Partial purification and characterization of the DNA polymerase from the cyanelles of *Cyanophora paradoxa*

Melissa A. White, J. Clinton Bailey II, Gordon C. Cannon, Sabine Heinhorst*

Department of Chemistry and Biochemistry, the University of Southern Mississippi, Hattiesburg, MS 39406-5043, USA

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Abstract A DNA polymerase was partially purified and characterized from the photosynthetic organelles (cyanelles) of the protist, *Cyanophora paradoxa*. While cyanelles have several cyanobacterial features, such as a lysozyme-sensitive cell wall, unstacked thylakoids and light harvesting phycobilisomes, their genome size and structure resemble those of chloroplasts, suggesting that cyanelles occupy a unique intermediate position between chloroplasts and their phylogenetic ancestors, the cyanobacteria. When comparing the biochemical characteristics of the cyanelle DNA polymerase to those of its counterparts from higher plant chloroplasts and from a cyanobacterium, it is clear that the cyanelle enzyme resembles chloroplast DNA polymerases which are eukaryotic γ-type enzymes.

Key words: DNA polymerase; Cyanelle; *Cyanophora paradoxa*

1. Introduction

The unicellular biflagellate protozoan *Cyanophora paradoxa* contains photosynthetic organelles which are commonly referred to as cyanelles. These structures are surrounded by a rudimentary, penicillin sensitive cell wall [1] of which the peptidoglycan structure has recently been elucidated [2]. The fact that cyanelles have unstacked thylakoids which are lined with light harvesting phycobilisomes [3,4] and possess a central body that resembles a cyanobacterial carboxysome [5] had earlier led to the assumption that cyanelles are endosymbiotic cyanobacteria, yet despite many biochemical and physiological similarities between cyanelles and cyanobacteria (reviewed in [6]), both differ drastically in the size of their genomes. While the chromosome of the simplest unicellular, free living cyanobacterium is comparable in size to that of other eubacteria (10^6 bp range) [7], with 135 kbp the circular cyanelle genome is slightly smaller than the highly reduced plastome (150–160 kbp) of plant chloroplasts [8,9]. Cyanelle and chloroplast DNA possess two inverted repeat regions that harbor the genes for the ribosomal RNA species and separate the genome into a large and a small single copy region. The limited coding capacity of these organelle genomes is not sufficient to provide all the information necessary for an independent existence and explains why all attempts to culture cyanelles and chloroplasts extracellularly have failed.

In light of the decidedly cyanobacterial characteristics of cyanelles on one hand and the resemblance of their genome to that of higher plant chloroplasts on the other hand, the organelles of *C. paradoxa* appear to represent an intermediate stage between free living, unicellular cyanobacteria and modern photosynthetic organelles. According to the widely accepted endosymbiont hypothesis of organelle evolution, cyanobacteria are the presumed phylogenetic ancestors of chloroplasts [10,11]. Having invaded a primitive, heterotrophic eukaryote, presumably with time a symbiotic relationship between invader and host cell ensued which, through loss or transfer of genes from the endosymbiont to the host genome, gradually led to the loss of independence for the symbiont, which ultimately evolved into a photosynthetic organelle. Supporting this presumed gene transfer from organelle to nuclear genome are many multi-subunit proteins that function in the chloroplast. Only some subunits of these proteins are encoded by the organelle genome, while the genes for others reside on nuclear DNA. A case in point are the genes for the two subunits of ribulose-1,5-bisphosphate carboxylase (RuBisCO). In most photosynthetic eukaryotes, the small subunit of RuBisCO is encoded by a multigene family in the nucleus and the gene for the large, catalytic subunit of the enzyme is located on the plastome [8,12,13]. In cyanobacteria, the genes for both subunits of RuBisCO are located adjacent to each other and are transcribed from a common promoter [14,15]. Interestingly, the cyanelle genome of *C. paradoxa* also harbors both genes for RuBisCO [16], which are separated by only a small spacer and co-transcribed as well [17].

Because of the unique phylogenetic position of its cyanelles, *Cyanophora paradoxa* is an ideal organism for studies of the phylogenetic relationship between plastids and cyanobacteria and to probe the evolution of molecular mechanisms that operate in photosynthetic organelles. In the course of investigating the DNA replication machinery of soybean chloroplasts and the unicellular cyanobacterium *Anacystis nidulans* R2, we have previously purified and characterized DNA polymerases from both organisms [18,19]. While most higher plant organelle enzymes are members of the eukaryotic γ-type DNA polymerases (reviewed in [20]), the most abundant DNA polymerase from the cyanobacterium is similar in its biochemical properties, confirmed by partial amino acid analysis [19,21], to *E. coli* DNA polymerase I. Here, we describe the characteristics of the DNA polymerase from the cyanelles of *C. paradoxa* as a first step towards elucidating the evolution of genome replication mechanisms in photosynthetic organelles.

