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PARTIAL PURIFICATION OF MITOCHONDRIAL AND SUPERNATANT DNA POLYMERASE FROM SACCHAROMYCES CEREVISIAE

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SUMMARY

Yeast mitochondria incorporate [^{3}H]TMP into DNA that has a buoyant density of mitochondrial DNA. The reaction does not require additional DNA and is not inhibited by deoxyribonuclease. DNA polymerase has been solubilized and purified about 50 fold from mitochondria and about 150 fold from the supernatant fraction of the cell. Enzymatic properties of the two enzyme preparations are similar. Density gradient centrifugation indicates that the supernatant and mitochondrial enzymes have a different molecular size.

INTRODUCTION

It is now firmly established that mitochondria contain species of DNA which differ from that present in the nuclei[1-3]. Mitochondrial DNA appears to be synthesized in a semiconservative manner[4] independent of synthesis of nuclear DNA (ref. 5). DNA polymerase activity has been reported to be present in mitochondria isolated from yeast[6] and from rat liver[7,8]. KALF AND CH'IH[9] and MEYER AND SIMPSON[10] recently described the partial purification of mitochondrial DNA polymerase from rat liver. Their studies suggest that the enzymes differ from nuclear DNA polymerase in physical and enzymatic properties. This paper describes the solubilization and partial purification of DNA polymerase from mitochondrial and supernatant fractions of *Saccharomyces cerevisiae*. The cell supernatant fraction contains most of the DNA polymerase derived from the nucleus. The enzymatic properties of the enzymes are compared. The data suggest that the mitochondrial and supernatant enzymes may differ in molecular size.

METHODS

Strain D243-R1 (a, ad, ϱ^+), a haploid "grande", kindly provided by Dr. P. P. SLONIMSKI, Laboratoire de Génétique Moleculaire, Gif-sur-Yvette (France) was used in the study. Cultures were grown aerobically at 28° in a New Brunswick rotary shaker in a medium consisting of 1% yeast extract, 1% bactopeptone and 2% glucose.

Cultures used in the isolation of supernatant DNA polymerase were collected in the mid-exponential phase of growth. Mitochondrial DNA polymerase was purified from yeast harvested in the stationary growth phase. Yeast was washed 3 times with cold distilled water prior to disruption.

Preparation of supernatant enzyme

100 g (wet wt.) of cells were suspended in 300 ml of 0.02 M glycyl glycine buffer (pH 7.0) containing 0.65 M sorbitol. All procedures were carried out at 4°. The cells were disrupted in a Ribi-Cell fractionator (Model RF-1 Sorvall) at 20 000 lb/inch^2 at 2–6°. The suspension was centrifuged at 12 000 × *g* for 15 min and the slightly turbid supernatant was collected (Fraction I).

0.1 vol. of 2% protamine sulfate, Nutritional Biochemical Corp., Cleveland, Ohio, was slowly added to the supernatant. After standing for 10 min the precipitate was collected by centrifugation at 12 000 × *g* for 10 min. The precipitate was extracted for 10 min with 150 ml 0.1 M potassium phosphate (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA. The suspension was centrifuged at 46 000 × *g* for 20 min and the supernatant was collected (Fraction II). To each 100 ml of Fraction II, 31.4 g of $(NH_4)_2SO_4$ was added and the precipitate was collected by centrifugation at 12 000 × *g* for 10 min. The precipitate was dissolved in 65 ml of 0.01 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA (Fraction III). Fraction III was diluted with 2 vol. of 0.01 M potassium phosphate (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA, and absorbed on C_γ alumina gel (final concentration 5 mg/ml). After stirring for 10 min, the gel was separated by centrifugation and eluted twice with 100 ml of 0.3 M potassium phosphate (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA. The protein was precipitated with 43 g/100 ml of $(NH_4)_2SO_4$, the precipitate separated by centrifugation at 12 000 × *g* for 10 min and dissolved in 10 ml 0.05 M potassium phosphate (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA (Fraction IV).

