Mammalian Proliferating Cell Nuclear Antigen Stimulates the Processivity of Two Wheat Embryo DNA Polymerases

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Multiple DNA polymerases have been described in all organisms studied to date. Their specific functions are not easy to determine, except when powerful genetic and/or biochemical tools are available. However, the processivity of a DNA polymerase could reflect the physiological role of the enzyme. In this study, analogies between plant and animal DNA polymerases have been investigated by analyzing the size of the products synthesized by wheat DNA polymerases A, B, C1, and CII as a measure of their processivity. Thus, incubations have been carried out with poly(dA)-oligo(dt) as a template-primer under varying assay conditions. In the presence of MgCl₂, DNA polymerase A was highly processive, whereas DNA polymerases B, C1, and CII synthesized much shorter products. With MnCl₂ instead of MgCl₂, DNA polymerase A was highly processive. DNA polymerases B and C1 were moderately processive, and DNA polymerase CII remained strictly distributive. The effect of calf thymus proliferating cell nuclear antigen (PCNA) on wheat polymerases was studied as described for animal DNA polymerases. The high processivity of DNA polymerase A was PCNA independent, whereas both enzyme activity and processivity of wheat DNA polymerases B and CII were significantly stimulated by PCNA. On the other hand, DNA polymerase C1 was not stimulated by PCNA and, like animal DNA polymerase 8, was distributive in all cases. From these results, we propose that wheat DNA polymerase A could correspond to a DNA polymerase a, DNA polymerases B and CII could correspond to the δ-like enzyme, and DNA polymerase C1 could correspond to DNA polymerase β.

Prokaryotic and eukaryotic cells are characterized by the presence of multiple DNA polymerases. Five major DNA polymerases, a, b, γ, δ, and ε, have been characterized in animal cells, essentially with respect to the effect of inhibitors, template specificity, mol wt, subcellular localization, and their proposed role (Burgers et al., 1990; Wang, 1991).

DNA polymerase a is tightly associated with DNA primase (Kaguni and Lehman, 1988). This complex plays an important role in the replication of the lagging strand of the replication fork (Prelich and Stillman, 1988). DNA polymerase b is the main DNA repair enzyme in the nucleus (Wilson et al., 1988). DNA polymerase γ is involved in the replication of the mitochondrial genome (Fry and Loeb, 1986). DNA polymerase δ has been described in animal cells and yeast and is involved in nuclear DNA replication. DNA polymerase δ and α are distinguished by the 3'-5' proofreading exonuclease activity associated with the former enzyme (Byrnes et al., 1976; Lee and Toomey, 1987; Lee, 1988; Lee et al., 1991). An auxiliary protein (Tan et al., 1986), which was found to be identical with the PCNA (Lee and Whyte, 1984; Bravo et al., 1987; Prelich et al., 1987a, 1987b), is necessary in the case of DNA polymerase δ for processive DNA synthesis. Based on these properties, DNA polymerase δ appears to be a good candidate for the replication of the leading strand of the replication fork (Downey et al., 1988). More recently, a PCNA-independent DNA polymerase, carrying a 3'-5' exonuclease activity, was isolated from animal cells (Crute et al., 1986; Wahl et al., 1986; Focher et al., 1988, 1989; Lee et al., 1991a, 1991b). The function of this enzyme, currently termed DNA polymerase ε, is unknown, but it is probably involved in both nuclear DNA repair and replication (Morrison et al., 1990; Araki et al., 1992).

The situation is somehow different when considering lower eukaryotic cells. Five distinct species of enzymes have been described in yeast. No low mol wt nuclear DNA polymerase (δ-like) has been found in budding yeast, whereas two forms of DNA polymerase ε (II and I) have been studied (Hamatake et al., 1990; Wang, 1991; Araki et al., 1992). Yeast DNA polymerases I and III were found to be similar to animal DNA polymerases a and δ (Burgers et al., 1990; Wang, 1991).

