DNA-DEPENDENT RNA POLYMERASE FROM MITOCHONDRIA OF A CYTOPLASMIC "PETITE" MUTANT OF YEAST

Erhard Wintersberger

Department of Physiological Chemistry, University of Würzburg, D-87 Würzburg, Germany

Received July 31, 1970

SUMMARY: Mitochondria from a cytoplasmic "petite" mutant of Saccharomyces cerevisiae are functionally inactive and morphologically altered but contain a RNA polymerase like normal mitochondria. This enzyme can be solubilized by mechanical disruption of the mitochondrial membrane. The activity of the solubilized mitochondrial RNA polymerase is dependent upon externally added DNA; it requires all four nucleoside triphosphates, is inhibited by actinomycin D but is insensitive to rifampicin and α-amanitin.

INTRODUCTION

A considerable amount of evidence indicates that mitochondrial DNA is transcribed inside the organelle by a mitochondrial DNA-dependent RNA polymerase (1). RNA synthesis has been studied in whole mitochondria of a variety of organisms, including yeast, Neurospora and rat liver. Mitochondrial RNA polymerase is rather strongly bound to the inner membrane of mitochondria and thus cannot be solubilized by gentle destruction of this membrane (2). A more drastic disintegration of mitochondria, on the other hand, leads to an almost complete loss of RNA polymerase activity (2,3). This precluded a more detailed study of mitochondrial RNA polymerase and did not, for example, allow for a comparison of the properties of the mitochondrial enzyme with those of the enzymes active in nuclei.

Experiments with intact mitochondria from yeast and Neurospora have shown that RNA synthesis in these organelles is insensitive to rifampicin (3,4) This is in agreement with the recent finding that mitochondrial RNA synthesis measured in hamster cells in vivo is also not affected by the antibiotic (5).

On the basis of this criterion, RNA synthesis in mitochondria would seem to be indistinguishable from that occurring in nuclei but different from that in bacteria. However, experiments with the inhibitor α-amanitin gave different results:RNA synthesis in isolated yeast mitochondria was found to be insensitive to this inhibitor (E. Wintersberger, unpublished results) while yeast RNA polymerase of nuclear origin is inhibited (6).

Although these drugs may be useful to exclude a contamination of the mitochondrial fraction with bacteria or with nuclear polymerases, their effects on intact mitochondria give no definitive information relevant to the mitochondrial DNA-dependent RNA polymerase itself.

In this communication, I report studies on RNA polymerase from mitochondria of a respiration deficient "petite" mutant of yeast. In "petite" mutants mitochondrial DNA has lost its normal functions (1). They no longer carry out mitochondrial protein synthesis (7) and they have an altered, more fragile mitochondrial membrane (1). Using a mutant of this kind I asked whether "petite" mutants still contain a mitochondrial RNA polymerase, and if this were so, whether it would be possible to solubilize this enzyme due to the absence of a fully developed, rigid mitochondrial membrane.

MATERIALS AND METHODS

The haploid strain D 273-10 B-1, a respiration deficient, cytoplasmic mutant of Saccharomyces cerevisiae, was used. Cells were grown to late log phase in a medium containing 0.3 % yeast extract, 0.5 % peptone and 1 % glucose. They were disrupted mechanically and mitochondria were isolated and purified as described earlier (8). Purified mitochondria (10 to 15 mg mitochondrial protein) obtained from 80 g (wet weight) of cells were mixed with 2 volumes of alumina (Alcoa 305) and disintegrated by grinding in the cold for 2 to 3 minutes. The mixture was extracted with 0.5 ml of a buffer containing 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM ß-mercaptoethanol, 5 mM $MgCl_2$ and 10 % glycerol, and centrifuged for 30 minutes at 20,000 rpm in the SS-34 rotor of the Sorvall centrifuge. The clear supernatant, containing between 1 and 1.5 mg protein, was used as source of soluble mitochondrial DNA-dependent RNA polymerase.

RNA polymerase activity was measured in an assay mixture containing:

50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM ß-mercaptoethanol, 10 mM $MgCl_2$, 1.6 mM $MnCl_2$, 4.2 mM phosphoenol pyruvate (sodium salt), 100 µg/ml pyruvate kinase, 100 µM each of ATP, GTP and CTP, 20 µM UTP, 4 µCi/ml ^{3}H-UTP (Radiochemical center, Amersham; specific activity: 3 Ci/mmole), 100 µg/ml of heat denatured calf thymus DNA and enzyme. No external DNA was usually added for measurements of RNA polymerase in intact mitochondria because in this case mitochondrial DNA serves as template. The total volume of the assay mixtures was 0.125 ml. After incubation for 15 minutes at 35°C samples were cooled in ice and 100 µl aliquots were removed, applied to discs (2.4 cm diameter) of Whatman GF/C filters and treated further essentially as described by Bollum (9). Controls lacking enzyme were included in each set of determinations.

