Biochemistry

 Volume 39, Number 14 April 11, 2000

New Concepts

Physical Constraints in the Condensation of Eukaryotic Chromosomes. Local Concentration of DNA versus Linear Packing Ratio in Higher Order Chromatin Structures†

Joan-Ramon Daban*

Departament de Bioquímica i Biologia Molecular, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain

Received November 15, 1999

ABSTRACT: The local concentration of DNA in metaphase chromosomes of different organisms has been determined in several laboratories. The average of these measurements is 0.17 g/mL. In the first level of chromosome condensation, DNA is wrapped around histones forming nucleosomes. This organization limits the DNA concentration in nucleosomes to 0.3−0.4 g/mL. Furthermore, in the structural models suggested in different laboratories for the 30−40 nm chromatin fiber, the estimated DNA concentration is significantly reduced; it ranges from 0.04 to 0.27 g/mL. The DNA concentration is further reduced when the fiber is folded into the successive higher order structures suggested in different models for metaphase chromosomes; the estimated minimum decrease of DNA concentration represents an additional 40%. These observations suggest that most of the models proposed for the 30−40 nm chromatin fiber are not dense enough for the construction of metaphase chromosomes. In contrast, it is well-known that the linear packing ratio increases dramatically in each level of DNA folding in chromosomes. Thus, the consideration of the linear packing ratio is not enough for the study of chromatin condensation; the constraint resulting from the actual DNA concentration in metaphase chromosomes must be considered for the construction of models for condensed chromatin.

The density of DNA confined to small volumes in viruses, bacteria, and eukaryotic cells is high (*1*−*3*). Since the length of the chromosomal DNA molecules exceeds the dimensions of the cellular structures in which they are contained, the linear packing ratio (defined as the ratio between the length of extended DNA and the length of the structure that contains it) is widely used to measure the degree of DNA compaction (*4*, *5*). Kellenberger et al. (*6*) have shown that the local concentration of DNA (defined as the mass of DNA per unit volume of the biological structure that contains it) is also useful for the quantitative study of the DNA compactness. Here I show that the local concentration of DNA is more appropriate than the DNA packing ratio to validate structural models for chromatin condensation in metaphase chromosomes.

Local Concentration of DNA in Chromosomes. The concentration of DNA in the bacterial nucleoid is about 0.02 g/mL (i.e., 0.02 pg/μm^3) (*6*). The nuclei of eukaryotic cells have different volumes [their diameters range from 3 to 20 μm (*1*, *7*, *8*)]; cells having a small nucleus (~5 μm diameter)

† This work was supported in part by Grants PB95-0611 and PB98-0858 (Dirección General de Enseñanza Superior e Investigación Científica) and by Grant 1998SGR-00072 (Generalitat de Catalunya).

* Correspondence should be addressed to this author. E-mail: JoanRamon.Daban@uab.es; Fax: 34-93-5811264; Phone: 34-93-5811616.

10.1021/bi992628w CCC: $19.00
Published on Web 03/16/2000

Table 1: Local Concentration of DNA in Metaphase Chromosomes of Different Organisms and in Sperm Heads Containing Histones[a]

organisms and cells	DNA concn (g/mL)
human[b]	
fibroblast (*10*, *11*)	0.16[c]
lymphocytes (*13*)	0.12[d]
monocotyledon plants (*10*)	0.18[e]
dicotyledon plants (*10*)	0.18[f]
Euglena (*12*)	0.20
S. Purpuratus sperm (*16*)	0.19

[a] DNA concentration obtained in different laboratories by low-dose ratio-contrast scanning transmission electron microscopy (*12*), and from the mass of DNA and the volumes of metaphase chromosomes obtained from electron micrographs (*16*), three-dimensional reconstruction from electron micrographs of serial sections of embebded metaphase cells (*10*, *11*), and scanning force microscopy (*13*). [b] The DNA concentration in human cells was calculated considering 3.5 pg (i.e., 3.2×10^9 bp) of DNA per haploid genome (*1*, *2*, *71*). [c] Mean of the values obtained by Bennet et al. (*10*) and Heslop-Harrison et al. (*11*). [d] Average value obtained with hydrated methaphase chromosomes. [e] Average of 10 different species. [f] Average of 4 different species.

