Volume 10 Number 18 1982 Nucleic Acids Research

Circular single stranded phage M13-DNA as a template for DNA synthesis in protein extracts from *Xenopus laevis* eggs: evidence for a eukaryotic DNA priming activity

Hans-Dieter Riedel, Herbert König, Hans Stahl and Rolf Knippers

Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, FRG

Received 21 July 1982; Revised and Accepted 31 August 1982

SUMMARY

Unfractionated protein extracts from activated Xenopus laevis eggs contain all functions required for the chain elongation reactions in replicative DNA synthesis (A.Richter, B.Otto and R.Knippers, 1981, Nucl.Ac.Res. 9, 3793-3807). In order to further explore the DNA synthesizing capacity of this in vitro system and to obtain information on the DNA priming activity in these extracts single stranded phage M13-DNA was used as template for in vitro DNA synthesis. The main results of this investigation are: (i) single stranded circular template DNA is converted to a double stranded DNA form in an α-amanitin-insensitive reaction which is absolutely dependent on ribonucleoside triphosphates; (ii) the in vitro synthesized complementary strands are DNA fragments of 1000-2000 nucleotides lengths; (iii) the DNA primase activity copurifies through several column steps and sucrose gradient centrifugation with a DNA polymerase α.These activities may therefore be closely associated in a quarternary enzyme complex.

INTRODUCTION

Closed circular double stranded DNA is semiconservatively replicated after microinjection into eggs from Xenopus laevis (1,2,3). Protein extracts, prepared from frog eggs, are rich sources for DNA polymerases (4), DNA binding proteins (5), DNA ligases (6), RNase H (6), topoisomerases (7) and probably other biochemical factors for eukaryotic DNA replication and should therefore be a promising system to study in vitro semiconservative DNA replication. In fact, recent experiments (8) have shown that, in concentrated protein extracts from frog eggs, replicative intermediates of Simian Virus 40 (SV40) chromatin are readily converted into mature viral chromatin. Since replicative chain elongation in Xenopus egg extracts is discontinuous (8) we expect to find a function in egg extracts which performs

0305-1048/82/1018-5621$ 2.00/0

a priming reaction for polydeoxynucleotide synthesis on circular single stranded DNA.

To assay for such a priming reaction and to further explore the DNA synthetic potential of unfractionated extracts from Xenopus eggs we have used circular single stranded phage M13-DNA as a template for DNA synthesis. We found that a considerable fraction of M13-DNA is converted to a double stranded DNA in an ATP requiring reaction. We attempted to isolate the putative primase from the crude egg extracts and found a close association of this enzymatic activity with one of the two forms of DNA polymerase α detectable in Xenopus egg extracts (9).

Experimental Procedures

Preparation of egg extracts

Unfertilized as well as fertilized eggs from hormone stimulated Xenopus laevis were collected on ice, processed and extracted as described (6,8). The protein extracts were filtered through Sephadex G 50 columns, equilibrated with 20mM Hepes (pH 7.5), 100mM potassium acetate, 5mM $MgCl_2$, and 1mM dithioerythritol, to remove small molecular weight material such as nucleotides. We like to emphasize, however, that results, identical to those described below, were obtained when untreated extracts were used instead of Sephadex filtered extracts.

The protein concentrations of different egg extracts were between 10-20 mg/ml.

Preparation of DNA

Phage M13mp5 (10) was cultivated and purified as described (11). Phages M13-2 and M13-6 contain the SV40-Hind III-C fragment in each one of both orientations. The recombinant phages were constructed by A.Dorn in this laboratory. Phage M13-DNA was prepared as described (12). (^{3}H) labeled phage DNA was prepared from bacterial cultures grown in Tris-minimal medium (13), containing 0.2μCi/ml thymidine added at the time of optimal viral DNA synthesis.

DNA synthesis in vitro

In the standard reaction mixture, 0.1μg DNA in a 0.01 ml volume of 10mM Tris-HCl, 1mM EDTA (pH 8) were added to 0.03 ml egg extract (10 mg/ml protein). The final reaction volume was 0.05 ml and contained 150mM potassium acetate, 40mM Hepes (pH 7.5), 5mM $MgCl_2$, 0.5mM dithioerythritol, 0.1mM each of dATP, dCTP, dGTP,

0.01mM $\alpha(^{32}P)$ dTTP (spec.act. 2000-3000 cpm/pmol), 0.1mM each of CTP, GTP, UTP, 1.0mM ATP, 5mM phosphoenol pyruvate and 2.5µg ml pyruvate kinase (14). Incubations were performed at 30°C for the times indicated below. Incorporated radioactivity was determined in trichloroacetic acid precipitates as described (14). If required for further analysis, DNA was extracted from the incubation mixtures by phenol and chloroform-isoamylalcohol (24:1) and concentrated by ethanol precipitation.

