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# Properties of mitochondrial DNA polymerase in mitochondrial DNA synthesis in yeast*

Tapan K. Biswas**, Pritam Sengupta***, Renee Green, Paul Hakim, Bani Biswas and Sribir Sen

*Department of Pathology, University of Chicago, Chicago, IL 60637, U.S.A.*

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**Mitochondrial DNA polymerase from *Saccharomyces cerevisiae*, purified 3500 fold, was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis into three polypeptides. The major 150 kDa polypeptide was probably the catalytic subunit of the mitochondrial (mt) DNA polymerase and the other two polypeptides could be either proteolytic cleavage products of the polymerase, other subunits of the enzyme or protein contaminants. The mtDNA polymerase preferred an A+T-rich DNA template and did not require any RNA primer for DNA synthesis, at least under *in vitro* reaction conditions. It showed higher processivity on a double-stranded linear DNA template than on a single-stranded circular DNA template, and was capable of synthesizing at least about 1200 nucleotide primer-extended products without any major pause on a double-stranded DNA template.**

The mitochondrial DNA synthesis is independent from the nuclear DNA synthesis [1, 2], and continuous throughout the cell cycle [3]. The understanding of mtDNA replication has been achieved mainly from the study of vertebrate mtDNA [4]. In vertebrates, the heavy-strand DNA synthesis starts first at the D-loop region from 3'-end of RNA primer which is synthesized from a L-strand promoter. After synthesis of two-thirds of the daughter H-strand DNA, the parent H-strand is displaced as a single-stranded DNA and forms an intramolecular hairpin structure where an RNA-primed L-strand synthesis begins [4].

The mechanism of yeast mtDNA replication is thought to be quite different from others since a petite mitochondrial DNA consisting of many repeats of a 35 bp A-T sequence, is stably maintained in yeast mitochondria [5]. All *trans*-acting gene products involved in yeast mtDNA replication are encoded by the nuclear DNA. Among these proteins, only the mtDNA polymerase was isolated [6, 7]. The *cis*-acting DNA sequence involved in mtDNA replication has not been well defined, either. To define the biochemical and genetic requirements for yeast mtDNA synthesis, we have recently started an investigation on mtDNA polymerase [7]. In this raport, we describe the polypeptide structure

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**To whom correspondence should be sent

***Present address: University of Illinois, Chicago, Illinois, U.S.A.

Abbreviations used: BSA, bovine serum albumin; bp, base pair; DTT, dithiothreitol; dNTP, deoxyribonucleotide; mt, mitochondrial; NEM, *N*-ethylmaleimide; nt, nucleotide; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.

of the polymerase, *in vitro* run-off DNA synthesis and fidelity of the polymerase, and the role of mtDNA polymerase in mtDNA synthesis.

## MATERIALS AND METHODS

***Chemicals***, oligonucleotides, and enzymes were the same as described previously [7]. Phosphocellulose was purchased from Whatman, heparin-agarose and single stranded DNA-cellulose were from Sigma Chemical Company. Deoxyribonucleotides were purchased from P-L Biochemicals. Radiolabeled nucleotides were from ICN Biochemicals or from New England Nuclear. Protease inhibitors, protein markers, salmon sperm DNA and poly[dA-dT] were purchased from Sigma. Poly[rA]$_{305}$·poly[dT]$_{167}$ was from Pharmacia. DNase I, $T_4$ polynucleotide kinase, and restriction endonucleases were obtained from Bethesda Research Laboratories.

***DNA polymerase assay.*** The mtDNA polymerase activity was assayed according to Wintersberger & Blutsch [6] as described in the previous paper [7]. One unit of the polymerase was defined as the amount of enzyme that catalyzed the incorporation of 1 pmol of TMP/min into acid-insoluble materials at 37°C.

***Protein measurement.*** Protein concentration was determined colorimetrically by the method of Bradford [8] using γ-globin as a protein standard or by comparing with standards of known protein concentrations after gel electrophoresis and silver staining.

***SDS-polyacrylamide gel electrophoresis.*** SDS-polyacrylamide (7.5%) gel electrophoresis was carried out in Tris/glycine buffer [9], and then protein was stained with silver according to the method of Wray *et al.* [10]. Myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97.5 kDa), bovine serum albumin (66 kDa), chicken egg albumin (45 kDa), and carbonic anhydrase (29 kDa) were used as protein molecular mass markers.

