THE SYNTHESIS OF RIBOSOMAL-TYPE RNA BY ISOLATED RAT LIVER MITOCHONDRIA

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SUMMARY

Isolated rat liver mitochondria have been shown to synthesize RNA which has an electrophoretic mobility of "21S" and "12S" on agarose-acrylamide gels. The synthesis of these RNA species was inhibited by ethidium bromide. From these observations it is suggested that mammalian mitochondrial DNA codes for mitochondrial ribosomal-type RNA.

Recently, distinctive species of ribosomal-type RNA from mitochondrial fractions of hamster (BHK-21) cells (1, 2), human (HeLa) cells (3, 4) and mouse (L) cells (5) have been described. The RNA has a sedimentation coefficient of about 17S (1-4) or 15 and 13S (5) and has an electrophoretic mobility of about "21S" and "12S" compared to "28S" and "18S" for cytoplasmic ribosomal RNA. It has not been rigorously shown that the "21S" and "12S" RNA species are from mitochondria and not from a contaminant such as a virus, mycoplasma or another cellular organelle, but their presence in highly purified mitochondria from rat liver (5) strengthens the suggestion that they are true mitochondrial components. It has not been shown whether they arise from mitochondrial ribosomes. Since ethidium bromide inhibits the synthesis of the "21S" and "12S" mitochondrial RNA (4, 5) and since ethidium bromide reacts preferentially with circular DNA (6, 7) such as that from mitochondria, it has been suggested the mitochondrial ribosomal-type RNA is coded for by mitochondrial DNA. In contrast, it has been claimed that the main species of RNA coded for by mitochondrial DNA from HeLa cells sediments at from 9-16S (8) and there is one report of a 23S species of RNA from regenerating rat liver mitochondria (9). The nature of RNA synthesized by isolated rat liver mitochondria has been examined by agarose-acrylamide gel electrophoresis in an attempt to resolve these differences and to demonstrate

more clearly the origin of mitochondrial ribosomal-type RNA. It has been shown previously that isolated rat liver mitochondria synthesize RNA (10, 11) and that this RNA hybridizes with the heavy strand of mitochondrial DNA (12). The nature of the RNA synthesized in this system has been described as heterodisperse sedimenting at from 4-10S (13) or from 8-14S (10). We now find that isolated rat liver mitochondria synthesize "21S" and "12S" RNA and that this synthesis is inhibited by ethidium bromide.

METHODS

Mitochondria were isolated from livers of hooded rats using 0.25M sucrose–2mM EDTA, pH 7.2, as the isolation medium. All equipment and the isolation and incubation media were sterilized. Nuclei were removed by 2 centrifugations at 1,000 x g for 10 min and mitochondria were recovered at 6,500 x g for 10 min and washed twice. Mitochondria were incubated at 30° as described by Parsons and Simpson (14) with ribonucleoside triphosphates, excluding UTP, [^{3}H] UTP (15 μC/ml, specific activity 3.7 C/mmole) as the radioactive precursor and 2 mg mitochondrial protein/ml. After incubation, mitochondria were centrifuged, RNA extracted by a hot phenol-SDS method (3), precipitated with trichloracetic acid and counted by liquid scintillation (Table 1). RNA analyzed by sucrose density gradient centrifugation was precipitated by alcohol after the hot phenol-SDS extraction. RNA analyzed by agarose-acrylamide gel electrophoresis (5, 15, 16) was extracted by a modification (5) of the method of Kirby (17).

RESULTS

Rat liver mitochondria, incubated as described in the Methods section, incorporated [^{3}H] UTP into RNA at a linear rate for 1 hr. The incorporation was sensitive to Actinomycin D, acriflavin, and ethidium bromide, partially sensitive to ribonuclease and insensitive to deoxyribonuclease as is shown in Table 1. Also shown are the effects of these compounds on the synthesis of RNA by rat liver nuclei. The lack of sensitivity of the synthesis of RNA by the mitochondrial fraction to ribonuclease and deoxyribonuclease

Table I Effect of inhibitors and enzymes on [^{3}H] UTP incorporation into RNA by isolated mitochondria and nuclei

