Isolation and Characterization of a DNA Primase from Human Mitochondria*

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A family of enzymatic activities isolated from human mitochondria is capable of initiating DNA replication on single-stranded templates. The principal enzymes include at least a primase and DNA polymerase γ and require that rNTPs as well as dNTPs be present in the reaction mixture. Poly(dC) and poly(dT), as well as M13 phage DNA, are excellent templates for the primase activity. A single-stranded DNA containing the cloned origin of mitochondrial light-strand synthesis can be a more efficient template than M13 phage DNA alone. Primase and DNA polymerase activities were separated from each other by sedimentation in a glycerol density gradient. Using M13 phage DNA as template, these mitochondrial enzymes synthesize RNA primers that are 9 to 12 nucleotides in size and are covalently linked to nascent DNA. The formation of primers appears to be the rate-limiting step in the replication process. Replication of M13 DNA is sensitive to N-ethylmaleimide and dieoxyxynucleoside triphosphates, but insensitive to rifampicin, α-amanitin, and aphidicolin.

The mitochondrial genome of animal cells represents a versatile subject for studying eukaryotic DNA replication and gene expression. In mammals, the mitochondrial genome is a closed circular DNA of about 16.5 kilobases. This relatively small size and a compact gene organization render it more amenable to experimental manipulation than the complex chromosomes of the nucleus (1). The nucleotide sequences of three mammalian mtDNAs have been determined (2-4). It has become apparent from this sequence information and from biochemical data that most, if not all, of the enzymes that are involved with transcription and replication of mtDNA must be imported from the cytosol. Hence an understanding of the biosynthesis of mitochondrial nucleic acids and its regulation should provide useful insights into the mechanism of nuclear-cytoplasmic interactions.

Previous work in this laboratory has resulted in the isolation of a transcription activity from human mitochondria (5). The ability to reproduce the transcription process in vitro has allowed the identification of the major promoters for transcription of human mtDNA (6). We have also undertaken an effort to study in vitro replication of mtDNA. Our first goal is to isolate mitochondrial enzymes that are capable of initiating DNA replication. Here we report the identification and characterization of a fraction of mitochondrial proteins, isolated from human tissue culture cells, that can initiate replication on single-stranded DNA templates.

EXPERIMENTAL PROCEDURES

Reagents—Nucleoside triphosphates, poly(dA), poly(dC), poly(dG), poly(dT), and polyA-oligo(dT) were purchased from P-L Biochemicals. α-32P-labeled dATP, dCTP, dGTP, and TTP were from Amersham Corp. RNase-free DNAase I was from Miles Laboratories. Rifampicin was obtained from Calbiochem-Behring. 2′-Ethylmaleimide was purchased from Sigma. Aphidicolin was a gift from Dr. T. S.-F. Wang of Stanford University. DE52, phosphocelulose (P-11), and GF/A filters were from Whatman. The large fragment of Escherichia coli DNA polymerase I was from New England Nuclear. M13 DNA Templates—The single-stranded DNA template, M13KBL0"H, contains a 226-nucleotide HincII fragment (nucleotides 5694-5919) of human mtDNA and was cloned in M13mp7 by Maureen J. Bibb of this laboratory. The insert contains H-strand DNA sequence of the origin of L-strand replication, flanked by trNA genes. Plasmid DNA was prepared as previously described (6) and dialyzed against 1 liter of buffer that contained 50% glycerol, 20 mM Tris-HCl, pH 8.0, 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 14 mM 2-mercaptoethanol to about 0.3 M NaCl. The column was washed with buffer A containing 10% glycerol, 20 mM Tris-HCl, pH 8.0, 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 14 mM 2-mercaptoethanol to about 0.3 M NaCl. Mitochondria were resuspended in buffer A (10% glycerol, 20 mM Tris-HCl, pH 8.0, 0.2 mM EDTA) and incubated for 1 hour with 1 mg/ml of rifampicin, 50 μg/ml of DE52 cellulose, and 25 μg/ml of rifampicin. Mitochondria were washed with buffer A containing 0.3 M NaCl. The column was washed with 2 column volumes of the buffer A containing 0.3 M NaCl. Mitochondria were resuspended in buffer A containing 0.3 M NaCl. The column was washed with 1 liter of buffer that contained 50% glycerol, 20 mM Tris-HCl, pH 7.5, 0.2 mM EDTA, and 14 mM 2-mercaptoethanol to yield fraction III. Fraction III was stored at −20 °C.