***Purification of the mtDNA polymerase.*** Yeast (*S. cerevisiae* strain D273/10B) was grown to a late log phase in a medium containing 3% glycerol, 0.1% glucose, 1% yeast extract, and 1% peptone. Cells were harvested by centrifugation, and approximately 500 g of wet paste of cells was used for purification of yeast mtDNA polymerase. All operations for the enzyme purification were carried out at 4°C.

***Isolation of mitochondria***, preparation of mitochondrial extract, chromatography on phosphocellulose, heparin-agarose, and on single-stranded DNA-cellulose were performed as described previously [7].

***Glycerol gradient centrifugation.*** A 10–30% glycerol gradient was prepared with buffer B (10 mM Tris/HCl, pH 7.6, 1 mM DTT) containing 0.1 M KCl and protein inhibitors in a 4 ml Beckman polyallomer centrifuge tube. The concentrated enzyme fraction from the DNA-cellulose column was applied onto the glycerol gradient and centrifuged in a Beckman SW-60Ti rotor at 50 000 r.p.m. for 40 h at 4°C. Fractions of 0.2 ml were collected from the bottom and assayed for polymerase activity. The active fractions were pooled and 1 mg/ml BSA was added as an enzyme stabilizer, and then the enzyme preparation was frozen at –70°C. Without BSA, the enzyme was found to be unstable.

## RESULTS AND DISCUSSION

### Enzyme purification

Yeast was grown to a late-log phase in the medium containing glycerol as a carbon source to get the maximum amount of mtDNA polymerase [6]. The enzyme preparation was obtained as previously described [7], and then loaded onto a glycerol gradient. The mtDNA polymerase in the peak fraction of the glycerol gradient (Fig. 1A) was purified about 3 500 fold (Table 1) and SDS-polyacrylamide gel electrophoresis showed three predominant polypeptide bands in the size range of 116 kDa–200 kDa (Fig. 1B). The size of yeast mtDNA polymerase previously determined from the amino-acid sequence derived from the cloned mtDNA polymerase gene was found to be 145 kDa [11]. Therefore, the approx. 150 kDa protein band in the peak fraction of glycerol gradient was presumably the catalytic subunit of the mtDNA polymerase. However, it is not clear whether the other polypeptides in the peak fractions are the proteolytic cleavage products of the polymerase, protein contaminants or other subunits of the polymerase.

