REVIEWS OF MODERN PHYSICS VOLUME 31, NUMBER 2 APRIL, 1959

36
Fine Structure of Cell Nucleus, Chromosomes, Nucleoli, and Membrane

H. STANLEY BENNETT

Department of Anatomy, University of Washington School of Medicine, Seattle 5, Washington

THE most important concepts of the structure of the nucleus are based on information drawn from three rather separate approaches which are now converging. These approaches had their origins in the nineteenth century. The first of these—the optical approach—had its origin with Robert Brown's first recognition of the nucleus by the use of the light microscope in 1833. This distinguished botanist was also the discoverer of what is now called Brownian movement. The amplification of his original observation, as carried out with various optical tools, forms an exceedingly important part of present knowledge of the nucleus.

The second stream of knowledge is that of genetics. It can be regarded as beginning with Mendel's observation that inherited characteristics are transmitted from generation to generation in ratios of simple whole numbers. During the early part of the present century, derivatives of this discovery, correlated with optical observations on the nucleus, led to the field of cytogenetics. Subsequently, the information derived from genetic sources has played an essential part in concepts of nuclear structure. Indeed, the genetic data provide the only evidence regarding certain important structural features of the nucleus.

One can trace the third stream, the chemical one, to the work of Miescher, who published an important compendium in 1897 on the chemical characteristics of extracts derived from pus. Lamentably, this raw material was abundantly available in those days, before aseptic surgery had become popular. From this convenient product of human suffering, Miescher extracted substances which he named nucleic acids.

One can ask if the nucleus is an essential part of the cell. One can recall the red cell of the mammal, which is active for about 120 days without a nucleus. But how do other cells fare if the nucleus is removed? This has been investigated in a relatively small number of forms. It is possible to remove the nucleus from an amoeba by surgical means, as Mazia, for example, has done. It is possible also to amputate and study non-nucleated fragments of certain large algae, such as *Acetabularia*. One can summarize much by saying that a non-nucleated cell or cell fragment can survive for a certain period. An amoeba can eat, it can move about after enucleation, but in due time its synthetic capacities appear to degenerate, and, like the red cell, it perishes without reproducing. The red cell is perhaps the most successful of cells in functioning for a long period without a nucleus.

It is possible to isolate nuclei and to separate them from cytoplasmic components. If such isolated nuclei are studied biochemically, their metabolic behavior can be followed. The most elegant work along these lines has been carried out by Mirsky and Allfrey, based on earlier work by Dounce. The nuclei contain protein, DNA, RNA, and small amounts of lipid. Allfrey and Mirsky have found that isolated nuclei are capable of carrying out amino-acid incorporation into proteins and that certain other enzymatic activities are associated with the nucleus.

If one examines the unstained nucleus with a light microscope, either in the living cell or after fixation with ordinary reagents, one sees regions of varying density. This inhomogeneity of structure becomes even more striking following staining, since the nuclear components vary in their affinity for dyes. The components which bind the dyes have been called chromatin. As a rule, the term "chromatin" is used primarily to designate strongly-staining materials in the nucleus of the cell. It is a general term without any specific chemical significance. The term "nuclear chromatin" is applied to two distinct structures within nuclei. One comprises the chromosomes themselves; the second consists of nucleoli which are accumulations containing considerable quantities of RNA. Many of the staining reactions of chromosomes and of nucleoli are very similar or identical, but there are a few methods for distinguishing them from each other. These depend on chemical differences between DNA on the one hand, which dominates many of the properties of chromosomes, and RNA on the other, which has an important role in the nucleoli.

The appearance of nuclear chromatin can change greatly under different functional states of the cell. As the cell approaches a mitotic division, the chromatin material rearranges itself. At that time, one easily can see well-defined bodies, the chromosomes, so called because they can be stained so as to assume very vivid colors. It is well established that the chromosomes are present in the intermitotic nucleus, where they usually occur in a rather tenuous form which makes it difficult to recognize them morphologically. Yet chromosomal activity can be detected by a number of different means. The genetic method is perhaps the most powerful.

In certain specialized cases, the chromosomes may be present in a form quite different from the dispersed state which they assume in the normal intermitotic cell. For example, in the salivary gland cells of the fruit fly, *Drosophila*, the intermitotic chromosomes occur as dis-

crete bodies known as "giant" chromosomes, which may be over 200 μ long. Under the light microscope, they show a longitudinal fibrillar structure and a distinct series of aperiodic crossbands. These crossbands are unique in the sense that they exist in distinct and recognizable patterns in each chromosome. These patterns are important for correlating the genetic influence of the chromosomes with its structure.

