of p180 and p68 is required for their nuclear localization, since both subunits are cytoplasmic when expressed separately.

MOLECULAR ARCHITECTURE OF POL δ Five subunits have been identified in *Schizosaccharomyces pombe* pol δ (p125, p55, p54, p40 and p22) (24) four in mammalian cells (p125, p66, p50, and p12) (25, 26), and three in *Saccharomyces cerevisiae* (p125, p58, and p55) (27) (see Table 2, pp 142–43). Recently, a unified nomenclature for these subunits was proposed (28). Pol δ is present within the eukaryotic cell in high-molecular-weight complexes and the small subunits might be critical for their maintenance. In *S. cerevisiae* it has been shown that a complex of the p125 and p58 subunits is a dimer in solution, whereas addition of the third p55 subunit generates a high-molecular-weight complex that is a dimer of the heterotrimer p125/p58/p55. In *S. pombe* it has been shown that the complex of the p125, p55, and p22 subunits is a trimer in solution and that addition of the p54 subunit generated a dimer of the heterotetramer. Both dimeric forms of the *S. cerevisiae* heterotrimer (27) and of the *S. pombe* heterotetramer (29) were more efficient and processive in DNA synthesis than the corresponding monomeric forms. In mammalian cells, the three small subunits identified so far, p66, p50, and p12, are homologous to, respectively, the p55, p40, and p22 subunits of the *S. pombe* enzyme. A study of purified pol δ from mammalian tissue revealed that the native form of the enzyme had a

molecular weight between 250,000 and 500,000, which suggests that in mammalian cells pol δ also forms a dimeric pol (26).

MOLECULAR ARCHITECTURE OF POL ε Pol ε is composed of four subunits, p261, p59, p17, and p12 in human cells (30, 31) and p256, p80, p23, and p22 in *S. cerevisiae* (32) (see Table 2). In both cases the two smaller subunits interact with the larger two, forming a heterotetrameric complex. Biochemical analysis revealed that the p80 subunit alone or the heterodimer p256/p80 can form dimers, which suggests that p80 might be responsible for the formation of a dimeric pol.

Coordinated Leading and Lagging Strand Synthesis and the DNA Polymerase Switch Mechanism: Distinct Roles for DNA Polymerases α, δ, and ε

Pol α/primase associates with the initiation complex at the DNA origin (33) and starts to synthesize a short RNA/DNA hybrid of approximatively 10 RNA nucleotides followed by 20 to 30 DNA nucleotides. This oligonucleotide is then utilized by pol δ or ε for processive elongation on both the leading and the lagging strand (34). Replication on the lagging strand is characterized by small DNA pieces called Okazaki fragments, with a length of about 200 bases. In mammalian cells an initiation event has to happen 4×10^4 times on the leading strand (approximately the number of origins of DNA replication in a mammalian cell), but it has to be repeated at the beginning of each Okazaki fragment (about 2×10^7 times in mammalian cells).

The substitution of pol α/primase by the more processive pol δ holoenzyme is called pol switch and is dependent upon the synthesis of the RNA/DNA primer by pol α. The pol switch is coordinated and regulated by an ATP switch catalyzed by the auxiliary protein RF-C (35) and involves a complex network of interactions among pol α/primase, pol δ, RF-C, and the protein that binds single-stranded DNA, replication protein A (RP-A) (reviewed in 5).

Both pol α/primase and pol δ are perfectly suited for their respective roles: Pol α/primase can initiate synthesis de novo, whereas pol δ, through its interaction with proliferating cell nuclear antigen (PCNA, a processivity factor), has the ability to synthesize long stretches of DNA. The proposed dimerization of pol δ (27, 29) might play a role in the coordination of leading and lagging strand synthesis (like in the pol III holoenzyme in *E. coli*) and in establishing an asymmetric replication fork, possibly through association of pol α/primase to one of the two halves of the dimeric pol δ.

Genetic analysis in budding yeast has shown that pol ε is required for DNA replication (36). In addition, UV cross-linking studies with replicating chromatin in mammalian cells detected pol ε along with pol α and pol δ (37). Experiments in human cells (38) and in *Xenopus* egg extracts (39) further suggested that pol ε is involved in DNA replication. The N-terminal part of yeast pol ε, including the conserved core domains with all the catalytically important residues, is

TABLE 2 Subunit structure of DNA polymerases α, γ, δ, and ϵ

Subunit composition (kDa)			Chromosomal localization			
Human	***S. cerevisiae* (gene, SGDID[a])**	***S. pombe* (gene)**	**Human**	***S. cerevisiae***	***S. pombe***	**Main function**
DNA polymerase α						
180	165 (*POL1*, S0005046)	170 *(pol1)*	Xq21.3–q22.1	XIV	I	Catalytic subunit
68	86 (*POL12*, S0000131)	66 *(pol12)*	11	II	III	Structural, protein-protein interactions
55	58 (*PRI2*, S0001528)	55 *(pri2)*	6p11–p12	XI	II	Primase
48	49 (*PRI1*, S0001447)	45 *(pri1)*	12q13	IX	I	Primase
DNA polymerase δ						
125	125 (*POL3*, S0002260)	125 *(pol3)*	19q13.3–q13.4	IV	II	Catalytic subunit
	58 (*POL31*, S0003766)	55 *(cdc1)*		X	I	Structural
66	55 (*POL32*, S0003804)	54 *(cdc27)*	11q14	X	II	Multimerization, interaction with PCNA
50		40	7		I	Structural, protein-protein interactions
12		22 *(cdm1)*	11q13		II	

