Purification of a Mitochondrial DNA Polymerase from Crithidia fasciculata*

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The mitochondrial DNA polymerase from Crithidia fasciculata has been purified to near homogeneity. SDS-PAGE analysis of the purified enzyme reveals a single polypeptide with a molecular weight of approximately 43,000. The protein is basic, with an isoelectric point between 7.6–8.0. Its Stokes radius of 22 Å and its sedimentation coefficient of 4.1 S suggest a native molecular weight of 38,000, indicating that the protein is a monomer under our experimental conditions. Western blots and immunoprecipitations of crude extracts reveal a cross-reacting protein of 48 kDa, suggesting that the purified enzyme may be an enzymatically active proteolytic product. The mitochondrial origin of the polymerase was confirmed by cell fractionation. Our results indicate that the C. fasciculata enzyme may be among the smallest known mitochondrial polymerases.

Five classes of DNA polymerase, designated α, β, γ, δ, and ε, have been isolated from eukaryotic cells (1–4). DNA polymerases α, δ, and ε are nuclear enzymes associated with chromosomal replication, although their precise roles have not been deduced. DNA polymerase β is a low molecular weight, nuclear enzyme involved in DNA repair (2, 5, 6). DNA polymerase γ is the only DNA polymerase that has been isolated from mitochondria (2, 4, 7). Although this enzyme has also been detected in nuclear and cytosolic fractions, it is believed to be responsible for mitochondrial DNA replication.

DNA polymerase γ has been isolated from several eukaryotic organisms, and there are substantial differences in enzymes from different species. In chick embryo the enzyme is a homotetramer consisting of 47-kDa subunits (8), in Tetrahymena thermophila it is a heterodimer of 52 and 47 kDa (9), in Drosophila melanogaster it is a heterodimer of 125 and 35 kDa (10), and in Xenopus laevis it is a monomer of 140 kDa (11). Characterization of the partially purified mitochondrial DNA polymerase of Saccharomyces cerevisiae suggests similarities to DNA polymerase γ (12). The yeast nuclear gene MIP1, which encodes the catalytic subunit of the enzyme, possesses an open reading frame of 3762 nucleotides that would encode a basic protein of 143 kDa (13).

Although polymerase γ is one of the most poorly characterized eukaryotic DNA polymerases, several distinctive features of the enzyme have been used to identify the activity (2, 4, 14, 15). This enzyme can utilize the primer-template poly(rA)·poly(dT), and there are reports that in the presence of Mn²⁺ the enzyme actually prefers this template over either poly(dA)·poly(dT) or activated DNA. It is inhibited by sulfhydryl agents such as N-ethylmaleimide and by dideoxynucleoside triphosphates. It is normally stimulated by salt (200 mM KCl), and it is resistant to aphidicolin.

We are studying the enzymatic mechanism of kinetoplast DNA replication in Crithidia fasciculata. C. fasciculata are protozoan parasites in the family Trypanosomatidae that contain a single mitochondrion (cell 16). Within each mitochondrion is a DNA network known as the kinetoplast DNA, consisting of about 25 maxicircles (37 kilobases) and 5000 minicircles (2.5 kilobases), all of which are topologically interlocked. The maxicircles, which are functionally analogous to mitochondrial DNAs in other eukaryotes, replicate by a rolling circle mechanism. The minicircles, which encode guide RNAs involved in editing of many maxicircle transcripts (17), replicate by a Cairns-type mechanism. Minicircle replication occurs after the circles detach from the network and the progeny are reattached to the network periphery (kinetoplast DNA replication is reviewed in Ref. 18).

There is little known about the enzymatic reactions involved in kinetoplast DNA replication. The only homologous replicative enzyme that has been conclusively shown to be mitochondrial is a topoisomerase II (19). In an attempt to understand the mechanism of kinetoplast replication at the biochemical level, we are currently investigating various mitochondrial proteins that may be involved in the process. Previous studies have reported the identification and partial purification of DNA polymerases from Trypanosoma brucei and C. fasciculata (20–22). Characterization of these DNA polymerases suggested that they were α-like and β-like enzymes, and there was no indication as to whether they were mitochondrial in origin. We describe here the purification and initial characterization of a C. fasciculata mitochondrial DNA polymerase.