Multiple DNA polymerases from higher plants also have been isolated and characterized (Bryant and Fitchell, 1986; Litvak and Castroviejo, 1987). Caution must be taken when comparing plant and animal DNA polymerases because, although the cellular functions may be similar, the biochemical properties of enzymes from the two sources may be different. Four extramitochondrial DNA polymerases from wheat germ have previously been characterized (Litvak and Castroviejo, 1987; Table I). Wheat DNA polymerase A has some puzzling properties (Tarrago-Litvak et al., 1975; Laquel et al., 1990b): this polymerase is able to copy natural and synthetic RNA templates and thus shares some properties with the retroviral reverse transcriptase (Laquel et al., 1990b). On the other hand, as in the case of animal DNA polymerase a, DNA polymerase A copurifies with wheat DNA primase, the enzyme involved in the synthesis of short RNA primers of the lagging DNA strand (Graveline et al., 1984; Laquel et al., 1990a). Moreover, the efficient use of an RNA-primed DNA template by DNA polymerase A suggests a role of this enzyme in the initiation of DNA replication. Concerning DNA polymerase B, some biochemical properties such as
template specificity, sensitivity to inhibitors of DNA replication (aphidicolin, butyl-phenyl dGTP, etc.), and the copurification of DNA polymerase B with a 3′-5′ exonuclease activity indicate that DNA polymerase B can be considered to be a δ-like DNA polymerase (Richard et al., 1991). The low mol wt DNA polymerase CI is a wheat DNA polymerase that is very similar in size and has some properties in common with the low mol wt animal DNA polymerase β, except that, unlike the animal enzyme, it is strongly inhibited by N-ethylmaleimide (Castroviejo et al., 1991). Properties of DNA polymerase CI are similar to those of animal DNA polymerase α (Castroviejo et al., 1982).

The degree of processivity of a DNA polymerase, which is related to its capacity to synthesize DNA products of varying size, depends on the ability of the enzyme to dissociate from the template during chain elongation, has been shown to be a crucial parameter in defining the possible role of a DNA polymerase. In general terms, replicative DNA polymerases are more processive than repair DNA polymerases. Processivity may be an intrinsic property of a DNA polymerase, or it could be contributed by an extrinsic protein factor. For this reason, we have investigated the processivity of the different wheat DNA polymerases previously characterized in our laboratory. The effect of varying pH and divalent cation concentration and the effect of calf thymus PCNA on the processivity of DNA polymerases A, B, CI, and CII have been studied. This approach may be helpful in determining the function(s) of wheat DNA polymerases in DNA replication.

**MATERIALS AND METHODS**

**Enzymes**

DNA polymerases A, B, CI, and CII were purified from commercial wheat germ as described by Castroviejo et al. (1982), Laquel et al. (1990b), Castroviejo et al. (1991), and Richard et al. (1991). Rat liver DNA polymerase β was a generous gift from Dr. J. M. Rossignol (Institut de Recherche Scientifique sur le Cancer du Centre National de la Recherche Scientifique, Villejuif, France), and Xenopus laevis oocyte DNA polymerase α was a gift from Dr. L. Tarrago-Litvak. PCNA and DNA polymerase δ from calf thymus were a generous gift from Dr. Marietta Y. W. T. Lee (University of Miami School of Medicine, Miami, FL).

**Nucleic Acids and Nucleotides**

Poly(dA) and oligo(dT) were purchased from Sigma. The oligonucleotide-sizing markers (composed of oligomers of 8 to 32 bases in length) and ultrapure deoxynucleotide triphosphates came from PL Pharmacia. All radiochemicals were obtained from Amersham.

