THE BIOLOGICAL BASIS OF MEDICINE

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Preface

This work in six volumes aims at providing a balanced treatment between contemporary medical science and the applications of cellular biology in medicine. Contributions by more than 100 investigators, including zoologists, biochemists, physiologists, pharmacologists, geneticists, cytologists, pathologists and clinical investigators, serve to illustrate the increasing importance of the interdisciplinary approach. Volume I contains sections on the dynamic state of the cell, growth, cell injury and ageing. Volume 2 covers material on hormones, the control of intracellular processes and the effects of both biological and physical agents. The remaining 4 volumes deal with major biological phenomena such as atherosclerosis and hypertension, cancer and immunology of tissue transplantation, as well as with the ultrastructure, chemistry and pathophysiology of individual organ cells and subcellular organelles.

It is now generally appreciated that cell biology occupies not only a key position among the biological sciences, but also serves as the meeting-ground for the physiologist, biochemist, cytologist, pathologist and others. One of the underlying intentions of the present work is to demonstrate afresh that cell biology offers the basis upon which life in health and disease can be properly understood. However daring it may seem, our belief is that ultimately cellular biology will be the discipline unifying the basic medical sciences. One hopes both students and teachers of medicine and human biology at all levels will find these volumes stimulating and of unique value.

Our indebtedness to those who have written the six volumes goes without saying. We should also like to thank the staff of Academic Press for their unfailing efficiency and courtesy.

E. EDWARD BITTAR

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PART I

Molecular Genetics

A

CHAPTER 1

Molecular Organization of Chromosomes

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I. Introduction

The subject-matter of this chapter, the molecular structure of chromosomes, may be disposed of very simply with the admission that almost nothing is known of the molecular structure of this cell organelle. In spite of intensive work in this area, from approaches including microbial genetics, purely physical techniques such as X-ray diffraction, and the more morphological studies with the light and electron microscopes, very little of a definite nature has been added to the general picture of chromosome structure as it had developed mainly as a result of light microscopy in the early part of this century. This initial statement must be qualified, it is true, with respect to the molecular basis of heredity, which is now known to reside in the linear sequence of bases in the DNA double helix. The probable structure of DNA, derived from X-ray diffraction and other physical analyses (for a survey of this subject see Peacocke, 1960), is well known and will not be considered in detail here.

Once past this level the structure of the chromosome is less clearly understood. In some manner, as yet unknown, the group of proteins classified as "histones" is bound to the DNA in the chromosome, along with

RNA, and other proteins, variously termed "acidic", "insoluble", "neutral", or "residual" proteins. The precise binding of these additional components to the DNA and the molecular interactions of these components in replication, RNA synthesis, recombination, and coiling would properly constitute the "molecular structure" of the chromosome. These interactions lie in a scale of dimensions delimited at one end by physical studies of the DNA molecule, and at the other by the light microscopy of whole chromosomes. This scale of dimensions lies precisely with the resolving power of the electron microscope. It is no surprise, therefore, that much of the following discussion will deal with the results of electron microscopy of chromosomes.

Certain aspects of the subject will of necessity be omitted. The prokaryote (Ris and Chandler, 1963) chromosome will not be considered, nor will the general question of "strandedness" be given more than a brief review. Primary emphasis will be placed on the structure of the chromosomes of higher organisms, on a level as close to the molecular as possible. To this end, a survey will be made of the results of both thin sectioning and isolation of chromosomes for studies by electron microscopy.

II. The Structure of Thin Sectioned Nuclei

The appearance of nuclei in the electron microscope after fixation and thin sectioning is one of a more or less evenly distributed array of dots or short lengths of fibers, approximately 100 Å or slightly less in diameter, which represent aggregations of the electron stain or fixative which have been bound or deposited in a linear array. The appearance of these fibers in the electron microscope is much the same, whether osmium tetroxide is used as the sole fixative or is used as a postfixative after primary fixation with an aldehyde. Potassium permanganate, although providing a somewhat less dense distribution of fibers in the nucleus, leads to essentially the same result.