2. Materials and methods

2.1. Cell cultures

Cyanophora paradoxa (from the Paris Culture Collection, Institut Pasteur) was maintained in a modified Allen's medium [16,22] under continuous illumination from cool white fluorescent light banks on a shaker. Large scale 4-liter batch cultures in carboys were aerated

*Corresponding author. Fax: 601-266-6075.
E-mail: heinhrst@whale.st.usm.edu

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through an aquarium sparger and harvested by centrifugation 7 days after inoculation.

2.2. Preparation of cyanelles and cyanelle extracts

Cyanelles were purified by freezing and thawing of the *C. paradoxa* cell pellet [16], and stored at −70°C until further use. Several cyanelle preparations were thawed on ice and pooled by resuspension in cold buffer A (30 mM Tris-HCl, pH 8, 20 mM NaCl, 10% glycerol, 1 mM DTT, 0.3 mM PMSF, 0.3 mM PTSF). After addition of an equal volume of cold buffer A containing 300 mM NaCl, the cyanelles were broken by passage through a French pressure cell at 1000 psi and kept on ice for 15 min. Centrifugation at 14000 rpm (Beckman JA 14 rotor) for 20 min at 4°C produced a cleared extract that was subsequently dialyzed against two changes of buffer A.

2.3. Purification of the cyanelle DNA polymerase

All column chromatography steps were performed on a BioRad Econosystem at 4°C. The dialyzed cyanelle protein extract was loaded onto a DEAE cellulose column and eluted with a linear NaCl gradient (20–500 mM) in buffer A. Active fractions were pooled, dialyzed against buffer B (20 mM potassium phosphate, pH 8.0, 0.5 mM DTT, 10% glycerol, 0.1 mM PMSF, 0.1 mM PTSF) and purified further by chromatography on phosphocellulose. The column was developed with a linear potassium phosphate gradient (20–400 mM) in buffer B. Those fractions exhibiting DNA polymerase activity were pooled, dialyzed against buffer C (50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 10% glycerol, 100 mM ammonium sulfate, 0.1 mM PMSF, 0.1 mM PTSF) and loaded onto a heparin agarose column (pre-packed cartridge, BioRad). DNA polymerase was eluted from this column with a linear gradient of 100–500 mM $(NH_4)_2SO_4$ in buffer C.

2.4. DNA polymerase assay

The standard assay for the DNA polymerase from *C. paradoxa* cyanelles contained in a reaction volume of 50 μl: 50 mM Tris-HCl (pH 8), 12 mM $MgCl_2$, 1 mM DTT, 0.14 mg/ml BSA, 200 mM KCl, 0.12 mg/ml activated DNA and a mixture of dGTP, dCTP, dATP at 30 μM concentration, including 3 μM ^{3}H-TTP at a specific radioactivity of 3700 cpm/pmol nucleotides. Assay reactions were incubated for 30 min at 37°C and 45 μl aliquots spotted onto 1 cm^2 DE81 paper (Whatman). The paper squares were washed twice for 7 min each in 0.5 M sodium phosphate, pH 7, and once with ethanol. Bound radioactivity was determined by scintillation counting of the dry paper squares in Scintiverse BD (Fisher Scientific). One unit of activity is defined as 1 pmol of deoxyribonucleotides incorporated into DE81 paper-bound material per hour.

2.5. Exonuclease assays

Activated DNA was 5′-end labeled as described in [23]. To 3′-end label the DNA, the procedure of [24] was followed. Exonuclease assays were performed in 25 μl reactions in the absence of deoxynucleotides, but otherwise as described for the polymerase assays. The DE 81 paper-bound total cpm of the undigested DNA sample (without added enzyme) were subtracted from the remaining bound cpm of a sample with added enzyme, divided by the total cpm of undigested DNA and multiplied by 100 to give % excision.