TABLE 2 *Continued*

Subunit composition (kDa)			Chromosomal localization			
Human	***S. cerevisiae* (gene, SGDID[a])**	***S. pombe* (gene)**	**Human**	***S. cerevisiae***	***S. pombe***	**Main function**
DNA polymerase ϵ						
261	256 (*POL2*, S0005206)	250 *(pol2/cdc20)*	12q24.3	XIV	II	Catalytic subunit
59	80 (*DPB2*, S0006379)	67 *(dpb2)*	14q21–q22	XVI		Multimerization
17	23 (*DPB3*, S0000482)		9q33	II		Structural, protein-protein interactions
12	22 (*DPB4*, S0002528)	23 *(dpb4)*	2p12	IV	II	Structural, protein-protein interactions
DNA polymerase γ						
139.5	143.5 (*MIP1*, S0005857)	116.3 *(polG)*	15q25	XV	III	Catalytic subunit
55			17q			Processivity

[a]SGDID, Saccharomyces cerevisiae genome data base identification.

dispensable for viability, whereas the C-terminal part, which is involved in protein-protein interactions and checkpoint control, is essential in *S. cerevisiae* (40). *S. pombe* mutants with N-terminal deletions in pol ϵ are viable, as in *S. cerevisiae*, but show accumulation of DNA damage and need expression of the checkpoint genes *rad3, hus1,* and *chk1* (41). These data do not necessarily mean that the catalytic activity of pol ϵ is not involved in DNA replication, but rather suggest than it can be substituted for the basic synthetic function and not for the specialized checkpoint function. During evolution pol ϵ could have acquired specialized functions as a "sensor" pol for quality control of DNA replication, whereas the function of pol δ remained as a DNA synthesizing machine exclusively.

Fidelity of DNA Synthesis: Novel Roles for Accessory Proteins

As fidelity of pols has been reviewed in depth recently (42), we concentrate on a few recent developments. Genetic analysis has shown that fidelity is relevant for DNA replication (43), since mutations affecting the exonuclease activity of pol δ and pol ϵ result in high mutation rates in vivo. Moreover, transgenic mice with *exo* mutations of alleles for pol δ showed a striking increase in cancer susceptibility within 12 months of age (44). Because pol α is essential for DNA replication in vivo but lacks a proofreading activity, its infidelity poses a risk to the cell of accumulating dangerous mutations during DNA replication. Biochemical studies have revealed that RP-A can physically interact with pol α and stabilize its binding to a primer end, at the same time reducing the ability of pol α to incorporate a wrong nucleotide (45). Thus, it is conceivable that both the error-free Okazaki fragment maturation and fidelity clamp function of RP-A cooperate to prevent misincorporation by pol α. Interestingly, the same biochemical parameter (increased affinity of the enzyme for the DNA template) can have two opposite effects: increase in fidelity in the case of the RP-A/pol α interaction and decrease in fidelity in the case of the PCNA/pol δ interaction (46). From an evolutionary point of view, it is meaningful that maximization of processivity of pol δ might have been the main goal to be achieved. The higher rate of misincorporation could be compensated for by the associated proofreading exonuclease activity. In the case of pol α, the crucial target may have been to achieve a higher level of fidelity, whereas the increase in processivity was less important because of the limited size of the products synthesized.

The Matchmaker Concept for Establishing a Moving Platform: Replication Factor C and Proliferating Cell Nuclear Antigen

REPLICATION FACTOR C Replication factor C (RF-C) is a heteropentameric complex composed of one large subunit (p140/RFC1) and four smaller ones

(p40/RFC4, p38/RFC5, p37/RFC2, and p36/RFC3), which share considerable sequence similarity with each other as well as with their bacterial clamp loader counterparts in the pol III γ-complex (reviewed in 5). RF-C is a clamp loader and a matchmaker ATPase, which can load the sliding clamp PCNA onto DNA. RF-C-catalyzed PCNA loading is obligatory for the assembly of pol δ onto the DNA template to form the processive holoenzyme that acts during DNA synthesis of both leading and lagging strands at the replication fork (reviewed in 47). RF-C dissociates from PCNA after loading it onto the DNA and does not remain directly associated with the pol δ core (48). Electron microscopic studies suggested that in the absence of ATP, RF-C has a closed two-finger structure called the U form. This U form is converted into a more open C form upon binding of ATP. PCNA can be held between these two fingers, and a structural change in the RF-C conformation can open the PCNA ring so that it can encircle the DNA (49). Studies of the homologous system in *E. coli* (50), as well as the crystal structure of the δ′ subunit of the *E. coli* γ-complex (51), have given insights into how this may take place. The γ-complex can, upon ATP binding, associate with and open up the β-clamp (the structural and functional homolog of PCNA). The γ-complex/β-clamp/ATP complex then associates with the primer terminus and forms a ternary complex with the DNA. The DNA binding stimulates ATP hydrolysis, which ejects the γ-complex and leaves β on the DNA. In addition, the roles of the five subunits in the γ-complex (γ, δ.δ′, χ and ψ) have been identified to a great extent, and it seems that sequential ATP hydrolysis can drive the exact assembly of the β-clamp around the DNA (52). The δ-subunit of the γ-complex was identified as the clamp unloader (53) and might have an analogous role as the RF-C p40 subunit, which is also capable of unloading PCNA from the DNA (54). Moreover, as discussed above, RF-C is likely responsible for the polymerase switch.

DNA replication is not the only pathway requiring RF-C. As discussed in more detail below, PCNA is also involved in a number of DNA repair pathways, distinct from DNA replication, and since PCNA loading is required for all these functions, RF-C is also an important component of these pathways. Two well-documented examples are nucleotide excision repair (NER) (55) and long-patch base excision repair (BER) (56). Finally, RF-C may have a role in checkpoint control, since the *S. cerevisiae* Rfc5p has been shown to interact with Spk1p, an essential protein kinase for the transition from S phase to mitosis (57), whereas the *S. cerevisiae RFC2* gene is required for an S-phase checkpoint (58) and the *S. pombe* Rfc2p has been shown to play a key role in a DNA replication checkpoint (59).

