0031-6997/79/3004-0469$02.00/0
PHARMACOLOGICAL REVIEWS Vol. 30, No. 4

Levels of Organization of the DNA in Eucaryotic Chromosomes*

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Mitosis is the stage of the cell cycle at which the chromatin fiber of the nucleus becomes organized into distinct structural entities visible in the light microscope. Metaphase chromosomes are complexes containing the DNA packaged with histones and nonhistone proteins for distribution into daughter cells during mitosis. Knowledge of the structure of the chromosome, as bearer of the hereditary material, is fundamental to an understanding of diverse aspects of biology.

The DNA of eucaryotic chromosomes is compacted by at least three levels of organization. The first level is brought about by the fundamental structural unit of the chromatin fiber, the so-called nucleosome. The assembly of the chain of nucleosomes into the chromatin fiber is the second level. These two basic levels of folding seem to be due solely to the interaction of DNA and histones. The third level of organization is the folding and/or coiling of the chromatin fiber to form the compact chromosomes.

The Nucleosome

The chromatin fiber has been studied with the electron microscope for a number of years. As observed in early studies this fiber has a rather irregular, knobby appearance with a diameter varying between 200 to 300 Å (9, 11, 27). Recently, a wealth of data has shown that the chromatin fiber is composed of a repeating structural particle called the nucleosome.

If the basic chromatin is unfolded at low ionic strength the nucleosome can be observed in the electron microscope as a particle with a diameter of about 100 Å arranged along the DNA like beads on a string (21, 41). Studies of chromatin digestion with staphylococcal nuclease show that the "spacer" DNA that connects the repeating units is attacked first. Such treatment releases nucleosome oligomers and monomers, which allows a determination of the number of base pairs of DNA and a characterization of the protein components of the fundamental repeat. The nucleosome contains an octamer of histones (14) composed of two each of the lysine-rich histones H2A and H2B and the arginine-rich histones H3 and H4. The octamer of histones protects a DNA segment of 140 base pairs in length (3, 29, 30) against digestion with staphylococcal nuclease. The nucleosome containing the 140 base pair of DNA is referred to as the nucleosome "core" and is invariant in all chromatin samples studied (16, 18, 31). Variability exists in the spacer DNA (10 to 70 bp) that links the nucleosome cores (18).

How is the DNA arranged in the nucleosome? In the electron microscope the nucleosome has a roughly spherical appearance, about 100 Å in diameter. However, a combination of neutron and X-ray scattering data indicates that the nucleosome is disk-shaped, about 100 Å in diameter and 50 Å in height with the DNA being wrapped around the periphery (24). More direct and detailed information about the nucleosome core and the exact path of the peripheral DNA is expected from X-ray diffraction

* This research was supported by grant PHS GM 25111 from the National Institute of Health and by the National Science Foundation grant PCM76-18517.

studies of crystallized nucleosome cores (10). At the present resolution the crystallographic data are in good agreement with the model derived from the neutron scattering data.

Several lines of evidence suggest a possible 2-fold axis of symmetry in the nucleosome structure. Dissociation of the DNA-histone interaction with 2 M NaCl appears to liberate two tetramers from each unit containing one molecule of each histone (38) (heterotypic tetramer). Although this observation is in conflict with other data (34), the idea of the "half" nucleosome is supported by electron microscopic observations (22) and studies of histone-histone interaction (7). The results of digestion of nuclei with pancreatic DNase I also support this idea. This enzyme generates in the DNA protected by the nucleosome single-stranded cuts that are seperated by integral multiples of 10 nucleotide residues (19). Kinetic data show that the relative accessibility of the potential cleavage sites vary and that these sites are symmetrically distributed in the 140 base pair fragment, consistent with a dyad axis. The existence of such a dyad axis in the nucleosome is not completely established but has been included in some models (10, 39).

