This is shown diagrammatically in Figure 3B. One consequence of this behavior is the aggregation of proteins in patches. If all membrane lipids assume a frozen state, membrane proteins become aggregated in large patches. Figure 3C shows this condition. Some proteins are depicted as having points of attachment to a cytoskeletal structure. The movement of these proteins is probably restricted.

We have taken into account only the lateral movements of proteins in the plane of the membrane. As membrane lipids freeze and the bilayer thickens, proteins normally exposed at the membrane surface may become buried, or they may be extruded as a consequence of their exclusion from frozen lipid regions.

Jost and coworkers[77-79] and others[80,81] have performed experiments which suggest that a finite amount of lipid is intimately associated with the surface of a membrane protein. This lipid is immobilized in comparison with "free lipid" in a fluid phase, and is depicted in Figure 3 as lipid with open circles for head groups. Several investigators have successfully extracted and purified certain membrane proteins. A defined lipid requirement for full activity of some proteins has been demonstrated,[82,83] but the mechanistic nature of the lipid requirement is not yet understood.

To date, there is no compelling evidence to indicate that frozen or partly frozen lipid phases play a physiological role. The ability of divalent cations to trigger lipid phase transitions through interactions with polar head groups and the importance of such cations in many membrane mediated functions suggest, however, that localized changes in membrane lipid physical state could be physiologically significant.

Work in the authors' laboratory was supported in part by U.S.P.H.S. research Grants GM 18233 and AI 10733. C.D.L. was a U.S.P.H.S. predoctoral trainee supported by Grant GM 1531. C.F.F. is the recipient of U.S.P.H.S. Research Career Development Award GM 42359.

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Histone Interactions and Chromatin Structure

Kensal E. Van Holde* and Irvin Isenberg

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

Received March 7, 1975

One of the most perplexing mysteries in molecular biology is how the deoxyribonucleic acid (DNA) is organized in the nuclei of eukaryotic cells. A typical cell contains an amount of DNA which, if extended, would be roughly 1 m long, somehow folded into a nucleus about 10^{-3} cm in diameter. The problem is the more acute because this DNA is not inert, but must be capable of being faithfully replicated, and transcribed (at least in part) into ribonucleic acid (RNA). The mystery becomes even deeper when we realize that these processes must be under subtle and complex control, for while all of the different kinds of cells in a eukaryotic organism contain the same nuclear DNA, only a small fraction of that DNA is expressed by transcription in any given tissue.[1] It seems likely that these two facets of the problem, packaging and the control of transcription, are related. However, we do not yet know enough to be able to state that this is so.

It has long been known that the DNA does not exist in eukaryotic nuclei as the free polynucleotide, but as a complex with proteins and some RNA. This complex contains only a portion of the total protein and RNA of the nucleus and is referred to as *chromatin*. Operationally, chromatin represents that portion of the nuclear content that may be extracted as a unit and be accessible to in vitro study. The assumption is made that this complex is also of significance in vivo; this belief is supported by the fact that isolated chromosomes have a DNA:protein ratio not greatly different from that of chromatin.[2,3]

The proteins of chromatin are of two general classes. First, there are a group of basic proteins called *histones*. In most eukaryotic nuclei there are

Kensal E. Van Holde was born in Eau Claire, Wisconsin, in 1928, and received both his B.S. degree and the Ph.D. from the University of Wisconsin. After 3 years as research chemist at E. I. du Pont de Nemours, 1 year of postdoctoral work at Wisconsin, and 1 year at University of Wisconsin, Milwaukee, he moved to University of Illinois, where he was on the faculty until 1967. He was then appointed to his present position as Professor of Biophysics at Oregon State University. Professor Van Holde's research concerns macromolecular structure studied by physical methods, and nucleic acid–protein interactions.

Irvin Isenberg was born in Philadelphia. He studied for the B.A. degree at Temple University, and received the Ph.D. in physics from University of Pennsylvania in 1950. He has been an investigator at the Institute for Muscle Research, Woods Hole, as well as a research associate in the Department of Anatomy and an Instructor in the Physics Department at the University of Chicago. He is now Professor of Biophysics at Oregon State University. Professor Isenberg's research interests are in fluorescence methods and physical properties of chromosomal proteins.

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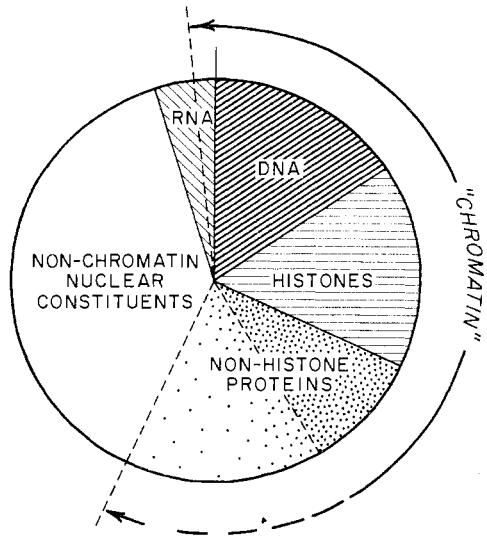

Figure 1. A schematic representation of the composition of chromatin, as contrasted to that of the whole nucleus. Most of the data are derived from the studies of Pomerai et al.,[9] who have compared different chromatin preparation.

only five principal types of these, although each may exist in a number of phosphorylated, acetylated, or methylated variants. The histones are almost always present in an amount roughly equal in weight to that of the DNA.[4-7]

The remaining chromatin proteins are usually called *nonhistone proteins*. Their distinguishing feature is diversity; in contrast to the small number of histones, there are apparently hundreds of different nonhistone proteins in chromatin, many of them present in very small amounts.[7] They include polymerases and other enzymes, perhaps some proteins of regulatory function,[8] and many of unknown role. Figure 1 compares the composition of chromatin prepared in different ways[9] with that of whole nuclei. Obviously the nonhistone proteins represent the most variable component in chromatin, and the amount of these nuclear proteins that should be considered an integral part of chromatin is at present unknown. This uncertainty, however, is not very important for our discussion.

