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Hydrolysis of 3′-Terminal Mispairs in Vitro by the 3′ → 5′ Exonuclease of DNA Polymerase δ Permits Subsequent Extension by DNA Polymerase α†

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Received March 2, 1990; Revised Manuscript Received March 30, 1990

ABSTRACT: Purified DNA polymerase α, the major replicating enzyme found in mammalian cells, lacks an associated 3′ → 5′ proofreading exonuclease that, in bacteria, contributes significantly to the accuracy of DNA replication. Calf thymus DNA polymerase α cannot remove mispaired 3′-termini, nor can it extend them efficiently. We designed a biochemical assay to search in cell extracts for a putative proofreading exonuclease that might function in concert with DNA polymerase α in vivo but dissociates from it during purification. Using this assay, we purified a 3′ → 5′ exonuclease from calf thymus that preferentially hydrolyzes mispaired 3′-termini, permitting subsequent extension of the correctly paired 3′-terminus by DNA polymerase α. This exonuclease copurifies with a DNA polymerase activity that is biochemically distinct from DNA polymerase α and exhibits characteristics described for a second replicative DNA polymerase, DNA polymerase δ. In related studies, we showed that the 3′ → 5′ exonuclease of authentic DNA polymerase δ, like the purified exonuclease, removes terminal mispairs, allowing extension by DNA polymerase α. These data suggest that a single proofreading exonuclease could be shared by DNA polymerases α and δ, functioning at the site of DNA replication in mammalian cells.

DNA polymerase α is thought to be one of the principal enzymes that replicates the mammalian genome (Fry & Loeb, 1986; Lehman & Kaguni, 1989). Purified DNA polymerase α is most frequently, but not invariably (Chen et al., 1979; Skarnes et al., 1986; Ottiger et al., 1987; Cotterill et al., 1987; Bialek et al., 1989), devoid of exonucleolytic proofreading activity (Chang et al., 1984; Wahl et al., 1984; Wang et al., 1984; Reyland & Loeb, 1987; Nasheuer & Grosse, 1987). In procaryotes and bacteriophage, a 3′ → 5′ exonuclease activity is a major contributor to the fidelity of DNA replication. The 3′ → 5′ exonuclease either is an integral part of the DNA polymerase polypeptide or is a separate subunit of the DNA replication complex (Brutlag & Kornberg, 1972; Scheuermann & Echols, 1984). It has been estimated that exonucleolytic proofreading enhances the fidelity of DNA synthesis in vitro 10-1000-fold (Kunkel, 1988). Considering the evolutionary conservation of enzymes involved in DNA replication (Wang et al., 1989), it seems possible that an exonuclease is associated with DNA polymerase α in mammalian cells but is separated during purification. However, the identity of such an exonuclease has not been clearly established.

We designed a biochemical assay to detect a putative proofreading exonuclease that functions in concert with DNA polymerase α in vitro and searched for this activity in calf thymus tissue. Our results show that a 3′ → 5′ exonuclease activity that preferentially removes terminally mispaired nu-

† This work was supported by National Institutes of Health Grants T32-CA-09437 (F.W.P.) and R35-CA-39903 (L.A.L.).
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0006-2960/90/0429-5226$02.50/0

cleotides can be isolated from cell extracts by this assay. Furthermore, our results indicate that the isolated 3′ → 5′ exonuclease is the exonuclease activity associated with DNA polymerase δ.

Materials and Methods

Materials. Radiolabeled deoxynucleoside triphosphates (dNTPs) were purchased from Du Pont-New England Nuclear, and unlabeled dNTPs and bovine serum albumin (RIA grade) were from Sigma. The T4 polynucleotide kinase was purchased from Promega. Exonuclease III was from Life Technologies. The oligonucleotide template and primer were synthesized and HPLC purified by Operon Technologies. DEAE-cellulose (DE-52) and phosphocellulose (P-11) were from Whatman, and hydroxylapatite (Bio-Gel HT) and Bio-Rex 70 were from Bio-Rad Laboratories. Purified calf thymus proliferating cell nuclear antigen (PCNA) was a gift of Drs. T. Myers and R. Bambara (University of Rochester). Butylphenyl-dGTP (BuPhdGTP) was a gift of Dr. G. Wright (University of Massachusetts Medical School).

