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# DNA primase activity associated with DNA polymerase α from *Xenopus laevis* ovaries

(RNA priming/DNA chain elongation/NTP-dependent DNA synthesis/gel electrophoresis)

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ABSTRACT **One of the two forms of DNA polymerase α from ovaries of the frog *Xenopus laevis* catalyzed ribonucleoside triphosphate-dependent DNA synthesis on single-stranded circular fd phage DNA templates. DNA synthesis was dependent on ATP and added template. CTP, GTP, and UTP stimulated DNA synthesis but were not required and could not substitute for ATP. DNA synthesis was not inhibited by α-amanitin. Neither poly(dT) nor double-stranded DNA served as template. Analysis of [$^{32}$P]-dTMP-labeled product by neutral and alkaline agarose gel electrophoresis showed that 0.1- to 1-kilobase DNA fragments (average size of ≈0.25 kilobase) were synthesized. The fragments were not covalently linked to the template. Either [α-$^{32}$P]NMP, [γ-$^{32}$P]ATP, or [γ-$^{32}$P]GTP were incorporated also into the product. Analysis of the product after hydrolysis by KOH, alkaline phosphatase, or bacteriophage T4 3′ → 5′ exonuclease showed the presence of a small oligoribonucleotide primer at the 5′ end of the newly synthesized DNA. NTP-dependent DNA-synthesizing activity copurified on six columns and cosedimented during glycerol gradient centrifugation with one form of DNA polymerase α activity but not with the other form. These results suggest that DNA primase activity is associated with one of the two forms of *X. laevis* DNA polymerase α.**

Neither prokaryotic nor eukaryotic DNA polymerases have been reported to catalyze *de novo* DNA synthesis (1). In prokaryotes an enzyme known as DNA primase has been shown to synthesize small oligoribonucleotide primers that normally are extended by DNA polymerase to form Okazaki fragments (2). Small stretches of primer RNA also have been reported at the 5′ end of Okazaki fragments in eukaryotes (3–8). DNA primase activity has been detected in nuclei isolated from polyoma-infected 3T6 cells (9), but this activity has not been further characterized.

Several very recent reports have suggested that DNA primase activity may be associated with DNA polymerase α activities. Association of an RNA polymerase activity that prefers $Mn^{2+}$ with partially purified DNA polymerase α activity has been reported in the sea urchin (10). A novel RNA polymerase activity that was stimulated by a specific protein factor has been reported to be associated with a form of Ehrlich ascites DNA polymerase α that was resolved by DEAE-cellulose column chromatography (11). Conaway and Lehman (12) also have described a DNA primase activity that appeared to copurify with a DNA polymerase α activity from *Drosophila* embryos.

Benbow *et al.* (13) have described an *in vitro* DNA replication system prepared from unfertilized eggs of the frog *Xenopus laevis*. This system catalyzed efficient initiation of DNA replication on double-stranded DNA templates. DNA polymerases $\alpha_1$, $\alpha_2$, β, and γ have been identified in the active fractions of this system (14–16). During an extensive study of primer template preferences of the two DNA polymerase α activities, we noted that one activity catalyzed ribonucleotide-dependent DNA synthesis on single-stranded circular DNA templates. In this paper we document the unusual finding that DNA primase activity copurifies with only one of the two forms of *X. laevis* ovarian DNA polymerase α activity.

## MATERIALS AND METHODS

**Materials.** *X. laevis* were obtained from the South African Snake Farm (Cape Providence, South Africa). Unlabeled deoxynucleoside triphosphates, ribonucleoside triphosphates, poly(dT), and oligo(rA)$_{10}$ were purchased from P-L Biochemicals. All radioactive precursors were obtained from ICN. α-Amanitin was from Sigma. Bacterial alkaline phosphatase, restriction endonuclease *Taq* I, and double-stranded φX174 DNA were from Bethesda Research Laboratories. Homogeneous bacteriophage T4 DNA polymerase with an associated 3′ → 5′ exonuclease activity (17) was a gift of M. J. Bessman (The Johns Hopkins University). Calf thymus DNA was obtained from Sigma and activated as described (16). fd106 and fd107 phage DNA were prepared according to Herrmann *et al.* (18) and further purified by sucrose gradient centrifugation (19).

