MITOCHONDRIAL DNA-DIRECTED RNA POLYMERASE FROM SACCHAROMYCES CEREVISAE MITOCHONDRIA

A. H. Scragg

National Institute for Medical Research, Mill Hill, London NW7 1AA

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SUMMARY. A DNA-directed RNA polymerase activity has been detected in yeast mitochondrial extracts which is sensitive to rifampicin. This activity is distinct from that found in the nucleus and cytoplasm, and is possibly coded for, or under the control of the mitochondrial genome.

Isolated mitochondria of animal cells and microorganisms are capable of independent RNA synthesis (1-4). It has been established that this synthesis is catalyzed by an RNA polymerase located within the mitochondrion (5).

Rifampicin, a potent inhibitor of bacterial RNA polymerase (6) has been shown to affect RNA polymerase activity in whole rat liver mitochondria (7), and extracts (8). However, RNA polymerase activity in yeast (9,10), and Neurospora crassa mitochondria (11) appears to be insensitive to the action of rifampicin. Recently, an RNA polymerase extracted from yeast mitochondria has been shown insensitive to rifampicin (12), confirming previous experiments with extracts from "petite" mitochondria (13). In contrast to this KUntzel and Schäfer (14) have isolated an electrophoretically pure mitochondrial RNA polymerase from N. crassa which is sensitive to rifampicin, and a rifampicin sensitive RNA polymerase fraction has been detected in Blastocladiella anersonii (15). Evidence is presented here for the presence of a rifampicin sensitive enzyme in Saccharomyces cerevisae.

MATERIALS AND METHODS: S. cerevisae (strains 239 N.C.Y.C; αDV 147; αDV 147 EB) were grown to stationary phase in 2°/o peptone, 1°/o yeast extract and 1°/o glucose at 25° C. Mitochondria were prepared as described (16), and sonicated at full power for 30 sec in 0.01 M tris-HCl buffer, pH 7.8, containing 0.002 M $MgCl_2$, 0.5 mM EDTA, 5 mM dithiothreitol, 0.5 M KCl and

15°/o glycerol. Whole mitochondria and debris were removed by centrifugation at 15,000 g for 30 min and the supernatant referred to as crude enzyme.

The nuclear RNA polymerase was prepared by ammonium sulphate fractionation (25-50°/o saturation) of the postmitochondrial supernatant. Mitochondrial and nuclear DNAs were prepared by hydroxylapatite chromatography (17) from DNA extracted from whole cells (18).

RESULTS: Initial experiments indicated the presence of RNA polymerase activity in sonicates of yeast mitochondria, but this activity did not survive ammonium sulphate fractionation and was reduced in activity by nucleases. Polymerase activity could be separated by Sephadex G-200 chromatography. Figure 1 shows the separation of three peaks of polymerase activity. Peak I eluted at the void volume, indicating a molecular weight in the region of 200,000. Peak II eluted close behind peak I, appearing as a shoulder on Sephadex G-100.

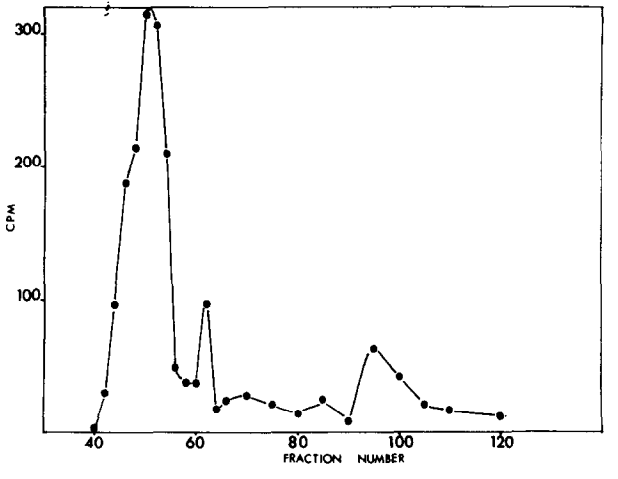

Fig. 1. Sephadex G-200 chromatography of mitochondrial RNA polymerase. 20 mg of the crude enzyme was applied to a (1.6 x 90 cm) column equilibrated with 0.05 M tris-HCl buffer, pH 7.5, containing 0.005 M magnesium acetate, 0.01 M EDTA and 5 mM dithiothreitol. The enzyme was eluted with the same buffer and 1.2 ml fractions collected. 25 µl aliquots were removed and assayed for RNA polymerase activity. RNA polymerase assay: the reaction mixture (65 µl) contained 50 mM tris-HCl buffer (pH 7.5), 20 mM magnesium acetate, 50 mM KCl, 1 mM each of ATP, GTP, CTP, 0.17 µCi ^{3}H-UTP (specific activity 10.5 Ci/mmole), 4.6 mM $MnCl_2$, enzyme and 6 µg denatured calf thymus DNA. Incubation was at 37°C for 10 min. 50 µl aliquots were removed, applied to Whatman No. 1 filter paper discs (2.4 cm dia.) and treated as described by Bollum (20).

