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A 5′ to 3′ exonuclease functionally interacts with calf DNA polymerase ε

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ABSTRACT **Analysis of fractions containing purified DNA polymerase ε from calf thymus has revealed the presence of a 5′ to 3′ exonuclease activity that is specific for a single strand of duplex DNA. This activity is capable of degrading a 3′-labeled oligonucleotide hybridized to M13mp18 DNA. When a second oligonucleotide primer is annealed 3 bases upstream, degradation of the downstream primer is strictly dependent on DNA synthesis from the upstream primer. Replacement of the downstream primer by an oligoribonucleotide of identical sequence results in a similar pattern of exonucleolytic activity. The activity has been highly purified and found to cosediment in glycerol gradients with a peptide of 56 kDa as judged by SDS/PAGE analysis. Effects of calf DNA polymerase α and δ on exonuclease activity are also observed but with differences in the pattern of products.**

We have recently demonstrated the presence of two forms of DNA polymerase ε in calf thymus extracts, designated ε and ε* (1). Both forms possess intrinsic DNA polymerase and single strand-specific 3′ to 5′ exonuclease activities. DNA polymerases ε and ε* have molecular masses of 210 and 145 kDa, respectively. DNA polymerase ε* can be proteolytically derived from DNA polymerase ε during purification. We considered that this situation might be analogous to DNA polymerase I and the Klenow fragment from *Escherichia coli* (2, 3). In this case, limited proteolysis of DNA polymerase I results in removal of a domain containing the 5′ to 3′ exonuclease. The remaining DNA polymerase retains the capacity to exonucleolytically degrade single-stranded DNA in the 3′ to 5′ direction. However, we (4) and others (5) have examined the single-stranded exonuclease activity of DNA polymerase ε from different sources and found it to demonstrate exclusively 3′ to 5′ directionality.

Recent work on the 5′ to 3′ exonuclease of the DNA polymerase from *Thermus aquaticus* indicated that this activity is specific for one strand of double-stranded DNA (6, 7). Utilizing a template similar to that described by Longley *et al.* (6), we have detected a 5′ to 3′ exonucleolytic activity in fractions of DNA polymerase ε. This activity has been purified to near homogeneity and its molecular mass has been determined to be 56 kDa as judged by SDS/PAGE. Thus, it appears that this activity represents a polypeptide other than polymerase ε. Based on enzymatic and physical properties of the exonuclease, it may be a homolog of similar enzymes found in mouse cells (8) and HeLa cells (9). If so, in addition to the enzymologic similarity with the proteins described in these two reports, we find that the exonuclease specifically interacts with homologous DNA polymerase ε, and not polymerases α and δ, in a two-primer assay described here. Based on these data, we propose a role for the complex of exonuclease and polymerase in DNA metabolism.

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MATERIALS AND METHODS

Materials. Synthetic oligodeoxyribonucleotides were from Genosys (The Woodlands, TX) and were purified by PAGE. The synthetic oligoribonucleotide corresponding to the −20 universal sequencing primer (5′-GUAAAACGACGGCCAGU-3′) was from National Bioscience Incorporated (Minneapolis). All nucleotides were Ultrapure from Pharmacia. The −20 universal sequencing primer (20 pmol) was 5′ phosphorylated with T4 polynucleotide kinase (United States Biochemical) and 1 mM ATP in the buffer supplied by the manufacturer. The exonuclease substrate was created by annealing the 5′-phosphorylated primer to 50 μg of M13mp18 plus-strand DNA at 90°C for 5 min followed by slow cooling to room temperature. $MgCl_2$ was added to 10 mM in addition to 50 μCi of [α-^{32}P]dGTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham). Labeling was accomplished by addition of 5 units of T7 DNA polymerase (Pharmacia), followed by incubation for 30 min at 37°C, which results in incorporation of a single dGMP. Unreacted dNTPs were removed by centrifugation through 0.5-ml spun columns of Sephadex G-50. To complete the substrate, 20 pmol of the upstream primer (5′-GTTTTCCCAGTCACGAC-3′) was added and annealed at 65°C for 5 min, followed again by slow cooling. DNA polymerase ε was purified as described by Siegal *et al.* (1). Calf thymus DNA polymerase α was purified by the method of Nasheuer and Grosse (10). Calf thymus DNA polymerase δ and proliferating cell nuclear antigen (PCNA) were kindly provided by C. K. Tan (University of Miami).

