ORIGIN OF THE MITOCHONDRIAL RNA POLYMERASE OF YEAST

A. H. SCRAGG*
National Institute for Medical Research, Mill Hill, London NW7 1AA, England

Received 11 March 1976

1. Introduction

It is now clear that mitochondria contain a specific DNA-directed RNA polymerase [1–7]. Isolation of a number of mitochondrial RNA polymerases has shown that these enzymes have a number of properties distinguishing them from their respective nuclear RNA polymerases, and those from bacteria. A particular difference is the mitochondrial enzymes single low mol. wt. subunit with values of 63 000 for *Neurospora crassa* [3], 65–70 000 from rat liver [1,2,4], 46–50 000 for *Xenopus laevis* [6] and 59–67 000 for yeast [5,7]. This is in sharp contrast to the high mol. wt. complexes consisting of a number of components found in both the nuclear and bacterial RNA polymerases [8,9]. The only similar RNA polymerases are those found associated with bacteriophages such as T_3 and T_7, having single polypeptides of 100–120 000 mol. wt. [10,11].

Similar to the bacterial RNA polymerases a number of the isolated mitochondrial RNA polymerases have been shown to be sensitive to rifamycin [1–4,7]. However, this property does not appear to be universal as rifamycin insensitivity has been reported for enzymes isolated from yeast [5], and *X. laevis* [6] mitochondria.

Many of the mitochondrial components although specific for mitochondria are nuclear coded and cytoplasmically synthesized [12]. It was therefore of interest to determine whether the yeast mitochondrial RNA polymerase was nuclear or mitochondrially coded, as it must be responsible for messenger RNA production within the mitochondrion. Evidence has been presented for the nuclear origin and cytoplasmic synthesis of the *N. crassa* enzyme [13].

2. Materials and methods

Saccharomyces cerevisae (strains 239, N.C.Y.C.; 239 LUV p^-; 239 p^-, a DNA zero strain lacking mitochondrial DNA [14]) was grown to stationary phase in 2% peptone, 1% yeast extract, and 1% glucose at 25°C.

Mitochondrial RNA polymerases were extracted and isolated with a modification of the method previously described [7].

RNA polymerase activity was determined in a standard reaction mixture containing, per ml; 50 μmol Tris-HCl (pH 7.5), 20 μmol magnesium acetate, 1 μmol $MnCl_2$, 1 μmol each of GTP and CTP, 2 μmol ATP, 0.05 μmol UTP, 6 μCi [^{3}H]UTP (10.5 Ci/mmol), 1 mg denatured calf thymus DNA, and 0.1 to 0.4 ml enzyme. Standard assays (55 μl) were incubated for 20 min at 37°C, a 50 μl sample removed, and applied to a Whatman No 1 filter paper disc (2.4 cm diameter). The discs were treated as described previously [7].

Mitochondrial RNA polymerase was labelled in vivo by incubating cells, prepared as described previously 15, with [^{3}H[- or [^{35}S]methionine using two methods of preparation. Method I; [^{3}H]methionine was added to a concentration of 2 μCi/ml (specific activity 250 mCi/mmol) 20 min after addition of chloramphenicol or cycloheximide (final concentration 100 μg/ml). Incubation was at 25°C for 2 h, after which mitochondria were isolated and the RNA polymerase isolated by DEAE-cellulose chromatography, and glycerol gradient centrifugation

* Present address: The Microbiological Research Establishment, Porton, Salisbury, Wiltshire SP4 OJG, England.

as described [7]. Method II; [^{35}S]methionine was added to a concentration of 10 μCi/ml (specific activity 160 Ci/mmol) 20 min after addition of chloramphenicol (final concentration 100 μg/ml) or ethidium bromide (final concentration 10 μg/ml). Incubation was at 25°C for 2 h after which the cells were disrupted mechanically [15]. After removal of cell debris the whole cell extract was treated in a similar manner to isolated mitochondria [7], and the RNA polymerase isolated in a similar manner.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was carried out according to the method of Weber and Osborn [16].

