OCCURRENCE OF A DNA-POLYMERASE IN ISOLATED YEAST MITOCHONDRIA[1]

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Mitochondria of yeast, like those of mammalian cells, contain small amounts of DNA (Schatz et al.,1964; Tewari et al.,1965; Carneo et al.,1966) which is used as a template by a mitochondrial DNA-dependent RNA-polymerase (Wintersberger and Tuppy 1965; Wintersberger,1966). It has been discussed (Gibor and Granick,1964) that mitochondrial DNA carries genetic information which is of importance in the process of biogenesis of this organelle. If this is the case, mitochondria must have a mechanism for an identical replication of their DNA. Autoradiographic studies (Chèvremont,1962; Guttes and Guttes,1964) suggest that mitochondrial DNA is turning over and it has been shown (Neubert et al.,1965; Schneider and Kuff,1965) that after intraveneous injection of labeled thymidine the radioactive label can be found in mitochondrial DNA of rat tissues.

In this paper data will be presented to show that isolated yeast mitochondria contain a DNA-polymerase with properties similar to those of the DNA-polymerases of bacteria and mammalian nuclei.

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MATERIALS AND METHODS

A locally isolated wild type strain of Saccharomyces cerevisiae was grown in a medium containing 0.8% glucose for 22 h at 26° as described previously (Wintersberger and Tuppy,1965). The preparation of yeast homogenates and the purification of the mitochondria by flotation in density gradients were the same as in the earlier work except that in the homogenization medium sucrose was replaced by mannitol. Yeast mitochondria prepared by this method contain on the average 4 µg DNA/mg protein. DNA isolated from these particles shows only one sharp thermal transition (Tm=75°) with no indication of a contamination by nuclear DNA (Viehhauser, Wintersberger and Tuppy, unpublished). DNA-polymerase was measured by the incorporation of ^{3}H-dATP into an acid-insoluble product. The reaction mixture contained, in 1 ml: 20 µmoles Tris.HCl (pH 7.5); 10 µmoles $MgCl_2$; 50 mµmoles each of dATP,dGTP,dCTP and dTTP; 1 mg phosphoenolpyruvate; 100 µg pyruvate kinase; 4 µc ^{3}H-dATP (Schwarz BioResearch Inc., 1.0 c/mmole) and mitochondria containing 1-1.5 mg protein. After 15 min of incubation at 37° 1 ml of cold 10% TCA was added and the precipitate was collected by centrifugation, washed twice with cold 5% TCA and once with alcohol/ether (3:1). It was then air-dried, dissolved in formic acid and its radioactivity measured in a scintillation counter.

Mitochondrial RNA-polymerase was measured as described earlier (Wintersberger,1966). The incubation mixture was identical with that described above except that deoxyribonucleoside triphosphates were replaced by ribonucleoside triphosphates.

Protein was estimated by the method of Lowry et al.(1951) with bovine serum albumin as a standard. DNA determinations

were performed according to the method of Burton (1956) using salmon sperm DNA as a standard.

RESULTS AND DISCUSSION

Properties of the incorporation reaction. Incorporation of radioactive dATP into an acid insoluble product requires the simultaneous presence of dGTP, dCTP and dTTP. Ommission of any one of them substantially reduces the level of incorporation. If these deoxyribonucleoside triphosphates are replaced by ribonucleoside triphosphates the amount of ^{3}H-dATP incorporated is negligible. The presence of Mg^{++} is an absolute requirement for the incorporation of the DNA precursor (Table 1).

Table 1

Requirements for dATP incorporation

Components	μμmoles dATP incorporated/ μg DNA/15 min
Complete system	20.2
Minus dGTP,dCTP and dTTP	0.5
Minus dGTP	2.8
Minus dCTP	0.7
Minus dTTP	0.6
Minus dGTP,dCTP and dTTP, plus GTP, CTP and UTP (50 mμmoles of each)	0
Minus $MgCl_2$	0
Complete system	22.9
Mitochondria pretreated with DNase	22.0
Plus 50 μg of yeast DNA	23.1

Mitochondria pretreated with relatively large amounts of pancreatic DNase (100 μg/ml, 20 min at 25° in the presence of 0.01 M $MgCl_2$) and subsequently washed free of enzyme have an unimpaired incorporating ability and an undiminished DNA content (Table 1). This indicates that the DNA primer is not removed by the action of DNase under these conditions. It is, therefore, likely that the nuclease does not penetrate the mitochondria, et least not to the site of DNA. The insensitivity

of the DNA-dependent RNA-polymerase activity of Neurospora crassa mitochondria (Luck and Reich,1964) and of yeast mitochondria towards DNase is in agreement with this assumption. Addition of DNA does not affect the level of incorporation of dATP (Table 1). Apparently DNA, like DNase, fails to penetrate to the site of the incorporating enzyme. These results suggest, therefore, that the DNA-polymerase is located inside the mitochondria.