	Mitochondria	Nuclei
	percent of control	
Actinomycin D	11.2 (40 µg/ml)	27.4 (9.5 µg/ml)
Acriflavin	15.4 (40 µg/ml)	-
Ethidium bromide	38.2 (0.33 µg/ml)	-
	19.8 (1.0 µg/ml)	-
Ribonuclease	82.6 (40 µg/ml)	11.7 (36 µg/ml)
Deoxyribonuclease	98.9 (82.6 µg/ml)	25.2 (25.2 µg/ml)

Rat liver mitochondria were incubated at 30°C for 1 hour. The control incorporation was 600cpm/hr/mg protein. Rat liver nuclei were incubated at 37°C for 20 min (18).

clearly differentiates this from RNA synthesis by nuclei. The sensitivity to the antibiotics indicates a DNA-dependent synthesis of RNA by mitochondria. These results are in general agreement with earlier studies (10, 11). In the first 10 min only of the incubation, the incorporation was partly insensitive to the antibiotics perhaps indicating a second type of incorporation or a permeability barrier.

RNA extracted after a 1 hr incubation by a hot phenol-SDS method and analyzed by sucrose density gradient centrifugation showed about 90% of the counts at about 4S and 10% of the counts in the 9 to 30S region. The higher molecular weight RNA was further characterized by agarose-acrylamide gel electrophoresis. In this case the RNA was extracted by a cold phenol-SDS method. Two major peaks with mobilities of "20.5S" and "11.5S" were observed (Fig 1). These will be referred to as "21S" and "12S". In addition there was a smaller peak at "28S" and a considerable amount of heterodisperse RNA. The synthesis of the "21S, "12S" and heterodisperse RNA was depressed about

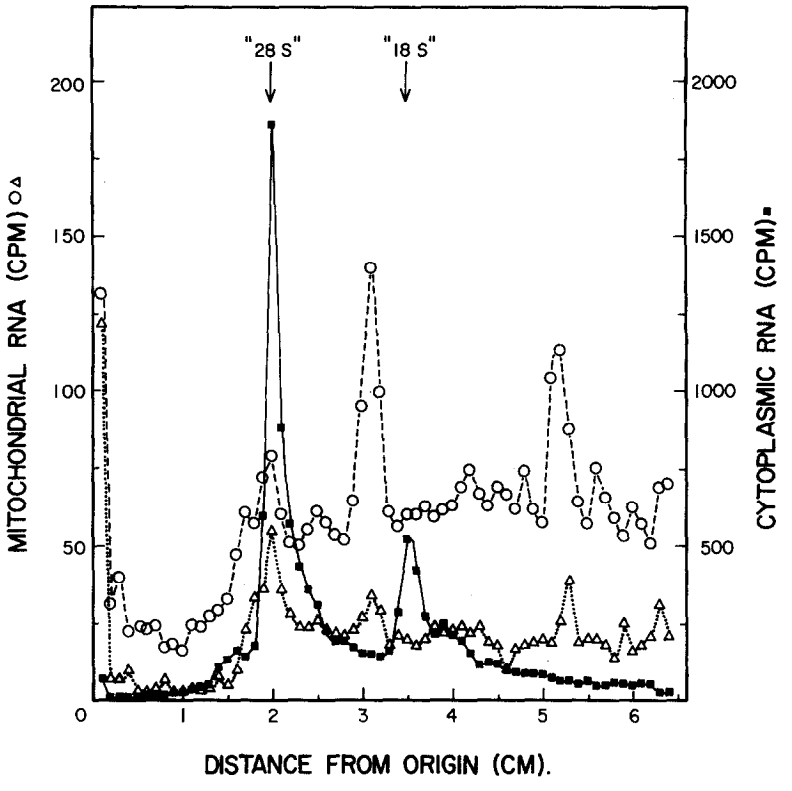

Fig. 1 Electrophoretic profile of RNA synthesized by isolated rat liver mitochondria. Electrophoresis on agarose-acrylamide gels was for 45 min at 30 volts/cm and 20 mA/tube. ■ , cytoplasmic RNA; o, mitochondrial RNA; Δ, mitochondrial RNA synthesized in the presence of ethidium bromide (0.33 µg/ml).