Chromatin Fiber

Although a wealth of evidence indicates that nucleosomes are the basic repeat unit of the chromatin fiber, much less is known about the packing arrangement of the string of nucleosomes in the thick (200 to 300 Å) fiber. Neutron diffraction studies indicate that the nucleosomes are arranged into a solenoid with a pitch of 100 Å and a diameter of 300 Å. Some electron micrographs of nucleosome oligomers are in agreement with such a model (4), while others indicate that the nucleosomes form clusters called "superbeads" rather than cylindrical solenoids, each containing about 6 to 10 nucleosomes (26, 32). The controversy regarding this conflicting morphological evidence is not settled, but it has to be kept in mind that the variations of structure may be due to uncontrolled parameters of sample preparation.

The most lysine-rich histones, H1, are not part of the nucleosome core particle, but bind to the spacer DNA linking the units. H1 liberation from chromatin correlates with the digestion of the spacer region by staphylococcal nuclease (20, 23, 36, 40). Good evidence is also available that suggests that the H1 histones are involved in stabilizing the assembly of nucleosomes in the thick fiber. Extraction of H1 from chromatin with salt unfolds the thick fiber into the thin (100 Å) fiber composed of a linear array of beads (5, 13, 37). The exact mode of the contribution of H1 to the assembly of oligonucleosomes is not clear, and various models have been discussed; as one possibility, H1 could stabilize the structure by cross-linking nonadjacent nucleosomes.

Folding of the Chromatin Fiber in Metaphase Chromosomes: Evidence for Radial Loops

Packaging of DNA in the nucleosome results in condensation of the DNA length by a factor of about seven, and a further condensation is achieved by assembly of the string of nucleosome into the thick fiber. All the different models for the arrangement of nucleosomes in this fiber result in a total linear shortening of the DNA by a factor of 30 to 40, whereas the ratio of total DNA length of the metaphase chromosomes is about 8000. Hence, whatever the precise arrangement of nucleosomes in the thick fiber, it is clear that higher levels of packaging are needed to explain the compactness of mitotic chromosomes.

Elucidation of the higher-order folding of the nucleoprotein is difficult, because of the complexity and compactness of the chromosomes. We have for this reason developed an approach that permits us to discriminate between the structural contribution made by the histones and certain nonhistone proteins that we have named scaffolding proteins (15). We found that it is possible to gently remove all histones and many nonhistone proteins from metaphase

FIG. 1. Electron micrographs of histone-depleted metaphase chromosome from HeLa cells. Histone-depleted chromosomes were isolated and prepared for electron microscopy by the monolayer technique described by Paulson and Laemmli (25). The chromosomes consist of a central fibrous scaffolding to which the DNA is bound, forming a halo. The scaffolding is composed of nonhistone proteins (1, 2). The individual DNA loops are clearly seen at the edge of the DNA halo (bar, 2 μm). In a few favorable micrographs these loops can be traced as complete radial loops starting from adjacent points on the scaffolding.

chromosomes by competition with polyanions (such as dextran sulfate and heparin) and thereby to isolate a structure containing a highly folded DNA chain held together by the so-called scaffolding proteins. These histone-depleted chromosomes can be isolated on sucrose gradients as fast sedimenting structures (4000 to 7000 S). They contain 5% to 8% of the chromosomal proteins (in about 30 bands on sodium dodecyl sulfate-polyacrylamide gels) and are virtually free of histones (1). This finding opened up the possibility of identifying and studying the role of the scaffolding proteins in the long-range order of mitotic chromosomes.

Quite unexpectedly we found that these histone-depleted chromosomes have a highly organized structure (25). Although expanded in size by a factor of two to three, the histone-depleted chromosomes retain the characteristic shape of the metaphase chromosome. This is because of the existence of a central network (scaffolding) composed of nonhistone proteins, which extends through most of the length of the chromatid to which the DNA is bound, forming a halo of DNA (fig. 1). Because of the large size of the DNA halo, only part of the original micrograph is shown. The DNA is clearly attached to the scaffold, but it is impossible to trace individual strands in most cases. In a few favorable micrographs, areas can be found of sufficiently low DNA density so that some strands can be traced (25). In all these cases we find perfect loops, which start at a point on the scaffolding and return to an adjacent point. They provide strong evidence for a radial loop arrangement of the DNA in the halo. The loops vary in size between 10 and 30 μm.