MATERIALS AND METHODS

DNA Polymerase Assay—DNA polymerase was assayed by the method of Wernette and Kaguni (10), with some modifications. The reactions (50 µl) contained 50 mM Tris-HCl (pH 9.0), 5 mM MgCl₂, 0.1 mg/ml BSA, 30 µM [α-³²P]dTPP (1000–3000 Ci/mmol), and 0.15 mg/ml activated calf thymus DNA (23). As will be discussed below, only a single deoxynucleoside triphosphate is used in this assay. After 15 min at 37 °C, the DNA was precipitated by the addition of 960 µl of 2 M HClO₄, 0.1 M Na₂HPO₄. The samples were collected under vacuum on GF/C filters (Whatman) and washed, first with 1 M HCl,

1 The abbreviations used are: BSA, bovine serum albumin; PMSE, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MOPS, 4-(morpholinopropanesulfonic acid); FPLC, fast protein liquid chromatography.
0.01 M NaP_2O_4 and then with 95% (v/v) ethanol. The filters were counted with scintillation fluid. One unit is the amount of enzyme that catalyzes incorporation of 1 pmol of dTMP into acid-insoluble material in 15 min at 37 °C. With the purified protein, the assay is linear with enzyme concentration in the range from 0 to 0.5 units.

Large Scale Growth of *C. fasciculata*—Parasites were grown in a fermenter containing 150 liters of 3.7% (w/v) brain heart infusion supplemented with 20 µg/ml hemin, 100 units/ml penicillin G and 0.1 mg/ml streptomycin. The cells were grown at 26 °C with aeration for 20–24 h to a density of 6 × 10^7/ml and then harvested by sedimentation in a Sharples continuous flow centrifuge. The cells were washed in SBG (150 mM NaCl, 0.5% (w/v) glucose, 200 mM sodium phosphate, pH 7.9) and then recovered by sedimentation (5000 × g, 10 min, 4 °C). The yield was approximately 650 g of cells (wet weight).

Percoll Purification of Mitochondria—*C. fasciculata* were grown with vigorous shaking (200 rpm) at room temperature in 8 liters of 3.7% (w/v) brain heart infusion supplemented with 20 µg/ml hemin using two 6-liter flasks. The cells were harvested by centrifugation (5000 × g, 10 min, 4 °C) and lysed mechanically as described below. The "crude mitochondria" were further purified by isopycnic centrifugation in Percoll gradients (24). The mitochondrial pellet was resuspended using a Dounce homogenizer in 200 ml of STE (250 mM sucrose, 20 mM Tris-HCl, pH 7.9, 20 mM EDTA, 0.2 mM PMSF, 1 µg/ml leupeptin, 4 °C) containing 50% (v/v) Percoll and centrifuged in a Ti-70 rotor (40,000 rpm, 30 min, 4 °C). The mitochondria form a band approximately midway in the gradient. After recovery of the band (50 ml), the mitochondria were separated from the Percoll by three washes with STE (500, 100, and 20 ml). The mitochondria were resuspended after each wash by sedimentation (25,000 × g, 30 min, 4 °C), and the final pellet was resuspended in mitochondrial lysis buffer (MLB; 0.5 M KCl, 200 mM Tris-HCl, pH 8.0, 20 mM EDTA, 10 mM DTT, 40% (v/v) glycerol, 10% (v/v) Nonidet P-40, 4 mM PMSF, 20 µg/ml leupeptin, 4 °C). Debris from the mitochondrial lysate was removed by sedimentation (27,000 × g, 30 min, 4 °C).

Production of Antibodies—Antibodies to *C. fasciculata* mitochondrial DNA polymerase were prepared by immunization of either mice (C-57) or a rabbit (New Zealand White). Mice were immunized monthly by intraperitoneal injections with approximately 5 µg of protein that was approximately 50% pure as determined by SDS-PAGE analysis. The rabbit was immunized every 2 weeks with 20 µg of the highly purified enzyme (Fraction 10) with injections distributed along a series of subcutaneous sites. The initial inoculations were prepared with Freund's complete adjuvant, and all subsequent boosts were in Freund's incomplete adjuvant. Both mouse and rabbit antibodies recognize the polymerase (and no other polypeptides) on Western blots. In addition, the mouse antibodies immunoprecipitate the native protein and will deplete enzyme activity in the presence of protein-A Sepharose.