70% by ethidium bromide (0.33 µg/ml) in the incubation medium (Fig 1). This was about that found for the synthesis of total RNA (Table 1). The synthesis of the "28S" RNA was not inhibited by ethidium bromide.

DISCUSSION

The results demonstrate that rat liver mitochondria contain and synthesize ribosomal-type RNA having an electrophoretic mobility of "21S" and "12S". The synthesis is not due to nuclei as indicated by the insensitivity to deoxyribonuclease and ribonuclease. The sensitivity to ethidium bromide further indicates that the synthesis is not an end addition of nucleotides to pre-existing RNA. The mitochondrial DNA, therefore, codes for the mitochondrial ribosomal-type RNA. This has also been found for mitochon-

drial DNA of Neurospora crassa (19), yeast (20) and Tetrahymena pyriformis (21). In addition, an ethidium bromide sensitive synthesis of heterodisperse RNA was observed. Its significance is not known.

The synthesis of a small amount of "28S" RNA was not inhibited by ethidium bromide. This could be an end addition of nucleotides to 28S cytoplasmic ribosomal RNA contaminating the mitochondria and could be the antibiotic-insensitive incorporation of [^{3}H] UTP that occured in the first 10 min of the incubation.

The synthesis of "21S" and "12S" RNA by isolated mitochondria confirms and extends previous studies on the nature of mitochondrial RNA (1-5) in which the RNA was labelled in mammalian cells in tissue culture and extracted from mitochondrial fractions. They are not in agreement with reports that mammalian mitochondrial RNA is 9-16S (8) or 23S (9).

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REFERENCES

1. Dubin, D. T., Biochem. Biophys. Res. Commun. 29, 655 (1967).
2. Dubin, D. T. and Montenecourt, B. S., J. Mol. Biol. 48, 279 (1970).
3. Vesco, C. and Penman, S., Proc. Nat. Acad. Sci., Wash., 62, 218 (1969).
4. Zylber, E., Vesco, C. and Penman, S., J. Mol. Biol. 44, 195 (1969).
5. Bartoov, B., Mitra, R. S. and Freeman, K. B., Biochem. J. in press.
6. Bauer, W. and Vinograd, J., J. Mol. Biol. 33, 141 (1968).
7. Vinograd, J., Morris, J., Davidson, N. and Dove, W. F. Jr., Proc. Nat. Acad. Sci., Wash., 49, 12 (1963).
8. Attardi, B. and Attardi, G., Nature, Lond., 224, 1079 (1969).
9. Kroon, A. M. and Aaij, C., in Biochemical Aspects of the Biogenesis of Mitochondria, p. 207. Ed. by Tager, J. M., Papa, S., Quagliariello, E. and Slater, E. C., Bari: Adriatica Editrice, (1968)
10. Neubert, D., Helge, H. and Merker, H.-J., Biochem. Zeit. 343, 44 (1965).
11. Sacconne, C., Gadaleta, M. N. and Gallerani, R., Eur. J. Biochem. 60, 61 (1969).
12. Borst, P. and Aaij, C., Biochem. Biophys. Res. Commun. 34, 358 (1969).
13. Neubert, D., Helge, H. and Merker, H.-J. in Biochemical Aspects of the Biogenesis of Mitochondria, p. 251. Ed. by Tager, J. M., Papa, S., Quagliariello, E. and Slater, E. C., Bari: Adriatica Editrice, (1968)
14. Parsons, P. and Simpson, M. V., Science 155, 91 (1967).
15. Watanabe, Y., Prevec, L. and Graham, A. F., Proc. Nat. Acad. Sci., Wash., 58, 1040 (1967).
16. Peacock, A. C. and Dingman, C. W., Biochem. 7, 668 (1968).

17. Kirby, K. S., Biochem. J. 96, 266 (1965).
18. Widnell, C. C. and Tata, J. R., Biochem. J. 92, 313 (1964).
19. Wood, D. D. and Luck, D. J. L., J. Mol. Biol. 41, 211 (1969).
20. Wintersberger, E. and Viehauser, G., Nature, Lond. 220, 699 (1968).
21. Suyama, Y. and Eyer, J. J., Biol. Chem. 243, 320 (1968).