After dialysis against 0.02 M phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA the dialysate was applied to a column (3 cm × 18 cm) of DEAE-cellulose equilibrated with the same buffer. The column was washed with 200 ml of 0.02 M potassium phosphate buffer (pH 7.4), 300 ml 0.05 M 2-mercaptoethanol and 200 ml 0.1 M potassium phosphate buffer (pH 7.4) containing 2-mercaptoethanol and EDTA. The enzyme was eluted with the last buffer (Fraction V).

Fraction V was applied directly to a column (1.5 cm × 3.0 cm) of hydroxylapatite (hypatite), Clarkson Chemical Co., Williamsport, Pa., which had been equilibrated with 0.02 M potassium phosphate and the enzyme was eluted at 0.3 ml/min with 0.2 M potassium phosphate (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA (Fraction VI). An equal volume of glycerol was added and the enzyme was stored at −20°. In the presence of glycerol, enzyme activity was stable for at least a month.

Purification of mitochondrial DNA polymerase

Formation of protoplasts. Protoplasts were prepared by modification of the methods of DUELL, INOUE AND UTTER[11] and KOVAC, BEDNAROVA AND GREKSAK[12]. 200 g cells (wet wt.) harvested in the stationary phase of growth were incubated in 600 ml of 0.5 M mercaptoethylamine (Calbiochem Corp., Los Angeles, Calif.), Grade B, 0.1 M Tris (pH 9.3) for 30 min at 30°. The cells were then washed twice with 400 ml

0.9 M sorbitol, 0.01 M citrate phosphate buffer (pH 5.8), 0.001 M EDTA, and suspended in 400 ml of the same buffer. 25 ml of glusalase, Endo Laboratories, Garden City, New York, was added and the suspension was incubated with shaking at 30° for 90–120 min. When protoplast formation had reached over 80% completion as indicated by lysis of the cell in H_2O, the suspension was centrifuged at 10 000 × *g* for 10 min. The protoplasts were washed twice with 300 ml of 0.9 M sorbitol, 0.002 M potassium phosphate (pH 7.0), 0.001 M EDTA.

Isolation of mitochondria. Protoplasts were suspended in 600 ml of 0.8 M sorbitol, 0.02 M glycyl glycine buffer (pH 7.0), 0.001 M EDTA, disrupted in a Waring blendor for 15 sec at top speed, and centrifuged at 1200 × *g* for 5 min. The supernatant was centrifuged again at 1200 × *g* for 5 min. The supernatant was centrifuged at 10 000 × *g* for 10 min and the crude mitochondrial pellet was suspended in the same volume of buffer. The suspension was centrifuged twice more at 1200 × *g* for 5 min to remove unbroken protoplasts and cells with fragments. The mitochondria were sedimented again from the supernatant at 12 000 × *g* for 10 min, and washed twice more in the same way. Further purification of mitochondria was carried out when indicated by discontinuous sorbitol density gradient centrifugation according to the method of JAYARAMAN, COTMAN AND MAHLER[13].

Solubilization and purification of mitochondrial DNA polymerase. Mitochondria (Fraction I) (218 mg protein) were suspended in 10 ml of 0.02 M glycyl glycine buffer (pH 7.0) containing 1 M KCl, 0.01 M 2-mercaptoethanol and 0.001 M EDTA, and stirred for 20 min. The suspension was centrifuged at 50 000 × *g* for 15 min and the pellet was extracted twice more with 10-ml portions of the same buffer. The combined supernatant was centrifuged at 55 000 × *g* in the Spinco Model L centrifuge (rotor 30) for 1 h, and the clear amber supernatant (Fraction II) was collected.

To 30 ml of Fraction II was added 10.5 g of $(NH_4)_2SO_4$. After 20 min the precipitate was collected by centrifugation at 12 000 × *g* for 10 min and dissolved in 5 ml of 0.05 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA (Fraction III).

Fraction III was dialyzed against 1 l of 0.05 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA with 2 changes of buffer. The dialysate was applied to a DEAE-cellulose column (2 cm × 11 cm) that had been previously equilibrated with 0.05 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA. The column was washed with 100 ml of the same buffer and the enzyme was eluted with 70 ml of 0.1 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA (Fraction IV). The flow rate was 1 ml/min, and 5-ml fractions were collected.

