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Mammalian DNA Polymerases α and δ: Current Status in DNA Replication†

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Early attempts to understand the replication of chromosomal DNA at the level of the DNA polymerases that catalyze the template-directed polymerization of nucleotides were confounded by the need to accommodate both the unidirectional movement of the replication fork and the obligate 5′ to 3′ direction of synthesis of all DNA polymerases. This conundrum was resolved when it was found that only one of the new DNA strands, the leading strand, was synthesized continuously in the 5′ to 3′ direction coinciding with the overall movement of the growing point, and the other strand, the lagging strand, was synthesized discontinuously in the 5′ to 3′ direction opposite to the overall direction of movement of the fork. Discontinuous synthesis of the lagging strand was found to involve RNA priming, primer removal, gap filling, and the eventual joining of the lagging strand segments (Okazaki fragments) by a ligase (Kornberg, 1980, 1982).

The elucidation of leading strand and lagging strand synthesis reconciled the unidirectional nature of fork movement with the obligate 5′ to 3′ direction of nucleotide polymerization; however, it raised a new problem. Although a single DNA polymerase is thought to catalyze both leading strand and lagging strand synthesis in prokaryotes, the functional properties of a replicase that catalyzes leading strand synthesis would be expected to be quite different from those required of a lagging strand replicase. For example, a leading strand replicase should be highly processive and, once bound to a replicon, remain associated until it is completely replicated, whereas a lagging strand replicase should have a significantly lower affinity for the template as it must be able to dissociate from the DNA after synthesizing each Okazaki fragment. These considerations have given rise to replication fork models in prokaryotes in which synthesis of both the leading and lagging strands is carried out by a replication complex that contains an asymmetric dimer of the catalytic subunit of the DNA polymerase (Sinha et al., 1980; Kornberg, 1982; McHenry, 1985). Each catalytic subunit is associated with different auxiliary or accessory proteins that modify the properties of the catalytic subunit to allow either highly processive leading strand synthesis or moderately processive lagging strand synthesis. Synchronous synthesis of both strands in these models is accommodated by introducing a loop into the lagging strand, which results in both leading and lagging strands having the same 3′ to 5′ orientation at the fork.

A similar model has been proposed for replication of the mammalian genome on the basis of the functional properties of chromatographically separable forms of DNA polymerase α that differ from each other in the nature of the accessory proteins associated with the catalytic core. It has been postulated that different forms of DNA polymerase α form a dimeric replication complex that is capable of synthesizing both leading and lagging strands (Ottiger & Hubscher, 1984). However, the results of several studies have suggested that replication of the mammalian genome may require two distinct DNA polymerases, i.e., that in addition to the known replicase DNA polymerase α, DNA polymerase δ may also play a role in DNA replication in higher eukaryotes (Lee et al., 1981); Dresler & Frattini, 1986; Decker et al., 1987; Hammond et al., 1987; Prelich et al., 1987b; Downey et al., 1988; Prelich & Stillman, 1988). In this review we will consider the properties of mammalian DNA polymerases α and δ and discuss the functional roles of both enzymes in DNA replication.

DNA Polymerase α. DNA polymerase α was the first mammalian DNA polymerase described (Bollum, 1960). It was found to be a high molecular weight enzyme that, unlike prokaryotic DNA polymerases, had no 3′ to 5′ exonuclease activity and was therefore unable to proofread errors of incorporation (Fry & Loeb, 1986). DNA polymerase α was initially implicated as a replicative DNA polymerase because of its sensitivity to a variety of inhibitors of DNA replication, e.g., aphidicolin, dideoxynucleotides, and arabinosyl nucleotides

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(DePamphilis & Wassarman, 1980), and this hypothesis was confirmed when the availability of monoclonal antibodies to DNA polymerase α allowed examination of the role of this enzyme in nuclear DNA replication by the use of permeabilized cells (Miller et al., 1985).

Recent studies on the in vitro replication of SV40 DNA have further supported the role of DNA polymerase α as a replicative enzyme. Plasmids that contain the origin of replication of SV40 can be replicated in vitro by extracts from human or monkey cells, with the only viral protein required being the large T antigen (Li & Kelly, 1984, 1985; Stillman & Gluzman, 1985; Wobbe et al., 1985). Preabsorption of cellular extracts with anti-DNA polymerase α antibodies results in the loss of replication activity (Murakami et al., 1986).