**Measurement of Processivity**

The assay mixture in a final volume of 50 μL contained either 50 mM Tris-HCl (pH 8.0) or 50 mM Hepes (pH 5.9) and 10 mM MgCl₂, 5 mM DTT, 2 A₂₆₀₃₀/mL of poly(dA), 0.01 A₂₆₀₃₀/mL of oligo(dT)₁₀ (ratio of primer:template = 1:20), and 5 μCi of [α³²P]dTP (3000 Ci/mmol) as the labeled substrate. Reactions were initiated with 1 unit of each DNA polymerase (corresponding to 1–7 μg of protein) and incubated at 30°C for 60 min unless otherwise stated. The reaction was stopped by the addition of EDTA to a final concentration of 20 mM. Proteins were extracted twice with phenol, and nucleic acids were precipitated at −20°C with 2 volumes of 96% ethanol in the presence of 5 μg of unlabeled calf thymus DNA as carrier. After the mixture was centrifuged, the pellets were dried and redissolved in formamide containing 0.5% xylene cyanol, 0.5% bromophenol blue, and 40% Suc. After the mixture was denatured at 85°C for 1 min, the size of the

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*Not, Not inhibited. Inh, Inhibited.
reaction product was determined by electrophoresis on 14% denaturing polyacrylamide gels containing 100 mM Tris-borate (pH 8.3), 7 M urea, and 2 mM EDTA. The oligonucleotidesizing markers (8–32 nucleotides long) were 5'-end labeled with [γ-<sup>32</sup>P]ATP and T4 polynucleotide kinase (Maniatis et al., 1983). Gels were 28 cm wide, 38 cm long, and 0.4 mm thick. Electrophoresis was carried out at 1200 V until the bromophenol blue tracking dye had migrated to about three-quarters of the length of the gel. After electrophoresis, the gels were covered with Saran wrap and exposed to Kodak X-AR film at -80°C, with an intensifying screen. When indicated, 1.5 µg of calf thymus PCNA was added to the reaction mixture before the addition of radiolabeled substrate.

DNA Polymerase Assay

Assays of DNA polymerase activity were carried out in 50-µL reaction mixtures that contained 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 5 mM MgCl<sub>2</sub>, 2 A<sub>260</sub>/mL of poly(dA), 0.01 A<sub>260</sub>/mL of oligo(dT)<sub>10</sub>, and 10 µM [<sup>3</sup>H]dTTP. Reaction mixtures were incubated for 30 min at 37°C unless otherwise indicated. Reactions were stopped by the addition of 1 mL of 10% ice-cold TCA in 0.1 M sodium PPi. The precipitate was filtered on nitrocellulose membranes and dried, and the radioactivity was counted in a 2,5-diphenyloxazole-POPOP-toluene (4 g, 0.1 g, 1 L<sup>−1</sup>) scintillation mixture.

Pulse-Chase Experiment

The reaction was done as described above, except enzymes were incubated at 30°C for a short time (pulse of 1 or 5 min) in the presence of 5 µCi of [α-<sup>32</sup>P]dTTP (3000 Ci/mmol). Samples were either immediately stopped with 20 mM EDTA and phenol extracted for electrophoretic analysis or 150 µM of unlabeled dTTP was added and incubated for 5, 20, or 60 min at 30°C before phenol extraction. The product size was determined by electrophoretic separation on a 14% denaturing polyacrylamide sequencing gel as described above.

RESULTS

Pioneering studies of the stimulation of animal DNA polymerase activity by PCNA, formerly called cyclin, showed the importance of parameters such as pH and divalent cation on the effect of PCNA in the processivity of DNA polymerases. Thus, the effect of PCNA on animal DNA polymerase δ has been described specifically at pH 5.9 in the presence of MgCl<sub>2</sub> and poly(dA)-oligo(dT) as the template-primer (Lee and Whyte, 1984; Bravo et al., 1987; Bauer and Burgers, 1988; Lee, 1988; Lee et al., 1991a). However, wheat enzymes are generally more active at a basic pH (8.0) when tested in the presence of activated DNA or synthetic templates. For this reason, wheat DNA polymerase activities were followed at two pH values with the template-primer poly(dA)-oligo(dT). DNA polymerase A and CII were more active at pH 8.0 than at the acidic pH. DNA polymerase B activity was not changed at either pH value, and DNA polymerase CII was more active in DNA synthesis at pH 5.9 when compared with pH 8.0. Both animal DNA polymerases α and δ were more active at pH 8.0, whereas animal DNA polymerase β was more active at pH 5.9. Processive DNA polymerases were tested in the presence of the sparsely primed poly(dA)-oligo(dT)<sub>10</sub> and [α-<sup>32</sup>P]dTTP as the labeled substrate as described in “Materials and Methods.” Incubations were for 5 min (lanes 1, 4, 7, 10, 13, 16, 19, and 22), 30 min (lanes 2, 5, 8, 11, 14, 17, 20, and 23), or 60 min (lanes 3, 6, 9, 12, 15, 18, 21, and 24) at pH 5.9 (lanes 1–12) or at pH 8.0 (lanes 13–24). The labeled nucleic acids were electrophoresed on a 14% polyacrylamide sequencing gel. Lanes 1 to 3 and 22 to 24 correspond to DNA polymerase CII, lanes 10 to 15 to DNA polymerase CI, lanes 7 to 9 and 16 to 18 to DNA polymerase B, and lanes 4 to 6 and 19 to 21 to DNA polymerase A.

