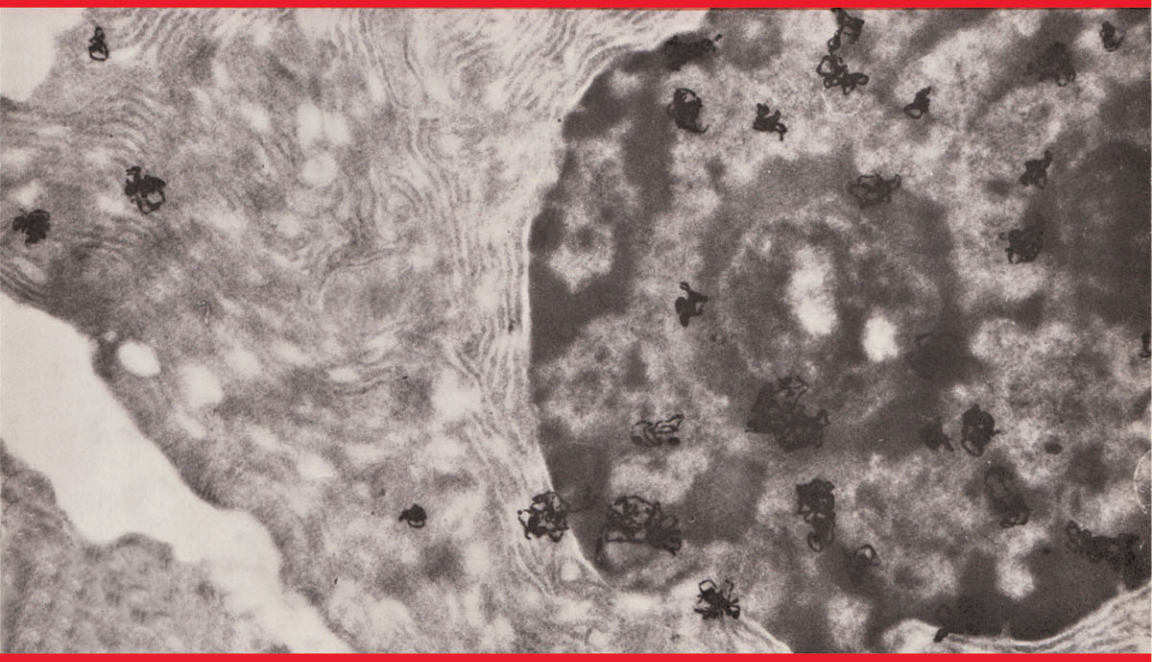
VOLUME II

THE CELL NUCLEUS

Edited by HARRIS BUSCH



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Volume II

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Preface

Although the cell nucleus is such an integral part of cell function, it has not been the subject of an extensive review in recent years. There have been important monographs on this subject including a conference on "The Cell Nucleus" chaired by J. S. Mitchell (Academic Press, 1960) and a conference on "The Nucleus of the Cancer Cell" (H. Busch, ed., Academic Press, 1963). When the monograph on "The Nucleolus" (H. Busch and K. Smetana, Academic Press, 1970) was undertaken it became apparent that there were so many contributory fields to nuclear and nucleolar function that a thorough review of the subject would be worthwhile. This three-volume treatise is designed to provide such a work.

It should be clear to researchers and students of the cell nucleus that there is such an enormous gap between our present information and the complete understanding of nuclear composition and function that this work represents only a small portion of the knowledge still to be developed in this field. It was simply not possible to cover the complete literature. Interested readers are urged to consult primary sources or special reviews.

I am indebted to my many colleagues around the world who have contributed to the actual writing of this work and particularly to the editorial advisors for their many suggestions that have brought this work to fruition. In addition, we are grateful for the aid provided for much of the basic research from the National Institutes of Health, the National Cancer Institute, the American Cancer Society, the National Science Foundation, and the Welch Foundation.

The cell nucleus is so important to the basic understanding of biological and medical problems that it holds a fascination for one and all. It is hoped that this treatise will provide a useful guide for research and study of this very exciting area of human endeavor.