**Purification of DNA Polymerase α Activities.** DNA polymerase α activity was prepared and separated as two forms by DEAE-cellulose column chromatography as described (14, 16). DNA polymerases $\alpha_1$ and $\alpha_2$ were further chromatographed by using the columns described by Joenje and Benbow (15), pooling each α peak separately. This procedure will be described in more detail elsewhere (20). Briefly, DNA polymerases $\alpha_1$ and $\alpha_2$ were eluted at 0.5 M Tris·HCl on carboxymethyl-Sephadex, at 65-Å Stokes radius on Sephadex G-200, at 60 mM NaCl on single-stranded DNA agarose, at 50 mM potassium phosphate on hydroxylapatite, and at 300 mM KCl on phosphocellulose (fraction X). The most purified DNA polymerase $\alpha_1$ had a specific activity of 50,000 units/mg of protein, and polymerase $\alpha_2$ had a specific activity of 75,000 units/mg of protein, where 1 unit was defined as 1 nmol of dAMP incorporated per 60 min at 37°C, with poly(dT)·oligo(rA)$_{10}$ as template primer, and where protein was measured by the assay of Lowry *et al.* (21). Specific activities based on Coomassie brilliant blue staining gave considerably higher specific activities. The specific activities of the most highly purified *X. laevis* DNA polymerase α activities were comparable to the highest specific activities for other eukaryotic DNA polymerase α activities (22–24). Both DNA polymerase $\alpha_1$ and $\alpha_2$ migrated as single peaks on nondenaturing gels. DNA primase activity was detected at all stages of DNA polymerase $\alpha_1$ purification after the initial separation from DNA polymerase $\alpha_2$. All DNA primase assays in this paper

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Abbreviation: kb, kilobase(s).

were carried out with 2.8 units of fraction X DNA polymerase $\alpha_1$ unless otherwise stated.

**Assay of NTP-Dependent DNA Synthesis.** The reaction mixture contained 25 mM Tris·HCl (pH 7.5), each dNTP (dATP, dCTP, dGTP, and dTTP) at 50 $\mu$M, 10 mM $MgCl_2$, 0.25 mM EDTA, 5 mM 2-mercaptoethanol, bovine serum albumin (200 $\mu$g/ml), 20% glycerol, 1.0 $\mu$g of fd107 phage single-stranded circular DNA (unless otherwise stated), 4 $\mu$Ci (1 Ci = $3.7 \times 10^{10}$ becquerels) of [$\alpha$-$^{32}$P]dTTP (4,000 cpm/pmol), 1 mM ATP, and CTP, GTP, and UTP each at 500 $\mu$M (unless otherwise stated) in a total volume of 50 $\mu$l. By using these conditions, template single-stranded DNA is in great excess. Equal DNA synthesis was also obtained by using 1/100th the amount of DNA (0.01 $\mu$g) per assay (unpublished data). After incubation at 30°C for 60 min, 25 $\mu$l of 2 mM dTTP/20 mM ATP/50 mM EDTA/20 mM *N*-ethylmaleimide and 200 $\mu$l containing 100 $\mu$g of calf thymus DNA were added, followed by 500 $\mu$l of 10% (wt/vol) trichloroacetic acid containing 1% sodium pyrophosphate. After 10 min at 0°C, acid-insoluble radioactivity was determined (16). NTP-independent DNA synthesis was measured by using the above assay condition but omitting ATP, CTP, GTP, and UTP.