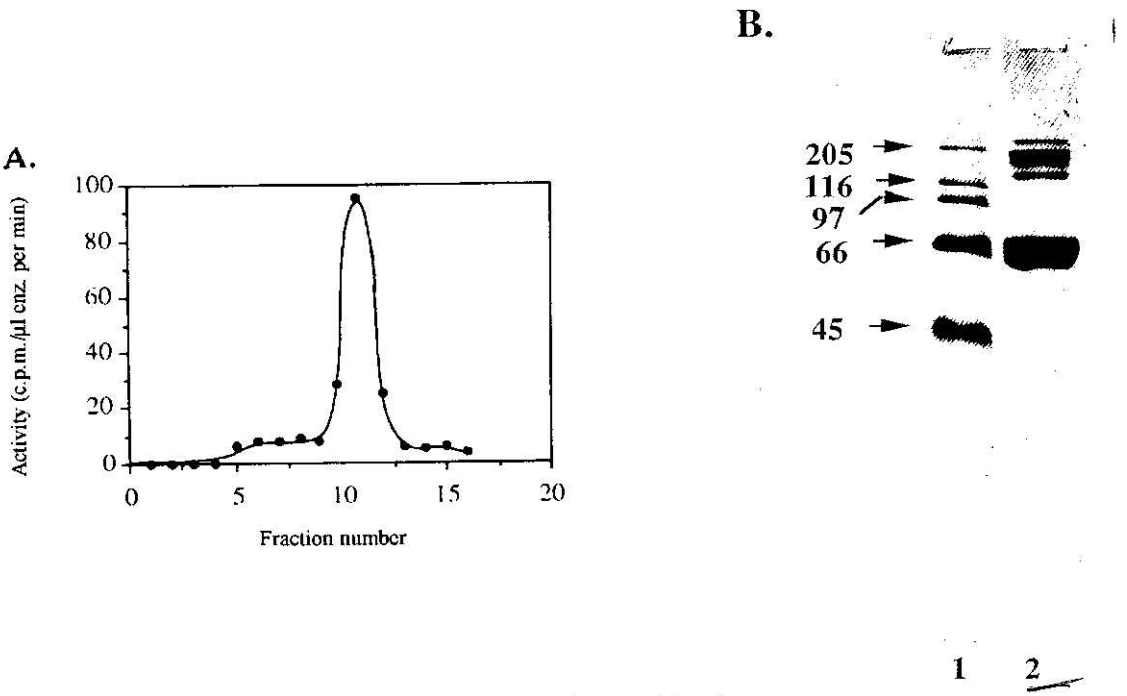



*Fig. 1. Fractionation profile and electrophoresis of mtDNA polymerase.*
A, Fractionation profile of the mtDNA polymerase activity on a 10–30% glycerol gradient. B, SDS-polyacrylamide gel (7.5%) electrophoresis of the glycerol gradient peak fraction, a final step in purification of the enzyme. Five hundred nanograms of protein were electrophoresed and then visualized by silver staining. Lane 1, protein molecular mass markers (top to bottom × $10^{-3}$): myosin, β-galactosidase, phosphorylase *b*, bovine serum albumin, and chicken egg albumin. Lane 2, glycerol gradient peak fraction. The 67 kDa protein band in the enzyme fraction corresponds to bovine serum albumin, which was added as an enzyme stabilizer after elution from the gradient.

Table 1
*Purification of mitochondrial DNA polymerase from S. cerevisiae.*
The mtDNA polymerase was purified from 500 g of yeast, and assayed under standard conditions as described under "Methods". One unit is defined as the amount of enzyme that incorporates 1 pmol of nucleotide per minute.

| Purification steps | Total protein | Total activity units (pmol/min) | Specific activity (units/mg protein) | Purification factor | Recovery (%) |
|---|---|---|---|---|---|
| 100 000 × *g* supernatant | 240 mg | 480 | 2 | | 100 |
| Phosphocellulose column | 2.2 mg | 285 | 130 | 65 | 60 |
| Heparin-agarose column | 94 μg | 214 | 2 277 | 1 138 | 45 |
| DNA-cellulose column | 33 μg | 166 | 5 030 | 2 515 | 35 |
| Glycerol gradient | 10 μg | 71 | 7 100 | 3 550 | 15 |

**Time course**

The rate of DNA synthesis was determined on activated calf-thymus DNA over a period of 30 min at 37°C (Fig. 2A). For comparison, the yeast nuclear DNA polymerase I was used in a parallel experiment. The maximum activity of the nuclear DNA polymerase I was found within 10–15 min, whereas the mtDNA polymerase activity increased with increasing time of incubation throughout the 30 min reaction period. It has been also found that deoxyribonucleotide polymerizing activity of the nuclear DNA polymerase I was twice as fast as that of mtDNA polymerase.

**Stability of the mtDNA polymerase**

Since the isolated mtDNA polymerase was found to be unstable, heat sensitivity of the glycerol gradient purified enzyme preparation

was tested by preincubation of the enzyme at 45°C for various time periods (Fig. 2B). The yeast nuclear DNA polymerase I was also used in a parallel experiment. The nuclear polymerase I activity decreased gradually with increasing time of preincubation, as expected. In contrast, one half of the mtDNA polymerase activity was inactivated within 3–5 min of preincubation at 45°C whereas the other half of the polymerase activity remained active even after 25 min of preincubation. Since three polypeptides are present in the enzyme preparation, one of the possible explanations of this observation would be the presence of a heat sensitive and a heat stable form of the polymerase. One of them would be the intact enzyme and the other would be the proteolytic product of the polymerase. The temperature sensitivity of yeast enzyme might be one of the possible factors for lower optimal growth temperature of yeast than of other organisms.

