Ribosomes

Ribosomes are cellular organelles serving for the protein synthesis. They consist of ribosomal ribonucleic acid (r-RNA) and proteins of cytoplasmatic origin. The subunits consisting of large and small particles are larger in eucaryote cells (80 S) than in procaryotes (70 S). For the functional ripening, Messenger ribonucleic acid (m-RNA) and transfer-ribonucleic acid (t-RNA) supplied from the nucleolus are necessary. The synthesis products of the ribosomes are polypeptide chains and proteins, which are secreted through vesicles into the inter-

spaces of the endoplasmatic reticulum.

The ribosomes originate probably from the nuclear membrane or from the nucleus, and perform their function either free or at the surface of the endoplasmatic reticulum in the cytoplasm.

Ribosome aggregates are called polysomes.

Historical data

After BRACHET's (1933) first assumptions that the ribonucleic acid (RNA) occurred chiefly in the cytoplasm, cytochemical methods (FEULGEN et al., 1937, BEHRENS, 1938) located the DNA in the nucleus and the RNA in the cytoplasm. Until then, it had been supposed that RNA was the nucleic acid of the plants, DNA that of the animal cells. BRACHET (1940, 1941) demonstrated significantly a correspondence between the protein-synthesis rate and the quantity of RNA in the cell. CLAUDE found by differential centrifugation (1938–1941) that «infectiosity» of the Rous-sarcoma was bound to small particles, which were traceable in the dark-field microscope and contained ribonucleoprotein and lipids. Only the electron-microscopical studies by PALADE (1955) demonstrated significantly the occurrence of these particles large about 200 Å in all tested cells and therewith the existence as real cell organelles. The terms «small granules» and «microsomes» used by CLAUDE were later changed for «ribosomes» , probably in consideration of the constituent of 50% RNA (PETERMAN, HAMILTON and MIZEN, 1954).

By tagging with amino-acid, BORSOOK (1950) found the highest concentration in the «microsomes» 30 minutes after the injection. LITTLEFIELD and KELLER furnished by different experiments (between 1955 and 1957) the conclusive proof that amino-acids are actually incorporated first into the ribosomes.

The statistical significance was established for the existence of ribosomes by various studies on bacteria. The ultracentrifuge provided fractions of particles of 40 S, 29 S and 5 S sedimentation coefficients. These spherical particles of the 40 S fraction contained 40% RNA. CHAO (1957) recognized the elementary significance of the Mg^{++} for the stability of the two ribosome elements with a constant of sedimentation of about 80 S. By the middle of the 1950s, definitive statistical significance (A. TISSIERES, 1974) was established for the existence of the spheric particles 200–300 Å in diameter, which consist about half of RNA and half of protein (i.e. of the ribosomes). After a meeting of the Biophysical Society, D. ROBERTS introduced the term «ribosome».

Structure of the ribosomes

The studies conducted on various species have not yet provided a uniform aspect, less so because the dehydrating processes in electron-microscopical preparations make it difficult to assess the circumstances in vivo. According to AMELUNXEN and SPIESS (1971) as well as NOMOMURA (1971), the smallest subunit is about 230 Å × 120 Å × 140 Å (X-ray model 55 Å × 220 Å × 220 Å in E. Coli),

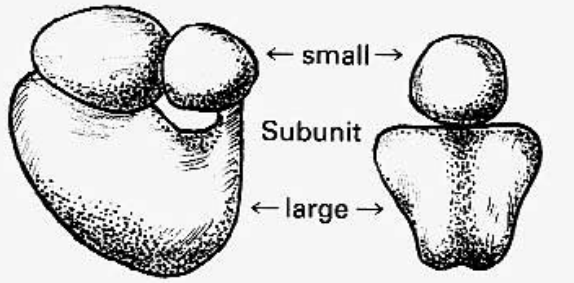

Fig. 30:
Model of *ribosome*

divided into two unequal segments by a notch of 40 Å in diameter. The «large subunit» has a diameter of about 230 Å (see fig. 30).

The best documentations of physicochemical findings on the structure and function were obtained with ribosomes of E. Coli (K. E. VAN HOLDE and W. E. HILL). Here, the 70 S-ribosomes consist of a 30 S-, a 50 S- subunit and Mg^{++}. The 70 S-particles contain quantities of Messenger RNA (mRNA), polypeptides and protein cofactors varying probably according to the functionary power of the primary cell. The unwashed 30 S subunits have a molecular weight of 1.0×10^6, those washed with NH_4CL or units precipitated with $(NH_4)\ 2SO_4$ a molecular weight of 0.90×10^6. Six proteins seem to be lost by washing (VOYNOW and KURLAND, 1971). The 16 S – RNA constituent (0.53 to 0.64×10^6) is estimated to make 64% in unwashed

Fig. 31:
Free ribosomes (r) and aggregated to endoplasmatic reticulum. M = mitochondria, N = nucleus. Immunocyte, 1:20,000.