Harris Busch

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Introduction

Why a cell nucleus? Although there is little doubt that the presence of a cell nucleus has permitted a great extension of the numbers of combinations and permutations of cellular phenotypes in both single and multicellular species, the origin of the cell nucleus is so ancient a part of evolution that it is uncertain what circumstances induced its origin and its development. What appear to be primitive "nuclear structures" have been found in both yeast and bacterial cells, but further evolutionary development produced a much more complex and functional structure in higher organisms. At present, it is not clear whether in its current state of development in the most specialized animal species, the cell has yet achieved its total potential for functionality. Some advantages of the cell nucleus may relate to a variety of characteristics of eukaryotic cells; for example, many eukaryotic cells are extremely long-lived and specifically differentiated, particularly cells of the central nervous system and the endocrine glands.

It seems reasonably clear that the development of a cell nucleus carried with it significant new chemical and physical properties of cells. Included among these are such obvious features as the nuclear envelope (nuclear membrane, or the bileaflet nuclear shell). This structure has three interfaces: one with the cytoplasm, another with the internal nuclear structure, and the third the space between the two layers of the nuclear envelope. This nuclear envelope not only serves as a geographic marker between the nuclear and cytoplasmic boundaries but in addition contains pores that give it more of a "Swiss cheese" or "Wiffle ball" appearance than a solid membrane between two heterogeneous masses. Through these pores migrate not only nuclear products that are "gene readouts" on their way to the cytoplasm but also the "cytonucleoproteins" and other elements that may serve as communication mechanisms between

the cytoplasm and the nucleus. The role of hormone protein receptors in nuclear function is an exciting current chapter in mechanisms of gene activation.

By scanning microscopy, the cell nucleus resembles a ball studded with small bodies since it is covered with ribosomes and probably with polysomes. Although it is not certain that synthesis of the nuclear proteins occurs on the outer nuclear surface, it seems likely that they are formed either there or close by, and rapidly penetrate the nuclear mass.

Nuclear constituents. The presence of nuclear DNA which is almost all of the genetic complement of the cell is the key characteristic of the nucleus, but there are other structures that are specialized nuclear constituents. Among these are the histones, whose evolutionary origin seems to be very close to that of the nuclear envelope itself. Although the histones are now extremely well defined in terms of structure and number, their functions are shrouded in almost as much mystery as ever. There are so many histone molecules per nucleus (10^8) and they are so few in types that their role has been currently relegated to that of either structural support for DNA or as a general gene repressor system which can be activated by combination of the histones with "acidic nuclear proteins" or nonhistone nuclear proteins. In any event, their presence in association with DNA is sufficiently universal in nucleated cells and even in the chromosomes that the rule is "where there is DNA, there are histones."

The nucleus contains defined structural elements which seem to increase in number as technical advances increase in electron microscopy. The largest of these structures and the most universal is the "nucleolus" which contains an intense concentration of RNA and is now known to produce most of the total RNA of the cell, especially the rRNA species which are the backbones of the ribosome. Its role in the production of other types of RNA, such as mRNA, remains to be defined. The ultrastructure of the nucleolus varies markedly in various cell types but its responsiveness to the variations in cell function is both ordered and harmonious with the other events and requirements of the cell.

Among the other structural elements of the nucleus are the *interchromatin dense granules* that are probably parts of the processing elements of the nucleus; *perichromatin dense granules,* dark RNP particles surrounded by a light halo (by usual electron microscopic studies); *intranuclear rodlets;* and other structures of which the juxtanucleolar channel system is one of the most intriguing. The functional roles of such "nucleus-specific" bodies are not defined, and manifest the very great requirement for research for understanding of the nuclear "government" of the cell.

The nucleus produces polysomes for export but retains for itself certain RNA molecules. Of these, the low molecular weight nuclear RNA species (LMWN RNA) are now being analyzed chemically, and the nucleotide sequences for three are defined. One of these, the U3 RNA, is "nucleolus specific." Others appear to be limited to the chromatin, and may exist juxtaposed to proteins in small RNP particles.

The nuclear proteins are composed of the histones, already noted above, many enzymes including the polymerases for RNA synthesis, structural proteins for ribosomal precursor elements, and other specialized processing elements of the nucleolus and nuclear nonhistone proteins (NHP) some of which may be "gene derepressor" proteins. Although the "gene derepressors" are clearly of enormous interest and objects of intensive research interest at present, it is only recently with the development of two-dimensional gel systems that the overall number of nuclear proteins has been approximated as several hundred. It is not yet clear which of these serve specific regulatory functions. It remains to be seen whether in individual chromosomes one or more of these nonhistone proteins (NHP) is specifically present. At present, methods for chromosome isolation seem to be improving to the point where it may be possible to ascertain whether any proteins have a special chromosome localization.

