Meetings

Nucleolus: Structure and Function

One of the most conspicuous structures in cells of higher animals and plants is the nucleolus. This organelle, because of its prominence and ubiquity, has long been an intriguing subject of study for those interested in biological structure-function relationships. Recently there has accumulated sufficient data concerning its fine structure, molecular composition, and metabolism to allow some insight into the role of the nucleolus in various cellular activities. Especially stimulating is its implication in the biosynthesis of ribosomes.

To summarize and correlate these facts and to achieve a continuity with historically developed concepts of the nucleolus, over 100 nucleolophiles gathered in Montevideo, Uruguay, 5–10 December 1965, at an international symposium entitled: The nucleolus—its structure and function. Among those attending were representatives of Argentina, Belgium, Brazil, Canada, Chile, France, Germany, Italy, Peru, Sweden, the United Kingdom, the United States, and Uruguay.

In the opening session, "Perspectives for the conference," J. Schultz (Institute for Cancer Research, Philadelphia) illustrated with numerous examples how, since the time of Montgomery, advances in our knowledge of the nucleolus have occurred in several bursts correlated with developments of particular methodologies in genetics, cytology, and biochemistry. W. Vincent (Pittsburgh) hurled a challenge to the conferees by pointing out several properties of starfish nucleoli that defy some of the currently proposed theories of nucleolar structure and function.

Clemente Estable (Montevideo), honorary president of the symposium, summarized the observations and criteria used to characterize the nucleolonema. Operationally defined as the filamentous component of the nucleolus, the nucleolonema is often observable with the light microscope in living or appropriately stained material, and can be related to a fibrillar network seen with the electron microscope. Its morphology varies according to the cell type and stage of the mitotic cycle, certain elements persisting throughout cell division. It was proposed that these persistent elements be identified with the nucleolar organizer. L. Chouinard (Laval) presented a correlated light and electron microscope study of the nucleolonema in plants, emphasizing in particular the occurrence of vacuoles during specific stages of the mitotic cycle.

Some general aspects of nucleolar fine structure, revealed by cytochemical techniques and electron microscopy, were discussed by H. Swift (Chicago) and W. Bernhard (Villejuif). The basic elements common to most nucleoli are: (i) fibrils, about 50 angstroms in diameter, which contain RNA; (ii) granules, which also contain RNA and are somewhat irregularly shaped and slightly smaller than the cytoplasmic ribosomes; and (iii) nucleolus-associated chromatin, portions of which penetrate deeply into the nucleolar body. Using high-resolution autoradiography, Bernhard and his colleagues observed that the incorporation of nucleoside into RNA occurs first in the fibrous and later in the granular portions of the nucleolus, thus suggesting a possible precursor-product relation between these components.

Swift pointed out that the granules associated with extranucleolar loci such as the puffed regions of salivary gland giant chromosomes are larger and less uniform in size than nucleolar granules, and thus are usually readily distinguishable in electron micrographs. Consideration of autoradiographic data on the rates of RNA synthesis attributable to nucleolar and extra-nucleolar elements has led to the idea that these two types of particles are produced independently of each other. Moreover, in *Maize* nuclei, which lack a nucleolar organizer, RNA-containing bodies accumulate which possess fibrous components but no well defined granular elements. Since these bodies do not occur in normal cells, an interaction between the nucleolar organizer and other chromosomal loci is also indicated. This concept was further elaborated on by C. Tandler (Buenos Aires) who argued that some of the material of the fibrillar zone represents products of other chromosomal loci fused together at the organizer during reconstruction of the nucleolus in telophase.

C. Pelling's (Tubingen) work on the giant chromosomes of *Chironomus* indicates that RNA is synthesized immediately adjacent to the nucleolar organizer and then migrates toward the outer nucleolar zones. Pelling reviewed some cytogenetic experiments of W. Beermann which demonstrated the complex nature of the organizer locus. After x-irradiation a mutant was obtained in which the organizer was split into two segments. Each was capable of producing a nucleolus.

