Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10548–10555, October 1996

Review

What determines the folding of the chromatin fiber?

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ABSTRACT **In this review, we attempt to summarize, in a critical manner, what is currently known about the processes of condensation and decondensation of chromatin fibers. We begin with a critical analysis of the possible mechanisms for condensation, considering both old and new evidence as to whether the linker DNA between nucleosomes bends or remains straight in the condensed structure. Concluding that the preponderance of evidence is for straight linkers, we ask what other fundamental process might allow condensation, and argue that there is evidence for linker histone-induced contraction of the internucleosome angle, as salt concentration is raised toward physiological levels. We also ask how certain specific regions of chromatin can become decondensed, even at physiological salt concentration, to allow transcription. We consider linker histone depletion and acetylation of the core histone tails, as possible mechanisms. On the basis of recent evidence, we suggest a unified model linking targeted acetylation of specific genomic regions to linker histone depletion, with unfolding of the condensed fiber as a consequence.**

The belated discovery by molecular biologists that chromatin structure might be of major importance in regulating DNA transcription and replication has sparked a renewed interest in some old questions: How does a chain of nucleosomes fold to produce the condensed fibers observed in the eukaryotic nucleus? What makes it unfold to allow transcription or replication? The latter question has been especially perplexing. The earliest electron microscopy studies of isolated chromatin fibers showed that at low salt concentrations an extended string of nucleosomes could be observed, whereas raising the ionic strength to levels close to physiological led to the formation of an irregular, highly condensed fiber about 30 nm in diameter. Speculations concerning the mechanisms of such folding and the structure of the "30 nm fiber" were rife in the early postnucleosome years (for review, see refs. 1 and 2, and references therein). Although many models for the condensed fiber structure were proposed, and hotly debated, the "solenoid" model of Finch and Klug (3) or variants thereof (4, 5) gained acceptance by most researchers. In such structures, nucleosomes adjacent on the DNA strand are packed cheek-by-jowl into a regular helix.

A corollary of the solenoid model is that the linker DNA between adjacent nucleosomes must, at least at physiological salt concentration, be bent or curled in some fashion to allow adjacent nucleosomes to contact one another. This salt-dependent bending or coiling was postulated to be facilitated by the interaction of linker DNA with "linker histones" (H1, H5, and the like), for these proteins have been demonstrated to be essential for proper chromatin fiber condensation (see below). In this review, we will try to critically evaluate the data on (*i*) linker DNA bending, (*ii*) nucleosome-nucleosome interactions, (*iii*) the role of linker histones, and (*iv*) the role of the core histone tails and their acetylation in the folding of the chromatin fiber. In addition, we present some speculations concerning the unfolding of the fiber in transcription-related processes.

DOES THE LINKER DNA BEND?

The solenoid model appeared to gain substantial support from the studies of Yao *et al.* (6), which provided evidence that the linker DNA in dinucleosomes did in fact contract or fold in some fashion as the salt concentration was raised from 0 to 20 mM. The picture (and in particular, the putative role of linker histones) was complicated by a subsequent study (7), which showed that the same changes could be observed with dinucleosomes from which linker histones had been removed. Yao *et al.* (6, 7) used two techniques to provide evidence for linker contraction: (*i*) direct visualization of fixed dinucleosomes by transmission electron microscopy (EM) and (*ii*) measurement of the translational diffusion coefficient of dinucleosomes by dynamic light scattering. The latter measurements showed an increase in the translational diffusion coefficient (D) with increasing salt concentration (Fig. 1*A*, dashed line). This is most easily explained by a compaction of the particle, for such compaction should produce a decrease in the frictional coefficient, f, and hence an increase in D, since D is inversely proportional to f. The results from hydrodynamic experiments were supported by EM studies, in which dinucleosomes in 20 mM Na^+ or 2 mM Mg^{2+} were observed to be more compact than those in 2 mM Na^+. However, in a recent publication, Bednar *et al.* (9) have repeated both experiments with quite different results. As Fig. 1*A* shows, they observe the diffusion coefficient to be independent of salt concentration over the same range. In addition, Bednar *et al.* (9) find by cryo-EM that the center-to-center distance between nucleosomes in dimers does not decrease as salt concentration is raised. Interestingly, they do, like Yao *et al.* (6, 7), find a contraction at higher salt when the dinucleosome is studied by conventional transmission EM. This indicates that either transmission EM or cryo-EM is giving an artifactual result.

Thus, we have, at the focus of an important issue, a clear contradiction: Does the linker DNA contract with increasing salt or does it not? On the resolution of this question depends how we may visualize the condensed chromatin fiber, and what role the linker histones may play in that condensation. To approach this issue, we have first asked: Are there other data in the literature that might support one view or the other?

In fact, there exists a wealth of relevant evidence, both in the earlier literature and from more contemporary work. Consider, for example, the measurement of diffusion coefficients of dinucleosomes by dynamic light scattering. In an earlier study, Marion *et al.* (8) find no change in D in up to 80 mM salt; their data are in almost exact quantitative agreement with those of Bednar *et al.* (9) (Fig. 1*A*) and are inconsistent with those of Yao *et al.* (6).

There is, in addition, extensive evidence from sedimentation studies of dinucleosomes. If a dinucleosome contracts with increasing salt concentration, the sedimentation coefficient s should increase, for like D, s is inversely proportional to the

Abbreviations: EM, electron microscope/microscopy; SFM, scanning force microscope.