This Account will treat histone–DNA interactions and the interactions of histone molecules with one another, with the idea that such interactions may hold the key to at least the *structural* organization of chromatin. The reader must realize that we are dealing with only one small part of a vast field; for a more general overview he might turn to recent reviews by Ris and Kubai,[10] Comings,[11] or Huberman.[12] We feel that our concentration is justified, however, because it is through the study of these histone interactions that new insights have developed in the last few years. These, together with new information about the internal structure of chromatin, have led to a radical rethinking of earlier models of this complex.

Table I
Properties of the Histones[a]

Histone[b]	Mol wt	Mol % Lysine	Mol % Arginine	
f1, I, KAP, H1	~21,000[c]	24.8	2.6	"Lysine rich"
f2b, IIb2, KAS, H2b	13,774	16.0	6.4	"Slightly lysine rich"
f2a2, IIb1, LAK, H2a	13,960	10.9	9.3	"Slightly lysine rich"
f3, III, ARE, H3	15,273	9.6	13.3	"Arginine rich"
f2a1, IV, GRK, H4	11,236	10.8	13.7	"Arginine rich"

[a] The data given (molecular weights, lysine and arginine contents) are for histones from calf thymus. From "Atlas of Protein Sequence and Structure", M. Dayhoff, Ed., National Biomedical Research Foundation, Silver Spring, Md., 1972. [b] There are a number of nomenclatures in use; in this paper we use that on the left. [c] Histone f1 has not yet been completely sequenced.

The Histone Stoichiometry of Chromatin

The histones are well-characterized proteins; in many cases the amino acid sequence has been determined. Table I presents some fundamental data on the five histones common to most higher eukaryotes. As can be seen, the histones are always very basic proteins, and can be divided into three classes, "lysine rich", "moderately lysine rich", and "arginine rich". Lower eukaryotes, such as the yeasts and some protozoa, appear to exhibit a somewhat different histone composition in their chromatins. It has been reported,[13] for example, that yeast has neither f1 nor f3.

Reasonably precise data on the amounts of the different histones are available for a few chromatins. Table II presents such data obtained by Garrard et al.[7] for rat liver chromatin. These results are in general agreement with those reported for other chromatins of higher eukaryotes,[14-17] although the percentages for f1 and f2a2 are somewhat lower than have been reported in some cases. Excepting f1, there is roughly 1 molecule of each of the histones per 100 base pairs of DNA. The quantity of f1 is especially uncertain, however, because it is very prone to proteolysis.[18] The last column in Table II shows that there are not enough basic residues in the histone complement of chromatin to neutralize all of the phosphates in DNA. This means that at least part of the DNA phosphates must either be exposed to the solvent or complexed to nonhistone protein, inorganic ions, or small organic molecules. In fact, a number of lines of evidence[19-22] indicate that only about

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Table II
Histone Stoichiometry of Rat Liver Chromatin[a]

Histone	mg of histone/ mg of DNA	Molecules of histone/100 base pairs	Basic residues[b]/ 200 phosphates
f1	0.110 (10.5%)	0.33	21
f2b	0.270 (25.7%)	1.25	39
f2a2	0.157 (14.9%)	0.72	22
f3	0.312 (29.2%)	1.31	43
f2a1	0.202 (19.2%)	1.15	31
Total	1.051 (100.0%	4.76	156

[a] Data adapted from Garrard et al.[7] [b] Basic residues include arginine, lysine, and histidine. There are 200 phosphates in 100 base pairs.

100–120 phosphate residues out of every 200 are not available to metal ions, dyes, or polylysine. This suggests that not all of the basic residues on the histone molecules are involved in neutralizing DNA phosphates.

The Individual Histones

Studies of the physical properties of histones have long been plagued by a number of severe problems. These problems stem basically from the procedures used to prepare pure histone fractions. Such methods use either acids, or nonaqueous solvents, or mixtures of these. The widely used methods of Johns,[23] for example, use perchloric acid and mixtures of ethanol and hydrochloric acid during extraction procedures. Such solvents almost always denature proteins, and there is every reason to believe that the histone fractions obtained by using them are indeed unfolded, and in a more or less random coil conformation.[24–34]

Recently, histone solutions have been prepared by using relatively mild extraction procedures.[35–37] These methods do not yield pure histone fractions. They yield histone complexes which, as will be seen, are important.

Let us now suppose that we have prepared pure

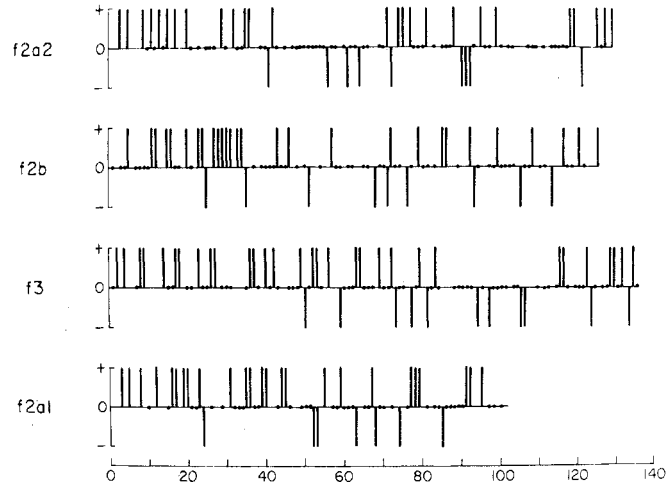

Figure 2. Diagrammatic representations of the amino acid distributions of four of the calf thymus histones. Upward-projecting bars denote lysine and arginine, downward-projecting bars denote aspartic and glutamic acids, and dots denote the hydrophobic residues, Val, Met, Ile, Leu, Tyr, Phe, Pro, and Ala.

