RAPID EVOLUTIONARY DIVERGENCE OF PROTEINS IN MAMMALIAN MITOCHONDRIAL RIBOSOMES

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Received 12 November 1977

1. Introduction

Compared to cytoplasmic ribosomes (cytoribosomes), the mitochondrial ribosomes (mitoribosomes) of distantly related organisms are surprisingly diverse with respect to their physical and chemical properties [1]. Among more closely related organisms, such as the vertebrates, very little variation is seen in the overall structural properties of mitoribosomes. Nevertheless, in view of the general trend toward greater phylogenetic variation among mitoribosomes than among cytoribosomes, it seems likely that systematic differences among the mitoribosomes of these closely related species could be detected at a sufficiently detailed level of analysis, as for example in an electrophoretic analysis of the ribosomal proteins. Comparisons of the proteins of cytoribosomes by two-dimensional polyacrylamide gel electrophoresis have revealed that most or all of these proteins are electrophoretically indistinguishable in mammals, birds and reptiles [2–4]. Electrophoretic analyses of mitoribosomal proteins from rat [5,6] and bovine [7] liver have also been performed. However, different electrophoretic systems were used in these studies, so it is impossible to compare the electrophoretic properties of rat and bovine mitoribosomal proteins from these results. The present experiments permit a direct comparison, and a test of the proposition that mitochondrial ribosomes show a higher rate of evolutionary divergence than cytoplasmic ribosomes.

2. Methods

Mitoribosome subunits were prepared using modifications of the procedures in [8,9]. Purified bovine and rat (Sprague-Dawley) liver mitochondria were resuspended to concn 20 mg protein/ml in buffer A (0.1 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10 mM Tris, pH 7.5) and lysed by Triton X-100 addition to 1.6%. After analysis of the ribosomes on linear 10–30% sucrose gradients (buffer A), mitochondrial monoribosomes were collected from the 55 S peak and dissociated into subunits by incubation with puromycin (1 mM) and heparin (50 μg/ml) in buffer C (5 mM $MgCl_2$, 0.5 M KCl, 5 mM β-mercaptoethanol, 20 mM triethanolamine · HCl, pH 7.5); derived subunits were separated by centrifugation in sucrose gradients containing buffer C.

The proteins were extracted with urea (6 M) and LiCl (3 M) and subjected to two-dimensional polyacrylamide gel electrophoresis, following the general procedure in [10]. Ribosomal proteins (5–10 μg) were labeled with [^{14}C]formaldehyde (New England Nuclear, 44 Ci/mol) by reductive methylation [11]. After labeling, ribosomes containing 200–300 μg protein were added as carrier and the proteins were extracted for electrophoresis. Stained gel slabs were prepared for fluorography [12] and exposed to Kodak RP/R-54 medical X-ray film as in [13].

3. Results

Two-dimensional electropherograms of the proteins from large and small subunits of bovine and rat mitochondrial ribosomes are shown in fig.1. The overall patterns of the large-subunit proteins from these two species are rather similar, and several individual protein spots appear to correspond closely in electro-

