Meetings

Histone Biology and Chemistry

Recent advances in the biophysics and biochemistry of the nucleic acids have clearly shown that these are the molecules entrusted by nature with the task of storage and transfer of genetic information. Rapid developments in the status of the coding problem provide us with the promise that the day is near when we shall have full understanding of the genetic language. Attention is gradually being focused upon the mechanisms which control the transcription of the genetic message, and which, by the exertion of such control, bring about organized biological activity of the cell and orderly development of a single cell into a multicellular organism. What substances in the cell could be expected to serve in the control of genetic activity; to program the transcription and expression of the genetic language?

Chromosomal DNA of higher organisms, unlike that of bacteria, is not essentially free within the cell, but is usually bound in some degree with the histones, the biology and function of which have, until recently, remained obscure. Because of their quantity, their exclusive location in the nucleus, and their strong interaction with DNA, histones may play a key role in organizing DNA into the superstructure of the chromosome, and thus regulate the properties and function of DNA. To try and discover this role, to discuss and think about histones, not only from the standpoint of histone chemistry, but also from the standpoint of their interaction with DNA and their place within the framework of molecular biology, the first World Conference on Histone Biology and Chemistry was held 29 April to 2 May 1963 at Rancho Santa Fe near San Diego, California.

Histones as obtained from the nucleus are mixtures of many individual protein species. Calf thymus histone, for instance, contains at least two dozen separate entities, no one of which is, as yet, of assured homogeneity. Present evidence indicates the probable existence in thymus of a hundred, or a few hundred, chemically individual species of histone, a large number to be sure, but fewer by far than the number of genes in the calf genome. Plant histones are typically different from those of calf thymus, containing fewer components rich in arginine. Histones of different specialized cell types (including pathogenic cells) in the same organism can and do differ. There is a need for histone preparative procedures more gentle than the acid extraction ordinarily used, and for more effective procedures for separation of the constituent histone species (*Speakers*: K. Murray, E. W. Johns, D. M. P. Phillips, J. A. V. Butler, H. Busch, K. Iwai, L. Hnilica, J. M. Neelin, and H. S. Cruft).

Discussion on the structure of nucleohistone and its components by x-ray diffraction, infrared spectroscopy, deuterium exchange, and optical rotary dispersion indicates that histones as they are present in the nucleohistone complex possess about 50 percent of the maximum possible helical content. When dissolved in water in the absence of DNA, histones lose their helical conformation. The denatured histones can partially reform the helical structure if caused to form a surface film, or if transferred to a solvent such as ethylenechlorhydrin in which they are sparingly soluble. No firm proposal for the structure of the nucleohistone complex was presented. Among the variety of tentative suggestions made were: (i) histone molecules may be wrapped around one or the other of the grooves of the DNA molecule; (ii) the structure may be sheet-like with DNA molecules running in one direction and histone molecules at an acute angle to them, an angle appropriate to cause the histone molecules to match up every other large groove of the DNA molecules (this arrangement would help to make understandable the coiling of DNA in the chromosomal structure); and (iii) the histone molecules may be localized between pairs of DNA molecules. Other compounds, steroids (the sex hormones) and polycyclic aromatic hydrocarbons, were shown to be strongly bound by denatured, but not by native, DNA. RNA also has much less affinity for these materials. The important contribution of hydrophobic and stacking interactions of the bases in nucleic acids and in binding of these substances was stressed.

Stereoscopic electron microscopy has revealed an apparently uniform unit of the structure of interphase chromatin. This unit consists of a rod approximately 160 Å in diameter, which in turn consists of two DNA strands, each 20 Å in diameter, and wound together in a paranemically coiled helix. The histone molecules of the nucleohistone complex presumably occupy the space between and around the DNA strands (*Speakers*: G. Zubay, E. M. Bradbury, B. Richards, B. Hyde, N. Davidson, and P. O. P. Ts'o).

It has been demonstrated, with tissue culture of hamster cells, that histones are not partitioned semi-conservatively at mitosis as are DNA molecules. During the course of DNA replication, the individual histone molecule can apparently depart from one DNA molecule and reappear associated with a different one. There appears to be a rearrangement of the position of histones even in cells in which there is no DNA multiplication. In fact, although histone synthesis ordinarily accompanies DNA synthesis, the two are not necessarily coupled, since in the presence of 5-fluorodeoxyuridine which blocks DNA replication, histone synthesis proceeds unimpaired. From pulse-labeling experiments in vivo and incubation studies in vitro the seat of histones synthesis appears to be the nucleolus. The mechanism by which histone is transferred from the nucleolus to the chromosome is not known (*Speakers*: J. Schultz, D. Prescott, A. Dounce, G. Rudkin, H. Busch, G. Flamm, M. Birnstiel, and R. Vendrely).

