HETEROGENEITY OF RETICULOCYTE RIBOSOMES

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SUMMARY. The proteins of free and membrane-bound reticulocyte ribosomes, when analyzed by polyacrylamide gel electrophoresis, are distinctly different. This physical heterogeneity may be related to the function of these two classes of ribosomes, which synthesize different types of protein in the eukaryocytic mammalian erythroid cell.

The complex structure of ribosomal protein was initially demonstrated by Waller (1). Subsequent stoichiometric analyses of the protein extracted from the 30S ribosomal subunit of E. coli indicate that ribosomes are physically heterogeneous (2), although the functional implications of these differences in ribosomal protein still remain obscure. Evidence suggests that im mammalian reticulocytes the two major classes of protein, globin and nonglobin protein, are preferentially synthesized by free and membrane-bound ribosomes, respectively (3). Analysis of the proteins of these two classes of reticulocyte ribosomes is therefore of importance in understanding the relationship between physical heterogeneity of ribosomes and their function in protein synthesis. We now report the existence of gross structural differences between the ribosomal protein of free and membrane-bound ribosomes of mammalian reticulocytes.

Free and membrane-bound ribosomes were isolated in the presence of 0.2% sodium deoxycholate from rabbit reticulocytes (4) and were rendered free of hemoglobin by sedimentation through a 5 to 20% sucrose gradient at 201,000 g for two hours at 4°. Four types of protein were prepared for acrylamide gel analysis from cells lysed in 7.37×10^{-3}M phosphate buffer (5): 1) an acid-acetone precipitate of ribosome and membrane-free lysate (P); 2) pro-

tein from washed cell membranes (M); 3) protein from isolated free ribosomes (IR); and 4) protein from isolated membrane-bound ribosomes (MR). The ribosomes from which proteins were isolated were capable of synthesizing protein in the cell-free system (4). All proteins were solubilized by incubation for three hours at 37° in 0.1M phosphate buffer, pH 7.1, containing 1% mercaptoethanol and 1% sodium lauryl sulfate. After dialysis against a 1:10 dilution of the same buffer containing 5 x 10^{-3}M EDTA overnight the proteins were electrophoresed in 7.5% acrylamide gels. After fixation in 20% trichloracetic acid the gels were stained with 0.25% Coomassie Blue and destained in 7% trichloracetic acid.

Figure 1 shows the electrophoretic patterns of equal amounts of the four

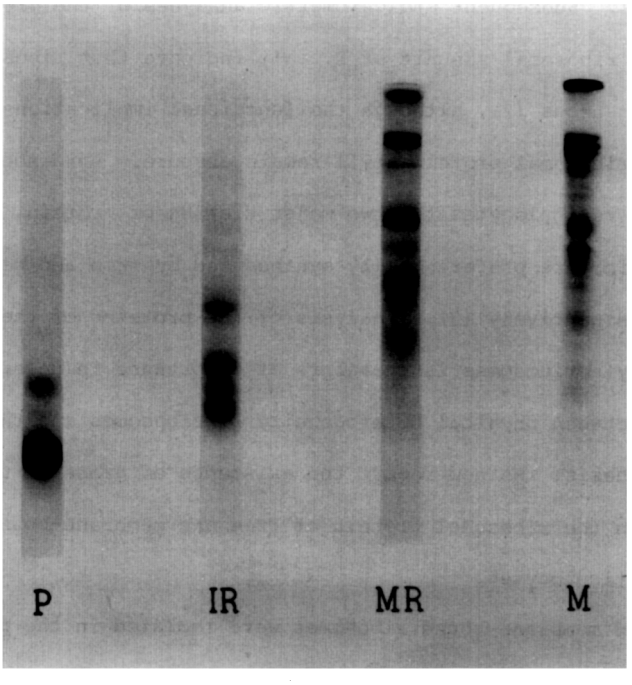

Figure 1 - Polyacrylamide gel electrophoresis of proteins from reticulocytes. P, acid-acetone precipitate of ribosome and membrane-free hemolysate; IR, free ribosomes; MR, membrane-bound ribosomes; M, membranes. Between 75 and 88 micrograms of each solubilized protein was electrophoresed by the method of Shapiro, Vinuela, and Maizel (7) in a 5 by 600 mm 7.5% polyacrylamide gel with 5% cross-linking at room temperature for 210 minutes using a current of 6 milliamperes per tube. Horizontal arrows by the side of the gels indicate differences between protein-free and membrane-bound ribosomes.