Table 2: Local Concentration of DNA in Nucleosomes and Different Structural Models Proposed for Chromatin Fibers

structure	DNA concn (g/mL)
nucleosome core particle	0.29[a]
nucleosome	0.37[b]
chromatin fiber models[c]	
solenoid (*25*, *35*, *36*)	0.15[d]
zigzag ribbon (*41*)	0.07−0.19[e]
three-dimensional zigzag (*44*−*46*)	0.06−0.15[f]
crossed-linker double helix (*29*, *43*)	0.20[g]
irregular three-dimensional zigzag (*32*, *49*)	0.04−0.14[h]
compact interdigitated solenoid (*40*)	0.27[i]

[a] 146 bp in a disk 5.7 nm thick and 11 nm in diameter (*20*−*22*). [b] Core particle plus 60 bp linker DNA modeled as a cylinder of 2 nm in diameter and 0.34 nm per bp. [c] The concentration of DNA was estimated considering that fibers are cylinders having the diameters and the number of nucleosomes per unit length indicated by the different authors; the results shown were obtained considering nucleosomes containing 206 bp. [d] 6 nucleosomes per 11 nm in a fiber of 32 nm diameter (*36*). [e] Fibers of 31 and 40 nm diameter with 2.5 and 11.6 nucleosomes per 11 nm, respectively, at low and intermediate ionic strength (*41*). [f] 1 nucleosome per 11 nm in a fiber of 20 nm diameter at low ionic strength (*44*); at intermediate ionic strength, the number of nucleosomes per unit length and the fiber diameter are identical to those of the solenoid (*45*, *46*). [g] 9.1 nucleosomes per 11 nm in a fiber of 34 nm diameter (*29*). [h] Fiber of approximately 33 nm in diameter containing 1.6 and 5.9 nucleosomes per 11 nm, respectively, at low and intermediate ionic strength (*32*). [i] 13.6 nucleosomes per 11 nm in a fiber of 36 nm diameter (*40*).

reach relatively high DNA concentrations in interphase, about 0.10 g/mL (*2*, *8*, *9*). Condensed chromosomes have a higher DNA density. The DNA concentration in metaphase chromosomes of different organisms has been obtained in several laboratories using different microscopy techniques (*10*−*13*). The values obtained in these studies are presented in Table 1. The average of these measurements is 0.17 g/mL. The local concentration of DNA in the head of bacteriophages is much higher, about 0.8 g/mL (*6*, *12*). The DNA concentration in the sperm head of mouse and insects is similar to that found for bacteriophages (*14*, *15*). In contrast, the local concentration of DNA in the sperm head of the sea urchin *S. Purpuratus* is similar to that found for condensed chromosomes [see Table 1 (*16*)].

The Structure of Nucleosomes Limits the Maximum DNA Concentration Attainable in Folded Chromatin Structures. The high DNA concentration in bacteriophages and other viruses allows the folding of DNA forming liquid-crystalline domains (*17*−*19*). The DNA in mouse and insect sperm is associated with protamines or other basic nonhistone proteins that form close packed structures with a high DNA density. In sea urchin sperm, as in metaphase chromosomes, DNA is wrapped around core histone octamers forming nucleosomes (*20*−*22*). As pointed out by Pogany et al. (*14*), the nucleosomal organization limits the DNA concentration to values that do not allow the tight packaging found in viruses and sperm cells containing nonhistone proteins. In fact, it can be seen in Table 2 that the estimated local concentration of DNA in nucleosome core particles is 0.29 g/mL. It has been found (*23*) that liquid crystals formed by hexagonally packed columns of nucleosome core particles have a concentration of approximately 0.4 g of chromatin/mL (i.e., ∼0.2 g of DNA/mL). The local concentration of DNA in complete nucleosomes depends on the length and conformation of linker DNA; this concentration can be estimated by considering simple models including linker DNA (see Table 2). For nucleosomes with a 60 bp linker, the estimated local concentration of DNA is 0.37 g/mL; values ranging from 0.32 to 0.39 g/mL correspond to nucleosomes with linkers of 20 to 80 bp. When comparing these values with those found for viruses and sperm heads containing nonhistone proteins, it is obvious that in any structure containing nucleosomes there is a significant fraction of space that cannot be occupied by DNA due to the particular structure of the nucleosome. Furthermore, since proteins and DNA have a substantial amount of tightly bound water molecules (*24*), the water associated with nucleosomes should reduce even more the actual volume fraction of DNA. Thus, assuming that nuclesomes do not change significantly their shape inside different chromatin structures, the above estimates show that the DNA concentration in structures formed from nucleosomes is limited to a maximum value ranging from about 0.3 to 0.4 g/mL.

