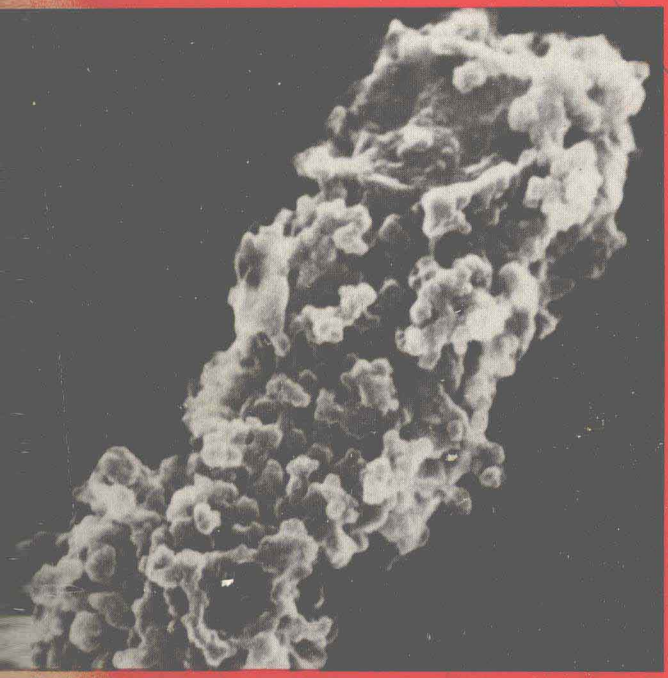
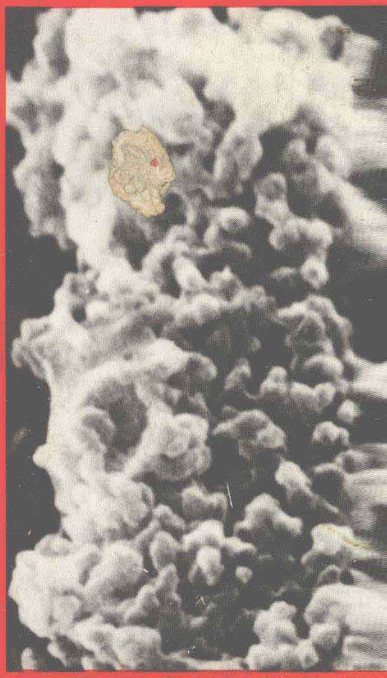
VOLUME V

THE CELL NUCLEUS

CHROMATIN PART B

Edited by HARRIS BUSCH





ACADEMIC PRESS, INC.
A Subsidiary of Harcourt Brace Jovanovich, Publishers

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CHROMATIN, Part B

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ACADEMIC PRESS New York San Francisco London 1978

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data
Main entry under title:

The Cell nucleus

Includes bibliographies.
1. Cell nuclei. I. Busch, Harris, ed. [DNLM: 1. Cell nucleus. QH595 B977c]
QH595.C44 574.8'732 73–18944
ISBN 0–12–147605–7 (v. 5)

PRINTED IN THE UNITED STATES OF AMERICA

THE CELL NUCLEUS

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CHROMATIN, Part B

细胞核 第5卷

染色质 第二部分

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Introduction

When "The Cell Nucleus" treatise was originally conceived in the early 1970's, the general idea was to present an overview of the subject with emphasis on areas of special interest. Very important advances are now being made in the field of chromatin chemistry and function. For this reason the current volumes have been developed with the aid of our able advisary Editorial Board.

The chromatin field is very complex, and it has suffered both because of the complexity of the definition of chromatin and the even greater complexity of its structure and function.

Definition of chromatin: Over the years the statement has been made that "chromatin is whatever a given author defines it to be in an operational sense." All agree that chromatin contains a DNA "backbone" which includes both the DNA and its "tightly bound proteins." These proteins consist of histones and some tightly bound nonhistone proteins. Thus, the "bare-bones" elements of chromatin were identified early, but even the functional roles of these important elements require much further study.

By 1963 workers in our laboratory and in a number of others had sufficient analytical information to make it clear that many species of nonhistone proteins existed in the nucleus and that some had very high turnover rates. It was also clear that many nonhistone proteins were structurally related to proteins of the cytoplasm. Their potential roles in gene control seemed likely to be greater than the potential of the histones which were few in number and had low turnover rates. However, the nonhistone proteins contain a large group of enzymes which include DNA synthetase(s) and modification enzymes as well as RNA synthetase(s) and processing enzymes. These structural and "carrier" proteins complicate the problem of understanding chromatin.

