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

polyribosomal patterns show a further shift towards the lighter forms also in reversibly damaged liver cells: protein synthesis stays at the low rate reached at the end of ischaemia.

The return of blood flow in a formerly ischaemic tissue seems to be a critical stage for the state of polyribosomes in the liver cell.

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Ribosome Tetramers from Cold-Treated Chick Embryos

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Byers (1967) has described a structure occurring in cold-treated chick embryo cells that is composed of ribosomes arranged in a crystal-like lattice. The smallest repeating unit of the lattice is a group of four ribosomes. From the work of Humphreys & Bell (1967) it was concluded that these tetramers were stable to the processes of homogenizing and centrifuging as applied to the preparation of ribosomes for analysis on sucrose density gradients. It seemed probable that further investigation of them would provide information about ribosome structure and function.

Ribosomes were prepared from cold-treated chick embryos by the method of Blobel & Potter (1967) and analysed on sucrose density gradients in the Spinco SW 40 rotor, by either 10–40% (w/v) sucrose or the isokinetics described by Noll (1967). A ribonuclease-stable peak has been found in the region of the tetramer, but the overall pattern of peaks found in the TKM buffer of Blobel & Potter (1967) is complex, with a number of minor peaks in addition to the easily recognizable monomer, dimer etc. The tetramer region has been resolved into two peaks with sedimentation coefficients 183S and 194S.

Lowering of the Mg^{2+} concentration caused a reversible dissociation of the higher aggregates into monomers and subunits. Increasing Mg^{2+} (or other bivalent cation) concentration caused non-specific aggregation. Ca^{2+} can replace Mg^{2+} , but in some circumstances an increased tendency to dimer formation was noticed. Mn^{2+} also caused dimer formation.

Increasing K^+ concentration caused disaggregation and at high concentration (0.35M) extensive

subunit formation. The 'tetramer' peak, however, was not lost, i.e. a peak equivalent in size to the tetramer remained, but with a stepwise alteration to positions with lower sedimentation coefficient.

Results of attempts to fractionate the ribosome peaks and data on amino acid-incorporating activity will be reported.

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Factors Affecting Separation of Free Polyribosomes from Membrane-Bound Polyribosomes

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Accumulating evidence suggests that free and membrane-bound polyribosomes from rat liver synthesize different non-overlapping classes of proteins (e.g. Hallinan, Murty & Grant, 1968a,b; Takagi & Ogata, 1968; Redman, 1969). A necessary prerequisite for such investigations is of course the separation of the two polyribosome classes from each other. A standard method used in this study consists of centrifuging postmitochondrial supernatant at 105000g_{av} through steps of 1.5M- on 2.0M-sucrose; free polyribosomes form a pellet at the bottom of the tube while most membranes, having a lower density, remain in the 1.5M-sucrose layer. It has been shown (Blobel & Potter, 1967; D. Lowe, T. Hallinan & E. Reid, unpublished work) that centrifugation time is very important. After only 4–5h (the centrifugation time used by many authors) sedimentation of free polyribosomes is incomplete. This phenomenon has now been further investigated.

Rats were either fed *ad libitum* or starved overnight. Polyribosome pellets obtained after centrifugation for various times were analysed for RNA content. Not only was the 4–4½h free polyribosome yield a small percentage of the 20–21h yield (which was similar for fed or starved rats), but this percentage was much smaller for fed (average 5% of the 21h value) than for starved rats (average 28%). However, this low yield at 4–5h could be increased tenfold by using diluted postmitochondrial supernatant, suggesting interference by concentration-dependent interaction of polyribosomes with non-sedimenting components.

In other experiments RNA was labelled with