One of the more amazing aspects of the cell nucleus is the variety of changes that occurs during cell division. Not the least remarkable is the disappearance of the nuclear envelope. In metaphase there is the precise and equal separation of chromosomes of the daughter cells. It must be remembered, however, that there are other events accompanying metaphase that are of great importance and that all of the cellular components are distributed to the daughter cells approximately equally. Aspects of the formation of spindles and other nuclear elements are dealt with as specific topics in these volumes.

Although a definitive answer as to "why a cell nucleus" requires some consideration of its components, one may ask whether the functions subserved within this structure could not as well be served in a "nucleus-free" system? One may ask many other questions. Does the nuclear envelope protect delicate nuclear structures from enzymatic attack? Does the nuclear segregation provide for multilog specialization of function? Does the segregation of specific reactions for gene control and gene readout provide improved concentration of reactants and increased efficiency of these reactions? Does the nuclear envelope provide for penetrance of specific cellular elements into the nucleus? At present one can only speculate on these questions.

History of the cell nucleus. Improvements in light microscopy in the

early nineteenth century permitted Robert Brown to discover the cell nucleus in 1830. The finding of "one nucleus per cell" led to the cell theory of Schleiden and Schwann in 1838. This concept provided a base for many developments including the understanding of Virchow that cells are all derived from pre-existing cells (*omnis cellula e cellula*) by extraordinary complex molecular events. The biological and clinical sequelae to the development of this concept have been truly astonishing in the last century and a half.

Definition of the nuclear contents emerged from development of staining methods and the improvements for isolation and analysis of nuclear products. After Miescher found DNA, the Dische stain established that DNA was largely localized to the nucleus in mammalian cells. By the use of appropriate staining techniques it was also found that the nucleus contained a nucleolus and, further, that the nucleolus contained vacuoles and nucleolini. With the Unna and other RNA stains, Brachet showed that RNA was concentrated in the nucleolus and cytoplasm. Development of microscopic spectrophotometry enabled Caspersson to show that the nucleolus is an island of RNA in a nuclear sea of DNA and histones.

Readily visualized by specific staining procedures, the chromosomes were observed in metaphase. Initially observed in 1873 by Butschlii, Flemming, Schneider, and others, they were named "chromosomes" by von Waldeyer–Hartz in 1888. Their separation into daughter cells was visible support for the concepts of Mendelian segregation. Chromosomal aberrations in special diseases and alterations in membranes and type of chromosomes in cell hybridization are topics of intensive current studies. Almost all of the elegant light microscopic studies on nucleoli that were beautifully reviewed by Montgomery were subjected to the criticism that staining procedures produce many artifacts. It remained for the development of light and electron microscopy to confirm and extend many features of the nucleus including the fascinating characteristics of the nucleolus and nuclear envelope. Not only were the characteristics of these structures defined by Bernhard, Swift, Smetana, and others but, in addition, important new structures were found that included nucleolar vacuoles, granular and fibrillar elements, perichromatin granules, interchromatinic granules, a variety of cytoplasmic invaginations, rodlets, and intranuclear tubular structures.

The preoccupation of biochemists with nuclear structures began in earnest after the finding of DNA by Miescher and the very rapid evolution of information of protamines and histones by Kossel, Lilienfeld, Mirsky, and others. After the Stedmans suggested that gene control might be exerted by nuclear proteins, an extensive series of investigations

on nuclear proteins developed that continue with increasing excitement at present.

"The Cell Nucleus" is designed to mark the state of our understanding in the mid 1970's at a time when an enormous number of new and exciting developments are occurring in morphological, biochemical, and biological comprehension of nuclear function. While the nucleus is generally regarded as the "governor" of the cell, information is still accumulating on what it governs, how it governs, and the input that produces specific responses. Although our understanding is incomplete, the great enthusiasm in the field is well supported by its many accomplishments. The Tables of Contents of these volumes show the breadth of our current concepts and information.