Centrifugation

Sucrose gradient centrifugation was performed in the Beckman SW55 rotor at 48,000 rpm and 0°C usually for four hours. Neutral sucrose gradients were linear from 25% to 5% sucrose made up in 1 M NaCl, 50mM Tris-HCl, 5mM EDTA (pH 7.5) and 0.1% Sarkosyl. Alkaline sucrose gradients (25% to 5%) contained 1 M NaCl, 0.3 M NaOH, 10mM EDTA, 0.1% Sarkosyl and 100µg/ml calf thymus DNA.

For alkaline CsCl equilibrium centrifugation, enough CsCl was added to DNA samples (in 0.2 M K_3PO_4; pH 12.5) to give an initial density of 1.77 g/ml. Volumes of 2.5-3.5 ml were centrifuged for 60-70 hrs at 22°C and 35,000 rpm in the SW55 rotor.

Electrophoresis

Agarose gels (1 - 1.2%) were run in 10mM Tris-acetate buffer (pH 8.0), containing 1mM EDTA. The gels were dried after electrophoresis and evaluated by autoradiography (15).

Isolation of DNA polymerase and associated primase activities

DNA polymerase activity was determined using activated calf thymus DNA under the conditions described previously (14). To isolate DNA polymerase from Xenopus egg extracts, we first passed the unfractionated protein extract through a double-stranded calf thymus DNA cellulose column. All detectable enzymatic activity remained on the column. The column was washed with five column volumes of Tris-buffer (50mM Tris-HCl;5mM $MgCl_2$; 1mM dithioerythritol; 10% glycerol). The bound enzyme was then eluted with 0.4 M KCl in Tris-buffer. The eluate was dialysed against Tris-buffer to give a final concentration of 50mM KCl. The dialysed sample was added to a DEAE-cellulose-column, equilibrated with Tris-buffer, containing 50mM KCl. About 50% of

the detectable DNA polymerase activity (fraction A) passed through the column while the remaining DNA polymerase (fraction B) was retained. The column bound fraction B was eluted in a linear KCl gradient at about 0.3 M KCl. Further purification was achieved by phosphocellulose chromatography. The polymerase fractions were first dialysed against Tris-buffer. Fraction A polymerase was eluted with KCl concentrations of about 0.05 and fraction B polymerase at 0.3 M KCl. (Details of the purification scheme will be published elsewhere). The final step of the current purification procedure was sucrose gradient centrifugation. The sucrose gradients were linear from 0-15% sucrose in 20% glycerol and 0.25 M ammonium sulfate, buffered by Tris-buffer. The centrifugation was carried out in the Beckman SW55 rotor for 24 hours at 48,000 rpm and 0°C. . Fraction A polymerase sedimented with about 8.2S and fraction B polymerase with about 6.7S relative to horse hemoglobin (4.3S) and IgG (7S) as sedimentation markers (16) (see below: Fig. 6). Fraction A polymerase contains the associated primase activity (see below).

RESULTS

Single stranded phage M13-DNA as template for DNA synthesis

DNA templates, extracted from phages M13mp5, M13-2 and M13-6, were added to aliquots of a *Xenopus laevis* egg extract and incubated in the presence of $\alpha(^{32}P)$ labeled desoxynucleoside triphosphate as described above. The results, shown in Fig.1, demonstrate that all three DNA preparations were effective templates for DNA synthesis *in vitro* indicating that the SV40 origin sequences, present in each one of both orientations in M13-2 and M13-6, did not influence the template function of single stranded circular DNA. We have also found that the products of *in vitro* DNA synthesis (see below) were identical regardless of whether M13mp5-DNA, M13-2-DNA or M13-6-DNA were used as templates in the *in vitro* system (data not shown). For this reason we present in the following sections only data obtained with M13mp5-DNA as a template.