**Effect of selective metal ions and NEM on mtDNA polymerase activity**

The earlier study determined the inhibitory effect of $Mn^{2+}$, EtBr and dTTP on the mtDNA polymerase activity [7]. The effect of some other metal ions (i.e., $MgCl_2$, NaCl, $ZnCl_2$) and NEM on mtDNA polymerase was tested for further information on the enzyme (Table 2). It has been found that 20 mM $Mg^{2+}$ was required for optimal activity of the mtDNA polymerase. At 50 mM NaCl concentration the mtDNA polymerase activity did not change whereas 37% or 71% of the polymerase activity was inhibited in the presence of 100 mM or 200 mM NaCl, respectively. Since $Zn^{2+}$ is an important co-factor for generation of metal-binding finger of many RNA-binding or DNA-binding pro-

Table 2

*The effects of metal ions and NEM on mitochondrial DNA polymerase activity.*

Metal ion or NEM was added to standard assay mixture, and the polymerase activity was measured as described under "Methods". Activity was expressed as percentage of the activity in the absence of metal ions or NEM.

| Metal ions or inhibitors | Concentration | mtDNA polymerase activity (%) |
|---|---|---|
| None | | 100 |
| $MgCl_2$ | 10 mM | 90 |
| | 20 mM | 100 |
| | 30 mM | 80 |
| NaCl | 50 mM | 100 |
| | 100 mM | 63 |
| | 200 mM | 29 |
| $ZnCl_2$ | 10 mM | 100 |
| | 30 mM | 150 |
| | 60 mM | 60 |
| NEM | 0.5 mM | 61 |
| | 1.0 mM | 41 |
| | 2.0 mM | 40 |

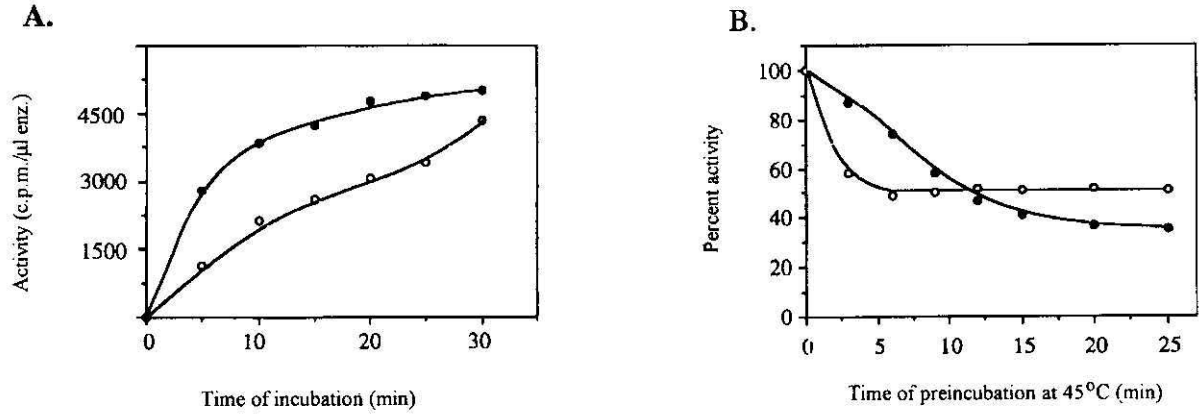



*Fig. 2. mtDNA polymerase activity and temperature sensitivity of the yeast DNA polymerase.*

A, DNA polymerase activity at different time periods. The reaction conditions were the same as those described in "Methods" section. Two microliters of enzyme from final preparation was added, the reactions were terminated at different time points. The acid-precipitable radioactivity was measured by a scintillation counter. B, Temperature sensitivity of the yeast DNA polymerases. The polymerases were preincubated at 45°C for different time periods and then DNA synthesis was started by the addition of 4 dNTPs including [α-$^3$H]TTP and continued for 20 min at 37°C as described above. ○, mtDNA polymerase; ●, nuclear DNA polymerase I.

teins [12], the effect of $Zn^{2+}$ on mtDNA polymerase activity was examined. In the presence of 30 mM $ZnCl_2$, the mtDNA polymerase activity increased by 50% whereas 60 mM $ZnCl_2$ inhibited the enzyme activity. The sulfhydryl blocking agent NEM at 1.0 mM concentration inhibited the mtDNA polymerase activity by 60%. Interestingly, the remaining 40% of activity was insensitive to NEM even at higher concentration (2.0 mM) pointing to the presence of two molecular forms of the mtDNA polymerase, as suggested above. The *Drosophila* mtDNA polymerase was also found to be sensitive to NEM [13].

