width given in the preceding paper of this symposium by Doty, from whom the sample for electron microscopy was obtained. The average length is smaller than that obtained from light-scattering data, but this method gives weight to the longer particles and the number average can be expected to be smaller. The distribution of lengths may be regarded as a disturbing result; but if the constituent helices can slide over one another as Doty has suggested, single helices protruding as "tails" from the main body of the molecule would very probably be too small to be observed by the present technique.

The examples presented above demonstrate that by this improved method a new class of biologically important molecules can be observed directly with the electron microscope. Some improvement would be desirable, particularly in the direction of reducing the ultimate graininess of the shadowing metal. Otherwise, the principal difficulties encountered at present are those of obtaining the molecules suitably isolated from one another on the mica surface, undamaged by drying artifacts and free of contaminants.

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*PATTERNS OF INTERACTION OF BIOLOGICAL MACROMOLECULES IN RELATION TO CELL FUNCTION**

BY FRANCIS O. SCHMITT

DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS

In the first paper in this symposium series, Dr. Paul Doty[1] discussed some of the newer knowledge concerning the physical and chemical properties of proteins, nucleic acids, and other biological macromolecules in solution. It is now known that, as in the case of the fibrous materials with which Dr. Doty dealt, the component macromolecules of many types of biologically important substances are very long (several thousand Ångstrom units) and very thin (10–20 A). The individual

macromolecules may themselves be composed of one or more covalent strands which coil about each other in characteristic helical fashion. The stability and type of structure characterizing each macromolecule is determined very importantly by the manner in which the covalent chains interact with each other. When the thin, highly elongated macromolecules combine to form fibers, they do so in a highly specific fashion which is determined in part by the temperature and by factors such as the pH, ionic strength, and concentration of the macromolecules in the system in which fibrogenesis is occurring.

In the case of proteins, when a substantial fraction of the amino acid constituents possess long side chains, the lateral interaction between macromolecules may give rise to regions of relative order and disorder, depending on the way in which the side chains can adjust themselves to the fairly closely packed fibrous configuration. These regions of relative order and disorder occur at very precise locations along the fiber and reflect the linear distribution of the side chains or amino acid types which is generally supposed to be very constant and specific for each protein. As a result of this type of specific interaction of side chains, the fibrils which are formed by macromolecular interaction may appear banded in the electron microscope, the bands and interbands being regions in which the interacting side chains pack in a relatively disordered or ordered fashion, respectively. The axial period and the intraperiod band pattern are characteristic both of the macromolecules and of the conditions under which the fibril formation occurs.

In certain especially favorable cases, such as that of collagen, it is possible to prepare solutions of purified macromolecules and, by varying the composition of the solution, reversibly to pass from one band type to another, representing different modes of interaction of the macromolecules. From an analysis[2-4] of the fine structure of the band patterns shown in electron micrographs of the collagen fibrils it has been possible to deduce that the native structure is probably the result of aggregation of the collagen macromolecules ("tropocollagen") oriented all in the same direction (parallel array) but staggered with respect to macromolecular ends by specific fractions of macromolecular lengths. Under other conditions, particularly of high ionic strength, there may be no regularity of packing of the macromolecules laterally, hence no banding in the reconstituted fibrils or tactoids. Under still other conditions, particularly after the addition of small amounts of highly charged materials (such as acid glycoproteins, chondroitin sulfate, etc.), the macromolecules may array themselves in antiparallel array (with ends approximately in register), producing the so-called "fibrous long-spacing" pattern. Under still other conditions the macromolecules may aggregate in parallel array but with ends in register; this leads to the formation of the so-called "segment long spacing."

It has not yet been possible to determine which specific side chains are involved in the formation of the various bands and interbands seen in native and reconstituted collagen fibrils, although it may eventually prove possible to obtain information on this subject by the use of reagents which combine specifically with certain of the side chains. Some very preliminary evidence suggests that when ATP combines with tropocollagen to form segment long spacings, the combination occurs primarily with the terminal groups of the lysine residues.