Assays. Exonuclease assays (25 μl) were performed in the following buffer: 60 mM [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane (Bistris), pH 6.5/5 mM $MgCl_2$/30 ng of exonuclease substrate/5% (vol/vol) glycerol/100 μg of bovine serum albumin per ml/5 mM 2-mercaptoethanol. When *E. coli* single-stranded DNA binding protein (SSB) (United States Biochemical) was present, it was used at a 10-fold mass ratio to DNA. In this case, reaction mixtures were preincubated for 5 min at room temperature to allow equilibrium binding. Incubation was for 30 min at 37°C followed by termination with 25 μl of 90% (vol/vol) formamide with bromophenol blue and xylene cyanol dye markers. Reaction products were separated on 12% denaturing polyacrylamide gels cast according to Sambrook *et al.* (11). Quantitation of exonuclease activity was performed as described by Goulian *et al.* (8). One unit equals 1 nmol of dNMP released in 30 min at 37°C. Polymerase assays were performed as described by Crute *et al.* (4).

Quantitation of Relative Amounts of Enzyme and Substrate. Experiments were performed to determine the relative stoi-

Abbreviations: SSB, single-stranded DNA binding protein; SV40, simian virus 40; RF-C, replication factor C; PCNA, proliferating cell nuclear antigen; BuPdGTP, N^2-(*p-n*-butylphenyl)-2′-dGTP.

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chiometry of enzymes and substrate termini. The first reaction assessed the percentage of 3′ termini to which polymerases were bound. It was designed to allow each polymerase to elongate only one primer. To accomplish this, increasing amounts of DNA polymerase ε were prebound to a constant amount of the two-primer substrate in 5 mM $MgCl_2$ for 2 min at 4°C. Reactions were initiated with 50 μM each dATP, dGTP, and dCTP and a minimum 100-fold excess (with respect to 3′-OH) of activated calf thymus DNA prepared according to Joyce (12). Consequently, when each polymerase finishes elongating the primer to which it was originally bound, it becomes sequestered from the two-primer substrate by binding to the calf thymus DNA. Reaction mixtures were incubated for 30 min at 37°C and the products were separated as described above. Less than 5% of the primers were extended, indicating that the substrate was in ≈20-fold stoichiometric excess over polymerase under the experimental conditions (data not shown). A control experiment was performed in the absence of added calf thymus DNA, allowing each polymerase to extend many primers. Under these conditions about half of the primers were extended (data not shown).

Similar experiments were performed utilizing the purified 5′ to 3′ exonuclease in the absence of added calf thymus DNA. Titration with increasing amounts of exonuclease indicated that <25% of the annealed downstream primer experienced any degradation under experimental conditions (data not shown). A proportion of the labeled primer is not susceptible to degradation, even at high levels of exonuclease. We presume this to be labeled primer that dissociated from the template during annealing of the upstream primer. Results presented below demonstrate that only annealed primers are susceptible to degradation.