### *In vitro* run-off DNA synthesis

The primer-specific activity of mtDNA polymerase on a mitochondrial DNA template has been tested by an *in vitro* run-off reaction. This reaction was performed using an M13 primer and a linear double-stranded M13 DNA template carrying a mtDNA insert (i.e., 140 nt, 442 nt or 1154 nt long mtDNA fragment). The mtDNA fragment was inserted into the polycloning sites of M13 plasmid to provide a mtDNA sequence in the template DNA as well as to increase the length of replicating DNA sequence. The template DNAs were linearized by restriction endonuclease *Eco*RI, and then hybridized with the primer by heating at 90°C for 5 min followed by cooling to room temperature. The reaction conditions were the same as described above except that the reaction volume was 25 µl, and 5 µM dATP and 5–10 µCi of [$\alpha$-$^{32}$P]dATP were used. The concentration of primer-template was in excess. The reaction was carried out at 37°C for 20 min, and then stopped by the addition of 25 µl of stop solution (0.3% SDS, 200 µg/ml tRNA) followed by phenol extraction. After centrifugation, DNA in the aqueous layer was precipitated by ammonium acetate/ethanol. The precipitated DNA was collected by centrifugation and electrophoresed on a 5% polyacrylamide-urea (8 M) gel. The primer-extended products were visualized by autoradiography (Fig. 3A). The DNA products of 160, 462 or 1174 nucleotides were obtained from these templates, as expected. This result demonstrated that mtDNA polymerase can synthesize at least 1174 nucleotides long DNA without any major pausing. It seems that the yeast mtDNA polymerase is moderately processive on a double-stranded DNA template. The results from the present and earlier studies [7] suggest that the mtDNA polymerase highly prefers A-T-rich DNA template which could be one of the reasons for selection of yeast mtDNA carrying about 85% A-T nucleotides in evolution.

The mtDNA polymerase activity was also tested on a topologically different single-stranded DNA template. Two different end-labeled M13 primers, a single-stranded M13 DNA template and a double-stranded linear M13 DNA template (for control experiment) were used in these reactions. The primer-extended DNA products from 20 min reaction are shown in Fig. 3B. In a negative control experiment, either the primer was omitted (Fig. 3B, lane 1) or a noncomplementary primer was provided (Fig. 3B, lane 4) and DNA product was not obtained, as expected. This result suggests that neither the mtDNA polymerase has its intrinsic priming activity nor DNA synthesis occurs without primer on these templates. However, there was a remarkable difference in primer-specific activity of the mtDNA polymerase on two different templates. A discrete 460 nucleotide run-off product was obtained from a double-stranded linear DNA template (Fig. 3B, lane 2) whereas many unexpected DNA products in the size range of 100-1500 nucleotides were obtained on a single-stranded circular M13 DNA template (Fig. 3B, lane 3). The continuous array of partially replicated DNA molecules is expected from a template primed/or paused at random positions. Since mtDNA synthesis started at a primer-specific location of the DNA template in these experiments, different DNA products on a single-stranded DNA template might occur due to random pausing. The multiple pausing could occur due to any of the following reasons: i) the mtDNA polymerase might not be processive on a single-stranded template and falls off easily, ii) the mtDNA polymerase might be very sensitive to the secondary structures of the single-stranded DNA template as has been found for the vaccinia virus [14] and T4 [15] DNA polymerases on single-stranded $\phi$X174 and fd DNA templates. The further analysis of processivity/fidelity of the mtDNA polymerase needs to be done using a direct mutational assay procedure capable of detecting substitution and frame shift mutations as described by Thomas *et al.* [16].

### The role of mtRNA polymerase in mtDNA synthesis

According to the observation of preferential transmission of some petite mtDNA in yeast, four putative origins of replication (*ori*) were identified in yeast mitochondria which are located in both mtDNA strands (two origins for each strand) [17]. Each *ori* carries a 300 bp conserved DNA sequence which consists of a mitochondrial promoter, a G-cluster endoribonuclease cleavage site and a hair-pin structure (Fig. 4A). This information on *ori* sequence suggests that in yeast mitochondria an RNA primer is made from the promoter, cleaved at the G-cluster, and then used as a primer for mtDNA synthesis. However, the previous [7] and present studies demonstrated that the mtDNA polymerase used poly[dA-dT] template but not the duplex RNA : DNA template (i.e., poly-$[rA]_{305}$.poly$[dT]_{167}$). To further elucidate the role of mitochondrial RNA or RNA polymerase in mtDNA synthesis, *in vitro* mtDNA syntheses were performed along with mitochondrial transcription (Fig. 4B). First, the efficiency of *in vitro* mitochondrial transcription was tested by the run-off transcription assay as described previously [18]. Mitochondrial RNA was made *in vitro* on a linear mtDNA template carrying a mitochondrial promoter by mtRNA polymerase in the presence of all four rNTPs, and an expected 72 nt RNA was obtained (Fig. 4B, lane 1). Similarly, an *in vitro* DNA synthesis was tested in the absence of primer and no run-off DNA product was obtained (Fig. 4B, lane 2) suggesting that mtDNA polymerase, like other

