MITOCHONDRIAL AUTONOMY: SYNTHESIS OF DNA FROM RNA TEMPLATES IN ISOLATED MAMMALIAN MITOCHONDRIA*

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1. Introduction

RNA-dependent DNA polymerases, since their initial identification [1, 2], have been identified in a variety of cells and viruses [3–10] including the bacterium *E. coli* [11–13]. The presence of RNA directed DNA synthesis in *E. coli* and in particular the information flow from RNA to DNA documented earlier [11] raises the question as to whether such information flow occurs in mammalian mitochondria. *E. coli* and mammalian mitochondria are similar in several respects [14] (autonomous synthesis of some macromolecules; response to certain antibiotics such as chloramphenicol, cycloheximide and camptothecin; and possession of DNA without a "nuclear" membrane) and mitochondria are thought by some to have arisen from an evolutionary standpoint from an invasion of eukaryotic cells by prokaryote bacteria and subsequent formation of symbios. The present report describes low levels of an enzyme which could be termed an RNA-dependent DNA polymerase in crude homogenates of mammalian mitochondria.

2. Methods

Sterile, essentially pure mitochondria of rat liver [15] and cerebral cortex [16] were prepared as described. A "nucleic acid free extract" was prepared from the liver or cortex mitochondria as described previously [7]. A 0.1% Triton X-100 extract of the mitochondria also exhibited activity but control activity, presumably due to endogenous DNA template, was prohibitively high.

3. Results and discussion

The data in table 1 indicate that both the rat liver and cerebral cortex mitochondria synthesized DNA dependent on RNA added to the assay. The activity is dependent on RNA since treatment of the rat liver RNA with RNase (but not DNase) greatly decreased the activity; furthermore two synthetic co-polymers rAdT and rArU functioned as "templates". That, in every instance, the product was DNA is evidenced by lack of RNase degradation, susceptability to DNase degradation, alkali resistance, and trichloroacetic acid insolubility. The reaction was dependant on Mg^{2+}, deoxynucleotides, temperature and time of incubation. Yeast RNA was not as effective a "template" as rat liver RNA (table 1).

The results should be interpreted with caution [17], since the RNA or synthetic polymers added to the reaction mixture may merely be cofactors accelerating a DNA–DNA reaction. Furthermore, the *in vitro* situation may not reflect the *in vivo* situation and the present data may be a consequence of a DNA-dependent DNA polymerase functioning very inefficiently with an RNA template. Also it is not known whether true "circular DNA" is being synthesized by the enzyme. In any event, the fact that an RNA-

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Table 1
Identification and properties of an RNA-dependent DNA polymerase from isolated rat liver and cerebral cortex mitochondria.

System	I	II	III	IV	V	VI
Complete	872	1130	1290	723	1222	1424
0° (incubate)	118	107	216	92	86	104
0 time (immediate termination)	0	0	0	0	0	0
minus 'enzyme'	0	0	0	0	0	0
minus 'enzyme' plus boiled 'enzyme'	14	29	32	41	32	24
minus 'template'	0	0	0	0	0	0
minus 'template' plus RNase, treated 'template'	71	–[a]	–	36	–	–
minus 'template' plus DNase treated 'template'	798	–	–	724	–	–
Product: DNase treated	111	142	163	108	142	201
Product: RNase treated	880	1041	1197	708	1124	1342
Product: 0.5 N NaOH	821	1103	1123	802	1014	1440
minus dATP, dCTP, dGTP	508	612	496	281	414	421
minus $MgCl_2$	216	104	221	122	131	141
minus 'template' plus yeast RNA	391	411	414	362	364	368
incubate 1 hr 37°	419	592	711	384	721	808
minus TTP-^{3}H plus 5 μCi TTP-^{3}H	516	621	621	406	611	707

Conditions: I. The complete system contained in a final volume of 1 ml the following: 1.50 mg protein from the 'nucleic acid free' extract of rat liver mitochondria ('enzyme'); 1.0 μmole each of dATP, dCTP, and dGTP; 50 μmoles Tris-HCl buffer pH 8.4, 5.0 μmoles $MgCl_2$; 10 μCi ^{3}H-methyl TTP (7 Ci/mmole, New England Nuclear); 20 μmoles dithiothreitol; 50 μmoles NaCl; and 36 μg of rat liver RNA ('template'). Assays were incubated at 37° for 2 hr, after which 1 mg of yeast RNA was added and the assay was made 10% in trichloroacetic acid. The resultant precipitates were washed 3 times with 10% trichloroacetic acid, dissolved in 1 N NaOH at 100°, plated on a glass fiber filter and counted in a liquid scintillation counter. Rat liver RNA ('template') was purchased from General Biochemicals and treated with DNase and the enzyme removed by phenol extraction. DNase and RNase were present at 4 mg per ml and were purchased from Worthington. All data are given with endogenous activity (about 10%) determined with water substituted for the 'template' subtracted. Yeast RNA was purchased from Worthington and was present at 36 μg per assay. Data are CPM per assay. II. As I, except 0.01 A_{260} rAdT was substituted for the rat liver RNA as 'template'. III. As I, except 0.01 A_{260} rArU was substituted for the rat liver RNA as 'template'. IV. As I, except 1.5 mg protein from the nucleic acid free extract of rat cerebral cortex mitochondria was substituted for the rat liver mitochondria extract. V. As II, except 1.5 mg protein from the nucleic acid free extract of rat cerebral cortex mitochondria was substituted for the rat liver mitochondria extract. VI. As III, except 1.5 mg protein from the nucleic acid free extract of rat cerebral cortex mitochondria was substituted for the rat liver mitochondria extract.

[a] Dash (–) indicates experiment was not performed.

dependent reaction occurs means that information in RNA from other mitochondria, extramitochondrial cytoplasm, nuclei or RNA viruses could become a part of the mitochondrial DNA and perhaps the mitochondrial genome. Such information transfer could play a role in mitochondrial autonomy and mitochondrial biogenesis through gene amplification.

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