ON THE INTERACTION OF MITOCHONDRIAL PROTEIN SYNTHESIZING SYSTEM WITH TEMPLATE RIBONUCLEIC ACID OF NUCLEAR ORIGIN

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1. Introduction

There are some indirect data on the participation of template RNA's of nuclear origin in protein synthesis in mitochondria [1] although the major fraction of mitochondrial mRNA is a transcript of mitochondrial DNA [2, 3]. Our preliminary experiments [4] showed uptake of labeled nRNA* by isolated mitochondria in a reconstituted system, some characteristics of the process being described. Similar results were obtained in model experiments with synthetic polyribonucleotides [5].

The present work was done to study the interaction of nuclear RNA with the structures responsible for protein synthesis in isolated intact mitochondria. The data obtained showed that penetration of nuclear RNA into mitochondria is followed by its binding to mitochondrial ribosomes and by its template activity in the mitochondrial translation system. The established compatibility between mitochondrial translation machinery and exogenous mRNA's is discussed in the light of the problem of the foreign genetic information expression in animal and human cells.

2. Materials and methods

Isolated rat liver mitochondria and total nRNA were used in the experiments. Pulse-labeled nRNA was isolated 40 min after intraperitoneal injection of 100 μCi [^{14}C]orotic acid. For nRNA isolation 'hot' phenol–detergent extraction [6] was used with final RNA purification by centrifugation through a cesium chloride density gradient [7].

Mitochondria were incubated with nRNA aerobically in the medium which had been shown to be optimal for oxidative phosphorylation and for template synthesis in mitochondria [8]. In most experiments actinomycin D was added in high concentration (50 μg per 1 ml) to prevent RNA resynthesis in mitochondria from radioactive breakdown products [1].

The amount of RNA taken up by mitochondria was expressed as RNAase-resistant trichloroacetic acid-insoluble radioactivity of the mitochondrial pellet after incubation. RNAase treatment of mitochondria was at 20° for 20 min (pancreatic RNAase EC 2.7.7.16, Calbiochem, 50 μg per 1 ml). Mitochondrial membrane fractions were obtained by differential centrifugation after digitonin treatment [9] which is also the most reliable procedure for purification of mitochondria from contaminating cytoplasmic membrane-bound ribosomes [10]. The inner membrane-matrix fraction was lysed with 2% Triton X-100 and the lysate obtained was used for sedimentation in a sucrose gradient in a fixed-angle rotor [11] and for equilibrium banding in cesium chloride density gradient.

Analytical methods: Protein content in mitochondria was estimated by the biuret reaction, RNA content spectrophotometrically [12]. Trichloroacetic acid insoluble radioactivity was measured after precipitation onto nitrocellulose filters (HUFS, CSSR, 0.3–0.5 μm) in a Nuclear Chicago liquid scintillation counter, Mark II.

*Abbreviations:
mRNA, messenger (template) RNA; nRNA, nuclear RNA.

Table 1
The distribution of nuclear [^{14}C]RNA in mitochondrial membrane fractions.

Subfractions	TCA-insoluble radioactivity	
	(cpm)	(%)
Intact mitochondria	3600	100
Inner membranes + matrix	2100	60
Fragments of inner membranes	400	11
Outer membranes	360	10
Intermembrane space material	370	10

Mitochondria (80 mg protein) were incubated with nuclear [^{14}C]RNA (400,000 cpm) in the presence of cell sap (5 mg protein per 1 ml) and actinomycin D (50 μg per 1 ml) at 32° for 20 min. After incubation mitochondria were treated with RNAase and fractionated (see Methods).