In vitro DNA synthesis was greatly reduced when ribonucleotides were omitted from the incubation assay (Fig.1). ATP alone as well as GTP and CTP (optimal concentrations: 2-4mM) could

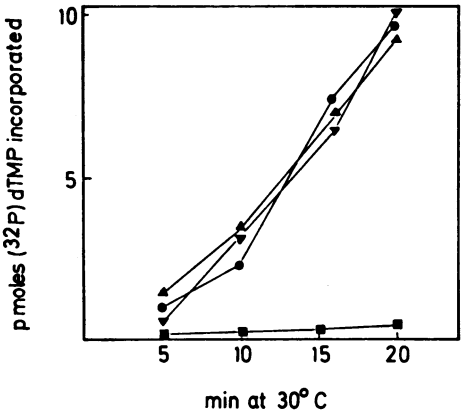

Figure 1. M13-DNA as template for in vitro DNA synthesis. M13mp5-DNA (●), M13-2 (▲) and M13-6 (▼) were used under standard reaction conditions, including ribonucleoside triphosphates (see: Experimental Procedures) to assay for the incorporation of α(^{32}P) dTTP into trichloroacetic acid precipitable material. M13mp5-DNA (■) was also used as template for DNA synthesis in the absence of ribonucleoside triphosphates.

replace the ribonucleoside triphosphate mixture of the standard assay. UTP alone was less effective (about 50%) than the mixture of four ribonucleotides. The drug α-amanitin in concentrations known to inhibit RNA polymerase II and III (10^{-4} M) did not influence the reaction.

No DNA synthesis was observed when a DNA template was omitted from the standard incubation mixture (8).

The conditions for optimal synthesis, including the concentrations of monovalent and divalent cations and pH, correspond essentially to the conditions determined previously (8) for replicative chain elongation in crude Xenopus egg extracts.

Products of in vitro DNA synthesis

(^{3}H) labeled M13mp5-DNA was used to investigate the products of in vitro DNA synthesis. Fig. 2 shows the results of an analysis by zone velocity sedimentation through neutral sucrose gradients. (^{32}P) labeled DNA, synthesized in vitro in the presence of ribonucleoside triphosphates, usually appeared in three parts of the gradient (Fig. 2a): (i) one part of the incorporated (^{32}P) radioactivity sedimented with or slightly slower than

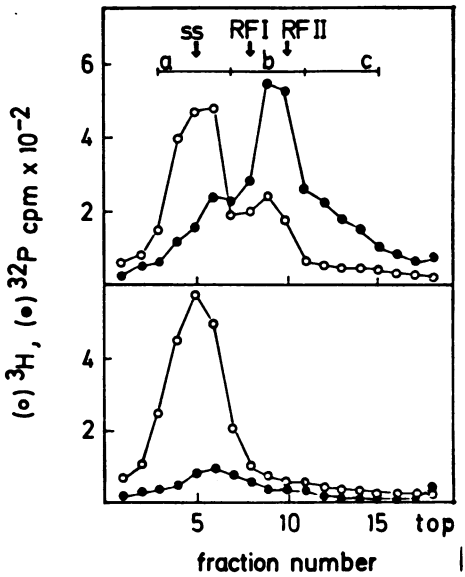

Figure 2. Analysis of DNA synthesis products by zone velocity sedimentation at neutral pH.
(a) (^{3}H) labeled M13mp5- DNA (o) was incubated for 20 min at 30°C under standard reaction conditions with ribonucleoside triphosphates. The DNA was then sedimented through neutral sucrose gradients. Incorporated (^{32}P) dTMP (•) was determined in 0.02 ml aliquots from each gradient fraction after trichloroacetic acid precipitation. The remainder of fractions 3-7 (a), 8-11 (b) and 12-15 (c) were combined as indicated. The DNA in the pooled samples was precipitated by ethanol and resuspended in 0.05 ml Tris-acetate-EDTA buffer for gel electrophoresis (see: Fig. 3). The vertical arrows indicate the position of authentic M13-DNA, centrifuged under identical conditions, in a parallel tube of the same centrifugation run. (SS: single stranded M13-DNA ; RF I: circularily closed, double stranded, supercoiled replicative form M13-DNA; RF II: nicked double stranded circular replicative form M13-DNA).
(b) The experiment was exactly performed as described under (a) except that ribonucleotides were omitted from the reaction mixture.

the peak of remaining single stranded (^{3}H) M13-DNA (fractions 3-7, Fig. 2a); (ii) most <u>in vitro</u> polymerized (^{32}P) radioactivity sedimented with 20-30 percent of the (^{3}H) template DNA like replicative form II-DNA (open circular double stranded M13-DNA) (fractions 8-11; Fig. 2a); (iii) the third part of the recovered (^{32}P) radioactivity sedimented as a trailing shoulder of the major replicative form II peak (fractions 12-15) (Fig.2a).