Figure 1. Influence of pH on the processivity of wheat DNA polymerases. DNA polymerases were tested in the presence of the sparsely primed poly(dA)-oligo(dT)<sub>10</sub> and [α-<sup>32</sup>P]dTTP as the labeled substrate as described in "Materials and Methods." Incubations were for 5 min (lanes 1, 4, 7, 10, 13, 16, 19, and 22), 30 min (lanes 2, 5, 8, 11, 14, 17, 20, and 23), or 60 min (lanes 3, 6, 9, 12, 15, 18, 21, and 24) at pH 5.9 (lanes 1–12) or at pH 8.0 (lanes 13–24). The labeled nucleic acids were electrophoresed on a 14% polyacrylamide sequencing gel. Lanes 1 to 3 and 22 to 24 correspond to DNA polymerase CII, lanes 10 to 15 to DNA polymerase CI, lanes 7 to 9 and 16 to 18 to DNA polymerase B, and lanes 4 to 6 and 19 to 21 to DNA polymerase A.
a weak degree of processivity at pH 8.0 could be observed primarily for DNA polymerase B.

A very different situation was obtained with DNA polymerase CI, which exhibited a low processivity in the presence of poly(dA)-oligo(dT)10. Only a few residues of \([\alpha-\text{32P}]\text{dTMP}\) were incorporated, giving rise to the small labeled bands. This illustrates a distributive mechanism of polymerization by DNA polymerase CI. The homogeneity of the oligo(dT)10 primer used was confirmed by \([\gamma-\text{32P}]\text{ATP}\) labeling with polynucleotide T4 kinase and electrophoresis on a sequencing gel. A major band of 10 nucleotide residues (95%) and a minor band of 12 residues (5%) were observed. Bands from 11 to 13 residues correspond to the incorporation of one nucleotide monophosphate in these major oligonucleotides present in the primer fraction. Nevertheless, some smaller bands were observed with DNA polymerase CI at pH 8.0, as well as with DNA polymerase CII at both pH values, suggesting the presence of a 5'-3' exonuclease activity. DNA polymerases A and B did not show such nuclease activity. Nevertheless, some bands just smaller than the primer were observed with DNA polymerase B, mainly at pH 5.9 and with Mn as the divalent cation (Fig. 2A), which may result from the 3'-5' exonuclease activity copurifying with this enzyme (Richard et al., 1991). The primer may be shortened by this activity and further extended with labeled nucleotides.

