THE OCCURRENCE OF RIBOSOMAL PROTEINS IN NUCLEOLI OF STARFISH OOCYTES *

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Numerous studies on the nucleoli and ribosomes from various cell types have shown a similarity between the morphological (fine structural) (Stevens, 1964) and biochemical (by analysis and in in vitro protein synthesizing systems) (Birnstiel, et al., 1963) properties of the ribosome and a ribonucleoprotein particle fraction of the nucleolus. Chemical analyses have confirmed the existence of a ribosomal-like RNA in nucleoli (Vincent, 1964). Little evidence has been presented on the similar and/or dissimilar proteins of these organelles. The studies presented below are an extension and refinement of pilot studies presented earlier (Vincent, et al., 1965). The high resolution of acrylamide disc gel electrophoresis has been applied to the protein fractions of these two organelles with the result that a number of proteins are found to be common to the two. The analyses also demonstrate that this similarity does not extend to all of the proteins of either organelle. Some proteins are distinctly ribosomal; others distinctly nucleolar. In addition, quantitative differences exist among the proteins which are common.

Experimental and Results

Ribosomes and nucleoli were prepared from starfish oocytes as described earlier (Vincent, et al., 1965). Variable amounts of purified lyophilized ribosomes were extracted with a solution of 2 M LiCl and 4.5 M Urea to give

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a final concentration of from 100 to 200 ug protein in 0.05 ml solution. Similar solutions of nucleolar proteins were extracted from whole lyophilized nucleoli. Studies proving that the nucleoli are free from ribosomal contamination are described in the previously mentioned paper (Vincent, et al., 1965).

These protein solutions were applied to columns of polyacrylamide gels and subjected to electrophoresis at a current of 5 mA per tube on a Canalco Model 6 Disc Electrophoresis apparatus, powered by a Canalco Model 200 constant current power supply. The gels used for separation were pH 4.5, 7½% with respect to acrylamide and 4 M with respect to urea. Electrophoresis was carried out at a room temperature for 90 minutes. Gels were immediately chilled and removed from their glass columns. They were stained in 0.5% Amidoschwartz 10B in 7% acetic acid for one hour minimum time. Destaining was accomplished at 12.5 mA per tube for approximately 90 minutes.

The split gel technique involved the creation of a water-tight partition at the sample end of the gel tubes to prevent admixture of jointly run samples. The efficacy of the separation of the two chambers was routinely established by running either of the protein samples against a blank. In this situation no stainable protein was found on the side of the gel column opposite the applied protein. Without this guarantee of separation the occurrence of the same bands on both sides of the gel would be meaningless.

When ribosomal proteins were run under the above conditions, a definitive pattern of at least twenty-two bands was observed in the gel. These are shown photographically in Fig. 1a. The nucleolar proteins which migrated toward the cathode under conditions of low pH similarly represented a complex pattern, in this case representing a minimum of twenty-five distict bands (Fig. 1b). In the pilot study reported earlier (Vincent, et al., 1965) it was concluded that only one of the basic proteins of the nucleolus could be established as being common to the two organelles. This was principally based on the evidence acquired by comparing the ribosomal proteins with the "residue" fraction of the nucleolus (dilute HCl-soluble or cold M NaCl-insoluble protein). The

studies carried out in this set of experiments show that the method of handling the nucleolar protein may have been instrumental in causing the difficulty in obtaining satisfactory gel patterns.

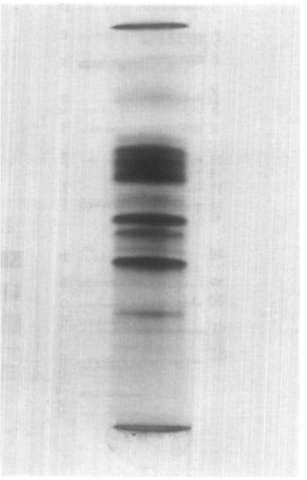

Fig. 1a. Photographic representation of typical ribosomal protein pattern.

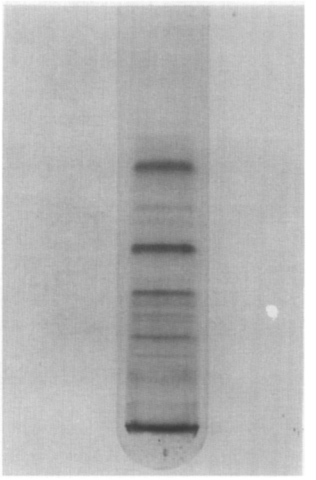

Fig. 1b. Photographic representation of typical nucleolar protein pattern.