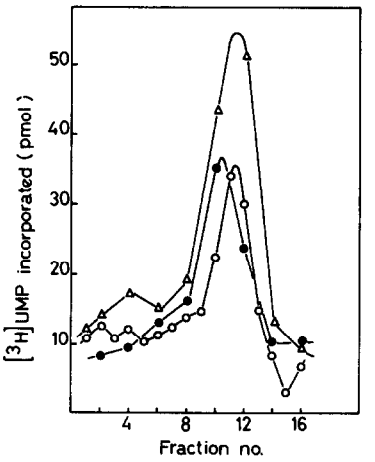

Fig.1. Glycerol gradient centrifugation of mitochondrial RNA polymerases. The peaks of activity from DEAE-cellulose chromatography were applied to 10 to 30% (v/v) glycerol gradients (5 ml volume) in 0.01 M Tris-HCl buffer, pH 7.8 containing 2 mM $MgCl_2$, 0.5 mM EDTA, 5 mM dithiothreitol, and 0.5 M KCl. Centrifugation was at 169 000 *g* for 1.5 h at 4°C, at which time 0.31 ml fractions were collected and assayed as described under Materials and methods. (-○-○-) strain 239 'wild type', 136 μg protein added; (-●-●-) strain 239 DNA-zero 'petite', 350 μg protein added; (-△-△-) strain 239 LUV 'petite', 340 μg protein added.

3. Results

In the study of the origin of various components of yeast mitochondria, yeast have the advantage of having available mutants which either lack mitochondrial DNA or have a reduced mitochondrial DNA content ('petites'). A method for the rapid isolation of yeast mitochondrial RNA polymerase has been developed [7]. This method has been applied to three yeast strains, a wild type, a 'petite', and a DNA-zero 'petite'. Fig.1 shows the final stage of purification, glycerol gradient centrifugation, of preparations from these three strains. A single peak of activity was found in each case in a position normally found in previous mitochondrial RNA polymerase preparations.

Table 1 shows the specific activities of the peak fractions from fig.1, and the effect of addition of rifamycin upon the activity. The specific activities of the wild type and 'petite' preparations were similar, but there was 50% reduction in activity from the DNA-zero 'petite' preparation. This reduction may perhaps be due to loss or leakage during preparation, as DNA-zero strains contain somewhat incomplete mitochondrial structures. All three enzymatic activities were sensitive to rifamycin, and it is clear that these represent similar enzymes. Thus it would appear that the mitochondrial RNA polymerase is not coded for by mitochondrial DNA.

Whether a protein is synthesized by the mitochondrion or in the cytoplasm can be determined by use

Table 1
Specific activities and effect of rifamycin SV in various mitochondrial RNA polymerases

Strain	Specific activity (nmoles UMP incorporated/mg protein/20 min/37°C)	% inhibition by rifamycin SV (20 μg/ml)
239 'wild type'	1.84	73
239 LUV 'petite'	1.77	63
239 DNA-zero 'petite'	0.78	53

The enzymes were assayed as described under Materials and methods.

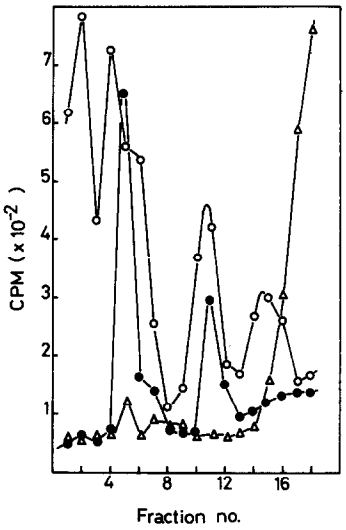

Fig.2. Glycerol gradient centrifugation of [^{3}H]methionine labelled mitochondrial RNA polymerases. As described under Materials and methods labelled mitochondrial RNA polymerase was prepared (Method I) and centrifuged as described in the legend to fig.1. Fractions of 0.28 ml were collected, 0.18 ml was used for reading the optical density at 280 nm and 0.1 ml was counted in a dioxane-based scintillant. (-o-o-) Control, strain 239; 11 150 cpm added (-•-•-) plus chloramphenicol, 100 μg/ml, 5000 cpm added; (-△-△-) plus cycloheximide, 100 μg/ml, 18 200 cpm added.

of antibiotics which selectively inhibit mitochondrial or cytoplasmic protein synthesis [12]. Mitochondrial RNA polymerase was isolated using Method I, (see Materials and methods) from cells labelled in the absence or presence of chloramphenicol or cycloheximide. Fig.2 shows the final stage of purification of these three preparations. The control culture gave a peak of radioactivity corresponding to the position found for the enzyme activity (fig.1). The same was true for the chloramphenicol-treated culture, in contrast to the cycloheximide-treated culture which only exhibited radioactivity at the top of the gradient. The protein profiles for all three preparations were essentially the same, the lack of label in the cycloheximide preparation not being due to degradation. Thus, the mitochondrial RNA polymerase appears to be made upon cytoplasmic ribosomes.