These short fibers visible in thin sections represent the basic macromolecular complex of the chromosome, or at least reflect an interaction of some part of the chromosome complex with heavy metals. For a time the identity of these fibrils with the chromosome unit fiber was disputed, because the early studies of Bahr (1954) indicated that OsO_4 does not interact with DNA. However, further evidence, largely circumstantial, has led to general acceptance of these fibers as representing the basic structural fiber of the chromosome.

With the exception of the nucleolus, which can be identified as a dense collection of granules in an irregularly outlined mass, no structures other than the thin fibers are regularly found in thin sectioned nuclei. In most cases the thin fibrils are distributed evenly throughout the nucleus, with no localized clumps or aggregations which can be distinguished. In nuclei from some sources, however, aggregations of the fibers occur which are more dense in certain regions of the nucleus (see, for example, Huxley and Zubay, 1961; Marinozzi, 1963). This differentiation into tightly coiled and more thinly dispersed areas appears to be enhanced or revealed by aldehyde fixation (Marinozzi, 1963). A granular component is often visible in these nuclei, dispersed among the thin fibers in the less densely aggregated areas of the nucleus. In the more condensed regions no structures are visible except thin fibers.

These observations have led to a number of attempts to characterize the fibers in the densely clumped regions by cytochemical means. Using autoradiography of thin sections, Moses and Meek (1962) and Hay and Revel (1963) were able to show that incorporation of tritiated thymidine occurred in these areas during interphase. These areas in thin sections correspond to regions which are stained intensely by the Feulgen reaction in adjacent thick sections for light microscopy (Moses, 1960; Huxley and Zubay, 1961). Digestion with deoxyribonuclease results in disappearance of the thin fibrils from thin sections and concomitant loss of Feulgen staining in adjacent thick sections (Dales, 1960).

These studies show that DNA is localized in quantity in areas which contain dense aggregations of thin fibrils in thin sectioned nuclei, and to this extent equate the thin fibers with the chromosome. However, it is not likely that all of the thin fibrils of the nucleus represent chromosome fibers. In an interesting study, Moses (1960) fixed nuclei in prophase of mitosis and made adjacent thick and thin sections of this material. Thick sections stained by the Feulgen technique showed prometaphase chromosomes clearly in the light microscope; adjacent thin sections showed an evenly distributed array of fibrils with no distinction between chromosomal and interchromosomal areas. Obviously, in this case, fibrils representing macromolecules of the "nuclear sap" could not be distinguished from chromosome fibers.

The identity of the fibers in thin sectioned nuclei could be more directly settled if specific stains for DNA were available to the electron microscopist. Unfortunately, there are no techniques available which can be described as more than selective for nucleic acids. The most successful of these, the indium trichloride method of Watson and Aldridge (1961), increases the contrast of the thin fibrils of the nucleus. Staining with the uranyl ion, which under certain conditions binds selectively to nucleic acids (Wolfe *et al.*, 1962), also results in the production of greater density in the thin fibrils of the nucleus. At best, these methods merely complement the results obtained by indirect means which show that the thin fibers of the nucleus in some way represent the structure of the chromosome.

A. *The diameter and substructure of the chromosome fibers in thin sections*

The basic fibers seen in thin sections are indefinite in outline and have been reported as varying in diameter from 20–30 Å expected for the DNA-histone complex (Zubay and Doty, 1959) to as much as 500 Å (for recent reviews see Kaufmann *et al.*, 1960; Ris, 1962; Hyde, 1965). The great majority of investigators describe the average diameter of these fibers as 100 Å or slightly less (Chardard, 1960; Gay, 1963*a*; Maggio *et al.*, 1963; Moses, 1960, 1964; Hyde, 1965; Sadowski and Steiner, 1968). In one of the few cases in which a statistical approach has been used in an estimation of the diameter of the fibers in thin sectioned nuclei, Kaye and McMaster-Kaye (1966) found the fiber diameter in meiotic cells of the house cricket to average 80–85 Å, with a standard error of 1·6–2·6 Å. The fibrillar structure in micrographs of thin sectioned nuclei presented by these authors is especially clear (Fig. 1). On the basis of the statistical analysis

