IMMUNOLOGICAL DIFFERENCE OF MITOCHONDRIAL AND CYTOPLASMIC RIBOSOMES OF *NEUROSPORA CRASSA*

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1. Introduction

The RNA of mitochondrial ribosomes is coded for and synthesized within the mitochondrion, whereas the proteins of the mitochondrial ribosomes are synthesized and most probably coded for outside the mitochondrion, i.e. at the cytoplasmic ribosomes and in the nucleus, respectively (for review see [1–3]. The nuclear genome therefore appears to include the information for the proteins of both mitochondrial and cytoplasmic ribosomes.

The question arises whether the proteins belonging to these two kinds of ribosomes are related in there amino acid sequences. One possibility to find an answer is to check the existence of sequence homologies by immunological techniques. This was done with antisera against whole mitochondrial and cytoplasmic ribosomes.

Antisera against cytoplasmic ribosomes do not react with mitochondrial ribosomes, and antisera against mitochondrial ribosomes do not react with cytoplasmic ribosomes. This indicates that there are no significant sequence homologies in the corresponding ribosomal proteins.

2. Materials and methods

Mitochondrial and cytoplasmic ribosomes from *Neurospora crassa* were prepared and analysed as described previously [4]. For radioactive labeling of the ribosomes, 1 μCi/ml L-^{3}H-leucine (specific radioactivity 52 Ci/mmol) (Radiochemical Centre, Amersham, England) was added to the cultures at the time of inoculation. Ribosomes from *E. coli* B were prepared according to the method of Spirin et al. [5]. For radioactive labeling, *E. coli* cultures in the logarithmic growth phase were incubated with 1 μCi/ml L-^{3}H-leucine for 3 hr.

Antibodies against mitochondrial and cytoplasmic ribosomes were raised in rabbits by injecting ribosomal suspensions emulsified with equal volumes of complete Freund's adjuvant (Difco, Detroit, Michigan). The rabbits received four subcutaneous injections of 3 mg of ribosomes at intervals of 10 days.

Quantitative immunoprecipitation was carried out in the presence of 1% Triton X-100 and AMT (0.1 M NH_4Cl, 0.01 M $MgCl_2$, 0.03 M Tris–HCl, pH 7.2). Increasing amounts of antiserum were added to a constant amount of antigen (2 OD_{260nm} units). The reaction mixtures were incubated at 4°C for 15 hr. After centrifugation, the precipitates were washed twice with 1% Triton X-100 in AMT and dissolved in 0.2 ml 1% sodium dodecyl sulfate, 0.1 M Tris–HCl, pH 8.0, for 15 hr.

Radioactivity was determined in a Packard Tricarb scintillation counter using butyl-PBD scintillator (6 g/l in toluene–2-methoxyethanol 3:2, v/v).

3. Results

Complete immunoprecipitation of ribosomes occurs, when cytoplasmic ribosomes are mixed with antiserum against cytoplasmic ribosomes and when mitochondrial ribosomes are mixed with antiserum against mitochondrial ribosomes (table 1 and fig. 1).

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Table 1
Precipitation of ribosomes by antisera

Ribosomes from	Antiserum	% Of ribosomes precipitated
Cytoplasm	Anti-c	99.8
	Anti-m	1.6
	Control	1.5
Mitochondria prepared with SET	Anti-c	10.8
	Anti-m	97.7
	Control	4.7
Mitochondria prepared with SMT	Anti-c	70.1
	Anti-m	18.2
	Control	5.0
E. coli	Anti-c	0.2
	Anti-m	0.3
	Control	0.3

The reaction mixture contained 0.3 ml antiserum, 2 $OD_{260m\mu}$ units of radioactively labeled ribosomes, 1% Triton/AMT. The final volume was 1.5 ml.

SET: 0.44 M sucrose, 0.002 M EDTA, 0.01 M Tris–HCl, pH 7.2.

SMT: 0.44 M sucrose, 0.01 M $MgCl_2$, 0.01 M Tris–HCl, pH 7.2.

Anti-c: antiserum against cytoplasmic ribosomes.
Anti-m: antiserum against mitochondrial ribosomes.
Control: serum from non-immunized rabbits.

