# ON THE INTRACELLULAR DISTRIBUTION OF POLYRIBOSOMES SYNTHESIZING MITOCHONDRIAL PROTEINS

V. S. GAITSKHOKI, O. I. KISSELEV, N. A. KLIMOV, N. K. MONAKHOV, G. V. MUKHA, A. L. SCHWARTZMAN and S. A. NEIFAKH

*Laboratory of Biochemical Genetics, Institute of Experimental Medicine of the USSR Academy of Medical Sciences, 197022 Leningrad, USSR*

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## 1. Introduction

Mitochondrial proteins are shown to be coded for by nuclear genes and by mitochondrial DNA (mtDNA) while their synthesis is known to occur on both cytoplasmic and mitochondrial ribosomes (see [1,2]). However the structural and functional interrelations between these translation systems within a cell as well as the contribution of different intracellular polyribosome (PRS) classes to biosynthesis of mitochondrial proteins remain to be studied.

We tried a new approach to this problem, which consisted of the identification of the PRS involved in the synthesis of mitochondrial proteins using $^{125}$I-labelled antibodies against mitochondrial antigens. The data obtained demonstrate that the immunochemical appraoch is valuable in the identification and quantitation of cellular PRS synthesizing proteins of mitochondria, chloroplasts and other organelles having dual intracellular origin. Such an approach could be also considered as a preliminary step in the preparative isolation of these PRS and of corresponding mRNA's. In particular, a membrane-bound cytoplasmic PRS fraction is described which is associated with mitochondria and engaged largely in the synthesis of mitochondrial proteins.

## 2. Methods

### 2.1. *Immunization and preparation of $^{125}$I-immunoglobulin (Ig)*

Rabbits were immunized intravenously with a suspension of purified rat liver mitochondria. The titer of antimitochondrial antibodies was determined in a complement fixation test. Ig fractions were isolated by ammonium sulphate precipitation from the sera of immunized and control rabbits. The fraction obtained was free of RNAse activity. Iodination was with carrier-free $^{125}$I ('Isotop', USSR, specific activity 8.5 Ci/mg) according to [3]. The specific radioactivity of iodinated Ig was 130 000–190 000 cpm per mg.

### 2.2. *Isolation and fractionation of cytoplasmic and mitochondrial PRS*

Total cytoplasmic PRS were isolated from post-mitochondrial supernatant lysed with 1.0% Triton X-100 by centrifugation through 1.75 M sucrose (Spinco L2 ultracentrifuge, rotor 30, 22 000 rpm, 16 hr). Free and membrane-bound PRS were separated according to Adelman et al. [4]. Mitochondria-associated cytoplasmic PRS were solubilized from the mitochondrial pellet with digitonin [5] followed by Triton X-100 lysis. The lysate was clarified at 20 000 *g* for 15 min. Purification of PRS was achieved as described for total PRS. Intramitochondrial PRS were obtained from an inner membrane–matrix fraction after digitonin treatment which was lysed with Triton X-100 and clarified at 20 000 *g*.

All the solutions contained heparin (100 IU per 1 ml) to prevent PRS breakdown.

### 2.3. *$^{125}$I-Ig binding to polyribosomes*

PRS were incubated with $^{125}$I-Ig in TKM buffer (0.03M Tris–HCl, pH 7.6, 0.025 M KCl, 0.005M $MgCl_2$) for 5 min at 37°C and then for 60 min at

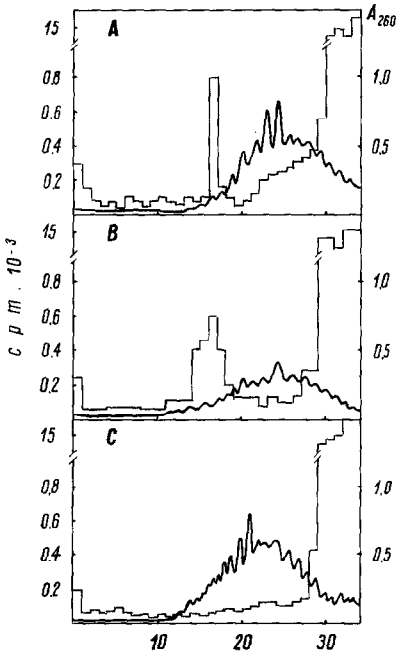


Fig. 1. The binding of $^{125}$I-antibodies with total, free and membrane-bound polyribosomes from post-mitochondrial supernatant. PRS after incubation with labelled Ig were fractionated in sucrose gradient (see Methods): Solid line – UV absorbance; bars–$^{125}$I-Ig radioactivity. A) Total PRS. B) Free PRS. C) Membrane-bound PRS.