15 ml of combined Fraction IV was applied to a column of hydroxylapatite (1.5 cm × 3 cm) which had been washed with 70 ml of 0.02 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA, and then washed with 80 ml of 0.1 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA. The enzyme was eluted with 0.2 M potassium phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA. The flow rate was 0.3 ml/min and 2-ml fractions were collected. An equal volume of glycerol was added to fractions containing enzyme activity and these were then stored at —20° (Fraction V).

DNA polymerase assay. Enzyme activity was assayed in a manner similar to the procedure of KORNBERG *et al.*[14]. The 0.3-ml incubation mixture contained 0.05 M

Tris (pH 7.5), 0.01 M $MgCl_2$, 0.001 M 2-mercaptoethanol, 0.167 mM dATP, dGTP, dCTP and [^{3}H]TTP (specific activity $1.4 \cdot 10^7$–$2.7 \cdot 10^7$ counts/min per μmole), 25 μg native salmon sperm DNA, and enzyme. The mixtures were incubated at 37° for 15 min. After adding 500 μg of salmon sperm DNA as carrier, the DNA was precipitated with cold 0.5 M perchloric acid and collected by centrifugation. The DNA was dissolved in 0.3 ml of 0.2 M NaOH and again precipitated with 0.5 M perchloric acid. The DNA was washed three times in this manner. Finally, the precipitate was dissolved in 0.5 ml of 0.2 M NH_4OH and the radioactivity was measured in BRAY's solution with a Tricarb liquid scintillation counter. A unit of enzyme activity is defined as the incorporation of 1 nmole of tritiated deoxynucleotide into acid-insoluble product during the 15-min incubation period. DNA polymerase activity in mitochondria was assayed in the same manner except that salmon sperm DNA was not included in the reaction.

Protein was determined by the method of LOWRY *et al.*[15] using bovine serum albumin as standard. Protein concentration in eluates from DEAE-cellulose and hydroxylapatite columns were measured spectrophotometrically by the method of WARBURG AND CHRISTIAN[16].

In order to characterize the product formed by mitochondria, DNA was isolated as previously described[17]. Preparative CsCl density gradient centrifugation was carried out as previously described[17].

Unlabeled deoxyribonucleoside triphosphates and [^{3}H]TTP were purchased from Schwarz BioResearch, Inc., Orangeburg, New Jersey. Salmon sperm DNA was obtained from Calbiochem Corp., Los Angeles, Calif., and calf thymus DNA from Sigma Chemical Co., St. Louis, Mo. *E. coli* (K 12) DNA was a gift from Dr. S. Liao. Denatured DNA was prepared by heating DNA dissolved in 0.01 M Tris (pH 7.5), 0.01 M NaCl, in a boiling-water bath for 10 min and quickly cooling in ice.

RESULTS

DNA polymerase in intact mitochondria

Intact yeast mitochondria incorporate [^{3}H]thymidylic acid into DNA (Table I).

TABLE I

REQUIREMENTS FOR CYTOPLASMIC AND MITOCHONDRIAL DNA POLYMERASE

Mitochondria were assayed using 1.0 mg of material purified by sorbitol gradient. 6 μg of Fraction V was used to assay partially purified mitochondrial DNA polymerase and 6 μg of Fraction VI to assay partially purified supernatant DNA polymerase. Activity is expressed as pmoles [^{3}H]TMP incorporated into DNA in 15 min.

	Mitochondria	*Mitochondrial polymerase*	*Supernatant polymerase*
Complete + added DNA	107	25.5	95.1
− added DNA	105	0.2	1.0
− $MgCl_2$	3.2	0.5	0.0
− 2-mercaptoethanol	103	25.3	4.8
− dATP	3.4	5.9	4.8
− dGTP	16.4	3.3	14.1
− dCTP	6.4	3.3	6.9
− dATP, dGTP, dCTP	1.0	2.8	
+ 30 μg deoxyribonuclease	88	0.0	0.0

The reaction requires bivalent cations, and is almost completely dependent on the presence of 4-deoxynucleoside triphosphates. The addition of DNA is not required and does not stimulate DNA synthesis, presumably because mitochondrial DNA is already available to the polymerase. The addition of 30 μg of pancreatic deoxyribonuclease to the reaction vessel results in only a small decrease in incorporation of [^{3}H]thymidylic acid into DNA. The mitochondrial membrane appears to protect the DNA template and product from the action of deoxyribonuclease. The small inhibition of incorporation by deoxyribonuclease may be due to disruption of some mitochondrial membranes or to the presence of small amounts of cytoplasmic polymerase.