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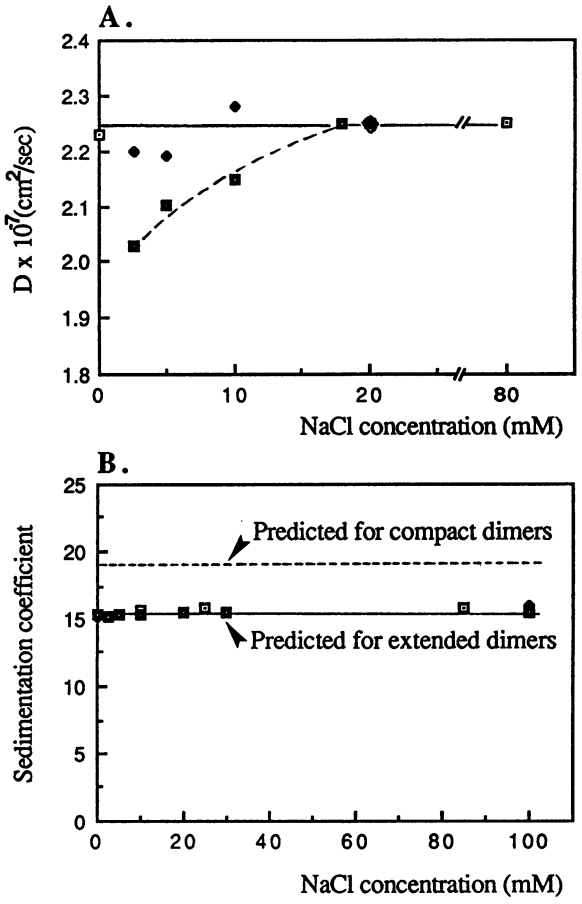

FIG. 1. Hydrodynamic studies of dinucleosomes as a function of salt concentration. (*A*) Diffusion coefficient measurements from quasi-elastic light scattering: □, data of Marion *et al.* (8); ■, data of Yao *et al.* (6); ◆, data of Bednar *et al.* (9). (*B*) Sedimentation coefficient measurements: ◆, data of Wittig and Wittig (10); ■, data of Strätling (11); □, data of Butler and Thomas (12).

frictional coefficient. Published results from a number of laboratories are shown in Fig. 1*B*; all lead to the same conclusion: there is no significant change in the sedimentation coefficient of dinucleosomes over the entire salt concentration range from 0 to 100 mM. All data points lie close to an average value of 15.4S. We can make a rough estimate of the change in s to be expected from contraction of the linker by using the Kirkwood (13) formalism as adopted by Bloomfield *et al.* (14). This expresses s_n, the sedimentation coefficient for an n-mer, in terms of s_1, the sedimentation coefficient of the monomer, the Stokes' radius of the monomer (r), and the set of distances (R_{ij}) between units i and j in the n-mer,

$$s_n/s_1 = 1 + r/n \sum_i \sum_j (1/R_{ij}). \qquad [1]$$

For an approximate calculation, we neglect explicit contributions of the linker DNA to the frictional coefficient, and take $s_1 = 11.3S$, *the value for a chromatosome* (11). *As shown in Fig. 2A*, the center-to-center distance depends slightly on the mutual orientation of the two histone cores. With an average extended linker length of 62 base pairs (bp) (as for chicken erythrocyte chromatin), we will have center-to-center distance ranging from 21 to 23 nm; we adopt 22 nm as an average value for R_{12}. On this basis, we predict an s of 15.4S for the dinucleosome. Given the approximations involved, the exact agreement with the observed value is probably fortuitous. However, the significant point is that decreasing R_{12} to 11 nm (for a condensed dimer, with the two nucleosomes touching each other) leads to a prediction of s_2 = 19S (Fig. 1*B*, broken line). The difference is clearly far greater than can be accounted for by experimental error.

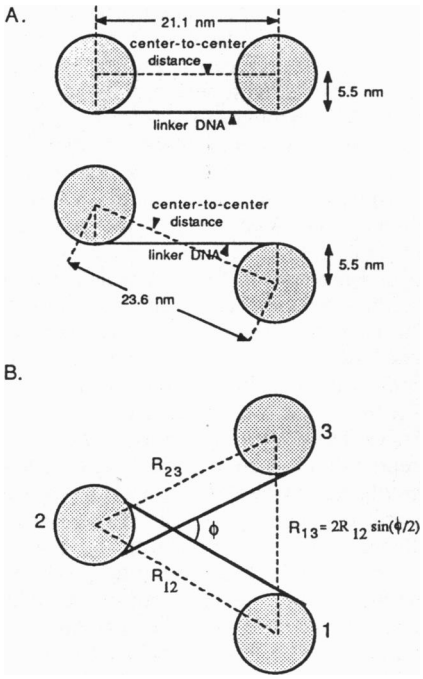

FIG. 2. Models of dinucleosomes and trinucleosomes. (*A*) Dinucleosome. The relationship between center-to-center distance and linker length depends slightly on relative orientation of nucleosomes. Two extremes are shown for chicken erythrocyte dinucleosomes. (*B*) Trinucleosome. The distance R_{13} depends on the angle ϕ between DNA duplexes entering and exiting nucleosome 2.

As a test for the credibility of the experimentally measured values for D and s, we may ask if the combination of the best estimates for D (2.25×10^{-7} cm^2/sec) and s (15.4×10^{-13} sec) gives a reasonable molecular weight for an average dinucleosome. It is realized of course, that individual dinucleosomes may vary in linker length, degree of external DNA trimming, and content of linker histones. Calculation of the molecular weight of a typical dinucleosome, based on reasonable structural parameters (total DNA length plus two histone octamers plus two linker histones) gives a value of $M = 5.13 \times 10^5$ g/mol. This is in almost exact agreement with that calculated from s and D from the Svedberg equation

$$M = \frac{RTS}{D(1 - \bar{\nu}\rho)} = 5.10 \times 10^5 \text{ g/mol} \qquad [2]$$

when we have used the value given by Wittig and Wittig (10) for the partial specific volume, $\bar{\nu}$.

Finally, we have recently performed measurements of center-to-center distance in dinucleosomes, imaged by scanning force microscopy (SFM) (G. Zuccheri, S. H. Leuba, C. Bustamante, J.Z., and K.v.H., unpublished work). No statistically significant differences as a function of salt concentration were observed: the average values at 0, 10, and 20 mM salt under various surface and fixing conditions were 20.1, 20.0, and 21.5 nm, respectively, essentially invariant with salt, and in agreement with the data given above.