DNA polymerase α is usually isolated as a complex with DNA primase, an oligoribonucleotide polymerase that synthesizes short ribonucleotide primers which can be extended by DNA polymerase α (Tseng & Ahlem, 1982; Hubscher, 1983; Wang et al., 1984; Yamaguchi et al., 1985). Primase and α-polymerase are tightly associated, although the activities have been shown to reside on different polypeptides (Suzuki et al., 1985; Holmes et al., 1986; Yagura et al., 1986). Recently the cDNA encoding the human DNA polymerase α catalytic subunit has been cloned and the primary structure determined (Wong et al., 1988).

DNA Polymerase δ. DNA polymerase δ was initially described as a mammalian DNA polymerase that differed from the other known species of mammalian DNA polymerase in having a tightly associated 3′ to 5′ exonuclease activity (Byrnes et al., 1976). Originally purified from rabbit bone marrow, DNA polymerase δ was subsequently isolated from calf thymus tissue and was shown to retain 3′ to 5′ exonuclease activity when purified to homogeneity by preparative gel electrophoresis (Lee et al., 1980). In addition to its proofreading capacity, DNA polymerase δ could also be distinguished from DNA polymerase α by its chromatographic behavior and template specificity. The preferred templates for DNA polymerase δ were found to be alternating copolymers such as poly(dA-dT), whereas activity on DNase-activated DNA templates was quite low (Byrnes et al., 1976; Lee et al., 1984).

Mammalian DNA polymerases with 3′ to 5′ exonuclease activity, and identified as DNA polymerase δ primarily on that basis, have been isolated from several tissues, e.g., calf thymus (Lee et al., 1980, 1984; Crute et al., 1986; Wahl et al., 1986), rabbit bone marrow (Goscin & Byrnes, 1982), and human placenta (Lee & Toomey, 1987). For the same reason DNA polymerase α_1 from mouse myeloma cells, which has a tightly associated 3′ to 5′ exonuclease, may be considered to be a DNA polymerase δ (Chen et al., 1979). The properties of these DNA polymerases vary, possibly as a result of differences in purification protocols. DNA polymerase δ isolated from fetal calf thymus in our laboratory, for example, is unable to utilize primed homopolymer templates such as poly(dA)/oligo(dT) except in the presence of a 37-kDa auxiliary protein (Tan et al., 1986), and a similar template specificity has been reported for DNA polymerase δ from human placenta (Lee & Toomey, 1987). In contrast, DNA polymerases δ I and II from calf thymus isolated in Bambara's laboratory (Crute et al., 1986; Wahl et al., 1986) and rabbit bone marrow DNA polymerase δ (Goscin & Byrnes, 1982) are very active with poly(dA)/oligo(dT) as template/primer. Immunoblotting of these enzymes with antibody to the auxiliary protein was negative, suggesting that activity on poly(dA)/oligo(dT) is not the result of an endogenous auxiliary protein (unpublished observations). Thus, the reasons for the difference in the template specificity of the DNA polymerase δ species isolated in different laboratories are not obvious.

Another difference in the properties of DNA polymerase δ species isolated in different laboratories is the association with DNA primase. Calf thymus DNA polymerase δ isolated in our laboratory is devoid of this activity (Downey et al., 1988), as is the human placenta enzyme (Lee & Toomey, 1987). Whether or not the rabbit bone marrow DNA polymerase δ has primase activity has not been reported; however, calf thymus DNA polymerases δ I and II isolated in Bambara's laboratory have associated primase activity (Crute et al., 1986; Wahl et al., 1986). Since DNA polymerase δ I is inhibited by monoclonal antibodies to KB cell DNA polymerase α and can be separated from 3′ to 5′ exonuclease activity, it appears likely that DNA polymerase δ I is a form of DNA polymerase α. However, DNA polymerase δ II has a tightly associated 3′ to 5′ exonuclease activity and is not inhibited by antibodies to DNA polymerase α (Wahl et al., 1986). It is possible that the different protocols used to purify DNA polymerase δ may result in loss of accessory proteins or that there are different forms of DNA polymerase δ. A property common to all DNA polymerase δ species isolated thus far is resistance to inhibition by butylphenyl-dGTP and/or butylanilino-dATP. Resolution of the differences in the properties of the DNA polymerase δ species will likely require collaborative studies among the various laboratories studying this enzyme.