RESULTS

Functional Interaction of the 5′ to 3′ Exonuclease with DNA Polymerase ε. The substrate used for the exonuclease assay consists of two synthetic primers annealed to M13mp18 plus-strand DNA (Fig. 1). Extension of the downstream primer (the −20 universal sequencing primer) by T7 DNA polymerase in the presence of [α-^{32}P]dGTP results in incorporation of a single labeled dGMP at the 3′ terminus. There is a 3-nucleotide gap between the downstream and the upstream primers. Extension of the upstream primer in the presence of dGTP and TTP would allow synthesis to a position equivalent to 2 nucleotides into the downstream primer. Similarly, inclusion of dATP (along with dGTP and TTP) would allow synthesis equivalent to 6 nucleotides into the downstream primer. Fig. 2*A* demonstrates that fractions of highly purified DNA polymerase ε are capable of degrading the downstream primer a length corresponding to the number of nucleotides incorporated at the upstream primer (Fig. 2*A*, lanes TG and ATG). With no, or limited, synthesis (lanes −, G, T, and A), only limited exonucleolytic degradation occurred. Reactions performed without the upstream primer present resulted in degradation of the downstream primer independent of added dNTPs (data not shown). Control experiments indicated that the substrate was present in at least a 20-fold stoichiometric excess over polymerase ε (see *Materials and Methods*). The fact that a functional dependence is observed under conditions of substrate excess

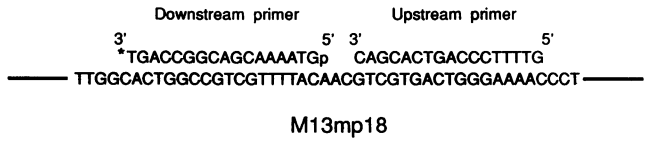

FIG. 1. Exonuclease substrate.

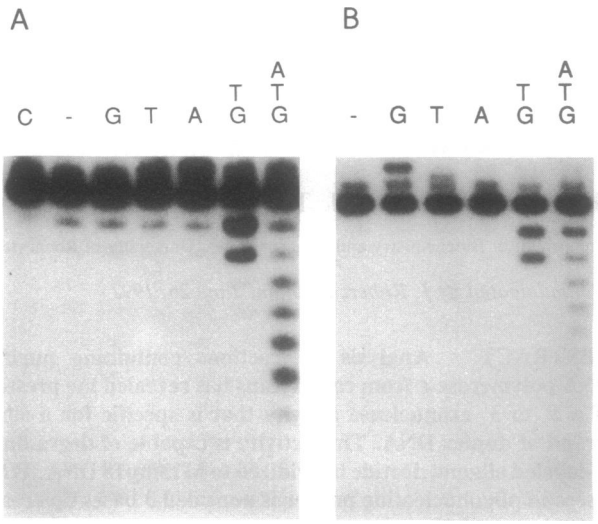

FIG. 2. 5′ to 3′ exonucleolytic degradation of DNA and RNA primers by purified fractions of DNA polymerases ε. Fractions of DNA polymerase ε (0.08 unit) were added to 30 ng of the doubly primed exonuclease substrate constructed with a 3′-labeled downstream DNA primer and saturating amounts of *E. coli* SSB (*A*). The 3′-labeled primer was replaced with an oligoribonucleotide of identical sequence, and polymerase was allowed to react with DNA in the absence of SSB (*B*). dNTPs were added to 50 μM as indicated (C, no enzyme control). Reaction mixtures were incubated at 37°C for 30 min and then separated on 12% acrylamide/urea sequencing gels. Autoradiography was performed at −70°C with Kodak XAR film and a DuPont Cronex Lightning Plus intensifying screen.

suggests that the two activities may associate on the same molecule. When the downstream oligodeoxyribonucleotide is replaced with an oligoribonucleotide of identical sequence, a similar pattern of degradation is observed (Fig. 2*B*).