**Incorporation of [$^{32}$P]NTP into Reaction Product.** Reactions were incubated as above except that [$\alpha$-$^{32}$P]dTTP was replaced by 20 $\mu$Ci of [$^3$H]dTTP (3,000 cpm/pmol) and 1 mM ATP was used with CTP, GTP, and UTP each at 200 $\mu$M and 25 $\mu$Ci of [$\alpha$-$^{32}$P]ATP, [$\alpha$-$^{32}$P]CTP, [$\alpha$-$^{32}$P]GTP, and [$\alpha$-$^{32}$P]UTP (17,300 cpm/pmol of NTP). In [$\gamma$-$^{32}$P]NTP incorporation, 1 mM ATP, 200 $\mu$M GTP, 100 $\mu$Ci of [$\gamma$-$^{32}$P]ATP, and 100 $\mu$Ci of [$\gamma$-$^{32}$P]-GTP (26,400 cpm/mol of ATP plus GTP) were used. After incubation, 10 $\mu$l of 250 mM EDTA, 5 $\mu$l of 200 mM *N*-ethylmaleimide, 2.5 $\mu$l of 10% sodium dodecyl sulfate, 10 $\mu$l of 5 mM dTTP, and 5 $\mu$l each of 100 mM ATP, CTP, GTP, and UTP were added, and the reaction product was dialyzed against 50 mM Tris·HCl, pH 7.5/200 mM NaCl/10 mM EDTA. The dialysate was extracted with phenol, followed by ethanol precipitation after adding 2 $\mu$g of fd107 phage single-stranded DNA. The precipitated DNA was washed three times with ice-cold 70% ethanol. After being dried, the $^{32}$P-containing precipitate was suspended in 25 mM Tris·HCl, pH 8.0/2 mM EDTA for further analysis.

**Gel Electrophoresis.** Gel electrophoresis was performed on a 1.0% agarose gel at neutral pH (25), on a 0.8% agarose gel at alkaline pH (26) or on a 12% polyacrylamide gel containing 7 M urea (27) as described. $^{32}$P-labeled or unlabeled DNA length markers were prepared from *Taq* I restriction fragments of $\phi$X174 double-stranded DNA (28).

## RESULTS

**NTP-Dependent DNA Synthesis.** When *X. laevis* ovarian DNA polymerase $\alpha_1$ was incubated with unprimed single-stranded circular fd106 or fd107 phage DNA under the DNA synthesis conditions described in *Materials and Methods*, DNA synthesis was stimulated >25-fold by the addition of NTPs (Table 1). No synthesis or stimulation was observed with poly(dT) or with double-stranded fd106 or fd107 DNA. Synthesis on primed DNA templates [poly(dT)·oligo(rA) or activated DNA] was depressed rather than stimulated by the addition of NTPs.

Synthesis with single-stranded circular fd107 DNA was dependent upon ATP (Table 2). CTP, GTP, or UTP were unable to substitute for this requirement, either singly or in combination. CTP, GTP, and UTP further stimulated ATP-dependent DNA synthesis. Maximal DNA synthesis was observed with all four NTPs and was not significantly inhibited by high concentrations of $\alpha$-amanitin. The $K_m$ for ATP was ≈500 $\mu$M (ATP only). The $K_m$ values for CTP, GTP, or UTP were ≈100–150 $\mu$M in the presence of 1 mM ATP. From the results in this section, we conclude that *X. laevis* ovarian DNA polymerase $\alpha_1$ catalyzed ribonucleotide-dependent DNA synthesis on unprimed DNA templates.

Table 1. Template specificity of NTP-dependent DNA synthesis

| Template, 1 $\mu$g | dNMP incorporated, pmol | |
|---|---|---|
| | No NTP | With NTP |
| None | 0.1 | 0.1 |
| fd106 ss DNA | 0.3 | 9.5* |
| fd107 ss DNA | 0.8 | 21 |
| fd106 RF DNA | 0.6 | 0.6 |
| fd107 RF DNA | 0.5 | 0.9‡ |
| Poly(dT)·oligo(rA)$_{10}$† | 2,800 | 950 |
| Poly(dT) | 0.4 | 0.4 |
| Calf thymus DNA | | |
| Activated | 160 | 120‡ |
| Native | 17 | 11 |

ss, Single-stranded; RF, replicative form I (supercoiled double-stranded DNA).
* Measured in the presence of 750 $\mu$M ATP; no CTP, GTP, or UTP were present.
† Instead of [$^{32}$P]dTTP, [$^3$H]dATP (1,500 cpm/pmol) was used.
‡ NTP concentration may regulate the choice between NTP-dependent priming of DNA synthesis and extension of preexisting DNA primers.

