THE FOLDING OF CHROMATIN

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I. INTRODUCTION

Chromatin is the nucleoprotein complex found in the nucleus of all eukaryotic cells. It undergoes obvious changes during the cell cycle and varies in form from the highly compact chromosomes of metaphase, which are readily visible even in the light microscope, to the much more diffuse structures present in the nucleus throughout much of the cycle. However even this "diffuse" structure must involve a considerable compaction of the cellular DNA for the whole length to be packed into the nucleus which is about 10 μm in diameter.

This compaction is brought about by a series of levels of folding and these form the subject of this review. At the lowest level, the DNA interacts with the histones to form a repetitive structure, which is the fundamental form of chromatin. While this underlying structure will be described briefly, it has been reviewed elsewhere recently[1] and most attention will therefore be paid to the further levels of folding or "higher order structure", which is also the more controversial.

II. UNDERLYING STRUCTURE

A. Gross Organization

Chromatin occurs in the nucleus with approximately twice the mass of protein as of DNA. The protein consists of roughly equal weights of histones, which are very basic proteins, and other, so-called "nonhistone", proteins which have a much wider range of properties and range from acidic to mildly basic in composition. There are five main types of histone and a complete primary sequence is available for each type in at least one organism. One striking feature is the extremely high conservation of sequence in both H3 and H4 throughout most eukaryotes in which the sequence has been determined. The general properties of the histones are shown in Table 1 (for full review see Reference 2).

Unlike the histones, there are many nonhistone proteins and individually they are present in much smaller amounts than any histone. The five histones are present in comparable molar amounts, but even the major nonhistone proteins are present at concentrations only about an order of magnitude lower and many of them are present in very small amounts indeed. Thus while the quantities of the histones make them obvious candidates for the general packing of the DNA in chromatin, the nonhistone proteins are more likely to be involved in catalytic or control functions and some of the major ones have been extensively studied (for recent symposium see Reference 3). Besides a possible control function in modulating the folding of chromatin, most probably at the higher levels, some of the nonhistone proteins may also be involved in the actual formation of the structure, but acting so that a single protein molecule may have an effect on a considerable length of chromatin. This possibility is discussed below (Section IV.A).

Table 1
GENERAL PROPERTIES OF THE HISTONES

Histone type	Histone	Molecular weight (M_r)	Degree of conservation
Core histones — can assemble DNA to particles	H3	15,400	Highly conserved
	H4	11,340	Highly conserved
Core histones — more pheripheral	H2A	14,000	Moderate variation between tissues and species
	H2B	13,770	Moderate variation between tissues and species
H1-like histones	H1	21,500	Varies markedly between tissues and species
	H1°	~21,500	Variable, mostly present in nonreplicating cells
	H5	21,500	Very variable, only present in transcriptionally inactive cells of some species

The size of the DNA in a chromosome suggests that it is impossible for it to be folded in a unique and specific fashion, but rather it must be packaged into a repeating structure. If this repeating structure is then packaged in an ordered fashion, this will achieve a high density of packing. This is supported by observations on the X-ray scattering from fibers.[4-6] which showed a simple series of low angle reflections or bands at about 11, 5.5, 3.7, 2.7, and 2.2 nm. This means that there is some repetitive substructure in chromatin, on the scale of about 11 nm. Biochemical evidence from the use of nuclease digestion to probe the organization of chromatin[7-10] also supported the hypothesis of a repetitive substructure as digestion of chromatin produced multimers of approximately 200 base pair lengths of DNA. The use of different nucleases showed that this was not a property of a specific enzyme,[8,10;] and it could be concluded that there was a basic repeating pattern in the organization of the majority of the chromatin. While the length of this repeat has since been shown to vary somewhat, not only between types of nucleus[11-14] but within individual nuclei,[15] so that all substructures are not identical even within a single nucleus, this does provide a general motif which can be used many times. This subunit is called the "nucleosome"[16] and, as originally suggested by Kornberg in 1974,[17] it consists of approximately 200 base pairs of DNA, 2 each of the 4 histones H2A, H2B, H3, and H4 and a single molecule of histone H1.

One of the problems in folding and compacting DNA lies in its stiffness, which is usually measured as the "persistence length". The more flexible the rod, the shorter will be the persistence length. For double helical DNA at ionic strength above 1 m*M*, the persistence length is about 50 nm,[18] corresponding to approximately 150 base pairs of DNA and correlating closely with the shortest lengths of DNA which can readily circularize by having their ends joined to give covalently closed circles.[19] A circle with circumference 50 nm will have a radius of about 8 nm and this therefore is the smallest radius of curvature around which free DNA will readily bend.

In practice, electron micrographs of chromatin show a variety of appearances from "beads-on-a-string", with the "beads" about 10 nm diameter,[20] through a fairly uniform fiber of 10 nm diameter[21] to a less uniform 30 nm fiber[22] (these are discussed in detail below). The compaction of the DNA in chromatin does not allow it to be stretched straight along the 10 nm fibers, and it must be folded with a maximum radius of curvature of 5 nm. This folding can only come about by a strong enough interaction with the histones to overcome the

histones'', H2A, H2B, H3, and H4.[23-26] H1 appears to be lost concomitantly with digestion below 165 base pairs[27] and the particle containing this length of DNA, the 8 core histone molecules, and 1 molecule of H1 has been described and called a ''chromatosome''.[28] The additional DNA in the nucleosome repeat, joining the nucleosome cores or, perhaps more precisely, the chromatosomes, is often referred to as ''linker DNA'', although under native conditions it is certainly not stretched out and the ''beads-on-a-string'' appearance is only seen at very low ionic strength or under partially denaturing conditions. Variations in the length of this linker DNA appear to account for the range of nucleosome repeat lengths, while the length on the chromatosome remains constant. Particularly if the linker is considered to be between chromatosomes, it may vary from essentially nil length for ''short repeat'' chromatin up to about 45 base pairs in erythrocyte chromatin.

The exact stoichiometry of H1, and its related variants such as H5, is somewhat more complicated, and the measurements have not always been accurate. However, careful quantitation of staining with histones from rabbit thymus nuclei[29] and use of in vivo radiolabeling of mouse myeloma cells,[30] both led to estimates of about 1 H1 molecule per core histone octamer. Recently in vitro radiolabeling of total histones from several sources[31] has shown some variation in the stoichiometry. While lymphocytes and glial cells were found to contain almost exactly 1.0 H1 per octamer, liver cells from several species contained approximately 0.8 molecules per octamer. In chicken erythrocyte nuclei, where H5 occurs as well as H1, there were 0.9 molecules of H5 and 0.4 molecules of H1 per core octamer. Hence although the stoichiometry has not been found to be constant, it appears to be close to 1 H1-like histone per nucleosome and no accurate measurement has given a value approaching 2.

2. Structure

a. The Nucleosome Core

Nucleosome core particles have been extensively studied by X-ray and neutron diffraction. The earliest available crystals of core particles were found to have partially proteolysed histones, but the physico-chemical properties of the particles were still very similar to those with intact histones. A low resolution picture of the nucleosome core was obtained using a combination of X-ray diffraction and electron microscopy on these crystals,[32] showing it as a flat circular disk, somewhat wedge-shaped and strongly divided into two layers. The overall dimensions were about 11 nm × 11 nm × 5.7 nm. This fitted with a model containing a central core of the histone octamer surrounded by DNA wound into about $1^3/_4$ turns of a shallow superhelix of pitch about 2.7 nm.

Crystals have since been obtained containing intact nucleosome cores, which diffract to higher resolution but otherwise have a unit cell very simlar to that for the proteolyzed material. The crystals are indistinguishable when the material comes from a wide variety of sources,[33] suggesting that the nucleosome core structure is universal throughout eukaryotic cells. The high angle diffuse X-ray scattering from the crystals shows that the DNA is in a B-type structure, and the data also show the presence of a dyad symmetry in the core particles.

The nucleosome core particles, containing approximately equal amounts of protein and DNA, are especially suitable for study by neutron scattering. The earliest work, using solutions of core particles, led to data which were consistent with the model derived from the X-ray work.[34-37] More recently, crystal diffraction studies have been carried out[38,39] using the contrast variation method where the D_2O content of the solvent is varied so that its scattering power for neutrons matches that of either the protein or the DNA, enabling the diffraction due to the other component to be measured. These studies have led to low resolution maps for both the DNA and the protein and these fit well with the model previously reported.

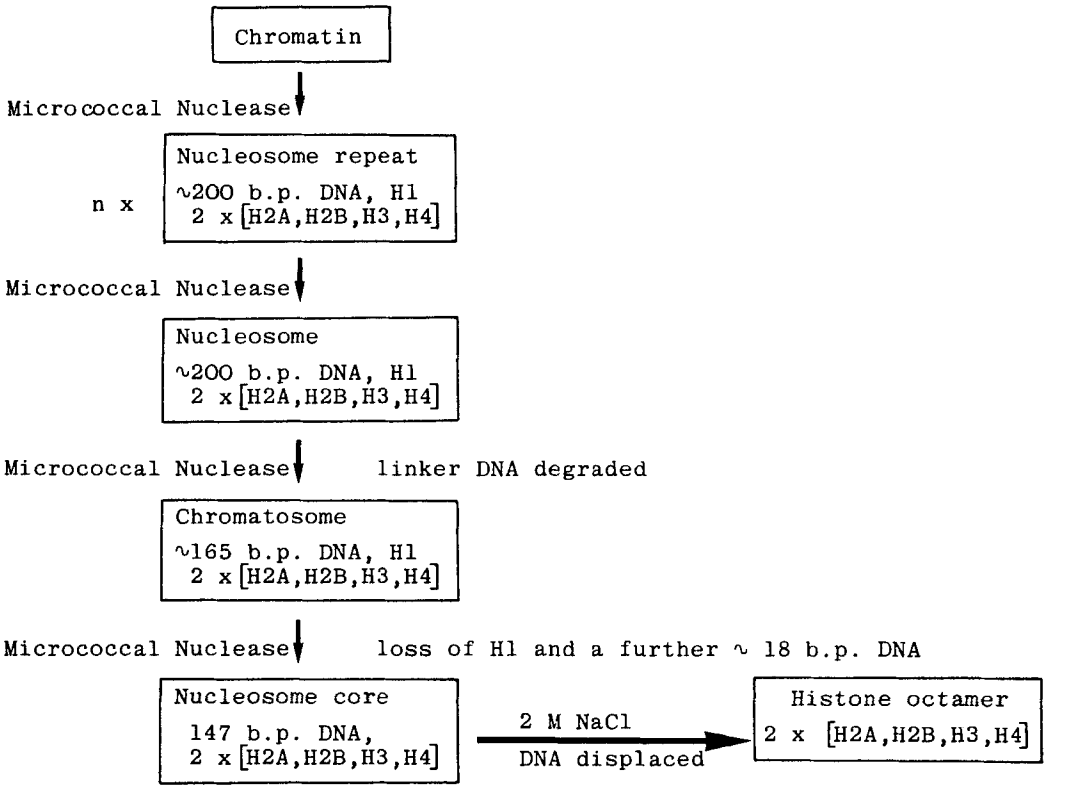

FIGURE 1. Schematic diagram of the breakdown of chromatin to subunits by digestion with micrococcal nuclease and dissociation with salt.

unfavorable bending of the DNA required and this has been estimated to require 21 to 28 kcal/mol of nucleosome.[22a] This bending will be minimized if the DNA lies on the *outside* of the fiber and, while this idea was unexpected when it was first suggested,[17] with hindsight it has the further advantage of allowing relatively unencumbered access to the DNA for the many proteins which must interact with it, even while the DNA is in its folded state.