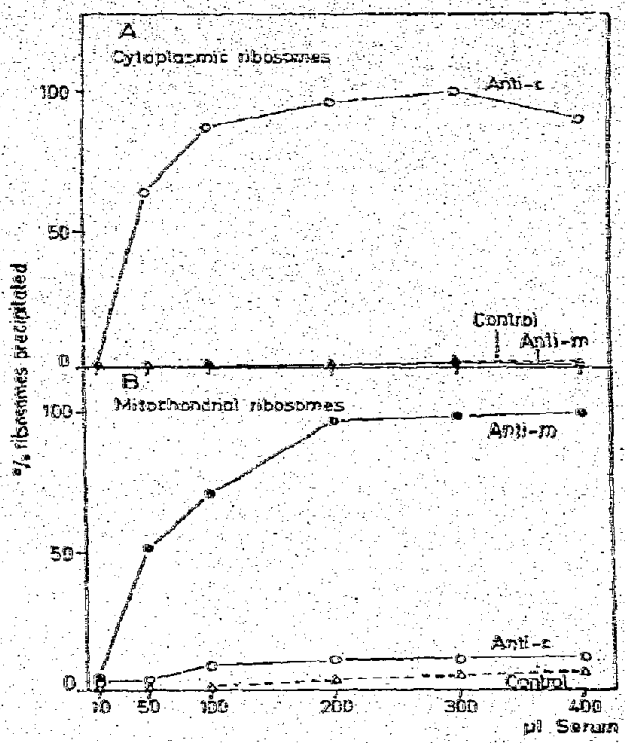

Fig. 1. Quantitative immunoprecipitation of mitochondrial and cytoplasmic ribosomes. A: Cytoplasmic ribosomes; B: Mitochondrial ribosomes. For abbreviations see table 1.

Cytoplasmic ribosomes are not precipitated by antiserum against mitochondrial ribosomes. The minute amount of ribosomes found in the immunoprecipitate in this case (ca. 1%) is the same as with control sera from non-immunized rabbits. On the other hand, antiserum against cytoplasmic ribosomes precipitates a small but significant amount of ribosomes from a preparation of mitochondrial ribosomes (ca. 11%). Also, with control sera an appreciable but lower amount of mitochondrial ribosomes is precipitated (ca. 5%). This latter effect can be explained by the particularly high tendency of mitochondrial ribosomes to aggregate [4, 6].

In order to decide whether the partial precipitation of ribosomes from mitochondria by antisera against cytoplasmic ribosomes represents a cross reaction or merely results from contamination of mitochondrial ribosomes by cytoplasmic ribosomes, mitochondrial ribosomes were subjected to density gradient centrifugation. This procedure resolves mitochondrial and cytoplasmic ribosomes, because they have different sedimentation coefficients [7]. This is shown in fig. 2. Fig. 2A represents a gradient profile of mitochondrial ribosomes (monomer 73S), fig. 2C a gradient profile of cytoplasmic ribosomes (monomer 77S). In fig. 2B a mixture of both types of ribosomes is centrifuged. The slightly faster moving cytoplasmic ribosomes are seen as shoulders on the mitochondrial monomer and dimer peaks.

Fig. 3A shows a radioactivity profile of a gradient on which ^{3}H-labeled ribosomes were centrifuged. The peak in fractions 12–13 represents the 73S monomeric ribosomes. When antiserum against cytoplasmic ribosomes is added to the gradient fractions and radioactivity is measured in the resulting immunoprecipitates, the pattern shown in fig. 3B is observed. Clearly, the main peak is in fractions 13–15 and therefore represents cytoplasmic 77S monomeric ribosomes. The second peak in fraction 11 represents cytoplasmic