30 S-particles and 71% in washed particles. 16 S-r RNA (= 564.000 daltons) correspond to 1650 nucleotides. 30 S-subunits are asymmetric, with a diameter nearly equal to the 50 S-particle, and highly hydrated.

The high amount of retained water makes it difficult to explain the findings obtained by the electron-microscope because the withdrawal of water causes the ribosome particles to shrink; lyophilisation seems to meet best the natural conditions.

50 S-subunit

The molecular weight of the unwashed 50 S-subunits comes to about 1.7×10^6, to 1.55×10^6 that of washed (NH4CL) or precipitated [(NH4)2 SO_4] 50 S-particles; the loss of mass does not reduce the sedimentation coefficient (K. E. VAN HOLDE and W. E. HILL, 1974). The molecular weight of the 23 S-r RNA was found to range from 1.0×10^6 to 1.1×10^6. The amount of RNA in unwashed 50 S-particles (subunits) is 65%, 71% in the washed; unwashed particles contain about 600.000 daltons of protein, the washed 450,000 daltons.

They seem to be more symmetrical than the 30 S-particles.

Small and wide angle X-ray powder diffraction studies of microcrystals of *Escherichia Coli* 70 S ribosomes revealed tetragonal centered macrocells of a = b = 43 nm and c = 52 nm with very strong reflections between 4 nm and 7 nm. This findings were confirmed by electron microscopy. The subcells were found by electron microscopy and X-ray diffraction to have orthogonal axes of a_s = 4.3 nm, b_s = 5.3 nm and c_s = 14.2 nm. (KUCKUK, E. D. 1982).

Ribosomes of eukaryotes have been studied less methodically but are apparently a little larger than those of prokaryotes.

Functionary associations

Ribosomes occur single *(monomeres)*, in groups *(polysomes)* and, obviously under unfavourable biological conditions, in aggregations of crystals (tetrameres, P422-crystals) (LAKE, SABATINI and NONOMURA, 1974).

Ribosomes seem to be capable of crystallising in various forms. Most of the studies have been conducted on ribosomes of chicken embryos treated with hypothermia (BYERS, 1967). The 166 S-particles in 5-day-old chicken embryos undercooled for 24 hours are ribosomes-tetrameres. Tetrameres are produced from ripe but inactive 80 S-ribosomes. The interribosomal binding seems to depend mainly on the concentration of ions, especially on the Mg^{++}-content of the medium. Tetrameres can deposit into laevorotatory or dextrorotary crystal-lattices of ribosomes. The three-dimensional structure of the monomeres as outlined in a model (fig. 30) from various techniques of investigation shows some criteria of the course of the function as far as they can be reconstructed from the lifeless artificial products of representation. A concise survey is given by fig. 32 (with legend).

In the association of polysomes, the Messenger RNA (m-RNA) cords, in conformity with biochemical findings, can be found among the subunits. The motility (rotation) of the ribosomes seems to be restricted by the connecting RNA cords if the cords traverse several ribosomes in the polysome unit. The diameter of the RNA cords of 15–30 Å suggests that secondary structures of RNA or protein-studded RNA are in question. Laterally, the RNA cord on either side of the ribosome can be followed to where the small and large subunits unite, frontally sometimes to the opaque spot at the boundary of the subunits. Here, probably, the entrance and exit of the ribosome are situated (fig. 30). At least 5 proteins (S4, S7, S8, S15, S20), in case even also S13, can be bound side-specifically to the 16 S-RNA cords of the 30 S-5 subunit of the ribosomes (KURLAND, 1974).

The synthetised polypeptide chains are probably conveyed through small channels in the 50 S subunits of the ribosomes and collected in vesicles (cistern-like bulges) of the endoplasmatic reticulum (fig. 32).

The *enzyme content* of the ribosomes is adapted to the high metabolic efficiency. Earlier enzyme analyses conducted on the «microsome fraction» substantiated the presence of the following enzymes (taking into account any possible impurities of the fraction): ribonuclease, amylase, dipeptidase, trypsin, catalase,

cytochrome C, co-enzyme A, ATP-ase, adenylic-acid-phosphatase, alkaline and acid phosphatase, lipase, arginase. The average content of the cytoplasm is exceeded by esterases, cytochrome-c-reductase, glucose-6 phosphate-phosphatase.

Vitamin B_2 and B_6 were identified in the microsome fraction (G. C. HIRSCH, 1955).

