PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 502nd Meeting of the Society was held at the University of Leeds on Monday and Tuesday, 12 and 13 January 1970, when the following papers were presented:

COLLOQUIUM ON 'THE STRUCTURE AND ACTIVITY OF POLYRIBOSOMES FROM ANIMAL CELLS'

The Activity of Polyribosomes from Liver

By A. KORNER. (School of Biological Sciences, University of Sussex, Brighton BN1 9QG, U.K.)

The protein-synthetic activity of already synthesized ribosomes can be altered in many ways. Alterations to mRNA, especially of the initiating and terminating ends, could change both the activity and profile of polyribosomes, though these two parameters are not necessarily directly correlatable. Changes could occur in the RNA or proteins of the ribosomes themselves, altering their ability to divide into the subunits, needed for initiation of peptide synthesis, or their ability to participate in other parts of the process. Several of these types of change have been observed with ribosomes from eukaryotic cells including liver.

Initiation is slow in many systems *in vitro*, perhaps because of damage to the 5'-hydroxyl terminus of mRNA, and there is evidence of blockage of protein synthesis because of the absence of a proper 3'-hydroxyl terminus on mRNA (Hunter & Korner, 1969), giving rise to large polyribosomes with low incorporating activity. Subunits are required for initiation in eukaryotic systems (Hogan & Korner, 1968), and it has been shown that the change from monomers to subunits and polyribosomes is controlled by amino acids. Hypophysectomy diminishes the protein-synthetic activity of the small ribosomal subunit of liver ribosomes (Barden & Korner, 1969), whereas diabetes diminishes the activity of the large ribosomal subunit of muscle (Martin & Wool, 1968). The difference here may be a tissue or a hormonal one. Hypophysectomy decreases the ability of whole ribosomes and of the 40S subunits to binding aminoacyl-tRNA molecules (C. C. Liew & A. Korner, unpublished work; N. Barden & A. Korner, unpublished work). The differences in incorporating activity between ribosomes from normal, hypophysectomized and growth-hormone-treated rats persist even when peptide chain initiation is inhibited (Korner, 1969).

The puromycin reaction has been used as an assay for inactive ribosomes (Wool & Kurihara, 1967), but the results must be interpreted with care.

Jefferson & Korner (1969) found that 11 amino

acids were necessary in perfused rat liver to incorporate radioactive amino acid into protein at high rates, to obtain ribosomes with high incorporating activity and to obtain non-degraded polyribosome profiles responsive to growth hormone (see also Clemens & Korner, 1969). Baliga, Pronczuk & Munro (1968) could restore activity to polyribosomes by amino acids and conclude that they help regulate attachment of ribosomes to mRNA. It has been found (M. J. Clemens, V. M. Pain & A. Korner, unpublished work) that amino acid mixtures enhance ribosome activity of normal and adrenalectomized rats, but not in hypophysectomized or diabetic rats, suggesting different lesions in the ribosomes of these animals.

The existence of fractional proteins (Kurland, Voynow & Hardy, 1969) in bacterial ribosomes offers a hypothesis to explain some of these observations.

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The Structure and Activity of Polyribosomes and Subparticles from Reticulocytes

By H. R. V. ARNSTEIN. (Department of Biochemistry, King's College, University of London, Strand, London W.C.2, U.K.)

In reticulocytes, most of the ribonucleoprotein particles are present in the cytoplasm as free polyribosomes, but a minor fraction occurs bound

to the cell membrane (Schreml & Burka, 1968). The predominant species active in haemoglobin biosynthesis contains five or six ribosomes (Warner, Knopf & Rich, 1963), and polyribosomes larger than tetramers synthesize relatively more β -chains, whereas smaller polyribosomes synthesize more α -chains (Hunt, Hunter & Munro, 1968a). There is a slight excess in the number of ribosomes engaged in making β -chains, the ratio of nascent β/α chains being approx. 1.1 (Hunt, Hunter & Munro, 1968b).

Electron microscopy shows the contour length of the pentaribosome to be 1600 Å, which is in agreement with the estimated minimum length of haemoglobin mRNA coding for single chains, on the assumption that the mRNA contains approx. 450 nucleotides stacked with a translation of 3.4 Å per nucleotide. More recent measurements of stacked and unstacked single-stranded polyribonucleotides indicate an interphosphate separation of 6.5 Å (Eisenberg & Felsenfeld, 1967), and on this basis haemoglobin mRNA could be as long as 2900 Å, which can accommodate 12 closely packed ribosomes of 240 Å diameter. Some larger polyribosomes containing 15–20 ribosomes have been demonstrated in reticulocytes (Rifkind, Luzzatto & Marks, 1964), and incubation of cells with L-O-methylthreonine inhibits the incorporation of isoleucine into protein and results in the appearance of aggregates containing 10–14 ribosomes, which contain almost exclusively nascent β -chain peptides, as well as small polyribosomes of two or three ribosomes that have an excess of nascent α -chain peptides (Kazazian & Freedman, 1968). These results suggest that, contrary to earlier views, the size of reticulocyte polyribosomes is governed not by the length of haemoglobin mRNA but by the availability of ribosomes.

Maturation of mammalian reticulocytes is accompanied by a progressive decline in protein synthesis and loss of ribonucleoprotein. A preferential decrease in polyribosome content has been reported by some investigators (Danon & Cividalli, 1968), whereas others have found no change in the proportion of polyribosomes and ribosomes (Burka & DeBellis, 1967). There is thus no general agreement and the mechanism responsible for the degradation of polyribosomes is uncertain.

