

*THE CELL NUCLEUS*  
Volume IV  
*CHROMATIN, Part A*

EDITED BY  
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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 Editorial Advisory 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 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 nucleus 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 "peribosomal 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 were a

series of regularly ordered chromatin elements. Since it was apparent that the DNA per se did not offer any particular bars to the cleavage reactions, it was not surprising that these limit digests were related to proteins bound to DNA.

A most significant advance in the field was made when it was found that nucleosomes or "nu bodies" were histone-containing particles bound to DNA like "beads on a string" or a necklace. This result totally altered prior concepts of the relationships of proteins to DNA. In earlier years a series of models had been offered which suggested that the relationships of proteins to DNA could be either as shells or as packets, but no meaningful evidence existed on this point. Within short order the suggestion was verified that the components of these nucleosomes or packets were histones, particularly histones 2A, 2B, 3, and 4, in duplexes, each containing 1:1:1:1 of these individual proteins.

Complex structures for chromatin have been developed to account for the ratios, packing, and rigidity of this structure. The need to include the H1 histone(s) is apparent; these molecules may be juxtaposed to each nucleosome. Since these histones are more easily extractable than the others problems exist in accounting for them and their many substituents.

Now that these basic elements of chromatin structure have been identified, much new information is forthcoming. Happily, a variety of visual measurements, including those obtained by crystallization of nucleosomes, supports the fundamental structure proposed, and now the nucleosome structure is established as a reality.

Although the nucleosomes are being extensively analyzed, few clues exist as to why they offer an evolutionary advantage to cellular function. The distribution of these microparticles seems to be quite random, and if there is ordering it is not yet apparent. It is now clear that the nucleosomes do not offer restrictions to gene readouts, i.e., apparently every gene can be read through the appropriate polymerases.

*Chromatin supercoiling:* The highest level of chromatin supercoiling is that observed in metaphase chromosomes (see Chapter 1 by Daskal and Busch, Volume IV). These chromosomes have packed DNA, nonhistone proteins, and histones into very small structures that are readily visible by light microscopy, and with the elegant Giemsa and quinacrine stains exhibit regular "banded" elements that serve not only to identify the chromosomes but provide a basis for the assessment of specific gene states (see "The Cell Nucleus," Volume II).

The general concept that highly activated chromatin consists of threads of double-stranded DNA fibers and that tightly coiled chroma-



tin is inactive as a template has emerged from many kinds of studies. In the nucleolus, as one example, it has been possible to identify 20 Å strands of DNA coursing through the structure in the center whereas the periphery contains heterochromatic DNA masses. These strands are the probable matrix of the active fibrillar region of the nucleolus (see Chapter 1 by Fakan, Volume V). What defines either supercoiling and uncoiling, i.e., of chromosomes and chromatin? Are these passive processes related to proteins associated with the chromatin or are both active processes?

New concepts are emerging to deal with these questions. Chromatin has "inherent elasticity" which gives it a "springlike" characteristic. Some believe that the unique shapes of chromatin and chromosomes may be governed by the elastic features inherent in chromatin. However, at the higher level of organization it hardly seems possible that such a simple explanation could be the critical one. "Condensing enzymes" or adherence factors are more likely to be involved in supercoiling. The recent finding of protein A24, a ubiquitin-containing derivative of histone 2A (see Chapter 5 by Goldknopf and Busch, Volume VI) which apparently is present in regular arrangement in nucleosomes, suggests that there is indeed a specific marker element that could serve as a basis for chromatin condensation.

It is equally possible that other proteins present in smaller amounts might also serve as a basis for micro- and macrochromatin condensation. In this sense it is notable that chromatin not only condenses into regular structures, but "microconvules" associated with these structures appear to have an approximately regular size.

Accordingly, it would seem that the supercoiled models proposed by Solari (see Chapter 10, Volume I), might well be characteristic of the lower order of organization of chromatin. The speculation would seem reasonable that both for coiling and uncoiling of chromatin forces are required that are operationally dependent on cellular demands and needs. It is an important problem of the future to assess these mechanisms in very precise chemical terms because of the potential for explanation of gene responses to cellular demands.

The volumes on chromatin in this treatise are designed to provide a comprehensive review of this field in the late 1970's and should serve as a basis for understanding the array of developments that are certain to come within the next decade. This field is both fundamental and exciting, and each advance offers a platform for many new research developments that may ultimately lead to a much clearer comprehension of cellular responses to environmental stimuli and stress.

Harris Busch