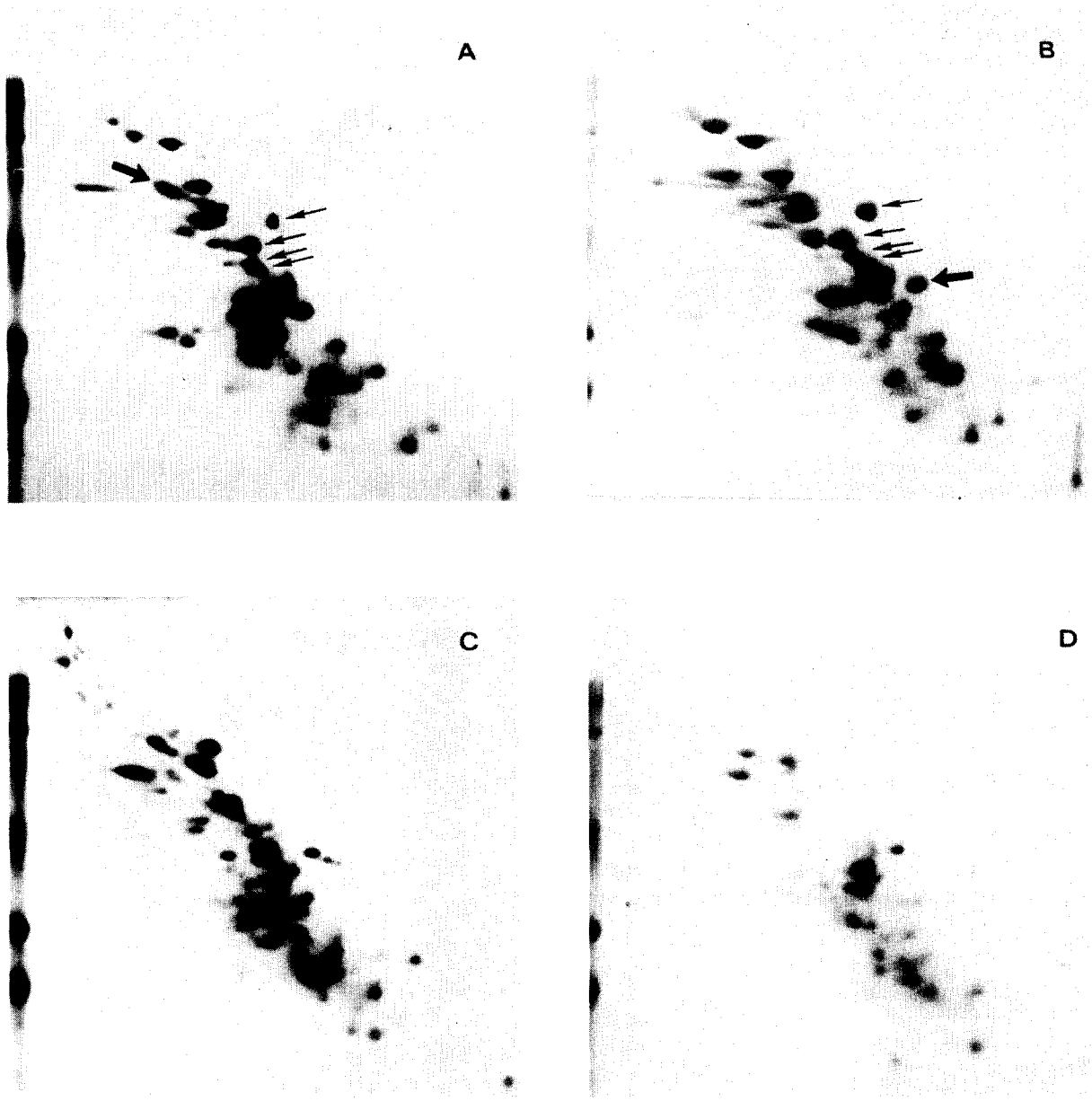

Fig.1. Electrophoretic patterns to the proteins of bovine and rat mitochondrial ribosomal subunits: (A) bovine large subunit; (B) rat large subunit; (C) bovine small subunit; (D) rat small subunit. The first dimension of electrophoresis (left to right) was performed in urea at pH 4.3, and the second dimension (top to bottom) in sodium dodecyl sulfate. At the left-hand sides of the photographs are several marker proteins (bovine serum albumin, γ-globulin heavy and light chains, ovalbumin, myoglobin and lysozyme), which were applied at a point immediately adjacent to the origin of the first dimension and electrophoresed in the second dimension only. Small arrows indicate examples of large-subunit proteins which appear to occupy similar positions, and large arrows indicate examples of proteins exhibiting mobilities obviously different in rat and bovine ribosomes.

phoretic mobility as, e.g., those indicated by small arrows in fig.1A,B. Some proteins appear to be shifted in position (e.g., large arrow in fig.1A), while others appear to have no obvious counterpart in the other ribosome (e.g., large arrow in fig.1B). Even less correspondence is noted for the small-subunit proteins in the bovine and rat patterns (fig.1C,D).