**TABLE I**

<table>
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<tr>
<th>Fraction number</th>
<th>Purification step</th>
<th>Total protein</th>
<th>Poly- merase activity</th>
<th>Specific activity</th>
<th>Purification</th>
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<td>1</td>
<td>Mitochondrial lysate supernatant</td>
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<td>1</td>
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<tr>
<td>2</td>
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<td>4</td>
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<td>3</td>
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<td>17,000</td>
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<tr>
<td>8</td>
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<td>2,100</td>
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<td>10</td>
<td>Superose 12</td>
<td>0.5</td>
<td>890</td>
<td>1,800</td>
<td>19,000</td>
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</table>

**RESULTS**

Purification of Mitochondrial Polymerase—Except where indicated otherwise, all operations were conducted at 4 °C using Buffer C (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT, 20% (v/v) glycerol, 0.1% (v/v) Nonidet P-40, 0.2 mM PMSF, 1 µg/ml leupeptin), and all dialyses were performed against Buffer C at 4 °C. All filtrations were accomplished...
with 0.2-μm membranes (Nalgene 500-ml or Millipore Millex-GV filter units). After obtaining C. fusciculata cells, the entire isolation was based on our previous method (25). Cells (325 g; about 4.5 \times 10^{10} cells) from a 75-liter culture were resuspended at 0 °C in 500 ml of digitonin lysis buffer (DLB; 0.25 M mannitol, 10 mM MOPS, pH 7.5, 250 μM MgCl₂, 250 μM EDTA, 5 μM L-ascorbic acid, 0.6 mg/ml polyvinylpyrrolidone, 0.3 mg/ml BSA, 0.2 mM PMSF, and 1 μg/ml leupeptin); they were then treated with a digitonin solution (0.1 g of digitonin/ml of N,N-dimethylformamide, diluted 1:10 in DLB). The digitonin solution was added slowly, with gentle mixing, until the final concentration was 1 g of digitonin/100 g of wet cell pellet weight. After swelling on ice for 2 h, the cells were centrifuged (5000 \times g, 10 min, 4 °C) and resuspended in 500 ml of fresh DLB without digitonin. The cells were then lysed with a Polytron (Brinkmann Instruments) at 4 °C (the lysate contained 2200 units of polymerase activity). The lysate was centrifuged (27,000 \times g, 30 min, 4 °C; the supernatant contained 400 units of polymerase activity), and the pellet was resuspended in 500 ml of STE. Following another centrifugation (27,000 \times g, 30 min, 4 °C; the supernatant contained 350 units of activity) the pellet, designated the crude mitochondria fraction (containing 1900 units of activity), was resuspended in 1000 ml of MLB to lyse the mitochondria. Debris from the mitochondrial lysate was removed by sedimentation (27,000 \times g, 30 min, 4 °C).

**Purification Scheme**—The mitochondrial lysate (Fraction 1, 1000 ml) was diluted 4-fold with Buffer C and treated batchwise with DEAE-Sephadex (Sigma, 500-ml packed volume), which does not bind the polymerase; however, this step was essential for efficient binding of the enzyme to phosphocellulose. The combined DEAE-Sephadex supernatant and wash (Fraction 2, 4000 ml) was treated batchwise with phosphocellulose (Whatman P-11, 500-ml packed volume) at 4 °C for 1 h. The buffer was transferred to a funnel with a glass wool plug, and protein was eluted with a KCl step gradient (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 M; 200 ml/step). The polymerase activity eluted in a broad peak between 0.3 and 0.7 M KCl (Fraction 3, 1000 ml).

Fraction 3 was loaded directly onto a 200-ml Bio-Gel HT column (Bio-Rad; 5 \times 15-cm column) at 0.8 ml/min, which was then eluted using a 1200-ml linear gradient from 0 to 1 M K₂HPO₄. The enzyme eluted at about 0.56 M, and the active fractions were dialyzed and filtered. Although there was a significant loss of activity during this step, the enzyme was concentrated to a smaller volume (Fraction 4, 150 ml).