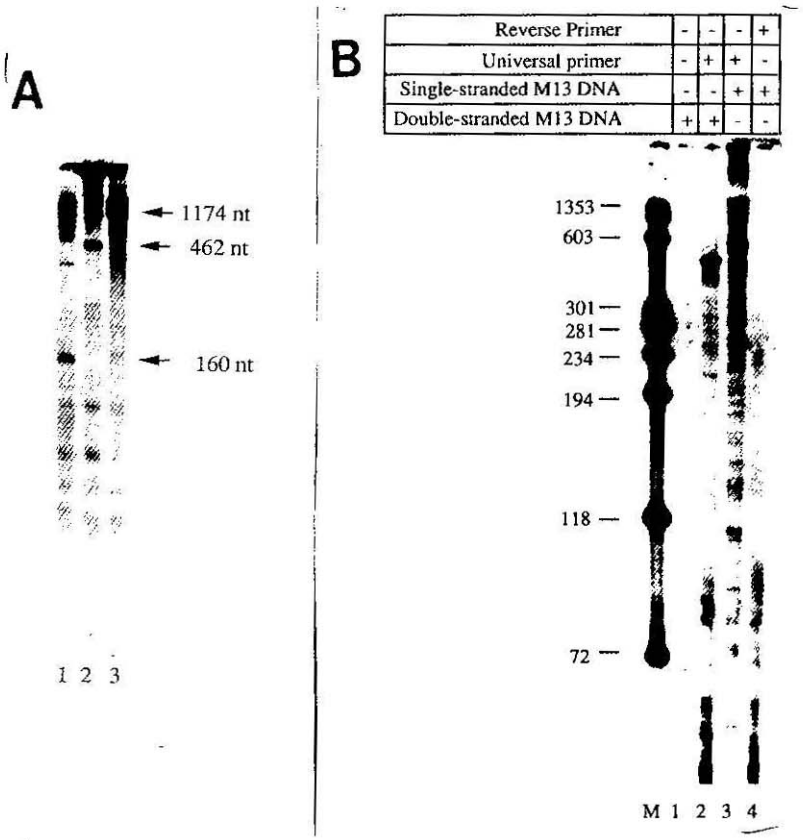
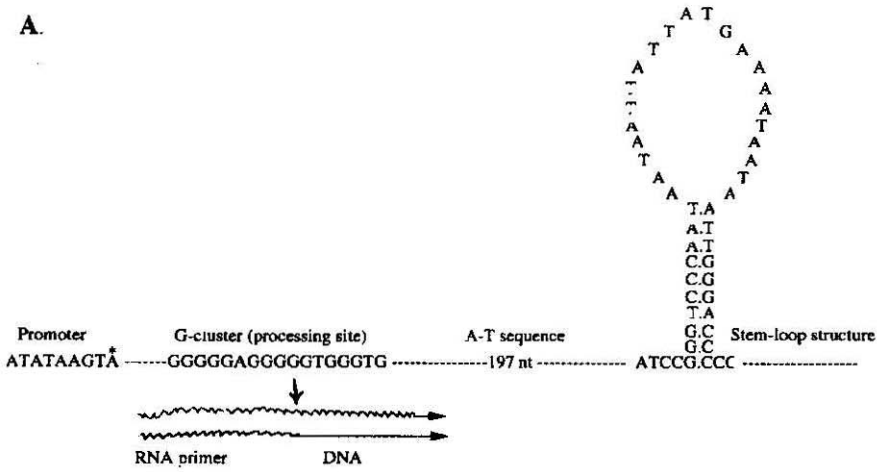