**Analysis of Newly Synthesized DNA by Agarose Gel Electrophoresis.** NTP-dependent DNA synthesis was carried out with fd107 phage single-stranded [$^3$H]DNA as template and [$^{32}$P]dTTP as precursor. The reaction products were analyzed by agarose gel electrophoresis (Fig. 1). In neutral gels, newly synthesized DNA (either NTP-independent or NTP-dependent synthesis) migrated between single-stranded DNA and nicked double-stranded circular DNA (Fig. 1 *A* and *B*). During longer (120 min) incubations, the $^3$H-labeled template was shifted toward the position of the double-stranded marker (data not shown). In alkaline gels, >98% of the input [$^3$H]DNA comigrated with fd107 single-stranded DNA markers (Fig. 1 *C* and *D*), suggesting that little if any template degradation occurred during incubation. The $^{32}$P-labeled product of NTP-independent DNA synthesis migrated close to linear single-stranded fd107 DNA (Fig. 1*C*). By contrast, 87% of the NTP-dependent reaction product migrated as small fragments of 0.1–1 kilobase (kb) in length with a mean length of ≈0.25 kb. Only 9% of the

Table 2. NTP-dependence of DNA synthesis

| Conditions | dNMP incorporated, pmol |
|---|---|
| Lacking enzyme | 0.2 |
| No NTP added | 1.1 |
| ATP (1 mM) | 5.1 |
| ATP (2 mM) | 5.0 |
| CTP (1 mM) | 1.5 |
| GTP (1 mM) | 1.4 |
| UTP (1 mM) | 1.6 |
| ATP (1 mM), CTP (500 $\mu$M) | 8.5 |
| ATP (1 mM), GTP (500 $\mu$M) | 8.4 |
| ATP (1 mM), UTP (500 $\mu$M) | 9.5 |
| CTP, GTP, UTP (500 $\mu$M each) | 1.4 |
| ATP (1 mM), CTP, GTP, UTP (500 $\mu$M each) | 18.0 |
| ATP (1 mM), CTP, GTP, UTP (500 $\mu$M each) + $\alpha$-amanitin, 240 $\mu$g/ml | 15.2 |

Assays were done as described in *Materials and Methods* except that the concentrations of NTPs were as indicated.