Fraction 4 was loaded onto a 10-ml single-stranded DNA cellulose column (ssDNA, Pharmacia LKB Biotechnology Inc.; 1 \times 10-cm column). The polymerase was eluted at 0.4 ml/min using a 100-ml linear gradient from 0 to 1 M KCl. The active fractions (50 ml, between 0.3 and 0.6 M salt) were pooled, dialyzed, filtered (Fraction 5, 130 ml), and loaded onto a 10-ml Cibacron Blue agarose column (Sigma; 1 \times 10-cm column) at 0.4 ml/min. Proteins were eluted with a 120-ml linear salt gradient from 0 to 1 M KCl, and the polymerase activity was recovered at 0.2 M salt. Active fractions were again pooled, dialyzed, filtered (Fraction 6, 50 ml), and loaded onto a 1-ml Mono S FPLC column (Pharmacia) at 0.5 ml/min. Using a 30-ml linear gradient from 0 to 1 M KCl, the active fractions were pooled (Fraction 7, 5 ml), adjusted to 1 M salt with 5 M K₂HPO₄, filtered, and loaded at 0.05 ml/min onto a 1-ml phenyl-Superose FPLC column (Pharmacia). A 30-ml linear negative gradient from 1 to 0 M K₂HPO₄ was used to recover the polymerase activity that eluted at 0.75 M salt. The active fractions were pooled, dialyzed against Buffer PL (25 mM triethylamine HCl, pH 11.0, 1 mM EDTA, 1 mM DTT, 20% (v/v) glycerol, 0.1% (v/v) Nonidet P-40), filtered (Fraction 8, 6 ml), and loaded onto a 4-ml Mono P FPLC column (Pharmacia) at 0.05 ml/min. Polymerase was eluted with Buffer PE (1 mM EDTA, 1 mM DTT, 20% (v/v) glycerol, 0.1% (v/v) Nonidet P-40), filtered (Fraction 9, 8 ml), and loaded onto a 4-ml Mono P FPLC column (Pharmacia) at 0.05 ml/min. The active fractions were pooled and loaded (Fraction 9, 5 ml). Fraction 9 was concentrated to 0.5 ml by binding to a 1-ml Mono S FPLC column (0.5 ml/min) and eluting with 1 M KCl. The final chromatographic procedure was gel filtration on a 30-ml Superose 12 FPLC column (Pharmacia) at 0.2 ml/min. The highly purified C. fusciculata mitochondrial DNA polymerase (Fraction 10, 2 ml) was adjusted to 50% (v/v) glycerol and stored at −20 °C.

Starting from the mitochondrial lysate supernatant, the DNA polymerase was purified 19,000-fold (Table I). The yield
from 325 g was about 0.5 mg of highly purified enzyme (Fraction 10). The enzyme is fairly stable under our storage conditions, maintaining over 50% of its activity after more than 4 months. Fraction 10 was used in all characterization experiments unless otherwise indicated.

**SDS-PAGE and Western Blot Analysis of Purification**—SDS-PAGE of aliquots from each step in the purification demonstrate that the final product is a single protein of 43 kDa (Fig. 1A). An identical gel was electrophoretically blotted onto Immobilon-P membrane, probed with rabbit anti-polymerase serum, and visualized using an anti-rabbit secondary antibody conjugated to alkaline phosphatase (Fig. 1B). These results confirm the enrichment of the 43-kDa protein at each step in the purification. Although the Western blot reveals that the 43-kDa protein is also present in the crude extract (lane 1), early fractions (lanes 1–4) also contain a cross-reacting protein of 47 kDa. It is possible that the 47-kDa protein is the native form of the enzyme; proteolysis during purification could result in formation of the 43-kDa form. No proteins larger than 47 kDa were detected by Western blot, although a smaller fragment of 35 kDa, which may be a proteolytic fragment, was seen in lanes 8 and 9.

**Immunoprecipitation of the Polymerase**—Cells labeled with [35S]methionine for 3 h were lysed with Nonidet P-40. After removal of debris by sedimentation, the lystate supernatant was incubated with mouse antiserum. Antibody-antigen complexes were recovered with protein A-Sepharose, analyzed by SDS-PAGE, and visualized by fluorography (Fig. 2). A protein of approximately 48 kDa was precipitated by the antibodies; this is probably the same polypeptide detected by Western blotting of Fractions 1–4. No other polypeptides were precipitated by the antibody; therefore, there is no suggestion that the polymerase exists in a stable complex with other 35S-labeled proteins.