3. Results

Table 1 demonstrates that [^{14}C]nRNA taken up by mitochondria during aerobic incubation was found mainly in the inner membrane-matrix fraction while

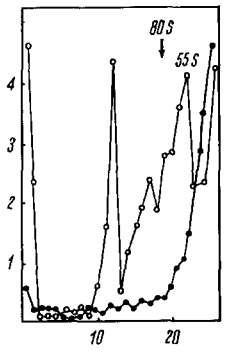

Fig. 1. The occurrence of [^{14}C]nRNA in ribonucleoprotein particles of inner membrane-matrix lysate. Mitochondria were incubated with nuclear [^{14}C]RNA for 20 min and then spun down by centrifugation. The pellet was treated with digitonin and the inner membrane-matrix fraction obtained was lysed with 2% Triton X-100. The lysate was layered on the top of a linear (10–30%, w/w) sucrose concentration gradient containing 0.01 M Tris-HCl buffer pH 7.4, 0.01 M magnesium chloride, 0.01 M potassium chloride. The centrifugation was performed in fixed-angle rotor No. 30 of a Spinco L2 ultracentrifuge at 29,000 rpm for 4.5 hr [11]. Abscissa: fraction numbers (from the bottom). Ordinate: trichloroacetic acid-insoluble radioactivity of fractions (cpm $\times 10^{-2}$). (o—o—o) Control; (•—•—•) lysate was treated with 20 mM EDTA.

Table 2
The effect of nuclear RNA on the incorporation of [^{14}C]-amino acids into protein by isolated mitochondria.

Additions	[^{14}C]amino acid incorporation, (cpm per 1 mg protein of mitochondria)
None	1430
RNA	1870
Actinomycin D	100
Actinomycin D + RNA	1480
Cycloheximide	1220
Cycloheximide + RNA	1750
Actinomycin D + cycloheximide	130
Actinomycin D + cycloheximide + RNA	1310

Mitochondria (1 mg protein per 1 ml) were incubated with cell sap (1.7 mg protein per 1 ml) and [^{14}C]amino acids (Chlorella protein hydrolysate, 2 μCi/ml) at 32° for 30 min). Additions: nRNA, 100 μg/ml; Actinomycin D, 50 μg/ml; cycloheximide, 50 μg/ml.

the other subfractions contained insignificant radioactivity. The detection of nRNA in the inner membrane-matrix fraction containing mitochondrial ribosomes (see [13]) suggested the binding of RNA which had penetrated to the mitochondrial protein-synthesizing structures.

After sedimentation analysis of the Triton X-100-lysed inner membrane-matrix fraction in a sucrose concentration gradient (fig. 1) the bulk of acid-insoluble radioactivity was found in ribonucleoprotein structures which could be tentatively identified as mitochondrial monoribosomes (55 S) and polysomes (~ 100 S) (see [14, 15]). The sensitivity of these structures to EDTA (fig. 1) which caused a shift of radioactive material to the top of the gradient could be considered as an evidence for this suggestion and allowed them to be distinguished from EDTA-resistant informosomes or artificial informosome-like ribonucleoprotein complexes formed after addition of exogenous RNA to cell extracts [16, 17].

In order to test the template activity of nuclear mRNA in the mitochondrial translation system a study on the effect of nRNA on [^{14}C]amino acid incorporation by isolated mitochondria was undertaken.

As can be seen from table 2, nRNA did produce a marked stimulation of mitochondrial [^{14}C]amino acid

