An Immunological Method of Fractionation of Ribosomes from the Chick Lens

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In a study of the regulation of protein synthesis during development and cell differentiation in the chick lens an attempt is being made to estimate the stability of mRNA molecules for specific polypeptides in the lens. A method has been developed that appears to separate those ribosomes that have attached to them nascent polypeptide chains with specific immunological determinants. Immunological methods have also been used to separate polyribosomes in muscle cells by Allen & Terrence (1968).

Chick lenses or mouse spleen were used to prepare ribosomes by the method of Korner (1961), with the modification that 1mg of yeast tRNA/ml was present at all stages to minimize the degradation of polyribosomes by ribonucleases.

The globulin fraction was precipitated from the antisera used by addition of ammonium sulphate to 50% saturation, and after two reprecipitations the globulins were made up in 0.9% NaCl to the original volume of serum.

The ribosomal pellet was resuspended in tris with salts and tRNA, and undispersed material was removed by centrifugation at 10000g for 10min. Then 0.5ml of ribosome suspension was mixed with 0.5ml of globulin solution. After 1h at 0°C the mixture was layered on top of centrifuge tubes containing 7ml of 50% (w/v) sucrose and 12ml of sucrose solution in a gradient from 20% to 5% (w/v), all solutions being in tris with salts and tRNA. The tubes were centrifuged at 22000g for 30min and fractions were collected by piercing the tube, the small pellet obtained also being taken.

Chick lens ribosomes and mouse spleen ribosomes were isotopically labelled by using either amino acid or uridine precursors. When a mixture of such ribosomes was treated with globulins from antiserum to total chick lens proteins, the lens ribosomes were displaced to the lower half of the gradient, leaving the spleen ribosomes in the upper half, whereas globulins against mouse serum displaced the spleen ribosomes and left the lens ribosomes in the upper half of the gradient.

Antisera that react with some fractions of the chick lens proteins, such as those reacting with α - or with β -crystallins, caused a displacement of a portion of the chick lens ribosomes, leaving a fraction that could subsequently be precipitated with antiserum to total chick lens proteins.

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Structure and Function of Polyribosomes in Ischaemic Liver Cells

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Dilation and vesiculation of the endoplasmic reticulum and dislocation of the ribosomes from the endoplasmic reticulum membranes have been observed in ischaemic livers (Bassi & Bernelli-Zazzera, 1964). Similar ultrastructural alterations, described in rat liver cells during carbon tetrachloride treatment, are associated with ribosomal changes and decreased capacity for protein synthesis (Smuckler, 1968).

In this communication we will report some preliminary results on the state and functioning of polyribosomes in ischaemic livers: polyribosomal patterns and incorporation of [¹⁴C]leucine into protein have been studied after various periods of ischaemia, known to cause reversible or irreversible damage to the liver cells, and in the recovery phase following the re-establishment of the normal blood flow (Bernelli-Zazzera & Gaja, 1964).

Postmitochondrial supernatants were obtained by centrifugation at 12000g for 20min of liver homogenates prepared in two different media: medium A for polyribosomal patterns, and medium B for protein synthesis (Ragnotti, Lawford & Campbell, 1969). The postmitochondrial supernatants obtained in medium A were treated with sodium deoxycholate (1.3%), layered over a 5ml linear gradient of 15–50% (w/v) sucrose in medium A, and centrifuged at 65000 rev./min for 60min in a Spinco SW 65 rotor. Extinction at 260nm was measured in a Unicam SP.800 spectrophotometer, fitted with a flow-cell attachment.

Incorporation and radioactivity were measured on postmitochondrial supernatants as previously described (Ragnotti *et al.* 1969).

Polyribosomal patterns show a progressive decrease of the heavier aggregates and a relative increase of the lighter fractions; on the whole, RNA content decreases. Protein synthesis is accordingly decreased; these changes are correlated with the duration of ischaemia. Within 4h of the re-establishment of blood supply both the aspect of the polyribosomal patterns and the capacity for protein synthesis return to normal only if the duration of ischaemia does not last beyond the 'point of no return' of the tissue. However, shortly after the re-establishment of the normal blood flow the