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Cellular DNA Polymerases

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ENZYMATIC PROPERTIES AND CHARACTERISTICS THAT DISTINGUISH EACH DNA POLYMERASE

During the past decade, five DNA polymerases (pol) have been characterized in eukaryotic cells. These enzymes are called α, β, γ, δ, and ε (Wang 1991). Their enzymatic properties, protein composition and structure, effectors, genetic relationships, genomic locations, expression during cell growth and proliferation, and their roles in DNA replication and repair have been described in previous reviews (Challberg and Kelly 1989; Stillman 1989, 1994; Hurwitz et al. 1990; Bambara and Jessee 1991; Wang 1991; So and Downey 1992). This chapter is an updated version of these reviews.

DNA pols α, β, δ, and ε are located in the nucleus, and DNA pol-γ is localized in the mitochondria. Therefore, pol-γ is thought to be the enzyme that replicates the mitochondrial DNA. The general enzymatic properties and characteristics that distinguish each DNA pol are summarized in Table 1, and the optimal assay conditions for each enzyme, such as pH, preferred primer template, and metal activators, are summarized in Table 2. The K_m values of their substrates and K_i values of their inhibitors vary widely depending on the purity of the enzyme, the integrity of the catalytic polypeptide, and the associated subunit components of the enzyme fraction used for the assay. Nonetheless, inhibitors can be used to distinguish one polymerase from another. Pols α, δ, and ε are sensitive to aphidicolin, but pols β and γ are not. Pols α, δ, and γ are sensitive to *N*-ethylmaleimide. Pol-α is particularly sensitive to butylphenyl-dGTP and butylphenyl-dATP. Pol-β and pol-γ are sensitive to dideoxynucleoside triphosphate (ddNTPs) (Edenberg et al. 1978; Krokan et al. 1979; Kaguni and Lehman 1988). Although carbonyldiphosphonate is a potent inhibitor of pol-δ, this compound does not inhibit pol-α and only moderately inhibits pol-ε. In addition, pol-δ is stimulated by 10% dimethylsulfoxide, whereas pol-ε is inhibited by it (Syvaoja et al. 1990).

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Table 1 Cellular DNA polymerases

	DNA polymerases				
	α	β	γ	δ	ε
Cellular location	nuclear	nuclear	mito	nuclear	nuclear
Protein structure					
mammalian cells catalytic polypeptides[a] (kD)	165[a]	40	140	125	255
associated subunits (kD)	70	none	35–55	48	55
	58				
	49				
Budding yeast					
catalytic polypeptides (kD)	165	68	140	124	250
associated subunits (kD)	86			48	80
					34/31
	58				29
	49				
Mammalian cDNA					
catalytic subunit					
human	yes	yes	yes	yes	yes
rodent	yes	yes	no	yes	no
associated subunits					
human	p70			p48	no
mouse	p58				
	p49				
Yeast genes					
budding yeast					
catalytic subunit	*POL1*	*POL4*	*MIP1*	*POL3*	*POL2*
associated subunits	*POL12*				*DPB2*
	Pri2				*DPB3*
	Pri1				*DPB4*
fission yeast					
catalytic subunit	polα+	—	polγ+	polδ+	—
associated subunits	—		—	—	—
chromosome localization					
mammal	Xq21.3-q22.1[b]	8p12-p11[c]	15q24[d]	19q13.3-q13.4[e]	12q24.3[f]
budding yeast	XIV[g]	III[h]	XV[i]	IV[j]	—
fission yeast	I[k]	—	III[l]	II[l]	—

[a]Molecular mass derived from deduced primary sequence. [b]Wang et al. (1985) and Adler et al. (1991). [c]Matsukage et al. (1986) and McBride et al. (1987). [d]W.C. Copeland (pers. comm.). [e]Chung et al. (1991) and Kemper et al. (1992). [f]Szpirer et al. (1994). [g]Lucchini et al. (1987) and Foiani et al. (1989). [h]Leem et al. (1994). [i]Foury (1989). [j]Boulet et al. (1989). [k]Singh and Klar (1993). [l]W.C. Copeland (pers. comm.).

Table 2 Properties of cellular DNA polymerases

	DNA polymerases				
	α	β	γ	δ	ε
Optimal asssay conditions					
pH	8.0	8.9	8.5	7.5	7.5
primer template	gapped DNA (60–150 nt)	gapped DNA (14–20 nt)	gapped DNA or poly(rA) oligo(dT)	poly(dA)-oligo(dT) or poly(dA-T)	poly(dA)-oligo(dT) or gapped DNA
preferred metal activator	Mg^{++}	Mg^{++}	Mg^{++} or Mn^{++}	Mg^{++}	Mg^{++}
Inhibitors					
aphidicolin	sensitive	not sensitive	sensitive	sensitive	sensitive
N-ethylmaleimide	sensitive	not sensitive	sensitive	sensitive	—
butylphenyl-dGTP or butylphenyl-dATP	highly sensitive	not sensitive	not sensitive	moderately sensitive	moderately sensitive
carbonyldiphosphonate	not sensitive	unknown	unknown	highly sensitive	not sensitive
dideoxynucleotide triphosphate (ddNTP)	not sensitive	sensitive	sensitive	not sensitive	not sensitive
Associated activities					
3′→5′ exonuclease	none	none	yes	yes	yes
primase	yes	none	none	none	none
Auxiliary proteins	none	none	none	PCNA	none
Processivity					
inherent	moderate	low	high	low	high
with PCNA	—	—	—	high	high
Fidelity	med	low	high	high	high

Pol-α is the only enzyme with an associated DNA primase (Wang 1991). Pol-β has no associated enzymatic activities, but pols γ, δ, and ε all have an intrinsic 3′→5′ exonuclease activity associated within their catalytic subunit. Pol-δ has an auxiliary protein, proliferating cell nuclear antigen (PCNA), that serves as a DNA sliding clamp and enhances the processivity of pol-δ, but pol-γ and pol-ε have intrinsic higher processivity. However, the processivity of these enzymes and the effect of PCNA on their processivity are both influenced by the Mg^{++} concentration in the reaction. The fidelity of DNA polymerases is reviewed by Roberts and Kunkel (this volume).

PROTEIN STRUCTURES AND SUBUNIT COMPONENTS

DNA Pol-α

DNA pol-α consists of four subunits: one catalytic subunit of 165–180 kD, one 70-kD subunit (also referred to as the B subunit or p70 subunit) with no detectable enzymatic activity (Collins et al. 1993; Foiani et al. 1994), and two subunits of 49 kD and 58 kD that contain the DNA primase activity (Wang 1991; Copeland and Wang 1993b). cDNAs for the catalytic subunit from human, mouse, *Drosophila melanogaster* embryo, fission yeast, and the gene from budding yeast have been isolated and characterized (Johnson et al. 1985; Wong et al. 1988; Damagnez et al. 1991; Hirose et al. 1991; Miyazawa et al. 1993; Park et al. 1993). Based on this sequence information, the catalytic subunit for pol-α is 165 kD. The catalytic subunit of human pol-α is glycosylated (Hsi et al. 1990); it is also phosphorylated in a cell-cycle-dependent manner (Nasheuer et al. 1991). Due to these posttranslational modifications, the apparent molecular mass is 180 kD (Hsi et al. 1990; Copeland and Wang 1991; Nasheuer et al. 1991). The fission yeast pol-α catalytic subunit is also phosphorylated in a cell-cycle-dependent manner (Park et al. 1995).

cDNAs for the pol-α p70 subunit have also been isolated from human, *D. melanogaster* embryo, and mouse, as well as from the gene from budding yeast (Cotterill et al. 1992; Collins et al. 1993; Miyazawa et al. 1993; Stadlbauer et al. 1994). Based on sequence data, the p70 subunit from human cells is 69.5 kD (Collins et al. 1993). The human p70 subunit, like the catalytic subunit, is phosphorylated in a cell-cycle-dependent manner (Nasheuer et al. 1991). The B subunit of budding yeast is 86 kD, whereas its apparent mass is present in two forms of 86 and 91 kD due to its posttranslational modification (Foiani et al. 1994).