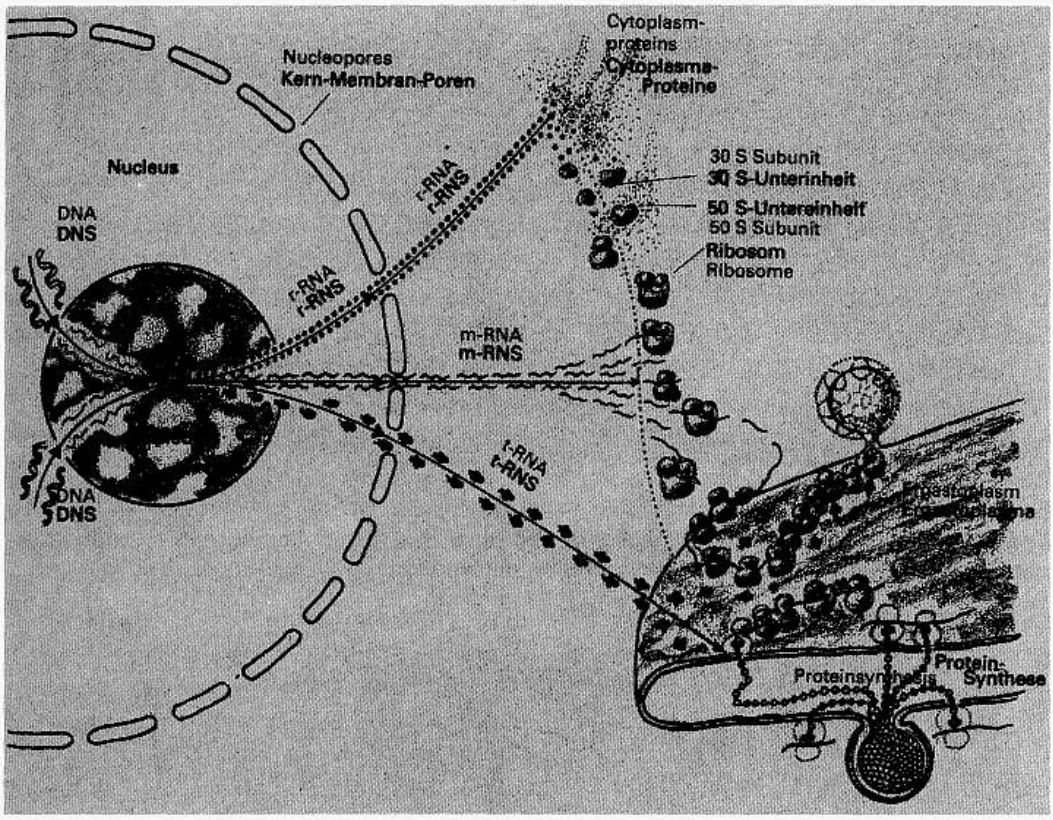

Fig. 32:
Functional cycle of the ribosomes (see text).
The DNA of the nucleus regulates via the intranucleolar DNA three different forms of ribonucleic acids of the nucleolus: r-RNA (ribosomal RNA), m-RNA (Messenger-RNA) and t-RNA (transfer RNA). The ribosomal RNA (r-RNA) gets through the nucleopores into the cytoplasm space and unites with cytoplasm proteins to form the ribosomes.
The Messenger-ribonucleic acid (m-RNA) penetrates through the nucleopores into the cytoplasm where it unites with the ribosomes, which temporarily are enabled to form polypeptide chains of a certain sequence.
The transfer ribonucleic acid (t-RNA) completes the functional unit so caused. The enzymes at the end of the trifoliate t-RNA molecules bind certain amino-acids, the genetic code of the m-RNA molecule is deciphered at the other end of the molecule. This assures the genetically fixed sequence of the amino-acids in the protein synthesis.
Ribosomes occur partly single in the cytoplasm (monomerics), partly – if highly efficient synthesis is necessary – in aggregates on the surface of the endoplasmatic reticulum (so-called polysomes), from where the synthetized proteins are evacuated into the interspaces of the reticulum. Examples of the electron-optical changes of the now following transportation processes are demonstrated in the immunoglobulin synthesis of fig. 31, 33, 34, 186–189.