B. The Nucleosome

1. Composition

The nucleosome appears to be the fundamental unit for the structure of chromatin. The picture is, however, complicated by variations between nucleosomes, even within a given nucleus. Not only are the histones modified post-synthetically at a sub-stoichiometric level as well as some of them occurring as allelic variants,[2] but also the exact length of DNA in nucleosomes varies. Thus while the average repeat length is about 198 base pairs, as found in many cells, it rises to 210 base pairs in some metabolically less active nuclei (e.g., the nucleated erythrocytes of birds or amphibia) and falls to 163 base pairs in more active nuclei (e.g., yeast cells or the neuronal cells from brain).[11-14] A similar range of repeat length has even been found within single nuclei, although the general repeat is still characteristic of the particular cell type.[15]

Whatever the cell type, however, digestion with micrococcal nuclease reduces the DNA length from that of the repeat, through a brief plateau around 165 base pairs, to an end product of 147 base pairs (Figure 1). These "nucleosome cores" are therefore relatively stable, each containing a discrete length of DNA and 2 molecules of each of the four "core

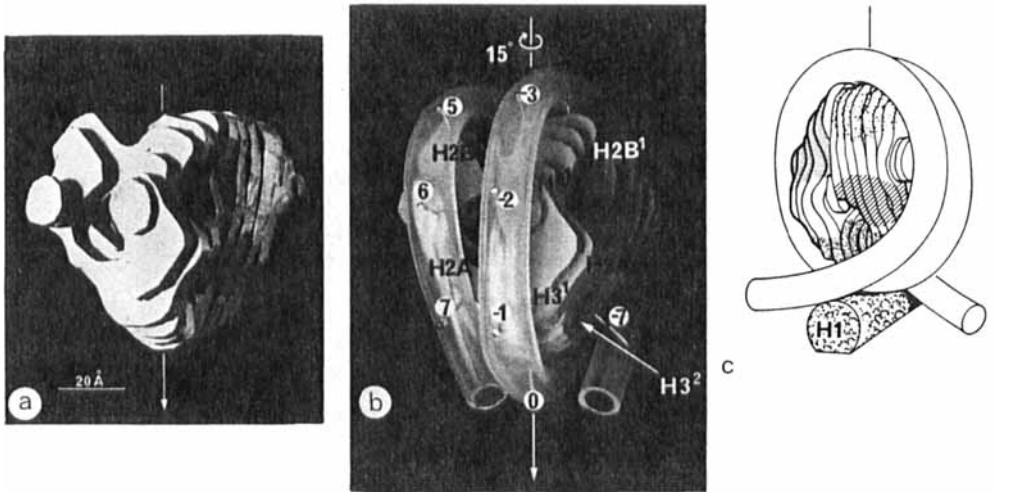

FIGURE 2. Pictures of the nucleosome core and the two-turn particle or chromatosome: (a) Model of the histone octamer obtained by 3-D image reconstruction from electron micrographs.[46] (b) The addition of $1^3/_4$ to 2 superhelical turns of DNA to form the nucleosome core. (For clarity the diameter of the tube is smaller than the true scale size of DNA.) Probable locations of individual histones and DNase I cutting sites on the DNA are marked.[46] (c) Potential binding site for single H1 molecule at the entry and exit points for the DNA, sealing off the chromatosomes. (From Klug, A., Rhodes, D., Smith, J., Finch, J. T., and Thomas, J. O., *Nature (London)*, 287, 509, 1980. With permission.)

The DNA dissociates from the histones with increasing salt concentrations and the histones form a number of polymorphic aggregates. If, however, the histones are prepared directly from chromatin in 2 *M* salt, a specific aggregate is formed which appears to be of similar structure to the aggregate present in intact chromatin, since chemical cross-linking between histones produces very similar patterns from either.[40] Moreover, the cross-linked aggregate can be reassociated with free DNA to regenerate many of the properties of chromatin.[41] The time course of the cross-linking showed the aggregate to be an octamer, while the use of a cleavable cross-linking reagent confirmed that the octamer contained 2 molecules of each of the core histones, H2A, H2B, H3, and H4, and that there was a specific pattern of dimeric contacts between them.[42] The existence of the octamer free in solution was confirmed by sedimentation analysis[43,44] and its pattern of dissociation was found to be into an $(H3)_2(H4)_2$ tetramer and dimers of H2A.H2B.[40,42,44,45]

During attempts to crystallize the octamer, hollow tubes were obtained which were sufficiently ordered in three-dimensions for image analysis by electron microscopy.[46] This showed that the histone octamer, like the nucleosome core, is a wedge-shaped particle of bipartite character (Figure 2a). On its periphery, a series of ridges forms an almost continuous helical ramp of external diameter 7 nm and pitch about 2.7 nm, exactly suitable to act as a former onto which $1^3/_4$ turns of a superhelix of DNA could be wound to give the appropriate dimensions for the nucleosome core (Figure 2b). On the assumption that histones are too small to be multidomain proteins, the histone-histone proximities determined by protein cross-linking[42] and the order of the histones along the DNA determined by chemically cross-linking histones to DNA[47,48] have been used to assign regions of density in the octamer map to particular histones,[46] giving a reasonably detailed model for the nucleosome core (Figure 2b).

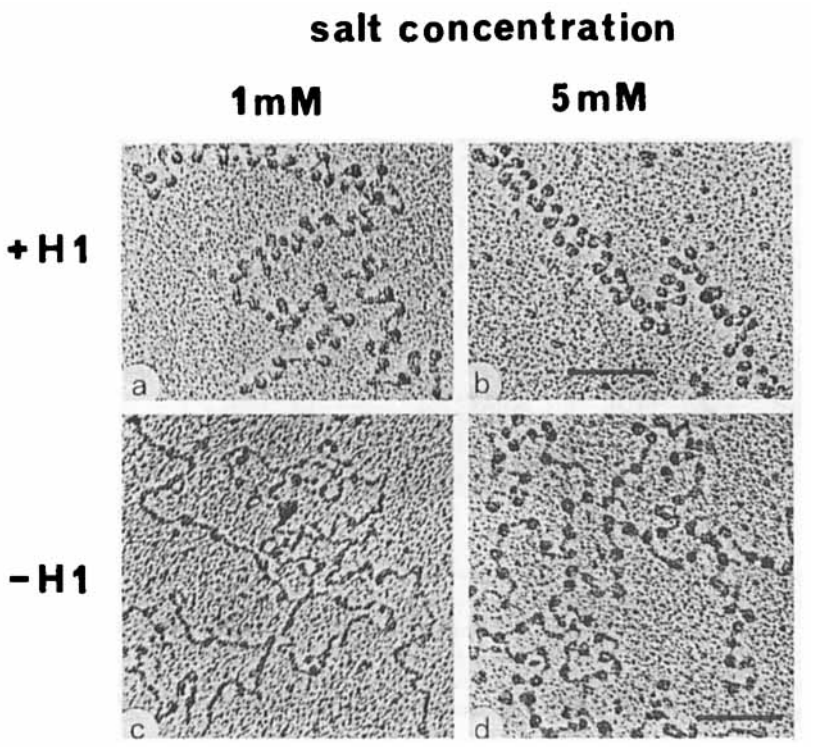

FIGURE 3. Electron micrographs of chromatin at low ionic strength, with and without H1.[50] In the presence of H1 (a and b) the structure is more ordered, with the DNA entering and leaving the nucleosome at sites close together; this order is lost without H1 and even when nucleosome beads are visible (d — at higher ionic strength), the DNA enters and leaves them in a random way. Bar indicates 100 nm.

b. Location of Histone H1 and the Nucleosome Filament

Histone H1 is the histone most readily dissociated from chromatin by salt, dissociating around 0.45 *M* while the core histones only start to dissociate around 0.85 *M*. Its removal appears to leave the underlying nucleosomal structure essentially unaltered, except that sliding of the nucleosomes may occur at the salt concentration involved, altering the nucleosomal spacing.[49] A commensurate observation is the ready migration of H1 from intact chromatin onto H1-depleted chromatin[30] and even between different nucleosome oligomers during nuclease digestion of chromatin, if care is not taken to avoid this. These suggest that H1 may be external to the main nucleosomal structure and this is supported by the absence of H1 from the nucleosome cores, with its release on digestion below 165 base pairs of DNA.[27]

H1 is much too small to be seen directly in the electron microscope, but it is still possible to infer its position on the nucleosome from its effect upon the appearance of chromatin in a moderately folded state. This is most clearly seen below ionic strength 5 m*M*, where the structures are very different in the presence or absence of H1 (Figure 3). In the presence of H1 at ionic strength 5 m*M* an ordered structure is seen,[50] with the nucleosomes flat on the grid in a fairly regular zigzag, arising from the DNA entering and leaving the nucleosomes at sites close together as would be expected for a particle containing about 2 superhelical turns. Following reval of H1, however, the entry and exit points are much less regular and frequently almost opposite each other, destroying the ordered structure. Moreover, at

ionic strength 1 m*M*, nucleosomes without H1 unravel into a rather linear structure in which beads are no longer visible, in contrast to beads with H1 which are still clearly visible.

These results are compatible with physico-chemical measurements made on chromatin in solution. Thus the zigzag structure is consistent with the mass per unit length measured under similar ionic strength.[51,52] Furthermore, only about 3 of the phosphate charges on the terminal 20 base pairs at each end of the DNA on a nucleosome core appear to be neutralized by interaction with the histone core[53] and loss of a few nucleotides from the ends of the DNA results in a marked destabilization of the nucleosome core.[54] At very low ionic strength nucleosome cores appear to be opened up in solution.[55-57a] H1 prevents this from happening and one can therefore conclude that at least part of it must be located at, and stabilize, the region where the DNA enters and leaves the nucleosome (Figure 2c).

The biochemical evidence is also consistent with such a particle with 2 full turns of DNA stabilized by an H1 molecule located on one side of it. Although the nucleosome core particle is the only relatively stable, and hence homogeneous, product which has been observed during digestion of chromatin by micrococcal nuclease, the chromatosome occurs as a transient intermediate, containing 165 base pairs of DNA and an H1 molecule as well as the core histone octamer,[28] and it is during its conversion to the core particle containing 147 base pairs of DNA that H1 is released.[27] As the 147 base pairs of the core particle correspond to $1^3/_4$ turns of a DNA superhelix it is probable that 165 base pairs will give 2 full turns and, since the pitch of the DNA superhelix is only slightly greater than the diameter of the DNA duplex, that the two ends of the DNA will be close together on the chromatosome, both readily being associated with the same single molecule of H1.[32]

The presence of H1 is essential for the formation of regular higher order structure in chromatin.[22,50,58-61] Its removal perturbs the salt-induced conformational changes of nucleosome oligomers in solution[58-61] and even loss of a small percentage produces significant changes in both the appearance[62] and behavior in solution[63] of long oligomers. Removal of H1 even prevents the formation of the lowest regular level of structure of oligonucleosomes, the zigzag,[50] as just described. This zigzag structure is probably the morphology adopted, under the particular conditions of fixation and specimen preparation employed, by what has otherwise been described as the "10 nm fibre" or the "nucleosome filament" and it shows particularly clearly that even in this lowest level of folding the H1 regions of adjacent nucleosomes are probably close together or touching. With increasing ionic strength, more of the H1 regions could interact with one another, to produce more compacted structures, as discussed below. Polymers of H1 have indeed been shown to exist by chemical cross-linking at both low and high ionic strength.[64-66] but it remains to be shown that they actually play the specific role just suggested.

An additional structural complication arises from the attachment of a *single* H1 molecule in the chromatosome, for while the 2-turn particle has dyad symmetry, this must be broken by the H1 molecule to produce a polar structure. It is not known how the H1 molecules in the chromatosomes are arranged within the nucleosome filament: the arrangement could be polar, with all the H1 molecules oriented in the same direction, or nonpolar, with the H1 molecules either randomly oriented or else arranged in some specific pattern, such as alternating in orientation. Whether polar or nonpolar, any specific pattern of orientation would require the incorporation of an incoming H1 molecule to be organized so that its orientation is determined by its neighbor. While a random orientation seems most probable, an alternating orientation would give a two-nucleosome repeat and might explain the observations of a ladder of DNA lengths based upon a two-nucleosome length which have been made under certain specific conditions of digestion with DNase I.[67,68] It is not yet possible to determine the arrangement of H1 orientation in native chromatin and more data are required to settle this question.

III. THE 30-nm FIBER

A. Structural Determination

1. Problems of Determining the Structure

The well-known "beads-on-a-string" appearance of nucleosomes along the DNA[20] only occurs when the structure has been stretched out, or at very low ionic strength[50] (Figure 3), when the electrostatic repulsions between nucleosomes may produce a similar effect. Native chromatin, containing histone H1, occurs as a continuous filament of nucleosomes of diameter about 10 nm in the presence of even low concentrations of salt (discussed above). It is the folding of the filament, to give the 30-nm diameter fibers or even more condensed structures, which is usually referred to as "higher order structure", but there is some disagreement about the form of this folding.

The problem in determining the structure lies in the very nature of the 30-nm fibre. X-ray diffraction studies show a 10-nm reflection corresponding to the formation of the 30-nm fibers,[69] for which an obvious explanation is that it is a repeat determined by the diameter of the 10-nm filament, but the bands in the X-ray pattern are relatively broad. This shows that although the structures present are ordered, they are not completely regular. Thus the nucleosome cores, and perhaps also the chromatosomes, appear to have an unvarying structure (apart from histone modifications) but the varying lengths of the linker DNA mean that the nucleosome repeat is not constant and the regularity falls off further on going to higher levels of structure, even while reasonable order is maintained.