Further confirmation of these results is shown in fig.3. A mitochondrial RNA polymerase enriched preparation was labelled using Method II, (Materials and methods) in the absence or presence of chloramphenicol or ethidium bromide. The appropriate peak from DEAE-cellulose chromatography [7] was pooled, and analysed by polyacrylamide gel electrophoresis. Fig.3 shows autoradiographs of these gels. Little or no difference can be seen in the mol. wt. region of the mitochondrial RNA polymerase, (68 000,

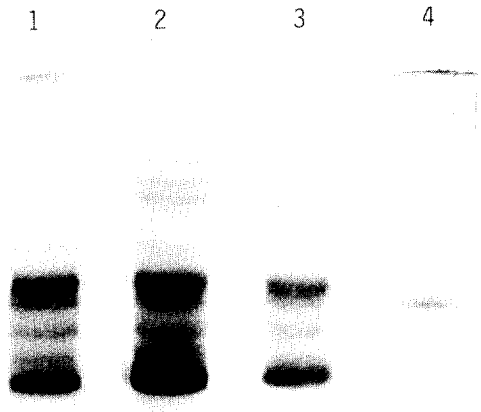

Fig.3. Autoradiographs of sodium dodecyl sulphate polyacrylamide gel electrophoresis of [^{35}S]methionine labelled preparations. As described under Materials and Methods labelled preparations (Method II) of mitochondrial RNA polymerase were prepared, electrophoresed, and autoradiographs prepared. (1) control, no additions, 50 000 cpm added to gel; (2) plus ethidium bromide (10 μg/ml), 42 000 cpm added to gel; (3) plus chloramphenicol (100 μg/ml), 55 500 cpm added to gel; (4) stained gel of purified enzyme run under similar conditions.

[7]. The same is true for densitometer traces of stained gels of the same preparations. Thus neither chloramphenicol or ethidium bromide appeared to affect the synthesis of the enzyme.

4. Discussion

From the results presented here the mitochondrial RNA polymerase, like many other components of the mitochondrion [12], is nuclear coded and synthesized in the cytoplasm. This is shown by the presence of the enzyme in a DNA-zero 'petite' (fig.1) and that its synthesis is unaffected by ethidium bromide or chloramphenicol, but inhibited by cycloheximide (figs.2 and 3). This appears to be similar to the mitochondrial RNA polymerase from *N. crassa* [13], and confirms the detection of polymerase activity in a 'petite' yeast [17], and the presence of messenger RNA in another [18]. However, no evidence was obtained for an increase in mitochondrial RNA polymerase labelling with short or long (results not shown) incubations with ethidium bromide in contrast to its effect upon the *N. crassa* [13] enzyme. Thus, although very similar, the control model proposed by Barath and Kuntzel [13] for the *N. crassa* enzyme does not appear to occur in yeast.

Acknowledgements

The author wishes to thank Dr D. H. Williamson for providing the yeast strains used, and to Mr B. Trinnaman for his expert technical assistance.

References

[1] Reid, B. D. and Parsons, P. (1971) Proc. Natl. Acad. Sci. US 68, 2830–2834.
[2] Mukerjee, H. and Goldfeder, A. (1973) Biochemistry 12, 5096–5101.
[3] Kuntzel, H. and Schafer, K. P. (1971) Nature New Biol. 231, 265–271.
[4] Gallerani, R. and Saccone, C. (1974) in: The Biogenesis of Mitochondria (Kroon, A. M. and Saccone, C. eds.) pp. 58–69, Academic Press, New York, London.
[5] Rogall, G. and Wintersberger, E. (1974) FEBS Lett. 46, 333–336.
[6] Wu, G. J. and David, I. B. (1972) Biochemistry 11, 3589–3595.
[7] Scragg, A. H. (1974) in: The Biogenesis of Mitochondria (Kroon, A. M. and Saccone, C. eds.) pp. 47–57, Academic Press, New York, London.
[8] Chambon, P. (1975) Annual Rev. Biochemistry 44, 613–638.
[9] Burgess, R. R. (1969) J. Biol. Chem. 244, 6160–6167.
[10] Chamberlin, M., McGrath, J. and Waskell, L. (1970) Nature 228, 227–231.
[11] Beier, H. and Hausmann, R. (1974) Nature 251, 538–540.
[12] Borst, P. (1972) Annual Rev. Biochemistry 41, 333–374.
[13] Barath, Z. and Kuntzel, H. (1972) Nature New Biol. 240, 195–197.
[14] Williamson, D. H. (1970) Heredity 25, 491–495.
[15] Thomas, D. Y. and Williamson, D. H. (1971) Nature New Biol. 233, 196–199.
[16] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[17] Wintersberger, E. (1971) Biochem. Biophys. Res. Commun. 40, 1179–1184.
[18] Halbreich, A., Di Franco, A., Groudinsky, O., Cosson, J. and Slonimski, P. P. (1975) Biochem. Biophys. Res. Commun. 64, 1286–1292.