PROLIFERATING CELL NUCLEAR ANTIGEN PCNA is a homotrimeric ring-shaped protein with a molecular mass of 29 kDa for each monomer that occupies 120° in the ring (reviewed in 60). Each monomer is composed of two domains. The crystal structure of *S. cerevisiae* (61) and human PCNA (62) revealed how PCNA can carry out its sliding clamp function on DNA by forming a trimeric ring that

encircles the DNA strand without making direct contact with it. In addition to its crystal structure being solved, a wealth of information about the involvement of PCNA in replication has accumulated and the list of PCNA interactors continues to grow rapidly. At least 30 proteins appear to interact with PCNA (60), although the exact function of most of these interactions has not been clarified. Here we discuss only the interactions of PCNA with pols and RF-C.

PCNA contacts the three multisubunit proteins pol δ, pol ε, and RF-C (63–65), and the common interaction domain has been mapped to a region on the outer front surface of PCNA, involving the loop that connects the two domains of each PCNA monomer and a loop immediately preceding the C terminus. In addition, pol ε has been shown to interact with a loop on the back side of PCNA (66). An interesting question involves the site of PCNA interaction in the pol δ holoenzyme, and apparently there are multiple sites of interaction. The structure of the prokaryotic and bacteriophage sliding clamps are very similar to eukaryotic PCNA and have almost superimposable structures. The β-subunit of pol III holoenzyme is a dimer with 180° for each monomer (67), and the gp45 of bacteriophage T4 is a trimer (68). An important role for the linker in the attachment of the pol to the clamp ring has been demonstrated by using a modeling approach to a molecular replacement search, combining the structure of the sliding clamp of gp45 of bacteriophage RB69 (a close relative of T4 and to the family of B pols such as pol α, pol δ, and pol ε), with the RB69 pol structure. This analysis indicated that the interaction between the pol and the sliding clamp resembles the interaction of the human kinase inhibitor p21 and PCNA, which suggests that the pol/sliding clamp interaction has been conserved in evolution over 10^9 years. Since the clamp structure is perfectly conserved from bacteria to human, two properties of the *E. coli* β-subunit could also be extended to human PCNA and pol δ. First, the γ-complex (the RF-C homolog) can load the β-subunit (the PCNA homolog) onto a primer with a length of 10 nucleotides only if no steric hindrance by another protein is there. In such a situation 14–16 nucleotides are required. The complete pol III holoenzyme needs 22 nucleotides for a successful loading followed by DNA synthesis (69). Second, clamps are dynamic in handling secondary structures. Small obstacles (stems, loops, flaps, and bubbles) can be overcome by the pol III holoenzyme; this is likely to occur since this enzyme has strand displacement activity (70). Because pol δ also has strand displacement activity (71), one could expect an analogous handling of frequently occurring obstacles in eukaryotic chromosomes.

DNA Polymerase γ, The Mitochondrial Replicase

Pol γ is a heterodimeric protein composed of a large subunit, responsible for the catalytic activities, and a small accessory subunit. The large subunit of human pol γ has been cloned and the gene mapped to the chromosomal location 15q25 (Table 2) (72). It contains 1239 amino acids, with a calculated molecular mass of 139.5 kDa. The amino acid sequence in human cells is 42%, 43%, 49%, and 78% identical to those of *S. pombe*, *S. cerevisiae*, *D. melanogaster*, and the C-terminal

half of *G. gallus*, respectively. The large subunit was recombinantly expressed and the enzyme (p140) demonstrated DNA polymerase and both 5′ → 3′ and 3′ → 5′ exonuclease activities (73). Pol γ is also able to catalyze the removal of a 5′-deoxyribose phosphate (74). The p55 subunit was shown to stimulate both the polymerase and exonuclease activities of p140. The crystallographic structure of the pol γ p55 subunit from mouse cells showed that the dimerization domain folded into a four-helix bundle and that dimerization was necessary for pol γ stimulation (75). Thus the p55 subunit contains separate domains for binding to p140, dimerization, and DNA binding.

Further Functions of DNA Polymerases α/Primase, δ, ε, and γ: A Coordinated Interplay

CELL-CYCLE REGULATION DNA replication and chromosome segregation are highly coordinated and interdependent. DNA replication enzymes (and their accessory factors) can trigger S-phase arrest when they are stalled at DNA lesions in damaged DNA. However, they are also the targets of inhibitory signals triggered by external stimuli, which can block S-phase progression to allow DNA repair or, alternatively, apoptosis. Increasing evidence points to a critical role for pol α in integrating DNA replication and cell-cycle regulation (reviewed in 76). The *S. cerevisiae* protein kinase Rad53 modulates the replication apparatus for the lagging strand by controlling phosphorylation of the pol α/primase complex in response to intra-S-phase DNA damage (77). According to these results, the *S. pombe* Rad53 homolog cdc1$^+$ was shown to be a suppressor of a temperature-sensitive mutant of pol α (78). Genetic studies in *S. cerevisiae* (79) and in *S. pombe* (80) suggested that pol α is likely also a target of checkpoint controls, through its two associated primase subunits. Additional evidence came from a study in *Xenopus laevis* extracts, where chromatin was found to be competent to initiate a checkpoint response only after the DNA was unwound and pol α had been loaded. Checkpoint induction did not require de novo DNA synthesis on the template strand, but did require RNA primer synthesis by primase (81).