The two DNA primase subunits from human, mouse, *D. melanogaster* embryo, and budding yeast all have apparent molecular masses of

58 kD and 49 kD in agreement with the molecular masses predicted from their respective cDNAs (Foiani et al. 1989; Prussak et al. 1989; Bakkenist and Cotterill 1994; Stadlbauer et al. 1994).

DNA Pol-β

Pol-β from human, mouse, rat, and calf is a single polypeptide of 39 kD, based on purified enzyme fractions or cDNA sequences (Wang 1991). In budding yeast, an open reading frame designated YCR14C corresponds to a protein of 67 kD and has been reported to be the gene encoding a DNA pol-β homolog named POL4 (Prasad et al. 1993b; Leem et al. 1994).

DNA Pol-δ

Pol-δ from both budding and fission yeast (also named POL3), as well as from mammalian cells, has two subunits, a catalytic subunit with an apparent molecular mass of 124 kD and a 48-kD subunit (Wang 1991). Both cDNAs and genes encoding the 124-kD catalytic subunit from budding and fission yeast, as well as from mammalian cells, have been isolated (Boulet et al. 1989; Pignede et al. 1991; Zhang et al. 1991; Yang et al. 1992; Cullmann et al. 1993; Park et al. 1993). The observed molecular mass of DNA pol-δ purified from various organisms is in agreement with the molecular mass deduced from the cDNA sequence, suggesting that pol-δ is not extensively modified. The cDNA encoding the small subunit of bovine and human pol-δ has been isolated. The deduced proteins, 50,885 D and 51,289 D, respectively, are 94% identical to each other. Although the primary sequence of the catalytic subunit of pol-δ shares substantial similarity with the herpesvirus family pol catalytic subunit, the small subunit of mammalian pol-δ shows no similarity to the small subunit of either herpes simplex type 1 (HSV1) UL42 protein or the Epstein-Barr virus BMRF1 protein. The small subunit of human pol-δ is located on chromosome 7 (Zhang et al. 1995).

DNA Pol-ε

The gene of pol-ε from budding yeast (also named *POL2*) and cDNA from human cells have been cloned and contain an open reading frame that encodes the 255-kD catalytic subunit (Morrison et al. 1990; Syvaoja 1990; Kesti et al. 1993). The exact subunit composition is not yet resolved. The most purified pol-ε enzyme fraction from cultured human

cells reproducibly contains a 255-kD protein and a 55-kD protein. The human 55-kD subunit is antigenically unrelated to similarly sized subunits in yeast. Partially purified human pol-ε contains a larger complex of proteins that include 85-kD, 70-kD, and 49-kD proteins (S. Linn, pers. comm.). In budding yeast, pol-ε was reported to contain a 256-kD catalytic subunit encoded by the *POL2* gene and four subunits of 80 kD, 34 kD, 31 kD, and 29 kD encoded by genes *DPB2*, *DPB3*, *DPB3*, and *DPB4*, respectively. Although *POL2* is an essential gene, *DPB2* and *DPB3* are not essential genes (Sugino 1995). Both pol-δ and pol-ε contain an intrinsic proofreading 3′→5′ exonuclease. A notable distinction between these two pols is not only their catalytic subunit size, but also their response to PCNA.

DNA Pol-γ

The gene of mitochondrial DNA pol, pol-γ, from budding yeast has been isolated and named MIPI. It encodes a protein of 143.5 kD (Foury 1989). The cDNAs of pol-γ from fission yeast, *D. melanogaster,* and human cells were recently isolated. The human DNA pol-γ cDNA encodes a protein of 1266 amino acids with a deduced molecular mass of 140 kD. The fission yeast pol-γ cDNA encodes a protein of 1018 amino acids with a deduced molecular mass of 116 kD (W.C. Copeland, pers. comm.). Studies of pol-γ purified from *D. melanogaster* embryos have revealed that the enzyme is a heterodimer comprising subunits of 125 kD and 35 kD (Kaguni and Lehman 1988).

CATALYTIC MECHANISMS

DNA pol-α interacts with its substrates in an ordered sequential manner, first interacting with template (single-stranded DNA), then with primer, and finally with dNTP. Specification of dNTP interaction is dictated by the template sequence. The minimum length of primer that can interact with pol-α effectively is eight nucleotides. The terminal three–five nucleotides of the primer must be complementary to the template. Pol-α interacts with primer terminated in either 3′-H or 3′-OH, but not with 3′-PO_4. (Fisher et al. 1981; Fisher and Korn 1981a,b). Pol-β, unlike pol-α, has a weak affinity for single-stranded DNA and no affinity for duplex DNA, but it has a high affinity for nicked duplex DNA with nicked termini bearing either 3′-OH or 3′-PO_4. Pol-β can perform limited strand-displacement synthesis at a nick (Wang and Korn 1980). Thus, this enzyme was shown to perform limited strand-displacement synthesis

at the 3′-OH site of a nick generated by AP endonuclease at the 5′side of an apurinic site (Mosbaugh and Linn 1983). Pol-β from either human cells or mouse cells interacts with its substrates in a rigidly ordered bi-bi kinetics (Tanabe et al. 1979; Wang and Korn 1982). Pol-β interacts with primer template concertedly followed by dNTP binding. For catalytically productive recognition of the primer template by pol-β, the primer template must be a primer base-paired to a short length of potentially single-stranded template. The metal activators, Mg^{++} or Mn^{++}, influence the minimum length of template requirement as well as the enzyme's processivity and error rate (Wang and Korn 1982).

Pol-α is a moderately processive enzyme in Mg^{++}-catalyzed reactions (Copeland and Wang 1991). Pol-β is a distributive enzyme with long gapped DNA as a substrate (Wang and Korn 1980, 1982), but with short gaps of up to six nucleotides, pol-β synthesizes DNA processively. Gap-filling activity requires 5′-PO_4-terminated polynucleotide downstream (Singhal and Wilson 1993). Pol-β prefers to bind downstream from the 3′-end of the gap (Prasad et al. 1994).

