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Preface

The ICN-UCLA conference on Molecular Mechanisms in the Control of Gene Expression, organized through the Molecular Biology Institute of UCLA, was held in Keystone, Colorado, March 21 to 26, 1976. The year preceding the meeting brought major advances in our understanding of the action of repressors on specific nucleotide sequences in DNA, of how DNA and histones are intertwined in eucaryotic chromosomes, and in the development of new techniques, restriction and cloning, seemingly able to lift genes from complex genomes. These three topics and the increasingly revealing studies on control of gene expression in both multicellular organisms and microbial systems provided the major substance for the conference and these proceedings.

The success of a meeting is largely dependent on those who speak. We sincerely thank all those who accepted our invitations, both those included in this volume and those who are not. We include the speakers in plenary sessions, those who took part in the roundtable discussions, and the participants who made poster presentations. We are also thankful to the session chairmen for their help and counsel. For this we particularly thank A. Worcel, M. Ptashne, B. Magasanik, J. Sambrook, B. McCarthy, G. Felsenfeld, and H. Boyer. We thank as well W. Gilbert, R. Burgess, B. Forget, B. Polisky, R. Meagher, and P. Leder for conducting roundtable sessions. We are also grateful to D. Hogness for his special conference address, and to B. Lewin for his willingness to write the conference summary.

We thank F. Stusser and C. Winter and their associates for their hard work in putting the conference together. We also thank ICN Pharmaceuticals, Inc., for their support of this conference series and the National Science Foundation for a grant that paid for a portion of the expenses for this conference. We specifically acknowledge L. Berlowitz of NSF for his interest. Finally, we thank a number of people who helped either with the conference or with this volume. These include P. Sullivan, L. Spector, J. Abcarian, M. Cleary, C. Landel, J. Grunstein, E. Chang, S. Suggs, J. Isaacson, B. Carlson, and L. Lasky, and the staffs at Wilshire West Travel, the Keystone Conference Center, and the Keystone Center for Continuing Education.

Donald P. Nierlich
William J. Rutter
C. Fred Fox

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THE STRUCTURE OF CHROMATIN AND ITS RECONSTRUCTION

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ABSTRACT. It is now generally accepted that most the DNA and histones of chromatin are organized into subunits, called nu bodies or nucleosomes, each containing about 190 base pairs of DNA and approximately an equal weight of histone (1-4). The existence of such subunits has been demonstrated by partial staphylococcal nuclease digestion of nuclei, which results in liberation of nucleosome monomers and oligomers.

Further nuclease digestion of nucleosomes or of purified chromatin results in attack on the nucleosome structure itself, yielding a discrete set of double-stranded DNA fragments ranging in size from about 160 to 40 base pairs (5). Similar sets of fragments can also be generated by digestion of nucleoprotein reconstituted from DNA and chromatin total histones. We have systematically examined the nuclease digestion patterns of DNA-histone reconstitutes containing all possible combinations of the histones H2A, H2B, H3 and H4. We find that single histones, and most combinations of histone pairs, do not give rise to discrete DNA fragments. The histone pair H3/H4 appears to be essential to generation of the characteristic chromatin digest pattern. Use of other probes of structure, such as DNase I and trypsin, confirms this conclusion. We are able to show that the H3/H4 pair stabilizes DNA segments that are almost as long as the nucleosome "core", and we conclude that the DNA of the nucleosome is to a large extent organized by this arginine-rich histone pair.

Chemical probes are a common tool of the physical chemist for the study of macromolecular organization. We have employed chemical probe methods for the study of chromatin structure. The most widely used of these probes is the enzyme staphylococcal nuclease, which makes double-strand cuts across DNA. The action of this enzyme on the DNA of nuclei is well known: It leads to the generation of a series of nucleoprotein particles of discrete sizes, containing DNA fragments of discrete lengths that are multiples of a fundamental subunit about 200 base

pairs in length (3). This is one of the major pieces of evidence for the organization of the bulk of chromatin in the form of nucleosomes or ν-bodies (1,2,6,7).