To compare the electrophoretic mobilities of the bovine and rat proteins more precisely, samples of proteins from both animals were mixed and co-electrophoresed on the same gel. This mixture contained a quantity of bovine mitoribosomal proteins adequate for detection by staining, and a much smaller quantity of the rat proteins which had been radioactively labelled by reductive methylation with [^{14}C]formaldehyde. Comparison of the autoradiograph of the dried gel with the pattern of stained proteins accurate positioning of the radioactive rat proteins relative to the bovine proteins. Control experiments in which reductively methylated mitoribosomal proteins were co-electrophoresed with stainable quantities of the same proteins showed that the electrophoretic mobilities of the proteins were not altered by the labelling reaction. Some of the rat proteins seen in stained gels failed to label (e.g., large arrow in fig.1B). It is not known whether their failure to label under these conditions is due to a lower lysine content [14], or simply to masking of available lysines due to incomplete denaturation of these proteins.

Figure 2 shows the stained pattern and the autoradiograph from a comparison of bovine and rat large-subunit proteins. The relative positions of the two

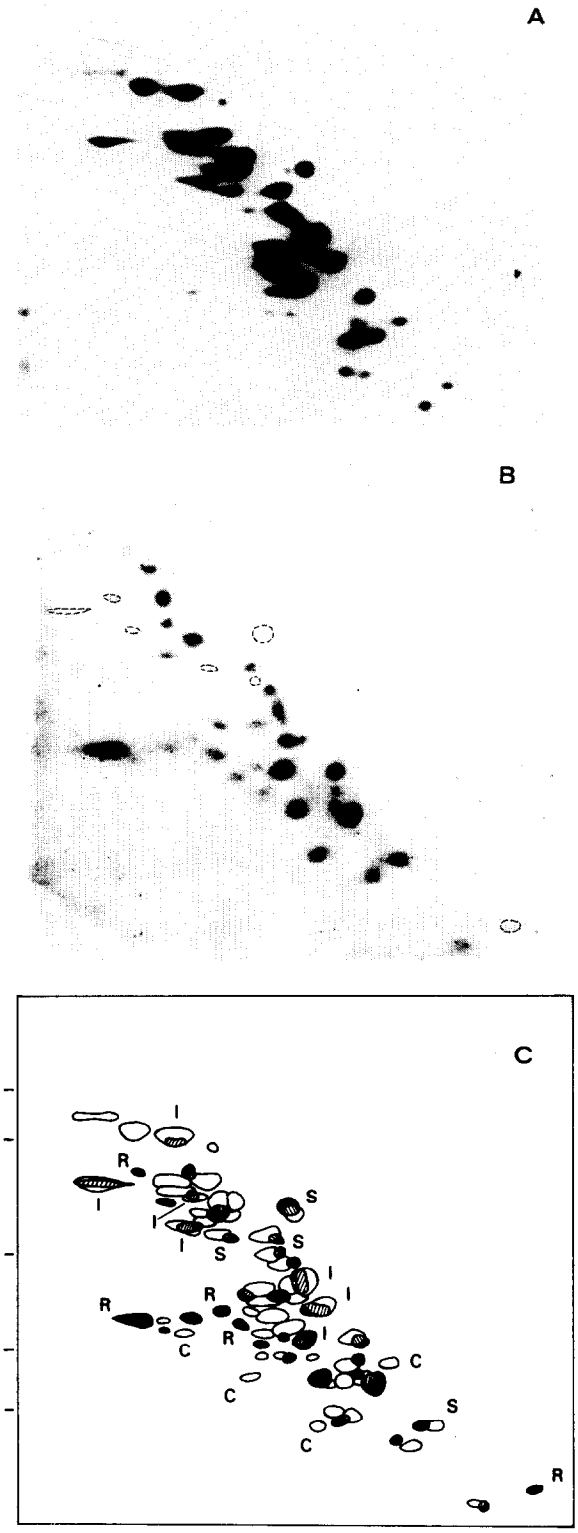

Fig.2. Co-electrophoresis of bovine and rat mitoribosomal large-subunit protein. (A) Photograph of the stained gel (bovine proteins). (B) Autoradiograph of the gel (rat proteins). Faint spots visible on the original autoradiograph are indicated by dashed circles. (C) Schematic diagram showing relative positions of bovine (open spots) and rat (filled spots) proteins. Regions occupied by both bovine and rat proteins are indicated by cross-hatching. The letters indicate examples of indistinguishable (I), similar (S), cow-specific (C) and rat-specific (R) proteins. The second-dimension electrophoretic positions of the marker proteins bovine serum albumin (mol. wt 68 000), ovalbumin (44 000), γ-globulin light chain (23 500), myoglobin (17 200) and lysozyme (14 400) are shown at the left of the diagram.