The catalytic mechanisms of pols δ and ε have not been investigated as extensively as those of pols α and β, but their response to PCNA has been investigated. PCNA can stimulate processive DNA synthesis for pol-δ on primed, single-stranded DNA primer template (Tan et al. 1986; Prelich et al. 1987; So and Downey 1988, 1992; Downey et al. 1990; Sabatino et al. 1990). Studies of mouse pol-δ showed that the 125-kD catalytic subunit of pol-δ alone is not stimulated by PCNA. The presence of the 48-kD subunit of pol-δ appears to be necessary for the stimulation of processivity by PCNA (Goulian et al. 1990). This finding was later confirmed by the study of pol-δ from *D. melanogaster* embryo (Chiang et al. 1993). Pol-ε was originally identified as a large exonuclease containing pol that is insensitive to PCNA stimulation (Syvaoja and Linn 1989; Morrison et al. 1990; Syvaoja et al. 1990; Syvaoja 1990; Weiser et al. 1991). However, pol-ε can form a stable complex with replication factor C (RF-C), ATP, and PCNA at the primer terminus (Burgers 1991; Lee et al. 1991a,b; Podust et al. 1992).

The polymerization and exonucleolytic mechanism of mtDNA pol-γ from embryos of *D. melanogaster* have been investigated (Olson and Kaguni 1992; Williams et al. 1993; Lewis et al. 1994; Williams and Kaguni 1995). The enzyme replicates with the highest substrate specificity on single-stranded DNA of natural DNA sequence in a quasi-processive manner with a high degree of nucleotide insertion fidelity. The intrinsic mispair-specific 3′→5′exonuclease hydrolyzes 3′-terminal mispair at approximately 15-fold higher efficiency than 3′-

terminal base pairs under nonpolymerization conditions. Under DNA polymerizing conditions, pol-γ does not extend a 3′-mispair even in the presence of a large excess of the next correct nucleotide. The mechanism of transferring template primer from the exonuclease active site to the pol-γ active site is intermolecular (Olson and Kaguni 1992). Maximal activity of pol-γ is obtained when assayed under moderate salt, although the highest processivity is observed under low-salt conditions (Williams et al. 1993). In the presence of single-stranded DNA-binding protein, pol-γ exhibits concurrently maximal DNA polymerizing activity and processivity (Williams et al. 1993; Williams and Kaguni 1995).

PRIMARY SEQUENCE AND STRUCTURAL CONSERVATION OF CELLULAR DNA POLS

The availability of new protein sequence data in the last few years allows the compilation and alignment of all the DNA pol protein sequences. According to their similarities to the three *Escherichia coli* DNA pols I, II, and III, Ito and Braithwaite classified the DNA-dependent DNA pols into four families: A, B, C, and X (Ito and Braithwaite 1991). The α-like DNA pols including the cellular pols α, δ, and ε (yeast *POL1*, *POL3*, and *POL2*, respectively) were classified in family B, whereas cellular pols β and γ were classified in families X and A, respectively (Ito and Braithwaite 1991). The deduced human DNA pol-α amino acid sequence from the cDNA has identified six highly conserved domains in the eukaryotic and prokaryotic pols (Wong et al. 1988; Wang et al. 1989; Wang 1991). Pols containing these six conserved regions are designated as "α-like" DNA pols (Delarue et al. 1990). The α-like pols include the yeast pol1, *E. coli* pol2, pols from the herpesvirus family, vaccinia virus, adenovirus, the bacterial phage pols such as the *E. coli* phage PRD1 and T4, and the *Bacillus* phage ϕ29. The similarity between these conserved regions is most striking in their linear spatial arrangement on each pol polypeptide. The regions are designated I–VI by their extent of similarity, with region I being the most conserved (Wong et al. 1988). Delarue et al. designated the three highly conserved regions II, III, and I named by Wang et al. (Wang 1991) as motifs A, B, and C, respectively (Delarue et al. 1990).

Conservation of these regions in cellular pols from prokaryotic to eukaryotic cells suggests that these enzymes may have diverged from a common ancestral gene and are conserved for maintenance of basic DNA polymerizing function. Sequence alignment of motif C and motif A of α-like DNA pols with the active site of *E. coli* pol1 and HIV-1 reverse transcriptase (RT) is almost nonexistent. Predicted secondary structures

of these highly conserved regions in α-like pols, however, show striking structural similarities with the secondary structures of the active sites of *E. coli* pol I and HIV-1 RT (Fig. 1). The predicted secondary structures of regions I and II (or motif C and A) of the α-like pols are similar to the secondary structures of the β strands 8, 12, 13, 9, and 14 of Klenow fragment, and to the five β strands 5, 9, 10, 6, and 11 of the "palm" subdomain of HIV RT (Kohlstaedt et al. 1992; Jacobo-Molina et al. 1993). These observations strongly support the notion that similar mechanisms in basic DNA polymerization by different pols from diverse phylogenetic sources dictate the structural conservation of the catalytic site. Thus, the three most conserved regions, I, II, and III (or motifs, C, A, and B), are components of the catalytic site of α-like DNA pols.

STRUCTURE-FUNCTION RELATIONSHIPS OF DNA POL-α AND DNA POL-β

DNA Pol-α

The overproduction of catalytically active recombinant human pol-α and the lack of an intrinsic 3′→5′ exonuclease of pol-α make this particular pol an ideal model for structure-function relationship study, particularly for identifying residues responsible for DNA synthetic fidelity. Results of the study can serve as a prototypic model for cellular pols δ and ε.

The most conserved region in the α-like pols, region I (or motif C), is centered around six highly invariant residues, Tyr-Gly-Asp-Thr-Asp-Ser (-YGDTDS-). The predicted secondary structure of this region is a pair of antiparallel β sheets with a sharp turn. With the overexpression of the recombinant human DNA pol-α catalytic subunit (Copeland and Wang 1991) and the development of a one-step immunoaffinity purification protocol (Wang et al. 1995), five site-directed mutations were introduced at the most conserved amino acids in region I (-YGDTDS-) (Copeland and Wang 1993a) based on the rationale described previously (Copeland and Wang 1993a; Copeland et al. 1995). Steady-state kinetic analysis of the mutants indicates that the effect of these conserved mutations is manifest in altered rates of catalysis, not in affinity to substrates. Moreover, two mutations, Asp-1002 to asparagine and Thr-1003 to serine, yielded mutant enzymes utilizing Mn^{++} more effectively in catalysis than Mg^{++}. This finding suggests that these amino acids (Asp-1004, Asp-1002, and Thr-1003) are involved in binding the activator metal that is critical for catalysis. The two aspartate residues, Asp-1002 and Asp-1004, may directly participate in chelating the metal ion like the aspartate residues in the active site of Klenow, HIV RT, and T7 RNA pol to form

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                                    IV          II  VI  III I   V
NH2 ================================■■■==========■■==■■==■■■■=■■===■■============= COOH
                                                A           B   C

                 --- REGION II ----        --------- REGION III ---------       ---- REGION I ---

                       *    *  *  *                   *          **                 •  ***  •
Human pol α     855-FILLL D FNSLYPSIIQ   944-IRQKAL K LTA.NSM YG CLGFSYSRFY   995-NLEVIYG DTD SIMIN

