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SECTION 12

BIOSYNTHESIS OF PROTEINS

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STUDIES ON THE INITIATION OF PROTEIN SYNTHESIS USING HAEMOGLOBIN mRNP

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When rabbit reticulocyte polysomes are treated with EDTA a RNP species containing 9s mRNA for haemoglobin is released [1]. We would like to report that this mRNP species can be incorporated into reticulocyte polyribosomes using a pH 5.4 precipitate [2] from a post mitochondrial lysate. Using this technique we have been able to demonstrate that an 80s initiation complex is formed; furthermore the cold sensitivity and action of inhibitors on this step have been investigated. Our results are consistent with current ideas on the initiation of protein synthesis in which the first step is the binding of mRNA to the small subparticle [3, 4]. This step can be inhibited by aurine tricarboxylic acid but is not cold sensitive. However the subsequent process of formation of an 80s initiation complex is cold sensitive and can not be prevented by the addition of cycloheximide.

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ISOLATION AND IDENTIFICATION BY BIOLOGICAL ACTIVITY OF GLOBIN SPECIFIC MESSENGER RNA AND MESSENGER RIBONUCLEOPROTEIN COMPLEX FROM DUCK ERYTHROBLAST.

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Hemoglobin specific messenger RNA (mRNA) and messenger ribonucleoprotein particles (mRNP) have been isolated from polyribosomes of duck erythroblast in which 80% of the protein synthesized is hemoglobin. mRNA was purified by SDS-sucrose gradient and ethanol precipitation; mRNP was prepared by EDTA dissociation, purification in a glycerol gradient and concentration by membrane ultrafiltration. Using EDTA concentrations, varying from 0.005 to 2 µMoles/OD_{260} polysomes, the dissociation of polyribosomes into subunits was complete, but the mRNP particle was only released between 0.05 and 2µMoles EDTA/OD_{260}. With weaker concentration of EDTA the mRNP was still attached to the small subunit and, under these conditions, the two dissociated subunits were able to reform polyribosomes after restoration of the original Mg^{++} concentration. These polyribosomes have been shown to be active in cell-free protein synthesis. The purified mRNA and mRNP particles are now being tested in heterologous "cell-free" systems from rabbit reticulocytes and HeLa cells to check their ability to direct the synthesis of duck globin and also to investigate the role of the protein moiety of the mRNP complex in the translation of the messenger RNA

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THE TRANSLATION OF 9S RNA FROM RETICULOCYTES TO GLOBIN CHAINS IN FROG OOCYTES AND EGGS.

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When 9S RNA from rabbit reticulocyte polyribosomes is injected with haemin into Xenopus oocytes or eggs, it induces the synthesis of rabbit haemoglobin in these cells
The synthesized rabbit haemoglobin has been characterized by molecular filtration on sephadex G100 columns, by polyacrylamide gel electrophoresis, by the separation of the α and β chains by CM-cellulose chromatography, and by ion-exchange chromatography of the tryptic peptides from isolated α and β chains.
This result proves that the 9S ribonucleic acid fraction from reticulocyte polyribosomes contains a mixture of the mRNAs for α and β globin chains. Furthermore, this finding shows that frog eggs and oocytes provide an excellent system for testing the translation of messenger RNA; globin mRNAs are very effectively translated for more than twenty four hours in the cytoplasm of these cells.

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