Our results suggest a scaffolding model for metaphase chromosome structure in which a set of nonhistone proteins form a central network cross-linking the DNA into a radial distribution of loops. The role of

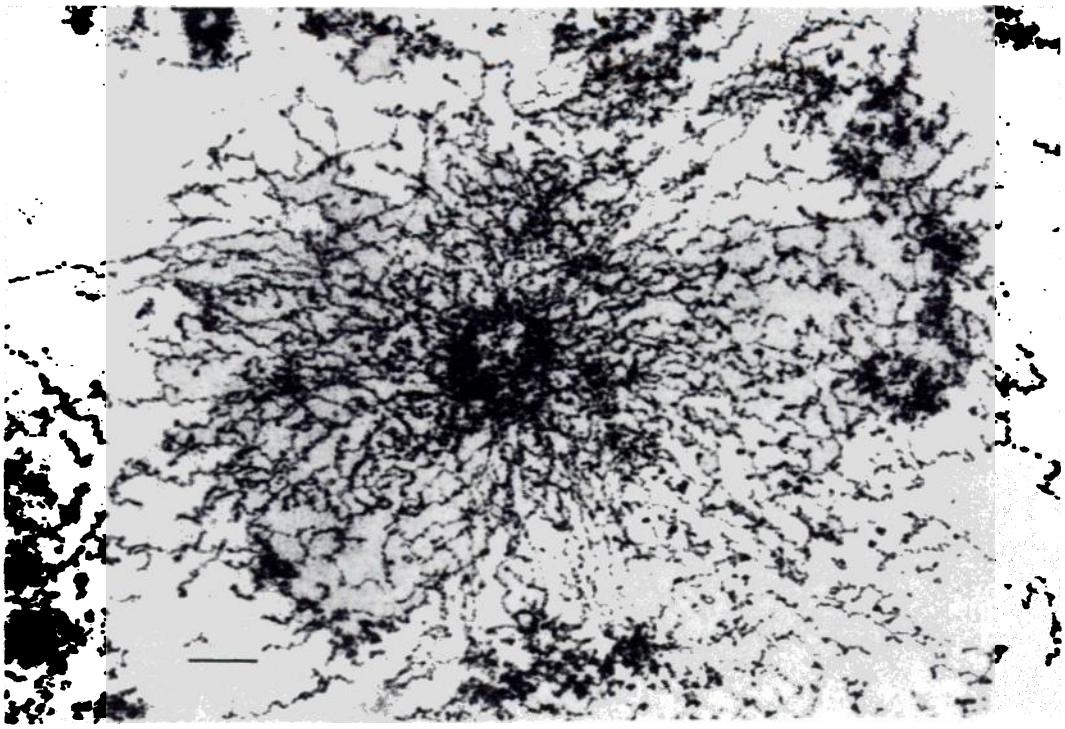

FIG. 2. Cross section through a chromatid of a swollen chromosome. Chromosomes were swollen with EDTA, fixed and embedded in elon as described by Marsden and Laemmli (17). The micrographs provide evidence of a radial arrangement of the chromatin fiber around a central axis (bar, 0.2 μm).

the histones in this model is to compact these DNA loops.

The idea of a backbone or core to help organize the nucleoprotein fiber is not new [e.g., see Taylor (33)] and has been incorporated in some early models as a means of organizing the chromatin fiber. Some doubt about the existence of cores has been expressed, principally on the ground that such a structure cannot be seen in thin sections of chromosomes [e.g., see Comings and Okada (6)]. This is not surprising since the fibrous scaffolding that extends through the chromatid may not be sufficiently distinct to be recognizable in the presence of all the other components of the compact chromosome. We reasoned that such a structure would be best observed in swollen chromosomes containing well dispersed chromatin fibers, and we have studied this question recently by thin-sectioning intact chromosomes. We found that a central scaffolding region and radially arranged loops can be clearly discerned in electron micrographs of swollen chromosomes (17).