It seems highly probable that similar factors of macromolecular specificity and of environmental conditions are involved in the formation of other types of fibrils

which appear banded in the electron microscope. Among these are the paramyosin fibrils of molluscan adductor muscles, the fibrin fibrils formed from soluble fibrinogen molecules in the process of blood clotting and in the myofibrils of striated muscle.

If the molecular machinery of cells, at least so far as the fibrous structures are concerned, is built of such elongated macromolecules having reactive side chains upon them, we may well expect that alterations of interaction patterns, induced by the presence of specific substances (such as ATP) or by change of the chemical environment, may well cause the alterations characteristic of tissue function (such as muscle contraction, ciliary motion, cell division, and so on).

Although it may be possible from electron optically resolvable band patterns to deduce something about the internal architecture and mode of interaction of the macromolecules which compose the fibrils, failure of any particular type of fibril to manifest a band pattern by no means signifies that such fibrils are not composed of long, thin macromolecules and that the latter may be in very specific array. If the type of fibril in question does not happen to possess side chains of such size and chemical properties as to produce regions of relative disorder when they interact with neighboring macromolecules, no bands may be seen in the electron microscope. Deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) are examples of such fiber types. They show no axial repeating band structure, but there is every reason to believe that these macromolecules interact with each other and with other types of macromolecules in highly specific fashion, particularly in the case of chromosomes. In no cellular constituent is specificity of structure, orientation, and chemical properties more significant than in chromosomes.

It has recently been suggested[4, 5] that application to the chromosome problem of the type of analysis, very briefly described above for the fibrous protein macromolecules, may lead to new and profitable ideas of the nature of the gene. The argument runs briefly as follows. It is known that when chromosomes are extracted under very mild conditions, the native DNA macromolecules may be extracted, isolated, and characterized by physical-chemical methods, as Dr. Doty has described. Such isolated DNA macromolecules are very long (contour length of about 30,000 A) and thin (20 A). In the chromosome these DNA macromolecules (together with some RNA) interact with certain as yet poorly defined fibrous protein macromolecules and with histones and protamines.[6, 7] The giant chromosomes of the salivary glands of Dipteran insects manifest characteristic band patterns when observed with the light microscope after appropriate staining or when examined in ultraviolet light. This band pattern is aperiodic; there is no true repeat over the length of the entire chromosome (over 100 μ). The positions of various genes have been localized with respect to these microscopically visible bands in genetic (linkage) maps. The bands and interbands, which have highly characteristic positions, densities, dimensions, and staining characteristics, represent regions of relatively high and low DNA-protein ratios, respectively. They must be explicable in terms of specific backing of elongated DNA and protein macromolecules.

The bonding between the chromosomal macromolecules must be relatively weak, since the constituents can be extracted by relatively mild treatment, such as elevation of ionic strength and presence of substances known to break hydrogen bonds.[8] It may not be unreasonable, therefore, to suppose that the characteristic band pattern

of these giant chromosomes may have been formed by a highly specific interaction of a finite number of "species" of DNA and protein macromolecules. Here "species" is used to indicate types of macromolecules which may be so similar chemically as to be difficult to distinguish by the methods of physical chemistry but which nevertheless have specific structural differences. Thus, by interaction of the various species of DNA and protein macromolecules in parallel and antiparallel array, with ends in register or staggered by specific amounts, as modulated by the presence of specific types of substances, it is possible that aperiodic patterns of even such enormous lengths might be produced.

On such a theory, it is in the native macromolecules, representing the various species of DNA and of protein, that the chemical and biological specificity primarily resides; the types of band patterns which will be produced when these constituents interact will depend, in turn, upon the chemical nature of the environment at the time. Given the synthesis of the various species of macromolecules, their aggregation in the fashion characteristic of the native chromosomes may occur spontaneously if the chemical nature of the environment is appropriate.