None of these data, however, can give direct information as to the conformation of the linker DNA *in situ*. An independent experimental approach to the issue of linker DNA bending has been developed that allows investigation of the state of the linker in nuclei (15, 16). The method is based upon the observation that the rate of photo-induced thymine dimer formation is affected by the direction and degree of DNA

bending. DNA from dinucleosomes isolated from irradiated nuclei was examined for its distribution of thymine dimers. It was found that whereas dimer formation in the core region of the nucleosome occurred with periodicity of approximately 10 bp, the distribution through the linker region was nearly uniform, indicating that linker DNA in nuclei is relatively straight. Support for this contention comes from recent EM imaging of chromatin in sections of starfish sperm nuclei, in which straight linker DNA between nucleosomes can clearly be observed (17, 18).

The organization of linker DNA in chromatin and nuclei has been also studied by monitoring the products of nuclease digestion. The 10 bp periodicity of nuclease cutting repeatedly observed (refs. 19, 20, and 68 and references therein) is usually taken as an indication that chromatin DNA forms a continuous superhelix, the linker DNA continuing the path of the DNA in the core particle. This notion may not be correct, however, in view of the report that the same periodicity of DNA cutting by nucleases was observed when DNA was laterally shielded by a flat surface (mica, calcium phosphate surface) (21). Thus, it seems equally appropriate to interprete the 10 bp periodicity in terms of some sort of lateral shielding (asymmetric accessibility) in the fiber rather than in terms of coiled linker DNA.

In summary, there is an unreconciled disagreement as to the probable conformation of linker DNA in condensed chromatin. In our opinion, the preponderance of data indicate that the linker remains extended even at elevated salt concentration. Yet, the contrary experiments of Yao *et al.* (6, 7) are carefully performed and documented, and cannot be lightly dismissed. Furthermore, it is very difficult, especially with the older data, to be sure that comparable structures and conditions are being compared.

Although we can see no easy way to resolve this apparent contradiction, we feel, upon weighing all the evidence, that the hypothesis that the linkers remain extended as chromatin condenses must be considered very seriously. If this is so, the solenoid model and some of its variants cannot be accepted. We and others have, however, argued that none of the several specific models that envisage the condensed fiber as a regular structure have any substantial experimental support (22, 23).

WHAT HAPPENS DURING CONDENSATION?

If the linker DNA neither bends nor coils during salt-induced chromatin fiber condensation, what change can occur? A strong candidate is collapse in the angle ϕ, made between DNA duplexes entering and exiting from the nucleosome (see Fig. 2*B*).

Indirect evidence for just this kind of change can be found in sedimentation studies of oligonucleosomes (10, 12). For example, the data of Butler and Thomas (12) (Fig. 3) show that the trimer and higher oligomers respond qualitatively differently to salt increases than do monomers and dimers. As we noted above, whereas monomers and dimers exhibit salt-independent sedimentation coefficients, the trimer and next several oligomers display an initial increase in s between 5 and 20 mM NaCl, followed by a leveling off at higher salt concentrations. This has to be attributed to changes in some structural feature of trimers and higher oligomers that is not found in dinucleosomes, and an obvious suggestion is that this is the angle ϕ. We can estimate the magnitude of this change by using Eq. **1**, with the R_{ij} parameters as defined in Fig. 2*B*. If linkers do not bend, R_{12} and R_{23} will be constants, but R_{13} will depend on ϕ according to the formula shown in the figure.

In the right ordinate of Fig. 4, we show the values of ϕ that would account for the trimer sedimentation coefficients shown as the left ordinate. A decrease in ϕ from about 100° to about 45° could account for the change in s observed as salt increases. A value of about 100° at low salt is in agreement with SFM observations (S. H. Leuba, K.v.H., and J.Z., unpublished data;

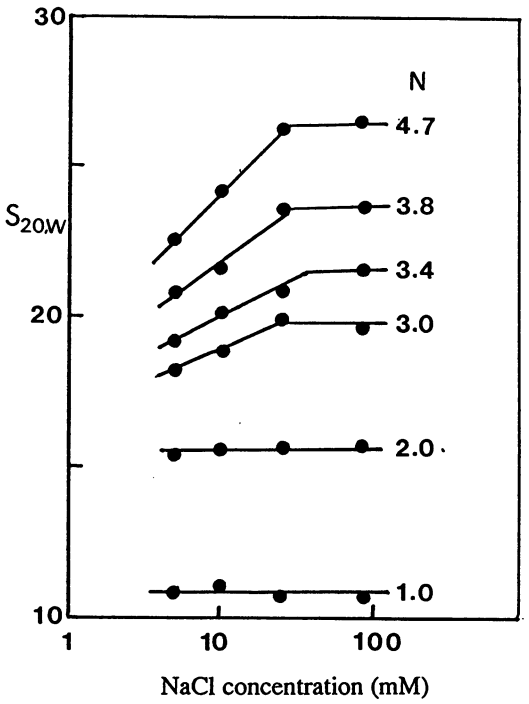

FIG. 3. The data of Butler and Thomas (12) for sedimentation of small oligonucleosomes as a function of salt concentration. The number N denotes the average number of nucleosomes in each oligonucleosome. Sedimentation coefficients are in Svedbergs. Data courtesy of J. Butler and J. Thomas (Department of Biochemistry, University of Cambridge, Cambridge, U.K.).

see Fig. 6). We postulate that angles smaller than about 45° are not permitted because of steric hindrance between nucleosomes 1 and 3. Note that the positions in which they are drawn in Fig. 2*B* are the most mutually avoiding; each can rotate about the line describing the linker, leading to greater interference. The physical basis of such nominal "rotation" could either be heterogeneity in linker lengths or be possible changes in the twist of the linker DNA. If the linker DNA is rigid, then the orientation of nucleosome $i + 1$ relative to that of nucleosome i will depend on the ratio of the linker length (in bp) to the helical twist of the DNA. Adding one extra bp to the linker, for example, will lead to a rotation of one nucleosome with respect to the other of 36°. Alternatively, changes in this "rotation" may be achieved by underwinding or overwinding of the linker DNA. However, since such changes in twist would be accompanied by a large free energy penalty, they may not

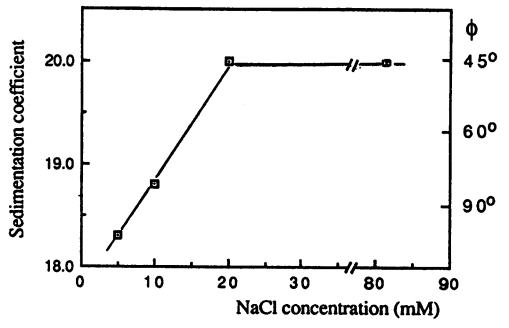

FIG. 4. Explaining the variation in trinucleosome sedimentation coefficient with salt on the basis of change in the angle ϕ. Sedimentation coefficients (in Svedbergs) of trinucleosomes are graphed versus salt concentration as in Fig. 3. On the right ordinate is given the angle ϕ that will account for different values, using Eq. **1** and the expression of R_{13} given in Fig. 2*B*.

generally occur, as has been pointed out by Widom and colleagues (24, 25).