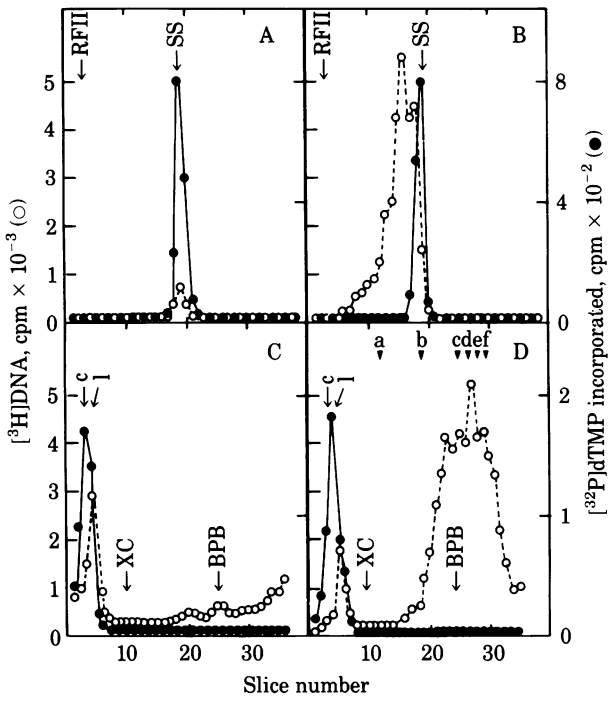


FIG. 1. Analysis of the reaction product by agarose gel electrophoresis. (*A* and *C*) DNA synthesis without NTP. (*B* and *D*) DNA synthesis with NTP. DNA polymerase $\alpha_1$ was incubated as described in the assay of NTP-dependent DNA synthesis except that template DNA was replaced by 1.5 μg of fd107 single-stranded [³H]DNA. After incubation and addition of a 10-μl solution of 6 mM dTTP/20 mM ATP/100 mM EDTA/60 mM *N*-ethylmaleimide/2% sodium dodecyl sulfate, samples were dialyzed against 25 mM Tris·HCl, pH 8.0/10 mM EDTA/10% glycerol. For neutral agarose gel electrophoresis (*A* and *B*), 1 μl of 2% bromophenol blue (BPB) was added, and the samples were applied onto a 1.0% agarose gel containing 40 mM Tris·HCl (pH 8.0), 80 mM sodium acetate, and 2 mM EDTA (25) and were electrophoresed at 50 V/14 cm for 12 hr. After being stained with ethidium bromide (5 μg/ml) and photographed, the gel lanes were sliced to 3.0-mm pieces, which were heated at 80°C for 10 min with 0.5 ml of 3.5% perchloric acid, and the radioactivity was determined by liquid scintillation spectrometry. For alkaline agarose gel electrophoresis (*C* and *D*), 1 μl of 2% bromophenol blue (BPB)/xylene cyanol (XC) and 5 μl of 4.5 M NaOH were added to samples, which were then applied on a 0.8% agarose gel containing 30 mM NaOH and 1 mM EDTA as described by Ikeda *et al.* (26). Electrophoresis was at 42 V/14 cm for 3 hr and 40 min. Radioactivities were measured as described above. RF-II, ss, c, and l, positions of fd107 phage replicative form II (nicked double-stranded circular DNA), single-stranded DNA, single-stranded circular DNA, and single-stranded linear DNA, respectively. a–f, Migration positions of the DNA length markers, which correspond to 2,916, 1,177, 406, 329, 233, and 143 nucleotides. Slice number 1 corresponds to the origin.

newly synthesized DNA migrated between 1 and 10 kb, and <4% migrated near the fd107 single-stranded linear [³H]DNA (Fig. 1*D*). From the results in this section, we conclude that the newly synthesized DNA was associated (presumably hydrogen-bonded) with the template DNA but was not covalently linked to the template DNA.

**Incorporation of [α-³²P]NMP or [γ-³²P]NTP into the Reaction Product.** To establish the role of the ribonucleotides in the NTP-dependent DNA polymerase $\alpha_1$-catalyzed DNA synthesis, the reaction products of NTP-dependent incorporation of either [α-³²P]NTPs or [γ-³²P]NTPs and [³H]dTTP with fd107 single-stranded DNA templates were isolated. By assuming that all four [α-³²P]ribonucleotides were incorporated with equal efficiencies, 0.49 pmol of NMPs were incorporated in a reaction involving the incorporation of 18 pmol of dNMP. The $K_m$ for GTP in the presence of 1 mM ATP was ≈100 μM. When [γ-³²P]ATP and [γ-³²P]GTP were used as precursors, 0.12 pmol of NTP was incorporated during the incorporation of 19 pmol of dNMP. Treatment with 0.3 M KOH for 18 hr degraded 90% of the α-³²P-labeled product and 96% of the γ-³²P-labeled material but <10% of the ³H-labeled material (Table 3). Only 14% of the α-³²P-labeled product was susceptible to degradation by alkaline phosphatase, whereas 87% of the γ-³²P-labeled material was released. Digestion of the reaction product with bacteriophage T4 3′ → 5′ exonuclease released only 16% of the α-³²P radioactivity but 97% of the ³H radioactivity. These results suggest that ribonucleotides were found at the 5′ end of the product, that most ribonucleotides were joined to other ribonucleotides, and that most of the deoxyribonucleotides were linked in extensive regions at the 3′ end of the product.

**Analysis of [α-³²P]NMP-Labeled Product After Digestion with Bacteriophage T4 3′ → 5′ Exonuclease.** To establish the size of the ribonucleotide region at the 5′ end of the product, [α-³²P]NMP- and [³H]dTMP-labeled reaction product was digested with T4 3′ → 5′ exonuclease for 23 hr at 37°C, by which time release of ³H had leveled off (Table 3). The reaction products before and after digestion were subjected to electrophoresis on 7 M urea/12% polyacrylamide gels (Fig. 2). Analysis of undigested reaction product showed that ³²P was heterogeneously distributed in large (>0.1 kb) fragments (Fig. 2, lane A). Mock digestions also were carried out to ensure that the DNA was not degraded during the extended incubations at 37°C. Analysis of the digested product showed that most of the ³²P was localized in a discrete band that migrated to a position calculated (27) to correspond to a fragment of about 10 nucleo-