Still another deduction which follows from this hypothesis is that the type of interaction between adjacent macromolecules (DNA-DNA, DNA-protein, or protein-protein) may condition the type of gene activity possible under each particular circumstance. Thus chemical groupings corresponding to genes may occur in juxtaposition in two or more adjacent macromolecules which may be of the same or of different species of DNA or protein. With the same population of macromolecules a wide variety of bicatalyzers or genes may be capable of "activation" or "inactivation." Slight changes in the chemical environment of the chromosomes may, on this hypothesis, cause a shift of macromolecular types with respect to each other, with consequent activation or inactivation of catalytic groups or genes without essential alteration of the constituent macromolecules themselves. It is attractive to suppose that changes in the chromosomal environment occurring during differentiation and morphogenesis may produce just such changed interaction of the macromolecules, providing the feedback type of mechanism which must somehow be involved in the highly regulatory processes of growth and development.

To attempt to put some of these speculations to experimental test, Dr. Herman W. Lewis and the author have made a preliminary study[9] of the reaction properties of the giant chromosomes of *Drosophila* when exposed to certain changes in the chemical environment. These experiments will be described in more detail elsewhere, but the essential experimental procedure was as follows: Each salivary gland was divided in two. Both halves were placed in a test solution for a specified length of time, after which one of the halves was fixed in acetocarmine, squashed, and prepared for chromosome examination. At the same time, the other half was returned to *Drosophila* Ringer solution for a specified period of time, after which it too was fixed in acetocarmine and prepared for examination of the chromosomes. The purpose of the test solution was to attempt, under relatively mild conditions, to dissociate the DNA macromolecules from their neighbors sufficiently to permit them to shift axially to such a degree as to result in appreciable alteration or complete loss of band structure. This was accomplished by using solutions at various ionic strengths of salts such as NaCl, NaI, and NH_4I, as well as solutions known to break hydrogen bonds, such as guanidine hydrochloride and urea. By varying the

length of time to which the chromosomes were exposed to these test solutions, it was possible to vary the degree of axial displacement of the DNA constituents and thus to avoid obviously irreversible changes. Photomicrographs were taken with phase-contrast and with bright-field illumination.

Under the most favorable circumstances it was possible to disrupt the band structure fairly completely by exposure to the test solution and then, by restoring the gland to *Drosophila* Ringer solution, to achieve a considerable degree of restoration of band structure. Whether this "reconstituted" structure is of the native or an artificial type is not yet known. So far as they go, these results are compatible with the macromolecular hypothesis of the chromosome structure described above. However, much remains to be done before any definitive conclusions can be reached. With the type of experiment described above, one attempts, after an exhaustive microscopic study of the entire preparation, to photograph what seems to be a type of band pattern characteristic of most of the cells in that particular preparation. Since in few cases are the chromosomes of all the cells of a given preparation similarly affected, this procedure is subject to errors of judgment of the observer. Methods are currently being developed by which it may be possible to photograph the chromosomes of individual cells before, during, and after exposure to the test solution. It may thus become possible to arrive at clear-cut conclusions as to whether or not the DNA and protein macromolecules did, in fact, shift reversibly with respect to each other in the intact chromosome as a result of exposure to the test solution. Efforts will also be made to produce light optically resolvable banded structures by appropriate chemical manipulation of extracted and purified DNA and protein macromolecules in vitro, along lines similar to those by which the type of interaction between collagen macromolecules can be experimentally altered, as has been demonstrated by the electron microscope studies mentioned above. Progress in these experiments will be reported in due course. Eventually, when advances in electron microscope techniques such as that described in this symposium series by Hall[10] permit, it may be possible to demonstrate specific types of interaction of individual thin, elongated macromolecules in pairs or small clusters without the necessity of dealing with entire fibrils and their band patterns, from which to deduce macromolecular interaction types.

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