**Activity Co-purifies with a 43-kDa Protein**—To determine whether polymerase activity co-purifies with the 43-kDa protein, fractions obtained from Cibacron Blue agarose (Fig. 3, A and B) and phenyl-Superose chromatography (Fig. 3, C and D) were examined. Fractions from each of the chromatographic elutions were assayed for polymerase activity and also analyzed by SDS-PAGE. These experiments indicated that the mitochondrial DNA polymerase activity co-purified with the 43-kDa protein. Furthermore, the level of enzyme activity in each fraction was proportional to the amount of the 43-kDa protein present as indicated by the intensity of the silver-stained band. Similar results were obtained when the enzyme was fractionated on Bio-Gel HT, ssDNA cellulose, Mono S, Mono P, and Superose 12 FPLC.

**Characterization of the Polymerization Reaction**—In assays of the DNA polymerase, higher incorporation of radioactivity was detectable when a single dNTP was added to the assay mix than when all four dNTPs were added. The addition of
the other three dNTPs actually reduced the incorporation of the $\alpha^{32}$P-labeled nucleotide. This effect could either be the result of very low enzyme processivity, or it could be due to the enzyme not polymerizing nucleotides in response to a template. To test its ability to copy a template, we used M13 DNA primed with a 5'-$^{32}$P-labeled oligonucleotide. As shown in the dideoxyribonucleotide chain termination reactions in Fig. 4, the polymerase precisely copied the template, allowing accurate reading of over 30 bases of its nucleotide sequence.

**Determination of Molecular Weight of the Native Protein**—
This value was calculated from the Stokes radius (determined by Superose 12 FPLC) and the sedimentation coefficient (determined by glycerol gradient centrifugation). Comparisons were made with two reference proteins, albumin (66 kDa) and carbonic anhydrase (29 kDa). The Stokes radius for the polymerase is 22 Å (Fig. 5A), and the sedimentation coefficient is 4.1 S (Fig. 5B). From these values, the molecular weight of the polymerase was calculated as 38,000 (using the Svedberg equation and assuming a partial specific volume of 0.725 cm$^3$/g). Since this was calculated using only two marker proteins, it may not be a precise value. Nevertheless, it is within 10% of the molecular weight obtained by SDS-PAGE, thus demonstrating that this enzyme is monomeric.

**Isoelectric Focusing of the Polymerase**—The isoelectric point of the C. fasciculata polymerase was determined by two-dimensional isoelectric focusing performed by the method of O'Farrell (26). The first dimension involved electrophoresis of the protein through a polyacrylamide gel with a pH gradient between 3.5 and 10.0. The second dimension was SDS-PAGE, and proteins were visualized by silver stain (Fig. 6). The purified polymerase contained two species (both 43 kDa) with apparent pl values of 7.6 and 8.0.
A C. fasciculata Mitochondrial DNA Polymerase

Fig. 6. Isoelectric focusing. Electrophoresis in the first dimension was in a tube gel using Ampholines (Pharmacia LKB Biotechnology Inc.) covering the pH range from 3.5 to 10. Electrophoresis in the second dimension was by SDS-PAGE (12% (w/v) gel), and the proteins were visualized by silver staining. The pH was estimated by cutting up a "mock" tube gel into 5-cm slices, soaking the slices in 3 ml of water, and measuring the pH directly (shown at the bottom of the gel). Molecular mass markers for the second dimension are shown to the left of the panel. The position of the polymerase is indicated by the arrow on the right.

Isolation of Polymerase from Mitochondria—As a first step in determining whether the DNA polymerase actually is mitochondrial in origin, we conducted an isolation from mitochondria purified on a Percoll gradient. The Percoll fraction was composed of vesicles along with some flagellar components. The presence of flagella is not surprising in light of a recent observation of a direct association between the flagella and the kinetoplast (27). Approximately half the vesicles contained the brightly staining kinetoplast DNA (as visualized by fluorescent microscopy in the presence of ethidium bromide) that is characteristic of Crithidia mitochondria. The Percoll-purified mitochondria were lysed, and the polymerase activity was partially purified using hydroxylapatite chromatography, single-stranded DNA cellulose chromatography, Mono S FPLC, and sedimentation on a glycerol gradient. After each purification step, the fractions were assayed for activity and analyzed by SDS-PAGE; the results of two of these comparisons are presented in Fig. 7. Panels A and C show the results for the glycerol gradient, whereas panels B and D give the results for the Mono S FPLC. In each case, the DNA polymerase activity coincided with the enrichment of a protein of 43 kDa on SDS-PAGE. Based on size, activity, and purification characteristics, the polymerase activity isolated from the Percoll-purified mitochondria appeared identical with that isolated from the crude mitochondrial fraction.