2.6. Glycerol gradient centrifugation

Cyanelle DNA polymerase, purified through the heparin agarose step, was sedimented through preformed 10–30% glycerol gradients (11 ml) in 50 mM Tris-HCl (pH 8), 225 mM KCl, 0.5 mM DTT, 0.1 mM EDTA, 0.1 mM PTSF, 0.1 mM PMSF. The samples were centrifuged for 60 h at 35000 rpm in a Beckman SW 40 rotor. Forty fractions per gradient were collected and assayed for DNA polymerase activity. Standards for an estimation of the molecular mass of DNA polymerase were β-amylase (M_r = 200000), alcohol dehydrogenase (M_r = 150000), bovine serum albumin (M_r = 66000) and carbonic anhydrase (M_r = 29000).

2.7. Protein determination

Using BSA as a standard, protein concentrations were estimated by the method of Bradford using a commercially available kit (BioRad).

2.8. Polyacrylamide gel electrophoresis

Denaturing gel electrophoresis for proteins was performed according to [25] using a Mini-Protean (BioRad) gel electrophoresis chamber and size standards from the same manufacturer. Polypeptides were visualized after staining with silver according to the manufacturer's (BioRad) instructions.

3. Results

3.1. Purification of the cyanelle DNA polymerase

Rupture of the *Cyanophora paradoxa* host cell at low pressure, as described in Section 2 and in [16], causes negligible breakage of the cyanelles, as judged by the lack of blue color (which would result from release of phycobiliproteins from the thylakoids) into the supernatant after clearing the crude *C. paradoxa* host cell lysate by centrifugation. DNA from cyanelles isolated in this manner had been previously shown to be free of nuclear contamination [16]. In addition, no aphidicolin sensitive DNA polymerase species of presumed nuclear origin were detectable in the organelles (not shown).

The cyanelle DNA polymerase eluted from DEAE cellulose as a broad peak on the trailing edge of the bulk protein at 250 mM NaCl (Fig. 1), with the pooled peak fractions representing a 30% yield. In the following step, typically 60–70% of the cyanelle DNA polymerase activity applied to phosphocellulose was recovered in a single peak of activity that eluted at 300 mM potassium phosphate and was well separated from the majority of the protein (Fig. 1). The specific activity of the pooled active fractions was 10–20-fold higher than that of the phosphocellulose load (Table 1). The enzyme eluted as a single peak of activity at 275 mM $(NH_4)_2SO_4$ from the third purification step, chromatography on heparin agarose (Fig. 1). At this stage of purification, an elution profile for bulk protein could no longer be established since the overall protein concentration was too low. A more than 100-fold purification over the previous chromatography step was achieved. The pooled, active peak fractions eluting from heparin agarose typically represented 5–7% of the activity in the crude cyanelle extract.

No DNA polymerase activity was detectable in the flow-through fraction of any of the three column chromatography steps, and the overall increase in specific activity resulting from these three purification steps was typically more than

Table 1
Purification of the cyanelle DNA polymerase

Purification step	Protein (mg)	Activity (units)[a]	Specific activity (units/mg)	Purification	Yield (%)
Cleared cyanelle lysate	613	26.1	0.043	1	100
DEAE cellulose	105	8.4	0.08	1.9	32
Phosphocellulose	6.4	5.5	0.84	19.8	21
Heparin agarose	0.013	1.7	135	3160	7

[a]A unit of activity is defined as 1 pmol of deoxynucleotides incorporated into filter-bound material per hour at 37°C using activated DNA as a template and Mg^{2+} as the divalent cation.