histone fractions and we have them in a denatured state. If we now wish to renature the histones, we are faced with a problem: what criterion should we use to recognize a native state? In studying the renaturation of enzymes there is the obvious criterion of enzymatic activity, but with histones we have no obvious direct test. We shall see, however, that an indirect criterion may be used, one that is satisfactory, at least in part.

The problems just described are joined to others, no less severe. Histones tend to aggregate to very large polymers in solution. (In this Account, we use the term "aggregation" to denote the formation of such large polymers. The formation of small specific oligomers, like dimers and tetramers, will not be called "aggregation".) Since the work of Cruft et al.[38] in 1958, it has been known that the aggregation depends on a variety of factors—salt concentration and temperature—but reasonable methods for avoiding the aggregation were not forthcoming at that time. Furthermore, in 1958 no one knew how many types of histones existed nor how to prepare pure histone fractions. With the realization that there were only five major classes of histones,[16,23,29–40] the groundwork was laid for basic physical studies.

The next major step forward was the determination of the amino acid sequence of f2a1;[41,42] since then, three other histones have been sequenced.[43–45] In Figure 2 the distribution of basic, acid, and hydrophobic residues is shown. One striking feature is immediately evident: the molecules have very heterogeneous distributions. The N-terminal end of each his-

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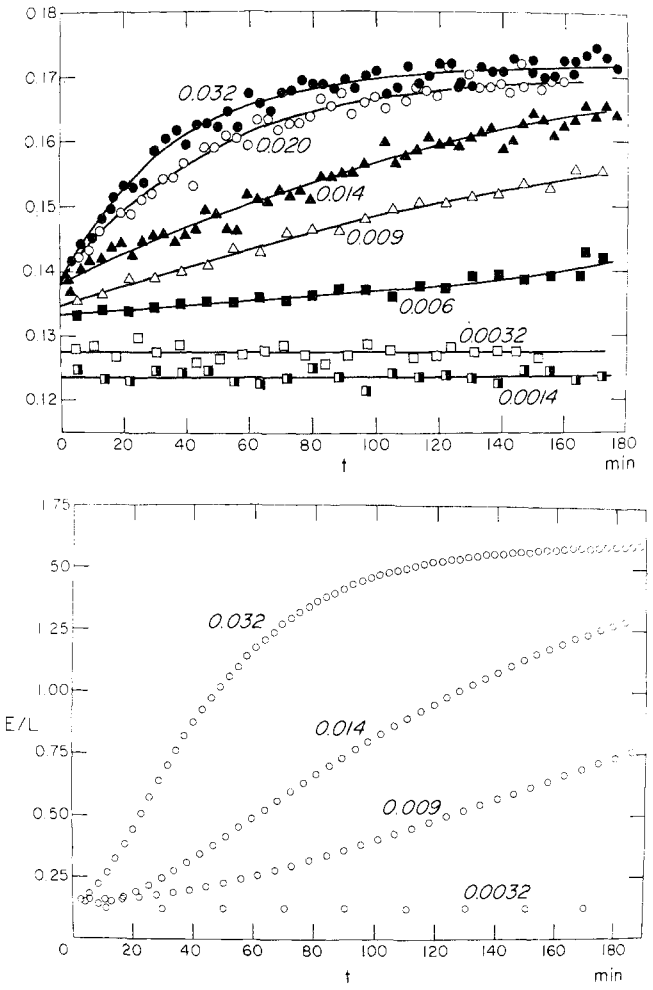

Figure 3. Fluorescence anisotropy, r, and light scattering, E/L, for histone f3 as a function of time at a number of phosphate concentrations (shown on curve), pH 7. The anisotropy without salt is 0.10. The anisotropy at zero time is a function of salt concentration; the scattered light is not. Aggregation occurs only during the slow step. The fluorescence was measured at 325 nm and excited at 279 nm. The light scatter was at 365 nm. The histone concentration is 10^{-5} M.

tone is highly basic. On the other hand the C-terminal half of the molecule has an amino acid composition and distribution that is similar to that usually found in globular proteins. It is as if histones f2a1, f2a2, f2b, and f3 were each composed of two different proteins, covalently linked into a single polypeptide chain. But from this it follows that, if salt were added to a histone solution in water, at least part of the molecule might refold in a highly cooperative way that would be similar to the renaturation of an enzyme. Studies on protein folding are best carried out in dilute solution, and we have employed techniques that are sensitive and capable of measuring small changes in dilute solution: fluorescence polarization, circular dichroism (CD), and light scattering.