The most informative micrographs were obtained after dispersion of chromatin fibers by chelation of the divalent cations present. The chromosomes swell during this process by a factor of about four as a result of unfolding of the thick (200 to 300 Å) chromatin fiber to the thin (100 Å) fiber. This latter structure is most likely a linear arrangement of nucleosomes. Figure 2 shows a typical cross-section of a swollen chromosome. This cross-section reveals a central, dense region from which many fibers are predominantly oriented in a radial fashion. Many fibers form loops that can often be traced for long distances in a radial direction. Most of the ends observed at the periphery are probably not free ends, but are loops that leave the plane of sectioning. There are good reasons to think that the DNA loops observed in the histone-depleted chromosome are structurally related

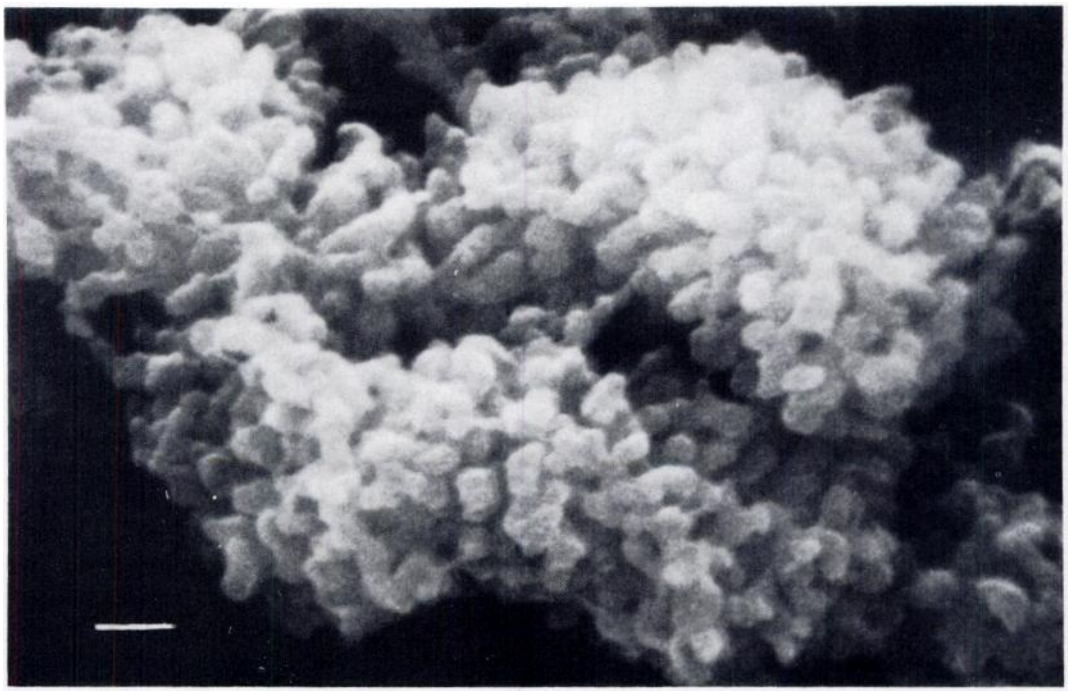

FIG. 3. Surface topology of chromosomes observed with the scanning electron micrograph. Isolated chromosomes were fixed, dehydrated, and coated with gold/paladium as described by Marsden and Laemmli (17). Note the compact projections as the major topological feature. These projections are thought to be the condensed loops of the thick nucleoprotein fiber (bar, 0.2 μm).