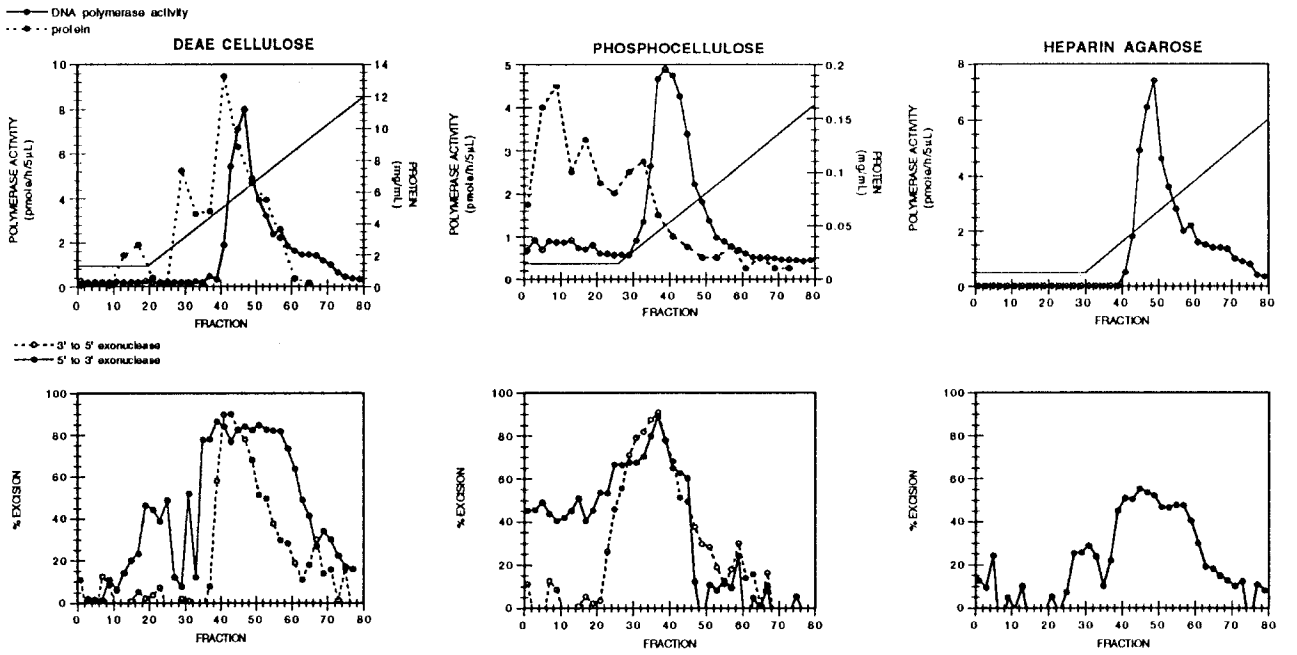

Fig. 1. Elution profiles of DNA polymerase, 3′ to 5′ and 5′ to 3′ exonuclease activities from DEAE cellulose, phosphocellulose and heparin agarose columns. Chromatography and assay conditions are described in detail in Section 2. The solid lines in the upper panels indicate salt concentration.

3000-fold (Table 1). Attempts to further purify the cyanelle enzyme on single stranded DNA cellulose were unsuccessful, since no DNA polymerase activity could be recovered. This could be related to the low protein concentration in the highly purified fraction which, coupled with the limited stability of the enzyme, might prevent recovery of active DNA polymerase. When stored at −70°C in the presence of 10% glycerol and 0.1 mM PTSF and PMSF, the enzyme gradually lost its activity within 6–8 weeks.

3.2. Characterization of the cyanelle DNA polymerase

The molecular weight of the native enzyme was estimated by sedimentation velocity centrifugation through glycerol gradients. DNA polymerase activity in fractions at various stages of purification was present as a single peak of approximately 85 000 molecular weight, as determined by comparison with size standards that were sedimented under identical conditions (Fig. 2).

Using the pooled peak fractions of DNA polymerase activity purified through the heparin agarose step, the assay requirements of this enzyme were assessed (Table 2). The cyanelle DNA polymerase was active over a broad pH spectrum from 7.0 to 9.5 with an optimum at pH 9.0. The temperature optimum was 42°C. The fact that the enzyme had lost 75% of its activity at 55°C indicates that it is heat labile. Like other DNA polymerases, the cyanelle enzyme required a divalent cation for activity and, depending on the template (Table 3), Mg^{2+} or Mn^{2+} was a suitable cofactor in vitro. The cyanelle DNA polymerase was essentially inactive in the absence of salt and displayed maximum activity at 100 mM KCl. By contrast, the enzyme did not tolerate phosphate well, being severely inhibited at phosphate concentrations above 5 mM. The sensitivity of the cyanelle DNA polymerase to *N*-ethylmaleimide (NEM) suggests that the enzyme depends on reduced sulfhydryl groups for activity.

Table 2
Properties of the cyanelle DNA polymerase

Parameter	*Cyanophora paradoxa* Cyanelle DNA polymerase
pH optimum	9.0, broad
Mg^{2+} optimum	12–14 mM
Mn^{2+} optimum	0.25–0.5 mM
KCl optimum	100 mM
Temperature optimum	42°C
KPO_4	Inhibited above 5 mM
NEM	Inhibited
Aphidicolin	Resistant
Ara-CTP (100 µM)	30% inhibited
Ara-CTP (200 µM)	35% inhibited
ddTTP : TTP = 1 : 1	20% inhibited
ddTTP : TTP = 10 : 1	45% inhibited

All assays were performed with activated DNA.