Structural Changes in Histones

Histones dissolved in water show little structure.[24,27,28,30-34,46-48] The CD spectrum resembles that of a random coil, and the polarization of the fluorescence of the tyrosines is very low, indicating that the tyrosines can rotate easily.[30-34]

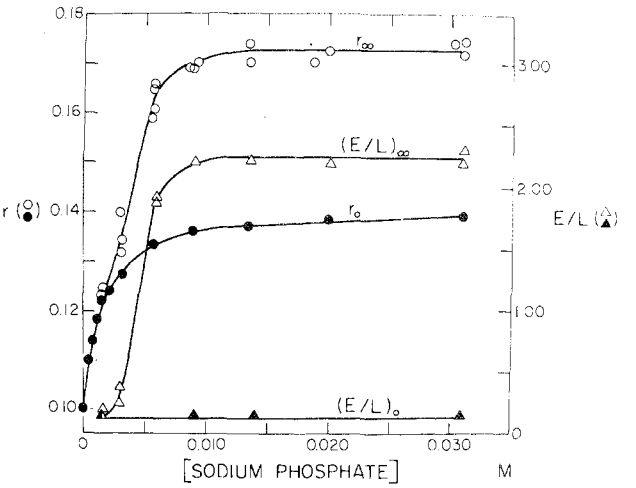

Figure 4. Limiting values of the anisotropy, r, and light scattering, E/L, at zero time and at infinite time.

Upon the addition of salt to approach concentrations characteristic of physiological conditions, both the CD and anisotropy of the fluorescence of all of the histones undergo immediate changes. At low protein concentrations, of the order of 10^{-5} M, the immediate change in histones f2a1 and f3 is followed by a slower change which takes minutes or hours, with the rate depending on the histone concentration, the type of salt used, the salt concentration, and the temperature. Histones f2a2 and f2b, at 10^{-5} M concentration, show only immediate changes.

These changes have been called the fast and the slow step, respectively. The decomposition of the salt-induced changes into fast and slow steps introduces a great simplification into the study of histones, since these steps are quite different processes, and by studying them separately it is possible to understand them.

Figure 3 shows typical data for the fluorescence anisotropy and scattered light intensity as a function of time. The anisotropy data show both the fast and slow steps. (The existence of the fast step is seen as a change, at $t = 0$, of the anisotropy as a function of salt). However, the light-scattering data have different character; they show only a slow step. Since the scattered light depends on the aggregation, it follows that there is no aggregation during the fast step. Aggregation occurs only during the slow step. This is verified in Figure 4 where limiting values of the anisotropy and scattered light, at zero time and at infinite time, are plotted as a function of the phosphate concentration. Below a critical value of the salt concentration, the anisotropy at infinite time, r_∞, equals the anisotropy at zero time, r_0; there is no slow step. There is, however, a fast step because r_0 rises with increasing salt. However, the scattered light at zero time does not change with salt.

It must be emphasized that the fast change is not simply the beginning of the slow change. These are completely different events. They are separable, at low concentrations of protein, because they occur on completely different time scales. The fundamentally different nature of the two processes is verified by CD studies.[30-34] In the fast step, there is α-helix formation; in the slow step, β sheet is formed.

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As noted above, from the amino acid sequences of the histones, we suspect that the molecules can undergo highly cooperative foldings, much like the renaturation of a denatured globular protein. The processes may differ in that in globular proteins the entire molecule folds, but in a histone perhaps only part of the molecule folds. We now identify this folding as the fast step. If this identification is valid, the folding would be highly cooperative and might be approximated by a two-state model—an equilibrium between a denatured and a renatured state. Such a model has been tested and the data do fit a two-state model for the fast change in three of the histones—f2a1, f2b, and f3.[30-32,34] Histone f2a2 is anomalous,[33] but the fast step is highly cooperative even here.

At any given salt concentration there is a simple equilibrium between the number of molecules in the denatured state and the number in a renatured state. The ratio of these numbers is the equilibrium constant, and this equilibrium constant may be measured both by fluorescence anisotropy techniques and by CD techniques. If a highly cooperative folding does indeed occur, then these two equilibrium constants should be equal to one another since both techniques would be measuring two aspects of the same folding. This is an important test because circular dichroism and the anisotropy of fluorescence are sensitive to two completely different properties of the protein. The CD is a function of the orientation of the peptide planes relative to one another, while the anisotropy is a function of the rigidity of the transition moments of the tyrosines. Experimentally, the folding when measured by CD is indistinguishable from the folding when measured by the anisotropy of the tyrosine fluorescence, which shows that the fast step folding is indeed highly cooperative.[30-32,34]

The fast step folding resembles in every way the renaturation of a denatured enzyme. While this, in itself, does not demonstrate that the fast step product is the native form of a histone molecule, it strongly suggests that it is or, at least, that it is close to it.

Appreciable fast change folding occurs at 0.15 *M* NaCl or 5 m*M* phosphate—both typically physiological values. Thus, it is reasonable to expect this folding in vivo.

Specific Histone–Histone Interactions

Histone f2a1 has a slow aggregation step; f2b has not. If f2b and f2a1 are mixed in 1:1 ratios, however, the aggregation of f2a1 is blocked.[49] Even more dramatic is the effect of f3 and f2a1 on one another. Both histones individually have slow aggregations. Together, neither does[50] (Figure 5).

All of the four histones, f2a1, f2a2, f2b, and f3, complex with one another.[50,51] However, the interactions are highly specific, and the binding constants and other properties show considerable variation from one complex to another.

The stoichiometry of the interaction may be examined by continuous variation methods[49-52] which show that optimal interaction occurs at 1:1 molar ratios. A continuous variation plot also helps determine the fraction of molecules that is complexed. To obtain a binding constant one must also know how many molecules of each type are in the complex; i.e., is the complex a dimer, tetramer, hexamer, or some other 1:1 species? This, of course, is answered by a molecular weight determination which, together with the stoichiometry, also determines how many molecules of each type are in the complex.