extracted proteins. The acid-acetone precipitated protein from the ribosome-free supernatant (P) consists primarily of globin and globin subunits, the two fractions which migrate most rapidly towards the anode, the lesser amounts of more slowly migrating non-globin proteins (3). The protein derived from free ribosomes (IR) could be resolved into more than 25 distinct bands on the densitometric scans of the gels (figure 2). Since none of these bands coincide with those of authentic globin (P in figure 1) they cannot be ascribed to contamination by soluble protein. Similarly, comparison of the protein derived from membrane-bound ribosomes (MR) with globin gels (figure 1) shows that membrane-bound ribosomes are not contaminated with soluble cytoplasmic proteins. Comparison of the gels does indicate, however, that membrane-bound ribosomes contain some protein of membrane origin although distinct proteins different from membrane are also present. This confirms the previous suggestion that the large amount of the protein in membrane-bound ribonucleoprotein particles of reticulocytes is of membrane origin (4).

The heterogeneity of reticulocyte ribosomes is evident from examination

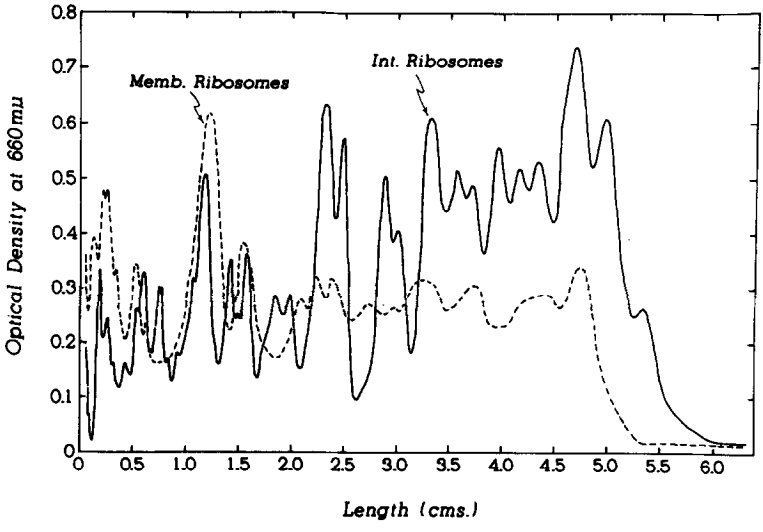

Figure 2 - Densitometric tracing of acrylamide gel electrophoreses of protein derived from free (solid line) and membrane-bound (dotted line) reticulocyte ribosomes. The gels, stained with Coomassie Blue, were scanned at 0.5 cm/min in a 6 mm width cuvette in a Gilford model 2410 linear scanning apparatus at a wavelength of 660 mμ. Origin of the electrophoresis is at left.

of figure 1. The horizontal arrows indicate representative protein fractions from free ribosomes which are not present in bound ribosomes and protein fractions in membrane-bound ribosomes which are not found in free reticulocyte ribosomes. The densitometric scans of the same gels (figure 2) indicate these differences more strikingly. These differences must represent ribosomal protein since comparison with soluble and membrane protein shows that they cannot be ascribed to contamination with either membrane or cytoplasmic proteins. This was confirmed by densitometry of the gels (not shown) since photographs do not accurately reproduce all of the protein fractions in the gels.

Although it has been conclusively demonstrated that E. coli ribosomes are heterogeneous (2) it is not clear whether this represents a static situation, in which subclasses of ribosomes differ permanently, or a dynamic situation in which ribosomal proteins are in an orderly state of flux related to function The knowledge of ribosomal proteins of eukaryocytic cells, compared to that of prokaryocytic cells, is scant. Gould, in analyzing the individual subunits of reticulocyte ribosomes, concluded that some proteins are present in multiple copies and others in less than stoichiometric amounts (6). She felt it unlikely that the presence of numerous minor components reflected requirements for different sets of messenger RNA's. This conclusion is not unexpected since free ribosomes, the only type which she examined, synthesize almost exclusively a single protein, globin, while nonglobin proteins are predominently synthesized on ribosomes bound to the reticulocyte cell membrane (3). The present findings of well-defined differences in the structural proteins of these two types of ribosomes lead us to conclude that such differences may indeed be related to the requirements for a specific messenger RNA, and make it less likely that such differences represent proteins which are only cyclically bound to the ribosome. A similar conclusion was reached by Fridlender and Wettstein in studying the proteins of free and membrane-bound ribosomes in chick embryo cells (8).

The location of these proteins in the larger or smaller subunit of re-

ticulocyte ribosomes has not been determined, nor has analysis of the molecular weights of the protein fractions been carried out. Nevertheless, the data clearly show heterogeneity of two types of reticulocyte ribosomes which synthesize different types of protein. The findings suggest that ribosomal proteins are intimately involved in the complex process which determines specificity synthesis.

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