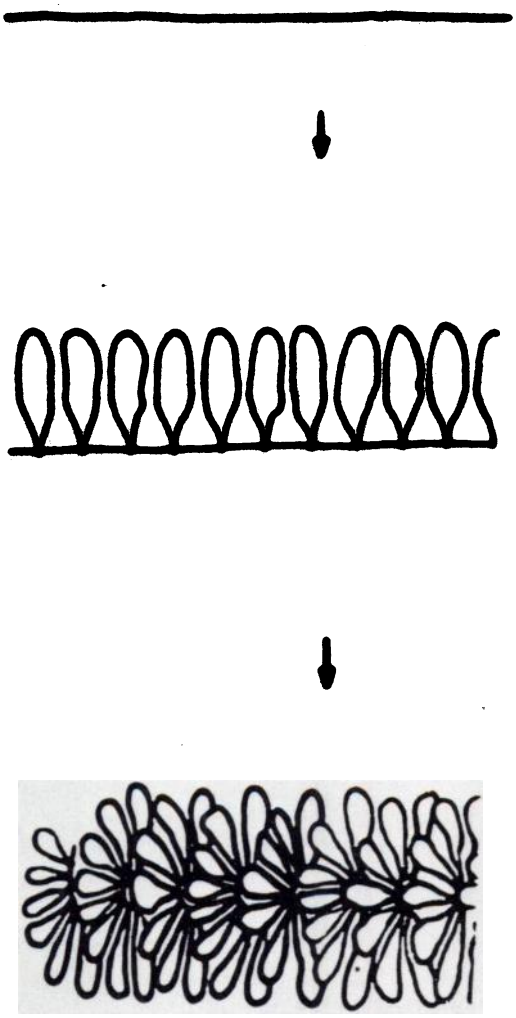

FIG. 4. Organization of the chromatin fiber in the radial loop model. In the radial loop model the basic fiber is first folded to form loops and subsequently wound such that the bases of the loops become the central axis of the chromatid.

to those observed in the swollen chromosomes. The DNA loops observed in the histone-depleted chromosomes have an average length of about 23 μm (25), which would be shortened to about 3.5 μm by the linear array of nucleosomes of the 100 Å fiber. The approximate loop size of the 100 Å fiber in the swollen chromosomes is approximately 3 to 4 μm, which provides strong support for the structural relatedness of these loops.

A radial loop arrangement is also consistent with appearance of chromosomes, stabilized either with intermediate levels of magnesium chloride or hexylene glycol. The latter chromosomes are particularly suited for examination of the compact configuration, because of the relative absence of cytoplasmic material that tends to adhere to the periphery of chromosomes. Thin sections of these chromosomes show regular projections of about 500 Å diameter, which are again tight loops of the thick fiber, possibly shortened by twisting the fiber into short supercoils. These projections, called convules by Daskal et al. (8), can also be seen in the scanning electron microscope. Figure 3 shows such a micrograph: the surface texture of these chromosomes is very "bumpy"; the "convules" have a diameter of about 500 to 550 Å and are the most prominent topological feature. The features of these projections from the periphery of hexylene-glycol-stabilized chromosomes suggest they are compacted forms of the more extended loops of swollen chromosomes or of the naked DNA loops of histone-depleted chromosomes.

We have recently proposed a model, depicted in figure 4, in which the chromatin fiber is first folded to form loops (fig. 1) and subsequently wound so that the bases of the loops form the central axis of the chromatid (17). The role of the scaffolding proteins is to stabilize and cross-tie the loops. We do not know how one loop is structurally related to a neighboring loop, but the simplest way would be to arrange them in a helical array.

Many chromosome models are available in the literature and most are based on helical arrangements in which the chromatin fiber is wound into a coil and the coil wound again one or possibly two times to achieve the compactness of the intact chromosomes (28). Our micrographs provide no

evidence for such a hierarchy of coils, but do give strong evidence for the type of model shown in figure 4.

We have briefly discussed here the information available pertaining to chromosome structure relevant to DNA packaging. The picture that emerges is a static one and provides no insights regarding the dynamic behavior of chromosomes. Chromosomes must condense and decondense in the course of being replicated and transcribed. Our structural and biochemical knowledge is so far too incomplete to allow fulfillment of the tenet of the molecular biologist that knowledge of structure leads to understanding of function, but recent progress in chromosome research is very encouraging and promises a more detailed understanding in the near future.

Acknowledgments. We wish to thank Mei Lie Wong for her valuable assistance and J. R. Fresco for reading the manuscript.