The "high concentrations of nonhistone proteins in the nucleus" and the fact that "turnover of some of the acidic nuclear proteins is far in

excess of that of the histones" (as quoted by Busch, 1965) led to an intensive series of investigations in isolation, purification, and functional analyses of these proteins. Along with these developments, two major advances served to add zest to this complex field:

1. *"Reconstitution:"* In the late 1960's schemes were evolved for recombining DNA, histones, and nonhistone proteins. They were based on the solubility of these macromolecules in concentrated salt and urea solutions. It was hoped that slow and steady dilution of the urea and the salt by dialysis or other means would provide a basis for "self-assembly," a process that had been remarkably successful for "reconstitution" of ribosomes.

There have been vehement, lengthy discussions of the validity of this approach. The idea that the myriad of genes could find or be found by special proteins or RNA over a period of prolonged dialysis seemed virtually miraculous. Of course, one is not shocked by miracles, and clearly in the cell such specific combinations must occur. The "reconstitution approach" suggested that "special carriers" or special energy reactions are not necessary since such reassociations apparently occurred by diffusion and ionic and hydrogen bonding.

2. *Variable DNA binding:* As time progressed it became clear that the binding constants of proteins for DNA were remarkably different. Some nonhistone proteins are very tightly bound to DNA, even more strongly than histones. Others are so loosely bound that they could be eluted from DNA by either solutions of low salt concentration or low concentration of buffers. Such loosely bound proteins did not seem likely to provide for meaningful chemical interactions unless the proteins were present in large amounts. Among the examples of the "loosely bound" proteins are "hormone–receptor complexes;" many thousands of copies are present in the nucleoplasm, and these apparently interact with DNA at selected sites by "mass action." This latter finding has required a further redefinition of chromatin. If a specific molecular species is important in cell function by virtue of its interactions with DNA and if these interactions with DNA, which are important because of concentration rather than high affinity, involve very loose binding, "chromatin" must include both the loosely and tightly bound elements. Thus, it is likely that many chromatin elements are not bound to DNA at any given time, and, accordingly, "insoluble chromatin" may represent only DNA–protein complexes of higher affinity.

Chromatin as the whole nucleus: Many workers now view chromatin as the nucleus stripped of the outer shell with the exception of the

inner layer of the nuclear envelope. This view makes it possible to include a number of nuclear elements involved in synthesis as well as the soluble nuclear elements that interact with chromatin by mass action. The concept is a logical extension of the idea that both loosely and tightly bound elements interact with the genome.

The key question that emerges from the concept that "chromatin" includes everything within the nuclear envelope is whether the concept goes further, i.e., Why not include the cytosol? This cellular fraction is capable of interacting with the genome through the nuclear pores and the nuclear envelope. Years ago the rapid penetration of inorganic elements into the nucleus and incorporation or modification of nuclear elements were pointed out. Exemplary in this respect are substrates such as uridine and drugs such as actinomycin D which are capable of entering the nucleous and inhibiting nucleolar function with great velocity. The rapid penetration of uridine and its incorporation into small and large RNA molecules were dramatically demonstrated by autoradiography.

Accordingly, it is clear that the nucleus and chromatin are in very dynamic equilibrium with the cytoplasm. The nucleolus and nucleus "sense" the ongoing molecular events in the cytosol and other organelles with great rapidity, and their reactions are integrated with the response of the cell nucleus in terms of production of such products as "preribosomal particles," mRNA, and the corresponding ribonucleoproteins which are transported to the cytoplasm where they affect the overall functions of the cell.

Included in the "information gathering process" of the cell nucleus are the critical elements of hormone action, the hormone–receptor complex. These complexes are part of the overall "stimulus–receptor" complexes of cell organization, and constitute important elements that "drive" genes to increased or decreased transcription rates or qualitatively alter the gene activity.

Nucleosomes and structure of chromatin: Ever since the clarification by Watson and Crick of DNA structure through X-ray crystallographic analyses, there has been an enormous accumulation of new information on the subject of DNA structure and function. It is not surprising, therefore, that X-ray crystallographic analysis of chromatin units was a logical step since such units represent a higher order of magnitude of gene organization.

However, great advances in this field emerged from other types of studies, i.e., partial degradation of chromatin utilizing DNases and other cleavage mechanisms which yielded unexpected results. A series of reports noted that in limit digests of chromatin there was a