DNA synthesized by intact mitochondria has a buoyant density identical to that of yeast mitochondrial DNA when analyzed by CsCl density gradient centri-

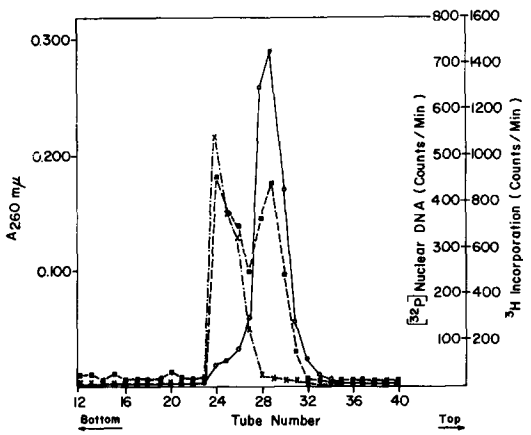

Fig. 1. CsCl density gradient analysis of [^{3}H]DNA synthesized by isolated yeast mitochondria. The reaction system was identical with that described in METHODS except that no DNA was added. Unlabeled yeast mitochondrial and nuclear DNA and ^{32}P yeast nuclear DNA were added prior to centrifugation. ○——○, [^{3}H]DNA synthesized by mitochondria; ■— — — ■, marker nuclear and mitochondrial DNA; × —·— ×, marker ^{32}P nuclear DNA, specific activity $2 \cdot 10^4$ counts/min per μg DNA.

fugation (Fig. 1). The [^{3}H]DNA bands almost exclusively with carrier mitochondrial DNA ($\varrho = 1.685$); while less than 5% of the radioactivity bands with carrier nuclear DNA ($\varrho = 1.700$). The mitochondria therefore appear to be almost free of contaminating nuclear DNA polymerase and template.

DNA polymerase activity in different phases of growth

Mitochondrial development is repressed by high glucose concentration in the culture medium[18,19]. When glucose is used as an energy source, oxidative enzyme activity is greatly diminished during the exponential phase of growth, but it markedly increased in the stationary phase of growth. Table II shows the DNA polymerase activity of mitochondria and of the cell supernatant in yeast harvested in the exponential and stationary growth phases. Mitochondrial polymerase activity was maximal during stationary growth, a period when it might be expected that mitochondrial replication was taking place, whereas supernatant DNA polymerase activity was maximal during the exponential phase of growth.

TABLE II

DNA POLYMERASE ACTIVITY IN SUPERNATANT AND MITOCHONDRIA IN DIFFERENT GROWTH PHASES
Specific activity is expressed as nmoles [^{3}H]TMP incorporated in 15 min per mg protein.

	Supernatant specific activity	*Mitochondria specific activity*
Exponential	0.13	0.012
Stationary	0.05	0.041

Purification of supernatant and mitochondrial DNA polymerase

The procedures used to purify supernatant and mitochondrial DNA polymerase are outlined in Tables III and IV. Both enzymes were relatively stable and could be stored in the frozen state until DEAE-cellulose chromatography. After this procedure, perhaps secondary to removal of nucleic acids, the enzymes were very labile and activity could be preserved only by addition of 50% glycerol and storage at −20°. It was necessary to carry out DEAE-cellulose and hydroxylapatite chromatography

TABLE III

PURIFICATION OF SUPERNATANT DNA POLYMERASE
Specific activity is expressed as nmoles [^{3}H]TMP incorporated per mg protein.

