Meetings

Ribonucleoproteins

Within the past year the amount of literature dealing with ribosome structure and function has increased so rapidly that the specialist receives each new issue of the *Journal of Molecular Biology* and the *Proceedings of the National Academy of Sciences* with mounting despair. Moreover, the worst is yet to come. To help increase the exchange of ideas and information among scientists active in this field, the Institute for Developmental Biology at the University of Colorado sponsored a conference on ribonucleoproteins. The conference was held on the Boulder campus 12–14 July 1967. The meeting was attended by approximately 80 persons, of whom 50 percent came from other states. Presentations were informal and no publication of the proceedings is planned. The conference was supported by funds from the National Institutes of Health and the University of Colorado.

No attempt was made to discuss all aspects of current research on ribonucleoproteins. The presentations emphasized ribosome assembly, and some problems dealing with ribosomal functions. Several types of RNA-protein complexes not involving ribosomal RNA (rRNA) were also described.

Relatively little is now known about the processes which lead to the association of ribosomal proteins (r-proteins) and ribosomal RNA's. Although it is generally conceded that the 18*S* and 28*S* RNA's of eukaryotes are synthesized in the nucleolus, no one has clearly shown either the site of synthesis of r-protein or the site(s) at which the various RNA's and proteins combine to form ribosomal subunits. One major obstacle to the search for ribosomal precursors has been the failure to break apart the nucleolus without completely dissociating RNA from protein. Recently, Jon Warner has obtained two major classes of particles from dissociated HeLa nucleoli. The larger, which sediments at 80*S*, contains most of the types of nucleolar RNA, and may be a mixture of several particles. Its proteins have not yet been analyzed. The smaller, which sediments at 55*S*, contains only 32*S* RNA. It is presumably a precursor of the cytoplasmic 50*S* subunit. Its proteins differ from those of the 50*S* subunit (derived from complete ribosomes) only in the absence of one band in the acrylamide gel patterns.

An interesting analogy can be drawn between the 55*S* particle from HeLa nucleoli and a 40*S* particle from *Escherichia coli,* described by Syozo Osawa. The latter is also a precursor of the 50*S* ribosomal subunit, which lacks several proteins that are found in the mature subunit.

In contrast, Robert Perry has observed that newly synthesized ribosomal subunits obtained from L-cell cytoplasm contain "extra" proteins, that is, proteins not found in the derived subunits. About 10 to 15 percent of the total protein of each subunit is in this category. The function of the extra proteins is unknown, as is their fate. Perry also notes that when polysomes are dissociated with ethylenediaminetetraacetate, the messenger RNA is released as a complex with protein. Whether this is the same as the "extra" protein found on new subunits has not been determined.

Syozo Osawa summarized the latest information on the assembly of *E. coli* ribosomes. By treating the bacteria with a low concentration of chloramphenicol (0.5 to 1.0 μg/ml), he has been able to produce increased concentrations of the normal ribosome precursors. It is now clear that there are at least two precursors of the 50*S* subunit (40*S* and 30*S*) and two precursors of the 30*S* subunit (26*S* and 22*S*). The 23*S* RNA found in the 40*S* and 30*S* precursor particles has 60 percent of the methyl groups found in mature 23*S* rRNA; the 17*S* RNA found in the 26*S* and 22*S* precursor particles has only 15 to 20 percent of the methyl groups found in mature 16*S* rRNA. There is no evidence for a common precursor of 16*S* and 23*S* RNA in bacteria. Studies are now being carried out on the proteins of the ribosome precursors.

An interesting by-product of Osawa's protein studies is the finding that CM-particles (immature "ribosomes" made in the presence of 40 to 50 μg of chloramphenicol per milliliter) have few proteins in common with normal ribosomes. CM-particles are probably not incomplete ribosomes, as previously thought, but an artifact. Indeed, Osawa cited evidence which suggests that they are fortuitous combinations of immature rRNA with proteins, formed during homogenization of the cells.

One prerequisite to almost any attempt to elucidate the functions of ribosomal proteins is the availability of large quantities of each protein free of significant contamination by the other ribosomal proteins. Since there are 30 to 40 major ribosomal proteins, effective separation has been a formidable technical obstacle. The technical problems have been mostly solved for *E. coli* ribosomal proteins in the laboratory of Charles Kurland, as reported by Simon Hardy at this conference. Using phosphocellulose columns, they have purified 18 out of 20 proteins from the 30*S* subunits, and nearly half of the 30 proteins from the 50*S* subunit. Each protein is at least 80 percent pure, as judged from acrylamide gel electropherograms. Peptide analyses have been performed by Gary Craven on several of these proteins, and each protein analyzed has been found to be unique. Osawa also reported excellent resolution of the proteins from *E. coli* ribosomes on carboxymethyl cellulose columns.

One of the most puzzling aspects of ribosome structure has been the basic fact that a ribosome consists of two subunits. Although it had seemed obvious to many persons that the subunits must separate at some time during ribosomal functioning, none of the known steps of protein synthesis required separation, and experiments designed to ask whether the subunits do separate had all been equivocal. Both questions have been settled by experiments reported at this conference.

By means of a transfer experiment, in which *E. coli* grown in the presence of the heavy isotopes N^{15} and C^{13} were shifted to medium containing N^{14} and

C^{12}, Ray Kaempfer has shown that subunit exchange definitely occurs. The heavy ribosomes, which were resolved from their light counterparts on sucrose gradients, sedimented at 86*S* instead of the usual 70*S*. After 3.5 generations of growth on light medium, all single ribosomes had become half-heavy. The 50*S* and 30*S* subunits were conserved.