Pol ε is another important sensor for UV damage and DNA replication blocks during S phase. This checkpoint function has been mapped to the C-terminal domain of pol ε (see above) (82). As already mentioned, a pol ε mutant with a deletion encompassing the entire N-terminal half is viable in yeast, which suggests that another pol can compensate for the catalytic role of pol ε (83). Interestingly, *S. pombe* mutants with an N-terminal deletion in pol ε were able to replicate but accumulated damages, which suggests a major defect during DNA synthesis. In contrast, mutations affecting the C-terminal region do not present an intact S-phase checkpoint (41). In *S. cerevisiae* cells, a physical complex containing the checkpoint protein Dpb11 and pol ε has been detected (84). Moreover, mutations affecting the small subunit Dpb4p of pol ε are lethal in a Dpb11 mutant genetic background (85). During the S phase of the cell cycle, Dpb11 associated preferentially with DNA fragments containing autonomous

replicating sequences (ARSs) at the same time as pol ε does. Association of Dpb11 and pol ε with these fragments was found to be mutually dependent. Moreover, Dpb11 was shown to be required for the association of pol α/primase with the ARS (86), providing a possible link between the checkpoint functions of these two pols.

Activity of replicative pols is also regulated in a cell-cycle-dependent manner (87). Posttranslational modification by phosphorylation is well documented for pol α/primase (88). *S. pombe* pol α/primase is a phosphoprotein; serine is its exclusive phosphoamino acid. Pol α/primase was found to be phosphorylated to a threefold higher level in late-S-phase cells compared with cells in the G_2 and M phases. Moreover, the phosphorylation sites of pol α/primase in late-S-phase cells were not the same as those in G_2/M-phase cells. The p180 catalytic subunit is phosphorylated throughout the cell cycle and the p66 subunit only in mitosis. The p68 subunit homolog in yeast undergoes a complex regulation (89). It is present in two forms, of 86 and 91 kDa: The p91 form results from cell-cycle-regulated phosphorylation of p86, whereas the p86 subunit present in G_1 arises by dephosphorylation of p91 while cells are exiting from mitosis, becomes phosphorylated in early S phase, and is competent and sufficient to initiate DNA replication. However, another pol of p86 is also transiently synthesized as a consequence of periodic transcription of the *POL12* gene and is phosphorylated no earlier than G_2. This phosphorylation event requires binding of p86 to the p180 subunit.

Pol δ is also a phosphoprotein and is most actively phosphorylated during the S phase. Physical interaction between pol δ and cyclin-dependent kinases has been detected and the interaction domains mapped (90).

DNA REPAIR Three types of excision repair are acting in the cell (91): BER, NER, and mismatch repair (MMR), each of them targeted to specific DNA lesions. A common feature of all these mechanisms is that DNA synthesis is required in order to replace the damaged DNA with a faithful copy of the intact strand. Genetic and biochemical studies are consistent with an involvement of both pols δ and ε in NER and BER (92). For example, either pol δ or ε can be used to reconstitute long-patch BER (56) and NER in vitro (55). Because of its limited activity in strand displacement, for in vitro reconstitution studies pol δ seemed to be more efficient in long-patch BER, whereas pol ε achieved a higher efficiency in gap-filling synthesis in NER reactions. In vitro complementation assays also showed the ability of pol δ to reconstitute MMR in deficient extracts (93). Finally, genetic evidence from *S. cerevisiae* suggested that pol α/primase is involved in double-strand break repair (DSBR), which could be accomplished through generation of a structure like a replication fork (94).

In contrast to the chromosomal DNA, the mitochondrial DNA (mtDNA) has a 10-fold higher rate of nucleotide substitution. Pol γ, with its associated $3' \rightarrow 5'$ proofreading exonuclease (95), participates in mtDNA repair. When mutagenic uracil residues are incorporated in mtDNA, subsequent to actions of

uracil-DNA glycosylase and apurinic/apyrimidinic endonuclease, human pol γ is able to fill a single nucleotide gap in the presence of a 5′-terminal deoxyribose phosphate flap (96). In addition, the p140 subunit of human pol γ, thanks to its intrinsic 5′-deoxyribose phosphatase activity, catalyzes the release of the phosphate residue from incised apurinic/apyrimidinic sites, thus producing a substrate for DNA ligase (74, 97).

DNA RECOMBINATION A particular kind of DNA repair is DSBR, which occurs through a recombination-type mechanism (98). Genetic studies of yeast with temperature-sensitive (ts) pol δ mutants suggested that pol δ might be involved in DSBR. As mentioned above, there is genetic evidence that pol α/primase is also important in DSBR (94). The repair of DSBs can be accomplished through a homologous recombination pathway termed break-induced replication (BIR), involving DNA synthesis initiated by the free end of the chromosomal fragment. The recipient chromosome has both strands newly synthesized, with the generation of structures like a replication fork, requiring coordinated leading and lagging strand synthesis. Thus, the indications for a requirement of different replicative pols in DSBR might reflect the need to establish a fully functional replication fork at the site of the lesion.

TELOMERE MAINTENANCE Maintenance of the physical integrity of chromosomal ends, the telomeres, is another critical event coupled to DNA replication (99). Although a specialized DNA polymerase, telomerase (Table 1), is responsible for the synthesis of telomeres in eukaryotic cells, recent evidence points to an active role of DNA replication enzymes as well in controlling telomere length (100). Mutations in the structural gene for pol α in *S. cerevisiae* caused an increase in telomere length. Telomeric DNA synthesis by telomerase should be tightly coregulated with the production of the opposite strand to prevent telomerase from generating excessively long single-strand tails, which may be deleterious to chromosome stability. Using a synthetic telomere DNA template, synthesis of the telomere complementary strand in whole mammalian cell extracts was shown to be inhibited by neutralizing antibodies to pol α. Purified pol α/primase was capable of catalyzing synthesis of the lagging strand with the same requirements as those observed in crude cell extracts. In addition, mammalian pol α/primase was shown to precisely initiate de novo synthesis of an RNA primer with adenosine, opposite the 3′-side thymidine in the G-rich telomere repeat 5′-(TTAGGG)(n)-3′ and to synthesize the nascent DNA fragments by extending the primer. Moreover, pol α/primase extends the product DNA far beyond the length of the template DNA, which suggests a role in telomere expansion. Further evidence for a role of pol α/primase in telomere metabolism comes from the observation that the *S. cerevisiae* telomere-binding protein Cdc13p interacts with the catalytic subunit of pol α and RP-A (101).