PCNA, Cyclin, and DNA Polymerase δ Auxiliary Protein. The proliferating cell nuclear antigen (PCNA) was initially described as a nuclear protein reactive with sera from a subset of patients with the autoimmune disease systemic lupus erythematosus (SLE) (Miyachi et al., 1978; Tahasaki et al., 1984) and was subsequently found to be identical with a cell cycle regulated protein called cyclin (Sadaie & Mathews, 1986). By the use of human autoantibodies, PCNA/cyclin, a 36-kDa polypeptide, was shown to be synthesized during the S phase of the cell cycle but not during G1, G2, or mitosis (Kurki et al., 1986; Sadaie & Mathews, 1986; Bravo & Macdonald-Bravo, 1985; Celis & Celis, 1985; Celis et al., 1987). The induction of PCNA synthesis is closely correlated with the induction of DNA synthesis in cells treated with a variety of mitogenic agents, preceding DNA replication by a short interval (Bravo, 1986). These findings led to the suggestion that PCNA/cyclin is involved in cellular DNA replication (Sadaie & Mathews, 1986; Bravo, 1986; Celis et al., 1986). Recently PCNA was found to be required for efficient replication of SV40 DNA in vitro, supporting a role for this protein in replication of the cellular genome (Prelich et al., 1987a).

An auxiliary protein for DNA polymerase δ that markedly affects the activity and processivity of DNA polymerase δ with template/primers containing long stretches of single-stranded template, e.g., primed homopolymers or single-stranded phage DNA, was recently purified from calf thymus tissue (Tan et al., 1986; Prelich et al., 1987b). This protein, which has a subunit molecular weight of 37 00, specifically affects the activity of DNA polymerase δ, and it has no effect on the activity of DNA polymerase α. The auxiliary protein for DNA polymerase δ has been found to be identical with PCNA/cyclin by several criteria: (1) both proteins comigrate on sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (Prelich et al., 1987b) and on two-dimensional gel electrophoresis (Bravo et al., 1987); (2) autoantibodies to PCNA neutralize the activity of the auxiliary protein, and monoclonal antibodies to authentic PCNA detect the auxiliary protein in immunoblots (Tan et al., 1987b); and (3) the calf thymus

auxiliary protein for DNA polymerase δ can substitute for human PCNA in SV40 replication in vitro, and conversely, human PCNA can effectively substitute for the calf thymus DNA polymerase δ auxiliary protein in markedly stimulating the activity and processivity of calf thymus DNA polymerase δ with primed homopolymer templates (Prelich et al., 1987b). The demonstration that the auxiliary protein for DNA polymerase δ is a cell cycle regulated protein that is required for SV40 replication suggested that DNA polymerase δ, in addition to DNA polymerase α, may be required for cellular DNA replication.

DNA Polymerases α and δ Are Distinct Enzymatic Species: Both Are Required for DNA Replication. DNA polymerases α and δ are alike in many properties; e.g., both are high molecular weight, acidic proteins that are sensitive to inhibition by *N*-ethylmaleimide. DNA synthesis with both enzymes is sensitive to inhibitors of replication such as aphidicolin and arabinosylnucleotides and resistant to dideoxynucleotides (Lee et al., 1981). Because of these similarities, the question has arisen as to whether DNA polymerase δ is a modified form of DNA polymerase α, or vice versa.

Several lines of evidence suggest that DNA polymerases α and δ are unique enzymatic species. The tight association of DNA polymerase δ with a proofreading 3′ to 5′ exonuclease activity and the lack of such an activity in highly purified preparations of DNA polymerase α has been a major distinction between these two enzymes. The recent report that the catalytic subunit of DNA polymerase α from *Drosophila melanogaster* has a cryptic 3′ to 5′ exonuclease activity (Cotterill et al., 1987) may have diminished the usefulness of this criterion for distinguishing DNA polymerases α and δ, although it may be the case that *Drosophila* DNA polymerase α is not representative of the α-polymerases of higher eukaryotes, as this phenomenon has not been reported for mammalian enzymes.

A second line of evidence suggesting that DNA polymerases α and δ are distinct enzymes is their response to the cell cycle regulated protein PCNA. Both the activity and processivity of DNA polymerase δ are markedly affected by the presence of PCNA (Tan et al., 1986; Prelich et al., 1987b), whereas it has no effect on either the activity or processivity of DNA polymerase α (Tan et al., 1986; Downey et al., 1988).

A third criterion by which DNA polymerases α and δ can be distinguished is their sensitivities to inhibition by the replication inhibitors butylphenyl-dGTP and butylanilino-dATP, DNA polymerase α being over 100-fold more sensitive than DNA polymerase δ (Byrnes, 1985; Lee et al., 1985; Wahl et al., 1986). This difference between these two DNA polymerases has recently been exploited to implicate DNA polymerase δ in cellular DNA replication. Studies on the effects of these inhibitors on DNA replication in permeabilized cells (Dresler & Frattini, 1986; Hammond et al., 1987) and on in vitro SV40 replication catalyzed by cellular extracts (Decker et al., 1987) have suggested that both DNA polymerases α and δ are required for DNA replication in mammalian cells.