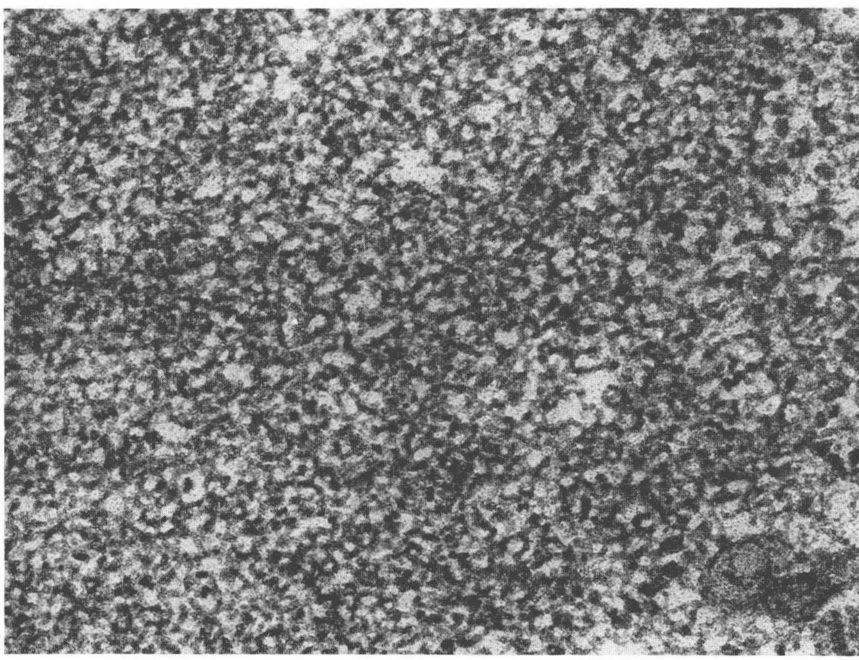

FIG. 1. Thin sectioned meiotic chromosome of the house cricket, *Acheta domestica*. Fibers in this chromosome average 80–85 Å in diameter. ×98 000. (Courtesy of Jerome S. Kaye.)

carried out by Kaye and McMaster-Kaye, the unit chromosome fiber should probably be described as the "80 Å fiber". However, since most of the current literature refers to the unit fiber as the "100 Å" fiber, the latter dimension will be used for convenience here.

If it is accepted that the 100 Å fiber is a basic unit of the chromosomes of higher organisms, an immediate problem is presented in relating this entity to the DNA-histone complex, which is expected to be of smaller dimensions, about 30 Å (Zubay and Doty, 1959). Since DNA has not been shown to interact with osmium, the density observed in the 100 Å fibers may represent an interaction of the osmium with residues on the proteins associated with the DNA molecule rather than with the DNA itself. In this case, the unexpectedly wide diameter of 100 Å could result from staining of a matrix of protein surrounding a DNA core. The DNA within the matrix could be extended, without further coiling of the DNA helix, or could be secondarily coiled within the limits of the 100 Å matrix. On the other hand, the 100 Å fiber could represent a super coil of a single DNA molecule complexed with protein, or two or more such complexes extended in parallel array or twisted together, with no sheath or matrix necessarily associated with the complex.

It is interesting that it has not been possible to distinguish between these possibilities with the electron microscope, even though all of the macromolecules expected to be present within the 100 Å fiber have dimensions which lie within the resolving power of this instrument. A number of observations exist of substructure within the 100 Å fiber (Amano *et al.*, 1956; Bopp-Hassenkamp, 1959; Nebel, 1959; Goodman and Spiro, 1962; Ris, 1962; Hyde, 1964; Wettstein and Sotelo, 1965). Fiber substructure has been described in these reports as consisting of single or multiple subunits which are parallel, twisted, or spiraled. In a few instances, the 100 Å fiber is represented as a part of a series of coils or spirals forming a hierarchy which extends from 30 Å to 500 and even 1000 Å or more (Bopp-Hassenkamp, 1959; Nebel, 1959). Hierarchies of this type might be expected to occur in metaphase chromosomes, since a high degree of order of coiling can be seen in the light microscope in the chromosomes of some organisms (see, for example, Taylor, 1958*a*). Although excellent preparations for electron microscopy have been made of chromosomes at metaphase (Chardard, 1960; Albersheim and Killias, 1963; Barnicot and Huxley, 1965), no order is discernible in the folding or coiling of the 100 Å fiber. While the possibility exists that this appearance results from poor fixation, the lack of order seen in the pattern of folding of the basic fiber does not support any scheme which postulates a highly ordered system of gyres to be present.