The effect of various inhibitors on the activity of the cyanelle DNA polymerase was determined. The cyanelle enzyme was completely unaffected by 20 µg/ml aphidicolin and largely resistant to 200 µM arabinosyl-CTP (ara-CTP), two inhibitors of DNA polymerase α [26]. Likewise, the chain terminating 2′,3′-dideoxynucleotide ddTTP only moderately affected the cyanelle enzyme. At a ddTTP to TTP ratio of 1 : 1, conditions which completely inactivate animal mitochondrial γ-type DNA polymerases [27,28], the cyanelle enzyme retained 80% of its activity.

Since different DNA polymerases vary in their ability to utilize a particular primed template in vitro [29], the template preferences of the cyanelle enzyme were evaluated in the presence of either Mg^{2+} or Mn^{2+} at their optimal concentrations. The cyanelle DNA polymerase clearly preferred activated DNA in the presence of Mg^{2+} over either poly(dA) or poly(rA) primed with dT-oligomers in the presence of either cation and was unable to utilize unprimed, single stranded circular DNA or double stranded, covalently closed circles (Table 3).

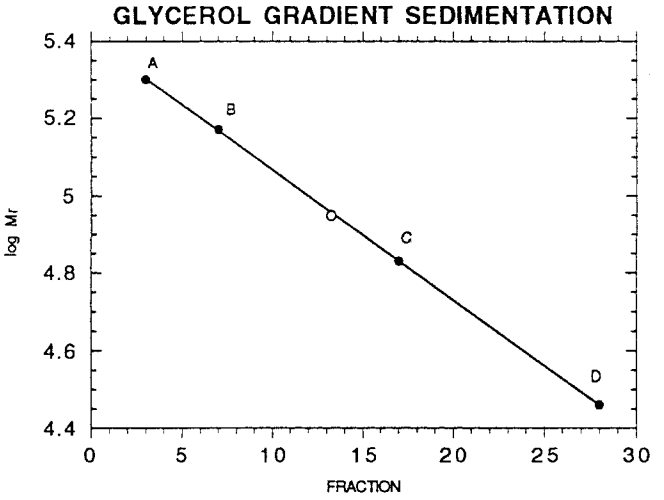

Fig. 2. Glycerol gradient sedimentation of the cyanelle DNA polymerase. The pooled, active fractions eluted from heparin agarose were sedimented through a 10–30% glycerol gradient as described in Section 2. Protein standards were (A) β-amylase (200 000); (B) alcohol dehydrogenase (150 000); (C) bovine serum albumin (67 000); (D) carbonic anhydrase (29 000). The open circle denotes the position of the highest DNA polymerase activity in the gradient.

Slight activity was detected in the presence of Mn^{2+} on oligo-dT primed, polyadenylated RNA.

3.3. *Co-purifying exonuclease activities*

To determine if the DNA polymerase from the cyanelles of *C. paradoxa* is associated with an exonuclease activity, column fractions were scanned with activated DNA radiolabeled on either the 3′- or the 5′-ends as nuclease substrate. The peak of DNA polymerase activity eluting from DEAE- and from phosphocellulose is clearly superimposed on, but not exactly coinciding with, peaks of 3′ to 5′, as well as 5′ to 3′ exonuclease activities (Fig. 1). While no 3′ to 5′ exonuclease activity could be detected in the fractions recovered from heparin agarose with the assay employed, a broad peak of 5′ to 3′ exonuclease activity was co-eluting with the DNA polymerase (Fig. 1).