DNA polymerases α and δ also differ in their sensitivity to inhibition by neutralizing antibodies. Several monoclonal antibodies to KB cell DNA polymerase α have been shown to neutralize the activity of DNA polymerase α species from a variety of cells and tissues (Tanaka et al., 1982; Byrnes, 1985; Wahl et al., 1986; Lee & Toomey, 1987; Downey et al., 1988). However, anti-DNA polymerase α antibodies do not inhibit the activity of DNA polymerase δ species from either calf thymus (Wahl et al., 1986;; Downey et al., 1988), human placenta (Lee & Toomey, 1987), or rabbit bone marrow

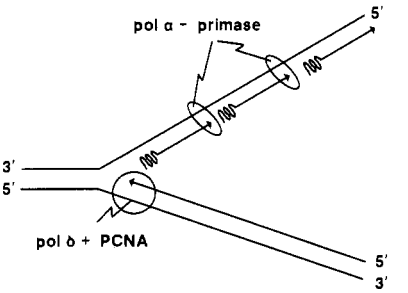

FIGURE 1: Schematic representation of the roles of DNA polymerases α and δ in DNA replication. This model was initially presented at the meeting on Eukaryotic DNA Replication held at Cold Spring Harbor on Sept 2–6, 1987 (Downey et al., 1988).

(Byrnes, 1985). More importantly, polyclonal antibodies to DNA polymerase δ from human placenta (Lee & Toomey, 1987) or calf thymus (Downey et al., 1988) do not inhibit the activity of DNA polymerase α from the same species, suggesting that the catalytic cores of these two DNA polymerases are immunologically distinct. Data on the primary structures of these enzymes are necessary to determine whether they share any homology.

Studies on the functional properties of DNA polymerases α and δ from fetal calf thymus also support the hypothesis that these two DNA polymerases are distinct enzymatic species. Measurements of the processivities of DNA polymerases α and δ on primed homopolymer templates have shown that, whereas the processivity of DNA polymerase α is moderate (less than 100 nucleotides incorporated per enzyme binding event) (Hohn & Grosse, 1987; Tan et al., 1987a) and unaffected by the presence of PCNA (Downey et al., 1988), the processivity of DNA polymerase δ is extremely high (greater than 1000 nucleotides incorporated per enzyme binding event) in the presence of PCNA, although it is essentially distributive in the absence of PCNA (approximately 10 nucleotides incorporated per enzyme binding event) (Downey et al., 1988). Recent studies using $(dA)_{4500}$ as template for processivity measurements have suggested that the processivity of DNA polymerase δ in the presence of PCNA is at least 4500 and is probably limited by template length (unpublished observations).

Another functional difference between DNA polymerases α and δ is their ability to carry out strand-displacement synthesis. By the use of a poly(dA)-tailed pBR322 template to determine the ability of a DNA polymerase to carry out strand-displacement synthesis it was found that, while both DNA polymerases α and δ were able to replicate the single-stranded poly(dA) tails, only DNA polymerase δ was able to replicate the double-stranded portion of the template (Downey et al., 1988). The inability of immunoaffinity-purified HeLa cell DNA polymerase α to carry out strand-displacement synthesis has previously been noted (Murakami et al., 1986). These results suggest that DNA polymerases α and δ have markedly different functional properties and suggest that their in vivo roles may be different.

Do Different Enzymes Catalyze Leading and Lagging Strand Synthesis? The lack of DNA polymerase mutants in higher eukaryotes makes it difficult to determine the function of either DNA polymerase α or DNA polymerase δ in replication. However, on the basis of the functional properties of DNA polymerases α and δ, we have suggested possible roles for these two enzymes at the replication fork (Figure 1), i.e., that DNA polymerase δ is the leading strand replicase and DNA polymerase α is the lagging strand replicase (Downey et al., 1988).

