The Production of Active Subparticles from Rabbit Reticulocyte Ribosomes

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Bacterial ribosomes dissociate into subparticles simply on lowering the Mg^{2+} concentration of the medium (Tissières, Schlessinger & Gros, 1960). Restoration of the original concentration of Mg^{2+} results in reconstitution of the subparticles into ribosomes active in protein biosynthesis in a cell-free system (Schlessinger & Gros, 1963).

In contrast, attempts to prepare biologically active subparticles from mammalian ribosomes have generally been abortive (Lamfrom & Glowacki, 1962; Gould, Arnstein & Cox, 1966). Miller, Hamada, Yang, Cohen & Schweet (1967) and Hamada, Yang, Heintz & Schweet (1968) have reported that exposure of rabbit reticulocyte ribosomes to high concentrations of KCl produces subparticles that, together with a supernatant fraction, are active in synthesis of haemoglobin *de novo*.

In this Laboratory the production of subparticles from polysomes was examined by dialysing concentrated solutions against 0.5M-KCl, by zone ultracentrifuging through a sucrose gradient in 0.5M-KCl or by differential centrifuging of solutions made 0.5M in KCl. The products of each treatment and their ability to support protein synthesis in a reticulocyte cell-free system in the presence and absence of polyuridylic acid were examined.

It was found that exposure to 0.5M-KCl is not a sufficient condition for the complete dissociation of ribosomes into subparticles and that the reaction shows a concentration-dependence, implying the existence of an equilibrium between the various ribosomal species. Disturbance of the equilibrium by removing certain products, as in zone centrifuging, can lead to complete dissociation.

The subparticles produced by dialysis and zone centrifuging were biologically inactive and very unstable. If the pellet obtained by differential centrifuging was suspended in a Mg^{2+} -free buffer it consisted entirely of particles having the sedimentation coefficients of the two ribosomal subparticles. Addition of Mg^{2+} converted about 30% of this material into heavier sedimenting species, which had 20–40% of the control activity in the cell-free system. Addition of the supernatant fraction resulted in further apparent reconstitution (about 70% of the ribonucleoprotein present sedimented as ribosomes and polyribosomes) and led to a 50–100% restoration of biological activity. Incorporations similar to or higher than those of the control were obtained when both polyuridylic acid

and supernatant factors were present. The effects of temperature and time on the yield and the integrity of the RNA and protein moieties of the subparticles are discussed.

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The Effect of Encephalomyocarditis-Virus Ribonucleic Acid on Amino Acid Incorporation in Two Mammalian Cell-Free Protein-Synthesizing Systems

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As a preliminary to experiments designed to study initiation of protein synthesis in mammalian systems we isolated RNA from mouse encephalomyocarditis (EMC) virus and added it to cell-free systems from rabbit reticulocytes and from mouse Krebs II ascites cells.

The incorporation of [^{14}C]phenylalanine by the reticulocyte lysate was consistently decreased by 10–60% in the presence of EMC-RNA. Isoleucine, arginine and phenylalanine incorporation were all decreased to approximately the same extent. Preincubation of the cell-free system decreased and finally abolished the endogenous incorporation without greatly affecting its ability to respond to poly U. EMC-RNA inhibited the residual incorporation of the preincubated system to a greater extent than in the non-preincubated system. The inhibition of isoleucine incorporated by poly U was also more profound after preincubation.

In contrast with these results with the reticulocyte system a cellfree system from ascites cells exhibited stimulation of incorporation by EMC-RNA, as found by Kerr, Cohen & Work (1966). Preincubation of this system rendered it almost completely dependent on added EMC-RNA. Liver ribosomal RNA, on the other hand, stimulated only in the non-preincubated system.

This difference between the ascites and reticulocyte systems was studied further in cross-over experiments employing ribosomes from one tissue with cell sap from the other. EMC-RNA inhibited amino acid incorporation whenever reticulocyte ribosomes were used irrespective of the source of the