4. Discussion

Considering that the cyanelle is 'almost a cyanobacterial chloroplast', an expression which was coined by Jaynes and Vernon [6] to acknowledge its unique cyanobacterial and, at the same time, plastid-like features, we are interested in determining the extent to which the cyanelle DNA replication apparatus resembles that of higher plant chloroplasts and which, if any, cyanobacterial attributes have been maintained. According to the endosymbiont hypothesis, the majority of the genes that once resided on the ancestral cyanobacterial genome of the endosymbiont have either been lost or been transferred to the host (nuclear) genome [10,11]. Previous studies have revealed that the majority of chloroplast and cyanelle proteins are, indeed, nuclear-encoded, synthesized on cytoplasmic ribosomes and imported post-translationally into the organelle [30,31]. Among these proteins seem to be most, if not all, of those that are involved in the replication of the higher plant plastome (reviewed in [20]). Since current knowledge of the molecular biology of plastome replication is sparse, and practically no information exists about this process in cyanelles and in cyanobacteria, we have begun a comparative study by examining the enzymes that synthesize DNA. Chloroplast DNA polymerases from several higher plant species have already been characterized and, while some dispute remains about their preferred in vitro template, they have, nevertheless, been classified as bona fide eukaryotic DNA polymerases of the γ-type [18,32,33]. Only one study describes a DNA polymerase from a cyanobacterium [19,21]. The enzyme from *A. nidulans* R2 is essentially identical in its biochemical properties to *E. coli* DNA polymerase I, and microsequencing of an internal peptide fragment has confirmed the close relationship between these two prokaryotic enzymes. Furthermore, the complete nucleotide sequence of the *Synechocystis* sp. strain PCC 6803 genome (accessible through www.kazusa.or.jp) revealed strong amino acid sequence homology of two open reading frames to *Pol*I and the catalytic subunit of *Pol*III of *E. coli*.

The DNA polymerase from the cyanelles of *C. paradoxa* was purified several thousand fold by a sequence of three chromatography steps. Although the extent of purification achieved by chromatography on DEAE cellulose was a modest 2-fold (Table 1), this step was maintained because it very efficiently removes endogenous DNA. Only one peak of DNA polymerase activity was eluted from each column matrix using the standard assay with activated DNA and Mg^{2+}, and no activity was detected in any of the column flow-through or wash fractions (not shown). According to its sedimentation velocity in glycerol gradients, the highly purified cyanelle DNA polymerase has an approximate molecular weight of 85 000, which is in the range of chloroplast DNA polymerases for which, depending on the plant species, molecular weights of 85 000–105 000 have been reported [18,32,33]. The most abundant DNA polymerase (*Pol*I) from the cyanobacterium *Anacystis nidulans* R2 has an approximate molecular weight of 107 000 [19]. Although the estimate for the molecular weight of the cyanelle DNA polymerase might be too low because of proteolytic degradation of the enzyme, this possibility is reduced since protease inhibitors were added to each buffer used in the purification procedure. Furthermore, a single peak of the same molecular weight was obtained from glycerol gradient centrifugation of a less purified enzyme fraction (pooled DEAE cellulose peak fractions).

Table 3
In vitro template preferences of the cyanelle DNA polymerase

Template	Cation[a]	% Activity[b]
Activated DNA	Mg^{2+}	100
	Mn^{2+}	49
Poly(dA) : oligo(dT)	Mg^{2+}	6
	Mn^{2+}	14
Poly(rA) : oligo(dT)	Mg^{2+}	14
	Mn^{2+}	22
M13mp18 single stranded circular DNA	Mg^{2+}	5
	Mn^{2+}	5
pUC19 double stranded circular DNA	Mg^{2+}	6
	Mn^{2+}	4
Polyadenylated RNA : oligo(dT)	Mg^{2+}	8
	Mn^{2+}	19

[a]The divalent cation concentrations are 12 mM Mg^{2+} and 0.25 mM Mn^{2+}, respectively.
[b]100% activity corresponds to 0.34 units.

While both DEAE- and phosphocellulose purified fractions of the cyanelle DNA polymerase contain measurable 3′ to 5′ exonuclease activity, this activity was no longer detectable in the fractions eluting from heparin agarose. Previous work from our laboratory has shown that a similar nuclease activity which co-purifies with one form of chloroplast DNA polymerase from soybean is strongly inhibited by $(NH_4)_2SO_4$ [34]. To rule out interference by the salt, the pooled, active fractions eluting from heparin agarose were dialyzed and the 3′ to 5′ exonuclease assay repeated. No activity of this enzyme was detectable (not shown). This most highly purified DNA polymerase fraction did, however, contain a 5′ to 3′ exonuclease activity, although at this point a distinction between specific and fortuitous association of both enzyme activities cannot be made. Attempts to detect this nuclease in glycerol gradient fractions have been unsuccessful. The long centrifugation times at 4°C that are necessary to sediment the enzyme, coupled with its apparent instability, result in almost complete loss of DNA polymerase and nuclease activities. While the chloroplast DNA polymerase from pea, when purified to homogeneity, is devoid of any nuclease activity [33], one form of the soybean enzyme [34] and the organellar DNA polymerase from spinach [35] appear to be associated with a 3′ to 5′ exonuclease activity. In the most highly purified fraction of the cyanobacterial DNA polymerase, both 3′ to 5′ and 5′ to 3′ exonuclease activities were detectable and, based on activity gel analysis, appeared to reside on the same polypeptide as the polymerase activity [19]. An assignment of polymerase or exonuclease activities to a particular polypeptide in the enzyme fractions from cyanelles was not possible since the cyanelle DNA polymerase, like its plant organellar counterparts [18,35,36], does not seem amenable to in situ activity analysis. No active polypeptides could be detected in SDS-polyacrylamide gels after renaturation.