The consequence of such a decrease in the angle ϕ can be seen from simple modeling studies of the kind shown in Fig. 5. In constructing these models, we have assumed a rigid linker with a twist of 10.4 bp per turn and a random distribution of linker lengths between 51 and 73 bp (for other parameters of the model, see refs. 26 and 27). The effect of decreasing ϕ from 90° to 45° is quite dramatic; it leads to compaction of what was a quite irregular, kinky, fiber-like structure to something much more recognizable as a "canonical" fiber (more regular in diameter along its length, with a smoother path of the axis). The average diameter does not change much (see Fig. 5); what has happened is an accordion-like folding similar to that postulated by Bordas *et al.* (28, 29).

It should be emphasized that the compact fiber generated in this way is still not a regular solenoid. Linker lengths and orientation of nucleosomes are nearly random. More important, there cannot in this structure be close contacts between nucleosomes i and $i + 1$; only between i and $i + n$ when $n \geq 2$ are contacts possible. In this respect, the model resembles some earlier models, in which linker DNA crosses the fiber interior (e.g., refs. 30 and 31), albeit without a regular structure. We must stress, however, that the lack of interaction between successive nucleosomes depends on just one condition: the linker DNA must be straight. One does not need to invoke criss-crossing by the linker DNA of the fiber interior, as suggested by some models. We also note that in regions of chromatin with uniformly spaced nucleosomes (satellite chromatin could be a possible example) regular helices should be generated; the structure of the helix would be exquisitely sensitive to the exact linker length (see ref. 17).

The kind of indirect evidence presented above for the collapse in the angle ϕ has been recently supported by direct measurements on trinucleosomes imaged by cryo-EM (9). The mean angle (as measured between entering and exiting linker DNA) changed from ≈56° in 5 mM to ≈39° in 20 mM salt, effectively bringing the two outer nucleosomes close to each other, thus compacting the particles.

Why might the angle ϕ decrease as salt concentration increases? We can imagine several different explanations, which follow.

***(i)* Decrease in Electrostatic Repulsion Between Linkers.** In a thoughtful analysis, Clark and Kimura (32) have considered the role of electrostatic interactions in determining chromatin structure, and single out linker–linker repulsion as a probable major source of condensed fiber destabilization at low ionic strength.

***(ii)* Decrease in Electrostatic Repulsion Between Nucleosomes, Perhaps Accompanied by Favorable Interactions when They Can Come into Close Contact.** The decrease of electrostatic repulsion between nucleosomes is held by Clark and Kimura (32) to be of lesser importance than that between linkers. The contribution of favorable interactions between nucleosomes is more difficult to evaluate, although the formation of defined aggregates ("arcs and helices") of isolated core particles at higher ionic strength (33) would suggest that such interactions may not be negligible.

The idea that nucleosome–nucleosome interactions actually take place in the condensed chromatin fiber has been implicitly