Interaction of underfocus diffraction images with the electron image of the 100 Å fiber must be kept in mind as a possible source of confusion in interpreting the substructure of the 100 Å fiber. This source is suspect in cases in which the fiber is seen to contain two parallel subunits which run side by side for long distances without twisting. It seems unlikely, given the highly irregular pattern of coiling or folding observed, that two precisely parallel and equidistant subunits could be visible for any distance along the length of the 100 Å fiber. Pronounced underfocus grain in the image, with a periodicity of 20–30 Å can give the impression that twisted 30 Å subunits exist in the 100 Å fiber. The importance of through-focal series cannot be overemphasized as a method for eliminating confusion resulting from this source. Some idea of the difficulties inherent in working at this level can be appreciated by the fact that Ris, perhaps the strongest proponent of substructure within the 100 Å fiber, has recently determined that his earlier observations of two 40 Å subunits within the 100 Å fiber are erroneous (Ris, 1967).

The work of Kaye and McMaster-Kaye (1966) on meiotic cells of the house cricket presents a detailed analysis of fiber diameter which has direct bearing on the question of fiber substructure. Fibers 80–85 Å were found by these authors to exist in chromosomes in this organism following a statistical analysis of measurements of nuclear fibers at various stages of meiosis and spermiogenesis. Although fibers smaller than 80 Å were visible, the range of diameter noted had a unimodal distribution, with no indication of the presence of a discrete class of smaller fibers. An increase in diameter to 110–170 Å, found to occur during spermiogenesis, was gradual, and thus failed to support the interpretation that side by side aggregation of smaller unit fibers was responsible for production of the larger diameter.

Fibers approximating 100 Å have also been found in thin sections of isolated nuclei (Maggio *et al.*, 1963; Sadowski and Steiner, 1968) or chromatin fractions (Frenster *et al.*, 1963), although thinner fibers have been additionally reported in such preparations (Crawley and Harris, 1963; Birnstiel and Hyde, 1963; Maggio *et al.*, 1963). It has been possible to distinguish between "active" and "inactive" chromatin on the basis of incorporation of RNA precursors in both intact and isolated nuclei or chromatin fractions (Frenster *et al.*, 1963; Littau *et al.*, 1964; Frenster, 1965). Active chromatin has been shown to correspond to less densely aggregated regions of the nucleus, and inactive chromatin to the densely clumped areas. Both regions have been shown to contain 100 Å fibers (Frenster, 1965), although there is some indication that "active" regions may contain fibers that are regularly thinner than 100 Å (Littau *et al.*, 1964).

The occurrence of thinner fibers in regions of the chromosome active in RNA synthesis or DNA replication is to be expected on intuitive grounds, since it is probable that DNA must separate or unwind from the double helical configuration, or dissociate from bound proteins for these activities to occur. In an attempt to utilize regions of the nucleus known to be active in DNA synthesis to investigate this possibility, a number of investigators (Phegan and Moses, 1967; Ringertz *et al.*, 1967) have turned to the ciliate protozoan *Euplotes*. In this organism, distinct bands of replication pass along the length of the elongated macronucleus. These regions can be readily identified in Feulgen-stained thick sections, and in adjacent thin sections for electron microscopy. In thin sections of these regions, the chromatin of the macronucleus can be seen to disperse into fine fibrils which are far less than 100 Å in diameter (Phegan and Moses, 1967) or to "disappear" (Ringertz *et al.*, 1967). With the exception of the interband regions of the synaptinemal complex (to be discussed below), these observations appear to be the only case in which very thin fibers, significantly less than 80–100 Å, regularly occur. These observations in *Euplotes* can be taken in support of the contention that the structural configuration of DNA and associated macromolecules, represented by the 100 Å fiber, dissociates or unwinds before replication.