Table 4 summarizes the salient features of the cyanelle DNA polymerase and compares its properties to those of the enzymes from higher plant chloroplasts and from the cyanobacterium *Anacystis nidulans* R2. The resistance of the cyanelle DNA polymerase to aphidicolin, its relative resistance to ara-CTP and to the potent DNA polymerase γ inhibitor ddTTP, its sensitivity to phosphate and NEM and its requirement for a monovalent salt mirror the characteristics of chloroplast enzymes from higher plants [18,32,33]. However, while the chloroplast enzymes from soybean and spinach are able to elongate the oligo-dT primer of a poly(rA) template in the presence of Mn^{2+} [18,32], the cyanelle enzyme, like the DNA polymerase from pea chloroplasts [33], used this primed ribohomopolymer very inefficiently and, instead, preferred activated DNA as a template in vitro. The cyanelle enzyme differs significantly from the most abundant DNA polymerase in the cyanobacterium *Anacystis nidulans* R2, which is resistant to NEM, unaffected by 100 mM phosphate and prefers poly(dA) primed with oligo(dT) in the presence of Mn^{2+} as a template in vitro [18]. Based on its biochemical

Table 4
Comparison of DNA polymerases from chloroplasts, cyanelles and a cyanobacterium

	Anacystis nidulans PolΠ[a]	*Cyanophora paradoxa*	Higher plant chloroplast
			Cyanelle DNA polymerase
DNA polymerases[b,c,d,e]			
Molecular weight	107 000	85 000	85 000–105 000
5′ to 3′ exonuclease	Yes	Yes	No[b,d]
3′ to 5′ exonuclease	Yes	No	Yes[b,e]; no[b,d]
Preferred template/cation	Poly(dA) : oligo(dT)/Mn^{2+}	Activated DNA/Mg^{2+}	Poly(rA) : oligo(dT)/Mn^{2+} [b,c]
Activated DNA/Mg^{2+} [d]			
KPO_4	Unaffected	Sensitive	Sensitive
KCl optimum	150 mM	100 mM	100–125 mM
NEM	Resistant	Sensitive	Sensitive
Aphidicolin	Resistant	Resistant	Resistant
Ara-CTP	Resistant	Largely resistant	Resistant
ddNTP : dNTP = 1 : 1	Resistant	Largely resistant	Largely resistant

References: [a][19]; [b][18]; [c][32]; [d][33]; [e][35].

characteristics, the cyanelle DNA polymerase should be considered a chloroplast-like, eukaryotic enzyme. The enzyme has less in common with its prokaryotic, cyanobacterial counterpart. An ultimate confirmation of phylogenetic relatedness to either its chloroplast or bacterial counterparts will have to await the cloning and sequencing of the first gene for a chloroplast DNA polymerase. To date, the only published sequence information is that for mitochondrial DNA polymerases from several yeasts and *Xenopus laevis* [37–39].