It has not been possible to crystallize anything larger than the nucleosome core, so the main technique which can be used to try to determine the actual higher order structure is electron microscopy. Although isolated nucleosomes and the zigzag that they form at low ionic strength are clearly visible in the electron microscope, even the 10-nm nucleosome filament and certainly the 30-nm fiber show very little detail, but rather appear to have relatively smooth surfaces and show little contrast along their lengths. Moreover the irregularity along the lengths of the 30-nm fibers together with the lack of ordered aggregates of them, which may well be a consequence of this, mean that it has not been possible to employ image analysis techniques on electron micrographs, to enhance the detail sufficiently for the structure to be distinguished directly. The only technique available has therefore been to follow the breakdown or formation of the higher order structure by varying the conditions, and to try to deduce the final structure from the appearance of partially condensed forms.

One essential feature for reliability in any such study is the obtaining of a continuum of forms as the condensation state is changed. Isolated images obtained from essentially "morphological studies" can be seriously misleading, as very slight changes in technique can produce totally dissimilar pictures.[70] These arise as a result of artifacts produced both during the sample preparation and on adsorption to the specimen grid, with the state of the grid substrate having a marked effect upon the final appearance of the specimen. For confidence to be reposed in any study, it is necessary that a complete series of micrographs be obtained with samples of the same chromatin preparation prepared for microscopy under the identical conditions, except for the variable (e.g., ionic strength) used to alter the condensation state. The only acceptable alternative is for control samples to be prepared in parallel with the experimental ones, in several of the previously characterized condensation states.

2. Models for the Compaction

a. A Solenoid

One obvious way of contracting the DNA further and forming a 30-nm fiber is for the nucleosome filament to be helically wound. A solenoid, or contact helix, of the 10-nm filament with the turns in apposition and about 6 nucleosomes per turn[22] fits with the electron

micrographs. It also gives an obvious explanation for the 10-nm X-ray reflection[69] as this would be generated by the helical pitch which will be determined by the filament diameter. There is no requirement for an exact number of nucleosomes per turn, as many regular helices have noninteger numbers of subunits per turn. Moreover, if the H1 is located towards the center of the solenoid and folding is brought about by interaction of increasing regions of H1 with each other, eventually producing a helical polymer and accounting for the geometric form of the solenoid, slight variations in these contacts could lead to a family of solenoids. Such variations might be similar to the "*quasi*-equivalent bonding" found between the protein subunits in the capsids of many isometric viruses[71] and would allow variation in the number of nucleosomes per turn of a solenoid. This polymorphism might occur not only under different conditions, in particular of ionic strength, but also conceivably along the length of an individual solenoid, leading to the observed irregularity. Variations in the linker length[13,15] might add to this irregularity.

A central location within the solenoid of the H1 whose aggregation is involved in, and may even control, the solenoid formation would have an important consequence. If the essential contacts are those of the H1 near the center of the fiber rather than those directly between the faces of the nucleosomes, then this might lead to the flexibility and irregularity of the 30-nm fiber. Such a mode of construction could also be easily deformed, so that the fiber could fold further to give a still more condensed state of chromatin (see Section IV).

b. Superbeads

An alternative type of higher order structure would be for groups of nucleosomes to aggregate together to form "superbeads" in an H1-dependent fashion and for these superbeads then to aggregate further to give the 30-nm fiber.[58-60] Such superbeads might have a diameter about 30 nm and, if they were not fully juxtaposed, might provide a facile explanation for the "bumpy" appearance sometimes seen for the 30-nm fiber. Unfortunately, however, no specific model has been proposed for the packing of nucleosomes into superbeads and it is not even clear whether they are thought to have any defined structure, or simply to be short nucleosome oligomers cut from the 30-nm fiber. Thus the estimates of the number of nucleosomes in a superbead have varied from 6 to 10,[59] 7 or 8,[72,73] up to as many as 12.[74] A parallel has even been drawn with a globular aggregate, containing 25 to 29 nucleosomes, which is said to be "in good agreement with the superbead model proposed for the 250- and 300-Å chromosomal fiber."[75]

The status of superbeads is further complicated by the recent suggestion,[74] from one of the originators of the idea of superbeads, that they may not have a *specific* structure together with the claim that "nucleosomes are somehow clustered in discrete globular particles (superbeads)."[75] Moreover the other main group whose data have been cited as supporting the existence of superbeads have now suggested that they may be "dissociation refractory particles"[76] which are found during nuclease digestion of chromatin, but leaving unclear whether they pre-exist in the chromatin or are formed during the digestion. Although the evidence concerning this model will be reviewed, reaching any firm conclusion about the existence of superbeads is complicated by the variations in the proposals concerning their composition and structure. Given the fourfold range of estimates of their size, this imprecision is probably necessary, but it does reduce the value of the model for making any prediction and, correspondingly, render it less testable.

B. Electron Microscope Observations

Chromatin has long been an obvious object for study with the electron microscope and the observation of the "beads-on-a-string" appearance[20] was one of the pieces of evidence giving early support to the model of repeating nucleosomes.[17] This was followed up by experiments to study the condensation of chromatin and divalent cations were found to cause

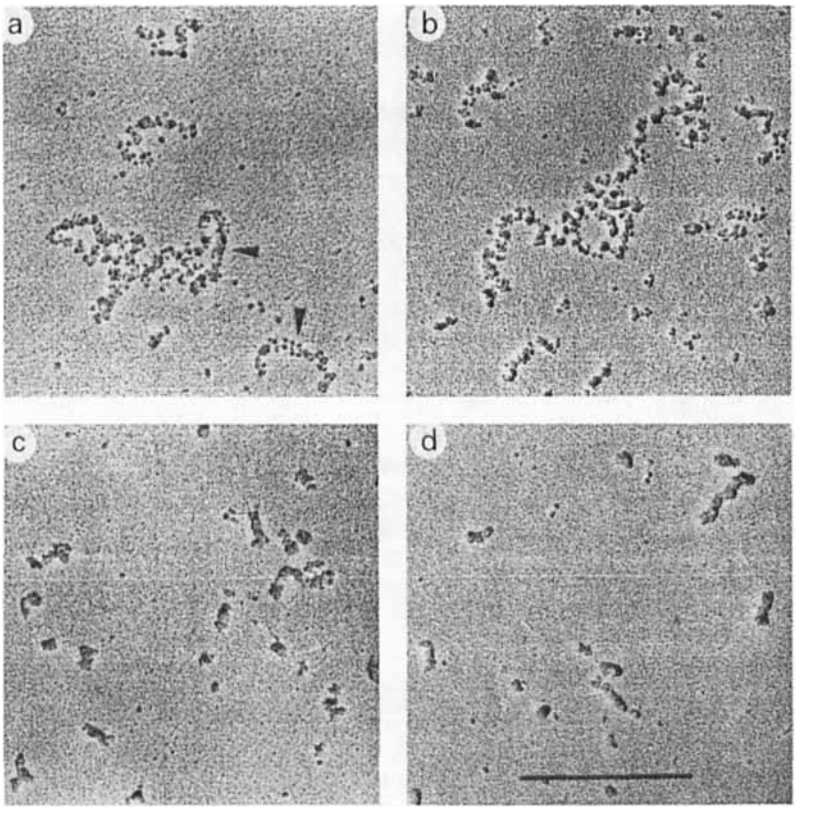

FIGURE 4. Electron micrographs of chromatin containing H1 at increasing ionic strength. Samples were fixed for electron microscopy at ionic strengths (a) 15 mM, (b) 25 mM, (c) 45 mM, and (d) 65 mM. The arrows in (a) point to regions where the nucleosomes approach closely and give the impression of a loose helical structure. Bar indicates 500 nm. (From, Thoma, F., Koller, Th., and Klug, A., *J. Cell Biol.*, 83, 403, 1979. With permission.)

the formation of 30-nm fibers even at very low concentrations[22] and, although no intermediate forms could be observed, the requirement for H1 was demonstrated. Since then a systematic study of the formation of the 30-nm fibers has been carried out, employing monovalent salts to drive the folding with increasing ionic strength, and it was possible to obtain a range of structures showing increasing degrees of compaction as the ionic strength was raised.[50]

From a filament of nucleosomes around ionic strength 1 m*M* (Figure 3a), the extent of structure increased through the zigzag filament around 5 m*M* (Figure 3b) and then a family of more compact intermediate structures, until by 60 m*M* a fully compact 30-nm structure was formed (Figure 4).[50] Between 40 and 60 m*M* the fiber was still compact, though with discontinuities in places, but it nevertheless had a clear three-dimensional appearance with cross-striations about 10 to 15 nm apart. For this reason the intermediate structures were interpreted as a family of helices, each having apparently increasing numbers of nucleosomes per turn with increasing ionic strength. This complete series of micrographs of chromatin compaction into the 30-nm fiber therefore provides strong support for the solenoidal model, and measurements of the mass-per-unit length in the scanning electron microscope also give values compatible with the solenoidal model.[77]

Taken in isolation out of the ionic strength series, the partially formed structures around 40 to 50 m*M* look similiar to the micrographs which have been cited as evidence for

superbeads.[72,73] Moreover, in recent experiments[78] chromatin was spread directly from nuclei, using essentially the Miller spreading technique,[79] and strings of rough, globular particles along the DNA, interpreted as superbeads, were observed over a range of ionic conditions. Perhaps rather ominously, for it contradicts the other published data, removal of H1 only caused some loosening in the structure, as if the ionic strength had been lowered, but otherwise gave structures essentially indistinguishable from apparently native chromatin. The explanation for these anomalous results probably lies in the specimen preparation for electron microscopy. In these micrographs,[78] the chromatin is almost entirely extended into relatively straight fibers showing variable-sized, beaded structures along their lengths. Such streaked fibers are characteristic of sample preparation with weak adhesion of the molecules to the specimen grid,[80] when either stretching or condensation of the fibers can occur during drying. Indeed the pictures described[78] have been mimicked, but with more careful control of the conditions so that it has been possible to demonstrate that the structures seen are probably artifactual.[70]

This highlights a major difficulty in the interpretation of electron micrographs of chromatin: namely the ease with which inadequate specimen preparation can generate artifacts. Thus the appearance of chromatin in the electron microscope is sensitive to the details of the fixation (as well as the conditions under which it is carried out); the adsorption onto the specimen grid; and any subsequent staining, washing, and drying procedures.[50,70,80] Even wnen these have been properly controlled, the selection of individual images is still hazardous, as exemplified by the partially formed structures around 40 to 50 m*M* ionic strength. While in isolation, these may seem to show superbeads; when seen in context, it is apparent that the clumps of nucleosomes are not discrete structures but only intermediates in a process of continuous aggregation, held together by the fixation necessary during the specimen preparation[50,80] and probably corresponding to one or two turns of a solenoid. It is therefore desirable to complement microscopy with physico-chemical studies in solution which, although they cannot determine the structure, are not prone to the same artifacts and can provide valuable confirmation of changes in the structure.

c. Solution Studies

1. Nuclease Digestion

Just as nuclease digestion first showed the nucleosomal repeat in chromatin,[7-10] so it might be hoped it could detect any repetitive superstructure into which the nucleosomes might be ordered. Such a hope rests on the implicit assumption that this superstructure will be discontinuous, as the winding of the DNA into nucleosomes is discontinuous, so that favored cutting sites are available to the nuclease. In the case of a continuous structure, such as a solenoid, there is no obvious reason, apart from propinquity effects, why specific pairs of sites should be preferred and thus generate discrete-sized fragments of the nucleosome filament. Rather it might be expected that there would be a tendency for the nuclease to make a second cut in a neighboring place along the solenoidal axis from the first cut, and so to generate fragments with a distribution of sizes whose mean and width would vary with the digestion conditions and nuclease employed. Because of the 3-dimensional aggregation in the 30-nm fiber, the cutting site distribution may therefore result in an apparent "preferred size" of fragment, corresponding to the most abundant fragment in a distribution whose mode will be related to the number of nucleosomes in a turn.

Thus the most convincing evidence for a regularly repeating supernucleosomal structure would be the detection of a series of multimers of such a structure rather than a single, broad peak. While there have been reports of such series,[73,81,82] it is not clear whether this interpretation is correct. Thus the series have only been observed as slight maxima on a broad peak of chromatin fragments in sucrose gradients and the sizes of the aggregates determined from the DNA extracted from fractions of the gradients containing these maxima.