DNA Polymerase β, The Prototype of a Repair Enzyme

Pol β is the smallest eukaryotic pol and is composed of a single 39-kDa polypeptide containing 335 amino acid residues (reviewed in 102). It consists of two domains: The 8-kDa N-terminal domain performs the 5′-deoxyribose phosphatase activity (to remove the 5′-deoxyribose phosphate) and single-stranded DNA binding, whereas the large 31-kDa domain performs the pol activity. Pol β is able to fill short gaps in a distributive way and these gaps contain a 5′-phosphate. The structure of pol β has been solved by X-ray crystallography (17, 103) and some of its structural characteristics were discussed in the section "A Mechanistic Perspective."

PHYSIOLOGICAL FUNCTIONS OF POL β More than 20 years ago pol β was proposed as a DNA repair enzyme (104). The constant danger of damaging DNA is counteracted by a variety of repair mechanisms such as NER, BER, DSBR, MMR, and recombinational repair (RR). Among these, BER as an essential mechanism relies to a great extent on pol β (105). BER involves the removal of a single base and its replacement. Efficient repair of a uracil-guanine base pair present in a duplex oligonucleotide can be achieved in vitro, via replacement of a single nucleotide (short-patch BER), by the sequential action of the human proteins uracil-DNA glycosylase, the apurinic/apyrimidinic endonuclease HAP 1, pol β, and either DNA ligase I or III. The 5′-deoxyribose phosphatase activity of pol β removes the 5′-phosphate and renders the unphosphorylated 5′-OH group into a substrate for DNA ligase. DNA synthesis as well as the 5′-deoxyribose phosphatase are coupled, and both are essential for short-patch BER (106). The second BER pathway, which involves filling a gap of several nucleotides (long-patch BER) specifically requires the clamp PCNA, its clamp loader RF-C, and the Flap endonuclease 1 (Fen1). The efficiency of long-patch BER detected in extracts from pol β–deleted mouse cells, as well as the PCNA dependency of this pathway, strongly suggested the involvement of either pol δ and/or ε in the resynthesis step (see above). In this pathway Fen1 is required to cleave a reaction intermediate generated by displacement of the template strand during gap filling. This Fen1- and PCNA-dependent pathway can be reconstituted with pol δ and ε (56), but recent experiments also suggested that pol β may act in this pathway in vivo, as also suggested by the reduced repair activity in pol β–deficient cells (107). Finally, Fen1 stimulates the strand displacement activity of pol β, which suggests a communication between these two enzymes (108). In sum, it appears that the vital BER pathway has developed several strategies to repair damaged DNA and these strategies involve different sets of DNA synthesis machineries (e.g. pol β alone, pol β with Fen1, or pol δ or pol ε holoenzymes with Fen1).

Pol β has other roles besides BER (102). First, it has been implicated in meiotic events associated with synapsis and recombination. Second, the 67-kDa *S. cerevisiae* homolog of mammalian pol β encoded by the nonessential *POL4*

gene has been implicated in DSBR; it probably utilizes a nonhomologous end-joining mechanism. Third, mice carrying a targeted disruption of the pol β gene had growth retardation and died of a respiratory failure immediately after birth (109). The increased apoptotic cell death observed in the developing central and peripheral nervous systems suggest that pol β plays an essential role in neurogenesis.

THE NOVEL DNA POLYMERASES

Discovery

The classical pols have inherent high fidelity and perform accurate DNA synthesis. During DNA replication, however, there are situations where lesions in DNA can impede the replication machinery. We have learned in the last few years that a variety of so-called lesion-replicating pols can overcome the replication blocks (reviewed in 4, 110, and also in this volume, 111). These pols belong to a group of four structurally related proteins that are found in all three domains of life, the prokaryotes, the archaea, and the eukaryotes. Initially, genetic studies in *E. coli* showed that UmuC and DinB are involved in translesion DNA synthesis, whereas in *S. cerevisiae*, Rev1 and Rad30 were found to have similar roles (reviewed in 6). These genes were found to code for novel pols that are able to replicate damaged DNA; this group is now called the Y-family of DNA polymerases (11). The first identified translesion pol was the Rev3 and Rev7 holoenzyme in yeast called pol ζ (112), which was shown to perform thymidine dimer bypass (113). Translesion synthesis required an additional enzyme called Rev1 (114), containing a template-directed deoxycytidyltransferase activity, mainly incorporating C in front of abasic sites. The product of Rev1 can be extended by pol ζ.

Later, the Rad30 pathway that facilitates translesion synthesis was identified in yeast. This gene belongs to the Rad6 epistasis group, which is involved in postreplication repair. Rad30 was identified as a pol and named pol η. Soon thereafter paralogs of pol η were identified in human cells: Rad30A for pol η, Rad30B for pol ι, and DinB1 for pol κ.