The incorporation of ^{3}H-dATP reaches a maximal level within 15 minutes. Prolonged incubation results in a slowly progressing decrease in acid insoluble radioactivity. Exhaustion of one of the externally added components necessary for the polymerase activity is unlikely to produce this effect, as the incorporation increases linearily with the amount of mitochondria used up to at least 2 - 2.5 mg of mitochondrial protein. Similar results have,however, been obtained in the RNA-polymerase reaction in mitochondria of Neurospora crassa (Luck and Reich, 1964) and yeast (Wintersberger,1966). It is possible that the newly formed polymerase products are sensitive to a nuclease present inside the mitochondria (Linn and Lehman,1966).

Actinomycin inhibits the DNA-polymerase activity. As observed in other systems (Hurwitz et al.,1963), this effect is not as pronounced as the inhibition of the DNA-dependent RNA-synthesis. In contrast, mitomycin C, a typical inhibitor of DNA synthesis (Shiba et al.,1959), inhibits the incorporation of dATP while the RNA-polymerase activity remains unaffected (Table 2). The relatively large amounts of mitomycin C necessary to inhibit the mitochondrial DNA-polymerase are probably due to a low permeability of the mitochondrial membrane for this antibiotic. Similar observations were made in vivo (Neubert et al.,1965).

Table 2

Effect of actinomycin D and mitomycin C on the synthesis of DNA and RNA

Incubation conditions	DNA synthesis (μμmoles dATP incorporated/ μg DNA/15 min)	RNA synthesis (μμmoles ATP incorporated/ 10 μg RNA/15 min)
Complete system	21.8	15
Plus 10 μg actinomycin D	16.0	6
Plus 20 μg actinomycin D	13.1	4
Plus 20 μg mitomycin C	17.7	15.5
Plus 50 μg mitomycin C	14.6	16

Characterization of the polymerase product. The entire acid precipitable radioactivity was rendered soluble by heating for 15 min at 90° in 5% TCA or for 20 min at 70° in 10% perchloric acid.

For a further characterization of the polymerase product, an incubation on a 20fold larger scale was performed. The mitochondria were re-isolated by centrifugation and DNA was extracted from the particles and purified using the method of Marmur (1961). The isolated DNA had almost the same specific radioactivity as the TCA precipitate. The purified DNA was incubated with pancreatic DNase, with snake venom phosphodiesterase or with a mixture of the two enzymes. The results obtained (Table 3) show that ^{3}H-dATP was incorporated into mitochondrial DNA.

On chromatography of mitochondrial DNA on a methylated albumin kieselgur column (Sueoka and Cheng,1962) most of the material was eluted by 0.6 to 0.7 M NaCl and the radioactivity was eluted together with the DNA. The specific activity of the DNA remained unchanged during this procedure.

Table 3

Enzymatic hydrolysis of labeled mitochondrial DNA

15 µg of purified, labeled DNA, dissolved in 1 ml 0.02 M Tris (pH 7.5), containing 0.01 M $MgCl_2$ were incubated for 40 min at 37° with either 5 µg of pancreatic DNase (Worthington), 5 units of snake venom phosphodiesterase (Calbiochem), or a mixture of the two enzymes. The radioactivity precipitable by cold 10% TCA (after addition of 2 mg yeast RNA) was then measured.

Incubation conditions	% Acid insoluble radioactivity
Control (no enzyme)	100
Pancreatic DNase	23
Snake venom phosphodiesterase	69
DNase plus phosphodiesterase	0

CONCLUSIONS

The studies on isolated yeast mitochondria reported in this communication give clear evidence for the presence in these particles of a DNA-polymerase. This finding is in agreement with a previous report on an *in vivo* incorporation of labeled thymidine into mitochondrial DNA of rat tissues (Neubert et al., 1965). The occurrence of a DNA-polymerase in mitochondria would enable these particles to replicate their DNA independently of the nucleus. Neubert et al. (1965) have shown that the synthesis of mitochondrial DNA is not strictly dependent on the mitotic index and does not proceed in phase with nuclear DNA synthesis. Parsons (1965), in autoradiographic studies with *Tetrahymena*, arrived at a similar conclusion. Results of this kind strongly support the view that mitochondria are endowed with a partial genetic autonomy.

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