**Effect of Divalent Cations**

Divalent cations, such as MgCl2 or MnCl2, are essential for DNA polymerase activity and may influence the degree of processivity of these enzymes. To study this parameter, experiments were performed in which the MgCl2 concentration was varied from 0.05 to 20 mm and the MnCl2 concentration was varied from 0.01 to 5 mm. Analysis of DNA polymerase B processivity at different cation concentrations revealed that this polymerase was significantly processive at pH 5.9 with an optimum processivity at 0.1 mm MnCl2 (Fig. 2A). No processive synthesis was observed at pH 5.9 with MgCl2. At pH 8.0, in the presence of both MnCl2 and MgCl2, the enzyme was essentially distributive. Results with DNA polymerase CII were similar to those obtained with DNA polymerase B (Fig. 2B). Only at pH 5.9 in the presence of MnCl2 (optimum at 0.5 mm) did this enzyme behave processively. DNA polym-

![Figure 2. Influence of divalent cation concentration on processivity of wheat DNA polymerases.](https://www.plantphysiol.org/doi/abs/10.1104/pp.102.110.60)

Enzymes were incubated for 30 min at 30°C in the presence of poly(dA)-oligo(dT)10 and \([\alpha-\text{32P}]\text{dTTP}\) (as described in “Materials and Methods”) in the presence of various concentrations of MgCl2 and MnCl2, at pH 8.0 and 5.9. Product length was analyzed by electrophoresis on 14% denaturing polyacrylamide gels. In each gel, lanes 1, 12, 5 mm MnCl2; lanes 2, 13, 1 mm MnCl2; lanes 3, 14, 0.5 mm MnCl2; lanes 4, 15, 0.1 mm MnCl2; lanes 5, 16, 0.05 mm MnCl2; lanes 6, 17, 0.01 mm MnCl2; lanes 7, 18, 20 mm MgCl2; lanes 8, 19, 10 mm MgCl2; lanes 9, 20, 5 mm MgCl2; lanes 10, 21, 1 mm MgCl2; lanes 11, 22, 0.05 mm MgCl2; lanes 1 to 11, pH 5.9; lanes 12 to 22, pH 8.0. A, DNA polymerase B. The upper band at the bottom of the figure corresponds to the monomer, and the fast migrating bands correspond to the degradation products of dTTP. B, DNA polymerase CI; C, DNA polymerase CII.
erase CII activity was greater in the presence of MnCl2 than with MgCl2 at both pH values.

As for DNA polymerase β, under all conditions studied, DNA polymerase CI behaved as a strictly distributive polymerase (Fig. 2C). The enzyme did not distinguish between the oligo(dT)10 and oligo(dT)12 primers, giving rise to the main labeled bands of 11 and 13 bases. However, polymerase CI at pH 5.9 was significantly more active with MnCl2 (optimum at 5 mM) than with MgCl2 (optimum at 20 mM), whereas no difference was observed at pH 8.0. The same optima of MnCl2 and MgCl2 were observed at both pH values.

The high degree of processivity of DNA polymerase A was not modified by either MgCl2 or MnCl2. The enzyme clearly preferred MgCl2 at both pH values tested (data not shown).

Effect of PCNA

The effect of calf thymus PCNA on the activity and processivity of wheat DNA polymerases was studied as described by Lee et al., 1991a. In control experiments, the ability of calf thymus PCNA to stimulate animal DNA polymerase δ was confirmed: at pH 5.9 in the presence of 10 mM MgCl2, PCNA stimulated DNA polymerase activity 8-fold. Under the same conditions, animal DNA polymerase α and β were not stimulated (data not shown). The effect of increasing amounts of PCNA on the activity of wheat DNA polymerases is shown in Figure 3. DNA polymerases B and CII were significantly stimulated (about 17-fold) by the addition of 6 µg of PCNA. However, under the same conditions, DNA polymerase A and CI were only slightly stimulated (less than 3-fold).

The effect of PCNA on enzyme processivity at 30°C and pH 5.9 with 10 mM Mg (measured as described in “Materials and Methods”) was analyzed under the conditions of minimum activity of the contaminating exonuclease activity. As shown in Figure 4A, DNA polymerase A was fully processive, in either the presence or absence of PCNA. In the case of DNA polymerases B and CII, both the enzyme activity and the degree of processivity were strongly stimulated by PCNA.