Consider now the question: How can one exploit the properties of the various chemical components of the nucleus in such a way as to gain information about their disposition, structure, interactions, and relationships? Both acid and basic dyes are bound by many of the components of the nucleus. From this, it is inferred that nucleoproteins behave as a mixture of anionic and cationic ion-exchange resins. These properties can be attributed to their negatively charged phosphate groups and positively charged protein groups. One can exploit these characteristics by setting up a suitable competitive system which introduces colored cationic molecules to bind to the nucleic-acid phosphate groups, replacing positively charged protein groups. This procedure tags phosphate groups of the nucleic acids with colored tracers or indicators. The capacity of anionic polymer groups, such as nucleic-acid phosphate groups, to bind basic dyes is spoken of as the property of "basophilia." Thus, nucleochromatin is often spoken of as being strongly basophilic. In this way, the nucleic acid can be tagged rather nonspecifically. Analogously, one can use anionic dyes such as azosulfonic acids to bind to polymers containing basic groups in tissue components. Such polymers are found in the nucleus in the form of the basic proteins which are associated with the nucleic acids to form nucleoproteins.

Most of these dye-binding methods do not distinguish between RNA and DNA. But, from the analytical data on isolated nuclei, there is reason to believe that both are present in the nucleus. If one turns to another property of nucleic acids—namely, the absorption spectrum in the ultraviolet of the purine and pyrimidine residues—it can be shown that the same structures which take up basic dyes also absorb strongly in the ultraviolet. This is consistent with the view that the nucleoprotein complex is responsible for the staining reaction and for the absorption in the ultraviolet. But the latter method does not permit a distinction between DNA and RNA components.

There is, however, a reaction which does distinguish between these two types of nucleic acids. It has proved to be exceedingly useful in characterizing the nucleic-acid distribution in cells. This reaction is known as the Feulgen reaction. It depends upon the presence in nucleic acids of linkages which are differentially susceptible to hydrolysis. Using appropriate conditions, dilute HCl will hydrolyze certain sugar-base linkages in DNA but not in RNA. The hydrolyzed sugars of DNA are thus converted to reducing groups resembling aldehydes. These can be labeled by coupling them to a suitable colored reagent which reacts with aldehydes. Each single aldehyde group formed by hydrolysis is then tagged with a colored chromophore. This permits the DNA to be distinguished sharply from RNA. This useful reaction can be used quantitatively.

There is another important approach to the problem of distinguishing the localization in cells of RNA and DNA. This involves the use of specific enzymes. The enzyme, ribonuclease, will depolymerize RNA but not DNA. If RNA is removed from the nucleus by action of this enzyme, a loss of dye-binding capacity may appear in certain structures. The material originally present which was removed by the ribonuclease is thus shown to be RNA. It is also possible to remove DNA by deoxyribonuclease, and thus to ascertain which dye-binding component contains DNA. Still another method, often less reliable and less rigorously specific than the others, can be used to stain DNA and RNA in contrasting colors in the same cell. These three methods —the Fuelgen reaction, the use of specific enzymes, and the use of differential staining—permit one to distinguish cytochemically between DNA and RNA and provide information about the localization of these substances within the cell.

It turns out that DNA is found characteristically in the chromosomes and frequently as a jacket surrounding the nucleolar material. RNA is abundant in the central portions of the nucleoli and small amounts can be found within the chromosomes or scattered about in the nucleus.

If a large number of nuclei from a given species are examined by the Feulgen or some other suitable method and the amount of DNA in the individual nuclei is measured by absorption microspectrophotometry, as carried out by Alfert and by Swift, a small number of cells (for example, sperm cells) display a certain unit quantity of DNA. However, the majority of cells contains approximately twice this unit amount, while a third group of cells yields values clustering around four times the unit amount. In certain organisms—in the human liver, for example—one may find nuclei with up to eight times the unit value. From this, it derives that the amount of DNA in individual nuclei tends to occur in integral multiples of some unit quantity.

The chromosomes in dividing cells can often be counted with precision. In most higher animals, spermatozoa, the spermatids, and fully mature ova are found to contain a certain number of chromosomes. This number is called the "haploid number," and the chromosomes making up this number comprise a single set of chromosomes. These cells contain a single unit quantity of DNA, as mentioned earlier. The great majority of cells in most multicellular organisms contains twice the number of chromosomes characterizing a single set, and contains twice the unit quantity of DNA. Such cells are said to contain the diploid number of chromosomes. In diploid cells, most of the chromosomes occur in homologous pairs. Each member of a pair resembles its

mate closely, but morphological and genetic differences between the members of a homologous pair are often found. One may also find cells with four, six, eight, or some other small integral multiple of the haploid number of chromosomes, and with the same multiple of the unit amount of DNA found in haploid cells. Such cells are called tetraploid, hexaploid, or octaploid, respectively. In mammalian liver, one can find many tetraploid and some octaploid cells in a population which is predominantly diploid.

Each set of chromosomes contains a single set of genes. Thus, in diploid cells, many genes are represented twice, once in each member of a homologous pair. Such cells are said to be homozygous for the characteristics represented by genes which occur in identical form twice in each cell. But some gene loci are not identical in each member of a homologous pair of chromosomes. Such genes are represented only once in each cell, which is then said to be heterozygous with respect to the characteristic represented in those dissimilar loci. These data have contributed to the concept that there is a definite amount of DNA characteristic of each chromosome set, each chromosome, and each gene locus.