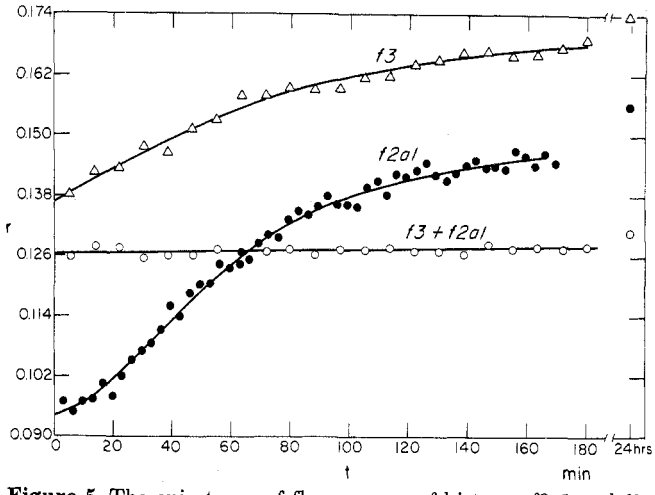

Figure 5. The anisotropy of fluorescence of histones f2a1 and f3, separately and in a 1:1 mixture; 0.016 *M* phosphate buffer, pH 7.0. Each histone had a concentration of 10^{-5} *M*.

Sedimentation-equilibrium data show that f2a1–f3 is a tetramer[53] and f2a2–f2b[51] and f2a1–f2b[54] are dimers. The determination of the molecular weights of the other complexes is not as easy. The pairs f2a1–f3, f2a2–f2b, and f2a1–f2b have the highest binding constants. In solutions of these histone pairs, aggregation is blocked. This is not true for the other complexes, and attempts at measuring the molecular weights must therefore be made in solutions that are slowly aggregating. This is technically difficult.

A number of other laboratories have also reported the existence of one or another histone complex. Skandrani et al.[55] showed that f2a2 and f2b interacted during guanidine hydrochloride gradient chromatography on Amberlite. Kelley[56] observed the same complex in studies of chromatographic fractions of mixed histones. Clark et al.[57] reported that the ^{13}C nuclear magnetic resonance of mixtures of f2a1 and f2a2 was not the sum of the resonances in the individual proteins. They concluded therefore that the histones interacted. Kornberg and Thomas[36] and Roark et al.[37] both reported the existence of the f2a1–f3 tetramer and Kornberg and Thomas showed that f2a2 and f2b could be cross-linked in solution by dimethyl suberimidate.

Table III and Figure 6 summarize the data currently known about the complexes. Perhaps the most important point to be seen is that the interactions are both strong and highly specific. Furthermore, in the three strongest complexes, the amount of α helix is

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Table III

Complex	α-Helix formation, residues/complex	*K*	β-Sheet formation?
f3–f2a1	9	$0.7 \times 10^{21}\ M^{-3}$	No
f2a2–f2b	15	$10^{6}\ M^{-1}$	No
f2b–f2a1	8	$10^{6}\ M^{-1}$	No
f2a2–f3	0	$0.1-1 \times 10^{6}\ M^{-1}$	Yes
f2a2–f2a1	1	$0.04 \times 10^{6}\ M^{-1}$	Partially
f3–f2b	0	?	Yes

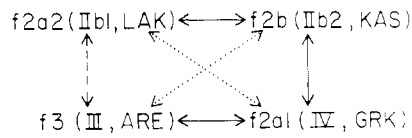

Figure 6. Histone cross-complexing pattern. Solid lines show very strong complexes. Dashed line shows a somewhat weaker complex, while dotted lines show considerably weaker complexes.

greater in the complex than it is in the individual histones. In other words, the secondary structure changes upon complexing. It is difficult to imagine the secondary structure changing without the tertiary structure also changing; a general alteration of the protein conformation probably occurs. This implies that some sort of induced fit occurs upon complexing, so that the subunits of the complex change their conformation to form a tight compact structure. The structure is a new, higher order, entity.

It has long been recognized that the histone molecules, by their basic nature, are well suited to interact with the polyanion, DNA. The discovery of this other class of interactions, strong specific complexing *between* histones, suggests that both types of interaction might be of importance in determining chromatin structure.

The Supercoil Model of Chromatin Structure

How are the DNA and protein structurally combined in chromatin?

The idea that has, until very recently, dominated the thinking of most investigators might be termed the "supercoil model". The model was proposed on the basis of low-angle X-ray diffraction studies of chromatin fibers and gels.[58,59] It should be emphasized that the diffraction rings observed are neither very sharp, nor do they ever exhibit evidence for any high degree of structural orientation, even in fibers. The intensities, positions, and even existence of some of the rings depend upon the mode of preparation of the sample, and in gels, upon the concentration of the gel.[60] Nevertheless, in many instances a series of reflections corresponding to spacings of about 110, 55, 38, 26, and 22 Å have been observed. In addition, there is seen a weakly oriented 3.4-Å reflection, corresponding to the base spacing in the DNA helix. It was proposed[41] that the 110-Å–22-Å set are successive orders of reflection from a supercoil of about 120-Å pitch and 100-Å diameter.

A somewhat different model, a nonuniform supercoil with similar diameter but smaller pitch, has been proposed by Bram and Ris.[61] Such models can account for the compact packing of the DNA in chromosomes, and have been interpreted as not inconsistent with a number of electron microscope studies of chromatin.[61-63] These show irregular bumpy fibers of a number of thicknesses, with regions about 100 Å in diameter being relatively common. For these reasons, and for want of any evidence for any other kind of structure, the supercoil model has dominated thinking for a number of years.

Nevertheless, it has been widely recognized that the available X-ray data do not dictate a unique solution. Very recently, neutron diffraction studies[64] have shown that the basic assumption of the supercoil model, the idea that the 110-Å–22-Å series represents successive orders of a single reflection, may be in error. The protein and DNA fractions of chromatin appear to contribute quite differently to the various reflections. Thus, a quite different kind of structure is indicated; this had already been demonstrated by experiments of another kind.