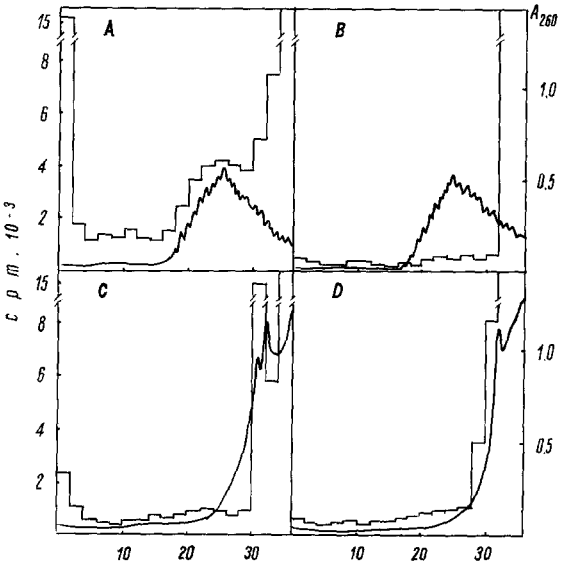


Fig. 2. The binding of $^{125}$I-antibodies with mitochondria-associated cytoplasmic PRS. Incubation and centrifugation conditions – see Methods: A) PRS + $^{125}$I-antibodies; B) PRS + $^{125}$I-Ig from control serum; C) PRS + $^{125}$I-antibodies after pre-incubation with 1M KCl and puromycin; D) PRS + $^{125}$I-antibodies after 0.03 M EDTA pre-treatment. Solid line – UV absorbance; bars – $^{125}$I radioactivity.

0°C. The incubation mixture was subjected to sucrose gradient centrifugation (10–34 %, w/w, sucrose with TKM buffer, SW 25.1 rotor, 22 500 rpm, 3 or 6 hr for cytoplasmic and mitochondrial PRS respectively). Gradients were fractionated using a peristaltic pump, the UV profile being recorded in a flow cell adapted to an 'Hitachi 356' double-beam spectrophotometer. $^{125}$I radioactivity was measured in a toluene-based scintillation mixture or in special gamma-vials (Koch Light) with Ashcroft scintillation mixture [6].

Two types of controls were made in all the experiments: (i) PRS binding with $^{125}$I-Ig from non-immunized rabbits and (ii) binding changes after treatments dissociating PRS and releasing nascent chains (puromycin alone or with 1 M KCl, EDTA treatment).

## 3. Results

An attempt was made to identify discrete PRS fractions involved in the synthesis of mitochondrial proteins and to determine their size from sedimentation pattern. It was demonstrated that a rather fraction of total post-mitochondrial PRS carried $^{125}$I-Ig after incubation of these PRS with $^{125}$I-Ig (fig. 1A). The size-class of these Ig-binding PRS corresponded to the structure containing 9–11 monomers according to [7]. The position of radioactive antibodies in the gradient seemed to reflect the actual size of antigen-containing PRS because the independent experiments with gradient fractionation of PRS and their subsequent incubation with antibodies revealed the same distribution of Ig-binding PRS as in fig. 1A. The distribution of bound antibodies in experiments with free PRS (fig. 1B) was just the same as in similar tests with unfractionated PRS from postmitochondrial supernatant. In both cases the significant $^{125}$I radioactivity was recovered from the bottom of tubes probably due to formation of heavy precipitate. On the other hand there was neither immune precipitate nor

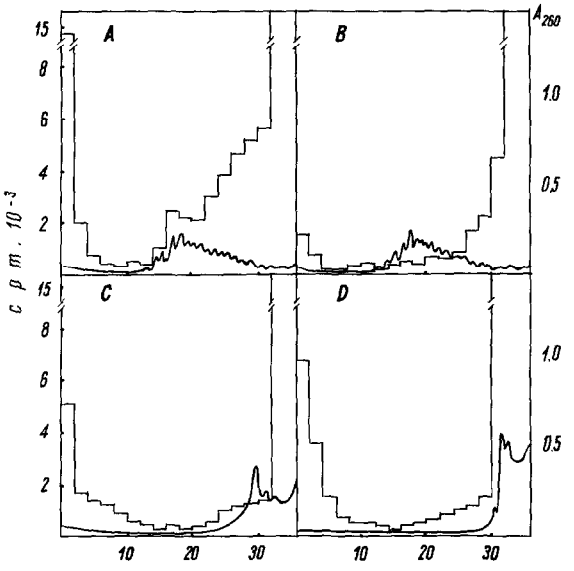


Fig. 3. The binding of $^{125}$I-antibodies with intramitochondrial polyribosomes. PRS fractionation – see Methods, other conditions and symbols – see legend to fig. 2.