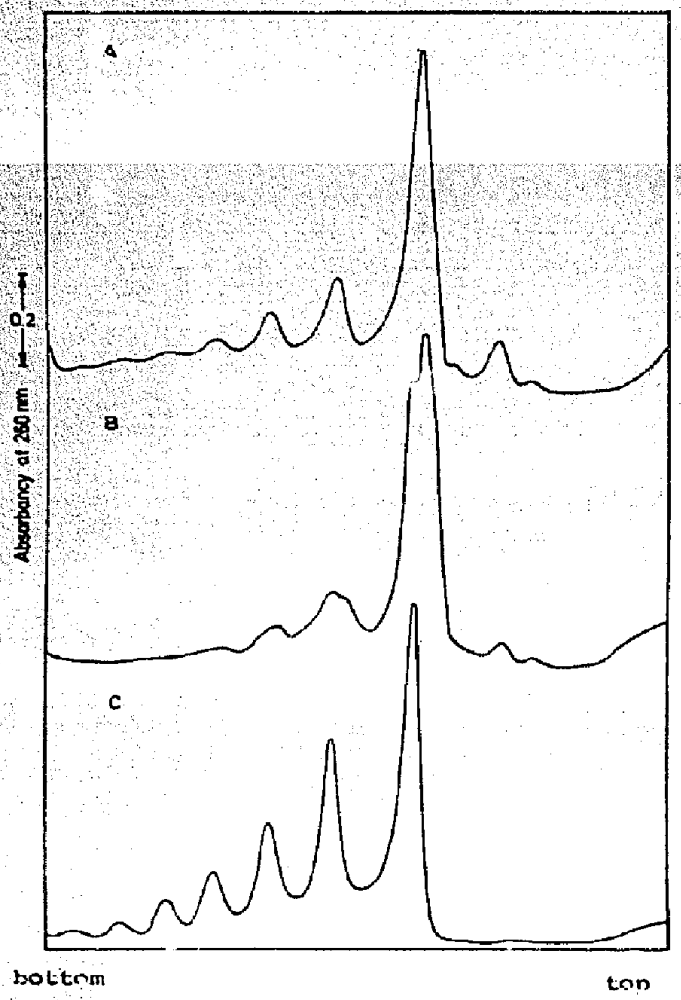

Fig. 2. Density gradient centrifugation of mitochondrial and cytoplasmic ribosomes. A: Mitochondrial ribosomes; B: Mitochondrial + cytoplasmic ribosomes; C: Cytoplasmic ribosomes. Gradient centrifugation was performed as described previously [4]. The gradients were pumped through a flow cell and the absorption at 260 nm was recorded.

60S large subunits. About 5% of the total radioactivity applied to the gradient is precipitated by antiserum against cytoplasmic ribosomes. When after removal of the precipitate, antiserum against mitochondrial ribosomes is added, precipitation of mitochondrial ribosomes occurs. This is indicated by the radioactivity pattern in fig. 3C. The position of the main peak (fraction 12) coincides with that in fig. 3A, whereas in this position in fig. 3B there is a minimum.

From these data it can be concluded that antiserum against cytoplasmic ribosomes does not react with mitochondrial ribosomes. Rather, in preparations of mitochondrial ribosomes about 5% of cytoplasmic ribosomes are present, though the mitochondria were washed three times with EDTA containing media before isolating the ribosomes.

Ribosomes from mitochondria which were prepared in the absence of EDTA and in the presence of 10 mM Mg^{2+}, display after gradient centrifugation an op-