Step	*Total units*	*Specific activity*
I. Crude extract	446	0.12
II. Protamine sulfate	216	0.22
III. $(NH_4)_2SO_4$ precipitation	192	0.30
IV. Alumina C_γ gel	118	1.17
V. DEAE-cellulose	62	4.1
VI. Hydroxylapatite	20*	16.7

* $A_{280\ m\mu}/A_{260\ m\mu} = 1.45$.

with a minimum of delay on the same day. Final yields of enzyme were about 5%. Supernatant polymerase was purified about 150 fold and mitochondrial polymerase about 50 fold.

Comparison of properties of supernatant and mitochondrial DNA polymerase

The partially purified mitochondrial and supernatant enzymes showed properties similar to other DNA polymerases. In both cases there was an almost absolute requirement for added DNA template, $MgCl_2$, and for addition of 4-deoxynucleoside triphosphates (Table I).

Incorporation of [^{3}H]thymidylic acid into DNA by both partially purified polymerases was proportional to enzyme concentration (Fig. 2) and continued almost linearly with time for at least 30–60 min (Fig. 3). The requirement for bivalent cations, Mg^{2+} or Mn^{2+} is shown in Fig. 4. The optimal concentration of the bivalent cations were similar for the two enzyme preparations.

The pH optima for both enzymes were similar (Fig. 5), being approx. 8.0–8.5 with Tris buffer and 6.5 with phosphate buffer.

TABLE IV

PURIFICATION OF MITOCHONDRIAL DNA POLYMERASE

Mitochondria were assayed in the absence of added DNA. The addition of DNA did not stimulate incorporation of [^{3}H]TMP into DNA. The addition of 30 μg deoxyribonuclease reduced incorporation by 28%. Specific activity is expressed as nmoles [^{3}H]TMP incorporated per mg protein.

Step	*Total units*	*Specific activity*	*Ratio activity denatured template/ native template*
I. Mitochondria	22	0.10	
II. Crude extract	8.8	0.17	1.9
III. $(NH_4)_2SO_4$ precipitation	7.7	0.23	1.6
IV. DEAE-cellulose	2.9	0.97	0.7
V. Hydroxylapatite	1.4*	4.8	0.7

* $A_{280\ m\mu}/A_{260\ m\mu} = 1.42$.

The activities of the polymerases with different templates are compared in Table V. *E. coli* DNA was a more effective template than salmon sperm DNA for both enzymes, and calf thymus DNA was least effective. There was little difference in relative activity of the two enzymes with native and denatured template; in both

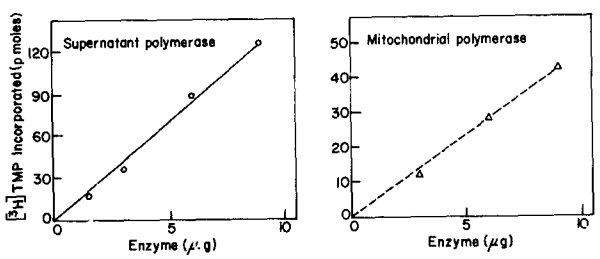

Fig. 2. Relation between the incorporation of [^{3}H]thymidylate into DNA and enzyme concentration. Fraction VI of supernatant enzyme and Fraction V of mitochondrial enzyme were used.

cases native DNA appeared to be somewhat more effective than denatured DNA. The degree of purification greatly altered the relative activity of the mitochondrial enzyme with native and denatured template (Table III). The crude enzyme preferred denatured template while the purer enzyme was more active with native template. The differences in response are possibly secondary to different amounts of contami-

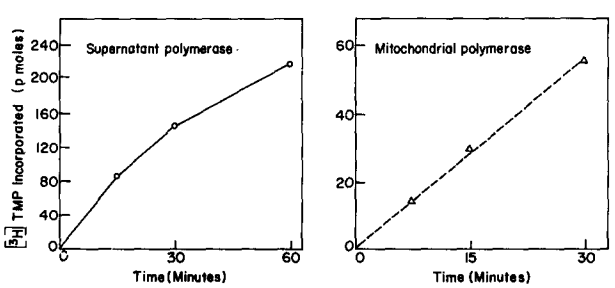

Fig. 3. Time course of incorporation of [^{3}H]thymidylate into DNA.