The nucleus of a mature salamander oocyte contains several hundred peripherally located clusters of spherical nucleoli unattached to the chromosomes. Each nucleolus is composed of a fibrous core surrounded by a granular cortex. O. Miller (Oak Ridge) described how exposure to low molarity saline causes the granular components to disperse and the cores to expand into structures resembling loosely beaded necklaces. From the resultant alterations produced by nucleases and proteases it was concluded that the necklace is a strand of DNA, 30 angstroms in thickness, beaded with ribonucleoprotein. This structure is of particular interest in view of the finding by J. Gall (Yale) that a vigorous synthesis of the 45*S* precursor of ribosomal RNA occurs in these oocyte nucleoli.

In some differentiating systems one can observe ultrastructural changes in the association of chromosomal and other nucleolar material, although no complete detachment occurs. Such cases were described for root tips by B. Hyde (Vermont) and for insect spermatocytes by J. Sotelo (Montevideo). An exceptionally vivid account of the behavior of the nucleolar structures in living cells was given in motion pictures shown by Renate Lettré (Heidelberg) and Estable.

Several papers dealt with the effects of various chemical and viral agents on the nucleolar constituents. Bernhard and U. Stenram (Lund) outlined how segregation of fibrillar and granular

components or diminution of granular components can be experimentally produced with such compounds as actinomycin D and nitroquinoline-*N*-oxide. R. Love (Jefferson Medical) showed that these compounds produce characteristic alterations in nucleolini (toluidine blue–molybdate staining intranucleolar bodies). Ruth Kleinfeld (Syracuse) described how thioacetamide causes hypertrophy of liver nucleoli and increased nucleolar RNA synthesis, but does not lead to increases in cytoplasmic RNA. Recovery from the drug treatment is characterized by rapid increases in cytoplasmic RNA and restoration of normal nucleolar size and RNA content. A hypothesis that thioacetamide blocks a step in the conversion of ribosomal precursor RNA to ribosomal RNA was confirmed by biochemical data presented later in the symposium by H. Busch (Baylor). These effects were compared to those produced by other stimuli, such as regeneration and starvation.

Recently, the characterization of nucleolar genes has been investigated by DNA-RNA hybridization. By studying the saturation levels reached when a particular sample of denatured DNA is allowed to form duplexes with varying amounts of ribosomal RNA, one can obtain an estimate of the number of cistrons coding for the ribosomal RNA. Furthermore, with appropriate genetic material one can use this technique to locate the ribosomal cistrons on the genome. Such an approach formed the basis of two of the symposium papers.

M. Birnstiel (Edinburgh) demonstrated that, whereas the wild-type genome of the toad, *Xenopus laevis*, contains approximately 1600 cistrons complementary to 28*S* and 18*S* ribosomal RNA, the DNA of the anucleolate homozygous mutant contains virtually none, and that of the heterozygote, about 800. Such results strongly support the idea that all the information for the synthesis of ribosomal RNA is encoded in the nucleolar portion of the genome. By means of buoyant density fractionation in CsCl, Birnstiel and his associates isolated a minor discrete DNA satellite band, relatively rich in guanine-cytosine, which is peculiar to DNA from organisms containing nucleoli and is believed to represent the ribosomal cistrons.

A direct proportionality between dosages of nucleolus-organizer chromosome and number of ribosomal cistrons was reported for *Drosophila melanogaster* by F. Ritossa (Naples and Urbana). In this case it was estimated that an organizer possesses on the order of 130 linearly inserted cistrons for the two ribosomal RNA's. When a similar study was made with transfer RNA, for which there are about 1000 cistrons per haploid genome, no correlation with the nucleolus organizer was found. An even finer dissection of the complex organizer locus is being carried out with various bobbed mutants which appear to represent a series of ribosome deficiencies.