Functions of DNA Polymerases ζ, η, ι, κ, and Rev1, The Lesion-Replicating Enzymes

Replicative pols stop before a DNA lesion; at that point translesion pols are likely attracted to damaged DNA. The duties to be covered by these pols include (*a*) the verification of the type of DNA damage [e.g. an abasic site, a thymine-thymine cyclobutane pyrimidine dimer, a *cis*-platinum adduct, an 8-oxoguanine adduct, or an *N*-2-acetyl aminofluorene (AAF) adduct], (*b*) the way the DNA is synthesized over the lesion, and (*c*) how a lesion-terminated primer is extended so that the

TABLE 3 The novel DNA polymerases and terminal deoxynucleotidyltransferase[a]

Pol	Genes	Functional tasks
ζ	*Rev3/Rev7*	Developmental pol (nonredundant)
POLZ[b]		Cell proliferation pol
B[c]		Mismatch extender pol
		Somatic hypermutation pol (low errors)
η	*Rad30A* (polV)[d]	Xeroderma pigmentosum variant pol
POLH		Accurate mismatch pol
Y		Somatic hypermutation pol (strand-biased hot-spot A mutations, less than pol κ)
θ		Repair of interstrand cross-links
POLQ		
A		
ι	*Rad30B*	Meiosis pol
POLI		Pol that can incorporate opposite to lesions
Y		Most error-prone pol
		Violates Watson-Crick base pair rule
		Somatic hypermutation pol (high errors)
κ	*DinB1* (polIV)[d]	Deletion and base substitution pol
POLK		Low fidelity and moderate processivity
Y		Somatic hypermutation pol (strand-biased hot-spot A mutations, more than pol η)
λ		Repair in meiosis
POLL		Homologous to pol β
X		
μ		Homologous to TdT
POLM		Lymphoid formation pol
X		Somatic hypermutation pol?
σ1	*Trf4-1*	Homologous to pol β
POLS1		Stimulated by PCNA
X		Fourth essential pol in yeast
		Sister chromatid cohesion pol
σ2	*Trf4-2*	Homologous to pol β
POLS2		Sister chromatid cohesion pol
X		
ϕ	*POL5*	Fifth essential pol in yeast
POLF		Stimulated by RF-C and PCNA
B		Not involved in replication

TABLE 3 *Continued*

Pol	Genes	Functional tasks
Eso1		Cohesion factor containing a domain similar to pol η
Rev1		Synthesis opposite an abasic site
REV1L		
TdT		Template-independent pol

[a]For details see text and references therein.

[b]Human Genome Organization (HUGO) nomenclature.

[c]DNA polymerase family A, B, X, or Y (see References 10 and 11).

[d]Correponding *E. coli* genes.

replication machinery of pol δ holoenzyme can resume DNA synthesis. See Table 3.

POL η Human pol η is the product of the *XPV* gene, which is mutated in patients with xeroderma pigmentosum variant (XP-V), who have a predisposition for skin cancer (115, 116). Cells from these patients are defective in the replication of DNA synthesis over damaged DNA. Even though pol η is a limited-fidelity pol (117), if compared to the more accurate pol α, pol β, pol γ, pol δ, and pol ϵ, it is a translesion pol with a high fidelity in replicating over damaged DNA with certain types of lesions (118). Pol η incorporates the correct nucleotide over lesions such as a thymine-thymine cyclobutane pyrimidine dimer (119), a *cis*-platinum adduct, or an AAF adduct and continues chain elongation, whereas replicative pols cannot, which suggests that pol η has a dual function in translesion synthesis and in elongation from a lesion (118). On the other hand, if the translesion synthesis by pol η is incorrect, elongation cannot be performed. This inability to elongate suggests that the fidelity of translesion synthesis includes the two steps of DNA synthesis per se and subsequent elongation from the lesion. Pol η is also very efficient in bypassing O^6-methylguanine formed by the action of alkylating agents (120). Pol δ can also bypass O^6-methylguanine, but this bypass is less accurate since the chance that the right C base is incorporated opposite O^6-methylguanine is twofold higher with pol η than with pol δ.

Pol η was reported to be capable of performing translesion synthesis that is error-free in general (121), but also of performing translesion synthesis that is error-prone on 8-oxoguanine (122). The latter study also compared its ability to synthesize over abasic sites and on (+)-*trans-anti*-benzo(a)pyrene-N^2-dGuanine, which suggests a second role for pol η in translesion synthesis of mutagenic bypass in mammalian cells (122, 123). Both synthetic activity and lesion bypass are stimulated by the physical interaction between pol η and PCNA, which is mediated by a consensus PCNA-binding motif (124).

Finally, it is interesting to note that pol η differs in the ability to bypass lesions if one compares the corresponding enzymes from the single cellular organism yeast to multicellular organisms. Whereas yeast pol η predominantly incorporates C opposite 8-oxoguanine, human pol η inserts C and A with similar efficiencies. Pol η from yeast favors incorporation of G opposite an apurinic site, whereas human pol η prefers A under the same conditions (120, 121).

Pol η is the first Y-family pol for which the crystal structure has been resolved (125) and the amino acids critical for activity and biological functions identified (126). This pol shows small and stubby fingers and thumb domains, with respect to the known pols.

Pol η has been found to colocalize uniformly in the nucleus and is associated with replication foci during S phase (127). When the cells were treated with DNA-damaging agents (UV, carcinogens), pol η accumulated at replication foci that stalled at DNA damages. Furthermore it was found that the C-terminal 70 amino acids are essential for nuclear localization and the next 40 amino acids for relocalization into the replication foci. These localization domains are important since two mutations in this region of pol η were found in XP-V patients. Inactivation of pol η could be a promising strategy to enhance the anticancer potency of alkylating agents in cancer chemotherapy. Moreover, pol η deficiency in XP-V uncovered an overlap between the S-phase checkpoint and double-strand break repair (128). It has also been postulated that replication errors introduced by pol η can be corrected by extrinsic exonucleases (129). Finally, a cohesion molecule from fission yeast *S. pombe* called Eso1p consists of a domain that is very similar to pol η (130) but its function is unknown.