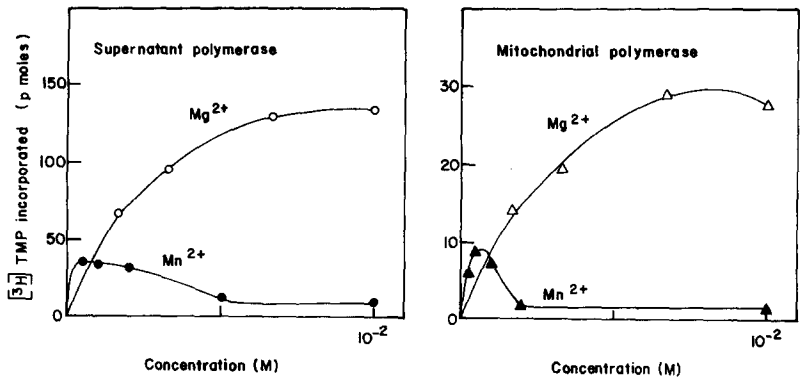

Fig. 4. Bivalent cation requirement of DNA polymerase.

nating nuclease in the preparations. Another factor that altered the relative activity of the enzyme with native and denatured template was ionic strength. At high concentrations of KCl or NH_4Cl incorporation of [^{3}H]thymidylate was greatly reduced when native DNA was template, but only slightly with denatured DNA (Table VI). The ratio of activity with denatured template to activity with native primer was therefore increased 3–4 fold at high ionic strength.

Density gradient centrifugation of the enzyme

In order to compare the molecular sizes of the enzymes, purified fractions of mitochondrial supernatant DNA polymerase were centrifuged in a glycerol density gradient. Fig. 6 shows the patterns obtained with the individual enzymes and with a mixture of the two enzymes. Two components appear to be present in the supernatant DNA polymerase preparation but only one can be seen in the mitochondrial DNA polymerase. The mitochondrial DNA polymerase sediments with the slower component present in small amount in the supernatant DNA polymerase.

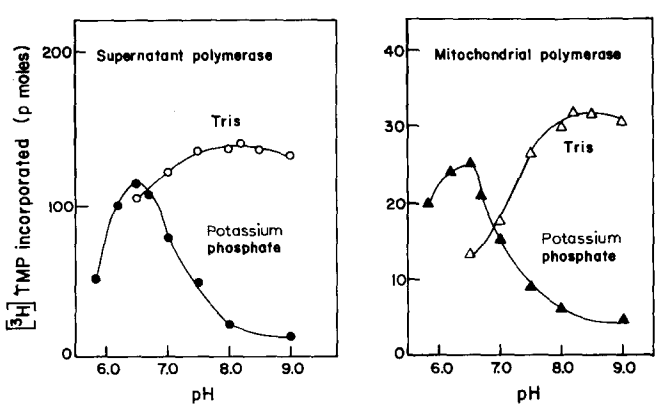

Fig. 5. pH optima for supernatant and mitochondrial DNA polymerases. The final buffer concentration was 0.083 M. Tris–malate was used at pH 6.5 and 7.0, and Tris–HCl at higher pH. 9 μg of Fraction VI of supernatant enzyme, and 6 μg of Fraction V of mitochondrial enzyme were used in the standard assay system.

TABLE V

ACTIVITY OF DNA POLYMERASE WITH DIFFERENT TEMPLATES
4.5 μg of Fraction V mitochondrial DNA polymerase and 9.0 μg of Fraction VI of supernatant DNA polymerase were used in the assays.

Primer	*pmoles [^{3}H]TMP incorporation in 15 min*	
	Mitochondrial	*Supernatant*
Salmon sperm DNA, native	25.2	152
Salmon sperm DNA, denatured	18.9	86
Calf thymus DNA, native	5.4	26.3
Calf thymus DNA, denatured	5.8	14.6
E. coli DNA, native	61.2	190
E. coli DNA, denatured	31.2	138

DISCUSSION

DNA polymerase activity in preparations of isolated yeast mitochondria appears to represent the activity present in these organelles rather than contamination by enzyme derived from the nucleus for the following reasons: (1) Activity does not require the addition of DNA template; (2) the DNA synthesized by isolated mitochondria has the buoyant density of mitochondrial DNA; (3) Deoxyribonuclease only slightly inhibits the reaction. The incorporation requires 4-deoxynucleoside triphosphates and therefore does not represent terminal addition or homopolymer formation.