The abbreviations used are: L-"H, heavy-strand template DNA sequence of light-strand replication origin; dNTP, deoxynucleoside triphosphate; rNTP, ribonucleoside triphosphate; dGTP, di-deoxynucleoside triphosphate; dA, heavy and light strand, respectively; O6, origin of L-strand replication.

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20-min period to reach 45% saturation. The suspension was stirred gently for another 30 min, and was centrifuged in a Beckman 75 Ti rotor at 25,000 rpm for 20 min. The pellet was resuspended in buffer B (10% glycerol, 20 mM Tris-HCl, pH 7.5, 0.2 mM EDTA, and 14 mM 2-mercaptoethanol) to a final concentration of 10 to 20 mg of protein/ml. The material was then dialyzed against two changes (1 liter each) of buffer B. Insoluble materials were removed by centrifugation and the supernatant (referred to as ammonium sulfate fraction in the text) was stored at -70°C. Protein concentrations were determined as described by Bradford (9).

DNA Replication Assay—DNA synthesis was assayed by measuring incorporation of [3H]-labeled TMP or dAMP into acid-insoluble materials. Each assay was carried out in a total volume of 25 μl and contained 20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, BSA at 0.1 mg/ml, 1 mM 2-mercaptoethanol, 2 mM ATP, 300 μM each of CTP, GTP, and UTP, 10 μM of [α-32P]dATP or [α-32P]TPP (specific activity of 5,000 to 20,000 cpm/μmol) and the other three dNTPs at 100 μM each. DNA template was either M13 phage DNA at 20 μg/ml or poly(dT) at 10 μg/ml. In assays of poly(dT) replication, ATP and dATP were the only nucleoside triphosphates in the reaction mixtures. The reaction was initiated by adding mitochondrial fractions and was terminated by adding EDTA to 50 mM and 30 μM of sonicated calf thymus DNA. Incorporation was assayed by precipitation of nucleic acids with 1 N HCl containing 1% (w/v) sodium pyrophosphate. Acid-insoluble materials were collected on Whatman GF/A filters, which were then washed, dried, and counted in a toluene-based scintillation fluid. One unit of DNA primase activity is defined as the amount that will catalyze polymerization of 1 pmol of dAMP on poly(dT) template at 37°C in 1 h.

Assay of DNA Polymerase γ Activity—Each reaction mixture was in a total volume of 25 μl and contained 20 mM Tris-HCl, pH 7.5, 0.5 mM MnCl₂, BSA at 0.1 mg/ml, 50 mM sodium phosphate, 0.1 M NaCl, polyA-oligo(dT) at 25 μg/ml, 10 μM of [α-32P]TPP (8,000 to 15,000 cpm/μmol), 1 mM 2-mercaptoethanol, and varying amounts of mitochondrial enzymes. Reaction mixtures were incubated at 37°C and incorporation was assayed by acid precipitation as described above. One unit of activity is defined as the amount that will result in incorporation of 1 pmol of TTP into acid-insoluble materials at 37°C in 1 h.

Assay of RNA Primer Synthesis—The reaction was carried out as described for assaying M13 DNA replication except that [α-32P]TPP (150,000 cpm/μmol) was used at 4 μM as the only radiolabeled substrate. The reaction was terminated by extraction of the mixture with aqueous phenol. Ammonium acetate was added to 2 M and nucleic acids were precipitated with ethanol. Reaction products were resuspended in 5 μl of sample buffer (80% formamide, 50 mM Tris-borate, pH 8.3, 0.02% xylene cyanol, 0.02% bromphenol blue, and 1 mM EDTA) and were incubated at 90°C for 3 min. They were then separated by electrophoresis in a 20% polyacrylamide gel containing 7 M urea as described (10). An alkaline hydrolysate of [γ-32P]ATP or [c-32P]TTP was used as size markers.