Subunit Structure in Chromatin

In the period from 1970 to 1973, a number of laboratories began doing experiments which eventually led to a quite different structural model for chromatin. These experiments involved the digestion of chromatin by endonucleases. The early work of Clark and Felsenfeld[20] showed that only about 50% of calf thymus chromatin could be readily digested by staphylococcal nuclease. Furthermore, the protected regions of DNA were surprisingly small; the most recent estimates report them to be about 110 base pairs.[65] While there has been some argument about the precise interpretation of the Clark and Felsenfeld experiments,[22,66,67] the existence of small protected regions suggested that chromatin might have some kind of repetitive structure. This concept was supported by the elegant studies of Hewish and Burgoyne.[68] They showed by digesting chromatin with endogenous nuclease that the DNA was released in a set of *discrete sizes,* which appeared to be integral multiples of the smallest. Intact nuclei were used; the very specific digestion cannot therefore be an artifact of chromatin preparation. Furthermore, endonuclease digestion of free DNA, as opposed to chromatin, does *not* lead to discrete sizes; a broad continuum results. Thus, the result must be explained by some feature of *chromatin* structure, rather than as a direct consequence of features in the DNA sequence.

The properties of these nucleoprotein complexes remaining after limited nuclease digestion of chromatin have been investigated by Rill and Van Holde[69] and Sahasrabuddhe and Van Holde.[70] The nuclease-

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Table IV
Properties of PS particles and Trypsin-Treated PS particles (TPS)[a]

	PS	TPS
$s_{20,w}$, S	10–12	6–7
Mol wt	176,000	158,000
f/f_0	1.1	2.0
Protein/DNA, mg/mg	1.53	1.25
Melting point, °C	84	56–60
$s_{20,w}$ of DNA, S	4.8–5.4	4.8–5.0

[a] Data of Sahasrabuddhe and Van Holde.[70]

resistant fragments, termed PS particles, were shown, in agreement with Clark and Felsenfeld, to contain about 50% of the DNA. The average DNA fragment size corresponded to 110–120 base pairs, or 70,000–80,000 daltons. Associated with this was about 100,000 daltons of protein. Hydrodynamic measurements indicated that the particles were very compact; the frictional coefficient, f/f_0 = 1.1, is a value typical of many globular proteins. Although these particles each contained a segment of DNA which, if extended in B form, would span about 400 Å, the Stokes law diameter of the particles was found to be only about 80 Å. It was further found that even a mild digestion of these particles by the protease trypsin, which resulted in only slight loss of protein, led to a pronounced increase in the frictional coefficient. This suggested that the compact structure of these particles was maintained by the protein. These results are summarized in Table IV.

The particulate structure of chromatin has also been demonstrated in electron microscope studies. Woodcock[71] and Olins and Olins[72,73] have reported "string of beads" structures of the kind shown in Figure 7. The globules, about 80 Å in diameter, are connected by thin strands. The particles have been termed "ν-bodies" by Olins and Olins. The similarities between ν-bodies and PS particles are obvious, and have been further confirmed by electron microscope studies of isolated PS particles.[74] Occasionally, particles in PS preparations were observed to carry single or double tails about 20 Å in diameter, and a number of doublet particles were observed, connected by thin strands. Langmore and Wooley[75] using a high-resolution scanning transmission electron microscope on unstained, vacuum dried preparations found similar structures, although the globules observed by this technique are flatter, being about 120 Å in diameter by 40 Å thick.

Recently, measurements have been reported of the sizes of DNA contained in the monomer and multimer fragments produced by very mild digestion of chromatin in nuclei. These results indicate a somewhat larger DNA piece in the monomer than the 120 base pairs found in the PS particles. Values in the neighborhood of 200 base pairs,[76,77] or 185 base pairs,[78] or "from 110 to 175 base pairs" [79] have been given. Similarly, studies of chromatin fragments prepared by ultrasonication[80] indicate a monomer fraction of molecular weight about 3×10^5, carrying a DNA piece of about 200 base pairs.

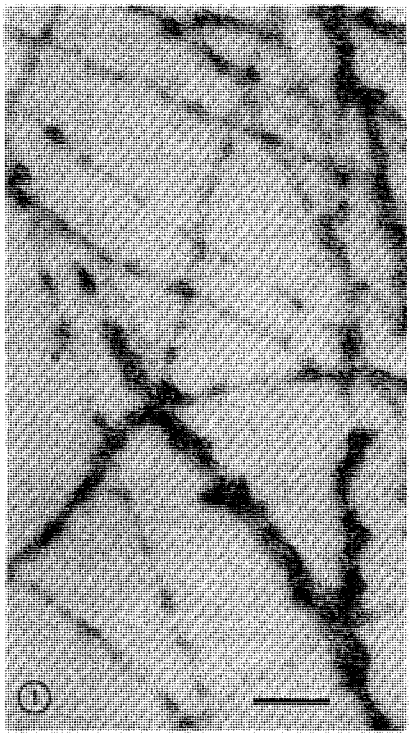

Figure 7. An electron micrograph of chromatin (C. L. F. Woodcock, unpublished results).

While there is some uncertainty in all of these numbers because of the present limitations in methods used for measuring the size of small DNA molecules, and because the subunits always appear to be hetergeneous, it is becoming clear that the "monomer" is larger than the "PS particle". What does this mean?