Table 3. Analysis of [³²P]NMP- and [³H]dNMP-labeled product by hydrolysis

| Treatment | Incorporated radioactivity,* % | | | |
|---|---|---|---|---|
| | [α-³²P]NMP product | | [γ-³²P]NTP product | |
| | ³H | ³²P | ³H | ³²P |
| KOH | 90 | 10 | 90 | 4 |
| Alkaline phosphatase | 95 | 86 | 92 | 13 |
| T4 3′ → 5′ exonuclease | 3 | 84 | — | — |

An aliquot of [α-³²P]NMP- and [³H]dTMP-labeled product or [γ-³²P]NTP- and [³H]dTMP-labeled product was treated as indicated. In KOH treatment, a 10-μl aliquot was treated with 10 μl of 0.6 M KOH and incubated at 37°C for 18 hr. After incubation, 6 μl of 1.0 M HCl was added, followed by addition of 30 μl of carrier solution containing 30 mM EDTA, 0.3 M ammonium formate, 10 mM sodium pyrophosphate, and ATP, CTP, GTP, UTP, and dTTP each at 2 mM. The sample solution was applied to DE81 filters, and radioactivity was determined after washing as described by Rowen and Kornberg (29). In alkaline phosphatase treatment, the product was incubated at 37°C in a 30-μl reaction mixture that contained 25 mM tRNA (Sigma), 200 μM poly(dT), 50 μM oligo(rA)$_{10}$, and 100 units of bacterial alkaline phosphatase (1 unit of enzyme hydrolyzes 1 nmol of ATP in 30 min at 37°C). After 60 min, 5-μl reaction samples were assayed as in the KOH treatment after addition of the carrier solution. The reaction reached equilibrium at 30 min after incubation. In exonuclease treatment, after denaturation at 90°C for 5 min, product was incubated at 37°C in a 30-μl reaction mixture that contained 25 mM Tris·HCl (pH 8.8), 8 mM $MgCl_2$, 5 mM 2-mercaptoethanol, bovine serum albumin (160 μg/ml), 10% glycerol, 40 μg of yeast tRNA, and 0.002 unit of T4 3′ → 5′ exonuclease (1 unit of the enzyme produces 40 nmol of dNMP from single-stranded DNA at 37°C for 30 min). After 23 hr, 10-μl reaction samples were assayed as in the KOH treatment after addition of the carrier solution. Hydrolysis of the reaction product by T4 3′ → 5′ exonuclease was essentially complete at 20 hr after incubation.

* Compared to incorporated radioactivity before treatment as 100%: in α-³²P-labeled product, ³H = 9,700 cpm and ³²P = 1,500 cpm; in γ-³²P-labeled product, ³H = 3,600 cpm and ³²P = 2,000 cpm.