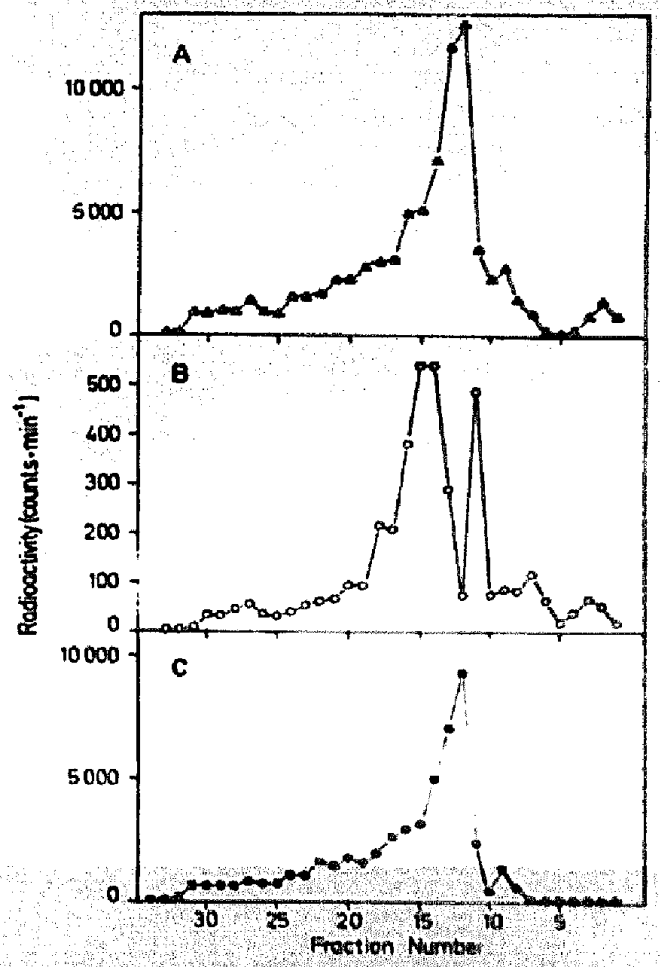

Fig. 3. Immunoprecipitation of mitochondrial ribosomes from density gradient fractions. *Neurospora* cells were labeled with ^{3}H-leucine and mitochondria were prepared in SET. The ribosomes from these mitochondria were subjected to gradient centrifugation as shown in fig. 2 and the gradient was divided into fractions: A: Radioactivity in the gradient fractions before addition of antisera; B: Radioactivity precipitated with antiserum against cytoplasmic ribosomes; C: Radioactivity precipitated with antiserum against mitochondrial ribosomes.

tical density profile similar to that in fig. 2C, with a small shoulder in the 73S region. Antibodies against cytoplasmic ribosomes are able to precipitate 70–80% of the ribosomes from such a preparation, whereas with antisera against mitochondrial ribosomes ca. 15–20% of the total ribosomes are precipitated (see table 1). In agreement, the yield of ribosomes (related to mitochondrial protein) is about 4–5 times higher from mitochondria which were prepared in the presence of Mg^{2+}, if compared to mitochondria prepared in the presence of EDTA. These observations demonstrate that cytoplasmic ribosomes contaminate mitochondrial ribosomes to a very high degree if Mg^{2+} is present in the isolation medium for mitochondria.

Table 1 includes data on the reaction of *E. coli* ribosomes with antisera against mitochondrial and cytoplasmic ribosomes from *Neurospora*. Obviously, there is no cross reaction between these antisera and *E. coli* ribosomes.

4. Discussion

The determination of immunological cross reaction is a sensitive method to detect sequence homologies in proteins. In the special case of ribosomal proteins this has been demonstrated with bacteria [8–10]. Antibodies against the individual ribosomal proteins can be raised in rabbits by injecting whole ribosomes [11].

The results presented here show that antisera against cytoplasmic ribosomes do not cross react with mitochondrial ribosomes and antisera against mitochondrial ribosomes do not cross react with cytoplasmic ribosomes. This suggests that there are no significant sequence homologies in the ribosomal proteins of the two types of ribosomes. This confirms and extends earlier findings that the gel electrophoretic patterns of mitochondrial and cytoplasmic ribosomal proteins from various organisms are different [12–15]. Also, proteins from mitochondrial and cytoplasmic ribosomes of *Neurospora* behave differently upon chromatography on carboxymethyl cellulose columns [16].

Furthermore, data in this report demonstrate that neither antibodies against cytoplasmic ribosomes nor antibodies against mitochondrial ribosomes cross react with *E. coli* ribosomes. Similarly, it has been shown that antisera against *E. coli* ribosomes do not cross react with mitochondrial ribosomes from *Neurospora* [9]. Although there are many functional similarities between mitochondrial ribosomes from eucaryotes and bacterial ribosomes (for review see [1]), the corresponding ribosomal proteins apparently do not share significant common sequences. Albergina and Suskind [17] have reported that in immunodiffusion tests spur formation between antisera against cytoplasmic ribosomal proteins of *Neurospora* and *E. coli* ribosomal proteins occurs. However, spur formation between antiserum against *E. coli* ribosomal proteins and *Neurospora* cytoplasmic ribosomal proteins was not seen. The data in the report seem not to be detailed enough to allow the conclusion that a cross reaction takes place. Further studies with immunodiffusion tests are necessary to clarify this point.

In conclusion, though the proteins of both mitochondrial and cytoplasmic ribosomes are made at the cytoplasmic ribosomes and are probably coded for in the nuclear genome, they appear not to be significantly related in structure. It cannot be decided whether the genes for mitochondrial and cytoplasmic ribosomal proteins are derived from two different sets of genes or have evolved from a unique set of genes in a way that no more common sequences are left.

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