E.coli pol I    700-VIVSA D Y.SQIELRIM   752-EQRRSA K AINFGLI YG MSAFGLARQL   875-RMINQVH D.E LVFEV

T7 RNA pol      532-LPLAF D GSCSGIQHFS   625-VTRSVT K RSVMTLA YG SKEFGFRQQV   805-ESFALIH D.S FGTIP

HIV-1 RT        105-SVTVL D VGDAYFSVPL                                        178-IVIYQYM D.D LYVGS

Secondary structure
Hum pol α           bbbbb b btttttttaaa        aaaaaa a att.ttt tt tttttttttt     ttbbbbt ttt bbbbb
E. coli pol I       bbbbb b btttaaaaaa         aaaaaa a aaaaaaa tt tttttttttt     tbbbbbt t.t bbbbb
T7 RNA pol          bbbbb b btttaaaaaa         aaaaaa a aaaaaat tt tttttttttt     bbbbbbb t.t tbbbb
HIV-1 RT            bbbbb b bbttaaaaaa                                            bbbbbbt t.t bbbbb

               a=α-helix; b=β-strand; t=turn.
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Figure 1 Sequence alignment and secondary structure comparison of the three most conserved regions of human DNA pol-α with the active sites of *E. coli* pol I, T7 RNA pol, and HIV-1 RT. A schematic diagram depicting the spatial relationship of the six conserved regions of human DNA pol-α is shown at the top. Amino acid residues in the three most conserved regions, I, II, and III (or A, B, and C motifs in α-like DNA polymerases) of human DNA pol-α are aligned with the amino acid residues in the active sites of *E. coli* pol I, T7 RNA pol, and HIV-1 RT. Amino acid residues of human DNA pol-α marked with asterisks are the residues targeted for mutagenesis. Predicted secondary structure of human DNA pol-α regions I, II, and III are compared with the secondary structures of the active sites of *E. coli* pol I, T7 RNA pol, and HIV-1 RT. (Reprinted, with permission, from Copeland et al. 1995.)

the "catalytic triad" (Kohlstaedt et al. 1992; Jacobo-Molina et al. 1993; Sousa et al. 1993). Results from the Thr-1003 mutation suggest that the β-methyl group of the threonine side chain might be locked in a hydrophobic pocket, preventing free rotation around the C_α-C_β bond, thus positioning the Thr-1003 hydroxyl group to form a crucial bond with the metal ion (Copeland and Wang 1993a).

Further investigation was performed with these mutants in the most conserved region on the fidelity of DNA synthesis with either Mg^{++} or Mn^{++} as the metal activator. Mn^{++} is known to cause polymerases to misinsert nucleotides and to utilize dideoxynucleotide. The two region I mutants, D1002N and T1003S, which utilize Mn^{++} in catalysis more effectively than Mg^{++}, actually have an improved misinsertion fidelity in Mn^{++}-catalyzed reactions as compared to that of the wild-type enzyme. The improved misinsertion fidelity of these two mutants in Mn^{++}-catalyzed reactions is due to K_m discrimination of the incorrect nucleotide.

Study of mispair extension showed that in reactions with Mn^{++} as metal activator, the wild-type enzyme readily extended the mispaired primer. In contrast, the two mutants, D1002N and T1003S, were unable to extend the mispaired termini in either Mg^{++}- or Mn^{++}-catalyzed reactions. These findings suggest that the side chains of these residues in region I play an essential role in the Mn^{++}-induced infidelity during DNA synthesis by human DNA pol-α (Copeland et al. 1993).

The second most conserved region of α-like DNA pols, region II (or motif A), spans a block of 40 amino acid residues centered at the core sequence -DFNSLYPSII-. The functions of Gly-841, Asp-860, Ser-863, Tyr-865, and Ser-867 in the region II (motif A) of the human DNA pol-α catalytic subunit were tested by conserved site-directed mutagenesis. Steady-state kinetic analysis of mutants G841A, D860A, D860S, D860N, Y865S, and Y865F demonstrated no significant difference in their K_m values for primer template compared to that of the wild-type enzyme. In contrast, mutants D860A, Y865S, and Y865F showed a 5- to 10-fold increase in the K_m for dNTP compared to the wild-type enzyme. DNA synthetic fidelity studies of these mutants showed that mutant Y865S but not Y865F had a much higher misinsertion efficiency than the wild-type enzyme in Mg^{++}-catalyzed as well as in Mn^{++}-catalyzed reactions. These results indicate that Asp-860 and Tyr-865 in region II (motif A) of human DNA pol-α are involved in incoming dNTP substrate binding. By using three deoxynucleotide structural analogs as probes, the structural requirement for dNTP binding with Tyr-865 is shown to be the nucleotide base. Results of these studies strongly suggest that the phenyl ring of Tyr-865 directly interacts with the nucleotide base moiety of the deoxynucleotide triphosphate and plays a critical role in the misinsertion fidelity of DNA synthesis. Mutation of Gly-841, which is highly conserved among the α-like DNA pols, appears to affect both catalysis and substrate deoxynucleotide binding. This suggests that Gly-841 is essential for the maintenance of the overall structure of the pol-α catalytic site (Dong et al. 1993a). The function of two serine residues, Ser-863 and Ser-867, in this core sequence was also investigated. Mutation of the two serine residues in the core sequence of region II to either alanine or threonine yielded mutant enzymes with similar K_m for dNTPs, k_{cat}, processivity, and misinsertion fidelity of DNA synthesis as the wild-type enzyme. Mutation of Ser-867 to alanine demonstrated a 30-fold increase in K_m for primer template and a 5-fold higher K_D for binding primer template, suggesting that Ser-867 is involved in interacting with primer template. Single-stranded DNA inhibition data suggest that removal of the hydroxyl side chain of Ser-867 affects the pol's interaction with

primer and not with template. Moreover, according to footprinting experiments, the structural feature of the primer recognized by Ser-867 is the 3′-OH terminus. Mutation of Ser-867 to alanine also decreases the mutant enzyme's K_m for dNTP to extend a mispaired primer and, thus, enhances its capacity to extend a mispaired primer terminus. These findings suggest that the hydroxyl side chain of Ser-867 of human DNA pol-α is involved in primer interaction during DNA synthesis and plays an essential role in mispair-extension fidelity of DNA synthesis (Dong et al. 1993b).

The function of a highly conserved lysine residue, Lys-950, of human DNA pol-α located in the third most conserved region in a predicted α helix was also analyzed by site-directed mutation. Lys-950 was mutagenized to arginine, alanine, and asparagine. All of the three Lys-950 mutants utilized Mn^{++} as metal activator more effectively than the wild-type enzyme. The mutant enzymes, K950R, K950A, and K950N, showed an increase in the K_m values for deoxynucleotide triphosphate in either Mg^{++}- or Mn^{++}-catalyzed reactions. Although mutations of Lys-950 residue caused an increase in the K_m value for dNTP, mutations of Lys-950 to arginine, alanine, or asparagine did not alter the mutant enzymes' misinsertion efficiency as compared to that of the wild-type enzyme in Mg^{++}-catalyzed reactions, suggesting that the nucleotide base of the incoming dNTP is not the structural feature interacting with the side chain of Lys-950. However, in Mn^{++}-catalyzed reactions, all of the three Lys-950 mutants have improved misinsertion DNA synthetic fidelity. Studies with inhibitor, structural analogs of dNTP, pyrophosphate, and comparison of the affinity of the Lys-950 mutant derivatives to dCTPαS versus dCTP suggest that this highly conserved Lys-950 is involved in interacting with the oxygen group of the α-phosphate moiety of the incoming dNTP (Dong and Wang 1995).