Although Kaempfer's data clearly establish the existence of subunit exchange, they do not permit one to determine how often the subunits separate. Masayasu Nomura reported experiments which suggest that separation of the subunits must occur each time a ribosome completes a polypeptide chain, since formyl-methionyl-tRNA will bind only to 30*S* subunits. Binding of f-met-tRNA is inhibited by 50*S* and 70*S* particles. Valyl-tRNA, on the other hand, would not bind to 30*S* subunits in the presence of poly-AUG, but would bind if both 30*S* and 50*S* subunits were present.

Additional evidence consistent with the concept of subunit exchanges comes from David Schlessinger's laboratory. Using "fragile" strains of *E. coli*, which can be lysed almost instantaneously, Schlessinger and his co-workers have shown that the 70*S* ribosome is almost nonexistent in vivo. Instead, ribosomes occur either as components of polysomes or as free 50*S* and 30*S* subunits. This distribution would be expected if the initiation of polypeptide chains could only be effected by means of free 30*S* subunits, as Nomura reports.

Another approach to the analysis of ribosome function is exemplified by Nomura's experiments on reconstitution. Nomura has recently fractionated the "meniscus proteins" from *E. coli* ribosomes, that is, the proteins which are stripped from ribosomes by treatment with CsCl and which band at the top of the CsCl gradient after equilibrium centrifugation. He then recombines the meniscus proteins with the ribosomal cores, separately and in various combinations. Assays for biological activity of these partially reconstituted particles have already revealed much information about the function of some of the proteins. For example, the basic meniscus proteins from the 30*S* subunit are required for amino acid incorporation and for tRNA binding, but the acidic meniscus proteins are not. In contrast, the acidic meniscus proteins from the 50*S* subunits are required for those functions, but the basic meniscus proteins are not. Many other combinations have been tested, as Nomura reported. The method of reconstitution is an extremely powerful tool, which is certain to play an important role in future research on ribosome structure and function.

Genetic approaches to the study of ribosome function have not been neglected; but, as summarized by Joel Flasks, the efforts to find ribosome mutants in microorganisms have met with surprisingly little success.

Several other aspects of ribosome function were considered by David Apirion, Jesse Salb, and Kivie Moldave. Apirion summarized genetic studies on lincomycin- and erythromycin-sensitive mutants of *E. coli*, which apparently affect the 50*S* subunit of the ribosome. He suggested that tRNA binds at the site that is sensitive to these antibiotics. He also reported that ribosomes from cells grown anaerobically appear to bind lysine tRNA in a different manner from that of the controls. Salb compared the ribosomes of mitotic HeLa cells, interferon-treated cells, and microsomes from normal rat liver. All have a relatively low ability to support protein synthesis in vitro, and all can be activated by treatment with trypsin. The possibility that an extra protein on the ribosomes is responsible for the inhibition is strengthened by the observation that 50*S* subunits derived from interferon-treated cells show one more protein band than the controls, when analyzed by acrylamide gel electrophoresis.

Moldave has continued his studies on the effect of ribosomes on RNA transcription. Using a crude preparation of DNA with endogenous RNA polymerase prepared from lysates of *E. coli*, he finds that ribosomes will increase both the amount of RNA made in vitro, the length of the reaction, and the average size of the product. Some of the newly synthesized RNA is freed from the DNA template, and becomes attached to the ribosomes. Chloramphenicol does not alter these effects of ribosomes. Similar effects can be obtained with 30*S* subunits alone. It is therefore clear that protein synthesis is not required for the stimulation of transcription mediated by ribosomes. Experiments are now in progress to determine whether the initiation factors required for protein synthesis are also required in this system.

The 3rd day of the conference was primarily devoted to discussion of nonribosomal ribonucleoproteins. Ru Chih Huang presented her latest data on the RNA which she finds covalently bound to protein from chick embryo nuclei. Animo acid analysis shows that the protein which is bound to RNA is not a basic protein. The presence of β-alanine in the hydrolyzates suggests that the RNA is linked to the protein through dihydrouracil. Studies are now being done on the protein-bound RNA made by chromatin in vitro.

Dai Nakada described the recombination of MS2 protein with MS2 RNA. The normal virus contains 180 molecules of protein per molecule of RNA. When purified RNA has been combined with 6 to 20 molecules of viral protein in vitro, it will still serve as a template for protein synthesis, but the ability to code for noncoat proteins has been preferentially suppressed. This system will undoubtedly be of value in studying the control of translation.

Hewson Swift summarized information on various types of nuclear ribonucleoproteins that have been observed with the electron microscope. The size of the large particles seen in the Balbiani rings of dipteran polytene chromosomes, for example, implies that they are not ribosomes. In two anucleolate mutants—one in the frog, *Xenopus*, and one in maize—many particles larger than ribosomes can be seen. The amorphous masses that form in place of true nucleoli contain no granules, but they do contain RNA. These observations are a useful reminder that the nucleolus may be a more complicated organelle than many scientists currently envision it to be.

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Dosimetry: High-Energy Radiation Therapy

Almost two decades have passed since the initiation of the therapeutic use of high-energy radiations with both the high-energy x-rays and electrons from the betatron. Radiation dosimetry, including the distribution of dose produced by these radiations in tissue, is central to their preferred use in therapy. The significance of this development was reflected in the response to the symposium on high-energy radiation