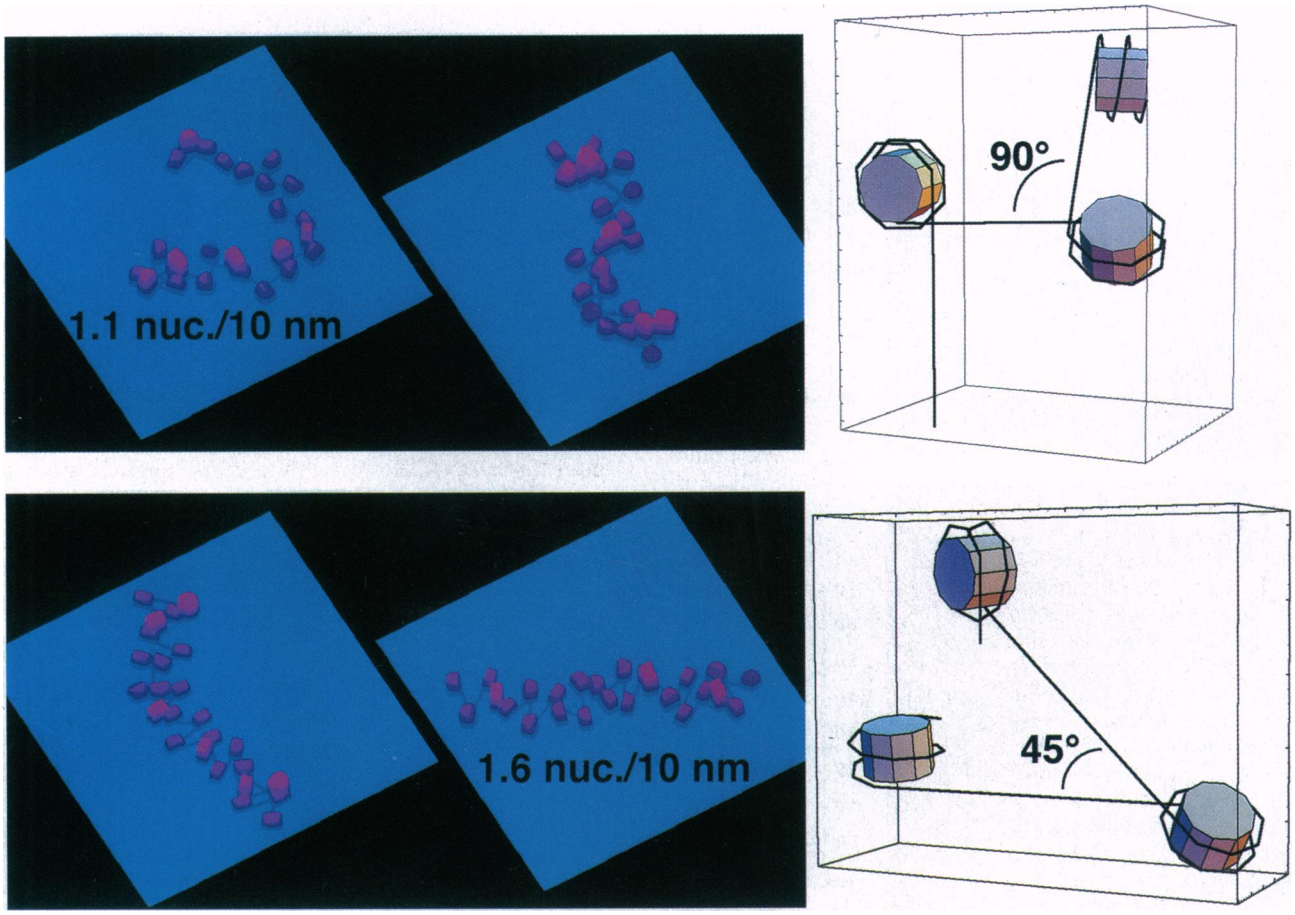

FIG. 5. The effect of angle ϕ on the conformation of modeled chromatin fibers. (*Upper*) Chromatin fibers have been modeled by assuming 1.75 turns of DNA about the histone octamer, giving an angle ϕ of 90°. (*Lower*) It is assumed that DNA is wrapped about 1.6 turns about each octamer, giving $\phi = 45°$. The lower value of ϕ gives a more regular and uniform fiber structure, which is also more condensed (number of nucleosomes per 10 nm of fiber length equals 1.6 for the 45° structure, in comparison with 1.1 for the 90° structure). Courtesy of S. Leuba.

assumed by many researchers, but the actual data to support it are, to the best of our knowledge, sparse and indirect. Early studies of nucleosome reconstitution on linear templates have found closely spaced core particles (34). EM vizualization of minichromosomes reconstituted on negatively supercoiled circular templates showed the coexistence of saturated minichromosomes and of naked DNA molecules at low histone/DNA ratios (35). Such results have been taken to imply cooperativity of nucleosome assembly via nucleosome–nucleosome interactions. The tails of the core histones have been implicated in these interactions, since their removal by trypsin led to loss of the cooperativity (36). The significance of these results is complicated by the observation that nucleosomes reconstituted on relaxed circles lose the cooperativity of assembly (36). More recently, Garcia-Ramirez *et al.* (37) reported studies on the salt-dependent folding of nucleosomal arrays reconstituted from trypsinized core histones onto a tandemly repeated 12-mer of the 5S rRNA gene. This complex failed to compact upon salt increase, in contrast to its counterpart reconstitute containing intact histones. Although such types of experiments are interpreted to indicate nucleosome–nucleosome interactions, in fact they may be just recording effects of binding of the core histone tails to linker DNA. That such binding occurs has been documented in a number of studies (see refs. 20 and 38, and references therein). It should also be noted that all above studies involved reconstitutes lacking linker histones, hence their relevance to *in situ* chromatin remains elusive.

***(iii)* Effect of Salt on the Way in Which Linker Histones Interact with Linker DNA.** As we shall see below, this becomes a most interesting possibility in view of recent advances concerning the binding of linker histones to mononucleosomes.

WHAT IS THE ROLE OF LINKER HISTONES?

Shortly after the discovery of the nucleosomal structure of chromatin, it was realized that the presence of linker histones was somehow essential for the proper folding of the chromatin fiber (for example, see ref. 39). The word "proper" is important here, for a number of workers have shown that some kind of condensation occurs even when linker histones are absent (for review, see ref. 40 and references therein). However, in cases where the structures of such condensates have been visualized by microscopy, they have been found not to closely resemble the native condensed fiber (e.g., ref. 39). Thus, it is perhaps better to say that linker histones facilitate, in part by charge neutralization, and help guide the proper folding of the chromatin fiber than that they are necessary for folding *per se*.

Even at low ionic strength, removal of linker histones from chromatin produces a dramatic change in fiber morphology. Fig. 6*A* contrasts SFM images of native chicken erythrocyte chromatin with the same material after the removal of histones H1 and H5. Conditions for preparation and microscopy are identical. The differences in the structures are both qualitative and quantitative. Since SFM images directly demonstrate three-dimensionality, it is clear that the native fiber exists as a very irregular helix-like structure, even at this low ionic strength (see also ref. 26). On the other hand, the H1/H5-depleted fibers lie almost flat on the surface. SFM lends itself well to measurement of center-to-center distances between nucleosomes. For the native and linker histone-depleted fibers,