There are no structural data for any member of the α-like DNA pols (family B). Given the structural similarity between the α-like DNA pol regions I, II, and III with the active sites of *E. coli* Klenow fragment and HIV-1 RT, it is reasonable to assume that the three most conserved regions are the components of the α-like DNA pol active site. Based on these mutational studies, a model for the way in which these highly conserved residues in the active site of α-like pols collaborate for catalysis was proposed (Fig. 2). In the active site of human DNA pol-α, two aspartic acid residues in region I, Asp-1002 and Asp-1004, chelate with the metal activator cation, Mg^{++}, which in turn chelates with the oxygen moiety of the β-phosphate and γ-phosphate of the incoming dNTP (Copeland and Wang 1993a). The phenyl ring side chain of Tyr-865

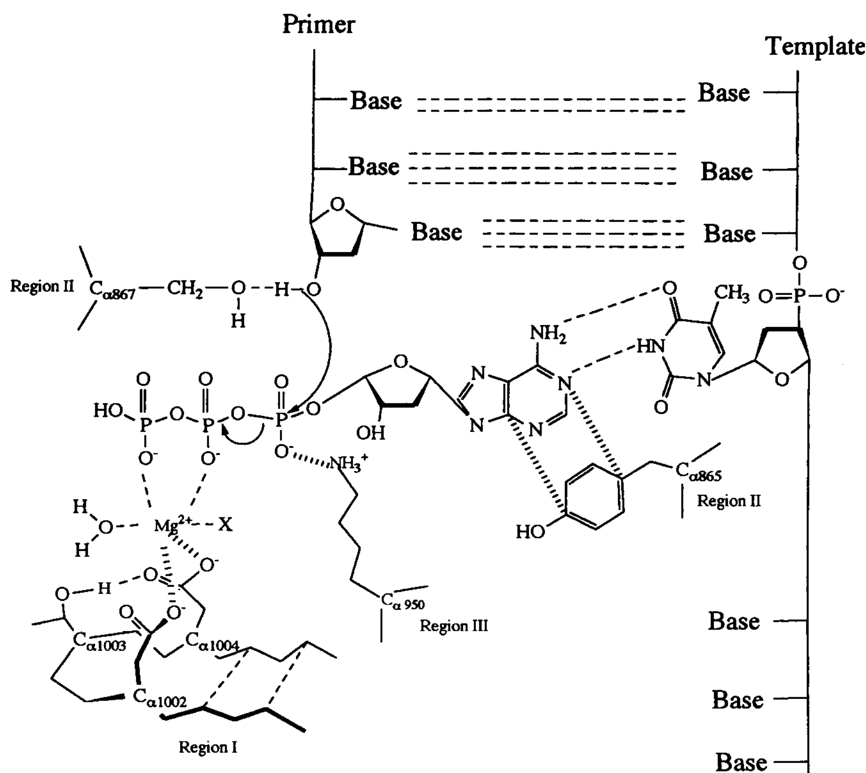

Figure 2 Model of the α-like DNA pol active-site residues that collaborate for nucleotidyl transfer. Shown are the proposed functions of residues of human DNA pol-α studied by site-directed mutagenesis. The three region I residues, Asp-1002, Thr-1003, and Asp-1004, bind to the metal-nucleotide complex. The phenyl ring of Tyr-856 in region II interacts with the nucleotide base moiety of the incoming dNTP. The hydroxyl side chain of the Ser-867 residue forms hydrogen bonds to the 3′-OH terminus of the primer. The positively charged side chain of Lys-950 of region III interacts with the oxygen group of the α-phosphate of the incoming dNTP. An X represents the unknown side chain(s) of residue(s) in the active site that might participate in chelating the Mg^{++} metal activator. (Reprinted, with permission, from Dong and Wang 1995.)

forms hydrophobic bonds with the nucleotide base of the incoming dNTP to properly position the incoming dNTP for Watson-Crick base-pairing (Dong et al. 1993a). The oxygen moiety of the Ser-867 hydroxyl side chain forms a hydrogen bond either directly or indirectly with the 3′-OH terminus of the primer. The hydrogen-bond formation might enhance the

oxygen moiety at the 3′-OH-primer terminus for nucleophilic attack at the α-phosphate of the incoming dNTP (Dong et al. 1993b). The positively charged side chain of Lys-950 interacts either directly or indirectly with the oxygen group of the α-phosphate moiety of the incoming dNTP to position the α-phosphate for the nucleophilic attack by the oxygen moiety at the 3′-OH-primer terminus.

DNA Pol-β

Pol-β consists of two domains connected by a protease-sensitive hinge region. The amino-terminal region 8-kD domain (residues 1–87) is required for template binding, and the carboxy-terminal 31-kD domain (residues 88–335) possesses the catalytic activity (Kumar et al. 1990a,b). These two independent domains can be separated by proteolysis. The 8-kD domain alone has no enzymatic activity, whereas the 31-kD domain has approximately 5% of the pol activity of the 39-kD pol-β (Kumar et al. 1990a). The amino-terminal 8-kD domain has been shown by affinity labeling and photocrosslinking studies to be the single-stranded DNA-binding domain (template-binding domain) (Casas et al. 1991, 1992; Prasad et al. 1993a).

The structure of rat pol-β in complex with a DNA primer template and ddCTP was determined at 2.9 Å and 3.6 Å resolution (Pelletier et al. 1994). The crystal structure has revealed that the 3′-OH terminus of the primer, the ddCTP phosphates, and two Mg^{++} ions are all clustered around Asp-190, Asp-192, and Asp-256 in the active site of pol-β. These three aspartic acid residues occupy positions spatially similar to the proposed "catalytic triad" aspartic acid residues in Klenow fragment, HIV-1 RT, and T7 RNA pol (Kohlstaedt et al. 1992; Jacobo-Molina et al. 1993; Sousa et al. 1993). These aspartic acid residues may have similar biochemical function as the two aspartic acid residues of α-like DNA pols in the most conserved region I described above (Copeland et al. 1993; Copeland and Wang 1993a). Mutations of Asp-190 and Asp-192 yielded pol-β mutants with 0.1% of the activity of the wild type (Date et al. 1990, 1991). The conserved geometric arrangement of these aspartic acid residues in the pol-β active site with the other structurally known pols provides the physical evidence that pols from vast phylogenetic origins share a common mechanism of dNMP transfer during catalysis. Thus, the active-site structures of these pols are conserved in order to maintain the basic mechanism of nucleotidyl transfer. In this ternary

complex structure of pol-β, however, the DNA template is bound to pol-β in a different manner from that proposed for the other pols.

The crystal structures of the 31-kD domain of rat pol-β and the 39-kD domain of rat pol-β were also determined at 2.3 Å resolution and 3.6 Å resolution, respectively. The structural data of the rat pol-β show that the subdomain structures can be compared to the Klenow fragment as the fingers, palm, and thumb subdomains of a right hand (Kohlstaedt et al. 1992). Although the palm region of pol-β can be superimposed on Klenow fragment and HIV-1 RT, there are notable differences in their folding patterns (Davies et al. 1994). The topology of the β sheet of the pol-β palm subdomain is different from that of Klenow fragment and HIV-1 RT. There is an additional strand in pol-β. The amino-terminal 8-kD domain is composed of two pairs of antiparallel helices and is attached to the 31-kD domain at the tip of the finger subdomain by a flexible hinge. The 31-kD domain has the structural dimensions compatible to bind B-form DNA (Sawaya et al. 1994). Since biochemical evidence has shown that the hinge is less sensitive to proteolysis when pol-β is bound to the DNA primer template (Kumar et al. 1990a), this suggests that the 8-kD domain may fold over the bound template. The structure of the ternary complex indeed has shown that the 8-kD domain moves closer to the palm subdomain than the 39-kD structure alone (Pelletier et al. 1994; Sawaya et al. 1994).