**RESULTS**

**DNA Replication Enzymes in Human Mitochondria**—Our attempts to study in vitro replication of mtDNA began with the isolation of mitochondrial enzymes that are capable of priming and elongating as yet uncultivated mammalian DNA molecules. In all of the eukaryotic and prokaryotic DNA replication systems that have been examined, RNA priming is the most common mechanism of initiating DNA synthesis (11). That a similar mechanism may be operative in mammalian mitochondria has been suggested by previous experimental data (12–14). For these reasons, we have chosen as criteria for DNA replication enzymes with the ability to catalyze the synthesis of RNA primers on a DNA template, and also to elongate these primers into daughter DNA strands. One of the DNA templates we have used is a 226-nucleotide fragment containing H-strand sequence of human mtDNA where L-strand replication initiates in vivo (15). The fragment was cloned in bacteriophage M13mp7 and phage DNA (M13KBL∞H) was used in assaying mitochondrial enzymatic activities. The use of single-stranded DNA was designed to mimic the physical state of the origin of L-strand replication (Ol) when replication begins at that origin (16).

Mitochondria were purified from KB cells and lysed with Triton X-100. Using the crude lysate, we were not able to demonstrate replication activity on any DNA template. However, an ammonium sulfate fraction of mitochondrial proteins was able to stimulate DNA synthesis in the presence of M13KBL∞ DNA, dNTPs, rNTPs, and Mg²⁺ (Fig. 1). DNA synthesis was dependent on the presence of rNTPs in the reaction mixture, although a reduced level of DNA synthesis also occurred in the absence of rNTPs. Also, the rNTP-dependent DNA synthesis on M13KBL∞H was more efficient than that obtained with M13mp7 DNA; maximal synthesis with M13KBL∞H was almost twice that with vector DNA alone. At present it is not possible to evaluate the significance of this apparent difference in the extent of DNA synthesis because of the crude nature of the enzyme preparation. Examination of the reaction mixtures by agarose gel electrophoresis revealed that greater than 90% of the template DNA was degraded in the course of the incubation (data not shown). A nuclease activity was present among the mitochondrial proteins and may account for the generation of 3'-hydroxyl ends that result in significant levels of rNTP-independent DNA synthesis with either template. The presence of nuclease in the ammonium sulfate fraction also makes it impossible to determine the nature of the Ol-specific DNA synthesis using this particular enzyme fraction. We therefore proceeded to...
The high-speed supernatant of the mitochondrial lysate was subjected to successive chromatography on DEAE and phosphocellulose columns. Fractions obtained throughout the purification were assayed for DNA polymerase \( \gamma \) and primase activity. The latter was assayed using the synthetic template poly(dT). The two enzyme activities were adsorbed on both columns and were recovered by elution at higher ionic strength. The final preparation, fraction III, was completely free of any DNase activity. The 3-fold enhancement in recovery of primase activity following chromatography on DEAE-cellulose is most likely due to removal of nuclease or other inhibiting activities (Table I). The simple chromatographic procedures resulted in an 85-fold and 760-fold purification of DNA polymerase and primase, respectively. When stored at \(-20^\circ C\), both activities were stable for at least 2 months.

**Purification of DNA polymerase**

The procedure was designed to allow maximal recovery of activities that may be involved in DNA replication, such as DNA polymerase and primase, and at the same time permit removal of all nuclease or other inhibitory activities. During purification by this scheme, we observed co-fractionation of DNA polymerase and primase, and at the same time permit removal of all nuclease or other inhibitory activities (Table I). The simple chromatographic procedures resulted in an 85-fold and 760-fold purification of DNA polymerase and primase, respectively. When stored at \(-20^\circ C\), both activities were stable for at least 2 months.

**Suppression of Mitochondrial Primase and DNA Polymerase**

The chromatographic fractionation of mitochondrial enzymes was designed to allow maximal recovery of activities that may be involved in DNA replication, such as DNA polymerase and primase, and at the same time permit removal of all nuclease or other inhibitory activities. During purification by this scheme, we observed co-fractionation of DNA polymerase and primase activities. A tight association between cytosolic DNA primase activity and the major nuclear DNA polymerase activity has been observed previously in the purification of other eukaryotic DNA primases (17-22). In order to determine whether or not the mitochondrial primase and DNA polymerase activities are tightly associated, we subjected fraction III to analytical centrifugation in glycerol density gradients. As shown in Fig. 2, mitochondrial primase activity appeared to be a very fast sedimenting entity, with an apparent sedimentation coefficient considerably greater than 11.3 S. On the other hand, DNA polymerase activity had an apparent sedimentation coefficient between 7.6 S and 11.3 S. While the bulk of the two enzymatic activities were well resolved from each other, there was a fraction (10 to 20%) of each activity that co-sedimented with the other activity. DNA polymerase \( \gamma \) has been purified from chick embryos and shown to be an oligomeric enzyme with subunits of 47,000 daltons (23). The sedimentation rate of the peak of DNA polymerase activity shown in Fig. 2 is therefore consistent with the physical property of DNA polymerase \( \gamma \). Mitochondrial RNA polymerase has also been identified in KB cells and was found to have a sedimentation coefficient of about 8 S (5). Thus it appears that the mitochondrial enzyme responsible for priming DNA replication on single-stranded templates is different from the major mitochondrial polymerase. 