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PART I

Chromosomes—General

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1

Mammalian Chromosomes

Frances E. Arrighi

I. Introduction

For several decades plant and insect materials practically dominated every phase of chromosome studies. Cytology of higher animals was considered a difficult field in which very few venturesome biologists struggled to satisfy their curiosity. The lack of advances in this area was the result of technical inadequacies. The chromosomes of mammals (and other vertebrates as well) clump at the metaphase plate and are

coated also with nucleolar material which tends to "glue" two or more chromosomes together.

Perhaps the first major advance in mammalian cytology was the hypotonic solution pretreatment (Hsu and Pomerat, 1953). When metaphase cells are subjected to a hypotonic environment, the cells swell, the nucleolar material disperses, and the chromosomes scatter, thereby leaving the individual chromosomes discernible. The second important technical advance should be attributed to the use of colchicine to arrest metaphases and to the squash technique which forces the chromosomes to lie on a single plane of focus (Tjio and Levan, 1956). Colchicine (or other agents with similar action) has an additional advantageous effect: it condenses the metaphase chromosomes to facilitate observation and to reduce errors. The air-drying technique (Rothfels and Siminovitch, 1958) and its many modifications further simplified the procedure because many investigators could not master the squash technique.

The discovery that aneuploid individuals exist in human populations and that the same aneuploidy exhibits the same phenotypic abnormalities (Lejeune *et al.*, 1959) marked the first time chromosome studies became important to medicine. Within a few years practically every hospital established a cytogenetic laboratory for diagnosis and research.

In the meantime students of mammalogy became interested in utilizing the more modern techniques to reexamine the karyological features of various taxa, not only as a taxonomic tool but also as a means to interpret phylogenetic relationships and evolutionary divergence.

The great improvements in the field of mammalian cell culture also contributed significantly to the studies of mammalian chromosomes. One can not only initiate cell cultures from a small piece of tissue without sacrificing an animal, but also manipulate the cell populations to achieve what could not be achieved with cells *in vivo*, e.g., cell synchronization or cell hybridization, which directly or indirectly receive assistance from chromosome studies. The more recent advances in *in situ* nucleic acid hybridization and various types of banding of mammalian chromosomes should further advance the field of mammalian cytogenetics as well as studies on chromosome structure.

II. DNA Content, Chromosome Number, and Chromosome Morphology

A. DNA Content

A prevailing view is that the DNA content (or genome size) of all eutherian mammals is the same regardless of the variation in the diploid

chromosome number. That is to say, chromosomes are packages of genetic material. Since the DNA content is the same, species with fewer chromosomes have longer chromosomes and species with more chromosomes have smaller chromosomes. This conclusion was evident from two types of experiments—the determination of DNA content by chemical methods and the determination of chromosome length by cytological methods.

A recent review (Rees and Jones, 1972) showed that at the 2-C or diploid level (before replication) the DNA content of mammalian cells varied from five to six picograms. Considering the errors in determining the cell number and those in determining the amount of DNA, the difference is minimal. Cytological studies made by Ohno *et al.* (1964) pointed to a similar conclusion. These authors measured the total length of metaphase chromosomes of several mammalian species which differ vastly in the diploid numbers. The total chromosome length was similar.

The DNA constancy concept also finds support from geneticists. All eutherian mammals possess similar complexities in anatomy and physiology, so conceivably they should require a similar number of genes, and thus similar amounts of DNA. However, there are some reports, though not too recent, of tetraploid species having less than double the amount of DNA of diploid species (Rees and Jones, 1972).

This hypothesis of DNA content is roughly correct, but as can be seen later in our discussion on heterochromatin and evolution, variability occurs.

B. Chromosome Number

The diploid chromosome number of mammals has an astonishing variation from the lowest of six in the Indian muntjac (Wurster and Benirschke, 1970) to 92 in an amphibious "fish-eating" ichthyomyine rodent, *Anotomys leander,* (Gardner, 1971). Between these two extremes any number is conceivable, including odd numbers. However, the majority of mammalian species possess a diploid number from the 20's to the 50's. This general rule is applicable. The chromosomes are longer when the diploid number is low and shorter when the diploid number is high. Table I presents the distribution of diploid numbers correctly determined by various cytologists. The table was compiled from 351 species of mammalian karyotypes collected in *An Atlas of Mammalian Chromosomes* (Hsu and Benirschke, 1967–1973). The reason for such a wide variation in diploid numbers among mammals is not understood. If one examines the diploid numbers of smaller taxa such as a family or a genus, the range then narrows. The extreme case is a family of