Crystal structure of rat pol-β soaked in 5 mM $MnCl_2$ was obtained at 2.8 Å resolution. An electron density map showed the presence of two large positive peaks near the side chains of Asp-190, Asp-192, and Asp-256 that were interpreted as two metal-binding sites (Davies et al. 1994). The two Mn^{++} metal ions appear to be coordinated by one aspartic acid residue, Asp-190. The side chains of Asp-192 and Asp-256 do not directly contact the metal ion, but form salt bridges with a nearby arginine residue, Arg-254. The finding of two Mn^{++} at the catalytic site of pol-β is consistent with the proposed two-metal reaction model proposed for Klenow fragment and HIV-1 RT (Joyce and Steitz 1994).

Results from extensive biochemical studies of the pol-α active site, high-resolution structure analysis of pol-β either alone or as a ternary complex, and comparison with the structure of Klenow fragment and HIV-1 RT clearly demonstrate the generality of a catalytic mechanism for nucleotidyl transfer among the template-dependent cellular pols. The presence of a few highly conserved aspartic acid residues as critical active-site structural elements to chelate metal activator for catalysis reflects a common active architecture of DNA-template-dependent DNA pols.

EXPRESSION OF DNA POL GENES

DNA Pol-α

Expression of human DNA pol-α is up-regulated during activation of quiescent cells (G_0) to enter the mitotic cell cycle at the levels of transcript, protein, and enzymatic activity (Wahl et al. 1988; Pearson et al. 1991). The expression of human pol-α at the levels of transcript, protein, and enzymatic activity is greatly reduced in cells rendered temporarily quiescent either by contact inhibition or by serum starvation (Wahl et al. 1988). The promoter sequence required for the expression of a heterologous gene in actively cycling cells and in serum-deprived cells following serum addition was investigated (Pearson et al. 1991). The upstream sequence is G+C-rich and lacks a TATA sequence but has a CCAAT sequence on the opposite strand. Studies have shown that efficient expression of the reporters in cycling cells requires 248 bp of sequence upstream from the cap site containing Sp1-, Ap1-, Ap2-, and E2F-binding sites. The promoter confers on the reporter an appropriate, late serum response, as observed in serum induction of pol-α transcript (Wahl et al. 1988). Multiple sequence elements appear to mediate the full serum response (Pearson et al. 1991).

When cells permanently exit from the cell cycle during terminal differentiation, the expression of human DNA pol-α at the level of transcript, protein, and enzymatic activity are down-regulated, and the two primase subunits are also both down-regulated at the transcript level. Down-regulation of DNA pol-α at the messenger RNA level is caused in part by a decrease of transcriptional initiation rate with no transcription elongation block (Moore and Wang 1994).

In actively cycling cells, however, the transcript, protein, and enzymatic activity of human DNA pol-α are constitutively expressed throughout the cell cycle. The protein and enzymatic activity show a nominal increase in the S phase (Wahl et al. 1988). Similar findings have been observed in several mammalian replication proteins (Sherley and Kelley 1988; Tseng et al. 1989; Morris and Mathews 1989). Although pol-α is expressed throughout the cell cycle, the human DNA pol-α catalytic subunit and the p70 subunit proteins are both phosphorylated in a cell-cycle-regulated manner (Nasheuer et al. 1991).

DNA Pol-β

Expression of DNA pol-β in cultured cells and tissues is considered to be constitutive. Pol-β enzymatic activity and steady-state transcripts are expressed at a relatively low level and are independent of cell growth and

cell cycle stage (Chang and Bollum 1972; Zmudzka et al. 1988; Nowak et al. 1989). However, in rodent cells, pol-β enzymatic activity and steady-state transcript appear to be regulated in a tissue-specific manner (Hirose et al. 1989; Nowak et al. 1989) and are induced by treatment of cells with some DNA-damaging agents such as alkylating agents MMS or MNNG (Fornace et al. 1989). The promoters of pol-β genes from human, bovine, and rodent sources are G+C-rich and contain binding sites of two well-known transcriptional activators, Sp1 and ATF/CREB (Widen et al. 1988; Widen and Wilson 1991). In studies of the human pol-β core promoter, the ATF/CREB site is shown to be functionally responsive to elevated levels of intracellular cAMP (Englander et al. 1991; Englander and Wilson 1992b), and the ATF/CREB site also mediates a positive response of the cloned promoter to treatment of transfected Chinese hamster ovary (CHO) cells with MNNG (Kedar et al. 1991). This up-regulation was not shown in genetically mutated CHO cells that were deficient in protein kinase A activity. Thus, this suggests that this particular signal transduction pathway is required for the response to MNNG (Englander and Wilson 1992a,b). The pol-β core promoter is also up-regulated by expression of the activated $p21^{ras}$ protein. This suggests that activation of the protein kinase C signal transduction pathway may also have a role in pol-β gene expression (Kedar et al. 1990).

DNA Pol-δ

The mRNA and protein of pol-δ appear to be present throughout the mammalian cell cycle with a slight increase at the G_1/S junction (Zeng et al. 1994a).

Yeast DNA Pols

In budding yeast, the transcripts of genes encoding most DNA replication proteins, including pol-α (*POL1* or *CDC17*), pol-δ (*POL3* or *CDC2*), pol-ε (*POL2*), the 86-kD subunit of pol-α (B-subunit; *POL12*), and the two subunits of DNA primase (*PRI*1 and *PRI*2) (Johnson et al. 1985; Johnston et al. 1987, 1990; Bauer and Burgers 1990; Araki et al. 1992; Foiani et al. 1989, 1994), are coordinately induced at the G_1/S-phase junction. A DNA element, 5′-A/TPuACGCGTNA/T-3′, containing an *Mlu*I restriction site (termed *Mlu*I cell cycle box, MCB) was found in the upstream regions in all of these genes. These findings suggest that the cell-cycle-dependent transcription of the DNA synthesis genes in *S.*

cerevisiae may be coordinated by a common MCB-binding factor (Andrews and Herskowitz 1990; Gordon and Campbell 1991; McIntosh et al. 1991). Although the *POL1* gene is transiently transcribed during the cell cycle at the G_1/S-phase boundary, budding yeast pol-α protein is present at all stages of the cell cycle with only a slight increase following the peak of *POL1* transcript. This suggests that the de novo synthesis of pol-α protein is not rate-limiting or required for entrance of S phase of the cell cycle (Falconi et al. 1993). The 86-kD subunit of budding yeast pol-α, like its homolog in human cells, is phosphorylated in a cell-cycle-dependent manner (Foiani et al. 1994). In budding yeast, a weakly expressed 3.2-kb transcript of pol-β (*POL4*) was detected at constant level throughout the cell cycle. During meiosis, a 2.2-kb *POL4* transcript was highly induced, whereas the 3.2-kb transcript remained at a constant level (Leem et al. 1994).