Purification of Enzymes. DNA polymerase α was immunoaffinity purified as the DNA polymerase–primase complex devoid of exonuclease or endonuclease activities (Perrino & Loeb, 1989a). DNA polymerase δ used in Figure 5 was purified through step 6 as described (Lee et al., 1984). The ε subunit of *Escherichia coli* DNA polymerase III was purified as described (Perrino et al., 1988).

Proofreading Reactions. A 16-nucleotide primer was hybridized to a 30-nucleotide template (identical with nucleotides 581–610 of φX174 *am*3 DNA) to produce a DNA duplex containing an A·G mispair at the 3′-primer terminus (see Figure 1, panel A). Reactions (20 μL) contained 20 mM HEPES, pH 7.3, 1 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin, 2% glycerol, template–primer at 2.2 nM 3′-termini, and 2.0 units of DNA polymerase α (1 unit catalyzes the incorporation of 1 pmol of [α-^{32}P]dTMP into 0.022 pmol of 15mer-primed φX174 *am*3 DNA in 30 min at 30 °C with all four dNTPs at 50 μM). Reactions were for 30 min at 37 °C. For polyacrylamide gel analysis of reaction products, the 16mer primer was ^{32}P labeled on the 5′-end with T4 polynucleotide kinase, and dNTPs (when present) were 5 μM. Reactions were eluted through 0.5-mL Sephadex G-100 columns to remove unincorporated deoxynucleoside triphosphates, fractionated on 15% polyacrylamide sequencing gels, and visualized by autoradiography. For nucleotide incorporation analysis, dCTP, dGTP, and dTTP were 5 μM, and [α-^{32}P]dATP was 0.13 μM. Incorporation of radiolabel was determined by collecting acid-insoluble material on glass fiber disks.

DNA Polymerase Reactions. DNA polymerase reactions (30 μL) contained 20 mM HEPES, pH 7.3, 1 mM $MgCl_2$, 0.1 mg/mL bovine serum albumin, 2% glycerol, 0.04 mM dTTP, 0.5 μM [α-^{32}P]dATP, and 0.2 OD_{260} of poly(dA-dT). Reactions were for 30 min at 37 °C. For purification of DNA polymerase δ by the procedure of Lee et al. (1984), [α-^{32}P]-dATP was 5.0 μM. One unit of DNA polymerase δ used in Figure 5 catalyzes the incorporation of 1 nmol of labeled nucleotide in 60 min at 37 °C.

Purification of a 3′ → 5′ Proofreading Exonuclease from Calf Thymus. All procedures were at 0–4 °C, and activity was quantitated by incorporation of [α-^{32}P]dAMP by DNA polymerase α into the mispaired template–primer as described above. Thymus tissue (100 g) was thawed in two volumes of buffer A (50 mM Tris, pH 7.5, 250 mM sucrose, 1 mM dithiothreitol, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 10 mM sodium metabisulfite, 0.5 mM phenylmethanesulfonyl fluoride, 1 μg/mL leupeptin, and 1 μg/mL pepstatin A) and homogenized in a blender for 60 s, and debris was removed by centrifugation at 25000*g* for 30 min (fraction 1). Solid ammonium sulfate was added to 45% saturation, and the insoluble proteins were collected by centrifugation (10000*g*, 20 min), resuspended in buffer A (90 mL), and dialyzed overnight against buffer A (fraction 2). Fraction 2 was loaded onto a DEAE-cellulose column (4.9 cm^2 × 23 cm) previously equilibrated in buffer A. The proofreading activity was collected as the flow-through fraction and dialyzed against buffer B (same as buffer A but with 0.1 M potassium phosphate, pH 6.8, and with Tris and sucrose omitted) (fraction 3). Fraction 3 was loaded onto a phosphocellulose P-11 column (4.9 cm^2 × 24 cm) in buffer B and washed with 240 mL of buffer B. Elution was with a 600-mL linear gradient of 0.1–0.4 M potassium phosphate, pH 6.8, in buffer B. The activity eluted as a single peak at 0.22 M potassium phosphate. Pooled fractions were dialyzed against buffer C (same as buffer B but with 20 mM potassium phosphate, pH 6.8, and 20% glycerol) (fraction 4). One third of fraction 4 was loaded onto a hydroxylapatite column (2 cm^2 × 5 cm) in buffer C and washed with 20 mL of buffer C. Elution was with a 100-mL linear gradient of 0–0.5 M KCl in buffer C. The single peak of activity (0.1 M KCl) was pooled and dialyzed against buffer D (same as buffer C but with 20 mM Tris, pH 7.5, instead of potassium phosphate) (fraction 5). The activity was loaded onto a Bio-Rex 70 column (2 cm^2 × 6 cm) previously equilibrated in buffer D; the column was washed with 30 mL of buffer D, and the activity was eluted with a 100-mL linear gradient of 0–0.6 M NaCl in buffer D. Fractions were dialyzed to remove NaCl.