**Fraction III Catalyzes RNA-primed DNA Synthesis**

Fraction III was characterized for its ability to support DNA replication on biological and artificial DNA templates. With single-stranded M13 DNA, fraction III catalyzed DNA synthesis that was dependent on the presence of rNTPs in the reaction mixture (Fig. 3A). The activity increased sigmoidally with increasing enzyme concentration up to about 85 \( \mu \)g/ml. Higher concentrations of enzyme resulted in a drastic decrease in DNA synthesis. A small but significant amount of DNA synthesis occurred also in the absence of rNTPs. Analysis of these latter reaction products by agarose gel electrophoresis showed that they co-migrated with linear single-stranded DNA. They are therefore most likely products of elongation of 3'-hydroxyl ends of fold-back structures present in nicked phage DNA. Unlike the ammonium sulfate fraction, fraction III did not stimulate mitochondrial-specific DNA synthesis when M13KBLmo-H DNA was used as template (data not shown). This apparent discordance may be because the fractionation on phosphocellulose resulted in a reduction in concentrations of factors and enzymes that are required for interaction with \( O_{2} \), see "Discussion".

With poly(dT) as template, fraction III was also able to support the synthesis of poly(dA) (Fig. 3B). The activity was absolutely dependent on the presence of ATP and no DNA synthesis was detected in its absence. The extent of DNA synthesis could be enhanced 7- to 10-fold by the addition of 0.4 unit of \( E. coli \) DNA polymerase I large fragment (data not shown). We also compared different homopolymers for their ability to serve as templates for fraction III. As shown in Table II, poly(dC) appears to be the most efficient template for DNA replication by the mitochondrial primase. The rate of DNA synthesis with poly(dT) was only 25% of that obtained with poly(dC). In contrast, both poly(dA) and poly(dG) were relatively ineffective templates for the primase activity. 

**Supplementation of the latter two reactions with ATP and GTP did not result in any increase in the extent of DNA synthesis.** At present the basis for this rather specific template preference is not understood.

The replication of M13 DNA using fraction III was assayed as a function of time of incubation and was found to exhibit an initial lag of about 15 min before achieving its maximal rate (Fig. 4, closed circles). The initial lag varied from 15 to 17 min, after which the reaction remained linear for more than 60 min. The lag time was not due to temperature re-equilibration because all components had been preincubated separately at 37 °C before the reaction was initiated. However, a 15-min preincubation of enzyme, DNA, Mg\(^{2+}\), and rNTPs together was able to reduce the lag time to less than 5 min (Fig. 4, squares). All four rNTPs had to be present in the preincubation in order for the shift in time course to be observed. In contrast, rNTP-independent DNA synthesis achieved a linear rate as soon as the reaction was started. These results suggest that the synthesis of primer DNA is a relatively slow process compared to DNA chain elongation. Primer formation may therefore be a rate-limiting step in single-stranded DNA replication involving the mitochondrial primase.

**Discrete Oligoribonucleotide Primers Are Synthesized by Mitochondrial Fraction III Enzymes**

Data presented above suggest that fraction III is capable of catalyzing the synthesis of RNA primers that are subsequently elongated by DNA polymerase to form nascent DNA. In order to determine the nature of these RNA primers, we carried out a replication assay with fraction III and M13 DNA. The reaction mixture contained also radiolabeled GTP and the other three rNTPs, but no added dNTPs. Upon fractionation in a denaturing polyacrylamide gel, the radiolabeled products were found to be mostly oligonucleotides (Fig. 5, lane 1). The sizes of these oligonucleotides exhibited a modal distribution ranging from...
FIG. 2. Glycerol density gradient sedimentation profile of fraction III. Mitochondrial enzyme fraction III (4.4 µg) was fractionated on a 10 to 30% glycerol gradient as described under "Experimental Procedures." Fractions were collected from the bottom of the centrifuge tube and 10-µl aliquots were assayed for DNA polymerase γ (O) activity using poly(A)-oligo(dT) primer-template and for primase (●) activity using poly(dT) template. Recovery of DNA polymerase activity was about 20%.