Table 1
The precipitation of $^{125}$I-Ig with different polyribosomes

| Polyribosomes | Radioactivity of precipitate, cpm/$A_{260}$ of polyribosomes | | | |
|---|---|---|---|---|
| | $^{125}$I-antibodies | | Control | $^{125}$I-Ig |
| | Polyribosomes | | | |
| | Native | KCl+puromycin | Native | KCl+puromycin |
| Total post-mitochondrial | 3105 | 720 | 928 | 724 |
| Membrane-bound post-mitochondrial | 660 | 620 | 680 | 635 |
| Free post-mitochondrial | 19 560 | 830 | 615 | 623 |
| Mitochondria-associated | 18 150 | 950 | 648 | 585 |
| Intramitochondrial | 16 256 | 1608 | 745 | 680 |
| Control (without PRS) | 0 | 0 | 0 | 0 |

discrete radioactive peaks after incubation of membrane-bound postmitochondrial PRS with $^{125}$I-antibodies (fig. 1C). In the experiments with mitochondria-associated cytoplasmic PRS (fig. 2) and with intramitochondrial PRS (fig. 3) the diffuse distribution of antimitochondrial $^{125}$I-antibodies throughout the gradient was observed which could be considered as an indication of the presence of nascent antigens in all the size classes of PRS. Very intensive immune precipitate formation was also demonstrated. It should be noted that various PRS-dissociating treatments resulted in the decrease of $^{125}$I-Ig radioactivity in bottom fractions and PRS region of sucrose gradient (see figs. 2 C–D and 3 C–D) and table 1).

## 4. Discussion

Two possible sources of artifacts should be taken into consideration in experiments using immunochemical identification of PRS synthesizing mitochondrial proteins: (i) non-specific binding of Ig with ribosomal proteins [8] and (ii) the antigenicity of ribosomal proteins in mitochondrial preparations used as antigens for immunization. This second possibility could be the most important in experiments with mitochondrial and mitochondria-associated PRS.

The experiments with $^{125}$I-Ig from non-immunized rabbits seem to exclude nonspecific Ig binding with ribosomes because the binding in these cases was negligible. The second possibility could be also excluded because puromycin-induced release of nascent polypeptides from PRS resulted in very marked decrease of Ig binding. Thus it seems reasonable to conclude that: (i) the binding of PRS with Ig is a type of antigen-antibody reaction and (ii) the antigenicity of PRS is mainly due to nascent polypeptides. In relation to this we could believe that the intensity of Ig binding measured as $^{125}$I-Ig radioactivity either in PRS-containing fractions of the sucrose gradient or in the precipitate after gradient centrifugation could be an indication of the relative proportion of nascent mitochondrial antigens in PRS. A correction is necessary for non-specific (puromycin-resistant) Ig binding which contribution did not exceed 10–20% of the total one (see table 1).

A conclusion could be drawn that PRS involved in the synthesis of mitochondrial proteins are distributed non-randomly within a cell. The nascent mitochondrial antigens were detected only in the distinct

size classes of free cytoplasmic PRS but not in the membrane-bound PRS from postmitochondrial supernatant. On the other hand, the mitochondrial PRS of each size class were carrying the nascent peptides of mitochondrial destination. Finally, the most important fact is that mitochondria-associated cytoplasmic PRS appeared to be not a contamination of mitochondrial fraction but a specialized population of intracellular PRS engaged in the synthesis of polypeptides for mitochondria. Thus we could suggest the existence of an integrated system of cytoplasmic and mitochondrial PRS involved in the concerted synthesis of mitochondrial problems.

The spatial vicinity of a certain fraction of cytoplasmic PRS to mitochondria or their association with the outer membrane of these organelles could serve as a structural equivalent of this functional integration providing a vectorial discharge of newly-formed polypeptides into mitochondria.

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