The expression of two fission yeast (*Schizosaccharomyces pombe*) pols α and δ during the cell cycle was investigated (Bouvier et al. 1992; Pignede et al. 1992; Park et al. 1993). The steady-state transcripts of *S. pombe* polα^+ and polδ^+ genes, like those of their mammalian cell homologs, are present throughout the cell cycle. Sequence analysis of the polα^+ and polδ^+ genes revealed the absence of the *Mlu*I motifs in their upstream sequences (Park et al. 1993). The proteins of *S. pombe* pols α and δ are expressed constantly throughout the cell cycle. The enzymatic activity of pol-α measured by in vitro assay was found in all stages of the cell cycle (Park et al. 1993). The *S. pombe* pol-α, like its homolog in human cells, is phosphorylated in a cell-cycle-regulated manner (Park et al. 1995).

GENETIC STUDIES OF YEAST DNA POLS

DNA Pol-α

Genetic studies of budding and fission yeast DNA pols have revealed some novel functions of cellular DNA pols. During initiation of mating-type switching at the *mat1* locus of fission yeast, there is a double-strand chromosomal break (DSB). Three mating-type switch genes of *S. pombe*, *swi1*, *swi3*, and *swi7*, have been identified, and mutations of these genes reduce the level of DSB and switch efficiency. A genomic sequence that complemented the switching-defective *swi7* strain is the DNA pol-α gene. Disruption of the *swi7/polα* gene is lethal both in switching and non-switching strains, as expected for an essential replication gene. Thus, *S. pombe* pol-α has a role in initiation of mating-type switching at *mat1*, suggesting generation of DSB coupled with DNA replication. This find-

ing also links DNA replication in dictating the program of cellular differentiation (Singh and Klar 1993).

In a normal cell cycle, entry into mitosis is dependent on the completion of S phase, and the dependency is maintained by a checkpoint control mechanism (Enoch et al. 1991, 1992, 1993; Enoch and Nurse 1991). Results of fission yeast genetic studies (Kelly et al. 1993; Saka and Yanagida 1993; Hofmann and Beach 1994) have suggested that proteins or enzymes involved in the formation of an initiation or replication complex have a role in mitotic entry checkpoint operation. Germinating spores derived from *S. pombe* with a disrupted DNA pol-α gene have a heterogeneous phenotype. This is different from germinating spores derived from a disrupted pol-δ gene, which has a cell cycle division (cdc) phenotype (elongated phenotype) (Francesconi et al. 1993b). Further analysis has shown that germinating spores derived from spores lacking the DNA pol-α gene showed abnormal nuclear morphology with 1N DNA content (D. Bhuamik and T.S. Wang, in prep.). A multicopy suppressor of a temperature-sensitive DNA pol-α mutant named $cds1^+$ (*c*heck *D*NA *s*ynthesis) encoding a protein with a typical protein kinase motif was reported recently. Genetic data suggest that the primary role of $cds1^+$ is to monitor DNA synthesis by interacting with DNA pol-α and sending a signal to restrain the onset of mitosis during the progression of S phase (Murakami and Okayama 1995). The observations of germinating spores with pol-α gene disruption or deletion, together with the genetic results of $cds1^+$, suggest that pol-α may be involved in cell-cycle-checkpoint control.

DNA Pol-ε

A recent study of budding yeast DNA pol-ε shows that *pol2* mutants are defective for the S-phase checkpoint. By interallelic complementation and mutation analysis, the budding yeast pol-ε protein can be divided into two separate domains, an amino-terminal pol domain and a carboxy-terminal checkpoint domain. The carboxy-terminal domain of pol-ε was shown to function as a sensor for DNA replication coordinating the transcriptional and cell cycle responses to replication block (Navas et al. 1995).

ROLES OF DNA POLS IN DNA REPLICATION

The proposed functional roles of the principal replicative cellular pols α, δ, and ε are deduced from either yeast genetic data or cell-free

reconstituted replication reactions of the simian virus 40 (SV40) origin containing DNA. Genetic studies of budding yeast and fission yeast have demonstrated that cellular pols α, δ, and ε are all essential for cell viability and nuclear DNA replication (Campbell 1993; Francesconi et al. 1993b). In contrast, strains harboring a deletion or mutation of POL4 (pol-β gene) are viable. Strains with POL4 gene deletion or mutation also exhibit no meiosis defect (Prasad et al. 1993b; Leem et al. 1994). Chromosomal disruption of budding yeast *MIP1* gene (pol-γ gene) is not lethal to the cell, but renders cells with total loss of mitochondrial DNA and pol-γ activity (Foury 1989). Thus, the functional role of the MIP1 gene product, pol-γ, is primarily for mitochondrial DNA synthesis.

DNA pol-α is thought to be the principal cellular pol responsible for initiation (Wang 1991). This is based on the fact that pol-α has an associated primase activity that resides in the 49-kD and 58-kD subunits. Evidence from cell-free and reconstituted in vitro SV40 DNA replication has demonstrated that pol-α with its primase subunits functions primarily for synthesis of initiator DNA (iDNA) (Waga and Stillman 1994; Waga et al. 1994). In the cell-free SV40 replication reaction, it has been shown that the catalytic subunit of pol-α (p180) physically interacts with SV40 large T antigen (Dornreiter et al. 1990, 1993). A domain of human pol-α catalytic subunit in the amino-terminal region from residue 195 to residue 313 physically interacts with T antigen. Transition of SV40 DNA replication from preinitiation stage to initiation stage requires the physical interaction of this amino-terminal domain of human pol-α catalytic subunit with T antigen in the preinitiation complex (Dornreiter et al. 1993). Furthermore, the catalytic subunit of pol-α has also been shown to physically interact with bovine papillomavirus (BPV) initiation protein E1 and is an essential component for the cell-free BPV replication reaction (Park et al. 1994). Besides the catalytic subunit of pol-α, the 70-kD subunit (B subunit) was shown to interact with the T antigen (Collins et al. 1993).

In reconstituted replication reactions of SV40 origin containing DNA, pol-α:primase functions primarily to synthesize RNA-DNA primers for initiation of DNA replication at the origin. Pol-α:primase also functions for priming each Okazaki fragment on the replication protein A (RP-A)-coated lagging-strand template (Waga and Stillman 1994; Waga et al. 1994). The synthesis of the nascent primer and the Okazaki fragment on the RP-A-coated lagging-strand template is thought to be mediated by the interaction between T antigen and pol-α:primase complex (Dornreiter et al. 1993). T antigen functions as a DNA helicase on the leading-strand template in a 3′ to 5′ direction (Borowiec et al. 1990). This al-

lows the primase activity of the pol-α:primase complex to synthesize a short RNA primer and then to be extended by pol-α by synthesizing an iDNA (Hu et al. 1984; Nethanel and Kaufmann 1990). In vitro evidence has shown that in reconstituted SV40 DNA replication reaction, the function of pol-α:primase cannot be substituted by any other DNA pol and primase (Tsurimoto et al. 1990). Likewise, despite the sequence conservation among the pol-α of budding yeast, fission yeast, and human cells, there is a stringent species-specificity in vivo. These in vivo data strongly argue that specific protein-protein interactions between pol-α and other proteins are important not only for initiation during SV40 DNA replication, but also during chromosomal DNA replication (Tsurimoto et al. 1990; Francesconi et al. 1993a).