Local Concentration of DNA in Chromatin Fibers. The first level of DNA folding in eukaryotic cells is the nucleosome. In the second level of folding, the chain of nuclesomes, at intermediate ionic strength or in the presence of Mg^{2+}, forms a chromatin fiber of 30−40 nm in diameter (*25*−*32*). The different models that have been proposed for this structure differ on the disposition of linker DNA. In solenoid models, linker DNA is folded and consecutive nucleosomes form a simple helix (*25*, *33*−*37*) or a more compact fiber in which successive turns of this primary helix interdigitate forming secondary helices (*31*, *38*−*40*). In the twisted-ribbon model, a zigzag ribbon of nucleosomes forms a helix with the linker parallel to the fiber axis (*41*). Linker DNA is straight in regular (*29*, *42*−*46*) and irregular (*32*, *47*−*50)* crossed-linker models.

The local concentration of DNA in different chromatin fiber models is estimated as described in Table 2. The concentration of DNA is dependent on the nucleosome density, which is limited by the spatial disposition of nucleosomes within each model. In all cases, the local concentrations are estimated considering the DNA mass corresponding to nucleosomes having a linker of 60 bp [i.e., approximately the linker length of chicken erythrocytes (*29*, *43*, *50*)] and the volume occupied by the fiber (modeled as a cylinder)

Table 3: Levels of Chromatin Folding in Different Models for Metaphase Chromosomes and Estimated Decrease of the Local Concentration of DNA in the Final Condensed Structure

models	folding levels *above* the 30−40 nm chromatin fiber[a]			DNA concn decrease[b] (%)
	first	second	third	
models including loops[c]				
scaffold/loop (*57*, *61*, *63*)	loops	chromatid fiber	coiled chromatid	25
minibands (*59*)	loops	chromatid with minibands		21
coiled fibers (*54*)	loops	coiled fiber (200−300 nm)	coiled chromatid	27
radial array of loops (*9*)	loops	radial array (240 nm)	coiled chromatid	37
rosettes (*62*)	loops	thick fiber with rosettes	coiled chromatid	29
multiple helical coiling				
tubular fiber (*58*)	400 nm hollow fiber	coiled chromatid		77
sequential coiling (*52*)	200 nm hollow fiber	coiled chromatid		53
coiled coil (*56*)	200 nm fiber	coiled chromatid		62
irregular folding				
chromonema (*60*, *64*)	60−80 nm fiber	100−130 nm fiber	chromatid	25

[a] Only the levels of folding that can originate changes in the local concentration of DNA (see text) are indicated; diameters of intermediate structures are indicated in the table for models that include them in the original references. [b] Values estimated assuming hexagonal close packing of the fibers that compose each folding level (see text) and considering the dimensions of the holes in the center of helically coiled structures according to the orinal references; unless otherwise indicated in the original references, the diameter of the folded chromatid is considered to be 600 nm (*72*) [this value corresponds to highly condensed chromatids (*53*)]. [c] In all these models, loops are formed from 30−40 nm fibers containing 50−100 kb of DNA.

that contains them. The three-dimensional randomly organized zigzag model of Leuba et al. (*50*) has 0.07 g of DNA/mL for chromatin at low ionic strength, a value which is similar to that obtained with other models for unfolded fibers [see Table 2 (*32*, *41*, *44*)]. Models for folded chromatin show higher concentrations of DNA. The solenoid model contains 6 nucleosomes per 11 nm in a 32 nm diameter fiber (*35*, *36*) and has a DNA concentration of 0.15 g/mL; fiber diameters of 30 and 34 nm give 0.17 and 0.13 g/mL, respectively. Similar values are obtained with the solenoid models proposed by McGhee et al. (*33*) and Butler (*34*) and the three-dimensional zigzag model of Koch and co-workers (*44*−*46*) for folded chromatin fibers. The irregular three-dimensional zigzag model of Woodcock and co-workers (*32*, *49*) at intermediate ionic strength has a concentration of DNA of 0.14 g/mL; presumably, the irregular crossed-linker model of Staynov (*47*) has approximately the same DNA concentration, and the layered irregular zigzag model of Subirana et al. (*48*) has a lower concentration of DNA because it includes discontinuities in the fiber. The crossed-linker double (*29*, *43*) and triple (*42*) helix models show a higher DNA concentration (0.20 g/mL). The zigzag ribbon model (*41*) has a relatively large number of nucleosomes per unit length (∼12 nucleosomes per 11 nm), but its large diameter (40 nm) yields a DNA concentration (0.19 g of DNA/mL; Table 2) similar to that found for models with a lower number of nucleosomes per unit length. The highest DNA concentration (0.27 g/mL) has been found in the interdigitated solenoid model [∼14 nucleosomes per 11 nm in a fiber of 36 nm diameter (*40*)]. This model produces a roughly 2-fold increase in the concentration of DNA as compared to the normal solenoid because the stacking of nucleosomes in the secondary helices gives rise to a tight packaging (*40*).