sets of proteins are illustrated in fig.2C. For purposes of comparison in this analysis, those proteins which appear coincident or nearly coincident were judged to be indistinguishable, and are indicated by 'I' in fig.2C. By this criterion, very few proteins of the rat large subunit, at most only 7 out of the 36 seen in this experiment, are electrophoretically indistinguishable from those of the bovine large subunit. Additional rat proteins have mobilities similar to those of the cow ribosomes; examples are labeled 'S' in fig.2C. The remaining rat proteins occupy positions far removed from any cow mitoribosomal protein. Examples of such proteins unique to rat (R) or cow (C) ribosomes are indicated in fig.2C.

Similar results were obtained in the comparison of the small-subunit proteins from rat and bovine mitoribosomes (fig.3). Again most of the rat proteins are different from the bovine proteins. At most only 3 out of the 30 radioactively-labelled rat proteins could not be clearly resolved from the bovine proteins. These three pairs of coincident or nearly coincident proteins are indicated by 'I' in fig.3C. While some of the remaining proteins (S) show only slight differences in their mobilities, most have very different mobilities.

4. Discussion

In this comparison of the mitoribosomal proteins from two mammals we find that 56 out of a total 66 rat proteins are resolved from cow proteins in our two-dimensional electropherograms (fig.2C,3C), Thus, most of the mitoribosomal proteins in these two mammals have different electrophoretic properties. This is a surprising result in view of the high degree to which these properties of the proteins of cytoplasmic ribosomes are conserved [2]. The mobility differences in the present study range from just discernible, to major shifts in electrophoretic position, suggesting that many amino acid changes have occurred in these proteins since the divergence of cows and rats.