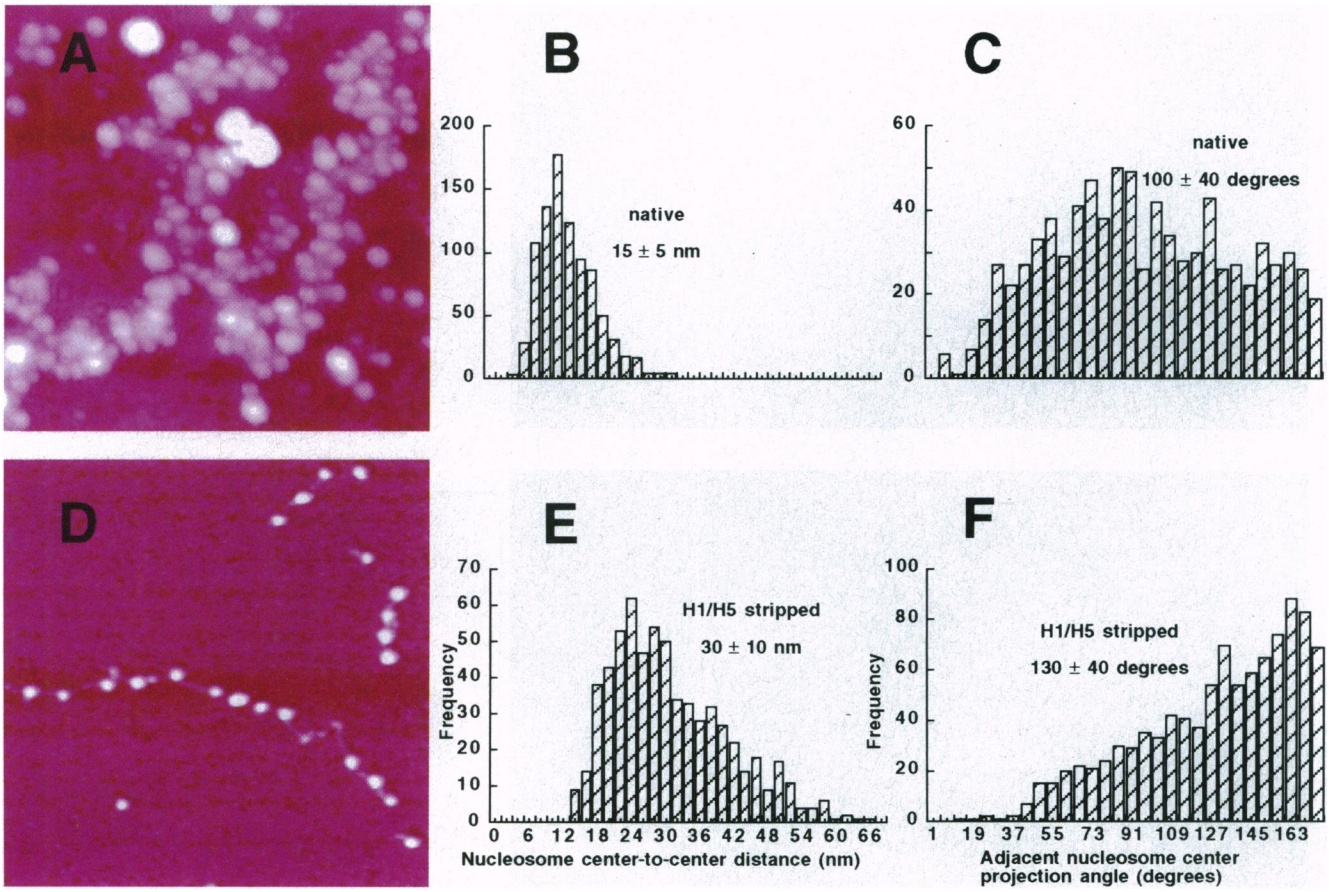

FIG. 6. SFM images and histograms of frequency distributions of center-to-center distances and projection angles between lines connecting consecutive nucleosome centers. (*A–C*) Native long chromatin fibers from chicken erythrocytes; (*D–F*) linker histone-stripped fibers. Because the fibers used in this particular study were glutaraldehyde-fixed, the mean center-to-center distance is slightly less than that found for unfixed fibers (20 nm). Courtesy of S. Leuba.

we find the distributions shown in Fig. 6 *B* and *E*. The mean value for the native fiber (20.1 ± 6.1 nm) is very close to what we would expect (62 bp × 0.34 nm/bp = 21 nm) if the linker is extended. There is, however, a significant dispersion about this mean, presumably resulting from differences in linker length. For the linker histone-depleted fiber, we observe a distribution that is strongly skewed to larger values; this can best be explained by the hypothesis (ref. 27 and see below) that a part of core DNA is actually peeled off to become linker when the linker histones are removed. Earlier biophysical and biochemical data supporting this view are discussed in our recent review (ref. 41; see also ref. 42).

Measurements of the distribution of angles (ϕ, see Fig. 6C) in SFM images of chromatin fibers reveal an even more dramatic effect of removing linker histones. As Fig. 6*C* shows, the angle is distributed about a mean of approximately 100° in native fibers at low ionic strength, in good agreement with Fig. 4. But when the chromatin is stripped of linker histones, a highly skewed distribution, biased toward 180°, is observed (Fig. 6*F*). A reasonable explanation, consistent with the increase in center-to-center distance (see above) and recent studies on mononucleosomes (43) is shown in Fig. 7*A Upper*.

The same studies (43) have demonstrated that when only the globular domain of linker histones in present, mononucleosomes adopt a conformation in which the DNA makes nearly two turns about the histone core (Fig. 7*A Lower Left*). However, the entering and emerging strands do not cross, but bend sharply away from one another. The same figure depicts what happens to the DNA upon binding of intact linker histone (Fig. 7*A Lower Right*). The presence of the C-tail of the linker histone causes the two DNA strands to pull together for form a "stem." As Fig. 7*B* shows, this will lead, in a trinucleosome,

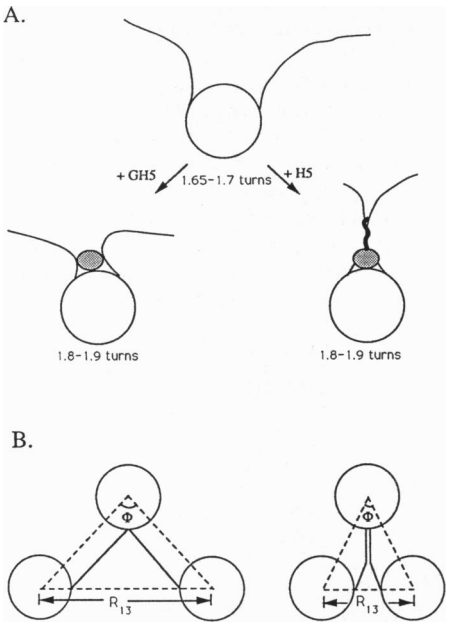

FIG. 7. (*A*) Recent models for the effect of linker histones on the DNA entering and exiting the nucleosome. These schematic drawings are based on the cryo-EM and EM studies of Furrer *et al.* (44) and Hamiche *et al.* (43). At the top is shown the kind of structure commonly observed for mononucleosomes not containing linker histones. The DNA tails are partially peeled off the core, and pushed apart by electrostatic repulsion. Addition of the globular domain of H5 pulls the DNA into nearly two complete turns about the histone core, but the tails still diverge. Addition of H5 molecules carrying the C-terminal tails, brings the two DNA duplexes together, producing a "stem" structure of about 30 nm in length. These structures were observed at 50 mM NaCl. (*B*) Schematic drawing of how the kind of stem structure depicted in *A* could result in a decrease in the value of ϕ calculated form nucleosome center positions.

to a decrease in the distance R_{13}, with a parallel decrease in the angle ϕ, as measured from nucleosome centers. It may be that this effect of the C-tail on linker DNA is why complete linker histones are required for the proper condensation of chromatin.