In Fig. 2b, we show the sedimentation properties of DNA, synthesized in vitro in the absence of ribonucleoside triphosphates. Most of the recovered incorporated (^{32}P) radioactivity cosedimented with single stranded (^{3}H) M13-DNA.

The three parts of the sucrose gradient in Fig. 2a were further investigated by agarose gel electrophoresis. The fractions, indicated in the legend to Fig. 2a, were combined to give three samples, concentrated by ethanol precipitation and electrophoresed in a 1% agarose gel. An autoradiogram of the dried gel is shown in Fig. 3: (i) most (^{32}P) radioactivity of the fastest sedimenting material traveled during electrophoresis like linear single stranded DNA while another part had the electrophoretic mobility of circular double stranded form II DNA; (ii) a considerable fraction of the (^{32}P) labeled material of fractions 8-11 (Fig. 2a) traveled in the agarose gel like double stranded replicative form II DNA, another part of the (^{32}P) labeled polymerization products migrated faster and may represent denaturation or degradation products; (iii) the slow sedimenting (^{32}P) labeled material of Fig. 2a appeared after gel electrophoresis as a broad distribution of small heterogenous DNA fragments.

An experiment was performed with (^{3}H)M13-DNA using 5-bromo-

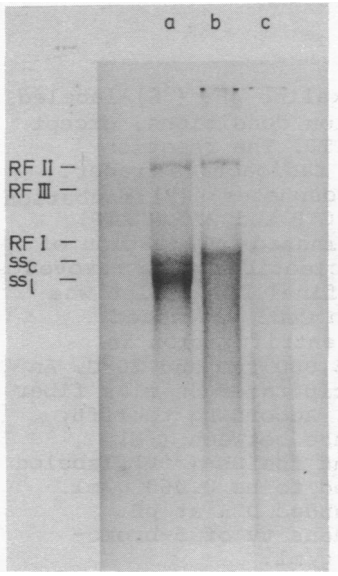

Figure 3. Autoradiogram of an agarose gel showing the (^{32}P) labeled products of in vitro DNA synthesis. The DNA samples were recoverd from the sucrose gradient of Fig.2A. The gel was stained after electrophoresis to identify the marker DNA run in different tracks: linear (SS_l) and circular (SS_c) single stranded DNA, double stranded circular form I (RF I) and form II DNA (RF II) as well as Eco RI-restricted double stranded, linear M13-DNA (RF III). The gel was dried and used for autoradiography.

Lane a: fractions 3- 7 of Fig. 2A;
lane b: fractions 8-11 of Fig. 2A;
lane c: fractions 12-15 of Fig. 2A.

desoxyuridine triphosphate (dBUTP) instead of dTTP in the standard reaction assay (with and without ribonucleoside triphosphates). The products of in vitro DNA synthesis were investigated by alkaline CsCl equilibrium centrifugation (Fig. 4).

In the absence of ribonucleoside triphosphates, all incorporated (^{32}P) radioactivity appeared in those fractions of the gradient where most (^{3}H) template DNA was recovered(Fig. 4a). The products