POL κ Pol κ is the product of the *DINB1* gene (131). Pol κ has a low fidelity of about 1:200 and performs a predominant T $\rightarrow$ G transversion mutation at a rate of about 1:147 (132). Moreover, pol κ creates mismatches with high frequency on undamaged DNA. Pol κ can neither bypass *cis-syn* or (6–4) thymine-thymine dimers nor *cis*-platin adducts. As for pol η, it was found that pol κ can pass certain lesions in an error-free and others in an error-prone way. Error-prone bypass was measured at abasic sites and at 8-oxoguanine lesions (132). In error-prone and error-free bypasses, A was preferentially incorporated. Error-free bypass is achieved with the AAF adduct, where pol κ incorporates either C or T and less efficiently A, and with the (−)-*trans-anti*-benzo(a)pyrene-N^2-dG adduct, where preferentially a C is incorporated opposite the lesion (133). Pol κ has another unique property: On the one hand it possesses a very low fidelity, but on the other hand it is moderately processive (25 or more nucleotides). This property suggests an important role in spontaneous mutagenesis (134).

POL ι Pol ι is the gene product of *RAD30B* (135, 136). In sharp contrast to pol η and to a certain extent pol κ, pol ι is a much less accurate translesion pol. It has been found that pol ι can even violate the Watson-Crick base pairing rule, since it preferentially incorporates a G instead of the correct A opposite a

template T (137, 138). This unexpected property might be of crucial importance to replicate over ^{5Me}C, which after deamination becomes a T. So the inaccurate pol ι can incorporate a G opposite a T that might have been produced from a ^{5Me}C (139). Moreover, pol ι can replicate over a thymine-thymine cyclobutane pyrimidine dimer and under certain conditions (e.g. GA instead of the correct AA) even extend wrongly synthesized lesions (140). This is in contrast to the accurate translesion pol η. In a new model for mutagenic bypass it was proposed that pol ι acts in concert with pol ζ (see below): Pol ι incorporates deoxyribonucleotides opposite DNA lesions, and pol ζ functions as a mispair extender (141) (see below), a property that the accurate replicative pols α, δ, and ϵ do not have.

Human pol ι can respond differently to various DNA lesions (138). First, it stops at an 8-oxoguanine lesion, and this is in contrast to pol η, pol κ, and even pol β. Second, it can preferentially incorporate G opposite an abasic site. Third, it can preferentially incorporate a C opposite an AAF-adducted G, and fourth, it is largely unresponsive to TT dimers.

Pol ι might contain a loose and flexible pocket as an active site, resulting in an extra low fidelity that can even violate the Watson-Crick base pair rule. On the other hand, this loose active pocket is able to fit certain types of damaged templates, resulting in the correct nucleotide incorporation opposite certain lesions. Finally, pol ι could have its role in BER since it contains a second enzymatic activity, the 5′-deoxyribose phosphatase, as do pol β (139), pol γ (74), and pol λ (see below).

POL ζ REV1 It was initially assumed that pol ζ is an error-prone translesion pol (113 and reviewed in 142). Subsequent experiments identified pol ζ as a mispair extending enzyme rather than a mispair inserting enzyme (141). It is likely that pol ζ will extend translesion products that are not accurate, such as the many misincorporations by pol ι and the few misincorporations by pol η (see above). Rev1, on the other hand, is an enzyme that can incorporate a C at an abasic site and has been shown to act in concert with pol ζ (114). Like pol η and pol ι, Rev1 is a translesion inserter and pol ζ again is the extender for the translesion products. A human homolog of the *S. cerevisiae REV3* gene was shown to encode the catalytic subunit of pol ζ (143), and a human homolog of the *S. cerevisiae REV1* also codes for a dCMP transferase that is dependent upon a DNA template (144). When the pol ζ catalytic subunit, the *REV3* gene, was disrupted in transgenic mice it resulted in early embryonic lethality between days 9.5 and 12.5 (145–147). One explanation for such an effect is that during propagation and differentiation through many cell divisions the cells might gradually accumulate DNA damages in the absence of pol ζ. In utero, moreover, DNA lesions constantly form in the genome as a result of oxidative and hydrolytic processes. The experiments with transgenic mice clearly indicate that pol ζ has a nonredundant function in development and this is likely the reason why genetic diseases of pol ζ have never been detected.

How Are the Functions of Lesion-Bypassing DNA Polymerases Coordinated?

Taking together the properties of all these pols, it is likely that pol η plays the pivotal role in error-free and accurate translesion replication. In wild-type cells the normal situation would first include accurate translesion replication by pol η with subsequent continuation of replication by pol δ holoenzyme. Second, efficient bypass of an apurinic site was demonstrated by the combined action of pol δ and pol ζ (148). Third, when pol η is inaccurate, pol ζ has to extend the strand before the replicative pol δ can resume. Finally, the very error-prone pol ι incorporates mismatches that are first extended by pol ζ, before replication by pol δ can continue. It is conceivable that the latter two pathways lead to error-prone bypasses. This could explain the predisposition of the XP-V patients to cancer, since they lack the accurate translesion bypass by pol η. Why has nature maintained pols that are inaccurate? It is quite obvious that DNA damage is deleterious to the cells. Therefore, it is not surprising that cells evolved multiple ways to repair damage, and some of these methods have redundant specialties. In experiments performed in *S. cerevisiae* it was found that the cell repairs spontaneous DNA damages by employing redundant functions of NER, BER, MMR, RR, DSBR, recombination, and translesion synthesis pathways. These experiments also suggested that the translesion pol ζ can introduce multiple mutations when bypassing spontaneous DNA damages in *S. cerevisiae* (149). As indicated above, the error-prone role of pol ζ is likely a consequence of its property to extend mispaired bases rather than to misincorporate opposite lesions (141).

