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

sap, whereas it stimulated when ascites ribosomes were used in the presence of their own sap. When reticulocyte sap was incubated with ascites cell ribosomes EMC-RNA had no significant effect.

One interpretation of these results is that ascites-cell sap contains a factor absent from reticulocyte-cell sap which is necessary for the translation of the added EMC-RNA. It seems that reticulocyte ribosomes are able to combine with added RNA in such a way that incorporation on the endogenous message is decreased.

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Variations in the Microstructure of Histones during Early Cleavage Stages in *Echinus*

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Earlier work on nuclear proteins from sea-urchins indicated that in the mature unfertilized egg the thiol group(s) on the histone(s) was present in the disulphide form and that an increase occurred in the proportion of thiol after fertilization, reaching a peak just before cleavage (Ord & Stocken, 1968). These findings have now been confirmed and extended by using *Echinus esculentus* and histone extracted from washed nuclei in ethanol-1.25M-HCl (4:1, v/v) (Johns, 1964). Fertilization and subsequent incubation of the eggs were performed at 8°; the first furrow appeared at 180min. after fertilization. The eggs were collected at 10-20min. intervals from fertilization to the second cleavage. The packed eggs were stored at -15° for about a fortnight before extracting the histones. The acid-ethanol extracts were precipitated immediately with 5vol. of acetone-HCl and were dialysed against 50mM-HCl and water at 2° before analysis. Only traces of lysine-rich histones are present in *Echinus* in early cleavage stages; the material analysed contained components migrating on electrophoresis on polyacrylamide gel in positions similar to that of the arginine-rich histone F3 and of the F2A group. Neither phosphate nor thiol is present in the latter histones so that the SH+S·S and phosphate contents are predominantly accounted for by histone F3 (Ord & Stocken, 1967).

The total SH+S·S content of the histones analysed did not vary (69.3 ± 13.0 nmol/mg. of protein), but the proportion of thiol increased from less than 10 to more than 50% at 140, 180 and 275min. after fertilization. From work on thymi-

dine uptake into *Strongylocentrotus purpuratus* at 15° (Hinegardner, Rao & Feldman, 1964), these times probably correspond to periods of DNA synthesis. As reported earlier there is also a sharp peak in the percentage of SH 30min. after fertilization that is not found in subsequent cycles. The phosphate content of the proteins (53.6 ± 5.9 ng.atoms of P/mg. of protein) remained constant for 10-90min. after fertilization; preliminary results indicate it may then vary reciprocally to the proportion of thiol.

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Histone Phosphorylation and Cell Division

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An approximate temporal correlation between the phosphorylation of histone F1 and DNA synthesis in regenerating rat liver has been reported by Ord & Stocken (1967). More detailed work has now shown that the increase in phosphorylation of histone F1 precedes the incorporation of ^{32}P into DNA by approx. 1hr.

Partially hepatectomized and control sham-operated animals were injected with $100\mu\text{C}$ of [^{32}P]phosphate/100g. body wt. intramuscularly 30min. before death. Groups of three or four rats were killed at $\frac{1}{2}$ hr. intervals over the period 15-19hr. after hepatectomy. Histone F1 was obtained from washed nuclei by extraction with perchloric acid (Johns, 1964) and its phosphate content determined by the method of Bartlett (1959). ^{32}P incorporation into DNA was measured by the method of DeLuca, Rossiter & Strickland (1953). Tissue phosphate soluble in 20% trichloroacetic acid was used as a reference standard for determining relative specific radioactivities.

A further indication of a link between DNA synthesis and phosphorylation of histone F1 comes from the finding that 1000 rads total body γ -irradiation at 12hr. after partial hepatectomy prevents the phosphorylation of histone F1 in a manner similar to the inhibition of DNA synthesis (Holmes & Mee, 1955).

Unilaterally nephrectomized rats have also been found to give increased phosphorylation of histone F1 24hr. after the operation when DNA synthesis is known to be taking place. 1000 rads total body