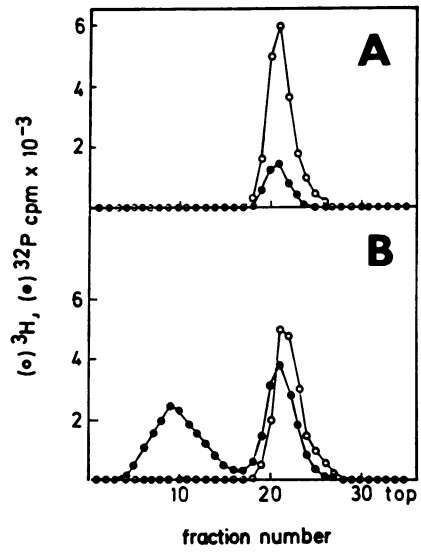

Figure 4. Equilibrium centrifugation at alkaline pH. (^{3}H)-labeled M13-DNA was incubated under standard reaction conditions, except that 12µmoles dBUTP were used instead of dTTP. The reaction mixture contained 1µmoles α(^{32}P) dTTP as a radioactive tracer. (A) Incubation without ribonucleoside triphosphates; (B) Incubation in the presence of 0.2mM each of CTP, UTP, GTP and 2.5mM ATP. After 20 min at 30°C, the reaction was terminated by addition of EDTA (final conc.: 20mM). Unincorporated nucleotides were removed by repeated precipitations in ethanol. The final DNA pellet was resuspended in 0.2 M K_3PO_4 (pH12.5). Enough CsCl was added to obtain an initial density of 1.77g/ml. Centrifugation in the SW55 rotor was performed for 65 h at 35,000 rpm and 20°C. An 0.03 ml-aliquot from each fraction was precipitated on glas fiber filter by trichloroacetic acid and counted. According to Ifft, Voet and Vinograd (17) the density difference between (^{3}H) labeled M13-DNA (fraction 21 of Fig. 4B) and the BU-(^{32}P) labeled synthesis product (fraction 9) was estimated to be 0.060 g/ml. If the buoyant density of light single stranded DNA at pH 11.5 is assumed to be 1.745 g/ml (18) the density of 5-bromo-uracil containing DNA should be about 1.81 g/ml.

of the standard reaction (including ribonucleoside triphosphates) appeared in two peaks: (i) about 50% of the recovered (^{32}P) counts had a mean buoyant density of about 1.8 g/ml as expected for denatured DNA in which most thymidine containing residues are replaced by 5-bromouracil nucleotides (17,18); (ii) another fraction of (^{32}P) labeled DNA had a mean density slightly higher than single stranded (^{3}H) M13-DNA (Fig. 4b).

We conclude from the data of Fig. 2-4 that two different DNA synthesis reactions occur in unfractionated Xenopus egg extracts with single stranded M13-DNA as template.

One reaction involves apparently a covalent addition of desoxyribonucleotides to backfolded ends of linearized single stranded phage DNA which may be present in the original preparation of phage M13-DNA or which may arise by breakage of circular DNA during incubation. This "repair type" reaction occurs in the presence as well as in the absence of ribonucleoside triphosphates.

Another DNA synthesis reaction was only observed in the presence of ribonucleoside triphosphates. This reaction produced DNA molecules which sedimented in neutral sucrose gradients and traveled during agarose gel electrophoresis like double stranded M13-RF II DNA. The double stranded nature of these DNA molecules was shown by alkaline cesium chloride gradient centrifugation which yielded light as well as heavy DNA strands containing 5-bromouridine monophosphate instead of thymidine monophosphate.

The rather broad distribution in alkaline cesium chloride gradients (Fig.4b) of (^{32}P)- and 5-bromouracil labeled DNA suggests that these DNA strands are of less than unit M13 length. This assumption is supported by sucrose gradient analysis at alkaline pH (Fig.5a): "heavy" (^{32}P)-DNA sediments with 8-10 S (compared to linear M13-DNA, S = 15; ref. 19), corresponding to DNA fragments of about 1000-2000 nucleotides lengths (20).

We have tried to isolate the DNA priming activity from the crude protein extract and found that it remained closely associated with one of the two chromatographically distinct (6) DNA polymerase activities.

Isolation of a DNA priming activity

The crude Xenopus egg extract was fractionated by affinity

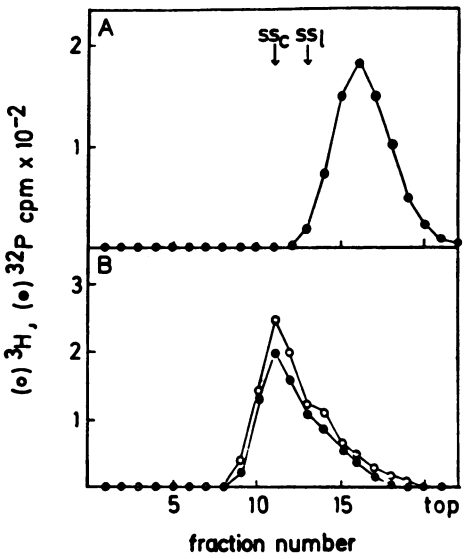

Figure 5. Alkaline sucrose gradients.
(A) The BU-(^{32}P) labeled denatured DNA was recovered from a CsCl equilibrium gradient (fractions 5-15 of Fig.4B), dialysed to remove salt and precipitated in ethanol. The precipitated DNA was resuspended in 30mM NaOH and sedimented through an alkaline sucrose gradient.
(B) The "light" DNA in fractions 19-25 (Fig.4B) was also combined, dialyzed and ethanol precipitated before alkaline sucrose gradient centrifugation. The vertical arrows indicate the fractions, containing circular (SS_c) and linear (SS_l) single stranded M13-DNA, in an alkaline sucrose gradient centrifuged under identical conditions in a parallel tube.