WHAT IS THE ROLE OF CORE HISTONE TAILS IN FIBER COMPACTION?

The N-terminal tails of the core histones do not seem to participate in the structure of the core particle itself (45) but to protrude out of it as disordered structures. If fully extended, these tails can reach relatively far to interact with other chromatin components. As noted above, some indirect data would indicate their possible involvement in nucleosome–nucleosome interactions. While it is still unclear whether such interactions actually exist *in situ*, interactions of the tails with linker DNA are well established (refs. 20 and 38, and references therein). Core histones have also been shown to interact with histone H1 (see ref. 41 and references therein) and it is not unreasonable to suggest that these interactions involve mainly the protruding tails of the core histones.

If the core histone tails interact with both the linker DNA and the linker histones, one might expect them to affect fiber structure. Indeed, numerous physical studies of partially trypsinized chromatin from which the core histone tails had been removed have reported such a connection (42, 46–51).

All these studies focus on the salt-induced ability of the fiber to condense and establish that the tails of the core histones are important for condensation. We have recently approached the effect of the core histone tails on the fiber structure at low ionic strength (S. H. Leuba, C. Bustamante, K.v.H., and J.Z., unpublished work). We have previously demonstrated that the fiber is three-dimensionally organized even at low ionic strength (26). We now used mild trypsin digestion of chicken erythrocyte fibers such that the only tail cleaved off was that of histone H3 (together with both tails of the linker histones) and SFM to image fiber morphology and quantitate the changes due to trypsinization in terms of nucleosome center-to-center distances, the angle ϕ, and fiber heights. The trypsinization experiments performed on native fibers were complemented with reconstitution experiments in which intact linker histones or their globular domains were added back to H1/H5 depleted fibers, containing either intact core histones or core histones lacking the N-terminal tail of histone H3. The results unequivocally showed that in addition to the globular domain of the linker histones, the fibers must contain either the unstructured tails of the linker histones or the N-terminal tail of histone H3, to three-dimensionally fold. Only flat, "bead-on-a-string" type morphology was observed when both the tails of the linker histones and that of histone H3 were missing. We believe that the dependence of fiber condensation on the tails of the core histones observed in the earlier studies (see above) reflects the inability of the "tailless" fibers to properly three-dimensionally organize at low ionic strength to start with; in the lack of such proper initial folding, further compaction cannot occur correctly.

HOW CAN CHROMATIN FIBERS UNFOLD TO ALLOW TRANSCRIPTION?

It is becoming increasingly evident that both the initiation of transcription and its elongation phase must require massive opening up of condensed chromatin fibers. Some of the factors involved in "chromatin remodeling" required for initiation are now known to be truly enormous, as is the polymerase complex itself; neither can possibly be incorporated into a condensed chromatin fiber structure.

There is, in fact, substantial evidence that transcriptionally active regions of chromatin exhibit a more "open" fiber structure than do those which are repressed (1, 2). In addition

to the existence of specific nuclease hypersensitive sites (locations at which nucleosomes seem to be missing or altered), there exist, associated with genes either competent for or engaged in transcription, extensive regions over which nuclease sensitivity is increased. Exactly how the chromatin conformation has been altered in such regions remains obscure. Several groups have reported experiments showing lower sedimentation coefficients for chromatin fragments from active genes than from inactive fragments of the same size, implying unfolding. For example, Fischer and Felsenfeld (52) have compared globin gene and ovalbumin gene chromatin in chicken erythrocytes and oviduct. In each case, the gene that is active in the tissue (or had been active, in the globin case) yielded chromatin fragments of larger size than inactive gene chromatin that sedimented to the same point in a sucrose gradient. The differences were not large and were observed only at higher ionic strength (100 mM); nevertheless, they could be indicative of a preferential partial unfolding of the active chromatin regions under physiological salt conditions. In the case of the erythrocyte globin genes, this unfolding appears to be very much localized to the hypersensitive sites. In a careful study, Caplan *et al.* (53) showed that the lower *s* value could be accounted for by complete unfolding at just these localities; indeed, restriction digestion of the whole globin domain led to subfragments that sedimented as condensed chromatin. Thus, it may be that chromatin in such "poised" (but inactive) genes remains largely folded in the coding regions. Nonetheless, we must expect that condensed fibers will be unraveled when transcription is actually occurring.

What are the chemical or compositional changes that could allow the chromatin fiber to decondense even under physiological ionic conditions? Two possibilities have been repeatedly suggested, both of which are consistent with what we have seen above concerning fiber stabilization. These are (*i*) depletion of linker histones and (*ii*) modification of histone tails.

Depletion of Linker Histones. There are many studies that show, or purport to show, a deficiency in linker histones in transcriptionally active regions of chromatin. This work has been described in a recent review (54) and need only be summarized here. In brief, the conclusion from diverse studies on many genes is that linker histones are present on transcribed or transcribable genes, but to a reduced extent. Many studies report about 50% reduction; others indicate an inverse relationship between transcriptional activity and linker histone content. However, there are also experiments that indicate little, if any, depletion (for review, see ref. 55). It should be noted that the potential for transcription and actual transcription may require different levels of linker histone depletion. Perhaps chromatin can be made accessible to the transcriptional machinery through only partial depletion (for example, in the promoter region), but requires complete depletion for the actual passage of polymerase (see refs. 56 and 57). Finally, results from protein–DNA cross-linking experiments indicate that in some cases gene activation may be accompanied by a change in the way the linker histone interacts with DNA rather than by histone removal (58). It was observed that the globular domain of H1 could not be readily cross-linked to the DNA in active genes, although the tails of the histone remained attached.