FIG. 3. Replication of single-stranded DNA templates catalyzed by mitochondrial enzyme fraction III. A, M13mp7 phage DNA (20 µg/ml) was incubated with fraction III in the absence (O) or presence (●) of added rNTPs. B, poly(dT) (10 µg/ml) was incubated with fraction III in the absence (O) or presence (●) of added ATP.

TABLE II

| DNA     | rNTP  | [α-32P]dNTP DNA synthesis
|---------|------|---------------------------|
|         |      | pmol/h                    |
| Poly(dA) | None | TTP                       |
|         | UTP  | TTP                       |
| Poly(dC)| None | dGTP                      |
|         | GTP  | dGTP                      |
| Poly(dG)| None | dCTP                      |
|         | CTP  | dCTP                      |
| Poly(dT)| None | dATP                      |
|         | ATP  | dATP                      |

DNA replication assays were carried out as described under "Experimental Procedures." Each reaction mixture contained DNA at 12 µg/ml, fraction III at 18 µg/ml, rNTP at 0.8 mM, and [α-32P]dNTP at 10 µM (10,000 to 20,000 cpm/pmol).

FIG. 4. Time course of DNA replication catalyzed by mitochondrial enzyme fraction III. M13mp7 phage DNA (20 µg/ml) was incubated in buffered reaction mixtures that contained 10 mM MgCl₂, mitochondrial fraction III at 26 µg/ml, and no additional reagent (O, ●), or 200 µM each of all four rNTPs (■). Following a 15-min incubation at 37 °C, DNA synthesis was initiated by adding either dNTPs (O, ■) or dNTPs with rNTPs (●).
4). These data indicate that fraction III synthesized a group of small RNAs, mostly between 9 and 12 nucleotides in size, that were covalently linked to nascent DNA. The existence of DNase-sensitive products in reaction mixtures that contained no added dNTPs is most likely due to the presence of contaminating dNTPs in commercial preparations of rNTPs. Similar phenomena have previously been documented by other investigators (17, 18, 22).

Characteristics of DNA Replication Activities in Fraction III—The rNTP requirements of M13 DNA replication catalyzed by fraction III were examined and the results are shown in Table III. While all four rNTPs are required for maximal DNA synthesis, ATP seems to be the most critical component; omission of ATP from the reaction mixture drastically reduces DNA replication. On the other hand, omission of any one of the other three rNTPs, such as GTP, resulted in only about a 50% reduction in DNA synthesis. We also examined the effects of a number of reagents on the replication reaction. The addition of rifampicin or α-amanitin to the reaction mixture did not have any deleterious effect on the replication of M13 DNA. These reagents are known to inhibit prokaryotic and eukaryotic nuclear RNA polymerases, respectively. Also, the reaction seemed to be insensitive to aphidicolin at concentrations that inhibit DNA polymerase α (26). Drastic inhibition of DNA replication was achieved with the use of high ionic strength, N-ethylmaleimide, or the chain terminator dideoxynucleoside triphosphate. Insensitivity of the replication reaction to aphidicolin, coupled with its sensitivity to N-ethylmaleimide and ddTTP, provide strong evidence that the DNA elongation process is catalyzed by DNA polymerase γ (27, 28).

DISCUSSION

In this report we have described the identification and characterization of human mitochondrial enzymes that are capable of initiating DNA replication in vitro. These enzymes, referred to as fraction III, support replication of single-stranded DNA templates such as M13 phage DNA and poly(dT). The replication process is absolutely dependent on the presence of rNTPs in the reaction mixture. We have presented evidence that the rNTPs are used to synthesize RNA primers of 9 to 12 nucleotides. These primers are elongated by an activity inferred to be DNA polymerase γ on the basis of its sensitivity to several diagnostic reagents. The assays that have been described in this report were not able to detect any specific initiation at Oα using fraction III and M13KBLα·H DNA. However, we have been able to achieve that goal using a more sensitive assay.2 Under the assay conditions employed here, fraction III is not able to support replication of duplex DNA.3 The use of single-stranded DNA templates has significant biological relevance because the region of mtDNA where L-strand replication initiates is exposed as a single-stranded region at the onset of L-strand replication (16).