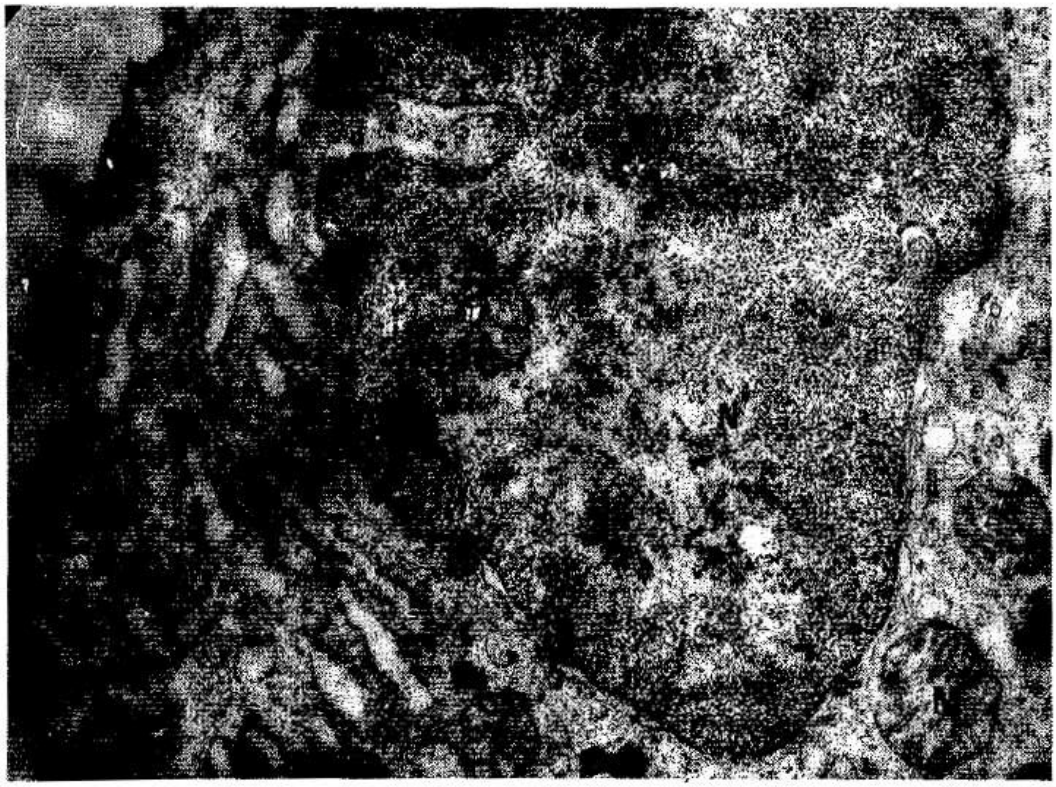

Fig. 33:
Endoplasmatic reticulum thickly studded with ribosomes in a mature immunocyte (peritoneal exudate, guinea-pig after BCG-immunisation). M = mitochondria, N = nucleus. 1:20,000.

Reconstruction of the ribosomes

The reconstitution of functional subunits of ribosomes is comparatively easy to conduct experimentally at 37° C within a few minutes (NOMURA and HELD, 1974). 16 S-RNA free from proteins (phenol extraction or precipitation of urea-LiCl) is mixed with 30 S-ribosome protein. The reconstitution is obtained with 20 mM Mg^{++} ions and with an optimum concentration of ions of 0.37. The 30 S-subunits reconstructed with 16 S-RNA and purified 30 S-ribosome proteins behave physically and functionally like natural ribosomes, only the binding of the S_1-protein seems to be weaker. Under these experimental conditions, only 7 of the ribosomal proteins bind primarily to the RNA cord, the rest follow secondarily.

Eukaryotes-ribosomes

Most of the experimental studies of ribosomes were conducted on prokaryotes. Eukaryotes-ribosomes are a little larger than prokaryotes-ribosomes and have about 80 S for the functional ribosome and 60 S or 40 S for the subunits (PETERMANN, 1964); they contain 3 (to 4?) molecules of RNA and somewhat more than 70 proteins. The small subunit consists of an 18 S-RNA cord and about 30 proteins having a total mass of 0.78×10^6 daltons. The RNA constituent comes to 45.5%.

The large subunit consists of a 28 S-, a 5 S-RNA molecule (1.7×10^6) and about 40 proteins (1.37×10^6 daltons); the total mass comes to about 3.0×10^6, the RNA constituent to about 59.4%. The growth of the mass of the eukaryotes-ribosomes is caused by the increase of the large subunit whereas the small subunit apparently does not partake of the evolution. Eukaryotes-ribosomes are not uniform and vary from 3.9×10^6 in plants to 4.55×10^6 daltons in mammals. In contrast to the prokaryotes-ribosomes, the reconstruction of eukaryotes-ribosomes from the subunits is difficult, presumably because sufficient quantities of 45S-RNA are not available.

Fig. 34:
Lamellae of endoplasmatic reticulum (r) thickly studded with *ribosomes in mature immunocyte* as the interlamellar interspaces are about to extend (= secretion of the synthetized proteins). 1:20,000.