A typical progression of events when nuclei are digested with staphylococcal nuclease is shown in Fig. 1. At early times in the digestion, the DNA demonstrates characteristic behavior described above. With increasing digestion, increasing amounts of monomer DNA (Band 1, 190 base pairs in length) are generated. The next step involves removal of about another 50 base pairs of DNA to produce a fragment (Band 1A) 140 base pairs long. This fragment appears as a relatively sharp and kinetically stable band on DNA electrophoretic gels. We and others have suggested that this corresponds to a nucleosome "core" particle, and that the 50 base pair segment between cores is more accessible to nuclease (4,8). This isolated nucleosome core lacks H1 and also largely lacks H5 (8,9).

The reaction does not stop with generation of this nucleosome core. The nuclease next attacks the DNA within the core, giving rise to a series of double stranded DNA fragments ranging in size from 140 base pairs to 40 base pairs (5,10). When these fragments first appear, they are exact multiples of ten base pairs in length; as digestion proceeds, each fragment loses two base pairs. Finally, some of the fragments lose an additional two base pairs just before the digestion reaction stops, at the point where half of the DNA has been hydrolyzed to acid soluble products.

The regularly spaced array of DNA electrophoretic bands certainly reflects some aspect of the regularity of nucleosome structure. In the approach we discuss here, however, we will simply use the appearance of such bands as an indicator of the presence of some nucleosome-like structural components. We can then ask the question: which histones are required to generate the observed pattern of nuclease digestion? Implicit in the question is the idea that histones that do generate such a pattern must also play an important role in nucleosome structure formation.

To answer this question, we have reconstituted DNA with essentially all possible combinations of histones H2A, H2B, H3 and H4. We find that the generation of strong discrete DNA bands in staphylococcal nuclease digests depends upon the presence of both arginine-rich histones, H3 and H4, and they alone with DNA are able to create much of this structure (10). All combinations of histones in which H3 or H4 is missing give rise to no discrete

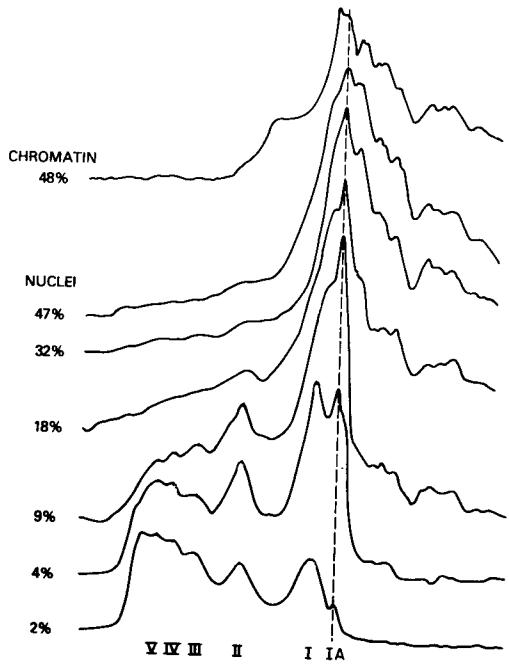

Fig. 1. Kinetics of nuclear digestion. Duck reticulocyte nuclei and duck reticulocyte chromatin were digested with 1-60 μg/ml of staphylococcal nuclease for 10 min to 1 hr in 1 mM Tris (pH 8)-0.1 mM $CaCl_2$ at 1 mg of DNA/ml. The fraction of the DNA soluble in 0.8M perchloric acid-0.8M NaCl was measured. The isolated DNA was run on a 4% polyacrylamide slab gel, stained, and photographed. Negatives were scanned with a Joyce-Loebl microdensitometer. These scans have been shown to be linear in DNA concentration. Migration is from left to right. From bottom to top are: nuclear digests at 2, 4, 9, 18, 32, and 47% (limit digest), and a chromatin digest at 48% acid-soluble DNA (limit digest). From (4).

fragments, with the exception that the histone combination H2A/H2B/H4 is observed to give fragments with low efficiency.