Effects of Inhibitors of DNA Polymerase ε. Fig. 3*A* demonstrates the effects of various inhibitors of DNA polymerase ε on exonuclease activity. When exonuclease-containing

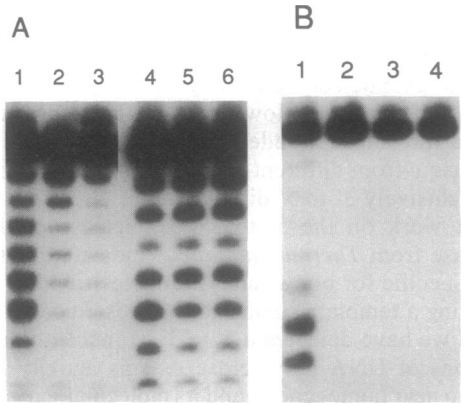

FIG. 3. Effect of polymerase inhibitors and strand specificity of the 5′ to 3′ exonuclease. (*A*) Fractions of DNA polymerase ε (0.08 unit) (lanes 1–3) or isolated exonuclease (0.5 ng; lanes 4–6) were incubated either with no inhibitor (lanes 1 and 4), with 40 μg of aphidicolin per ml (lanes 2 and 5), or with 100 μM BuPdGTP (lanes 3 and 6) in the two-primer assay. All assay mixtures contained dGTP, dATP, and TTP. (*B*) Specificity of the exonuclease for double-stranded DNA was assessed. Lanes: 1, complete reaction mixture plus DNA polymerase ε (0.08 unit); 2–4, substrate was first heat denatured at 95°C for 2 min and immediately placed on ice. A portion of the substrate was then incubated with saturating amounts of *E. coli* SSB for 5 min on ice and then reacted with DNA polymerase ε and the three dNTPs (lane 2), while the remainder of the DNA template reacted directly (lane 3) with polymerase ε without preincubation with SSB. Purified exonuclease (0.5 ng) was incubated with SSB-coated DNA in lane 4.

fractions of polymerase ε were assayed, both aphidicolin and N^2-(*p-n*-butylphenyl)-2′-dGTP (BuPdGTP) significantly inhibited exonucleolytic degradation of the downstream primer (lanes 1–3). When exonuclease that has been purified free of polymerase activity (see below) was assayed, these two polymerase inhibitors had a greatly diminished effect (lanes 4–6). Thus, inhibition of polymerase results in inhibition of the associated exonuclease, providing strong evidence for a direct interaction. Interestingly, addition of 40 μg of aphidicolin per ml resulted in an accumulation of products derived from exonucleolytic degradation of the 5′-terminal 1 or 2 nucleotides of the downstream primer and inhibition of products shortened by 5 or 6 nucleotides (lane 2). This is consistent with the hypothesis of Sabatino *et al.* (13) that aphidicolin sequesters inactivated polymerase ε at the primer/template terminus.

Exonuclease Is Specific for Double-Stranded DNA. The experiments described above did not distinguish whether the exonuclease was acting on the annealed primer or on primer that was displaced by the advancing polymerase. In the latter case, the activity might simply represent a contaminating single-stranded nuclease. To control for this possibility, the substrate was first denatured by heating, followed immediately by cooling on ice. The denatured substrate was divided in two, and either buffer or buffer plus *E. coli* SSB was added to each. As described later, SSB was shown to stimulate activity of the highly purified exonuclease on double-stranded substrates. After a brief incubation on ice, the polymerase fraction was added, along with the three dNTPs, and the reaction mixtures were incubated for 30 min at 37°C. Results indicate that there was no degradation of the single-stranded oligomer occurring in the 5′ to 3′ direction (Fig. 3*B*, lanes 2 and 3 vs. lane 1). Similarly, incubation of highly purified exonuclease (see below) with the denatured substrate coated with SSB (lane 4) resulted in no degradation of the downstream primer (in sharp contrast to results shown in Fig. 5).