Properties of the primase activity in fraction III closely resemble those of other eukaryotic primase activities (17–22, 24, 25). In all but one of these other cases, the primase activity was found to be tightly associated with the major nuclear DNA polymerase activity. This report represents the first description of a primase activity that is present in mitochondria and is presumably involved with replication of the mitochondrial genome. At present there is no indication as to whether the mitochondrial primase may be physically identical to the primase associated with DNA polymerase α in KB cell cytosol (24). We have provided evidence that even though the bulk of DNA polymerase γ and primase activities can be separated from each other, there exists a potential for the two

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2 T. W. Wong and D. A. Clayton, manuscript submitted.
3 T. W. Wong, unpublished results.
activities to participate in the formation of a multi-enzyme complex. The isolation of the replication enzymes should make it feasible to study the enzymatic interactions that are crucial to mtDNA replication and also to delineate the molecular mechanism of the replication process.

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REFERENCES
1. Clayton, D. A. (1982) *Cell* 28, 693–706
2. Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R., and Young, I. G. (1981) *Cell* 290, 457–465
3. Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, M. W., and Clayton, D. A. (1981) *Cell* 26, 167–180
4. Anderson, S., de Bruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F., and Young, I. G. (1982) *J. Mol. Biol.* 156, 683–717
5. Walberg, M. W., and Clayton, D. A. (1983) *J. Biol. Chem.* 258, 1268–1275
6. Chang, D. L., and Clayton, D. A. (1984) *Cell* 30, 635–643
7. Forsheit, A. B., and Ray, D. S. (1971) *Virology* 43, 647–664
8. Bogenhagen, D., and Clayton, D. A. (1974) *J. Biol. Chem.* 249, 7991–7995
9. Bradford, M. (1976) *Anal. Biochem.* 72, 248–254
10. Maxam, A., and Gilbert, W. (1980) *Methods Enzymol.* 65, 499–560
11. Kornberg, A. (1980) *DNA Replication*, pp. 347–411, W. H. Freeman, San Francisco, CA
12. Gillum, A. M., and Clayton, D. A. (1979) *J. Mol. Biol.* 135, 353–368
13. Brennicke, A., and Clayton, D. A. (1981) *J. Biol. Chem.* 256, 10613–10617
14. Chang, D. D., and Clayton, D. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 351–355
15. Tapper, D. P., and Clayton, D. A. (1981) *J. Biol. Chem.* 256, 5109–5115
16. Robbersen, D. L., Kasamatsu, H., and Vinograd, J. (1972) *Proc. Natl. Acad. Sci. U. S. A.* 69, 737–741
17. Chang, L. M. S., Rafter, E., Angl, C., and Bollum, F. J. (1984) *J. Biol. Chem.* 259, 14879–14887
18. Conaway, R. C., and Lehman, I. R. (1982) *Proc. Natl. Acad. Sci. U. S. A.* 79, 2523–2527
19. Gronostajski, R. M., Fled, J., and Hurwitz, J. (1984) *J. Biol. Chem.* 259, 9479–9486
20. Shiota, M., Nelson, E. M., Bayne, M. L., and Benbow, R. M. (1982) *Proc. Natl. Acad. Sci. U. S. A.* 79, 7209–7213
21. Singh, H., and Dumas, L. (1984) *J. Biol. Chem.* 259, 7936–7940
22. Wang, T. S.-F., Hu, S.-Z., and Korn, D. (1984) *J. Biol. Chem.* 259, 1854–1865
23. Yamaguchi, M., Matsukage, A., and Takahashi, T. (1980) *J. Biol. Chem.* 255, 7002–7009
24. Tseng, B. Y., and Ahlem, C. N. (1982) *J. Biol. Chem.* 257, 7280–7283
25. Yagura, T., Kozu, T., and Seno, T. (1982) *J. Biol. Chem.* 257, 11121–11127
26. Edenberg, H. J., Anderson, S., and DePamphilis, M. L. (1978) *J. Biol. Chem.* 253, 3273–3280
27. Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H., and Mano, Y. (1978) *Nature* 275, 458–459
28. Knopf, K. W., Yamada, M., and Weisebach, A. (1976) *Biochemistry* 15, 4540–4548