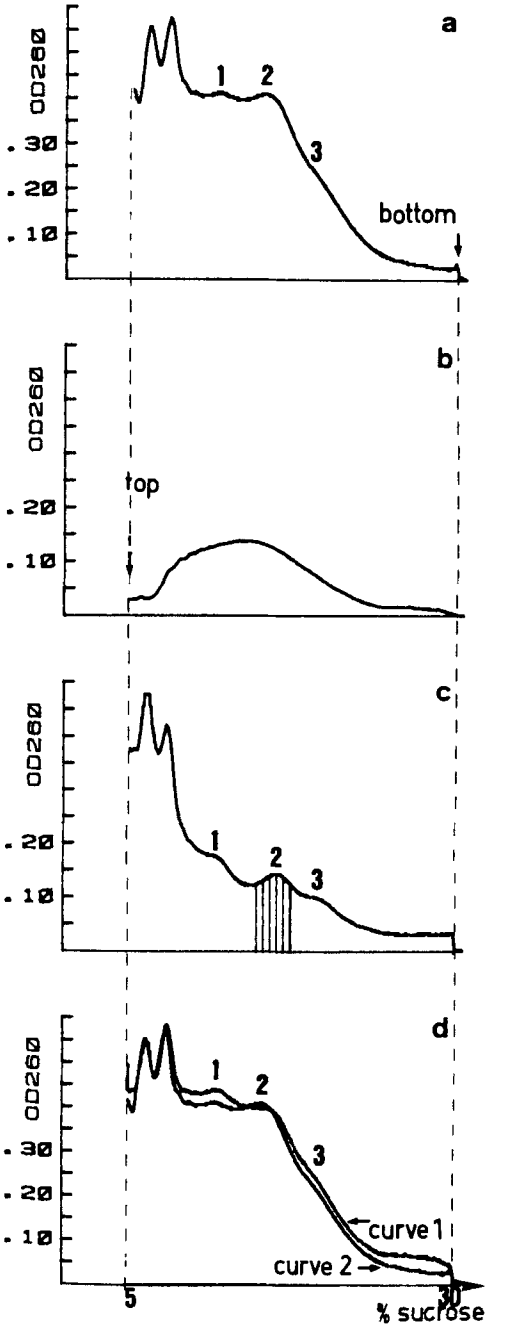

FIGURE 5. Generation of "superbead" profile in sucrose gradients by superposition of ribonucleoprotein particles on chromatin profile. (a) "Chromatin" extracted from nuclei after light digestion with micrococcal nuclease. (b) Mg^{++} insoluble part of (a) — chromatin is insoluble under these conditions. (c) Mg^{++} soluble part of (a) — containing few, if any, histones, but much nonhistone protein and RNA. (d) Sum of the Mg^{++} insoluble and soluble fractions (curve 1) compared to unfractionated material (curve 2 — same as a). (From Muyldermans, S. Lasters, I., Wyns, L., and Hamers, R., *Nucleic Acids Res.*, 8, 2165, 1980. With permission.)

If, however, the maxima were due to some component in the gradients other than the oligonucleosomes, the material extracted would simply reflect the size of the DNA in the continuous distribution of chromatin fragments underlying the peaks of this other material. There is evidence that the extraction techniques employed do release nuclear ribonucleoprotein particles, even without nuclease digestion of the chromatin, and that these sediment as a series of peaks overlying the single broad peak of the chromatin[81,83] (Figure 5). Moreover the estimates of the number of nucleosomes in the smallest peak, based upon the DNA size, have varied between 4[82] and 8,[73,81] a variation too wide to be readily ascribed to inaccurate measurement. More probably, the peaks are due to contaminating ribonucleoprotein particles[83] and the relative sedimentation rate of these, compared to that of nucleosome oligomers, varies with the exact sedimentation conditions, making the peaks overlie different regions of the chromatin peak in different experiments. There is not, therefore, any reliable evidence for the existence of any series of nucleosome multimers in the digests of chromatin, and thus no evidence for a *repeating* supranucleosomal structure.

A similar problem with the reproducibility of the data arises from attempts to estimate the size of any supranucleosomal particle. As already noted, the sizes estimated from the "series" of particles vary between 4[82] and 8[73,81] nucleosomes, while estimates based upon the single, broad peak which has been observed to persist in some digestions vary from 6 to 10,[59] 7 or 8,[72] up to as many as 12[74,76] nucleosomes. In fact, the very width of the "peak" observed in sucrose gradients strongly suggests that there is no discrete particle and it has been concluded that "these resistant regions (superbeads) are heterogeneous in size".[74]

Assignment of any structural significance to these apparent supranucleosomal particles is further complicated by the finding that the DNA between the nucleosomes need not be intact for them to exist,[76,84,85] but that they can be formed by aggregation of smaller oligonucleosomes. In all of these experiments, the amount of the aggregate was found to depend upon the concentration of oligomers, as would be expected for a reversible aggregation, and while it was suggested that the particles were "refractory" to dissociation,[76] there is in fact no evidence of any hysteresis in the aggregation equilibrium. Moreover, in the experiments where the histone composition of these aggregates was examined, the content of H1-type histones (including H5 since both experiments were on chromatin from chicken erythrocytes) was enhanced by up to 50%[84] or even nearly doubled in some cases,[75] compared to the native chromatin. Since binding of excess histones, and particularly H1, can even lead to precipitation of chromatin, this redistribution of H1 may well account for any enhanced stability of these particles and be occurring under the conditions used for the digestion and extraction of the chromatin. This is not improbable since H1 readily migrates between different chromatin strands, even at reasonably low ionic strengths.[30]

A different type of possible supranucleosomal structure, also revealed by nuclease digestion but with DNase I, is a dimeric repeat which appears to depend upon the higher order structure for its generation.[67,68,85] Two obvious possibilities which could cause this pattern would be either a folding into the 30-nm fiber which obstructed access by the nuclease to alternating DNA linkers, or an alternating orientation of the single H1 molecules in the nucleosomes, leading to structural distinctions between alternate linkers without restriction on the folding (see Section II.B.2.b). At present it is not possible to tell which, if either, of these hypotheses is correct.

2. Cross-linking

An alternative technique to probe for any supranucleosomal structure is by chemical cross-linking of the histones. Thus, just as cross-linking showed the existence of the octamer of core histones in the nucleosomal structure of chromatin,[40] so it might be possible to observe an oligomer whose size would characterize any supranucleosomal particle. Because of the number of protein molecules within an individual nucleosome, it is not realistic to hope to

follow the cross-linking of all of the histones in such a particle. However, since H1 molecules tend to be linked together more rapidly than they are linked to core histones, attempts have been made to use the size of H1 oligomers to determine the size of any oligonucleosomal aggregates.[65,66]

From one study it was concluded that there is a supranucleosomal structure containing 12 nucleosomes (and called a "clisone") which gave rise to a cross-linked aggregate of 12 H1 molecules and oligomers up to pentamer of this aggregate.[65] Most of the cross-linking was carried out by initial reaction of the protein amino groups with methyl-4-mercaptobutyrimidate hydrochloride and then subsequent oxidation of the resulting thiol groups in order to generate the cross-links. Unfortunately the protein was denatured and then subsequently exposed to conditions which would promote disulfide/sulfhydryl exchange before the remaining free sulfhydryl groups were blocked so, even if the oxidation conditions caused no structural alteration, it is not clear that the final aggregates will accurately reflect the original structures. Moreover, because of the technique, no time course of the cross-linking was possible and so only a single sample point is described.

Only a single experiment was reported in which a bifunctional reagent was used to generate the cross-links directly and, although it was concluded that the results were essentially similar, no significant quantity of the oligomers of the "clisone" was produced. Moreover, while the electrophoretic mobility of the broad single peak was indeed similar to that of the monomer of disulfide-linked material, it is not clear that the gel system employed would have much resolution for this material, since it appears to be running very near the front.

One result which raises further doubt about the reliability of this study is that the cross-linking leads to a 12 H1 aggregate not only from nuclei and compact chromatin, but also from chromatin at ionic strength 10 to 15 m*M*, when it will be in an extended configuration, largely as the zigzag.[50] Electron micrographs showing this extended configuration do not have any apparent periodicity at intervals of 12 nucleosomes along the filament, so there is no obvious structural feature to explain the cross-linking pattern. A further doubt about the general validity of these results arises from the report[65] that the material examined amounted to only about 5% of the H1 extracted with 3.5% perchloric acid from the cross-linked mixture. Hence at most 5%, and possibly much less, of the H1 in the original native chromatin is even being investigated and assigned into the "clisone" structures.

The results of the other experiments on cross-linking of H1 in long chromatin[66] are significantly different. In these experiments, cross-linking was carried out directly in a single reaction and both the time course of the reaction and the effect of a range of ionic strength are presented. By using 5% perchloric acid solubility, the cross-linked material was fractionated into an insoluble fraction, largely containing the core histones, and a soluble fraction which contained up to 85% of the total H1 as H1 oligomers, some of which were cross-linked to a single molecule of core histone. At ionic strength above 35 m*M* the H1 cross-linking proceeded steadily up to hexamer, but no larger oligomers were seen. Such a result could be compatible with supranucleosomal organization into groups of 6, but it is equally likely to result from the competition during cross-linking between an increasing size of H1 oligomer and the tendency for multiple core histones to be linked to the H1 oligomers, resulting in insolubility. The absence of dodecamer or any higher multiple of H1, which might be expected to result from linking together of hexamers if these were a stable end product, together with the steadily reducing fraction of H1 remaining soluble with increasing reaction, strongly supports this latter explanation.

Even at the lowest ionic strength investigated, 15 m*M*, H1 molecules are close enough together (i.e., within about 0.7 nm) to be cross-linked. However, increasing ionic strength does appear to promote greater H1-H1 interaction since the largest oligomer found at 15 m*M* is $(H1)_4$, whereas oligomers up to $(H1)_6$ are seen at 35 m*M* or above. The geometry of this increased interaction is not known but is, of course, compatible with increasing compaction.

the presence of H1 and it has been identified with the formation of the first stable turn of the solenoid.[61,63]

With sufficiently long oligomers, a further change in behavior is seen, with an abrupt jump in sedimentation coefficient occurring between ionic strengths 45 and 55 m*M* (Figure 7a). This jump only occurs for oligomers with an average size greater than 50 nucleosomes from rat liver,[88] but 60 nucleosomes from chicken erythrocytes.[63] Close examination of the dependence of the sedimentation coefficient upon size of the oligomer (Figure 7b) shows that whereas there is a simple power-law relationship at ionic strengths above that of the jump, there is a distinct break in the relationship at lower ionic strength, with this break occurring around the size where the jump is first apparent. The break is due to a slowing down of the large oligomers at low ionic strength rather than to the speeding up of small oligomers, i.e., it is a jump down and not a jump up. This is probably due to the solenoid having only weak axial bonding at lower ionic strengths and hence being susceptible to dynamic shearing if it is sufficiently long. Any transient interruption of the compaction of the uniform rod would increase the frictional drag, and hence result in a lowered sedimentation rate, as the rod would become more free-draining. This effect would correlate well with the larger size needed for the jump with chicken erythrocyte than with rat liver chromatin, since H5 is known to bind more strongly than H1, for which it is partially substituted,[31] and so could well provide a greater axial bonding interaction which would only break under the higher shear force resulting from a longer rod.

Further support for this interpretation comes from the effects of mild cross-linking, which mainly links H1 (see preceding section). If carried out at ionic strength above 55 m*M*, it led to loss of the jump,[61] but this could be restored virtually to normal if a cleavable cross-linker was used and the cross-links were cleaved. This shows that the effect was due to the cross-links per se and not to any effect of the chemical modification upon the lysine residues of the protein. Cross-linking at ionic strength well below the jump had no effect upon the sedimentation behavior, with the jump still occurring as the ionic strength was raised. Interestingly enough, if cross-linking was carried out at an ionic strength (40 m*M*) just below that at which the jump occurs, the results were similar to those obtained for cross-linking at high ionic strength, with maintenance of the tight configuration. While at first sight surprising, this is what would be expected from a fluctuating solenoid if the gaps between the turns had only a transitory existence, with any given neighboring turns in close contact most of the time. The effect of cross-linking would then be to hold each of the turns in contact with its two neighbors, thus stabilizing the compact solenoid even though the reaction was carried out at an ionic strength below that at which this structure would have been stable on its own.