It would be advantageous for the lagging strand replicase to have a tightly associtaed primase activity to catalyze the frequent de novo priming necessary for Okazaki fragment synthesis, as does DNA polymerase α. On the other hand, the leading strand replicase only has to initiate a new chain once per replicon, and would therefore not need an associated primase activity, and DNA polymerase δ has none. The differences in processivity between DNA polymerases α and δ are also consistent with their proposed functions. DNA polymerase α, which we suggest carries out discontinuous synthesis of the lagging strand, has a moderate processivity that may be sufficient to support synthesis of Okazaki fragments, i.e., 100–200 nucleotides in length in eukaryotes (DePamphilis & Wassarman, 1980; Denhardt & Faust, 1985), whereas DNA polymerase δ, which we suggest carries out continuous synthesis of the leading strand, is highly processive. Furthermore, a leading strand enzyme should be capable of strand-displacement synthesis since it must replicate through duplex regions of DNA at the fork, whereas a lagging strand enzyme does not have to displace the nontemplate strand; DNA polymerase δ is able to catalyze strand-displacement synthesis whereas DNA polymerase α cannot. Finally, it has been observed in prokaryotes that the leading strand moves ahead of the lagging strand by about the length of one nascent Okazaki fragment, suggesting that the controlling event in elongation of a replication fork is most likely the commencement of leading strand synthesis (Kornberg, 1980, 1982). Since the activity of DNA polymerase δ, but not DNA polymerase α, is regulated by PCNA, a protein synthesized during S phase and believed to be involved in regulating replication, this further implicates DNA polymerase δ as a candidate for leading strand replicase.

The results of recent studies by Prelich and Stillman (1988), in which single-stranded DNA fragments complementary to either the leading or lagging strand were used to characterize the products of in vitro SV40 DNA replication, support the hypothesis that DNA polymerase δ is the leading strand replicase. In the presence of PCNA, DNA synthesis proceeded bidirectionally on both leading and lagging strands to produce duplex daughter molecules. In contrast, in the absence of PCNA, which specifically affects the activity and processivity of DNA polymerase δ, only lagging strand synthesis was detected and the products synthesized had lengths similar to Okazaki fragments. Conversely, both monoclonal antibody to DNA polymerase α and butylphenyl-dGTP, a specific inhibitor of DNA polymerase α, have been shown to inhibit the discontinuous synthesis of Okazaki fragments in permeabilized cells (Miller et al., 1985; Hammond et al., 1987), suggesting that DNA polymerase α is the replicase for the lagging strand.

Although direct evidence of the involvement of DNA polymerase δ in replication is lacking, the proposal that the leading and lagging strands are synthesized by distinct DNA polymerases in higher eukaryotes, i.e., DNA polymerases δ and α, respectively, is nevertheless supported by circumstantial evidence. The model therefore provides a working hypothesis for further studies on the replication of the eukaryotic genome. Furthermore, by analogy with the replication fork models proposed for prokaryotes, it is possible that DNA polymerases α and δ are both components of a multiprotein replication complex. Although no evidence for such a complex has been obtained to date, some mechanism must exist to coordinate leading and lagging strand synthesis at the replication fork.

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Accelerated Publications

Chemical Cross-Linking of Sm and RNP Antigenic Proteins†

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ABSTRACT: Nuclear extracts, competent for in vitro premessenger RNA splicing, were chemically cross-linked with thiol-reversible reagents in order to study the organization of proteins within ribonucleoprotein particles (RNPs) containing uridine-rich small nuclear RNAs (UsnRNPs). The distribution of select UsnRNP antigens within cross-linked complexes was determined by Western blotting of diagonal two-dimensional gels. On the basis of calculations from the molecular weights of cross-linked complexes containing UsnRNP common proteins B′, B, and D, it is proposed that each of these proteins was associated with UsnRNP common proteins E and G. In addition, D′ is proposed to be positioned close to D. The spatial distribution of UsnRNP common proteins was such that B′ and B could not be cross-linked to D. The data also suggested that the 63-kDa U1 snRNP specific protein was cross-linked to other U1-specific proteins, particularly C, but not to the UsnRNP common proteins. We propose that part of the UsnRNP core of common proteins contains at least two asymmetrical copies of B′:B:D:D′:E:G with stoichiometries of 2:1:1:1:1:1 and 1:2:1:1:1:1.

Chemical cross-linking has been widely used for mapping extra- and intracellular protein interactions. The potential power of this technique in revealing the protein organization within ribonucleoprotein particles such as those that package premessenger RNA (hnRNPs)[1] or uridine-rich small nuclear RNAs (UsnRNPs) has not been exploited. Biochemical

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[1] Abbreviations: RNP, ribonucleoprotein particle; UsnRNP, RNP containing uridine-rich small nuclear RNA; hnRNP, RNP containing heterogeneous nuclear RNA; PMSF, phenylmethanesulfonyl fluoride; TEO, triethanolamine; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; DTBP, 3,3′-dimethyldithiobis(propionimidate); PAGE, polyacrylamide gel electrophoresis; 2D, two dimensional; Tris, tris(hydroxymethyl)aminomethane; NEM, *N*-ethylmaleimide; NaDodSO$_4$, sodium dodecyl sulfate.

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