An interesting difference was observed concerning the effect of PCNA on DNA polymerase CII and B. DNA polymerase CII was stimulated by PCNA after shorter incubation periods (5 min, lane 9), whereas for DNA polymerase B, the stimulation was noticeable only after 15 min of incubation (lane 2). Processivity of DNA polymerase CII was analyzed in the presence or absence of PCNA and compared to that of the animal DNA polymerase β. As shown in Figure 4B, the length of the products of these enzymes was not modified by PCNA. This common property of animal DNA polymerase β and wheat DNA polymerase CII was confirmed by a pulse-chase experiment in which enzymes were incubated for a short time in the presence of poly(dA)-oligo(dT)10 and [α-32P]dTTTP, followed by a chase of excess of unlabeled dTTTP, as described in “Materials and Methods.” Under these conditions, even with extended incubation periods, only a few residues were incorporated, giving rise to short-labeled primer.

In the case of a processive enzyme unable to dissociate from the elongating primer-template complex, the incorporation of unlabeled dTMP should chase the radioactivity of labeled primers to longer molecules. A kinetic illustration of this chase is shown in Figure 5. DNA polymerase CII, a processive enzyme when tested in the presence of PCNA, was able to elongate the labeled primer to larger-size products after different times of chase. In contrast, DNA polymerase CI was not able to elongate the primer to large-size products. The incorporation was limited to about 10 residues. Similarly, animal DNA polymerase β was poorly processive, because it was able to incorporate only about 20 residues.

DISCUSSION

To obtain a better understanding of the role of wheat DNA polymerases in DNA replication in plants, a study of their processivity as reflected by the size of the products synthesized under different conditions was conducted.

In our first report on this enzyme, we compared DNA polymerase A to animal DNA polymerase γ in terms of the efficient recognition of the synthetic template-primer poly(rA)-oligo(dT), stimulation by KCl, and resistance to aphidicolin (Tarrago-Litvak et al., 1975). Nevertheless, DNA polymerase A seems different from the wheat mitochondrial DNA polymerase (Christophe et al., 1981; Ricard et al., 1983) and the chloroplast DNA polymerase with regard to template specificity (P. Laquel, unpublished results).

On the other hand, some properties of DNA polymerase A suggest that this enzyme, although aphidicolin resistant, may be to some extent the wheat counterpart of DNA polymerase α: (a) it is the only wheat DNA polymerase found to be associated with DNA primase activity; (b) this enzyme is the wheat DNA polymerase capable of most efficiently rec-
Figure 4. Effect of PCNA on the processivity of wheat DNA polymerases. Enzymes were incubated in the presence of 0.4 μg of poly(dA)-oligo(dT)$_{10}$ (20:1), 10 mM MgCl$_2$, and [α-32P]dTTP as described in “Materials and Methods.” The extracted labeled DNA was electrophoresed on a 14% polyacrylamide sequencing gel. Enzymes were incubated for 5, 15, or 30 min at 30°C as indicated at the top of the figure in the presence (+) or in the absence (−) of PCNA (1.5 μg/assay). A, DNA polymerase B (lanes 1–6), DNA polymerase CII (lanes 7–12), DNA polymerase A (lanes 13–18), B, DNA polymerase CI (lanes 5–10), animal DNA polymerase β (lanes 1–3 and 11–13). Lane M corresponds to the 8 to 32 oligonucleotide-sizing markers.

The low mol wt DNA polymerase CI has been compared to the animal DNA polymerase β according to template specificity and the effect of inhibitors, except for N-ethylmaleimide (Castroviejo et al., 1991). In all conditions tested (pH, divalent cation concentrations, and effect of PCNA), DNA polymerase CI was strictly distributive, like the animal DNA polymerase β. In a pulse-chase experiment, both enzymes were still distributive, even in the presence of PCNA, thus demonstrating that this behavior was not dependent on substrate concentration. These results are in agreement with our previous hypothesis that DNA polymerase CI is the plant counterpart of the animal DNA polymerase β.

DNA polymerase CII shows some specific properties of DNA polymerase α, essentially the sensitivity toward aphidicolin (Table I). This enzyme was not processive when the pH (Fig. 1) or cation concentration (Fig. 2) was varied. A high degree of processivity was observed only when PCNA was present. Under these experimental conditions, DNA polymerase CII behaved as a DNA polymerase δ.