Some organisms live very well through most of their life cycle with haploid numbers of chromosomes in each nucleus. The fungus, *Neurospora*, for example, which has figured extensively in genetic studies, has a single set of chromosomes in each nucleus throughout long phases of the life cycle. The diploid phase may be very brief. In most metazoa, however, the diploid phase predominates.

One occasionally finds numbers of chromosomes that are *not* even multiples of the haploid number. Such cells are called "aneuploid" and contain amounts of DNA which are not integral multiples of the amount of DNA in haploid cells. Relevant examples are provided by certain cancers and leukemias. As a rule, the presence of aneuploidy and of morphologically abnormal chromosomes is interpreted as evidence for some genetic abnormality.

Levinthal (p. 227) remarks that information from genetics has led to the concept that the genetic carriers are arranged in linear sequence along some structure. The identification of the chromosome as this structure marked a major advance in the history of cytogenetics. Correlations between genetic and morphological data were most successfully worked out initially in *Drosophila*, where the chromosomes of the salivary glands are large and where genetic studies are readily carried out because of the short life cycle of the fly. Similar studies correlating genetic behavior with morphological abnormalities of chromosomes have now been carried out in a number of different forms. However, much of the corresponding work in virus and bacterial genetics is carried out conceptually, without direct visualization of chromosomes. The genetic data are used to construct a linear sequence, but no attempt is made ordinarily to correlate this sequence with morphological features of chromosomes—one important reason being that chromosomes have not been incontestably visualized in bacteria or in viruses. In these cases, the structural concept of linear sequence of units depends entirely upon genetic evidence.

At present, most of the important concepts of nuclear structure have been derived from genetic studies, from chemical analyses, and from examination with the light microscope. When combined ingeniously, these approaches have proved to be very powerful.

The electron microscope has yielded some additional information. It is evident that the intermitotic nucleus is surrounded by an envelope which consists of two unit membranes, an inner one and an outer one. These two membranes are joined to each other at certain sites. The lines of junctions surround and define pores several hundred Ångströms in diameter, perforating the nuclear membrane. Through these pores, the nuclear and cytoplasmic matrices communicate directly. The outer membrane of the nuclear envelope may also be continuous with membranes in the cytoplasm. The interior of the nucleus, however, is free from membranes, and is thus in sharp contrast to the cytoplasm, where membrane structures are frequent and often densely packed.

Electron micrographs usually show a rather irregular accumulation of granular material within the intermitotic nuclei. The granules presumably represent DNA and RNA, combined with protein. Very fine intranuclear helical threads have been described by some authors, but these are not seen with clarity and their significance is difficult to assess. No one has yet recognized structures in the nucleus corresponding to the nucleic-acid helices as studied by Hall and Doty, with the electron microscope, and pictured elsewhere in this volume (p. 107). One can find nucleoli in electron micrographs. They appear as rather irregular accumulations of granules more densely crowded together than elsewhere in the nucleus.

Mitotic cells show no nuclear envelope. The chromosomes are recognizable as dense accumulations of granular material without any membranous investment.

Thus, it appears that direct electron microscopy of the nucleus of ordinary cells has provided little information which is satisfying in the light of the physiological importance of the nucleus and its contents.

In certain specialized types of cells, organized structures have been detected with the electron microscope which are not characteristic of cells in general. Thus, in some sperm cells, dense striations running the length of the nucleus have been seen. Moses has detected more-delicate organized structures in certain spermatocytes. These appear as long thread-like elements with delicate side chains extending laterally. Efforts have been made to associate the filaments with some phase of chromosome structure. But, at the present time, it

would be hazardous to do more than point out that the dimensions of the threads of the side loops are not inconsistent with those of a nucleic-acid helical chain.

Although the electron microscope shows that the nucleus contains particles which closely resemble the RNP particles of the cytoplasm, it reveals relatively little concerning the mechanism of transfer of nucleic acid from nucleus to cytoplasm. Such a transfer would provide a means whereby the nucleus could transmit information to the cytoplasm. Yet one can see images which suggest transition stages in the course of outward movement of RNA from the nucleus. One may occasionally see accumulations of particles in the cytoplasm which very closely resemble the nucleoli. Similar appearances observed with the light microscope have led to the view that in some cells whole nucleoli may be discharged from the nucleus carrying large packets of RNA to the cytoplasm. On the other hand, it may be that much of the RNA escapes into the cytoplasm through the pores in the nuclear envelope. Another mechanism whereby RNA could pass from nucleus to cytoplasm involves a binding of the newly found RNA to the inner nuclear membrane. The latter then would flow into the cytoplasm, carrying the bound particles with it. Such a mechanism, however, is not well documented.

We are forced, then, to conclude that the task of correlating the fine structure of the nucleus with its function and with the chemical and genetic evidence at our disposal is largely before us.