We have used a number of other probes to investigate the role of the individual histones in nucleosome structure formation. Pancreatic DNase digestion of nuclei or chromatin gives rise to a series of single-stranded DNA fragments that are multiples of 10 nucleotides in length (11). Reconstituted nucleohistone behaves similarly. We find that partial reconstitutes also give the regular band pattern, provided that both H3 and H4 are present (12).

The proteolytic enzyme, trypsin, can also be used as a probe of histone organization in chromatin. It has been shown (13) that the core histones of intact chromatin are largely resistant to trypsin attack: about 20 amino acids from the N-termini of these histones are susceptible to digestion, leaving a set of well-defined resistant polypeptide products. Individual histones in solution or bound to DNA are not at all resistant to trypsin attack. We find once again that the presence of both H3 and H4 in reconstitutes is essential to the regeneration of trypsin-resistant histone structure (12).

Finally, we have examined the kinetics of staphylococcal nuclease digestion of various reconstitutes. We find that the kinetic constants fall into two classes: all reconstitutes lacking H3 or H4 (with the exception of H2A/H2B/H4, the anomalous combination discussed earlier) are digested at a rate like that of DNA. All reconstitutes containing both H3 and H4 are digested at a rate like that of intact chromatin (12).

All of these results very strongly suggest that histones H3 and H4 together play a principal role in organizing the nucleosome, and that they are essential to that organization. In contrast, the slightly lysine-rich histones, H2A and H2B, appear to augment the stability of the nucleosome but are not in themselves capable of generating those elements of structure that are sensed by the probes we have used.

These nuclease and protease probes are, of course, chosen to detect aspects of gross chromatin structure. During the past several years our laboratory has also been concerned with the relationship between the structure and transcriptional function of chromatin. From the point of view of the chemist, studies of the interaction between chromatin and DNA-dependent RNA polymerase can also be viewed as chemical probe experiments. Since we do not know the relationship between in vitro transcription and the in vivo process, this is perhaps the most realistic way to view in vitro transcription studies.

When E. coli RNA polymerase is allowed to transcribe chromatin from duck reticulocytes, it is found that the number of initiation sites available is about one per cent of the number available in protein-free DNA (14). It has been known for some time (15,16) that the transcript from reticulocyte chromatin is enriched in sequences that anneal to cDNA complementary to globin mRNA. The measured abundance of globin RNA sequences in the transcript is about one part in 10^4. Recently, Dr. Michael Zasloff in our laboratory has confirmed this result using UTP substituted with Hg in the 5 position (17) in the in vitro transcription system. The advantage of this technique is that it permits separation of the newly synthesized transcript from any contaminating endogenous message, and simplifies the assay considerably. Viewed strictly as a chemical probe, RNA polymerase can tell us a great deal about the organization of chromatin in the neighborhood of genes active in transcription, and may make it possible to isolate those factors responsible for the activity. Thus, chemical probe methods may eventually lead us to an understanding of the real mechanisms underlying this biological activity of chromatin.

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X-RAY DIFFRACTION FROM ISOLATED REPEAT UNITS OF CHROMATIN

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ABSTRACT. The X-ray diffraction patterns of whole chromatin in dilute solution and of isolated repeat units are essentially the same.

Recent work has established a repeating structure for the chromatin of eukaryotes. One repeat comprises two each of the four main histones surrounded by about 200 base pairs of DNA, forming a beadlike object about 100Å in diameter. Such beads occur in close apposition along the length of a chromatin fiber. The earliest indication of a repeating structure came from X-ray diffraction patterns of concentrated gels of chromatin (1-3), showing a series of rings at spacings of about 110, 55, 37, 27Å and beyond. With the advent of the bead model, the ring at 110Å was attributed to the center-to-center distance between beads along a fiber (4), but the origin of the rest of the pattern remained obscure.

An analysis of the diffraction from chromatin is complicated by the concentration dependence (2) : solutions and dilute gels (up to about 10%, w/w) give strong, diffuse scatter in the center of the pattern with a shoulder at about 55 to 60Å, a clear ring at 37Å, and