Results

A Biochemical Proofreading Assay for DNA Polymerase α. Immunoaffinity-purified DNA polymerase α–primase complex lacking a detectable 3′ → 5′ exonuclease has been purified from calf thymus by us and others (Chang et al., 1984; Wahl et al., 1984; Reyland & Loeb, 1987; Nasheuer & Grosse, 1987; Perrino & Loeb, 1989a). During in vitro DNA synthesis, this DNA polymerase α complex extends mismatched terminal nucleotides 10^3–10^6-fold slower than matched nucleotides (Perrino & Loeb, 1989a). Slow mispair extension by DNA polymerase α provides an opportunity for a separate 3′ → 5′ exonuclease to remove the terminal mispair before addition of the next correct nucleotide seals the mismatch into duplex DNA (Perrino & Loeb, 1989b). To attempt reconstitution of DNA polymerase α with a proofreading exonuclease, we designed a sensitive biochemical proofreading assay (Figure 1). This assay exploits DNA polymerase α's extremely slow rate of extension from a 3′-terminal A·G mispair. A template–primer was constructed by hybridizing an oligonucleotide primer of 16 nucleotides to a template of 30 nucleotides. Fifteen of the 16 nucleotides were complementary; the 3′-primer terminus formed an A·G mismatch (Figure 1A). We first investigated removal of the terminal mismatch using an established proofreading exonuclease from *E. coli*, the ε subunit of DNA polymerase III (Scheuemann & Echols, 1984). Less than 1% of the 5′-^{32}P radiolabeled 16-nucleotide primer is elongated to the predicted length of 22 nucleotides by DNA polymerase α in the absence of the ε subunit, as determined by electrophoresis of the reaction products through polyacrylamide gels (Figure 1B). Addition of increasing amounts of the ε subunit yields a progressive increase in elongation of the primer to the full-length 22-nucleotide copy of the template (Figure 1B). Elongation presumably occurs by initial hydrolysis of the terminally mispaired nucleotide by the ε subunit exonuclease and subsequent ex-