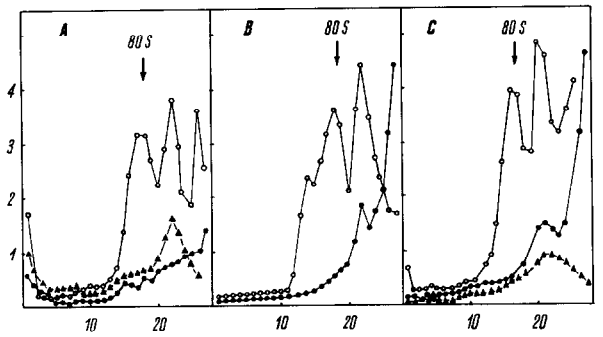

Fig. 2. The template activity of nuclear RNA in the mitochondrial system of protein synthesis. Mitochondria were preincubated for 20 min in different conditions and then pulse-labeled with a [^{14}C]amino acid mixture (5 μCi/ml, 5 min). Fractionation of mitochondria and sucrose gradient centrifugation – see legend to fig. 1. Abscissa: fraction numbers (from the bottom). Ordinate: trichloroacetic acid-insoluble radioactivity (cpm $\times 10^{-3}$). A) (○—○—○) Control lysate (without additions); (▲—▲—▲) chloramphenicol added (100 μg per 1 ml); (●—●—●) actinomycin D added (50 μg per 1 ml). B) (○—○—○) Control lysate (without additions); (●—●—●) puromycin (20 μg/ml) added after 5 min pulse labeling for additional 10 min chase. C) (○—○—○) Actinomycin (50 μg per 1 ml) and nuclear RNA (100 μg per 1 ml) added; (▲—▲—▲) the same, lysate treated with RNAase (50 μg/ml, 20 min, 20°); (●—●—●) actinomycin D and nRNA added (see above), puromycin chase – see legend to fig. 2B.

incorporation into protein. Several-fold enhancement of this stimulatory effect was observed in the presence of actinomycin D which itself produced a profound inhibition of endogenous incorporation. On the other hand cycloheximide affected neither endogenous incorporation nor activity of added RNA. Hence, the stimulatory effect of nRNA seems to be due to its template activity in the cycloheximide-resistant mitochondrial system.

The experiments on pulse labeling of isolated mitochondria with [^{14}C]amino acids and gradient analysis of the structures carrying nascent radioactive polypeptides supported the suggestion concerning template activity of nRNA within mitochondria.

Fig. 2A demonstrates that pulse-labeled structures in such experiments have sedimentation coefficients of 55 S (monoribosomes of mitochondria) and of about 100 S (polysomes). Sensitivity of the incorporation to chloramphenicol and to actinomycin D seems to prove the actual mitochondrial localization of these structures. The nascent character of pulse-labeled peptides was demonstrated in the experiments in which a puromycin chase caused the acid-insoluble radioactivity to move into the top fractions of the gradient (fig. 2B). On the background of actinomycin D-produced inhibition of amino acid incorporation unlabeled nuclear RNA caused activation of the process (see table 2) which correlated with re-appearance of nascent peptide-carrying structures in the sucrose gradient profile (fig. 2C). The sensitivity of these structures to a puromycin chase is an indication of their ribosomal character.

These data provide evidence for template activity of nRNA in the mitochondrial translation system.

4. Discussion

The data obtained demonstrated the potential possibility of both nuclear RNA transfer into mitochondria and its translation on mitochondrial ribosomes. These facts suggested that in a living cell the nuclear control over the mitochondrial structure and assembly can be mediated through the specific transfer of a certain group of mRNA's of nuclear origin and their translation within mitochondria. That this is indeed the case can be proved by hybridization of *in vivo* pulse-labeled mitochondrial RNA with nuclear DNA [18, 19].

The establishment of the nRNA template activity in isolated mitochondria suggests that mitochondria seem to have a special kind of RNA-dependent protein-synthesizing system which is able to form polypeptide chains on exogenous templates using endogenous energy for these processes. The availability of the mitochondrial system to exogenous mRNA-directed synthesis of 'complete' proteins possessing specific biological activity was demonstrated in our earlier experiments on the induction of virus-specific syntheses in mitochondria programmed by infectious viral RNA [20–23].

The 'simplicity' of the structure of mitochondrial mini-ribosomes from mammalian cells is probably the basis for the relative unspecificity of the mitochondrial translation system which appears to be compatible with different classes of template RNA's including mRNA's of 'prokaryotic' type – transcripts of mitochondrial DNA [1–3] and phage RNA's [24, 25] as well as templates of 'eukaryotic' type – animal viral RNA's [20–23] and nuclear RNA's (see above).