A possible explanation which may shed some light on the internal structure of the monomer comes from the observation that the monomer DNA band first obtained by limited digestion of nuclei is of somewhat higher molecular weight than the DNA band obtained from a longer digestion of the same chromatin[79] (see Figure 8). The chromatin appears to be first cut into multimers and monomers; the latter are then rapidly further degraded into a quite stable structure, the PS particle. Recent data (B. R. Shaw, T. Herman, and K. E. Van Holde, unpublished) indicate that the initial monomer size is about 170 base pairs; it is then degraded to a relatively stable unit similar to the PS particle. Prolonged digestion can eventually fragment these particles.

These results can be most simply explained by a model like that shown in Figure 9. Compact, condensed regions with coiled or folded DNA (the PS particles or ν-bodies) alternate with regions of extended DNA (which we call "spacer" regions) of

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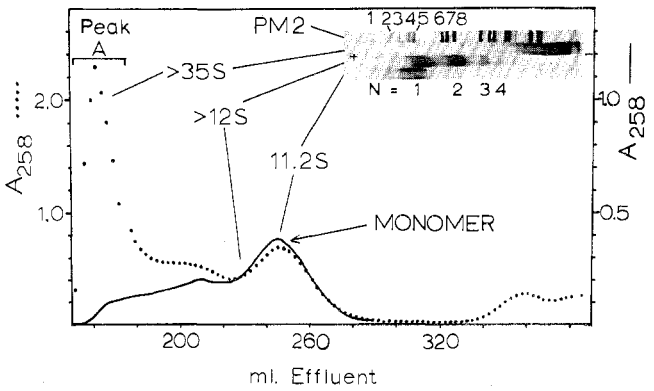

Figure 8. Chromatograms of nuclease-resistant chromatin particles on Bio-Gel A-5m. The dotted line shows results obtained when calf thymus nuclei (2×10^8 nuclei/ml) were incubated for 1.5 min at 37° in the presence of 185 units of staphylococcal nuclease per ml. After termination of nuclease activity and centrifugation, the supernatant was applied to the column and eluted with 10 m*M* Tris–0.7 m*M* EDTA, pH 7.3. Sedimentation coefficients are shown for three fractions. The electrophoretic profile of DNA isolated from these fractions is also shown (as negative prints). PM2 phage DNA cleaved by endonuclease R Hae III (from *Haemophilus aegyptius*) was used for molecular weight markers. Preliminary values for the numbered bands are (in base pairs): 1, 55; 2, 85–90; 3, 105–110; 4, 130–135; 5, 141–145; 6, 240–245; 7, 263–268; 8, 295–303. Note that the monomer band in the >12S fraction is somewhat larger than the corresponding band from the 11S fraction. The solid line depicts results of redigestion of peak A. The excluded chromatin particles were concentrated to 8 A_{260} units per ml and reincubated with 70 units of staphylococcal nuclease per ml for 30 sec at 37°. The redigested material was made 7% in sucrose, applied to the column, and eluted as above.

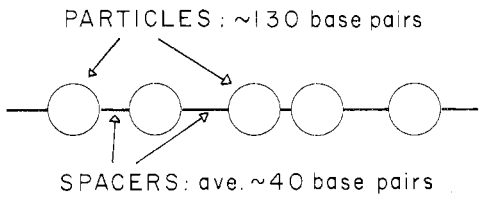

Figure 9. An attempted reconciliation of the various data on "subunits" and "PS particles" with electron microscope results. The size estimates are based on the most recent results (B. R. Shaw, T. Herman, and K. E. Van Holde, unpublished.

varying length. Very mild digestion or sonication will break the strands randomly in the spacer regions; the first monomers to be produced will then contain 150–200 base pairs of DNA. The exposed DNA "tails" can then be rapidly degraded, leading ultimately to individual PS particles or ν-bodies.

This picture is consistent with electron microscope studies of both whole chromatin[71–73] (see Figure 7) and PS particles[74] and with the studies of the time course of digestion. Furthermore, such a model can at least partially reconcile the disparities in estimates of the subunit size. Such a model does not require that the spacer DNA be devoid of proteins, but only that it be much less protected than the DNA in the particles.

What is the Internal Structure of the Subunits?

Studies of histone interactions and subunit structure in chromatin have suggested the idea: could not the specific histone–histone complexes be responsible for the repetitive protected regions in chromatin? Kornberg,[81] who with Thomas[36] had demonstrated the existence of tetramers of histones f2a1 and f3, and oligomers of histones f2a2 and f2b, proposed that an octamer, having two molecules each of these histones, per 200 base pairs of DNA, comprised the repetitive unit. Histone f1 was excluded from this list, and this is reasonable, since there are many lines of evidence to suggest that this histone plays a quite different role than the others. Removal of histone f1, for example, has no discernible effect on the characteristic low-angle X-ray pattern of chromatin.[59] In addition, Shaw et al. (unpublished) observed that f1 is present in multimers but not in monomers, a result consistent with the idea that f1 may be associated with the interparticle regions.

Recently, direct evidence has appeared for the existence of a specific histone pair in chromatin: f2b–f2a1. Martinson and McCarthy[82] used tetranitromethane (TNM) to cross-link histones. TNM is presumably a zero-length cross-linker binding two tyrosines together. For the methodology to succeed, a complex must have a tyrosine of one histone in close proximity to a tyrosine of another histone. Martinson and McCarthy found unambiguous evidence for an f2a1–f2b cross-linked complex. Such complexes were also found in reconstituted nucleohistones, but in order to obtain cross-linked f2a1–f2b it was necessary for f2a2 to be present also. This suggests that the existence of the f2a1–f2b complex is linked to the presence of the f2b–f2a2 complex.