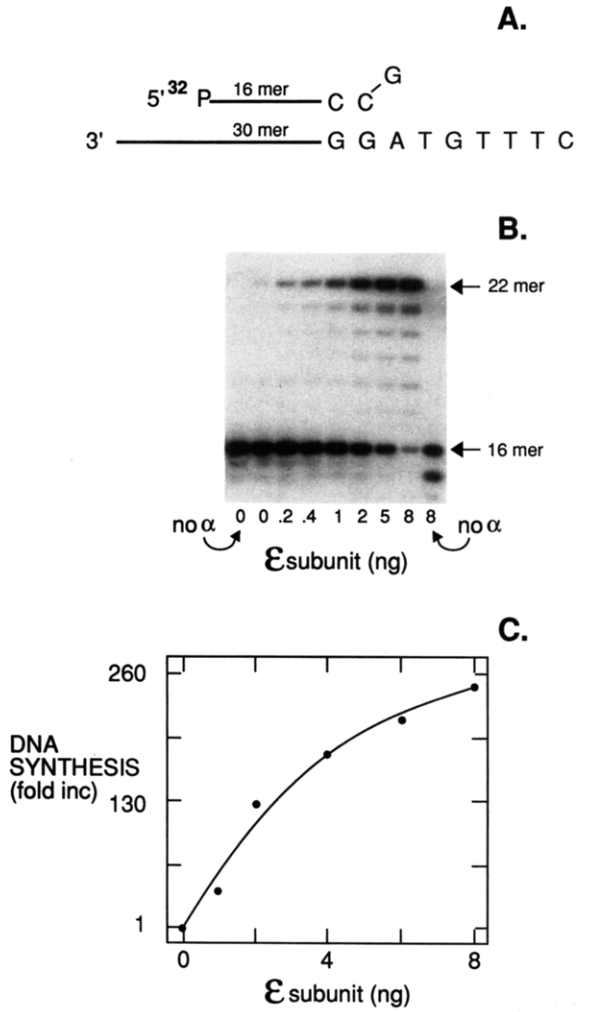

FIGURE 1: Biochemical proofreading assay. A 16-nucleotide primer (16mer) was hybridized to a 30-nucleotide template (30mer) to produce a DNA duplex containing an A·G mispair at the 3′-primer terminus (panel A). Proofreading reactions were carried out as described under Materials and Methods (panels B and C). DNA polymerase α was omitted in reactions shown in the terminal left and right lanes, and the positions of the 16mer primer and 22-nucleotide product (22mer) are indicated by arrows (panel B). DNA synthesis is expressed relative to that detected in the absence of added exonuclease (panel C).

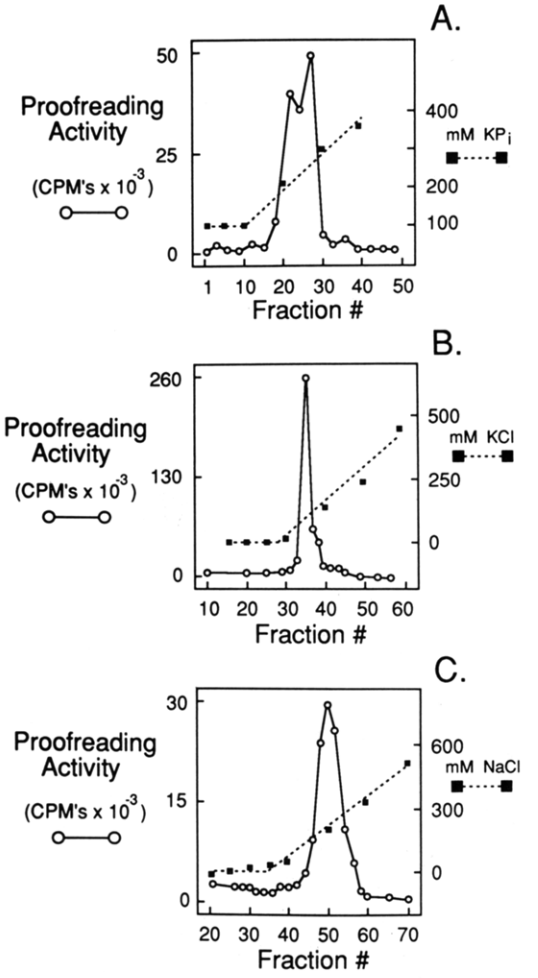

FIGURE 2: Purification of a 3′ → 5′ proofreading activity from calf thymus. The purification procedure was carried out as described under Materials and Methods. Elution profiles using the indicated linear gradients from the phosphocellulose P-11 (panel A), hydroxylapatite (panel B), and Bio-Rex 70 (panel C) columns are shown. Proofreading activity (O) was quantitated by incorporation of [α-^{32}P]dAMP by DNA polymerase α into the mispaired template–primer described in Figure 1.

tension from the new, correctly paired 3′-terminus by DNA polymerase α. In the absence of DNA polymerase α, specific hydrolysis of the 3′-terminal mispair by the ϵ subunit is detected by conversion of the 16-nucleotide primer to predominantly a product 15 nucleotides in length (Figure 1B). In parallel experiments, nucleotide incorporation by DNA polymerase α was examined with the mispaired template–primer. In the absence of added ϵ subunit, total nucleotide incorporation by DNA polymerase α is less than 1% of that detected after addition of 8 ng of the exonuclease (Figure 1C). These data establish that a separate 3′ → 5′ proofreading exonuclease can act in concert with DNA polymerase α and that an enzyme with this activity from mammalian cells might be identified with this incorporation assay.