REFERENCES

1. ADOLPH, K. W., CHENG, S. M., AND LAEMMLI, U. K.: Role of nonhistone proteins in metaphase chromosome structure. Cell **12**: 805–816, 1977.
2. ADOLPH, K. W., CHENG, S. M., PAULSON, J. R., AND LAEMMLI, U. K.: Isolation of a protein scaffold from mitotic HeLa cell chromosome. Proc. Nat. Acad. Sci. U.S.A. **74**: 4937–4941, 1977.
3. AXEL, R.: Cleavage of DNA in nuclei and chromatin with staphylococcal nuclease. Biochemistry **14**: 2921–2925, 1975.
4. CARPENTER, B. G., BALDWIN, J. P., BRADBURY, E. M., AND IBEL, K.: Organization of subunits in chromatin. Nucleic Acids Res. **3**: 1739–1946, 1976.
5. CHRISTIANSEN, G., AND GRIFFITH, J.: Salt and divalent cations affect the flexible nature of the natural beaded chromatin structure. Nucleic Acids Res. **4**: 1837–1851, 1977.
6. COMINGS, D. E., AND OKADA, T. A.: Some aspects of chromosome structure in eukaryotes. Cold Spring Harbor Symp. Quant. Biol. **38**: 145–153, 1974.
7. D'ANNA, J. A., AND ISENBERG, I.: A histone cross-complexing pattern. Biochemistry **13**: 4992–4997, 1974.
8. DASKAL, Y., MACE, M. L., WRAY, W., AND BUSCH, H.: Use of direct current sputtering for improved visualization of chromosome topology by scanning electron microscopy. Exp. Cell Res. **100**: 204–212, 1976.
9. DUPRAW, E. J.: DNA and Chromosomes, Holt, Rinehart and Winston, New York, 1970.
10. FINCH, J. T., LUTTER, L. C., RHODES, D., BROWN, R. S., RUSHTON, B., LEVITT, M., AND KLUG, A.: Structure of the nucleosome core particles of chromatin. Nature (London) **269**: 29–36, 1977.
11. GALL, J. G.: Chromosome fibers studied by a spreading technique. Chromosoma **20**: 221–233, 1966.
12. Deleted in proof.
13. KELLER, W., WENDEL, I., EICKEN, I., AND ZENTGRAF, H.: Biochemical and ultrastructural analysis of SV40 chromatin. Cold Spring Harbor Symp. Quant. Biol. **42**: 227–244, 1977.
14. KORNBERG, R. D.: Chromatin structure: A repeating unit of histones and DNA. Science **184**: 868–871, 1974.
15. LAEMMLI, U. K., CHENG, S. M., ADOLPH, K. W., PAULSON, J. R., BROWN, J. A., AND BAUMBACH, W. R.: Metaphase chromosome structure: The role of nonhistone proteins. Cold Spring Harbor Symp. Quant. Biol. **42**: 351–360, 1977.
16. LOHR, D., CORDEN, J., TATCHELL, K., KOVACIC, R. T., AND VAN HOLDE, K. E.: Comparative subunit structure of HeLa, yeast and chicken erythrocyte chromatin. Proc. Nat. Acad. Sci. U.S.A. **74**: 79–83, 1977.
17. MARSDEN, M., AND LAEMMLI, U. K.: Metaphase chromosome structure: Evidence for a radial loop model. Cell **17**: 849–858, 1979.
18. MORRIS, N. R.: A comparison of the structure of chicken erythrocyte and chicken liver chromatin. Cell **9**: 627–632, 1976.
19. NOLL, M.: Internal structure of the chromatin subunit. Nucleic Acids Res. **1**: 1573–1578, 1974.
20. NOLL, M., AND KORNBERG, R. D.: Action of micrococcal nuclease on chromatin and the location of histone H1. J. Mol. Biol. **109**: 393–404, 1977.
21. OLINS, A. L., AND OLINS, D. E.: Spheroid chromatin units (ν-bodies). Science **183**: 330–332, 1974.