Such a feature of the mitochondrial translation apparatus provides a good test-system for the study of foreign genome integration into an animal cell and their expression within a recipient cell. In particular, it is the mitochondrial protein-synthesizing system that seems to be responsible for the expression of the DNA phage genome in the experiments on the incorporation of transducing phages into human cells [26]. From this standpoint the interaction of nuclear mRNA with the mitochondrial translation machinery is of great interest not only as an approach to the analysis of nucleo-mitochondrial interrelations in a living cell but as a model for the study of the mechanisms underlying the expression of foreign genetic information in animal and human cells.

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References

[1] V.S. Gaitskhoki, O.I. Kisselev and Ja.D. Schaposhnikov, Biokhimiya 36 (1971) 60.
[2] C. Aaij and P. Borst, Biochim. Biophys. Acta 217 (1970) 562.
[3] V.S. Gaitskhoki, O.I. Kisselev and Ja.D. Schaposhnikov, Molekul. Biol. 6 (1972) 215.
[4] O.I. Kisselev and V.S. Gaitskhoki, Biokhimiya 37 (1972) N 6.
[5] R.F. Swanson, Nature 231 (1971) 31.
[6] K. Scherrer and J. Darnell, Biochem. Biophys. Res. Commun. 7 (1962) 486.
[7] W. Sauerbier and A. Bräutigam, Biochim. Biophys. Acta 199 (1970) 36.
[8] D. Szabados, Biokhimiya 36 (1971) 401.
[9] C. Schnaitman and J.W. Greenwalt, J. Cell Biol. 38 (1968) 158.
[10] L. Malkin, Biochem. Biophys. Res. Commun. 48 (1972) 1106.
[11] J. Coote and T. Work, European J. Biochem. 23 (1971) 564.
[12] A.S. Spirin, Biokhimiya 23 (1958) 656.
[13] D. Roodyn and D. Wilkie, Biogenesis of mitochondria (Methuen, London, 1968).
[14] S. Perlman and S. Penman, Nature 227 (1970) 133.
[15] P. Borst and L. Grivell, FEBS Letters 13 (1971) 73.
[16] A.S. Spirin, in: The mechanism of protein synthesis and its regulation, ed. L. Bosch (North-Holland, Amsterdam, 1972) p. 515.
[17] A.S. Voronina, A.S. Stepanov, A.A. Preobrazhensky and L.P. Ovchinnikov, Biokhimiya 37 (1972) 430.
[18] D.G. Humm and J.H. Humm, Proc. Natl. Acad. Sci. U.S. 55 (1966) 114.
[19] V.S. Gaitskhoki, O.I. Kisselev and Ja.D. Schaposhnikov, Molekul. Biol., in press.
[20] V.S. Gaitskhoki, F.I. Jershov, O.I. Kisselev, L.K. Menshikh, O.V. Zaitseva, L.V. Uryvaev, V.M. Zhdanov and S.A. Neifakh, Vopr. Virusol. N 3 (1971) 269.
[21] F.I. Jershov, V.S. Gaitskhoki, O.I. Kisselev, O.V. Zaitseva, L.K. Menshikh, L.V. Uryvaev, S.A. Neifakh and V.M. Zhdanov, Vopr. Virusol. N 3 (1971) 274.
[22] F.I. Jershov, V.S. Gaitskhoki, O.I. Kisselev, L.K. Menshikh, O.V. Zaitseva, L.V. Uryvaev, S.A. Neifakh and V.M. Zhdanov, Dokl. Akad. Nauk SSSR 200 (1971) 1452.
[23] V.S. Gaitskhoki, F.I. Jershov, O.I. Kisselev, L.K. Menshikh, O.V. Zaitseva, L.V. Uryvaev, V.M. Zhdanov and S.A. Neifakh, Dokl. Akad. Nauk SSSR 201 (1971) 220.
[24] A. Scragg, H. Morimoto, V. Villa, J. Neckhoroshev and H. Halvorson, Science 171 (1971) 908.
[25] D. Richter, P. Herrlich and M. Schweiger, Nature New Biol. 238 (1972) 74.
[26] C.R. Merril, M.R. Geyer and J.C. Petricciani, Nature 233 (1971) 398.