Isolation of a Proofreading Exonuclease from Calf Thymus. We isolated a 3′ → 5′ exonuclease from calf thymus tissue that proofreads for calf thymus DNA polymerase α. Greater than 90% of this activity was initially identified in the protein fraction that precipitated upon addition of ammonium sulfate to 45% saturation in a crude lysate (see Materials and Methods; results not shown). Upon chromatography on DEAE-cellulose greater than 95% of the activity was detected in the flow-through fractions (results not shown). This activity was further purified by sequential chromatography on phosphocellulose, hydroxylapatite, and Bio-Rex 70 (Figure 2). From each of these columns the proofreading activity eluted as a single peak, suggesting that only one exonuclease activity was detected. However, other exonucleases might be present but were either lost early in the purification or not detected with this assay.

To verify that the isolated activity hydrolyzes DNA in the 3′ → 5′ direction, we measured its activity directly using the polyacrylamide gel assay (Figure 3). In the absence of DNA polymerase α and dNTPs the exonuclease produces oligonucleotide products of 15 and 14 nucleotides in length (Figure 3A). Preferential hydrolysis of the 3′-terminal mispair is indicated by conversion of up to 25% of the 16mer primer to the 15mer; less than 5% of the 15mer is further hydrolyzed to produce 14mer or shorter. There is no detectable loss of the 5′-^{32}P label, indicating the absence of a 5′ → 3′ exonucleolytic activity. Concomitant with removal of the terminal mispair by this proofreading activity, there is increased extension by DNA polymerase α to yield a 22mer (Figure 3B).

Association of the Exonuclease with DNA Polymerase δ. Analysis of the purified proofreading activity indicated the presence of a DNA polymerase. To determine if these two activities chromatograph together, the coincidence of the two activities eluting in the Bio-Rex column fractions was exam-

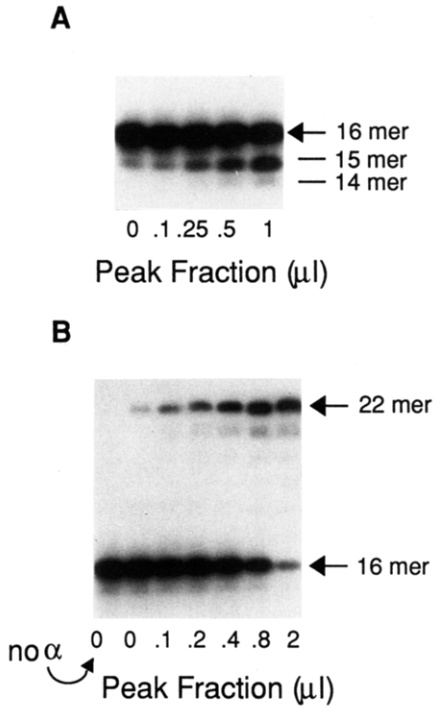

FIGURE 3: Polyacrylamide gel analysis of the exonuclease reaction products. Increasing amounts of the peak fraction (no. 50) from the Bio-Rex 70 column were incubated in proofreading reactions in the absence (panel A) or presence (panel B) of DNA polymerase α and dNTPs as described under Materials and Methods. DNA polymerase α was omitted from the reaction shown in the terminal left lane (panel B). The positions of the 16mer primer and oligonucleotide products are indicated.