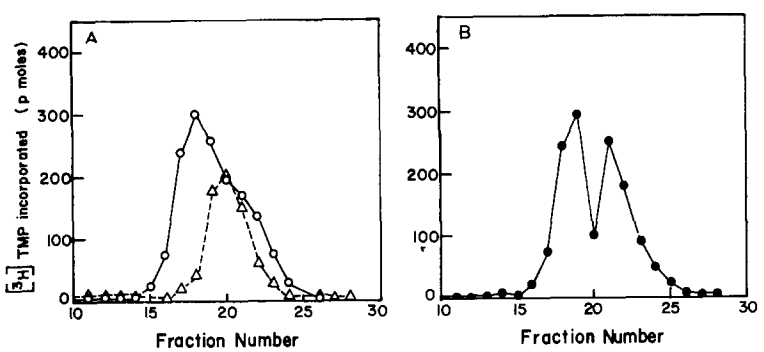

Fig. 6. Glycerol density gradient centrifugation of mitochondrial and supernatant DNA polymerase. 0.15 ml of the most purified fractions of polymerase were layered on a linear 6–25% glycerol gradient containing 0.1 M phosphate buffer (pH 7.4), 0.01 M 2-mercaptoethanol, 0.001 M EDTA and centrifuged for 13 h at 35 000 rev./min in the SW 39 rotor of a Spinco L-265 centrifuge. 12-drop fractions were collected and each fraction was assayed for DNA polymerase activity in the usual manner except that incubations were for 30 min. A. ○——○, supernatant DNA polymerase; △— — — △, mitochondrial DNA polymerase. B. Mixture of the two DNA polymerases.

These results are similar to those previously found in rat liver by HELGE AND NEUBERT[7] and PARSON AND SIMPSON[8] and in yeast by WINTERSBERGER[6]. It is also of note that mitochondrial DNA polymerase is most active during maximal mitochondrial development in the stationary growth phase, whereas nuclear DNA polymerase is most active during the exponential phase of growth.

TABLE VI

EFFECT OF SALT CONCENTRATION ON ACTIVITY OF PARTIALLY PURIFIED MITOCHONDRIAL DNA POLYMERASE WITH NATIVE AND DENATURED TEMPLATE

85 μg of Fraction IV enzyme was used in the experiment. The assays also contained 5% glycerol and 0.0125 M potassium phosphate (pH 7.4).

Addition	*Denatured template (pmoles)*	*Native template (pmoles)*	*Denatured template / Native template*
None	99	81	1.2
+ 0.08 M KCl	74	22	3.4
+ 0.15 M KCl	33	10	3.3
+ 0.08 M NH_4Cl	76	17	4.4
+ 0.15 M NH_4Cl	33	10.5	3.1

The DNA polymerase has been solubilized by extraction with solutions of high ionic strength, and partial purification of the enzyme has been achieved. At this level of purification, very little difference was observed in the enzymatic properties of the two enzymes. An apparent preference of crude mitochondrial DNA polymerase for denatured DNA disappeared when the enzyme was further purified. One must use great caution in interpreting preference for native and denatured template in a partially purified DNA polymerase since the difference may be related to contamination with nucleases specific for native or denatured DNA.

The lability of the enzyme prevented purification to a degree that would allow the unequivocal demonstration that the mitochondrial and supernatant enzyme were different molecular species. However, glycerol density gradient showed that the supernatant preparation contained a heavy component that was not present in the mitochondrial enzyme. The heavy component may represent enzyme derived from the nucleus whereas the lighter component may represent mitochondrial enzyme. The possibility of dimer formation[20], however, has not been eliminated. The recent work of KALF AND CH'IH[9] and MEYER AND SIMPSON[10] makes it highly probable that the mitochondrial and nuclear enzymes are different.