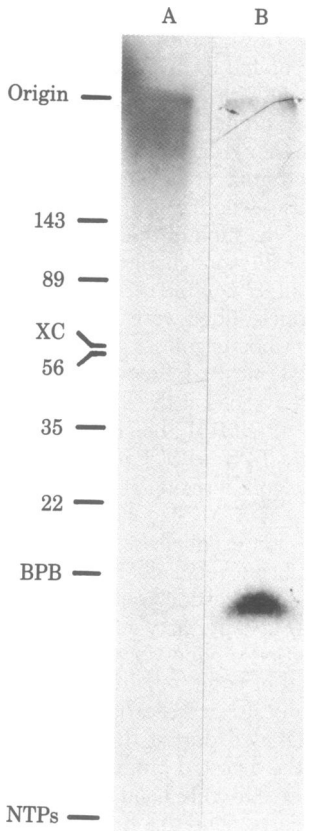


FIG. 2. Analysis of [$^{32}$P]NMP-labeled product by electrophoresis on a 7 M urea/12% polyacrylamide gel. Lanes: A, undigested [$^{32}$P]-NMP-labeled product; B, T4 phage 3′ → 5′ exonuclease-digested [$^{32}$P]NMP-labeled product. [$^{32}$P]NMP- and $^{3}$H-labeled product was prepared and digested with T4 exonuclease as described in Table 3. After addition of EDTA (20 mM), aliquots (3 μl) of the undigested and digested product, which contained 300 and 500 cpm of $^{32}$P, respectively, were concentrated by lyophilization and added to 25 μl of formamide with 2 μl each of 0.5% bromophenol blue (BPB) and xylene cyanol (XC). After heating at 65°C for 5 min, samples were applied to a 12% polyacrylamide gel containing 7 M urea as described by Maniatis *et al.* (27). Electrophoresis was at 140 V/14 cm for 2 hr. $^{32}$P-Labeled *Taq* I-digested φX174 DNA and [$^{32}$P]ATP, [$^{32}$P]CTP, [$^{32}$P]GTP, and [$^{32}$P]UTP (NTPs) were run as markers. After electrophoresis, the gel was subjected to autoradiography by using an intensifying screen. DNA size markers are shown in a parallel lane in nucleotides.

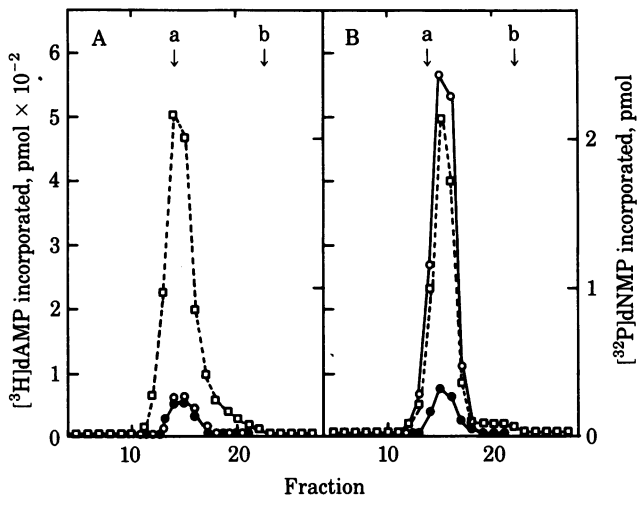


FIG. 3. NTP-dependent DNA synthesis associated with DNA polymerase $\alpha_1$. (*A*) DNA polymerase $\alpha_2$. (*B*) DNA polymerase $\alpha_1$. □, DNA polymerase α activity; ○ and ●, NTP-dependent DNA synthesis with (○) and without (●) NTP. DNA polymerase $\alpha_1$ and $\alpha_2$ (0.5 ml) was applied on an 11.5 ml of 10–30% glycerol gradient containing 50 mM Tris·HCl (pH 7.5), 200 mM KCl, 0.1 mM EDTA, 2 mM 2-mercaptoethanol, and 0.1 mM benzamidine·HCl and was centrifuged at 38,000 rpm for 40 hr in a SW 41 rotor. After centrifugation and fractionation (0.33 ml each; 35 fractions), 5-μl aliquots were assayed for NTP-dependent DNA synthesis. DNA polymerase α activity was measured as in NTP-dependent DNA synthesis without NTP except that DNA and [$^{32}$P]dTTP were replaced by 1.0 μg of poly(dT)·oligo(rA)$_{10}$ and [$^{3}$H]dATP (1,500 cpm/pmol), respectively. Arrows a and b, positions of rabbit muscle aldolase (7.4 S) and bovine serum albumin (4.4 S), respectively, which were centrifuged in parallel gradients. Sedimentation was from right to left.

tides in length. We conclude that oligoribonucleotides of about 10 nucleotides in length served as primers for DNA polymerase $\alpha_1$-catalyzed DNA synthesis.