Purification of the 5′ to 3′ Exonuclease. Utilizing the substrate with DNA primers described above, we were able to detect 5′ to 3′ exonuclease activity even in highly purified fractions of DNA polymerases ε (i.e., having specific activity for polymerization of 1500 units/mg or greater). To determine whether the exonuclease is intrinsic to the polymerase, both exonuclease and polymerase activity were simultaneously monitored through column chromatography. Chromatography on phosphocellulose, heparin-Sepharose, and Mono Q resins (1) resulted in a small separation, with exonuclease activity trailing polymerase activity by 10% of the gradient volume in each case (data not shown). However, even upon further chromatography of DNA polymerase ε, it was not possible to obtain a polymerase preparation that was entirely devoid of 5′ to 3′ exonuclease activity. Therefore, the alternative approach of purifying the exonuclease was taken. To accomplish this, DNA polymerase ε was prepared through phosphocellulose chromatography by the method described by Siegal *et al.* (1). Upon examination of exonuclease and polymerase activity profiles from this column, we pooled the exonuclease so as to contain a minimum of polymerase activity. This fraction was sedimented through isokinetic glycerol gradients (also described in ref. 1). Glycerol gradient sedimentation yielded a single peak of exonuclease activity, sedimenting at 3.4 S, with a specific activity of 57,600 units/mg (Fig. 4*A*). This fraction contained no detectable polymerase activity. This activity corresponds to a single band that migrates at 56 kDa upon SDS/PAGE analysis (Fig. 4*B*). DNA polymerase ε sedimented as a peak that was entirely separated from the exonuclease (data not shown).

Interaction of Isolated Exonuclease with DNA Polymerases α, δ, and ε. The nearly homogenous exonuclease activity obtained from glycerol gradient sedimentation was assessed

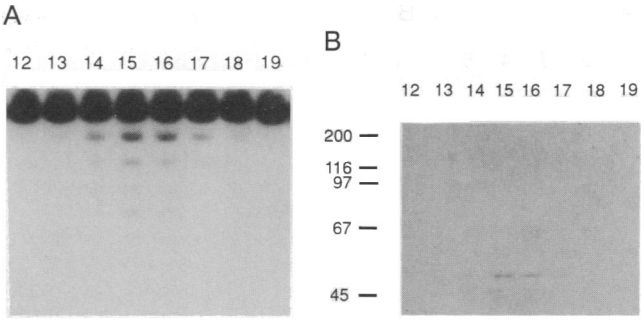

FIG. 4. Glycerol gradient sedimentation of 5′ to 3′ exonuclease. Isokinetic glycerol gradients were formed in SW 50.1 tubes with a 15% concentration of glycerol at the top. Two hundred micrograms of the phosphocellulose fraction of DNA polymerase ε was layered onto the gradients, which were sedimented at 50,000 rpm for 13.6 hr at 4°C. Gradient fractions are numbered from the top of the gradient; 24.5 μl of each was removed for exonuclease assays; 30 ng of the doubly primed exonuclease substrate was added and the reactions were processed as described in Fig. 2. (*A*) Autoradiograph of exonuclease assays performed on gradient fractions. (*B*) SDS/PAGE analysis of gradient fractions. A 10% slab gel was constructed as described (11). Proteins were visualized by Coomassie blue staining. Numbers on left are kDa.

for its ability to function on the doubly primed substrate in the absence of polymerase activity. Fig. 5 demonstrates that addition of increasing amounts of protein resulted in an increase in the amount of primer degraded and that this activity was efficient even in the absence of polymerase. Addition of a 10:1 mass ratio of *E. coli* SSB stimulated the exonuclease (lanes 1–5 vs. lanes 6–10). This stimulation is probably due to sequestration of single-stranded DNA by SSB, thus reducing nonspecific, and therefore unproductive, binding by the exonuclease (14, 15).

Titration of a constant amount of exonuclease with increasing amounts of DNA polymerase ε in the absence of deoxynucleoside triphosphates resulted in a decrease in the products resulting from degradation of the downstream primer (Fig. 6*A*, lanes 1–4). This could be a direct result of the presence of polymerase ε molecules on the primer template. It could also be an indirect effect—e.g., the shorter degradation products may dissociate readily and be preferentially degraded by the 3′ to 5′ exonuclease activity of polymerase ε. Addition of TTP, dGTP, and dATP restored degradation