The activities of both supernatant and mitochondrial DNA polymerases were inhibited by high salt concentrations (0.08–0.15 M KCl). The inhibition was greater when the enzymes were assayed with native template than with denatured template. Yeast mitochondrial DNA polymerase therefore differs from the enzyme isolated from rat liver by MEYER AND SIMPSON[10] which is markedly stimulated by high salt concentration. Rat liver nuclear DNA polymerase is not significantly stimulated by alteration of ionic strength. The difference in response to high ionic strength is therefore a means of distinguishing between liver mitochondrial and nuclear DNA polymerases.

The presence of a unique DNA polymerase localized in yeast mitochondria is in accordance with the previous finding of MOUNOLOU, PERRODIN AND SLONIMSKI[21] and of RABINOWITZ *et al.*[17,22] that labeled precursors are preferably incorporated into mitochondrial DNA by anaerobic yeast adapting to oxygen in a non-growth sustaining medium. Specific activities of mitochondrial DNA were 15–30 times greater than that of nuclear DNA during this period of rapid development of mitochondrial respiratory function.

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REFERENCES

1 D. B. RODYN AND D. WILKIE, *The Biogenesis of Mitochondria*, Methuen, London, 1968.
2 M. RABINOWITZ, *Bull. Soc. Chim. Biol.*, 50 (1968) 311.
3 S. GRANICK AND A. GIBOR, in J. N. DAVIDSON AND W. E. COHN, *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 6, Academic Press, New York, 1967, p. 143.
4 E. REICH AND D. J. L. LUCK, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 1600.
5 D. SMITH, P. TAURO, E. SCHWEIZER AND H. O. HALVORSEN, *Proc. Natl. Acad. Sci. U.S.*, 60 (1968) 936.
6 E. WINTERSBERGER, *Biochem. Biophys. Res. Commun.*, 25 (1966) 1.
7 H. HELGE AND D. NEUBERT, *Arch. Exptl. Pathol. Pharmakol.*, 251 (1965) 44.
8 P. PARSON AND M. V. SIMPSON, *Science*, 155 (1967) 91.
9 G. F. KALF AND J. J. CH'IH, *J. Biol. Chem.*, 243 (1968) 4904.
10 R. R. MEYER AND M. V. SIMPSON, *Proc. Natl. Acad. Sci. U.S.*, 61 (1968) 130.
11 E. A. DUELL, S. INOUE AND M. F. UTTER, *J. Bacteriol.*, 88 (1964) 1762.
12 L. KOVAC, H. BEDNAROVA AND M. GREFSAK, *Biochim. Biophys. Acta*, 153 (1968) 32.
13 J. JAYARAMAN, C. COTMAN AND H. R. MAHLER, *Arch. Biochem. Biophys.*, 116 (1966) 224.
14 M. J. BESSMAN, I. R. LEHMAN, E. S. SIMMS AND A. KORNBERG, *J. Biol. Chem.*, 233 (1958) 171.
15 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
16 O. WARBURG AND W. CHRISTIAN, *Biochem. Z.*, 310 (1941) 384.
17 M. RABINOWITZ, G. S. GETZ, J. CASEY AND H. SWIFT, *J. Mol. Biol.*, in the press.
18 P. P. SLONIMSKI, *Formation des Enzymes Respiratoires chez la Levure*, Masson, Paris, 1953.
19 P. P. SLONIMSKI, in C. LIÉBECQ, *Proc. 3rd Intern. Congr. Biochem., Brussels, 1955*, Academic Press, New York, 1956.
20 A. G. LEZIUS, S. B. HENNIG, C. MENZEL AND E. METZ, *J. Biochim.*, 2 (1967) 90.
21 J. C. MOUNOLOU, G. PERRODIN AND P. P. SLONIMSKI, in E. C. SLATER, J. M. TAGER, S. PAPA AND E. QUAGLIARIELLO, *Biochemical Aspects of the Biogenesis of Mitochondria*, Adriatica Editrice, Bari, 1968, p. 133.
22 M. RABINOWITZ, G. S. GETZ AND H. H. SWIFT, in E. C. SLATER, J. M. TAGER, S. PAPA AND E. QUAGLIARIELLO, *Biochemical Aspects of the Biogenesis of Mitochondica*, Adriatica Editrice, Bari, 1968, p. 155.