Considerable attention was given to the details of ribosome biosynthesis in diverse types of animal cells. It was generally agreed that the process begins with the synthesis in the nucleolus of large 45*S* precursor molecules. These are subsequently broken down to 18*S* components which are rapidly transferred to the cytoplasm, and to 30 to 35*S* components which remain for a longer time in the nucleolus. Eventually the 30 to 35*S* components are converted to 28*S* and also exported to the cytoplasm. This was shown for salamander oocytes by Gall, for Walker Tumor and rat liver by Busch, and for mammalian cell cultures by S. Penman (MIT) and R. Perry (Institute for Cancer Research, Philadelphia).

In cases where the probability of cytoplasmic contamination is low, such as the manually dissected oocyte nuclei described by Gall and the detergent purified nuclei of cultured cells used by Penman, very little or no 18*S* RNA is found to be present in the nucleus, thus suggesting that these types of nuclei do not contain any conventional ribosomes. This naturally led to concern as to whether there is any nuclear protein synthesis and, in particular, how ribosomal proteins are made. Although a system incorporating amino acids was derived from isolated nucleoli of guinea pig liver (Rachele Maggio, Palermo), it is not yet certain that in vivo there is any protein synthesis associated with the nucleolus. Vincent pointed out that when ribosomal and nucleolar proteins of starfish oocytes were compared by means of acid gel electrophoresis, there were more differences than similarities.

In spite of the fact that the problem of ribosomal protein synthesis was not resolved, it could be shown that the 18*S* and 28*S* ribosomal RNA's appear in the cytoplasm as 40*S* and 60*S* ribonucleoprotein particles which are believed to be the ribosome subunits. Perry demonstrated, however, that the newly formed particles are distinguishable from mature particles by virtue of possessing a lower buoyant density in cesium chloride.

Many of the incompletely resolved problems of ribosome biogenesis were discussed at length during a panel discussion by Birnstiel, D. Brown (Carnegie Institution of Washington), Gall, Penman, Perry, and Vincent. Among the topics discussed were whether a single 45*S* molecule contains both 18*S* and 28*S* components, the evidence for a pool of preformed ribosomal protein, and possible reasons why labeled 40*S* particles appear in the cytoplasm before the 60*S* particles.

The question of whether the nucleolus is a site of transfer RNA synthesis continued to be controversial, although most of the evidence seemed to indicate that it is not. P. Woods (Delaware) reported that differential extraction of labeled transfer RNA from *Vicia faba* meristems failed to alter the autoradiographically measured incorporation ascribable to the nucleolus, but significantly reduced the measured intensity of chromatin and cytoplasmic labeling. This was essentially in agreement with the previously cited hybridization evidence for the *Drosophila* system and some earlier work on mammalian cell cultures and *Xenopus*, summarized by Perry and H. Wallace (Amherst), respectively. On the other hand, Sirlin (Edinburgh) noted that the RNA synthesized by non-growing larval salivary glands of chironomids, which were treated with substituted benzimidazoles, is predominantly nucleolar. This RNA consists of a large proportion of 4*S* RNA, which contains ribothymidine and accepts methyl groups from methionine.

D. Comb (Harvard) and Brown showed that the nucleolus is also the site of synthesis of a species of 5*S* RNA which is capable of being methylated and is probably associated with the large ribosome subunit. This RNA, which is absent from the anucleolate mutants, can be distinguished from transfer RNA by chromatography on methylated albumin.

G. Siebert (Mainz and Baylor) gave an account of the various enzyme activities associated with isolated nucleoli. Especially striking was the marked nucleolar localization of RNA polymerase activity and the distinctive substrate specificities exhibited by nucleolar ribonuclease and ATPase.

One session concentrated on variations in nucleolar activities in the cell

cycle and during development. Brown summarized data from a variety of developmental systems in which early stages of embryogenesis are characterized by the absence of a nucleolus and lack of synthesis of ribosomal RNA. In these systems there appears to be a sufficient supply of maternal ribosomes to satisfy the early metabolic needs of the embryos. When nuclei from later stages are transplanted into unfertilized eggs, there is a disappearance of nucleoli and no further synthesis of ribosomal RNA until the embryo again reaches the later stage. Such experiments are believed to constitute evidence of a cytoplasmic control over the expression of the ribosomal cistrons. H. Barr (Wisconsin) speculated that this might be related to the much higher concentration of magnesium ions in unfertilized eggs as compared to the later stages. Adrienne Ficq (Brussels) described how the synthesis of cytoplasmic RNA, presumed to be predominantly ribosomal, occurs in the nucleolus of echinoderm oocytes prior to maturation and dissolution of the germinal vesicle.