chromatography on double-stranded DNA-cellulose and by ion exchange chromatography on DEAE- and phosphocellulose (see: Experimental Procedures). Chromatography on DEAE- und phosphocellulose revealed two chromatographically distinct forms of DNA polymerase when assayed under the usual conditions (14) with activated calf thymus DNA as primer-template. One of the two forms, fraction A polymerase, was also able to initiate DNA synthesis on closed circular M13-DNA as template in a reaction which was absolutely dependent on ribonucleoside triphosphates. The second DNA polymerase, fraction B, was unable to initiate DNA synthesis on single stranded DNA rings. We conclude that a primase activity copurifies through several column steps with fraction A polymerase. Furthermore, sucrose gradient analysis

showed that the ribonucleotide dependent primase activity co-sedimented with the fraction A polymerase (Fig. 6).

These results strongly suggest that the fraction A polymerase but not the fraction B polymerase is able to synthesize on circular single stranded DNA a primer which is then extended by deoxynucleotide polymerization to yield the DNA fragments described in Fig.5 and below. The dependency of this reaction on ribonucleoside triphosphates suggests that the primer is most likely RNA. This assumption is supported by an experiment in which (^{3}H) labeled dTTP and $\alpha(^{32}P)$ labeled ATP, CTP and GTP were used instead of non radioactive ribonucleotides. The reaction was carried out as described under Experimental Procedures

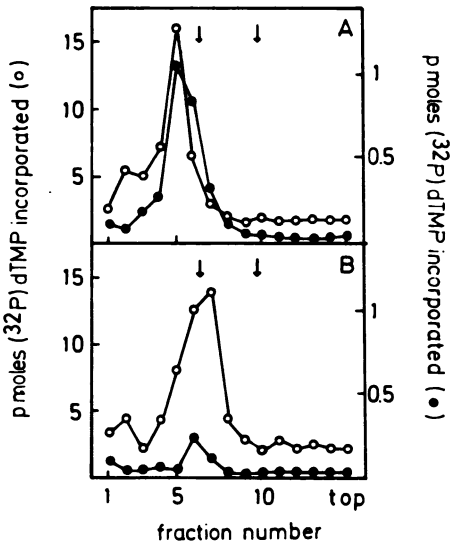

Figure 6. Sucrose gradient centrifugation of two chromatographically distinct DNA polymerases from Xenopus eggs. (A) Fraction A polymerase; (B) Fraction B polymerase. The conditions for centrifugation are described under Experimental Procedures. DNA polymerase activity was determined with activated calf thymus DNA (o) as described in (14) and with M13-DNA (•) as described above. The assay mixture with M13-DNA included ribonucleoside triphosphates. Incorporation of $\alpha(^{32}P)$dTMP was not detectable when ribonucleotides were not present in the reaction mixture.
The arrows indicate the position of Immunglobulin G (7 S) and horse hemoglobin (4.3 S), respectively.

except that sucrose gradient purified fraction A polymerase (Fig.6) was used as enzyme source rather than the unfractionated egg extract. We found a cosedimentation of the (^{3}H) and (^{32}P) labeled reaction products in a neutral sucrose gradient (Fig.7a). The reaction products were isolated from this sucrose gradient and divided into two parts. One part was heat denatured and centrifuged through a neutral sucrose gradient (Fig.7b); the second part was denatured in 80mM KOH and centrifuged through an alkaline sucrose gradient (Fig. 7c),

The data of Fig. 7, b and c demonstrate an alkali sensitivity of the (^{32}P) labeled polymerization products supporting the assumption that the primer is most probably a sequence of ribonucleotides as expected from numerous _in vivo_ studies showing that a fraction of Okazaki pieces, extracted from replicative intermediates of eukaryotic DNA, carries small RNA primers at the 5' ends (review in ref.nr. 21).