4. Scattering Studies

a. X-ray and Neutron Scattering

X-ray scattering was used at an early stage to try to determine the structure of chromatin, and showed a series of low-angle reflections which were interpreted as orders of an 11-nm repeat.[4-6] With the establishment of the nucleosome filament with a diameter of about 10 nm, it has been suggested that this may be giving rise to this reflection and interest has centered on trying to resolve the folding into the 30-nm fiber. Thus the appearance of a strong 10-nm reflection was found to accompany the condensation of the nucleosome filament and it was suggested that this reflection arose from the juxtaposition of turns of the filament within the higher order structure.[69]

In a more recent study, attempts were made to fit the angular dependence of the X-ray scattering at ionic strength slightly over 100 m*M* by analysis of Guinier plots and model building.[89] The data could be readily fitted either by a solid cylinder with a diameter of about 33 nm or by a cylinder with a central hole and an external diameter of about 30 nm.

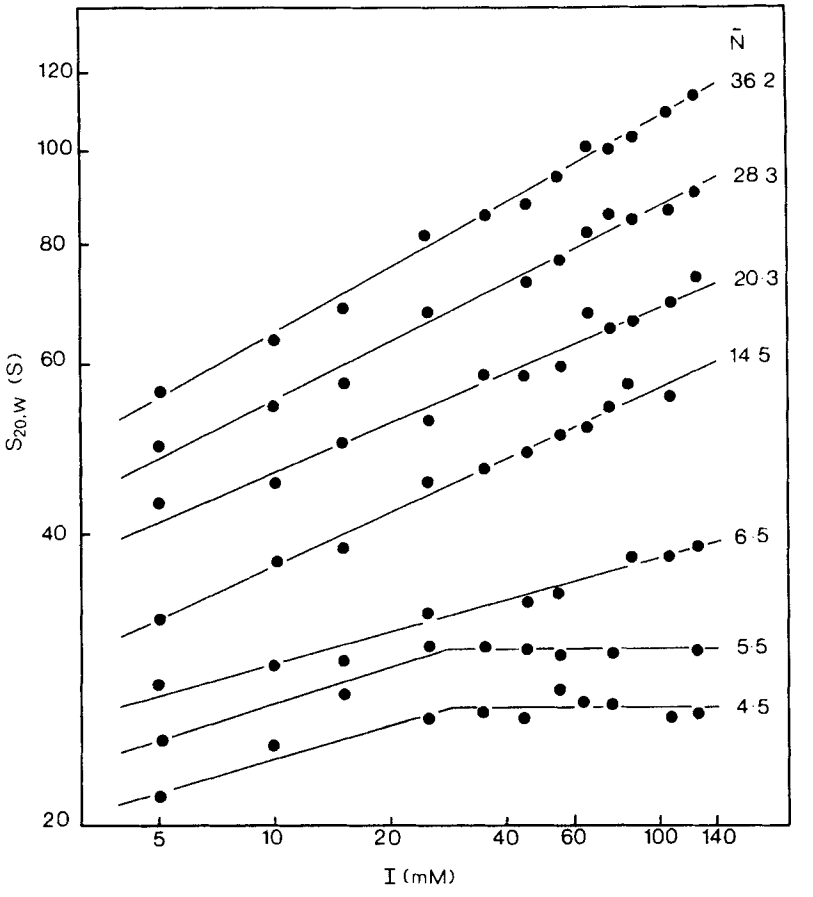

FIGURE 6. Dependence of sedimentation coefficient upon ionic strength for small nucleosome oligomers from chicken erythrocyte chromatin. Weight average sizes of the oligomers ($\bar{N}$) are expressed in numbers of nucleosomes and were determined from the DNA sizes.

3. Sedimentation Studies

The folding of the 10-nm filament towards and into the 30-nm fiber will cause changes in the diffusion coefficient which can readily be measured as alterations in the sedimentation coefficient. Many different measurements have been reported[59-61,63,73,86-88] and there are no major inconsistencies in the results. Most of this work has been performed using zonal sedimentation in linear or isokinetic sucrose gradients and sedimentation coefficients calculated by comparison with markers and this could well account for the minor differences in measured values, as the sedimentation coefficients of the markers might change with varying conditions, e.g., of ionic strength. A complete study has been made using the analytical ultracentrifuge, in which absolute sedimentation coefficients can be measured, of oligomers up to 100 nucleosomes long and over the range of ionic strength from 5 to 125 m*M*, with chromatin from both rat liver[61,88] and chicken erythrocytes.[63] Since these are the most detailed observations and cover the entire range of conditions where the 10-nm filament is folding into the 30-nm fiber, the following discussion will be concentrated on them. Moreover, the presence of H5 and the rather longer repeat length in chicken erythrocyte chromatin than in rat liver chromatin appear to have very little effect upon the overall behavior and the separate species of chromatin will only be distinguished when the regions where these differences do occur are considered.

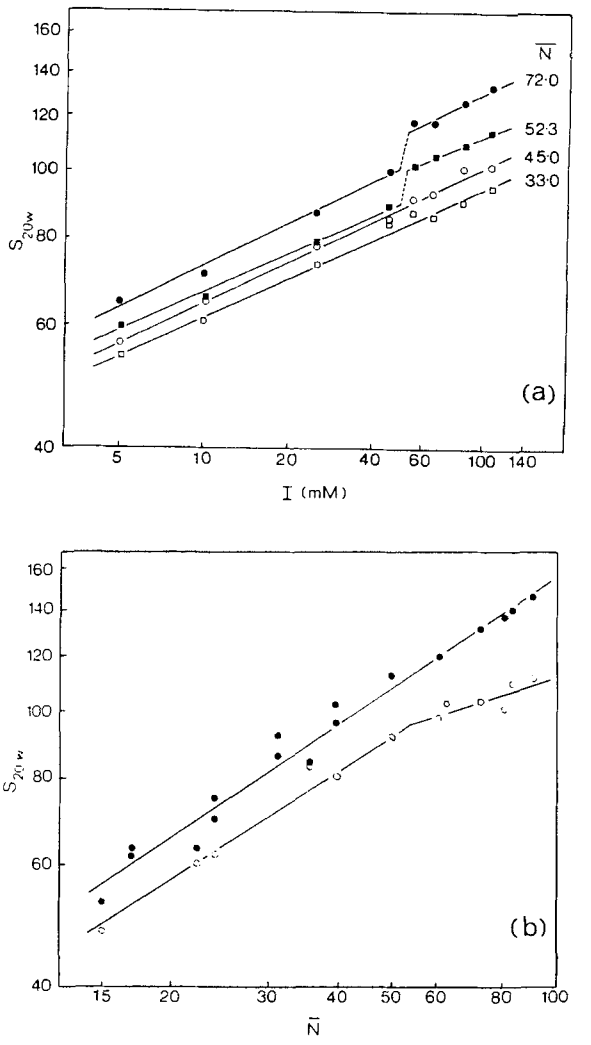

FIGURE 7. Dependence of sedimentation coefficient upon ionic strength and oligomer size for nucleosome oligomers from rat liver chromatin. (a) Ionic strength dependence for various oligomers (N is the weight average size expressed as number of nucleosomes. (b) Dependence upon the size of nucleosome oligomer at ionic strengths 25 mM (○ — below the jump) and 65 mM (● — above the jump). (From Thomas, J. O. and Butler, P. J. G., *J. Mol. Biol.*, 144, 89, 1980. With permission.)

For short oligomers, up to 5 nucleosomes long, the sedimentation coefficient shows a power-law dependence upon the ionic strength up to 25 m*M*, but is constant and independent of ionic strength above this value. Hexamer and larger oligomers show an abrupt change of behavior, with the power-law dependence extending over the entire range of ionic strength up to 125 m*M* (Figure 6). The change in behavior between pentamer and hexamer is compatible with the formation of some compact structure which requires at least six nucleosomes. However the absence of any further discontinuity of behavior around dodecamer or higher multiples of six nucleosomes is not compatible with the formation of a discrete structure but rather with the start of a continuous structure. This folding is dependent upon

There was no evidence for a repetitive structure larger than the 10-nm filament along the 30-nm fiber, as might be expected if the folding were into superbeads. One point of discrepancy with earlier results was the finding that although refolding did occur after dialysis to low ionic strength and then back up again, it was incomplete. However the chromatin employed had been fractionated by chromatography on Sepharose columns, and it has since been shown that this preparative procedure leads to partial loss of H1[63,90] and such loss could readily explain the difference from more native material.

Neutron scattering has also been used to follow the condensation into the 30-nm fiber over a range of ionic strengths.[52] These studies again showed the 10-nm filament around 15 m*M*, with an abrupt change in the radius of gyration as the ionic strength was increased above 20 m*M*, corresponding to the formation of a coil of external diameter about 34 nm. Increasing the ionic strength further led to the linear region of Guinier plots becoming more extended, probably indicating that the higher order structure becomes more regular. These results were interpreted in terms of a family of supercoils of nucleosomes of similar radius of gyration, but with different degrees of compaction, finally giving a fully compacted solenoid at the highest ionic strengths. Such a model would require open helices with the turns not in contact at lower ionic strengths, only giving a contact helix where the turns are in apposition in the most compact form. An alternative explanation, which would fit the data almost as well, is for a family of helices to occur with increasing numbers of nucleosomes per turn, as suggested from the electron microscopy.[50] Whatever model is taken, the packing ratio for the most compact supercoil requires 7 ± 1 nucleosomes per turn of the solenoid.[52]

b. Light Scattering

Similar models of open helices have been proposed to fit the data on diffusion coefficients from light scattering studies using laser light.[86,87,91] In one study carried out at approximately 15 m*M* ionic strength,[86,87] the sedimentation coefficient and diffusion coefficient for various lengths of chromatin were used to calculate the frictional ratio (f/f_o) as well as the radius of gyration and then models were tested to try to explain these. No single model could fit the data over the range from 2 to 20 nucleosomes, but there appeared to be a transition in behavior around 8 nucleosomes. While a detailed model, including the arrangement of the linker DNA was proposed, the detail is probably not reliable as the chromatin had been fractionated into oligomers by gel chromatography, which has since been shown to remove a fraction of the H1,[63,90] and so the structure is likely to have been somewhat perturbed.

Measurements of the molecular weights of a series of oligomers showed no variation of molecular weight over a range of ionic strengths,[91] showing that self-association was not occurring. Models were refined to give close fits to plots of the radius of gyration against the oligomer size at a number of ionic strengths. The preferred model involved a family of open helices, of diameter about 30 nm, whose pitch reduced with increasing ionic strength until a contact helix was formed at the highest ionic strength used. The overall properties of this contact helix were fully compatible with other observations on the 30-nm fiber described above. Although a superbead model could be found to fit the radius of gyration data, it was incompatible with electron micrographs of chromatin, particularly in requiring the existence of a 25-nm fiber even at low ionic strength.

In other experiments the relaxation times for the autocorrelation functions of nucleosome monomers to octamers were measured as a function of ionic strength.[92] These were used to calculate the translational diffusion coefficients for the oligomers and, despite only three ionic strengths being used, a discontinuity of behavior was apparent around the nucleosome hexamer. From the limited data available, it was not possible to tell whether this discontinuity resulted from a conformational change due to a change in size from hexamer to heptamer or whether it resulted from some specific structure containing six nucleosomes and would

recur at every sixth nucleosome even in larger structures. Some detailed hydrodynamic calculations were made, but, as these were based on an assumption of spherical nucleosomes rather than oblate ellipsoids, their value is doubtful.

5. Electric Dichroism

Electric dichroism measurements have been used to try to determine the orientation of the nucleosomes within the 30-nm fiber. A major problem which is encountered in the application of this technique is the need to apply high-voltage gradients across the sample, in order to obtain ordering of the electric dipoles of the molecules. It is therefore important to have a low conductivity in the solution to avoid excessive heating. Thus some of the experiments have been conducted at such low ionic strength that they have no relevance to the 30-nm fiber.[93,94] Two techniques have been employed to avoid this problem, either working with low concentrations of divalent cations to cause condensation without great increase in the ionic strength,[95] or chemically cross-linking the chromatin at high ionic strength to try to stabilize the structure when it is brought to low ionic strength for the electro-optical measurements,[96] although the cross-linking itself might cause further problems.