Table 1. Effect of rifampicin and α-amanitin on mitochondrial and nuclear RNA polymerase activity

Enzyme	Control	plus α-amanitin 6 μg/ml		plus rifampicin 38 μg/ml		76 μg/ml	
	Activity	Activity	% Inhibition	Activity	% Inhibition	Activity	% Inhibition
Nuclear	48	26.8	44	45	6	47	3
Strain 239 Peak I	6.7	5.9	12	2.42	64	1.34	80
Peak II	1.94	1.2	38	0.90	54	0.33	83
Peak III	0.55	0.50	9	0.40	27	-	-

RNA polymerase activity was measured as described in the legend to Figure 1. Activity is expressed as pmoles ^{3}H-UMP incorporated/mg protein/10 min. Peaks I, II and III refer to the peaks shown in Figure 1.

Table 2. Effect of rifampicin and α-amanitin on RNA polymerase activity from "wild type" and "petite" mitochondria

	Control	plus α-amanitin 6 μg/ml		plus rifampicin 38 μg/ml		76 μg/ml	
Enzyme	Activity	Activity	°/o Inhib.	Activity	°/o inhib.	Activity	°/o Inhib.
αDV 147 (crude enzyme)	1.6	1.5	6	1.2	25	1.1	32
αDV 147 EB (crude enzyme)	2.4	1.27	47	-	-	1.94	19
αDV 147 (peak 1)	9.9	9.1	8	5.65	43	5.9	41
αDV 147 EB (peak 1)	3.1	2.66	17	3.3	0	-	-

RNA polymerase activity was measured as described in the legend to Figure 1. Activity is expressed as pmoles ^{3}H-UMP incorporated/mg protein/10 min. αDV 147 is the "wild type" and αDV 147 EB is the "petite" (DNA zero).

This pattern of multiple peaks is similar to that obtained with N. crassa mitochondrial RNA polymerase (14) and probably represents aggregates.

Both the crude enzyme and all three peaks showed RNA polymerase activity sensitive to rifampicin (Table 1), although peak II appears to contain a significant amount of α-amanitin sensitive activity, probably due to nuclear contamination.

To obtain information on the origin of this polymeraase a mutant αDV 147 EB has been used which has no detectable mitochondrial DNA (19). Figure 2 shows the separation of the polymerases from both "wild type" and "petite" mitochondria. The "petite" contains some 25°/o of the activity of the "wild type". However, when this activity is compared with the "wild type" in response to rifampicin and α-amanitin (Table 2) the activity is nuclear in response and hence is probably nuclear contamination.

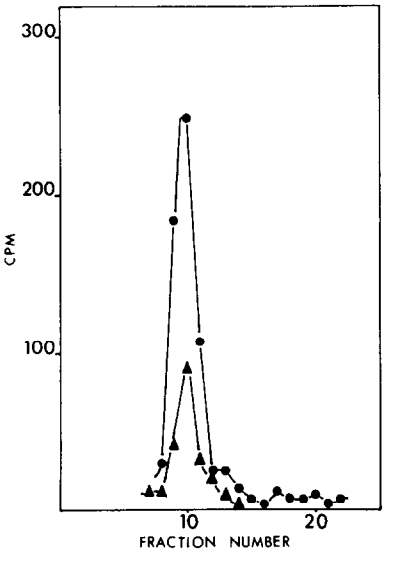

Fig. 2. Sephadex G-100 chromatography of mitochondrial RNA polymerase from "wild type" and "petite" mitochondria. 3.5 mg of "wild type" and 2.4 mg of "petite" crude enzyme equilibrated with sonication buffer (see Methods). The enzyme was eluted with the same buffer and 1.2 ml fractions collected. 25 µl aliquots were assayed for polymerase activity (see Legend to Figure 1). The specific activity of the two major peaks of the "wild type" and "petite" were 14.6 and 34 moles ^{3}H-UMP incorporated/mg protein/10 min respectively. (-O-O-) "wild type", αDV 147; (-Δ-Δ-) "petite", αDV 147 EB (DNA zero).

DISCUSSION: Clearly, yeast mitochondria contain a bacterial type of RNA polymerase which is sensitive to rifampicin and resistant to α-amanitin (6). Separation of three forms of polymerase activity indicates aggregates as found with the N. crassa mitochondrial polymerase (14), but in contrast has the ability to use denatured calf thymus DNA as a template. The rifampicin sensitive RNA polymerase activity is lost along with the mitochondrial genome. This could mean that the enzyme is coded for by the mitochondrial genome, although this could just be the loss of some fraction required for activity. The physical characteristics and requirements of the mitochondrial RNA polymerase are now under investigation.

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