*Fig. 3. DNA synthesis.*

A, Primer-specific run-off DNA synthesis by the mtDNA polymerase. The run-off assay was performed using an M13 primer and three M13 DNA templates carrying a mtDNA insert of 140, 442 or 1154 nt in size. For template-primer hybridization, the oligonucleotide primer and the linear DNA template were mixed together, heated at 90°C for 5 min, and then cooled to room temperature. The template-primer duplex (100 μg/ml) was added to the reaction mixture, and the reaction was carried out at 37°C for 20 min. The products were analyzed as described above. DNA products of 160 nucleotides (lane 1), 462 nucleotides (lane 2) or 1174 nucleotides (lane 3) were obtained from these templates. B, DNA synthesis on a double-stranded linear or a single-stranded circular DNA template. The reaction was carried out as above except that end labeled M13 primers and a single-stranded circular DNA template or a double-stranded linear DNA template were used. Lane M, end labeled *Hae*III-digested ϕX174 DNA marker; lane 1, no primer was added; lane 2, a primer and a double-stranded linear DNA template were added; lane 3, a primer and a single-stranded circular DNA template were added; lane 4, an M13 reverse primer and a single-stranded circular DNA template were added.

polymerases, absolutely requires a primer for DNA synthesis. Some minor DNA bands were found probably from nonspecific DNA synthesis on nicked templates. In a positive control experiment, an oligonucleotide primer was added and a 127 nt DNA product was obtained (Fig. 4B, lane 5) suggesting that the *in vitro* reaction conditions for the mtDNA polymerase were working. To test whether an RNA primer is used *in vitro* by the mtDNA polymerase, a 31 nt RNA was made by mtRNA polymerase in the presence of GTP, ATP and UTP (CTP-less transcription reaction) and then DNA synthesis was started by the addition of 4 dNTPs, [α-$^{32}$P]dATP and the mtDNA polymerase. The expected 72 nt product was not obtained. In a separate experiment, transcription/DNA synthesis were coupled by the addition of all rNTPs, dNTPs, [α-$^{32}$P]dATP, the mtRNA polymerase and the mtDNA polymerase together since both enzymes require similar reaction conditions. The predicted 72 nt product was not obtained, however, some radiolabeled products in the size range of 90–100 nt were found but their origins are not clear. These results suggest that RNA primer may not be necessary



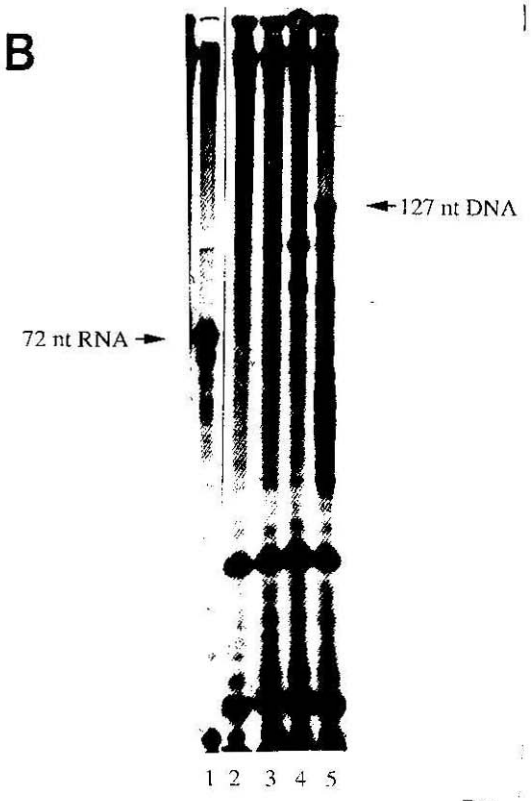




*Fig. 4. A, The conserved 300 bp ori sequence of yeast mitochondria.*
The 300 bp conserved sequence contains a mitochondrial octanucleotide promoter, a G-cluster (an endoribonuclease cleavage site) and a hair-pin structure. The wavy lines indicate the precursor primer RNA originated from the promoter. The transcriptional start site is shown by an asterisk. An arrow above the wavy lines shows the processing site of primer RNA. The solid line designates the DNA strand newly synthesized from the primer RNA. B, The effect of mitochondrial transcription on mtDNA synthesis. Lane 1, a 72 nt [$^{32}$P]UMP-labeled run-off RNA product from a truncated mitochondrial promoter in an *in vitro* transcription reaction. Lane 2, run-off DNA synthesis on the same template using 4 dNTPs, [α-$^{32}$P]dATP and the mtDNA polymerase but no primer. Lane 3, a 31 nt RNA primer was made from the same template by the mtRNA polymerase under CTP-less reaction conditions and then RNA polymerase activity was inactivated by incubation at 70°C for 10 min. DNA synthesis was started in the same tube by the addition of 4 dNTPs including [α-$^{32}$P]dATP and the DNA. Lane 4, both RNA and DNA synthesis were started together by the addition of 4 rNTPs, 4 dNTPs, [α-$^{32}$P]dATP, mtRNA polymerase and mtDNA polymerase. Lane 5, DNA synthesis was started by the addition of 4 dNTPs, [α-$^{32}$P]dATP, an oligonucleotide primer and the mtDNA polymerase. A 127 nt product was obtained which is larger than the 72 nt product due to the upstream location of the primer.