22. OUDET, P., SPADAFORA, C., AND CHAMBON, P.: Nucleosome structure. II. Structure of the SV40 minichromosome and electron microscopic evidence for reversible transitions of the nucleosome structure. Cold Spring Harbor Symp. Quant. Biol. **42**: 301–312, 1977.
23. PARDON, J. F., WORCESTER, D. L., WOOLEY, J. C., COTTER, R. I., LILLEY, D. M. J., AND RICHARDS, B. M.: The structure of the chromatin core particle in solution. Nucleic Acids Res. **4**: 3199–3214, 1977.
24. PARDON, J. F., COTTER, R. I., LILLEY, D. M. J., WORCESTER, D. L., CAMPBELL, A. M., WOOLEY, J. C., AND RICHARDS, B. M.: Scattering studies of chromatin subunits. Cold Spring Harbor Symp. Quant. Biol. **42**: 11–22, 1977.
25. PAULSON, J. R., AND LAEMMLI, U. K.: The structure of histone-depleted metaphase chromosomes. Cell **12**: 817–828, 1977.
26. RENZ, M., NEHLS, P., AND HOZIER, J.: Involvement of histone H1 in the organization of the chromosome fiber. Proc. Nat. Acad. Sci. U.S.A. **74**: 1879–1883, 1977.
27. RIS, H.: Chromosomal structure as seen by electron microscopy. Ciba Found. Symp. **28**: 7–15, 1975.
28. SADAT, J., AND MANUELIDIS, L.: A direct approach to the structure of eukaryotic chromosomes. Cold Spring Harbor Symp. Quant. Biol. **42**: 331–350, 1977.
29. SHAW, B., HERMAN, T., KOVACIC, R., BEAUDREAU, G., AND VAN HOLDE, K.: Analysis of subunit organization in chicken erythrocyte chromatin. Proc. Nat. Acad. Sci. U.S.A. **73**: 505–509, 1976.
30. SOLLNER-WEBB, B., AND FELSENFELD, G.: A comparison of the digestion of nuclei and chromatin by staphylococcal nuclease. Biochemistry **14**: 2915–2920, 1975.
31. SPADAFORA, C., BELLARD, M., COMPTON, J. L., AND CHAMBON, P.: The DNA repeat lengths in chromatins from sea urchin sperm and gastrula cells are markedly different. Fed. Eur. Biochem. Sco. Lett. **69**: 281–285, 1976.
32. STRATLING, W. H., MULLER, U., AND ZENTGRAF, H.: The higher order repeat structure of chromatin is built up of globular particles containing eight nucleosomes. Exp. Cell Res. **117**: 301–311, 1978.
33. TAYLOR, J. H.: Duplication of chromosomes. Sci. Amer. **198**: 36–42, 1968.
34. THOMAS, J.: The nucleosome core protein. Cold Spring Harbor Symp. Quant. Biol. **42**: 119–125, 1977.
35. Deleted in proof.

36. VARSHAVSKY, A. J., BAKAYEV, V. V., AND GEORGIEV, G. P.: Heterogeneity of chromatin subunits *in vitro* and location of histone H1. Nucleic Acids Res. **3**: 477–492, 1976.
37. VARSHAVSKY, A. J., BAKAYEV, V. V., AND GREGIEV, G. P.: On the structure of eukaryotic, prokaryotic and viral chromatin. Cold Spring Harbor Symp. Quant. Biol. **42**: 457–473, 1977.
38. WEINTRAUB, H., PALTER, K., AND VAN LENTE, F.: Histones H2A, H2B, H3 and H4 form a tetrameric complex in solutions of high salt. Cell **6**: 85–110, 1975.
39. WEINTRAUB, H., WORCEL, A., AND ALBERTS, B.: A model for chromatin based upon two symmetrically paired half-nucleosomes. Cell **9**: 409–417, 1976.
40. WHITLOCK, J. P., JR., AND SIMPSON, R. T.: Removal of histone H1 exposes a fifty base pair DNA segment between nucleosomes. Biochemistry **15**: 3307–3313, 1976.
41. WOODCOCK, C. L. F.: Ultrastructure of inactive chromatin. J. Cell Biol. **59**: 368a, 1973.