Native nucleohistones obtained from chromatin have limited capacity to support DNA-dependent RNA synthesis in systems containing plant embryo chromosomal RNA polymerase, or purified *E. coli* RNA polymerase. Removal of histone from nucleohistone liberates DNA and thus supports RNA synthesis. Addition of purified histones to the system leads to inhibition of RNA synthesis, and to the formation of nucleohistone complexes which have

higher thermal stability than does free DNA. Similar results have been observed with the isolated thymus nuclear system as well as with the thymus chromosomal-RNA polymerase system. Methods are available for the preparation of soluble (10,000*g* non-sedimentable) nucleohistones (native or reconstituted from purified histone and DNA); inhibition of RNA synthesis by histone in amounts stoichiometric to the DNA is not due to precipitation of DNA from solution. In the RNA polymerase system of *Micrococcus lysodeikticus*, however, diamines or protamines, at low concentration (and in amounts substantially less than stoichiometric to the DNA present) enhance the activity of RNA polymerase; this occurs only with native DNA as template. The presence of the polycation may cause the removal of RNA from the complex of DNA template and enzyme. The RNA newly synthesized by native chromatin in vitro is informational and supports protein synthesis. The DNA-dependent RNA synthesis in chromatin may be coupled to the RNA-dependent ribosomal protein synthesis. Preliminary information suggests that a specific protein, seed globulin, may have been synthesized by such a coupling system. Interesting observations were reported on the action of histones and of the antibiotic actinomycin D on the activity and morphology of the lampbrush chromosomes of *Triturus*. Actinomycin D, which is highly and structurally specific in its action upon such chromosomes, causes the loops to retract and simultaneously abolishes RNA synthesis. The action of histones mimics that of the antibiotic (*Speakers*: E. Stedman, A. Mirsky, J. Bonner, L. Hnilica, V. Allfrey, S. Weiss, R. C. Huang, and I. Leslie).

The band pattern of the giant chromosomes of fly larvae appears to reflect the genetic structure of the chromosome; a single band comprises a single genetic unit. Swelling associated with RNA synthesis by such a band (the "puffing" phenomenon) is generally regarded as a symptom of gene activity. That specific sequences of band puffing are evoked by application of the moulting hormone, ecdysone (a sterol), to such chromosomes strengthens this view. Puffing, which is inhibited by actinomycin D, is accompanied by a loosening of the chromosome structure and by the appearance of non-histone protein derived from some unknown precursor in the chromo-

some. This precursor is not histone itself, because histone, qualitatively at least exists in the puffing region throughout the duration of the puff. Further suggestions that steroid hormones act by intervention at the level of control of genetic activity are provided by the cases of evocation by estrogens of RNA and protein synthesis in uterine tissue, by testosterone in the prostate gland, and by the flowering hormone, also presumably a sterol, in the bud, which is the subject of floral induction. In the latter case, too, a dramatic reduction of the ratio of histone to DNA accompanies the inductive action of the hormone, suggesting that steroids can in some manner bring about the removal of histone from chromatin. A principal difficulty in the study of the role of histones in morphogenetic events is the quantitative and specific determination of histone. Cytochemistry of histones is still unsatisfactory. Acid fixation removes an unknown amount of histone from the test material. Fast Green, the standard histone stain, stains basic proteins in addition to histones, such as ribosomal structural protein. Better histochemical methods for histones are needed (*Speakers*: U. Clever, H. Swift, J. A. D. Zeevaart, E. Gifford, D. Bloch, W. Vincent, and M. Zalokar).

Histones constitute a principal constituent of the chromosome, and are associated intimately with the chromosomal DNA. In this association the histone renders the DNA inert in RNA-making. The way in which histone carries out its function appears to require understanding of the structure of nucleohistones. If the histones constitute the repressor of genetic activity which participates in and is responsible for the programming of the transcription of genetic information during the course of development, a high degree of specificity would be required of the histone-DNA interaction. In the past, attention has been focused mainly on the ionic nature of this interaction. There are other possible types of interaction of a weaker, but more selective, nature. One could, for example, think of the possibility of specific adapter molecules. Such adapters, if present, might enable histones to form specific complexes with DNA of specific information content (*Speakers*: K. Murray, J. Vinograd, R. L. Sinsheimer, H. S. Swift, I. R. Lehman, and R. Dulbecco).

The conference was held under the auspices of the division of biology, California Institute of Technology, with the joint fiscal support of the National Science Foundation, the Office of Naval Research, and a special gift from H. Kirke Macomber. It was attended by 56 invited participants representing Canada, England, France, Germany, Japan, Scotland, Switzerland, and the United States.

The proceedings of the conference will be published by Holden-Day, Inc., in the later part of 1963.

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Molecular Structure and Spectroscopy

Molecular forces, interactions, and structure and new spectroscopic techniques were the main topics of interest at the 17th annual symposium on molecular structure and spectroscopy at Ohio State University, 10–14 June.

In a critique on nonbonded forces, E. B. Wilson, Jr. (Harvard), cautioned that most of the present attempts at determining such forces do not lead to an unambiguous interpretation. He presented the results of some microwave studies of the forces involved in barriers hindering internal rotation. One result is that in 1-substituted propylenes, $CH_3C{=}CHX$, the *cis*-form has a much lower barrier than the *trans*-form. This presumably is a result of interaction between the methyl group and the substituent X. The cases where X=F, Cl, CN, and CH_3 have been studied.

Some very interesting work on energy transfer was described by G. W. Robinson (C.I.T.) and his co-workers in several papers dealing with exciton interactions in organic crystals. A direct measurement of triplet exciton interactions and information about the lowest singlet were gained from studies of mixed crystals of benzene and deuterobenzenes. By using isotopic species, preservation of the lattice symmetry and uniform mixing was assured. By varying the amount of deuteration in the isotopic species used, the depth of the exciton trap could be changed in a known manner. It was calculated that approximately 10^{12} nearest-neighbor excitation transfers could occur during the lifetime of the triplet.

W. H. Flygare (Illinois) proposed a