Martinson and McCarthy also found that the order of mixing is critical in reconstitution. If the histones are added sequentially to the DNA, no cross-linked dimer can be found. The histones must first be mixed and then added to the DNA. This demonstrates that histone complexes, and not individual histones, are needed for reconstitution.

A set of eight molecules, two of each of histones f2a1, f3, f2b, and f2a2, has a molecular weight of 109,000, quite close to the protein mass of 100,000 daltons found in each PS particle by Sahasrabuddhe and Van Holde.[70] Thus, the data seem to be consistent with a model in which this protein mass exists in only a portion of the subunit, the most nuclease-resistant segment. The picture is also generally consistent with the stoichiometric data given in Table II. Note that there are a little more than two each of histones f2a1, f3, and f2b per 200 base pairs of rat liver chromatin; there would be almost exactly two of each per 160 pairs. Histone f2a2 appears to be somewhat less frequent; if this difference is real, it may mean that not all subunits are alike. Other chromatins from higher eukaryotes show slightly different compositions; to what extent this reflects real differences or only difficulties of measurement is hard to judge at the present time. In any event, the stoichiometric data are generally consistent with a repeat in the range of 150–200 base pairs, but do not in themselves indicate how the histones are distributed over the subunit.

It is possible that the internal structure of the subunits may differ throughout the evolutionary scale, although the basic subunit pattern may remain. It has been reported[13] that yeast chromatin lacks histone f3 as well as f1. Nevertheless, upon digestion, either in nuclei or after extraction as chromatin, it

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yields a pattern of very sharp DNA bands.[83] The molecular weights of these bands correspond very closely to integral multiples of about 135 base pairs, with perhaps a small "spacer" of about 10 base pairs.

As to the details of structural organization, we can only speculate at the moment. Van Holde et al.[84] have proposed a model in which the globular C-terminal portions of the histones aggregate to form a core, about which the DNA is wrapped. The projecting N-terminal basic regions are then assumed to wrap into the major groove of the DNA, holding it to the core.

The idea that much of the DNA is wrapped on the outside of the subunit gains credence from the results obtained by Noll[85] with DNAse I, in which digestion to fragments which are multiples of 10 base pairs was found. Apparently the DNA in the particles is at least partially accessible to some nucleases.

It is clear that at this point our views of the structure of chromatin are changing rapidly. The next few years should be interesting.

The work of K. E. Van Holde was supported by NSF Grant GB-37307X; that of I. Isenberg was supported by U. S. Public Health Service Grant CA-10872. We thank Dr. C. L. F. Woodcock for permission to use an unpublished electron micrograph. Drs. Martinson and McCarthy permitted the authors to read their paper prior to publication, and we thank them.

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Antibodies: Analytical Tools to Study Pharmacologically Active Compounds

John J. Langone, Helen Van Vunakis, and Lawrence Levine*

Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

Received February 7, 1975

Competitive protein binding assays currently are being used to quantitate many different types of compounds.[1] They depend upon the affinity of a biological macromolecule for a particular ligand (e.g., intrinsic factor for vitamin B_{12}, thyroxine-binding globulin for thyroxine, a tissue receptor for a hormone, or an antibody for an antigen). Their sensitivity is made possible through the use of radioactive tracer molecules of high specific activity. The extent to which unlabeled ligand competes with radioactive ligand for a limited number of receptor sites serves as a basis for quantitation in this type of assay, and often picogram amounts of a particular compound can be estimated. Once the receptor molecule and labeled ligand are available, the assays are relatively simple to perform: mixtures containing labeled ligand, receptor molecule, and sample are incubated, free labeled ligand is separated from receptor-bound labeled ligand, and the extent of binding is determined.

Although natural receptors have been employed in several competitive binding assays, their use is limited by their availability and stability. The organic chemist, however, by designing proper antigens, often can produce antibodies in experimental animals that have the specificity and affinity required of a receptor molecule.

Radioimmunoassay (RIA). *Antibodies* are proteins found in the globulin fractions of blood that are produced by vertebrates in response to the presence of an *antigen,* i.e., a substance that is recognized by the host to be foreign.[2] Although antibodies are generally regarded as defensive weapons elicited for protection against invasion by pathogenic microorganisms, they also can be produced against specific low molecular weight compounds if these are covalently linked to macromolecules. Antibodies can show a remarkable ability to bind selectively the antigen that stimulated their production. Their *specificity* may be regarded as comparable to that of an enzyme for substrate. The binding constants between antibody and antigen are often of the order of 10^7 to 10^9 l./mol. This ability of antibodies to discriminate between the homologous antigen and the myriad of other compounds of widely diverse structure that are found in biological fluids, e.g., serum or urine, is of fundamental importance in their use as analytical tools.

The principle of the RIA technique was described by Berson and Yalow in 1960[3] (Figure 1). Constant amounts of free labeled antigen (Ag*) and a limited

John J. Langone is Senior Research Associate in the Biochemistry Department at Brandeis University. He received his Ph.D. degree in 1972 from Boston University in organic chemistry. His current interests include bioorganic chemistry, immunopharmacology, and immunochemistry of biologically active drugs.

Helen Van Vunakis is Professor of Biochemistry at Brandeis University. She received her Ph.D. degree in biochemistry at Columbia University, then held a postdoctoral position in the Department of Biochemistry at Johns Hopkins University, School of Hygiene and Public Health. Her research interests include structure and functions of proteins and nucleic acids, and immunochemistry and biochemistry of pharmacologically active compounds.

Lawrence Levine is Professor of Biochemistry at Brandeis University. He obtained a Sc.D. from the Johns Hopkins University, School of Hygiene and Public Health. His research utilizes immunochemical procedures for the study of biological problems.

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