Since the point at issue is to determine which of the ribosomal proteins are found in the nucleolus, it was decided in the studies to assay the total extractable proteins from the two organelles, rather than risk the introduction of other variables by treatment of the nucleolar fraction with other than the single solvent mixture. Consequently the fractions shown here include all of the proteins of the two organelles which are soluble in the LiCl - urea mixture, and which migrate toward the cathode at the specified pH. The results amply justify this approach since more of the nucleolar proteins are discernable as distinct bands and, more importantly, the proteins of the two organelles have been treated in an identical manner.

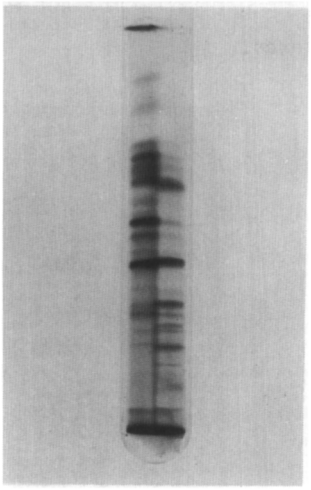

Fig. 2a. Photographic representation of the split gel. Ribosomal protein pattern is on the left; nucleolar protein on the right. Not all bands reproduce well in the photograph due to wide variation in densities and film latitudes.

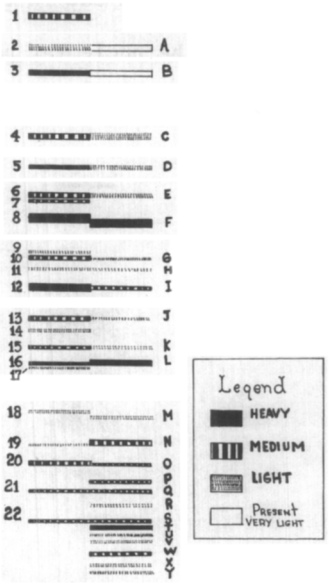

Fig. 2b. Diagrammatic representation of accompanying photograph. At least fourteen of the ribosomal bands are coincident with nucleolar bands. Some bands are exclusively ribosomal; other are exclusively nucleolar; still other are subject to question.

The results clearly demonstrate that at least fourteen of the twenty-two ribosomal proteins are electrophoretically similar to an equal number of nucleolar proteins. There are an additional two bands which overlap sufficiently to leave room for the possibility that similar proteins are common in these cases as well. The results of the split gel studies are summarized in Fig. 2a and 2b.

It is recognized that electrophoretic mobilities in acrylamide gels are influenced by numerous factors. Particle mass, net charge and shape (globular vs fibrillar) remain independent variables in the split gel technique. It is possible that two dissimilar proteins, by a coincidental combination of these factors, could achieve the same resultant electrophoretic mobility. The

resolution of the technique makes this possibility slight, and a coincidental identity of fourteen to sixteen proteins out of a total of twenty-two with a like number from a different protein fraction is inconceivably small. Since the other parameters of solute mobility in the gels are rendered identical by the technique (pH, current, time, average pore size of the gel, ohmic heating and diffusion), it is apparent that a coincidental identity of dissimilar proteins would necessarily reside with the parameters expressed by the protein molecules themselves. It is concluded that a majority of the ribosomal proteins are found in the nucleolus of the same cell in this biological system.

Several additional points should be brought to light. A portion of the ribosomal proteins which are not shown in the nucleolus may represent adsorbed protein of the cytoplasm, since nucleic acid-protein complexes such as ribosomes can adsorb such proteins. Similarly a number of the nucleolar proteins not seen in the ribosomal pattern may be nucleolar enzymes or adsorbed protein. It is possible, therefore, that an even greater similarity between these ribosomal and nucleolar proteins exists than is shown by the technique used. It is also possible that non-nucleolar proteins which are found in the ribosome may be of an extra-nucleolar origin. None of these prospects are contrary to the experimental findings nor to the concept that the nucleolus may be a site for the synthesis or assembly of ribosomes. They are, however, explicitly categorized as possibilities.

The conclusions which are borne out by the evidence are two. First, most of the ribosomal proteins of the starfish oocyte are represented in the nucleolus. Second, this study is in direct support of earlier immunological evidence suggesting a strong similarity between these two protein fractions (Vincent, et al., 1965).

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