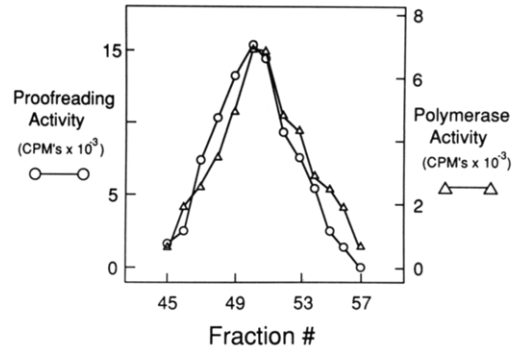

FIGURE 4: Coincident elution of DNA polymerase and exonuclease activities. Proofreading and DNA polymerase reactions were carried out for all fractions of the Bio-Rex 70 column as described under Materials and Methods. Proofreading (O) and DNA polymerase (Δ) activities were detected only in the fractions shown.

ined. DNA polymerase activity was measured with poly(dA-dT) as template–primer; the exonuclease activity was measured with the proofreading assay. The DNA polymerase and proofreading activities are nearly proportional throughout the peak fractions (Figure 4). The copurifying DNA polymerase activity has properties characteristic of DNA polymerase δ. It is stimulated 4-fold by PCNA with poly(dA)–oligo(dT) as a template–primer, and it is resistant to a 10-fold higher concentration of BuPhdGTP than is DNA polymerase α (results not shown). To verify this association, we purified the PCNA-dependent DNA polymerase δ from calf thymus using the procedure of Lee et al. (1984) and measured its ability to stimulate synthesis by DNA polymerase α in the proofreading assay. The addition of increasing amounts of DNA polymerase δ to the proofreading assay increases the amount of the 22mer product produced (Figure 5). This presumably results from the 3′ → 5′ exonuclease associated

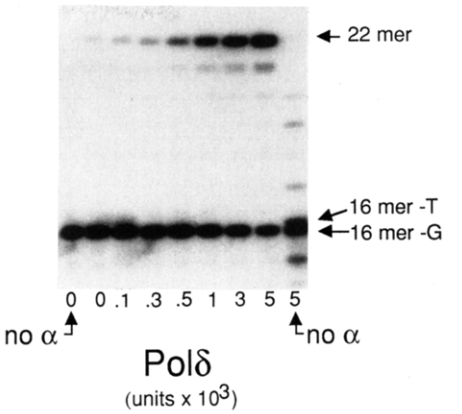

FIGURE 5: DNA polymerase δ removes mispairs for DNA polymerase α. Proofreading reactions were prepared for polyacrylamide gel analysis as described under Materials and Methods. Increasing amounts of DNA polymerase δ were added as indicated. DNA polymerase α was omitted in the reactions shown in the left- and right-most lanes. The positions of the starting 16mer primer (16mer-G), product 16mer oligonucleotide (16mer-T), and product 22mer oligonucleotide are indicated.

with DNA polymerase δ removing the 3′-terminal mispair and DNA polymerase α extending the now paired 3′-terminus. In the absence of DNA polymerase α, both the exonuclease activity and the DNA polymerase activity of δ are observed (Figure 5). The exonuclease removes the terminal mismatch, generating a band of 15 nucleotides. The DNA polymerase adds complementary nucleotides, producing extended products of 16 nucleotides or longer. As a result, two oligonucleotide bands are observed at the 16mer position. The two bands migrate to positions corresponding to 16mers with G or T 3′-termini, respectively. The 16mer-G corresponds to the original primer. The 16mer-T presumably results from exonucleolytic hydrolysis of the mispaired 3′-terminal G followed by incorporation of the correct nucleotide T. This interpretation is supported by the studies with purified ε subunit where, in the absence of DNA polymerase α, only a single band corresponding to the original primer was observed at the 16-nucleotide position. This result indicates that upon hydrolysis of the terminal mispair by the 3′ → 5′ exonuclease of DNA polymerase δ the now correctly paired terminus is accessible for elongation by DNA polymerase α.