On the basis of the results of reconstituted SV40 replication reaction, it is proposed that, once the iDNA is synthesized, an ATP-dependent structure-specific DNA-binding protein, RF-C, binds the iDNA and then loads PCNA and pol-δ into the replication fork to displace pol-α:primase from the DNA template. DNA pol-δ thus assumes the leading-strand synthesis at the SV40 origin (Tsurimoto et al. 1990). On the lagging strand, pol-α:primase synthesizes the RNA-DNA primers for each Okazaki fragment. A polymerase switching mechanism occurs by binding the RF-C to the 3′-end of the iDNA and then helps loading PCNA and pol-δ to complete the synthesis of each Okazaki fragment. Therefore, it has been proposed that a switching between DNA pol-α and DNA pol-δ occurs during each Okazaki fragment synthesis on the lagging-strand template like that on the leading strand (Waga and Stillman 1994). In this model, pol-α:primase is responsible for initiation and synthesis of iDNA on both leading and lagging strands of DNA. Pol-δ is responsible for elongating the leading-strand DNA as well as for the completion of each Okazaki fragment synthesis (Waga and Stillman 1994; Waga et al. 1994).

The role of pol-ε in replication is not yet clear. Genetic evidence in budding yeast has shown that pol-ε is essential for completion of S phase (Araki et al. 1992). On the basis of its processive biochemical property, pol-ε was suggested as the pol for leading-strand DNA synthesis (Morrison et al. 1990; Morrison and Sugino 1992). In contrast, in an in vitro SV40 replication reaction, substitution of pol-ε for pol-δ has yielded products of insufficient length as leading-strand products. In this reaction, pol-ε can synthesize moderate length products in the presence of pol-α:primase, implying pol-ε might have a role in Okazaki fragment synthesis (Lee et al. 1991b).

It should be noted that the current concept regarding the functional

role of pol-δ as the principal pol in nuclear DNA synthesis should be interpreted with caution, since the proposed model is only based on biochemical data derived from reconstituted in vitro SV40 DNA replication reactions. Numerous biochemical data have also implied that more than one kind of polymerase is involved in the completion of Okazaki fragment synthesis (Nethanel and Kaufmann 1990; Bullock et al. 1991; Burgers 1991; Nethanel et al. 1992; Podust and Hübscher 1993). Although production of form I SV40 DNA product in reconstituted reaction can be achieved with only two cellular DNA pols, α and δ, the efficiency of the form I DNA synthesis in the reconstituted replication reaction is not equal to the efficiency of DNA synthesis in either the cell-free replication system or SV40 viral DNA replication in vivo.

ROLES OF DNA POLS IN DNA REPAIR

Cellular DNA pols are also involved in nucleotide excision repair, postreplication repair, and recombination repair (Friedberg 1991, 1994; Friedberg et al. 1994). Biochemical and genetic studies of budding yeast base excision repair have suggested that pols α, δ, and ε effect excision repair, either directly or indirectly, under certain conditions. One report described that nucleotide base excision repair pathway is mainly catalyzed by pol-ε and further modulated by the presence of pol-α and pol-δ (Wang et al. 1993). In contrast, another study has shown that mutants of budding yeast pol-δ are defective in survival and repair of DNA methylation damage (Blank et al. 1994). In addition to the ongoing uncertainty regarding the role of pol-δ or pol-ε in base excision repair, another study of the cellular pol in nucleotide excision repair was performed by monitoring postirradiation molecular weight changes of cellular DNA in mutants defective in three cellular pols, α, δ, and ε, as well as Rev3 (Budd and Campbell 1995). This study has shown that a mutant defective in cellular pol-α, pol-δ, or pol-ε, as well as Rev3, is defective in repair. However, single mutations in any of these four genes do not show a repair defect. Pairwise combination of pol mutations demonstrated a repair defect only in a pol δ and ε double mutant. Since the extent of repair in this double mutant was comparable to the mutant defective in all four genes, pol-α and Rev3 appear to have only a minor, if any, role in the nucleotide base excision repair pathway. The data suggest that pols δ and ε both are able to perform repair synthesis, and that one can substitute for the other (Budd and Campbell 1995).

In mammalian cells, pol-ε was originally identified as a 10.2S and an approximately 220-kD polypeptide that complemented the repair syn-

thesis of UV-irradiated and permeabilized cultured diploid human fibroblasts (Nishida et al. 1988). Pol-ε transcript was found to be up-regulated during induction of quiescent cells to proliferate; however, after UV-irradiation, no significant difference in the level of pol-ε transcript in HeLa cells or fibroblasts was observed. Nonetheless, several lines of evidence have recently shown that pol-ε has a role in DNA repair. Using monoclonal antibodies against human pol-ε and immunoblot, Linn and his colleagues have identified >200-, 85-, and 70-kD polypeptides in a mammalian protein complex that was shown to be involved in repairing double-strand breaks and deletions by recombination in vitro (Jessberger et al. 1993). Pol-ε is also found in a large form of a mammalian damage DNA-binding protein (Keeney et al. 1993; Reardon et al. 1993). Moreover, the 85-kD and 70-kD proteins that were immunoreactive to the monoclonal antibody in the pol-ε fractions have been demonstrated to be the subunits of Ku protein, which is a DNA-binding protein with double-strand end specificity and is a helicase implicated in recombination, repair, and transcription functions. These findings suggest that pol-ε not only has a role in DNA repair or recombinational repair, but that pol-ε is also able to form a functional complex with Ku protein (S. Linn, pers. comm.). Complete repair synthesis has recently been reconstituted by 30 polypeptides of human cells in which two subunits of pol-ε, RF-C, PCNA, and DNA ligase I were found to be the essential components (Aboussekhra et al. 1995). Together, these results strongly implicate DNA pol-ε in nucleotide base excision repair.

Although the elegant demonstration of the reconstituted nucleotide excision repair system does not require pol-δ, the capacity of a human cell extract to repair UV-damaged plasmid DNA can be inhibited by antibodies against either pol-δ or PCNA. The mRNA levels of both pol-δ and PCNA were also stimulated after UV-irradiation of cultured cells, implicating pol-δ and its accessory protein PCNA in UV-irradiation DNA damage repair synthesis (Zeng et al. 1994b).

The biochemical properties of mammalian pol-β suggest that it is involved in DNA repair. Pol-β can fill gaps of six nucleotides in a processive manner if the gap has a 5′-phosphate (Singhal and Wilson 1993). The recent isolation of POL4 from budding yeast demonstrated that strains harboring a *pol4* deletion or mutation exhibit defects in neither mitotic growth nor meiosis. This suggests that pol-β does not have a role in replication and is involved in nonessential functions in DNA metabolism (Prasad et al. 1993b; Leem et al. 1994). Strains with deletion of *pol4* exhibit mild sensitivity to MMS treatment but do not exhibit UV sensitivity. Strains with *pol4* deletion also demonstrate a hyperrecom-

bination phenotype during intragenic recombination and a high frequency of illegitimate mating during a mating test (Leem et al. 1994). Together, these genetic results implicate that pol-β has a role in the double-strand-break repair pathway. Pol-β has been implicated in catalyzing DNA synthesis during base excision DNA repair (Matsumoto et al. 1994; Singhal et al. 1995). Pol-β also catalyzes release of 5′-terminal deoxyribose phosphate (dRP) residue from incised apurinic-apyrimidinic sites. The amino-terminal 8-kD domain of pol-β is the catalytic domain for this excision activity. The excision reaction appears to occur by β-elimination, not by hydrolysis (Matsumoto and Kim 1995).

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