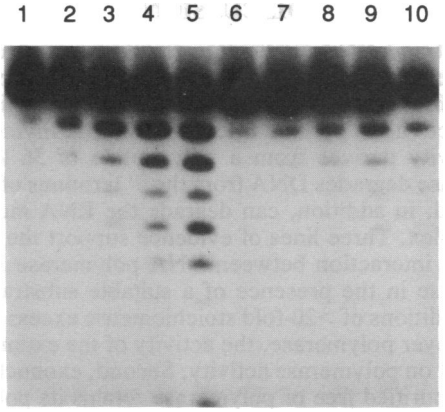

FIG. 5. Isolated 5′ to 3′ exonuclease can degrade DNA in the absence of synthesis. Increasing amounts of exonuclease isolated from glycerol gradients were incubated with SSB-coated (lanes 1–5) or naked (lanes 6–10) double-primed exonuclease template in the absence of deoxynucleotides. Samples were then treated as described in Fig. 2. Amounts of exonuclease are as follows: lanes 1 and 6, no enzyme control; lanes 2 and 7, 0.12 ng; lanes 3 and 8, 0.24 ng; lanes 4 and 9, 0.5 ng; lanes 5 and 10, 1 ng.

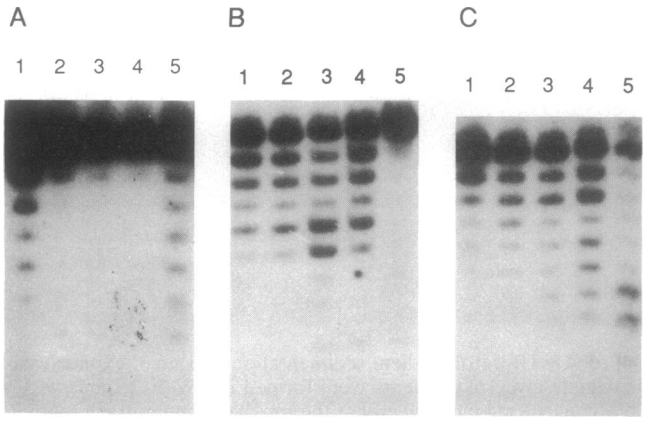

FIG. 6. Interaction of isolated exonuclease with various nuclear DNA polymerases. A constant amount of the 5′ to 3′ exonuclease (0.5 ng; glycerol gradient fraction) and the doubly primed exonuclease substrate were incubated with DNA polymerase ε (*A*), δ (*B*), or α (*C*) in the absence of dNTPs except as indicated. (*A*) Lanes: 1, no polymerase; 2, 0.04 unit of polymerase ε; 3, 0.08 unit of polymerase ε; 4, 0.16 unit of polymerase ε; 5, 0.16 unit of polymerase ε plus TTP, dGTP, and dATP. (*B*) All lanes contain 8 μg of PCNA per ml. Lanes: 1, 0.02 unit of polymerase δ; 2, 0.05 unit of polymerase δ; 3, 0.1 unit of polymerase δ; 4, 0.1 unit of polymerase δ plus the three dNTPs; 5, 0.1 unit of polymerase δ plus the three dNTPs, no added exonuclease. (*C*) Lanes: 1, no polymerase; 2, 0.1 unit of polymerase α; 3, 1.0 unit of polymerase α; 4, 2 units of polymerase α; 5, 2 units of polymerase α plus the three dNTPs.

of the downstream primer (lane 5). The pattern of degradation products was somewhat different with polymerase δ (Fig. 6*B*) and polymerase α (Fig. 6*C*). There is an apparent stimulation of exonuclease activity by polymerase α in the presence of the three dNTPs, but it was less evident with polymerase δ. This could be due in part to differences in polymerase activity. A similar stimulation was observed when either Klenow fragment or T7 polymerase was used to fill the 3-nucleotide gap between the primers (data not shown). It is not clear whether stimulation of exonuclease activity with the other polymerases is occurring by the same mechanism as with polymerase ε. For example, stimulation may result from generation of a better nuclease substrate through polymerization. In this case, differences observed among polymerases may reflect the capacity of each enzyme to fill the 3-nucleotide gap.