Evidence that the nucleolus is active in RNA synthesis throughout most of interphase, with the possible exception of a brief period in early S phase, was provided by F. Kasten (Pasadena Foundation for Medical Research). N. Das (Berkeley) found that in certain organisms nucleoli continued to actively synthesize RNA well into mitotic or meiotic prophase.

In the closing session C. H. Waddington (Edinburgh) provided an incisive and much appreciated summary of the symposium. Swift presented the report of a special nomenclature committee who undertook the task of attempting to assimilate the totality of morphological and biochemical data into a unified terminology. Final remarks were made by A. Hollaender (Oak Ridge) and F. Saez (Montevideo), president and vice president of the symposium.

This conference was the fifth in a series of biological meetings sponsored by various Latin American institutions with the cooperation of the Biology Division of the Oak Ridge National Laboratory. Sponsors for the Montevideo meeting were the Departmental Council of Montevideo, National Council of Scientific and Technical Research of Uruguay, Organization of American States, United States Atomic Energy Commission, United States National Science Foundation, and the University of Pittsburgh. Proceedings of the conference, including a full transcript of the discussions, will be published as a monograph of the National Cancer Institute. It is intended that this volume, which is scheduled to appear before the end of the year, will serve as a valuable reference source and guide for present and future generations of nucleolophiles.

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Forthcoming Events

August

10–11. European Assoc. for **Animal Production**, study commissions, mtgs., Edinburgh, Scotland. (K. Kállay, Corso Trieste 67, Rome, Italy)

10–12. Applications of **X-ray Analysis**, 15th annual conf., Denver, Colo. (J. B. Newkirk, Metallurgy Div., Denver Research Inst., Univ. of Denver, Denver)

11–18. **Animal Production**, 9th intern. congr., Edinburgh, Scotland (Congress Secretary, 5 Hope Park Sq., Edinburgh 8)

14–17. **Cryobiology**, intern. conf., Sapporo, Japan. (Z. Yosida, Inst. of Low Temperature Science, Hokkaido Univ., Sapporo)

14–17. **Soil Conservation** Soc. of America, Albuquerque, N.M. (H. W. Pritchard, 7515 NE Ankeny Rd., Ankeny, Iowa)

14–18. Canadian **Pharmaceutical** Assoc., 59th conv., St. John, New Brunswick. (P. W. Bell, 175 College St., Toronto 2B, Ont.)

14–19. American Inst. of **Biological Sciences**, 17th annual, Univ. of Maryland, College Park. (AIBS, 3900 Wisconsin Ave., Washington, D.C.)

The following societies will meet in conjunction with the AIBS. Additional information is available from AIBS or from the program chairmen listed below.

American **Bryological** Soc. (W. B. Schofield, Dept. of Botany, Univ. of British Columbia, Vancouver, Canada)

American **Fern** Soc. (I. Knobloch, Dept. of Botany and Plant Pathology, Michigan State Univ., East Lansing)

American **Fisheries** Soc. (L. E. Cronin, Natural Resources Inst., Administration Bldg., Univ. of Maryland, College Park)

American **Genetic** Assoc. (S. Burhoe, American Univ. Graduate School, Washington, D.C.)

American **Microscopical** Soc. (R. M. Cable, Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.)

American Soc. for **Horticultural Science** (A. H. Thompson, Dept. of Horticulture, Univ. of Maryland, College Park)

American Soc. of **Plant Physiologists** (R. S. Loomis, Dept. of Agronomy, Univ. of California, Davis)

American Soc. of **Plant Taxonomists** (L. R. Heckard, Dept. of Botany, Univ. of California, Berkeley)