Unfortunately these two studies yielded contradictory results for the limiting dichroism, measurements in the presence of Mg^{++} giving a value of -0.2,[95] while those on cross-linked material gave a value of $+0.06$.[96] One possibility is that this is due to perturbation of the structure by the extensive cross-linking employed with, typically, 7 additions of 1/10th volume of a concentrated solution of the cross-linking reagent. Thus it is reported[95] that cross-linking could result in somewhat erratic values, which even changed sign (from the negative value found with Mg^{++}), although typically averaging around -0.05 to -0.1. Possibly the more extensive cross-linking employed in the other study,[96] while ensuring reproducibility of the measurements, may have done this by always causing a similar distortion of the structure. It is indeed noticeable that the sedimentation coefficients, which do not change with ionic strength after the cross-linking, are rather higher than those measured for rat liver chromatin at the ionic strength used for the cross-linking.[61,88] While this could be due either to the increased mass with the cross-links or to differences between chromatin from rat liver and calf thymus, as employed in the electric dichroism study, it might also indicate that the structure had been altered. The direction of the difference is compatible with further compaction, as might be expected after very extensive cross-linking. An alternative explanation of the difference may be that divalent cations alone do not produce the most fully compacted structure. Thus a 25% increase in the sedimentation coefficient has been observed between Mg^{++} stabilized fibers and ones in the high ionic strength buffer used for the cross-linking.[97]

Interpretation of the electric dichroism is complicated by the large magnitude of the term due to naked DNA and, hence, the component which must be ascribed to the linker between chromatosomes. Moreover, there is the possibility of local segmental flexibility in any chromatin which is less than fully compacted. However calculations have been made leading to estimated angles between the flat faces of the nucleosome cores and the axis of the 30-nm fiber of between 0 and 20° for the Mg^{++} compacted fibers[95] and of about 40° for the cross-linked material.[96] This difference is again in the direction which would be caused by enhanced compaction in the cross-linked material. While it is therefore still not possible to decide on the structure for the 30-nm fiber, it is possible that the nucleosomes are varying between having their flat faces roughly parallel to the fiber axis, in fibers stabilized by Mg^{++} at low ionic strength, and being tilted almost half over at high ionic strength. Such a continuing compaction by compression of the 30-nm fiber would explain the continuous increase in sedimentation coefficient with ionic strength for long nucleosome oligomers even after the 30-nm fibers are formed (see Section III.C.3).

D. Conclusions

1. Preferred Model

It is possible to reach certain conclusions about the probable structure of the 30-nm fiber on the basis of the results described above. The superbead model was originally postulated to explain both the "knobbly" appearance of the 30-nm fiber as seen in certain electron micrographs and the series of peaks, apparently multimers of a specific supranucleosomal aggregate, found in sucrose gradient fractionation of digests of chromatin. It now appears that the apparent strings of superbeads seen in the electron microscope may well be morphological artifacts, brought about either by inadequate specimen preparation techniques or by choice of conditions where the underlying structure is not fully stable. Thus the very fixation necessary for specimen preparation could well fix in the "breaks" between turns of a solenoid if these temporarily separated due to fluctuations under conditions where the solenoid was not fully stable. The pictures of "superbeads" would then correspond to isolated turns, or groups of a few turns, of a solenoid.

The apparent multimers of the superbead have also been found to be an artifact, and measurements of the size of the main peak in nuclease digests have not shown any specific particle, but rather have shown a variation in size by a factor of over two. The rather broad peak and variations in size are just what might be expected during digestion of a continuous structure like the solenoid (see Section III.C.1) but not of an array of specific supranucleosomal particles. It therefore seems to be emerging that superbeads may not have any *specific* structure, in which case their significance is unclear. The inherent plasticity of solenoidal structures means that short lengths of the nucleosome filament could fold up into a variety of bead-like structures, while still possessing an underlying helical character. Whenever "superbeads" have been characterized (on sucrose gradients), they have been observed in preparations where extensive redistribution of H1 had taken place and frequently the fractions containing superbeads had an enhanced H1 content. This indicates that particularly stable turns of solenoid may be those which have bound additional H1 (or H5) molecules and that these may constitute much of a "superbead" preparation.

In contrast, the advent of more systematic studies has increased the data supporting the hypothesis of a solenoid structure for the 30-nm fiber. Thus the folding can be readily seen through the series of electron micrographs at increasing ionic strengths (Figures 3 and 4) and a distinct stabilization of the structure in solution is shown by the sedimentation studies at an ionic strength correlating with that at which the compact structure becomes visible.

The compaction of the nucleosome filament into the 30-nm fiber therefore seems to be brought about by increasing H1 (or H5) interactions folding the filament into a helical structure. At higher ionic strengths this fiber is a solenoid, with adjacent turns in close contact and about six nucleosomes per turn, although there may be some variation in this number between fibers and even within an individual fiber. At intermediate ionic strengths the folding might be either into open helices, still of 30-nm diameter and containing about 6 nucleosomes per turn, but with a pitch greater than 11 nm so that the turns are not in contact, or into contact helices with fewer subunits per turn (and hence a smaller diameter) but still with an 11-nm pitch so that the turns are touching. While it is not possible to exclude either possibility, the latter (pictured in Figure 8) would appear the more likely. Changing numbers of subunits per turn of a relatively compact structure could readily come about with increasing interaction energy, but a regular open helix with a continuously changing pitch is rather more difficult to envisage as the only force available, in this open state, to bring about the change would be torsion within the single filament.

The exact orientation of the nucleosome within the solenoid is not clear. However, the data are certainly compatible with them lying with their flat faces close to parallel to the solenoid axis and projecting more or less radially outwards (Figure 8). While various models have been proposed with the H1 molecules on alternate nucleosomes alternating between

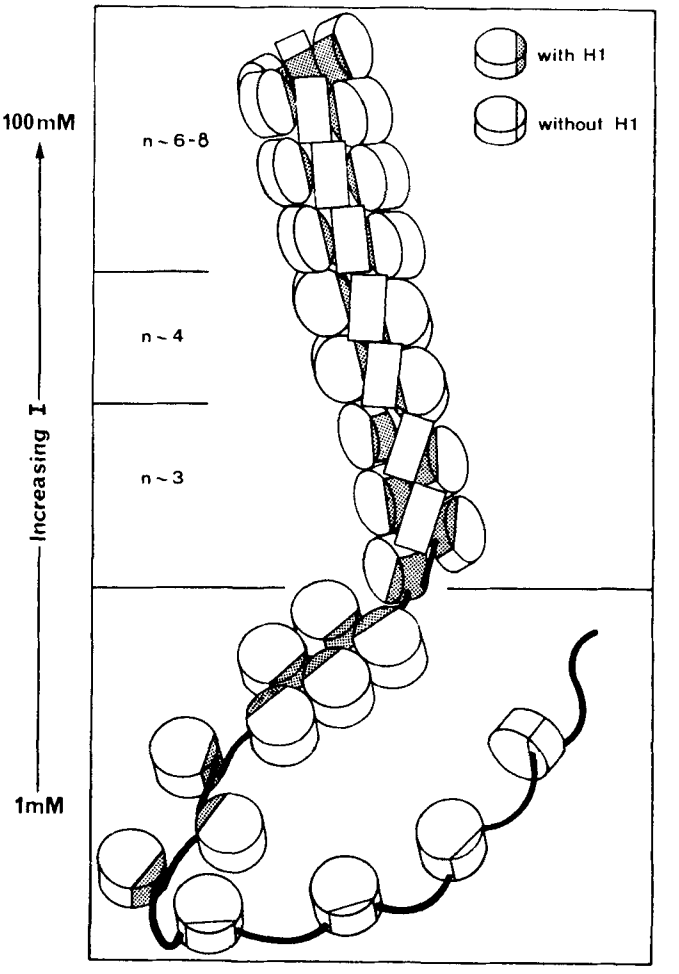

FIGURE 8. Hypothetical picture of helical folding of chromatin containing H1 with increasing ionic strength. The open zigzag closes up by interaction of the H1 molecules (shown shaded), to form helices with increasing numbers of nucleosomes per turn (n). In the absence of H1 (bottom right) no zigzags or other definite structures are found. Although for simplicity the helices are drawn as regular, they are in practice ordered but not perfectly regular (discussed in text). (From Thoma, F., Koller, Th., and Klug, A., *J. Cell Biol.*, 83, 403, 1979. With permission.)

the inside and outside of the solenoid, the readiness with which the majority of the H1 can be cross-linked into polymers and the similarity of the structure and stability of the solenoid with different linker DNA lengths makes it seem more likely that the H1 simply runs up the inner surface of the solenoid, in the central hole.

2. Relevance to Chromatin in Nuclei

One obvious question is how relevant the structure of the 30-nm fiber found for soluble chromatin may be to its state in the nucleus. There is little doubt that the nucleosome structure is largely unperturbed on extraction of chromatin, since the 200 base pair spacing is observed during digestion of intact nuclei and also direct lysis of nuclei on the specimen

grids for the electron microscope readily yields the "beads-on-a-string" appearance of chromatin. However the usual methods used for the preparation of long chromatin almost certainly disrupt the 30-nm fiber and the question here becomes how accurately it is being reconstructed.

Fibers of about 30-nm diameter are seen in electron micrographs of sections of fixed and embedded nuclei[21] and also spilling out of lysed nuclei,[70] where they have a very similar appearance to those formed by refolding of extracted chromatin at the same ionic strength.[50] Low-angle X-ray diffraction has also been used to relate the structure of chromatin in chromosomes, nuclei, and intact cells to the models derived for chromatin in solution.[98] This shows that a weak 40-nm periodicity is present in intact cells or nuclei and that this is directly related to the 30-nm, side-by-side packing of solenoidal chromosome fibers seen in the electron microscope. The change in spacing probably occurs because of drying, and a similar effect is seen in solution studies of isolated chromatin where diameters estimated from scattering measurements are consistently larger than those measured from electron micrographs. Thus it seems likely that the overall packing is similar in vivo to that observed in solution, and hence that the models derived from solution studies are relevant to chromatin in one of its natural states.

IV. FOLDING OF THE 30-nm FIBER

A. Metaphase Chromosomes

1. Packaging Unit

Although the 30-nm fiber may represent the state of much of the cellular chromatin throughout a large part of the cell cycle, it must still be folded further into even higher levels of structure, leading ultimately to the compact chromosomes in metaphase cells. Chromosomes are complicated structures and must certainly require additional components to organize them. By analogy with the formation of the solenoid from the nucleosome filament, one possible organization would be a helical coiling of the solenoid itself into a "supersolenoid" which could give a fiber of thickness compatible with chromosomes.[99] However, such a structure occurs for, at most, a small fraction of the chromatin and the evidence for its existence in vivo at all is weak; rather it may be an artifact formed by progressive denaturation in vitro. Moreover this simple model does not provide a ready explanation for the substructure which can be observed in chromosomes.

Indications of such a substructure, with the chromatin organized on a very large scale in packaging units, come from several distinct lines of evidence. First there is the finding of independently supercoiled "loops" of DNA containing about 80,000 base pairs in *Drosophila* chromatin: these appear to be supercoiled, even although they contain the core histones, and are independently relaxed by nicking with nuclease.[100] The preparations containing these loops were made under conditions of very low shearing, in 0.9 *M* sodium chloride, 0.4% Nonidet P40, and contained RNA and the core histones, H2A, H2B, H3 and H4, but not H1, which would be washed off by the salt. Secondly, detailed studies of the very early stages of digestion of nuclei from rat liver with both micrococcal nuclease and restriction endonucleases have led to the suggestion that this chromatin may also be organized into "domains" of between 34,000 and 75,000 base pairs.[101]

One interesting feature of these domains is that their estimated sizes are similar to those of the "chromomeres" described from cytological studies of chromatin. This correlation has led to the suggestion that each domain might correspond to a single staining band, or cytogenetic unit, on polytene chromosomes. While there is no direct proof either of this correspondence or of that between a band and a single transcriptional unit, such assumptions allow a picture to be drawn which integrates a number of the known levels of chromatin structure with its function.

