Migration of Nuclear RNA into Isolated Mouse Liver Mitochondria (36211)

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Little is known of the events leading to the biosynthesis of mitochondrial proteins. Since the mitochondrial genome is too small to code for more than a fraction of mitochondrial protein, it is apparent that nuclear DNA serves as template for the transcription of the messages. The crucial consideration now is whether translation of these messages is under the control of the mitochondrion and if so, how. This question has not attracted a share of the work commensurate with its importance. An obvious control mechanism would be for the messages to enter the mitochondria where they can be stored or propagated prior to translation, be it inside or outside the particle. While this manuscript was in preparation, Swanson (1) reported that synthetic polynucleotides may enter mitochondria and serve as templates for polypeptide synthesis. In the present study, evidence is presented that natural high molecular weight RNA, as well as RNA synthesized on a nuclear DNA template, can migrate into mitochondria, so that a control mechanism of the nature outlined above may in fact be operative.

Materials and Methods. Female mice of the imbred strain C3H/HeSy were used throughout.

Orotic acid-5-^{3}H (sp act 21 Ci/mmole) and carrier free orthophosphate-^{32}P were purchased from Amersham, Bucks England. Crystalline pancreatic DNase was a product of Sigma, St. Louis, MO.

Nuclei and mitochondria were isolated from livers of fasted ether-anesthetized animals by a slightly modified (2) method of Hogeboom (3). Nuclei were labeled in vivo by administering intraperitoneally 100 μCi of orotic acid-5-^{3}H/animal, 1 hr prior to sacrifice. Subfractionation of mitochondria into outer membranes, outer membrane-free mitochondria and contents of the intermembrane space was achieved by swelling and shrinking of purified mitochondria, followed by discontinuous density gradient centrifugation according to the method of Sottocasa *et al.* (4). Nuclear and mitochondrial RNA were isolated from the respective particles as previously described for mitochondrial DNA (2), except that the phenol-extracted nucleic acids were incubated with crystalline pancreatic DNase, rather than RNase, prior to gel filtration through Sephadex G-75.

^{32}P-Labeled RNA was synthesized on a liver nuclear DNA template with the *Micrococcus lysodeicticus* DNA-dependent RNA polymerase (5). α-^{32}P-UTP prepared by Dr. O. Antonoglou in this laboratory, according to the method of Symons (6, 7) was the label-carrying nucleoside triphosphate.

Results. Isolated liver mitochondria from 3 animals (2.1–2.4 mg total protein) were incubated for 1 hr at 37° in a total volume of 6 ml of Eagle's minimum essential medium, containing penicillin and streptomycin (100 units and 100 μg/ml, respectively), either with labeled nuclei (60–70 mg of total protein) or with RNA. The mitochondria were reisolated, washed three times, checked microscopically for the presence of nuclei and the RNA extracted. Table I gives a summary of the results from 5 individual experiments. Expts. 1 and 2 show that label can be transferred from nuclei labeled *in vivo* with orotic acid-5-^{3}H to mitochondria and furthermore that the label can be isolated from mitochondria as macromolecular RNA. In these cases, approximately 3% of the radioactivity liberated in the medium could be reisolated as macromolecular

TABLE I. Incorporation of Radioactivity into Mitochondrial RNA Following Incubation of Mitochondria with Labeled Nuclei or RNA.[a]

Expt. no.	Source of RNA	RNA radioactivity: Offered Total (cpm)	Offered Specific (cpm/A_{260})	Mitochondrial Total (cpm)	Mitochondrial Specific (cpm/A_{260})
1	Liver nuclei labeled *in vivo* for 1 hr with orotic acid-5-^{3}H	—	—	620	167
2	Liver nuclei labeled *in vivo* for 1 hr with orotic acid-5-^{3}H	—	—	195	126
3	RNA extracted from nuclei of Expt. 2	67,700	10,200	372	250
4	^{32}P-Labeled RNA synthesized on liver nuclear DNA template	20,000	100,000	665	618
5	Mixture of nucleoside triphosphates with α-^{32}P-UTP	20,000		5	2

[a] Incubation conditions as described in Materials and Methods.

RNA of specific radioactivity 347 and 756 for Expts. 1 and 2, respectively. Expts. 3 and 4 of Table I show that mitochondria can also take up RNA when they are incubated with labeled RNA whether isolated from *in vivo* labeled nuclei, or synthesized on a nuclear DNA template. When mitochondria are incubated with nucleoside triphosphates, one of which is α-^{32}P labeled, the radioactivity reisolated from mitochondria as macromolecular RNA is much less than the radioactivity of mitochondrial RNA when the particles are incubated either with labeled macromolecular RNA of equal total radioactivity or with nuclei (compare Expts. 4 and 5, Table I).

Table II presents evidence to the effect that labeled RNA incubated with mitochondria does not merely attach onto the outer surface of the particles, as cytoplasmic ribosomes do (8); but rather passes through the outer membrane, into the interior of the particle, where it can be detected.

Discussion. Goldstein and Trescott have shown that RNA can move from the nucleus to the cytoplasm (9), and vice versa (10). Attardi and Attardi (11) presented evidence that RNA can also move from the mitochondria to the endoplasmic reticulum. More recently, Cohen *et al.* (12) found that a portion of mitochondrial RNA in yeast hydridizes with nuclear DNA only and not with mitochondrial DNA. Goodenough and Levine (13) suggested that messenger RNA from the nucleus may be imported into the mitochondria

TABLE II. Distribution of Radioactivity in Mitochondrial Subfractions Following Incubation of Mitochondria with ^{32}P-Labeled RNA (19800 cpm).

Subfraction	Radioactivity (cpm)
Outer membranes	Not detectable
Outer membrane-free mitochondria	357
Intermembrane space	195

Incubation conditions and subfractionation of mitochondria as described under Materials and Methods.

and translated by the organelle's own ribosomes; while Swanson (1) reported that synthetic polynucleotides are taken up by mitochondria where they are utilized as templates for the synthesis of polypeptides. The present results show that RNA originating in the nucleus, as well as RNA formed by transcription of nuclear DNA *in vitro,* can enter the mitochondria. If this is the mechanism by which information is provided for the synthesis of some or all mitochondrial proteins, then the further fate of this karyogenic mitochondrial RNA should be investigated.

Summary. Macromolecular nuclear RNA can enter isolated mouse liver mitochondria, following incubation of the latter either with intact nuclei, labeled *in vivo* with orotic acid-5-^{3}H, or with ^{32}P-labeled RNA synthesized on a nuclear DNA template.

This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research. The author is thankful to Professor A. Synmeonidis for his unwavering support and to Mr. T. Karemfyllis for his excellent technical assistance.

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Received July 15, 1971. P.S.E.B.M., 1972, Vol. 139.