Acetylation of Core Histone Tails. We have noted above that removal of certain core histone N-terminal tails (especially those of H3, and possibly those of H4) inhibits the three-dimensional folding of the fiber at low ionic strength and the formation and/or maintenance of the condensed state of chromatin. This suggests that acetylation of lysines in these tails might also facilitate the relaxation of condensed chromatin fibers, since neutralization of cationic groups by acetylation should be expected to interfere with histone tail–DNA interactions. Direct evidence for such interference comes from recent DNA–histone cross-linking studies (59), which demonstrate a reduction in contacts between H3 tails and DNA in hyperacetylated nuclei. However, since not all contacts are lost, it is not clear how closely hyperacetylation will mimic proteolytic removal of tails in its effects on chromatin structure. Certainly, acetylation drastically weakens the interactions; quantitative studies of the binding of the N-terminal peptide of H4 to DNA show a reduction in affinity by a factor of 5×10^6 upon acetylation (60).

In any event, there remains a large body of correlative evidence to indicate that histone hyperacetylation is in some way associated with the transcriptionally competent state (for review, see ref. 61). That it is competence or "poising" for transcription, rather than transcription itself that correlate with hyperacetylation has been indicated by many studies, most recently and convincingly by the Crane-Robinson group (62). They have shown that transcriptionally active and "poised" chicken globin genes display equal levels of hyperacetylation, whereas inactive globin genes are much less acetylated. Moreover, this pattern extends beyond the transcribed regions, and, in fact, coincides with the pattern of generalized DNase I sensitivity (63).

Suggestive as all these correlations might be, the question as to how certain chromatin regions are specifically targeted for hyperacetylation has remained unanswered. Very recent results from Allis's laboratory (64) point toward an intriguing solution. Brownell *et al.* (64) have cloned the *Tetrahymena* histone acetyltransferase A, and find it highly similar in sequence to a known transcriptional coactivator from yeast, Gcn5p. Coactivators function as adaptor molecules that convey molecular signals from activators (the sequence-specific binding transcription factors that select genes to be activated) to the basal transcriptional apparatus (65). The crucial discovery of Allis and colleagues is that Gcn5p *itself* has type A acetyltransferase activity. Thus, although the evidence is still incomplete, it seems probable that the chain of events leading to specific acetylation of genes to be transcribed involves binding of transcription factors to specific gene sequences and recruiting histone acetyltransferases as coactivators, with the ensuing acetylation of core histone tails in this region (Fig. 8).

Are there data to link the two transcription-associated changes in chromatin described so far: the linker histone depletion and histone acetylation? Could it be that core histone hyperacetylation might itself induce linker histone loss or redistribution, with accompanying decondensation of the chromatin fibers? We believe that the answer is in the positive, since core histone tails have long been known to bind to linker

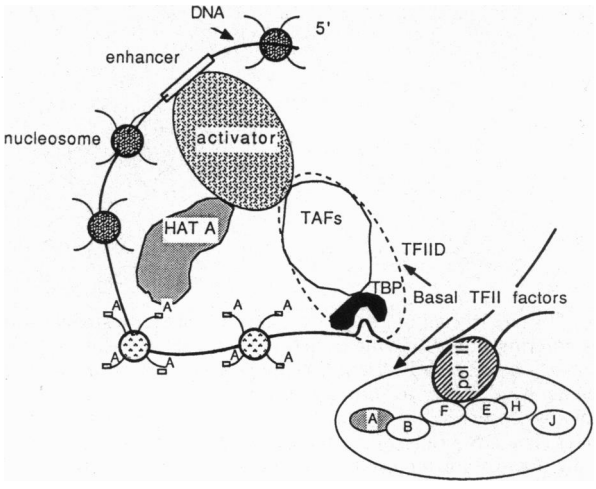

FIG. 8. Schematic presentation of how histone acetyltransferase A (HAT A) might be targeted to specific promoters. If HAT A is a coactivator protein, it will be expected to bind to certain transcriptional activators, which, by association with the TFIID complex, will activate transcription. HAT A will be thus be poised to hyperacetylate histones on nucleosomes selected by transcription factors for activation.

DNA (see above), the site of linker histone binding itself. If acetylation changes the way the tails of the core histones interact with the linker DNA, the altered interaction could trigger changes in the linker histone binding.

Some data in support of such a model exist. Evidence that H1 interactions with nucleosome cores lacking the N termini or containing acetylated histones may be less stable or altered, comes form studies showing lack of H1-mediated condensation in such chromatin (42, 66). Moreover, binding of H1 to such core particles did not produce the repression to binding of some transcription factors that is observed with core particle DNA, containing unmodified histones (67). The latter study also directly demostrated a reduction in the affinity of H1 for nucleosomal cores lacking the N-terminal tails of the core histones. If correct, this model will provide the first picture to incorporate chromatin modification, linker histone binding perturbation, and fiber decondensation into a logical scenario for preparing specific genes for transcription.

CONCLUDING REMARKS

We appear to be in a period when a number of lines of seemingly unconnected research are beginning to converge toward the solution of a major problem: How is transcription regulation related to the structure of chromatin? As we gain fuller understanding of the mechanics of the chromatin fiber, we begin to appreciate just what linker histones do in regulating its structure and accessibility to enzymes and regulatory protein factors. At the same time, the phenomenon of hyperacetylation, long known to be associated with chromatin activity, takes on renewed significance, as new data appear to show not only how it might be targeted to specific gens, but what it might do, in structural terms, when it is imposed therein. The next few years should be exciting, with new insights into chromatin structure and its participation in the regulation of gene expression.

We acknowledge the extensive help of Dr. S. H. Leuba (University of Oregon, Eugene) who provided unpublished data, SFM images, and measurements, and performed the mathematical modeling presented here. This work was supported by National Institutes of Health Grant GM50276.

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