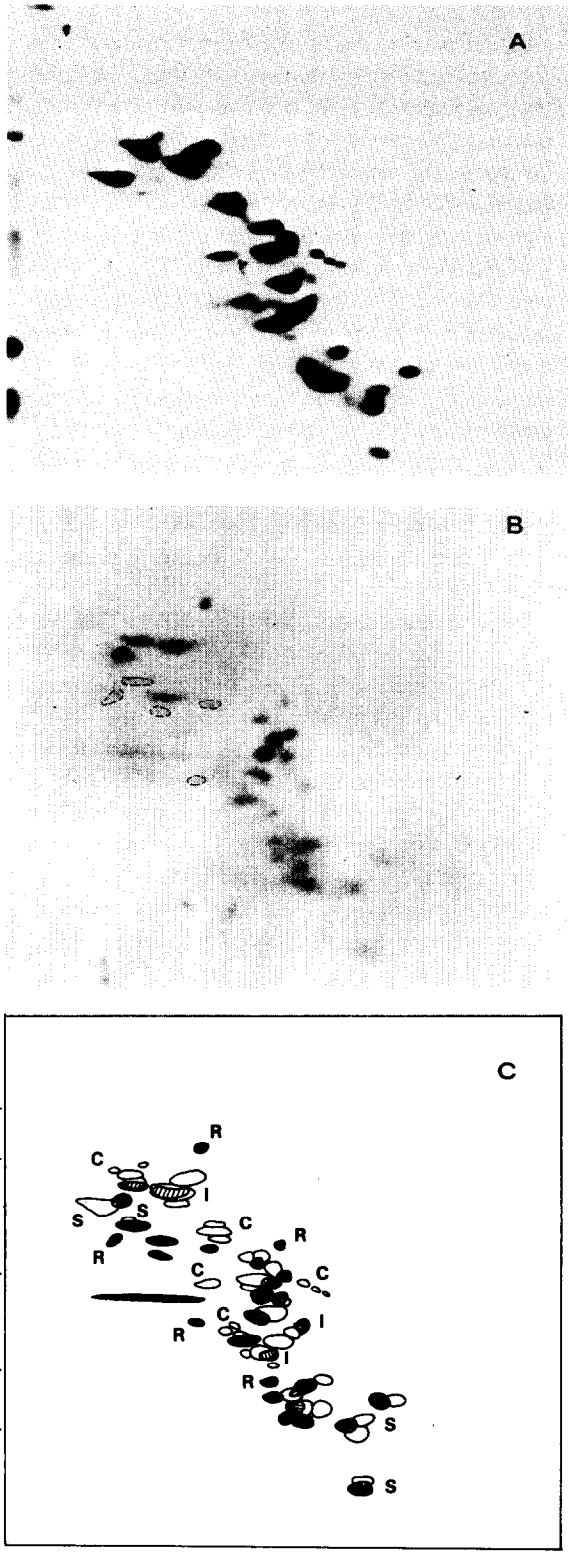

Fig.3. Co-electrophoresis of bovine and rat mitoribosomal small-subunit proteins. (A) Photograph of the stained gel (bovine proteins); (B) autoradiograph of the gel (rat proteins); (C) relative positions of bovine (open spots) and rat (filled spots) proteins. Labels and marker proteins are as described in fig.2 legend.

Differences among mitoribosomal proteins exist even in comparisons of more closely related organisms. The comparison [15] of large mitoribosomal subunit proteins from two species of toads showed at least 4 proteins in *Xenopus mulleri* absent from *X. laevis*, and at least 3 *X. laevis*-specific proteins. The number of species-specific proteins seen in this comparison of mitoribosomes from *X. mulleri* and *X. laevis* is much lower than we find in our analysis of the mitoribosomal proteins from two different mammals, using essentially the same electrophoretic system. In addition, greater electrophoretic differences exist between individual cow and rat mitoribosomal proteins. In this light it is not surprising that the mitoribosomal proteins from the phylogenetically distant organisms, rat and *Neurospora*, are so different that no obvious similarities are apparent in their electrophoretic properties [6].

These results contrast sharply with the findings for eukaryotic cytoplasmic ribosomes. No significant differences were found [2] among the cytoribosomal proteins of several mammalian species. Some differences have been detected, but relatively few. Only one such difference was found in comparisons of the cytoribosomal proteins of hamster and mouse [4], and only 3 were seen in comparisons of rat and HeLa cytoribosomal proteins [3]. Even more distantly related organisms show relatively few differences in the proteins of their cytoplasmic ribosomes: more than 90% of the proteins were electrophoretically indistinguishable in comparisons of mammalian and amphibian, or mammalian and fish, cytoplasmic ribosomes [2].

The evidence thus indicates that the degree of evolutionary divergence among mitoribosomal proteins is much higher than among the cytoribosomal proteins of the same organisms. This is especially interesting in view of the fact that essentially all of these ribosomal proteins are encoded in the nucleus and synthesized on cytoplasmic ribosomes [1]. These observations suggest that different structural or functional constraints are imposed on the proteins of mitochondrial and cytoplasmic ribosomes.

Acknowledgements

This work was supported by USPHS Grant GM-15438. R.A.H. and D.E.M. were supported by University of Florida Graduate Council Fellowships. We are grateful to Mark Critoph, Warren Clark and Mark Moffitt for their technical assistance.

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