Our conclusion was also supported by the following experiment. DNA synthesis products were purified by sucrose gradient centrifugation as shown in Fig. 7a. The products, composed of (^{32}P) ribonucleotides and (^{3}H) TMP residues, were collected by ethanol precipitation and incubated for 8 hours at room temperature in presence of phage T4 polymerase (from BRL) under conditions which are optimal for the 3'→5' exonuclease activity of this enzyme (22). During this incubation the acid precipitable (^{3}H) radioactivity decreased from about 20,000 cpm to 530 cpm while the (^{32}P) radioactivity decreased from about 320 cpm only to 280 cpm suggesting that most of the (^{32}P) ribonucleotides were located at the 5' ends of the reaction products and were resistant against an 3'→5' exonucleolytic desoxyribonuclease. The resistant (^{32}P) ribonucleotide primers traveled in a 20% polyacrylamide-urea gel (23) as expected for an oligonucleotide of less than eight residues (not shown).

CONCLUSION

Our data show that circular single stranded DNA is an effective template for DNA synthesis in unfractionated extracts from _Xenopus_ eggs.

We observe essentially two types of DNA synthesis. In a first

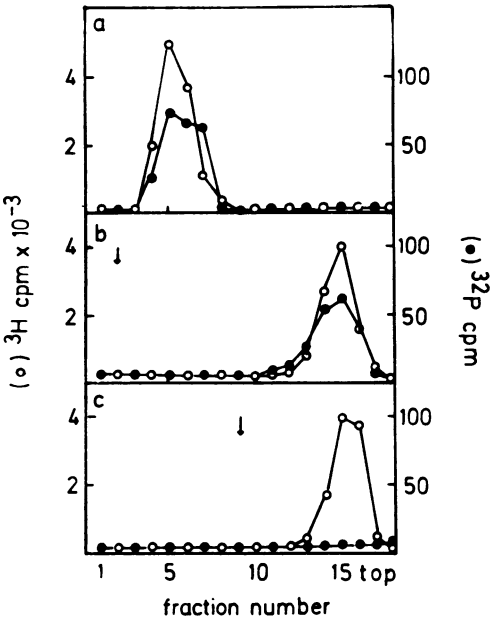

Figure 7. Sucrose gradient analysis of DNA synthesis products.
(a) The reaction mixture contained (^{3}H)dTTP (spec.act. ca. 2500 cpm/pmole) and α(^{32}P)ATP, α(^{32}P)GTP and α(^{32}P)CTP (total spec.act.: ca. 20,000 cpm/pmole) in addition to M13-DNA (see: experimental Procedures). The reaction was started by addition of 0.1 ml of sucrose gradient purified fraction A polymerase (Fig.6A). After 60 min at 30°C, the mixture was centrifuged through a neutral sucrose gradient. We show the distribution of acid precipitable radioactivity in 0.02 ml aliquots from each fraction. Fractions 4-7 were combined, dialysed and concentrated by ethanol precipitation.
(b) The synthesis products, shown in (a) were heat denatured and centrifuged through a neutral sucrose gradient.
(c) Alkaline sucrose gradient centrifugation of alkaline denatured synthesis products. The vertical arrows in (b) and (c) indicate the position of circular M13-DNA centrifuged under identical conditions in a parallel tube. From the position of M13-DNA in the alkali gradient we estimate that the in vitro synthesized DNA fragments sediment with 4-5 S .

reaction, which appears to be independent of ribonucleotides desoxyribonucleotides are covalently added to one end (presumably the 3'-OH end) of linearized M13-DNA. A second DNA synthesis reaction is absolutely dependent on ribonucleoside tri-

phosphates. The synthesis products are DNA fragments of 1000-2000 nucleotides lengths, hydrogen bonded to the single stranded DNA template. These findings indicate the presence of a DNA priming activity in Xenopus egg extracts. During our attempts to isolate this activity we found a primase in close association with one of the two detectable DNA polymerase α forms. It is conceivable that the primase is one component of a complex quarternary structure of this enzyme. We note that the active fraction A polymerase has a significant higher sedimentation coefficient (Fig.6) than the fraction B polymerase which is unable to initiate DNA synthesis on circular single stranded DNA. The fraction A polymerase synthesizes under optimal conditions DNA fragments which carry small RNA primers on their 5' ends and which sediment with 4-5 S through alkaline sucrose gradients. These fragments are clearly shorter than those synthesized in crude Xenopus egg extracts. This could be due, for example, to the presence of DNA binding proteins in the unfractionated protein extracts. It has been shown before that an eukaryotic single stranded DNA binding protein stimulates the activity of DNA polymerase α (24).