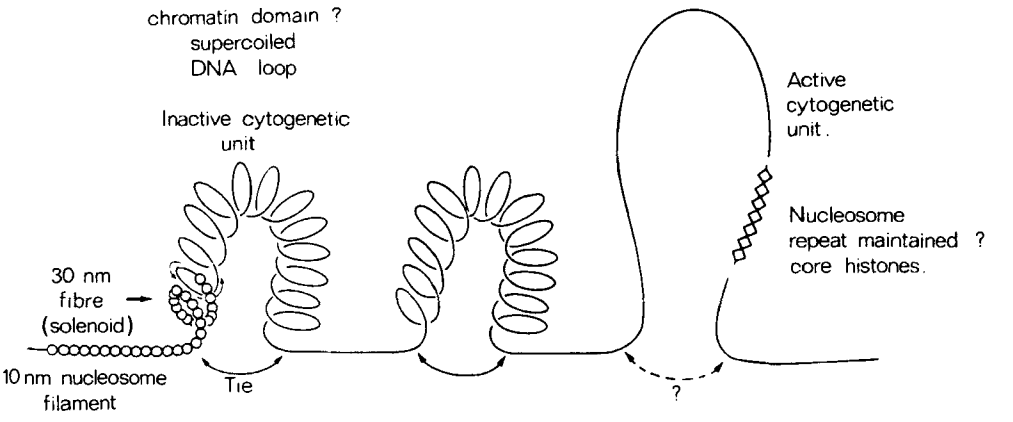

FIGURE 9. Highly schematic picture of possibly interacting levels of organization in chromatin. While the 10 nm nucleosome filament and its packing into the 30 nm fibre (or solenoid) are established, the connection between this, the "radial loops", and the cytogenetic units and also the unfolding of the 30-nm fiber throughout an active loop are speculative. (From Klug, A., *Phil. Trans. R. Soc. London*, B283, 233, 1978. With permission.)

2. Radial Loop Model

In one such picture[102] (Figure 9), the nucleosome filament of inactive chromatin is folded solenoidally into the 30-nm fiber, lengths of which are folded back on each other and "tied" at their bases by special RNA or protein to give the loops. When a given transcriptional unit becomes active, it is likely that the solenoid would unwind concomitantly with modification of the nucleosomes (see Section V). This picture is also compatible with the further compaction of chromatin into the condensed metaphase chromosomes, as the "ties" could interact together, either directly or through other nonhistone proteins, to give an organized structure which would hold the chromosome together. Such a structure has been suggested to lie along the axis of the chromosomes and called a "scaffold"[103,104] and, while there has been some question about its location there,[105] the organization of chromatin in metaphase chromosomes into 30-nm fibers with properties very similar to soluble chromatin prepared largely from interphase cells has been demonstrated.[106]

Despite the controversy about the nature of any scaffold at the center of the chromosomes, there is ample evidence that the 30-nm fiber is folded into "radial loops" emanating from near the center of the arms of the chromosome.[107-109] This evidence comes from electron micrographs, mostly of thin sections of stained chromosomes, but also in the scanning electron microscope with shadowed chromosomes,[107] and the specimens have been fixed under a variety of conditions. Variations in the fixation conditions in aqueous solution, in particular the concentrations of divalent cations, produce changes in the compactness of the chromosomes, but all of the pictures are compatible with a radial loop model as just described. Under the most compact conditions it appears that the loops of the 30-nm fiber may even be supercoiled, to give radially projecting stubby lengths of an apparent 50-nm fiber.[107]

The radial loops themselves are more obvious under less condensing conditions and consecutive serial sections, cut both transversely and longitudinally through chromosomes, have been obtained.[109] In these (Figure 10), the generally radial direction of the 30-nm fibers and their tendency to fold back at outer radii can be seen, although it is rarely possible to follow a given fiber throughout the entire loop, as they usually leave the plane of the section. As the serial sections become more grazing, there is a tendency for the fibers to appear as "dots" rather than an extended fiber (e.g., particularly noticeable in Figure 10d). Such a series of pictures establishes that the fundamental orientation of the fibers is radial and that they are uniformly distributed around and along the chromatid arms, although it remains possible that some small fraction of the fibers are longitudinally oriented near the centers of the chromatids.

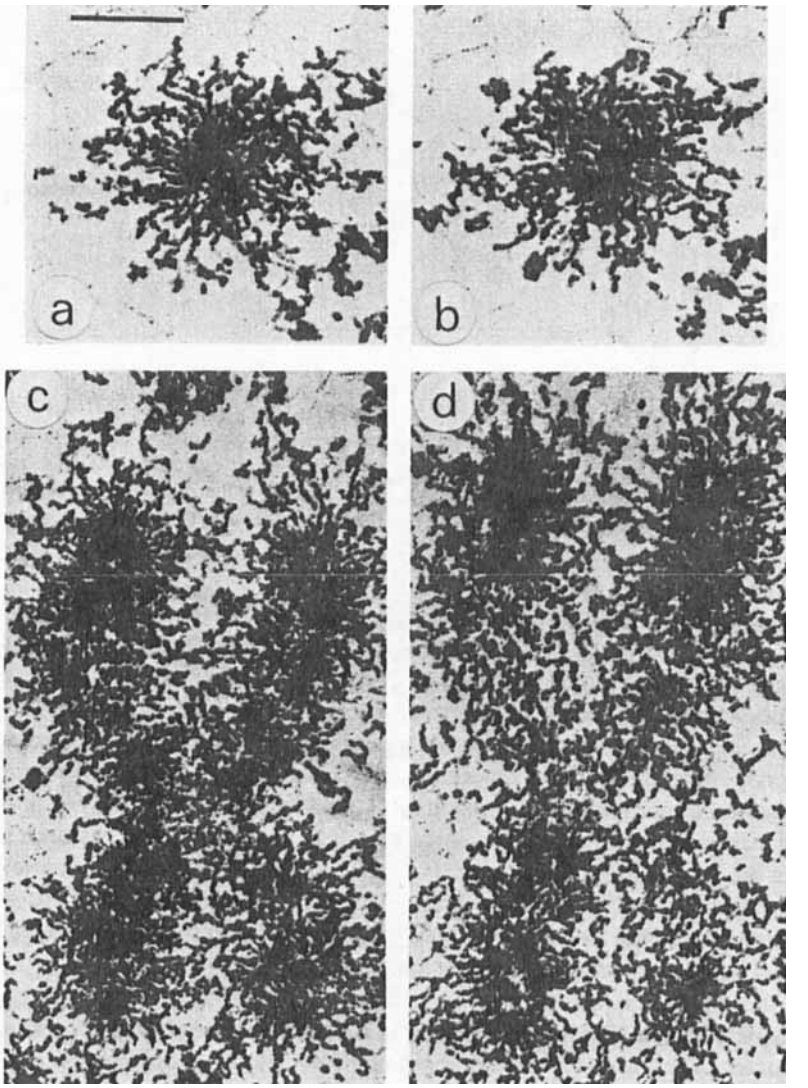

FIGURE 10. Electron micrographs of serial sections of chromosomes. The cells were fixed in hypotonic medium containing 1.5 mM Mg^{++} before staining, embedding, and sectioning. (a and b) show consecutive sections transversely through a chromosome, while (c and d) show consecutive longitudinal sections through both chromatids of a chromosome. Bar indicates 500 nm. (From Adolph, K. W., *Eur. J. Cell Biol.*, 24, 146, 1981. With permission.)

3. Chromosome Scaffolds

The most obvious and regular arrangement for the "ties" at the bases of the loops would be for them to interact together to give a regular, and possibly helical, array along the axis of the chromosome. Such arrays were observed at the core of chromosomes from which the histones had been removed, and called a "scaffold",[103,104] and this core structure was found to be stable even after nuclease digestion to remove the DNA. These results have been independently confirmed[110] and it was found that the scaffold remaining after 0.2 *M* hydrochloric acid extraction of the histones could still be stained to show the chromosome "banding". It was therefore suggested that this cytological property might be caused by the organization of the components of the scaffold, rather than by the chromatin in the loop.

In a more recent study,[111] images of a central core similar to those described as the scaffold were again found, but when the conditions were varied more widely this core becomes less dense and fibrous and under some conditions it was largely dispersed. In particular, nothing with the appearance of scaffolds could be seen when histone extraction was carried out either in the presence of sucrose, which has been found to reduce the aggregation of histones, or with chromosomes already adsorbed into electron microscope grids.[105] It was therefore suggested that the compact "scaffold" might be an artifact, although it still seems to be agreed that some form of core for the chromosomes does exist.

Despite this disagreement about the "native" morphology of the scaffold, and it is not surprising if such a high-level structure might display polymorphism under varying conditions, there is some information available about the biochemistry of the chromosome core and the interactions stabilizing it. Histone-depleted chromosomes sediment with sedimentation coefficients in the range of 4,000 to 7,000 S, but treatment with 1,10-phenanthroline, neocuproine, or 2-mercaptoethanol reduces their sedimentation coefficients to around 1,500 S.[112,113] The effect of 2-mercaptoethanol does not appear to be caused by reduction of disulfide bonds, since more effective reducing agents which will not chelate metal ions do not produce the same effect.

This loosening of the structure of the chromosome scaffold rather appears to be a consequence of the removal of specific divalent cations. This specificity is shown both by the failure of the general chelating agent, ethylenediaminetetra-acetic acid, to have any effect and by experiments on the use of divalent cations to reverse the effect of the chelating agents when added prior to removal of the histones. Thus a restoration of the native properties is found when Cu^{++} is added after the chelation and before removal of the histones, while Ca^{++} will also prevent the loosening of the scaffold, when incubated at 37°C, but in this case the effect is not reversible again and further treatment with one of the chelating agents no longer causes the swelling. Other divalent metal ions tested were not found to have any effect. The interaction of the Cu^{++} with the histone depleted chromosomes may well be via sulfhydryl groups. This is suggested not only by the effectiveness of sulfhydryl compounds in unfolding the scaffold, but also by the finding that treatment of the chromosomes with either mercuric acetate or iodoacetamide, after the initial treatment with a chelating agent, abolishes the ability of Cu^{++} or Ca^{++} to reverse the effect of this initial treatment.[113] If Cu^{++} ions are involved in the physiological condensation, they would be a variant from Ca^{++} or Mg^{++} and this might be important in allowing an independent control of the chromosome condensation by the Cu^{++} concentration, without major effects on other cellular processes.

Following isolation of the histone-depleted chromosomes, only about 10% of the original chromosomal proteins remain. Moreover, subsequent nuclease treatment to remove the DNA allows isolation of a residual "scaffolding" which has a very simple composition and constitutes 3 to 4% of the total chromosomal protein. The pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis appears reproducible and consists largely of two prominent bands, called "Sc1" and "Sc2", with apparent M_r 170,000 and 135,000 respectively.[113] Pretreatment with chelating agents prevents the sedimentation of these proteins in a residual scaffolding and, consistently with the results above, this effect can be reversed by Cu^{++} ions.

B. Interphase Nuclei

Although any very high level of structural organization is much less obvious in interphase nuclei than in metaphase chromosomes, and fewer attempts have been made to study it, there is evidence suggesting at least some degree of superstructure. Histone-depleted nuclei from HeLa cells have been studied by electron microscopy of thin sections[114] and show that after histone extraction material remains around the nuclear periphery as well as internally.

The DNA appears to be organized by a subset of the nonhistone proteins, and possibly also RNA, and, following nuclease digestion to remove the DNA, a "nuclear scaffold" still remains. Two main groups of proteins were seen from these nuclear scaffolds, one with M_r around 65,000 to 70,000 and the other with M_r of 50,000 to 55,000. Although it has since been suggested that this latter group may arise from some contamination with cytoskeletal material, the presence of the former group has been confirmed.[115]

As for the metaphase chromosomes, treatment with chelating agents converts histone-depleted nuclei from a faster-sedimenting form (about 18,000 S) to a slower form (about 8,500 S).[112,116] This destabilization by chelation is again prevented by subsequent treatment with Cu^{++} or Ca^{++} ions, with the latter producing an irreversible effect. The observation that these ions have an effect even at concentrations as low as 1 μM[116] suggests that they may well be binding at specific sites in the structure of the nuclear scaffold.

Concomitantly with the loosening of the nuclear scaffold on chelation of metal ions, nonhistone proteins are lost. Thus the more compact structure contains 10 to 15% of the total nuclear proteins, including the proteins of the nuclear lamina of M_r 60,000 to 70,000 and numerous high molecular weight species, and many of these are selectively lost until only 3 to 5% of the nuclear proteins remain in the expanded structure.[115] Among these remaining proteins are those of the nuclear lamina and also 2 minor proteins of M_r 64,000 and 200,000. Based on these observations, it has been proposed that the chromatin of interphase nuclei may be organized both by binding to the nuclear lamina and also to a further subset of nonhistone proteins, by a Cu^{++} (or Ca^{++}) dependent interaction, with this latter contact maintaining the more compact configuration. Much of this chromatin would, as already discussed, be organized in 30-nm fibers which are then further compacted by this still higher organization.