Discussion

With the use of a new biochemical proofreading assay, our results show that the 3′ → 5′ exonuclease of DNA polymerase δ can remove 3′-terminal mispairs to produce correctly paired 3′-termini that can be extended by DNA polymerase α. We isolated from calf thymus extracts an activity that stimulates synthesis by DNA polymerase α from an A·G terminal mispair. This stimulatory activity contains a 3′ → 5′ exonuclease that preferentially hydrolyzes mispaired 3′-termini over correctly paired 3′-termini. Thus, the most likely mechanism of its stimulation is production of a correctly paired 3′-terminus that is efficiently extended by the DNA polymerase α present in the reaction. A DNA polymerase activity was detected cochromatographing with the 3′ → 5′ exonuclease activity. The coincidence of DNA polymerase with 3′ → 5′ exonuclease, its stimulation by PCNA, and its relative resistance to BuPhdGTP suggested that it might be DNA polymerase δ. We tested this directly by purifying PCNA-dependent DNA polymerase δ using the method established by Lee et al. (1984). Like the proofreading activity isolated in the initial search, the PCNA-dependent DNA polymerase δ also stimulated synthesis by DNA polymerase α. In contrast to the 500-fold

stimulation of DNA polymerase δ by PCNA with poly(dA)–oligo(dT) as a template–primer reported by Tan et al. (1986), the DNA polymerase activity we isolated was stimulated only 4-fold by PCNA. Analysis of our preparation on SDS–polyacrylamide gels indicates the presence of multiple protein species (results not shown). It is possible that the moderate level of stimulation by PCNA that we observe is due to contaminating PCNA still present in our preparation.

A unique feature of the proofreading assay is the addition of exonuclease-free DNA polymerase α as a component to the reaction mixture that contains a mispaired template–primer substrate. The A·G terminal mispair was selected because it is the most poorly extended mispair by calf thymus DNA polymerase α (Perrino & Loeb, 1989a). Addition of an exonuclease to remove the terminal mispair results in a paired 3′-terminus that is efficiently utilized by DNA polymerase α. We first demonstrated the versatility of this assay using the *E. coli* ε subunit proofreading exonuclease, and conceivably, any 3′ → 5′ exonuclease that removes the terminally mispaired base could function in this assay. However, the preference for a mispair-specific 3′ → 5′ exonuclease in this assay is indicated by the inability of a second nuclease from *E. coli*, exonuclease III, to function efficiently, presumably because this enzyme preferentially hydrolyzes correctly paired 3′-termini (results not shown; Brutlag & Kornberg, 1972). Despite the apparent lack of specificity of the proofreading assay, it should be noted that only a single activity was detected early in the purification, and this activity chromatographed as a single species during subsequent purification steps.

The extent of exonucleotyic proofreading that occurs during DNA replication in animal cells is not known. The nature of single base substitution errors induced at the *aprt* locus in Chinese hamster ovary cells by nucleotide pool imbalances suggests that exonucleolytic proofreading is operable during DNA replication (Phear & Meuth, 1989). Because DNA polymerase α likely plays a central role in DNA replication in animal cells, it seems likely that a 3′ → 5′ proofreading exonuclease acts in concert with DNA polymerase α to achieve high fidelity during cellular DNA replication. However, the identification of such an exonuclease has been elusive. DNA polymerase α has been purified in association with 3′ → 5′ exonuclease activity from mouse myeloma cells (Chen et al., 1979), HeLa cells (Skarnes et al., 1986), calf thymus (Ottiger et al., 1987), and human lymphocytes (Bialek et al., 1989). The HeLa cell exonuclease was identified as a separate 69–kDa protein (Skarnes et al., 1986). Although it was not demonstrated directly, the exonuclease activity associated with the lymphocyte DNA polymerase α was attributed to the isolation of an "undegraded" DNA polymerase subunit (Bialek et al., 1989). The immunoaffinity-purified calf thymus DNA polymerase α complex used in this study is devoid of 3′ → 5′ exonuclease activity (Perriono & Loeb, 1989a). Furthermore, unlike the cryptic exonuclease activity detected in the *Drosophila* DNA polymerase α (Cotterill et al., 1987), we have so far detected no exonuclease activity upon dissociation of the polymerase and primase subunits of the immunoaffinity-purified calf thymus DNA polymerase α complex (unpublished results).