DISCUSSION

Lagging-strand DNA replication requires a nucleolytic activity to remove ribonucleotide primers that initiate Okazaki fragment formation. We have demonstrated the existence of such an activity in purified fractions from calf thymus tissue. This activity derives from a polypeptide of 56 kDa. The exonuclease degrades DNA from the 5′ terminus of a duplex region and, in addition, can degrade the RNA moiety of a heteroduplex. Three lines of evidence support the model of functional interaction between DNA polymerase ε and the exonuclease in the presence of a suitable substrate. First, under conditions of >20-fold stoichiometric excess of primer template over polymerase, the activity of the exonuclease is dependent on polymerase activity. Second, exonuclease that has been purified free of polymerase retains its polymerization dependence when purified polymerase ε is added back in stoichiometric quantities. Third, addition of DNA polymerase-specific inhibitors results in simultaneous inhibition of exonuclease activity only when polymerase is present.

The simian virus 40 (SV40) DNA replication system *in vitro* has been of great utility in understanding the enzymology of the eukaryotic replication fork. Results suggest that DNA polymerase δ is responsible for leading-strand synthesis primed by DNA polymerase α (16, 17). Okazaki fragments deriving from the lagging strand are primed and synthesized by DNA polymerase α (18–20).

Prokaryotic models have demonstrated that, even for bacteriophage species that are entirely dependent on activities supplied by the host for replication, only a subset of the host replication machinery may be used (21). We suggest that this may be happening during replication of SV40 *in vitro*. In fact, two recent reports suggest that more than one DNA polymerase is required to complete lagging-strand DNA synthesis in eukaryotic cells (22, 23). In addition, Morrison *et al.* (24) demonstrated that the gene encoding the yeast analog of polymerase ε, *POL2*, is essential for cell viability and that *pol2* haploids displayed a terminal morphology indicative of incomplete DNA replication, providing further evidence that polymerase ε is playing a role in DNA replication. This result prompted us to suggest that polymerase ε is responsible for completion of lagging-strand synthesis (25).

Ishimi *et al.* (9) purified a 5′ to 3′ exonuclease from HeLa cells that is required for formation of form I DNA in the SV40 system. This 44-kDa enzyme appears to be the human analog of the enzyme described here. When isolated from DNA polymerase ε, the enzymes from calf thymus and that from HeLa cells are identical enzymatically in terms of duplex specificity and preference for RNA. Evidently, the purification protocol of Ishimi *et al.* (9) results in early removal of polymerase ε. This probably occurs during gel filtration on Sephadex G-100. Recent work (26) has demonstrated that the addition of human polymerase ε, in conjunction with replication factor C (RF-C) and PCNA, to the SV40 system resulted in elongation of lagging-strand products. Our data indicate that the polymerase ε–5′ to 3′ exonuclease complex is capable of performing this extension and removing the initiator RNA from the downstream Okazaki fragment.

Goulian *et al.* (8) have described a system specifically designed to model lagging-strand synthesis. Use of this system with purified mouse cell enzymes has also identified a 5′ to 3′ exonuclease of ≈50 kDa that is required to remove initiator RNA from Okazaki fragments. The isolated exonuclease appears functionally and physically similar to the enzyme purified from calf thymus tissue. The authors have not investigated the properties of DNA polymerase ε in this system.

Tsurimoto *et al.* (20) suggest that DNA polymerase α initiates leading-strand synthesis in the SV40 system. At some point early in replication, RF-C and PCNA displace polymerase α at the 3′ terminus of the leading strand. This might easily occur due to the limited processivity of polymerase α under these conditions (27). We propose that a similar event occurs on the lagging strand, in which DNA polymerase α–primase, after synthesizing a short stretch of DNA, is displaced by RF-C and PCNA at the 3′ terminus. Next, complex formation occurs between RF-C/PCNA and polymerase ε–5′ to 3′ exonuclease. There is already evidence that RF-C/PCNA stabilizes polymerase ε binding to DNA (26). DNA synthesis is continued for ≈150 nucleotides and upon encounter of the downstream initiator RNA, polymerase ε–5′ to 3′ exonuclease performs a nick-translation reaction to remove the heteroduplex.