Previous results concerning the effect of butyl-phenyl dGTP, which is commonly used in animal cells to discriminate among DNA polymerases α, δ, and ε, have shown that both DNA polymerase CII and B are very resistant to this analog, whereas other wheat DNA polymer-
polymerases of plant PCNA after this protein has been interesting to study the effect on the homologous DNA (Bauer and Burgers, 1988). It will, nevertheless, polymerase mology found between the PCNA gene from plants and polymerases is not surprising given the high degree of ho-
purified from plants or expressed as a recombinant protein.

The ability of calf thymus PCNA to stimulate plant DNA similar, is less dependent on PCNA than on DNA polymerase a, which was previously linked to DNA polymerase 5 (or e) with regard to processivity and the effect of PCNA. Thus, DNA polymerase CII could play a role in the replication fork, in association with other plant DNA polymerases, or by intervening in DNA repair as described in mammalian cells (Morrisson et al., 1990; Araki et al., 1992). Work is in progress to obtain information concerning the primary structure of these DNA polymerases. Cloning and study of the genes and their regulation should provide crucial information for understanding DNA replication in plant cells.

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LITERATURE CITED

Araki H, Ropp PA, Johnson AL, Johnston LH, Morrison A, Sugino A (1992) DNA polymerase II, the probable homolog of mammalian DNA polymerase e, replicates chromosomal DNA in the yeast Saccharomyces cerevisiae. EMBO J 11: 733–740

Figure 5. Analysis of processivity of wheat DNA polymerases CII and CII compared to animal DNA polymerase 8 in a pulse-chase experiment. Enzymes were incubated for 1 min (lane 1) or 5 min (lanes 6 and 10) in the presence of poly(dA)-oligo(dT), plus 3 nm [α-32P]dTP at 30°C as described in “Materials and Methods.” After this labeling time, an excess (150 μM) of unlabeled dTPP was added to the reactional mixture, and incubation was pursued for 2 min (lane 2), 5 min (lanes 3, 7, and 11), 20 min (lanes 4, 8, and 12), or 45 min (lanes 5, 9, and 13). The product length was analyzed by electrophoresis on 14% denaturing polyacrylamide sequencing gel.

DNA polymerase CII, lanes 1 to 5; DNA polymerase CII, lanes 6 to 9; DNA polymerase 8, lanes 10 to 13. Wheat DNA polymerases and polymerase 8 were tested in the presence of 1.5 μg of PCNA.

In conclusion, experimental evidence has been obtained to confirm that wheat DNA polymerase B is the counterpart of the animal DNA polymerase 3 and thus could play a role in replicating the leading strand of the replication fork. The distributive behavior of wheat DNA polymerase CII, together with previous results (Castroviejo et al., 1991), supports our assertion that this enzyme is the plant counterpart of animal DNA polymerase 8. The situation is less clear, however, concerning the comparison of the two other plant DNA polymerases (enzymes A and CII) with animal DNA polymerases. The properties of DNA polymerase A are not orthodox. Thus, based on its properties concerning the recognition of some template-primer duplexes, the effect of some inhibitors, its high processivity, and its tight association with DNA primase, we speculate that it may be a plant α-like DNA polymerase and may play a role in the initiation and/or elongation of the lagging DNA strand of the replication fork in wheat.

The function of DNA polymerase CII is less clear. This enzyme, which was previously linked to DNA polymerase α, behaves like an animal DNA polymerase 8 (or e) with regard to processivity and the effect of PCNA. Thus, DNA polymerase CII could play a role in the replication fork, in association with other plant DNA polymerases, or by intervening in DNA repair as described in mammalian cells (Morrisson et al., 1990; Araki et al., 1992). Work is in progress to obtain information concerning the primary structure of these DNA polymerases. Cloning and study of the genes and their regulation should provide crucial information for understanding DNA replication in plant cells.

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