We finally point out that eukaryotic DNA sequences are not required for the priming reaction since unmodified M13-DNA was as effective as a template as M13-DNA carrying the SV40 origin sequences. This does, of course, not necessarily mean that the priming of complementary strands is completely sequence independent. This question as well as the detailed biochemistry of the DNA priming reaction are currently under investigation.

While this manuscript was prepared we learned that other research groups had also detected a polymerase-accociated primase activity in extracts from mouse ascites cells (25), from Drosophila embryos (26) and from Xenopus ovaries (27).

ACKNOWLEDGEMENT

This work was supported by a grant from Deutsche Forschungsgemeinschaft through SFB 138/B4.

REFERENCES

(1) Gurdon, J.B., Birnstiel, M.L. and Speight, U.A. (1969) Biochim.Biophys.Acta 174, 614-628
(2) Laskey, R.A. and Gurdon, J.B. (1973) Eur.J.Biochem. 467-471
(3) Harland, R.M. and Laskey, R.A. (1980) Cell 21, 761-771
(4) Benbow, R.M., Pestell, R.Q.W. and Ford, C.C. (1975) Dev.Biol. 43, 159-174
(5) Carrara, G., Gialtoni, S., Mercanti, D. and Tocchini-Valentini, G.P. (1977) Nucl.Ac.Res. 4, 2855-2870
(6) Benbow, R.M., Kraus, M.R. and Reeder, H. (1978) Cell 13, 307-318
(7) Matoccia, E., Gandini-Attardi, D., and Tocchini-Valentini, G.P. (1976) Proc.Nat.Acad.Sci.USA 73, 4551-4554
(8) Richter, A., Otto, B. and Knippers, R. (1981) Nucl.Ac.Res. 9, 3793-3807
(9) Fox, A.M., Breaux, C.B. and Benbow, R.M. (1980) Develop. Biol. 80, 79-95
(10) Heidecker, G., Messing, J. and Gronenborn, B. (1980) Gene 10, 69-73
(11) Yamamoto, K.R., Alberts, B.M., Benzinger, R., Lathorne, L. and Treiber, G. (1970) Virology 40, 734-744
(12) Knippers, R. and Hoffmann-Berling, H. (1966) J.Mol.Biol. 21, 281-292
(13) Knippers, R., Razin, A., Davis,R. and Sinsheimer, R.L. (1969) J.Mol.Biol. 45, 237-263
(14) Richter, A., Scheu, R. and Otto, B. (1980) Eur.J.Biochem. 109, 67-73
(15) Sundin, O. and Varsharsky, A. (1980) Cell 211, 103-14
(16) Sober, H.A. (ed.) (1970) Handbook of Biochemistry, CRC Press, Cleveland, Ohio
(17) Ifft, J.B., Voet, D.H. and Vinograd, J.R. (1961) J.Phys. Chem. 65, 1138-1144
(18) Baldwin, R.L. and Shooter, E.M. (1963) J.Mol.Biol. 7, 511-526
(19) Bouvier, F. and Zinder, N.D. (1974) Virology 60, 139-150
(20) Studier, F.W. (1965) J.Mol.Biol. 11, 313-390
(21) Ogawa, T. and Okazaki, T. (1980) Ann.Rev.Biochem. 49, 421-457
(22) Muzyeka, N., Poland, R.L. and Bessman, M.J. (1972) J.Biol.Chem. 247, 7116-7122
(23) Maniatis, T., Jeffry, A. and van de Sande, H. (1975) Biochemistry, 14, 3787-3797
(24) Otto, B., Baynes, M. and Knippers, R. (1977) Eur.J. Biochem. 73, 17-24
(25) Yagura, T., Kozu, T. and Seno,T. (1982) J.Biochem. 91, 607-618
(26) Conaway, R.C. and Lehman, I.R. (1982) Proc.Nat.Acad.Sci. USA 79, 2523-2527
(27) Shioda, M., Nelson, E.M., Bayne, M.L. and Benbow, R.M. (1982) Proc.Nat.Acad.Sci.USA, in press.