References

[1] Aitken, A. and Stanier, R.Y. (1979) J. Gen. Microbiol. 112, 219–223.
[2] Pfanzagl, B., Zenker, A., Pittenauer, E., Allmaier, G., Martinez-Torrecuadrada, J., Schmid, E.R., De Pedro, M.A. and Löffelhardt, W. (1996) J. Bacteriol. 178, 332–339.
[3] Giddings, T.H., Wasman, C. and Staehelin, L.A. (1983) Plant Physiol. 71, 409–419.
[4] Bryant, D.A., De Lorimier, R., Lambert, D.H., Dubbs, J.M., Stirevalt, V.L., Stevens Jr., S.E., Porter, R.D., Tam, J. and Jay, E. (1985) Proc. Natl. Acad. Sci. USA 82, 3242–3246.
[5] Mangeney, E. and Gibbs, S.P. (1987) Eur. J. Cell Biol. 43, 65–70.
[6] Jaynes, J.M. and Vernon, L.P. (1982) Trends Biochem. Sci. 7, 22–24.
[7] Herdman, M., Janvier, M., Rippka, R. and Stanier, R.Y. (1979) J. Gen. Microbiol. 111, 73–85.
[8] Sugiura, M. (1992) Plant Mol. Biol. 19, 149–168.
[9] Stirewalt, V., Michalowski, C.B., Löffelhardt, W., Bohnert, H.J. and Bryant, D.A. (1995) Plant Mol. Biol. Reporter 13, 327–332.
[10] Margulis, L. (1981) Symbiosis in Cell Evolution, W.H. Freeman, New York.
[11] Gray, M.W. (1989) Trends Genet. 5, 294–299.
[12] Berry-Lowe, S.L., McKnight, T.D., Shah, D.M. and Meagher, R.B. (1982) J. Mol. Appl. Genet. 1, 483–498.
[13] Dean, C., Van Den Elzen, P., Tamaki, S., Dunsmuir, P. and Bedbrook, J. (1985) Proc. Natl. Acad. Sci. USA 82, 4964–4968.
[14] Sinozaki, K. and Sugiura, M. (1983) Nucleic Acids Res. 11, 6957–6964.
[15] Nierzwicki-Bauer, S.A., Curtis, S.E. and Haselkorn, R. (1984) Proc. Natl. Acad. Sci. USA 81, 5961–5965.
[16] Heinhorst, S. and Shively, J.M. (1983) Nature (London) 304, 373–374.
[17] Starnes, S.M., Lambert, D.H., Maxwell, E.S., Stevens, S.E., Porter, R.D. and Shively, M. (1985) FEMS Mircobiol. Lett. 28, 165–169.
[18] Heinhorst, S., Cannon, G.C. and Weissbach, A. (1990) Plant Physiol. 92, 939–945.
[19] Lin, H.-J., Cannon, G.C. and Heinhorst, S. (1990) Nucleic Acids Res. 18, 59–6663.
[20] Heinhorst, S. and Cannon, G.C. (1993) J. Cell Sci. 104, 1–9.
[21] Lin, H.-J. (1993) Ph.D. Dissertation, University of Southern Mississippi.
[22] Allen, M.M. (1968) J. Phycol. 4, 1–4.
[23] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[24] Spanos, A., Sedgwick, G., Yarranton, G.T., Hhbscher, U. and Banks, G.R. (1981) Nucleic Acids Res. 9, 1825–1839.
[25] Laemmli, U.K. (1970) Nature 227, 680–685.
[26] Adams R.L.P., Knowler, J.T. and Leader, D.P. (1992) The Biochemistry of the Nucleic Acids, Chapman Hall, London.
[27] Zimmermann, W., Chen, S.M., Bolden, A. and Weissbach, A. (1980) J. Biol. Chem. 255, 11847–11852.
[28] Wernette, C.M. and Kaguni, L.S. (1986) J. Biol. Chem. 261, 14764–14770.
[29] Wang, T.S.-F. (1991) Annu. Rev. Biochem. 60, 513–552.
[30] Ellis, R.J. (1983) in: Subcellular Biochemistry (Roodyn, D.B., Ed.) vol. 9, pp. 237–261, Plenum Publishing Corporation.
[31] Burnap, R.L. and Trench, R.K. (1989) Proc. R. Soc. Lond. B 238, 73–87.
[32] Sala, F., Amileni, A.R., Parisi, B. and Spadari, S. (1980) Eur. J. Biochem. 112, 211–217.
[33] McKown, R.L. and Tewari, K.K. (1984) Proc. Natl. Acad. Sci. USA 81, 2354–2358.
[34] Bailey, J.C., Heinhorst, S. and Cannon, G.C. (1995) Plant Physiol. 107, 1277–1284.
[35] Keim, C.A. and Mosbaugh, D.W. (1991) Biochemistry 30, 11109–11118.
[36] Meissner, K., Heinhorst, S., Cannon, G.C. and Börner, T. (1993) Nucleic Acids Res. 21, 4893–4899.
[37] Foury, F. (1989) J. Biol. Chem. 264, 20552–20560.
[38] Ropp, P.A. and Copeland, W.C. (1995) Gene 165, 103–107.
[39] Ye, F., Carrodeguas, J.A. and Bogenhagen, D.F. (1996) Nucleic Acids Res. 24, 1481–1488.