V. TRANSCRIPTIONALLY ACTIVE CHROMATIN

A. Nucleosomal Structure

1. Electron Microscopic Studies

All of the foregoing discussion has been concerned with the structure of the bulk of the cellular chromatin which, in a typical eukaryotic cell, will not be active in transcription. The situation in transcriptionally active chromatin is more complicated and may even vary depending upon the actual transcription rate of a given region of the genome. Taken together with the selectivity inherent in much electron microscopy, this may explain the apparent conflict between a number of such studies and more biochemical experiments.

In an early study, which tended to concentrate upon the genes for ribosomal RNA, no evidence could be seen for a nucleosomal structure.[117] A similar conclusion was reached about actively transcribing chromatin from yeast,[118] and the result was generalized to apply to all of the active chromatin even though only ribosomal genes were identified. Moreover, the micrographs showed the chromatin fibers to be streaked out on the specimen grids and so the validity of the structural conclusions is doubtful because of the possible effects of such sample preparation techniques.[70] However one early study described the difference between ribosomal and nonribosomal chromatin during transcription, showing the former to be nonbeaded while the latter exhibited nucleosomes.[119] Two recent studies, concentrating on different, identified regions of the cellular DNA, have clarified the possible structures while showing the complexity of the position.

Nucleoli isolated from the germinal vesicles of oocytes of *Xenopus laevis* contain multiple copies of the DNA coding for ribosomal RNA which are very actively transcribed. When chromatin was isolated from such nucleoli, its appearance in the electron microscope was indistinguishable from that of naked DNA,[120] and there was no evidence for the presence of any protein other than the putative RNA polymerase molecules. Control experiments were

carried out to establish that the specimen preparation was not destroying any nucleosomal structure and also to show that the granular structures which could sometimes be observed were probably artifacts due to protein-DNA interaction after cell lysis. It is therefore likely that nucleolar DNA occurs as free DNA and not associated with histones or any other protein involved in chromatin organization.[120]

A different group of readily identifiable genes are those occurring in the Balbiani rings in salivary glands of *Chironomus tentans;* these are also involved in a high level of transcription and are normally heavily loaded with growing ribonucleoprotein particles, making it difficult to discern any other structural feature. However experiments have been performed using the RNA synthesis inhibitor, 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole, which blocks transcription at or near the initiation site, while allowing elongation and termination to proceed unhindered. In this way it has been possible to study the structure of these identified genes as the density of RNA molecules falls from very high to sparse.[121] In the fully active state, the genes appear as a 5-nm fiber and, together with the low level of DNA compaction, this suggests that the nucleosomes are structurally modified and opened out. As the RNA polymerases become more spaced out, the 10-nm, beaded nucleosome filament becomes visible between them and the histones must have been closely associated with the DNA to generate this. Probably the most likely explanation is that at least the core histones remain bound to the DNA during this transcription, but the core becomes unfolded during the process.

2. Chemical Studies

In contrast to the conclusions from much of the electron microscopy, nuclease digestion shows even actively transcribed chromatin to possess a periodic nucleosome structure,[122-124] although the observations on the ovalbumin gene in chick oviduct have since been called into question. In the earlier experiments,[124] hybridization was with cDNA to the messenger RNA, while the later experiments[125] were with genomic clones mostly to the 5′-side of the coding sequence, although also overlapping into the first intron. Whether the different probe locations account for the contradiction is not clear and further data are required to resolve this discrepancy. However, there is evidence that transcribed genes probably exist in an altered configuration. Transcribing DNA is preferentially susceptible to digestion by DNase I[126-128] and DNase II,[129] and in hen oviduct the ovalbumin gene is more rapidly cleaved by micrococcal nuclease than the globin gene.[124] Although the nucleosome repeat length appears to be inherited in somatic cells[130] and to correlate with the phenotypic expression,[131] the average length appears shorter in cells with an increased amount of RNA synthesis.[11-14] In addition, the repeat length on the actively transcribed DNA has recently been found to be shorter than the average for the cell.[132]

Besides the enhanced nuclease sensitivity of active regions of the chromatin, these also contain "hypersensitive" regions.[133,134] Unlike the general increased sensitivity, which appears both for some time before and for a period after the chromatin is transcribed, the hypersensitive sites occur only while a gene or set of genes is actually being expressed and may well involve some regions of protein-free DNA within the hypersensitive site.[135] A region of histone-free DNA has also been observed around the origin of replication in the minichromosomes of simian virus 40 and polyoma,[136,137] but here the DNA appears not to be protected from nuclease at any time. In this latter case the absence of nucleosomes could be due to a DNA sequence incompatible with packaging, but the hypersensitive regions in transcription must be due to some proteins which can recognize both specific regions of the DNA and also signals for when they should act.

The changes in properties of transcribing chromatin do correlate with an altered protein content. Active chromatin appears to have a reduced content of H1[138] and, since H1 is involved in the formation of the higher order structure, this could give rise to a looser and

less-folded structure in these regions. Various nonhistone proteins are concentrated on the active regions of the gene and, in particular, the high-mobility group proteins (HMG) 14 and 17 are not only found bound to actively transcribing chromatin, but also will selectively rebind to such regions after being dissociated by raised salt concentrations.[139,140]

B. Higher Order Structure

Although it is generally assumed that transcriptionally active chromatin will not display any higher order structure, this is not an essential conclusion from the available evidence. Thus it is certainly possible that the loops of chromatin might remain intact, even while the chromatin in a given loop might be unfolding and taking up a modified nucleosomal structure for transcriptional activity[102] (Figure 9). It seems rather less likely that the 30-nm fiber could continue to be present during transcription, but even this may be only a matter of degree.

In the experiments on the genes in the Balbiani rings, where the initiation of RNA polymerase was inhibited, a 25-nm fiber was observed as well as the 10-nm filament.[121] This thicker fiber certainly appeared after the last RNA polymerase molecule along a stretch of chromatin and it also appeared to be forming *between* two active polymerase molecules, if they were sufficiently far apart. Although it can be argued that this was only seen in these experiments involving the addition of a polymerase inhibitor, the Balbiani ring genes appeared as ordinary active genes, with an extended nucleofilament and densely packed nascent transcription products, in reversion experiments, so no irreversible effect had occurred. It thus appears that the formation of the 30-nm fiber does not require a gene to be devoid of RNA polymerase molecules along its entire length, but may rather depend upon their density.

This observation could lead to a model for active chromatin in which the nucleosome filament and even the solenoid are only transformed in a region around an RNA polymerase molecule and transformation of the entire gene depends upon the presence of sufficient RNA polymerase molecules. While this model is structurally plausible, it is not compatible with the generally held view that the entire gene is transformed in a determination step before activation. Whether the opening up of the hypersensitive regions alone, perhaps combined with some weakening of the interactions along the rest of the gene, might be sufficient to switch on a gene cannot be finally decided without further experimental evidence.

VI. CHROMATIN ASSEMBLY

A. Assembly of Nucleosomes

1. Histone Addition to DNA

An ordered structure for chromatin cannot be reassembled by simply mixing DNA and histones in stoichiometric amounts at ionic strength between 100 and 200 m*M* — conditions which are generally taken to correspond to physiological conditions in the cell. In such conditions the interaction between the DNA and the oppositely charged histones is so strong that nonspecific binding occurs and a precipitate forms rapidly. This problem can be overcome by mixing the components at sufficiently high ionic strength ($\geq$ 1 *M*) to reduce the interaction and then removal of the salt by dilution or dialysis (for review see reference 141). More recently it has been found possible to avoid the problem by a very slow addition of histones to the DNA[142] or even by allowing the precipitate to form and then the complex to rearrange during a prolonged incubation.[143] In both of these systems it was found essential to avoid any histone excess if satisfactory reassembly of nucleosome cores was to be obtained. Moreover, addition of H1 did not occur properly in these systems.

Neither of these systems appears to mimic in vivo assembly, which frequently occurs in the presence of excess histones, and there appears to be a requirement for some factor to reduce the overwhemingly strong interaction between the histones and DNA, slowing down the process so as to allow time for proper assembly. The first such "chaperone" to be

described was the protein "nucleoplasmin", which is a highly acidic polypeptide and catalyzes the addition of histones from a pool onto DNA.[144,145] Other polyanions also appear to be able to modulate the reassembly and, in particular, systems involving poly(glutamic acid)[143] and RNA[146] have been described. The complementary possibility is to complex the DNA so that it only becomes available for reaction at a slow rate and it has been reported that purified type I topoisomerase can catalyze the reassembly,[147] although the validity of this result has since been questioned because the enzyme preparation was found to contain RNA.[146]

One plausible candidate for holding the histones in a state in which they are available for assembly, but do not complex too rapidly onto the DNA, is nucleoplasmin. This protein has been described in the nuclei from many species[148] and has been shown to be present in sufficiently high concentrations in the embryos of *Xenopus laevis* to account even for the very high rate of chromatin assembly seen during early development.[149] Nucleoplasmin does not bind to DNA, but will interact with histones in vitro to render them in a state where they will no longer adhere to negatively charged surfaces.[149] Unusually for a protein, nucleoplasmin appears to be pentameric in solution and the aggregate with histones is also quite large. There is, however, no evidence of a sterically specific interaction with particular histones and its action appears to be by a general reduction in the binding to DNA rather than a more specific assembly of histone octamers.

While such factors can catalyze the assembly of nucleosomes, these are still not spaced at the same intervals as in naturally occurring chromatin and do not contain correctly positioned H1. The full assembly of chromatin can be obtained with an extract from oocytes of *Xenopus laevis*[144] which contains nucleoplasmin and possibly some other essential protein(s) which have yet to be described. Another factor involved in the assembly might be the state of modification of the histones. Although it is generally assumed that the many different modifications which have been described (for review see Reference 150) are involved in controlling the state of activity of the chromatin, some of the modifications could well be transiently present in nascent histones and be essential for their assembly into chromatin.[141]

B. Higher Order Structure

There is little information availble about the formation of the higher order structures in vivo. From the ease with which the 10-nm filament can be made to fold into the 30-nm fiber in vitro, it seems likely that this fiber could self-assemble, although it is possible that a process which occurs under some salt conditions may still require catalysis under different conditions. The observations of the rapid reformation of the 30-nm fiber after RNA synthesis[121] again suggest a relatively easy folding, but since this is occurring in vivo some additional factor could be involved.

The further folding of the 30-nm fiber into the structure of both interphase nuclei and metaphase chromosomes is still being investigated (see Section IV) and nothing is yet known about the process of assembly. It is, however, already clear that a number of nonhistone proteins are involved in these complex structures and other factors might be essential during assembly. These structures are also at a level to be involved in interactions with other significant structures in the cell, for instance the interaction of the chromosomes with the microtubules of the mitotic spindle during anaphase.

VII. OVERALL CONCLUSIONS

An overall picture of the folding of chromatin is emerging. although on going to higher levels of organization this picture gets fuzzy and less complete. The underlying structure is the nucleosome, containing 2 molecules of each of the 4 core histones, and most of these

also bind a single molecule of H1 (or H5). An interaction for which this H1 is essential then folds the nucleosomes into the 10-nm nucleosome filament, at low ionic strength, or into the 30-nm fiber at higher (and more physiological) ionic strengths. Loss of H1, or its displacement by nonhistone proteins, may well play a role in unfolding this level of the structure and allowing certain of the activities of the DNA to be expressed, but our knowledge about this control is very limited.

The 30-nm fiber is probably a solenoid which is stabilized by contacts between H1 molecules lying up the central hole. Because the major stabilizing interaction is at low radius, such a structure can readily bend and thus fold back upon itself allowing domains of chromatin to be formed, each represented by a single loop which is tied together at its base. The molecules involved in this tie can then interact together to form a scaffold holding together the chromatin. In interphase nuclei, this scaffolding appears to involve both the nuclear lamina and also some other component which maintains a more compact configuration. In metaphase chromosomes, however, the structure is largely stabilized from the center with the scaffold running along the arms of the chromosome and the chromatin organized into radial loops. The relation between these two forms of scaffold is not clear at present, nor is it known how the different structures of individual chromosomes are derived, although it seems certain that the information for this will lie in the DNA and will cause the binding of specific proteins to form both the centromere and the telomeres.

We are thus at an exciting stage where we understand the underlying chemistry and structure of chromatin and have some ideas about its assembly and the modifications which must occur during transcription. The next stages of our investigations should lead us to a more detailed picture in which we can begin to see the actual interactions bringing about the folding and how these are modified to change the structure as chromatin functions in the life of the cell.

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