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1. Siegal, G., Turchi, J. J., Jessee, C. B., Mallaber, L. M., Bambara, R. A. & Myers, T. W. (1992) *J. Biol. Chem.* **267**, 3991–3999.

2. Klenow, H. & Henningsen, I. (1970) *Proc. Natl. Acad. Sci. USA* **65**, 168–175.
3. Brutlag, D., Atkinson, M. R., Setlow, P. & Kornberg, A. (1969) *Biochem. Biophys. Res. Commun.* **37**, 982–989.
4. Crute, J. J., Wahl, A. F. & Bambara, R. A. (1986) *Biochemistry* **25**, 26–36.
5. Syvaoja, J. & Linn, S. (1989) *J. Biol. Chem.* **264**, 2489–2497.
6. Longley, M. J., Bennett, S. E. & Mosbaugh, D. W. (1990) *Nucleic Acids Res.* **18**, 7317–7322.
7. Holland, P. M., Abramson, R. D., Watson, R. & Gelfand, D. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7276–7280.
8. Goulian, M., Richards, S. H., Heard, C. J. & Bigsby, B. M. (1990) *J. Biol. Chem.* **265**, 18461–18471.
9. Ishimi, Y., Claude, A., Bullock, P. & Hurwitz, J. (1988) *J. Biol. Chem.* **263**, 19723–19733.
10. Nasheuer, H. P. & Grosse, F. (1988) *J. Biol. Chem.* **263**, 8981–8988.
11. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
12. Joyce, C. M. (1989) *J. Biol. Chem.* **264**, 10858–10866.
13. Sabatino, R. D., Myers, T. W. & Bambara, R. A. (1990) *Cancer Res.* **50**, 5340–5344.
14. Sapp, M., König, H., Riedel, H. D., Richter, A. & Knippers, R. (1985) *J. Biol. Chem.* **260**, 1550–1556.
15. Myers, T. W. & Romano, L. J. (1988) *J. Biol. Chem.* **263**, 17006–17015.
16. Prelich, G. & Stillman, B. (1988) *Cell* **53**, 117–126.
17. Weinberg, D. H. & Kelly, T. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9742–9746.
18. Lee, S. H., Eki, T. & Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7361–7365.
19. Eki, T. & Hurwitz, J. (1991) *J. Biol. Chem.* **266**, 3087–3100.
20. Tsurimoto, T., Melendy, T. & Stillman, B. (1990) *Nature (London)* **346**, 534–539.
21. Kornberg, A. & Baker, T. (1991) *DNA Replication* (Freeman, New York), pp. 553–636.
22. Bullock, P. A., Seo, Y. S. & Hurwitz, J. (1991) *Mol. Cell. Biol.* **11**, 2350–2361.
23. Nethanel, T. & Kaufmann, G. (1990) *J. Virol.* **64**, 5912–5918.
24. Morrison, A., Araki, H., Clark, A. B., Hamatake, R. K. & Sugino, A. (1990) *Cell* **62**, 1143–1151.
25. Bambara, R. A. & Jessee, C. B. (1991) *Biochim. Biophys. Acta* **1088**, 11–24.
26. Lee, S. H., Pan, Z. Q., Kwong, A. D., Burgers, P. M. & Hurwitz, J. (1991) *J. Biol. Chem.* **266**, 22707–22717.
27. Sabatino, R. D., Myers, T. W., Bambara, R. A., Kwon-Shin, O., Marraccino, R. L. & Frickey, P. H. (1988) *Biochemistry* **27**, 2998–3004.