At least two mechanisms are possible for the functional interaction of DNA polymerases and editing exonucleases. In previous studies we have shown that the exceptionally slow rate of mispair extension by calf thymus DNA polymerase α provides an opportunity for a separate proofreading exonuclease to remove misinserted nucleotides prior to subsequent elongation (Perrino & Loeb, 1989a,b). Upon misinsertion, the DNA polymerase could dissociate, providing access for the 3′ → 5′ exonuclease. Alternatively, the DNA polymerase could remain associated and change conformation, presenting the terminally misinserted base to the active site of the editing exonuclease. In either case the efficiency of editing misinserted nucleotides by a 3′ → 5′ exonuclease would be directly dependent on the DNA polymerase capacity to extend from a misincorporated nucleotide. The proofreading component could be located on a separate subunit of the replicative complex, or it could be an integral part of the same polypeptide. Association of a 3′ → 5′ exonuclease activity within the same polypeptide as the DNA polymerase activity does not necessarily dictate that misinsertion errors by the DNA polymerase would be hydrolyzed by the exonuclease activity of the same polypeptide. Recent studies with *E. coli* DNA polymerase I demonstrate that nucleotides misinserted during in vitro DNA synthesis are just as likely to be removed by the exonuclease of a separate enzyme as by the exonuclease activity residing on the same polypeptide (Joyce, 1989).

Recently, DNA polymerase δ, biochemically distinct from DNA polymerase α, has been implicated as a second replicative DNA polymerase (Bambara et al., 1989). Unlike DNA polymerase α, DNA polymerase δ has a tightly associated 3′ → 5′ exonuclease and lacks primase activity. DNA polymerase δ has been further distinguished from DNA polymerase α by (1) its preference for synthetic DNA templates, (2) its relative resistance to the nucleotide analogue BuPhdGTP, (3) its lack of inhibition by monoclonal antibodies prepared against DNA polymerase α, and (4) its distinct spectrum of peptides produced upon digestion with trypsin (Bambara et al., 1989; Wong et al., 1989). In addition, PCNA has been shown to stimulate in vitro DNA synthesis by one form of DNA polymerase δ (Tan et al., 1986). Other forms of DNA polymerase δ are affected less dramatically or not at all by PCNA (Bambara et al., 1989; Focher et al., 1988a; Syvaoja & Linn, 1989). However, definitive evidence that DNA polymerases α and δ are genetically distinct may await production of catalytically active DNA polymerases from the cloned genes for DNA polymerase α (Wong et al., 1988) and from the, as yet, unidentified gene(s) for DNA polymerase δ.

The mechanism of coordinate synthesis of the duplex DNA molecule at a replication fork is unknown. In procaryotes, there is evidence for a dimeric DNA polymerase (McHenry, 1988). It has been postulated that one of the DNA polymerases synthesizes processively on the leading DNA strand while the second DNA polymerase synthesizes discontinuously on the lagging DNA strand. By formation of a loop in the lagging strand, replication can progress in one direction (Alberts, 1987). This model for DNA replication has been extended to mammalian cells; it has been proposed that DNA polymerase α replicates one of the DNA strands and DNA polymerase δ the other (Downey et al., 1988; Focher et al., 1988b; Prelich & Stillman, 1988). It seems likely that both DNA strands are copied with equally high fidelity. However, DNA polymerase δ has a tightly associated 3′ → 5′ exonuclease that contributes to its high fidelity (Kunkel et al., 1987), while purified DNA polymerase α is devoid of this exonuclease activity. If DNA polymerase α replicates one strand and DNA polymerase δ the other, one would predict a disproportionately high number of errors on one DNA strand. It remains to be determined whether or not there are two distinct DNA polymerases at the replication fork in eucaryotes. Our results do not provide evidence for the physical association between DNA polymerase α and DNA polymerase δ. However, if these enzymes are associated during DNA

replication, our results suggest that the proofreading activity associated with DNA polymerase δ might also provide proofreading for DNA polymerase α, so that high-fidelity DNA synthesis might be achieved on both DNA strands. A shared proofreading exonuclease could provide a mechanism to synchronize synthesis on the leading and lagging DNA strands. Pauses by DNA polymerase due to misincorporation would be minimized and would not retard the orderly progression of replication forks.

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