Electron-Microscopic Study of Polyribosomes Extracted from HeLa Cells

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Many data suggest that the rapidly labelled heterogeneously sedimenting RNA extracted from the polyribosomes of animal cells is packaged into ribonucleoprotein particles (Penman, Vesco & Penman, 1968; Perry & Kelley, 1968; Cartouzou, Attali & Lissitaky, 1968; Temmerman & Lebleu, 1969).

Our fractionation on columns of methylated albumin on kieselguhr of the RNA extracted from polyribosomes of HeLa cells has shown that they contain (or are contaminated by) as much as 50% of rapidly labelled heterogeneously sedimenting RNA (Miller, Dhont, De Vreese & Van Nimmen, 1968; A. O. A. Miller, S. Bram, A. Miller-Faures & L. Van Nimmen, unpublished work). If this large amount of non-ribosomal RNA is in the form of ribonucleoprotein particles, it should be detectable in electron micrographs.

The preparation of the cytoplasmic extract and its analysis by sucrose-density-gradient sedimentation has already been described in detail (Miller *et al.* 1968). The fractions of the sucrose density gradient corresponding to the peak distribution of polyribosomes (about five to eight ribosomes) contain, besides polyribosome clusters, which are easily recognizable, some membranous materials. These look very much like the membranes of the smooth vesicles described by Palade & Siekevitz (1956), and it is known from experiments made by others that this contamination by phospholipid-rich membranes is the rule rather than the exception (Murty & Halliman, 1969; Priestley, Pruyn & Malt, 1969; Robinson, 1969).

Fractions further down the tube appear to contain nothing other than ribosomal material, but in the bottom fractions numerous particles arranged in rosettes are found. These resemble in shape and size the glycogen particles found in animal cells and the polysaccharide particles observed in bacteria (Heuson-Stiennon & Drochmans, 1967; Thiéry, 1967).

An experiment to decide whether these particles were composed of glycogen was inconclusive. The particles from HeLa-cell fractions and from

Escherichia coli polyribosome fractions were not digested away by α -amylase, but neither were the glycogen particles isolated from HeLa cells by a method devised for their isolation and purification from rat liver (Heuson-Stiennon & Drochmans, 1967).

No particles other than the polyribosomes and the glycogen-like particles have been detected that might correspond to the messenger-containing particles (messenger ribonucleoprotein particles) whose existence has been repeatedly postulated. The following possibilities are suggested to explain our failure to detect such particles.

(1) The particles are preferentially lost or destroyed during preparation of the specimens for electron microscopy.

(2) The particles resemble ribosomes so closely that the two are indistinguishable in mixed or separate aggregates.

(3) They do not exist and the heterogeneous RNA is in the form of non-resolvable strands attached to the ribosomes (Plagemann, 1969). On dissociation of the polyribosomes with EDTA and after fixation with formaldehyde (Perry & Kelley, 1968) it might be unspecifically adsorbed on the smooth membranes that contaminate the polyribosomes and therefore band in caesium chloride density gradients at a buoyant density slightly higher than that of pure membranes.

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