**Copurification of NTP-Dependent DNA-Synthesizing Activity with DNA Polymerase $\alpha_1$.** During early steps in the purification of the two *X. laevis* DNA polymerases (15, 16), high levels of NTP-independent DNA synthesis (presumably resulting from contaminating nucleases) interfered with precise measurement of NTP-dependent DNA-synthesizing activity. Nevertheless, NTP-dependent stimulation of DNA synthesis was observed coincident only with DNA polymerase $\alpha_1$ activity after DEAE-cellulose column chromatography (14–16). NTP-dependent synthesis was not observed with $\alpha_2$ activity or in other fractions through subsequent purification steps. As shown by others (12), NTP-dependent stimulation of DNA synthesis increased with progressive purification (from 1.5-fold in the $\alpha_1$ fraction after the first DEAE-cellulose chromatography to 25-fold in fraction X). During glycerol gradient centrifugation, NTP-dependent DNA-synthesizing activity precisely cosedimented with highly purified DNA polymerase $\alpha_1$ (6.5 S), but no NTP-dependent DNA-synthesizing activity was detected with DNA polymerase $\alpha_2$ (Fig. 3). To further confirm copurification, the assay for NTP-dependent DNA synthesis was carried out after addition of 2.8 units of DNA polymerase $\alpha_2$ activity to small aliquots of gradient fractions in DNA polymerase $\alpha_1$ (which lacked sufficient DNA polymerase $\alpha_1$ activity to catalyze detectable NTP-dependent DNA synthesis in the 10-min assay period). NTP-dependent DNA-synthesizing activity was again observed coincident with the peak of DNA polymerase $\alpha_1$ activity. This suggests that the primers formed by DNA polymerase $\alpha_1$ can be utilized by exogenously added DNA polymerase $\alpha_2$.

## DISCUSSION

Only one of the two forms of *X. laevis* ovarian DNA polymerase α that have been resolved by chromatography on DEAE-cellulose (16) catalyzed NTP-dependent DNA synthesis on circular single-stranded DNA templates in the presence of ATP (Tables 1 and 2; Figs. 1 and 3). Ribonucleotides also were incorporated into the reaction product (Table 3). The $K_m$ for NTP (100–150 μM) in NTP-dependent DNA synthesis was the same as the $K_m$ for GTP (100 μM) in ribonucleotide incorporation, suggesting a possible relationship between the DNA and RNA synthesis. Digestion with T4 3′ → 5′ exonuclease of the reaction product synthesized in the presence of [$^{32}$P]NTP and [$^{3}$H]dTTP generated a discrete oligoribonucleotide after nearly complete hydrolysis of the $^{3}$H-labeled material (Table 3 and Fig. 2). This suggested that a small piece of RNA was synthesized on the unprimed template and that DNA polymerase α subsequently used the RNA as a primer. γ-$^{32}$P label was also incorporated into the product, consistent with this interpretation.

The synthesis of RNA accompanying DNA synthesis does not

seem to be due to contamination by RNA polymerases I, II, or III (30), mitochondrial RNA polymerase (31), or terminal transferase (32). The evidence for this is (*i*) the template specificities (Table 1) were inconsistent with the known specificities of these enzymes, (*ii*) $K_m$ values for NTPs were an order of magnitude higher than has been reported for these enzymes, (*iii*) the synthesis was $\alpha$-amanitin-resistant (ruling out RNA polymerase II or III), and (*iv*) the NTP-dependent synthesizing activity sedimented as a peak of about 6.5 S (ruling out RNA polymerases I, II, and III).

NTP-dependent DNA-synthesizing activity copurified with DNA polymerase $\alpha_1$ activity during all stages of purification (data not shown) and cosedimented coincident with highly purified DNA polymerase $\alpha_1$ activity during glycerol gradient centrifugation (Fig. 3). No NTP-dependent DNA-synthesizing activity was detected at any stage of purification of DNA polymerase $\alpha_2$. Therefore, we also postulate the *X. laevis* DNA primase copurified and is associated with DNA polymerase $\alpha_1$ but not with *X. laevis* DNA polymerase $\alpha_2$. This is consistent with the results of Conaway and Lehman (12), if one assumes that *X. laevis* DNA polymerase $\alpha_2$ corresponds to the *Drosophila melanogaster* DNA polymerase $\alpha$ activity lacking DNA primase activity (cf. figure 4 in ref. 12).

All of the findings in this paper are most simply explained by the hypothesis that DNA primase activity is specifically associated with one form of *X. laevis* ovarian DNA polymerase $\alpha$ activity.

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