DISCUSSION

We have purified a mitochondrial DNA polymerase from C. fasciculata 19,000-fold to virtual homogeneity. The polymerase activity co-purifies with a protein of 43 kDa, as determined by SDS-PAGE, placing it among the smallest enzymes of this type from any species. The molecular weight of the native protein, as determined by gel filtration and glycerol gradient centrifugation, is about 38 kDa, indicating that the protein is monomeric under conditions used in our experiments. In this respect, the C. fasciculata enzyme differs from mitochondrial polymerases from other sources that are often multimeric or have larger subunit molecular weights. There are indications, however, that the enzyme we have purified...
could be a proteolytic fragment of the enzyme found in the cell. Western blots of cell lysates prepared in SDS reveal not only the 43-kDa protein but also a 47-kDa species. Western blots of purification fractions show that the 47-kDa species disappears after Fraction 4, whereas the 43-kDa species persists. Interestingly, it is also at this point, during the hydroxyapatite step, that there is a significant loss of enzyme activity. This raises the possibility that the 43-kDa protein is actually a degradation product of the 47-kDa species (our current efforts to clone the polymerase gene should reveal the size of its primary translation product). In support of this conjecture, immunoprecipitations of lysates of [35S]methionine-labeled cells, under non-denaturing conditions, revealed a single 48-kDa species on SDS gels. No other [35S]methionine-labeled protein was detected in this experiment, suggesting that the polymerase polypeptide does not exist in a stable complex with other proteins.

Although the purified enzyme may be a degradation product, it still exhibits polymerase activity. Our standard assay, used in its purification, contained only a single [32P]dNTP. To prove that the enzyme actually is a DNA polymerase that copies a primed template in the 5' to 3' direction, we used a ddNTP sequencing assay. The C. fasciculata polymerase accurately copied the template, the polymerase, however, does differ strikingly from y-polymerases found in other mitochondria. Unlike most mitochondrial polymerases, it does not efficiently utilize a poly(rA).poly(dT) primer-template, it appears to be very nonprocessive, and it is inhibited by 200 mM KCl. However, it is similar to other mitochondrial polymerases in that it is N-ethylmaleimide-sensitive (activity is inhibited 50% under standard assay conditions after pretreatment with 2 mM N-ethylmaleimide for 60 min at 37°C) and resistant to aphidicolin (up to 40 μg/ml under standard assay conditions). This DNA polymerase has some similarities to DNA polymerase B, one of the two polymerases previously identified in extracts of C. fasciculata (22).

Because the C. fasciculata polymerase differed from many mitochondrial DNA polymerases, it was essential that we carefully evaluate whether the 43-kDa enzyme was actually derived from mitochondria. The enzyme was isolated from a crude mitochondrial fraction, and its solubilization depended on extraction with detergent and high salt, consistent with it being an organellar enzyme. Furthermore, we could isolate the same polypeptide from a mitochondrial fraction purified on a Percoll gradient, minimizing the possibility that the enzyme derived from some other cellular location. Finally, immunofluorescence microscopy using either the rabbit or mouse antibody indicated that the polymerase polypeptide localized to the mitochondrion; little background fluorescence was detected in these experiments.

We are continuing our characterization of the enzyme, especially its processivity, fidelity, and precise localization in the mitochondria. We are currently screening a cDNA library for the polymerase; the clone will be used to deduce the protein’s amino acid sequence and to assess the regulation of the enzyme during the C. fasciculata cell cycle. We are also presently involved in a search for other proteins that may associate with the polymerase and facilitate its activity. Eventually we hope to establish an in vitro replication system to further analyze the enzymology of the replication process. These studies should reveal further details into the precise mechanism of kinetoplast replication.

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REFERENCES


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