Reticulocyte polyribosomes can be dissociated into ribosomal subparticles by several methods such as treatment with EDTA or concentrated salt solutions or exposure to moderately high pH (Gould, Arnstein & Cox, 1966; Yang, Hamada & Schweet, 1968; Bonanou, Cox, Higginson & Kanagalingam, 1968). In the absence of Mg^{2+} during dissociation with concentrated salt solutions a mixture of subparticles is obtained that loses activity in protein synthesis when further fractionated by sucrose-density-gradient centrifugation.

Treatment of polyribosomes with 1.0M-KCl in the presence of 10mM-MgCl₂, however, yielded subparticles that could be separated on sucrose density gradients with retention of full activity for polyphenylalanine synthesis in the presence of poly U (Bonanou & Arnstein, 1969). Residual polyribosomes and ribosomes carrying nascent protein always remain and can be converted into subparticles by a second treatment with salt, but dissociation is again incomplete, suggesting that there is an equilibrium between polyribosomes and subparticles. The subparticles are free of nascent protein, and treatment with concentrated KCl also removes protein that stimulates cell-free protein synthesis (Miller & Schweet, 1968), facilitates the reassociation of subparticles (Godin, Kruh & Dreyfus, 1969) and promotes the build-up of polyribosomes (Cohen, 1968). There is evidence that the KCl extract contains more than one factor (Gee-Clough, 1969). Although chain initiation in haemoglobin synthesis differs from bacterial protein synthesis in utilizing valyl-tRNA instead of N-formylmethionyl-tRNA (Rahamimoff & Arnstein, 1969), it is possible that initiation factors similar to those required in bacterial cell-free systems may be involved in haemoglobin synthesis.

The fate of mRNA when polyribosomes are dissociated is uncertain. The mixture of subparticles obtained by treatment with KCl retains the capacity to synthesize haemoglobin (Hamada, Yang, Heintz & Schweet, 1968) and therefore presumably contains mRNA. On the other hand, subparticles prepared by treatment of polyribosomes with 1.0M-KCl–10mM-MgCl₂ and fractionated on sucrose density gradients are almost inactive in polypeptide synthesis unless poly U is added (S. A. Bonanou, unpublished work), suggesting that mRNA has been removed or degraded under these conditions.

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Formation and Function of Polyribosomes in the Early Embryonic Development

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It has been shown that in the eggs of several animals the rate of protein synthesis is very low before fertilization, i.e. there is a small number of active polyribosomes. After fertilization the rate of protein synthesis increases rapidly and this is accompanied by a concurrent rapid build-up of polyribosomes (Rinaldi & Monroy, 1969; Firtel & Monroy, 1970). There is evidence that the structural state of the ribosomes of the unfertilized egg prevents their interaction with mRNA (Maggio, Vittorelli, Caffarelli-Mormino & Monroy, 1968). It is known that until approximately the blastula stage most of the protein synthesis is directed by mRNA that was synthesized during oogenesis (summary in Monroy, 1965). Nevertheless from the earliest post-fertilization stages some heterogeneous rapidly labelled RNA, which is neither rRNA nor tRNA, is synthesized and becomes associated with polyribosomes; most of this rapidly labelled RNA is found in a class of small polyribosomes (Rinaldi & Monroy, 1969; Kedes & Gross, 1969; Firtel & Monroy, 1970), which are the ones most sensitive to actinomycin (Infante & Nemer, 1967; Firtel & Monroy, 1970). Contrary to earlier assumptions (Spirin & Nemer, 1965) these polyribosomes are not inactive in protein synthesis; in fact there is evidence that they are the site of the synthesis of histones (Nemer & Lindsay, 1969; L. H. Kedes & P. R. Gross, personal communication; M. Crippa, personal communication). In the early stages of development the largest percentage of the new (i.e. of zygotic origin) mRNA thus appears to be translated by the small polyribosomes. Data on amphibians (Crippa & Gross, 1969) and on sea urchin (M. Crippa, personal communication) show that only about 50% of the total amount of mRNA present in the young embryo (cleavage stages to early blastula)

is associated with polyribosomes. DNA-RNA hybridization experiments suggest that both in the amphibians (M. Crippa & E. Costantino-Ceccarini, personal communication) and in the sea urchin (M. Crippa, personal communication) mRNA may be stored in rapidly sedimenting heavy particles. The molecular hybridization technique is being applied in an attempt to determine how the mRNA that is synthesized during oogenesis is stockpiled and rendered untranslatable in the unfertilized egg.

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The Function of Polyribosomes Attached to Membranes in Animal Cells

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The role of the membrane-bound polyribosomes in the synthesis of protein for export from animal cells is well established. In addition to facilitating the passage of protein from the cell, the containment of the protein within the membrane of the endoplasmic reticulum has other advantages. Thus in the assembly of immunoglobulin G from light chains and heavy chains the controlled environment provided by the membranes is important for the interaction of the four chains (Williamson, 1969).

Brew (1969) has described the role of membranes in the synthesis of lactose by the mammary gland. Thus α -lactalbumin is synthesized by the membrane-bound polyribosomes, released to the cisternal space (Brew & Campbell, 1967) and passed to the Golgi apparatus, where *N*-acetyl-lactosamine synthetase is located (Coffey & Reithel, 1968; Fleischer, Fleischer & Ozawa, 1969). The α -lactalbumin changes the substrate specificity of this enzyme and lactose is synthesized (Brew, Vanaman & Hill, 1968).

There is a marked variation in the rate of metabolic turnover of the proteins of the endoplasmic reticulum (see e.g. Kuriyama, Omura, Siekevitz & Palade, 1969; Arias, Doyle & Schimke, 1969). In particular there is a differential effect on the activity