RESULTS AND DISCUSSION

RNA synthesis in mitochondria of a "petite" mutant of yeast. - Table 1 shows that mitochondria from the respiration deficient "petite" mutant used in this work incorporate RNA precursors into an acid insoluble product. The specific activities measured with different preparations of mutant mitochondria were from 40 to 60 % of those observed with mitochondria from wild type yeast under comparable conditions. All four nucleoside triphosphates are required. Externally added DNA does not significantly increase the extent of incorporation; this indicates that synthesis occurs inside the mitochondria and that mitochondrial DNA is used as template. This is a common property of all systems used to measure RNA synthesis in intact mitochondria (1).

The fact that mitochondria from a cytoplasmic "petite" mutant still contain a functional RNA polymerase is by itself interesting. Since these mutants show no mitochondrial protein synthesis, the mitochondrial RNA polymerase, like mitochondrial DNA polymerase (8), must be synthesized on cytoplasmic ribosomes outside the organelle. This is also in accord with an earlier observation that yeast cells grown in the presence of chloramphenicol, when mitochondrial protein synthesis is inhibited, still contain mitochondrial RNA and therefore must be capable of RNA synthesis (10).

Solubilization and partial characterization of mitochondrial DNA-dependent RNA polymerase. - The assumption that by using mutant mitochondria with

Table 1: RNA synthesis in isolated mitochondria from a "petite" mutant of yeast. - The assay mixture (complete system) is described in MATERIALS AND METHODS. Each assay contained 70 ug mitochondrial protein.

Conditions	μμmoles UMP incorporated/ mg protein
Complete system	68
+ 100 μg/ml denatured calf thymus DNA	71
- ATP, GTP and CTP	12

Table 2: Properties of a soluble DNA-dependent RNA polymerase from mitochondria of a "petite" mutant of yeast. - The assay mixture (complete system) is described in MATERIALS AND METHODS. Each assay contained 25 μg protein.

Conditions	μμmoles UMP incorporated/ mg protein
Complete system	88
- ATP, GTP and CTP	9
- DNA	10
- denatured DNA + native calf thymus DNA	38
+ 50 μg/ml actinomycin D	18
+ 0.8 μg/ml rifampicin	83
+ 4 μg/ml rifampicin	85
+ 16 μg/ml rifampicin	92
+ 4 μg/ml α-amanitin	87
+ 8 μg/ml α-amanitin	90
+ 16 μg/ml α-amanitin	82

an altered mitochondrial membrane it would be possible to solubilize mitochondrial RNA polymerase proved to be correct. Mechanical disruption of mutant mitochondria by grinding with alumina did release from 30 to 50 %

of the original RNA polymerase activity in a soluble form. Some properties of the soluble enzyme are summarized in Table 2. As with intact mitochondria, the incorporation requires all four nucleoside triphosphates, but contrast to the reaction in whole mitochondria, the soluble enzyme is highly dependent upon externally added DNA. Denatured DNA is a better template than native DNA. The DNA dependency of the reaction is also evident from the inhibitory effect of actinomycin D. Neither rifampicin nor α-amanitin inhibit up to concentrations of 16 μg/ml. These results are in complete accordance with the previous observations on intact mitochondria (3,4). Thus, on the basis of the action of these inhibitors, mitochondrial RNA polymerase is distinctly different from bacterial RNA polymerase and from the yeast RNA polymerase of nuclear origin. The former enzyme is virtually completely inhibited by less than 1 μg/ml of rifampicin (11), whereas the latter is inhibited to the extent of about 70 % by 16 μg/ml of α-amanitin (6). It is interesting that recent experiments indicate that the RNA polymerase present in nucleoli of higher organisms is likewise resistant to both, rifampicin and α-amanitin (12). A similar enzyme has not yet been found in mitochondria-free extracts from yeast cells (H. Ponta and E. Wintersberger, unpublished experiments).

The small amount of RNA polymerase which can be obtained from mitochondria of the "petite" mutant unfortunately precludes further purification and characterization of the enzyme. Because mitochondria from wild type yeast are available in much larger quantities, we have applied the extraction method described above to normal mitochondria but thus far have no been able to solubilize a sizable amount of RNA polymerase from wild type mitochondria. We can therefore not as yet exclude the possibility that the RNA polymerase from mutant mitochondria is different in certain aspects from the enzyme of normal mitochondria.

This investigation was supported by Deutsche Forschungsgemeinschaft.

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