DNA Polymerases θ, λ, μ, σ, ϕ and Terminal Deoxynucleotidyl Transferase, Enzymes with Further Distinct Functions

The other novel pols identified so far and terminal deoxynucleotidyl transferase likely have roles other than translesion replication.

POL θ Pol θ is proposed to have a role in DNA repair of interstrand cross-links, but its mechanism is not known (150).

POL λ Pol λ shows homology to pol β. It has conserved critical amino acid residues for DNA binding, nucleotide binding and selection, catalysis, and deoxyribose 5′-phosphatase activity, which suggests a role in BER. Since pol λ was preferentially expressed in testis, and it appears to be developmentally regulated and associated to pachytene spermatocytes, a potential role in DNA repair during meiosis has been suggested (151).

POL μ The discovery of pol μ (152, 153) indicated that this pol has 41% identity to terminal deoxynucleotidyltransferase (TdT), but in contrast to TdT can

efficiently be stimulated by adding a template DNA. Moreover, pol μ is preferentially expressed in peripheral lymphoid tissues, and in human cells a large proportion of the expressed sequence tags correponding to this enzyme derived from germinal center B cells. This makes pol μ a candidate hypermutase, possibly involved in somatic hypermutation of immunoglobulin genes (152). Moreover, since the expressions of both pol μ and pol λ are down-regulated after DNA damage occurred, it has been suggested that these enzymes are unlikely to have a role in translesion DNA synthesis (154).

POL σ In *S. cerevisiae* the *Trf4* gene encodes for pol σ, which appears to be involved in DNA synthesis during sister chromatid cohesion, thus having a role in mitosis (155) and in chromosome segregation. Pol σ is required for building the connection between sister chromatids. Moreover, it is encoded by an essential gene, thus increasing the number of essential pols in eukaryotes to five (pol α, pol δ, pol ϵ, pol ϕ, and pol σ). Pol σ was initially called pol κ (155), but this name is now reserved for the *DINB1* gene product (see above). Finally, a second form of a *Trf4* gene product has been found so that these enzymes are now called pol σ1 and pol σ2.

POL ϕ Pol ϕ has been identified in *S. cerevisiae* and its function has in part been elucidated (A. Sugino, personal communication). The *POL5* gene is essential for yeast cell growth. It encodes a polypeptide of about 130 kDa, which has a weak similarity to family B pols. Its product, when purified from *S. cerevisiae* cells, has an aphidicolin-sensitive DNA polymerization activity. Pol ϕ is very much distributive, is error-free, but has no $3' \rightarrow 5'$ exonuclease activity. The activity is stimulated by addition of PCNA and RF-C on singly primed single-stranded DNA. The pol ϕ exclusively localizes in the nucleolus. The cell morphology of temperature-sensitive mutants of *POL5* does not show a dumbbell shape, which suggests that this pol is not required for chromosomal DNA replication. *S. pombe* has a homologous gene to *S. cerevisiae POL5,* but so far no homologs in other eukaryotic cells have been identified.

TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE This enzyme (abbreviated TdT) is a template-independent pol. It was first detected in connection with an unusual deoxynucleotide-polymerizing activity in pol preparation of calf thymus in 1960. TdT has been found only in lymphoid tissues and is proposed to have a role in somatic hypermutation of immunoglobulin genes (156).

How Many DNA Polymerases Are Involved in the Immune System?

Somatic hypermutation of immunoglobulin genes increases variability and thus leads to an increase in antibody diversity. Recent data from several groups

suggest that besides TdT (see above), at least the novel pol ζ, pol η, pol ι, pol κ, and pol μ share the task of creating mutations in the immunoglobulin loci (157).

This topic is covered extensively by another review in this volume (111) and is therefore only briefly summarized here. (*a*) Pol η is an A-T mutator in somatic hypermutation of immunoglobulin variable genes (158), and the mutation hot spots correlate with the pol η error spectrum (159). (*b*) Pol κ appears to have an even greater bias to the A-T mutation, which suggests that it could contribute even more to the variability of the immunoglobulin variable genes (159). (*c*) The translesion extender pol ζ was found to play an important role in hypermutation of the immunoglobulin and the *bcl*-6 genes, since it was found that pol ζ is up-regulated and pol κ down-regulated by the B-cell-receptor engagement (160). (*d*) Pol ι has an extremely low fidelity for nucleotide incorporation at the very end of a DNA template, which suggests its participation in hypermutation of immunoglobulin genes (161). (*e*) Double-strand break repair has been implicated in somatic hypermutation. DSBs are usually repaired by homologous recombination, which recruits an error-prone DNA polymerase (162). This model proposes that a nonprocessive, low-fidelity DNA polymerase, such as pol ι, performs the initial extension from hypermutation DSBR, resulting in clustering of mutations near the DSBR. Pol ι might then be replaced by a more lesion-processive DNA polymerase such as pol ζ or pol κ. (*f*) Finally, pol μ might act as a hypermutator in somatic hypermutation (152, 153).

FUTURE DIRECTIONS

In the last few years we have witnessed quite an enlargement of the pol family. In particular, the members of the novel pol family await the elucidation of their functional tasks. In the near future we would like to learn how the translesion pols can bypass the many lesions occurring in DNA, how pols are engaged in cohesion DNA synthesis, and how pols are involved in immunoglobulin recombination. Knockout transgenic mice may yield informative phenotypes that suggest roles for these pols. Additional structural studies will reveal the active sites and their surroundings for pols that can bypass DNA lesions. For the replicative pol α, pol δ, and pol ε, the exact subunit structures and their roles in DNA replication and the various DNA repair processes have to be worked out. The methods of choice include conditional knockout technologies and mutational and biochemical analysis of higher-order DNA replication complexes.

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