for mitochondrial DNA synthesis. In fact, the yeast mitochondrial petite genome is synthesized and maintained properly without having any mitochondrial promoter [5] or in the absence of mtRNA polymerase [19].

## REFERENCES


1. Kornberg, A. (1982) *DNA replication*. Freeman Publication, San Francisco.
2. Bogenhagen, D. & Clayton, D.A. (1977) Mouse cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. *Cell* **11**, 719–727.
3. Newlon, C.S. & Fangman, W.L. (1975) Mitochondrial DNA synthesis in cell cycle mutants of *Saccharomyces cerevisiae*. *Cell* **7**, 423–428.
4. Clayton, D.A. (1982) Replication of animal mitochondrial DNA. *Cell* **28**, 693–705.
5. Fangman, W.L., Henly, J.W., Churchill, G. & Brewer, B. (1989) Stable maintenance of a 35 bp yeast mitochondrial genome. *Mol. Cell. Biol.* **9**, 1917–1921.
6. Wintersberger, U. & Blutsch, H. (1976) DNA-dependent DNA polymerase from yeast mitochondria. *Eur. J. Biochem.* **68**, 199–207.
7. Sen, S., Mukhopadhyay, S., Wetzel, J. & Biswas, T.K (1994) Characterization of the mitochondrial DNA polymerase from *Saccharomyces cerevisiae*. *Acta Biochim. Polon.* **41**, 79–86.
8. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal. Biochem.* **72**, 248–254.
9. Weber, K. & Osborn, M. (1969) The reliability of molecular weight determination by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**, 4406–4412.
10. Wray, W., Boulikas, T., Wray, V. & Hancock, R. (1981) Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* **118**, 197–203.
11. Foury, F. (1989) Cloning and sequencing of the nuclear gene *MIP1* encoding the catalytic subunit of the yeast mitochondrial DNA polymerase. *J. Biol. Chem.* **264**, 20552–20560.
12. Berg, J.M. (1986) Potential metal-binding domains in nucleic acid binding proteins. *Science* **232**, 485–487.
13. Wernette, C.M. & Kaguni, L.S. (1986) A mitochondrial DNA polymerase from embryos of *Drosophila melanogaster*: purification, subunit structure, and partial characterization. *J. Biol. Chem.* **261**, 14764–14770.
14. Challberg, M.D. & Englund, P.T. (1979) The effect of template secondary structure on vaccinia DNA polymerase. *J. Biol. Chem.* **254**, 7820–7826.
15. Roth, A.C., Nossal, N.G. & Englund, P.T. (1982) Rapid hydrolysis of deoxynucleoside tri-phosphates accompanies DNA synthesis by T4 DNA polymerase and T4 accessory proteins 44/62 and 45. *J. Biol. Chem.* **257**, 1267–1273.
16. Thomas, D.C., Roberts, J.D. & Kunkel, T.A. (1991) Heteroduplex repair in extracts of human HeLa cells. *J. Biol. Chem.* **266**, 3744–3751.
17. Bernardi, G. (1982) Evolutionary origin and the biological function of noncoding sequences in the mitochondrial genome of yeast; in *Mitochondrial Genes* (Slonimsky, P., Borst, P. & Attardi, G., eds.) pp. 269–278, Cold Spring Harbor, NY.
18. Biswas, T.K. (1992) Transcriptional commitment of mitochondrial RNA polymerase from *Saccharomyces cerevisiae*. *J. Mol. Biol.* **226**, 335–347.
19. Fangman, W.L., Henly, J.W. & Brewer, B. (1990) *RPO41*-independent maintenence of r-mitochondrial DNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10**, 10–15.