The estimates presented in Table 2 show that, in the different structural models suggested for the 30−40 nm chromatin fibers, the local concentration of DNA is significantly lower than that estimated for isolated nucleosomes with the same linker length. Even in the most compact model [i.e., the interdigitated solenoid (*40*)], there is a significant fraction of space (∼27%) that is not occupied by nucleosomes. In the normal solenoid and other models having about 0.15 g of DNA/mL, the fraction of space not occupied by nucleosomes is approximately 59%. This raises important questions concerning the possibility of constructing metaphase chromosomes with a relatively high DNA concentration (see Table 1) with nucleosomes contained in fibers that, in principle, show a low efficiency in the use of space for DNA packaging. In fact, the average DNA concentration found experimentally for metaphase chromosomes is higher than the local DNA concentration estimated for several models considered above (see Table 2). Furthermore, these observations suggest that it is necessary to study the possible additional changes of DNA concentration that can be produced by the higher order structures (above the 30−40 nm fiber) that are required for the formation of metaphase chromosomes.

Levels of Chromatin Folding in Models for Metaphase Chromosomes: Possible Effects on the Local Concentration of DNA. X-ray diffraction (*51*) and microscopy (*52*−*56*) studies suggest that the 30−40 nm chromatin fiber is the basic structural unit that is further folded to form metaphase chromosomes. In keeping with this, several structural models proposed for the condensation of chromosomes consider a hierarchy of folding levels which start from the 30−40 nm fiber (*9*, *52*−*64*). As can be seen in Table 3, in the loop/scaffold model proposed by Laemmli and co-workers (*53*, *57*, *61*, *63*) and in other models including loops (*9*, *54*, *59*, *62*), the first level of condensation above the 30−40 nm fiber consists of the folding of this fiber into structural units containing 50−100 kb of DNA. Generally, in these models, the loops formed in the first level form wide fibers of 200−300 nm (second level), and, finally, these fibers are helically coiled in the metaphase chromatid (third level). The models that are based exclusively on successive helical coiling (*52*, *56*, *58*) only have two condensation levels: in the first level, the 30−40 nm fiber forms a wide superhelix, which in the second level coils into the chromatid. In the irregular model of Belmont et al. (*60*, *64*), there are at least three folding levels above the 30−40 nm fiber.

Each level considered in Table 3 corresponds to a structure that is formed from the folding of the structure that precedes

it. At least, there are two structural elements that can reduce the density of nucleosomes in the successive structures suggested for chromosome condensation. First, as observed for protein and DNA molecules in crystals (*65*, *66*), it seems reasonable to consider that the fibers that compose a structural level cannot penetrate each other. This will reduce the packing density of each structural level. In fact, packing analysis of protein crystals shows that protein molecules in the unit cell can occupy only about 75% of the total volume (*65*). The most usual packing found for pseudocylindrical objects (such as DNA molecules in partially ordered fibers and crystals) is hexagonal (*24*, *66*). The fraction of space occupied by fibers in a hexagonally packed structure is approximately 91%. This corresponds to a very close packing; square packing would reduce the volume fraction of fibers to about 79%. The internal structure of the helical coils suggested for different folding levels of metaphase chromosomes is the second structural element that may reduce the DNA concentration. Different helical structures of biological interest have a central hole (*1*), and, generally, metaphase chromosome models explicitly consider hollow fibers or tubs in several folding levels (*9*, *52*, *54*, *56*, *58*, *59*, *62*; see Table 3). These holes may represent a significant fraction of the total volume. For instance, it can be estimated that in the model of Manuelidis and Chen (*9*) the volume fraction of the hole in the coiled structure of the second folding level (Table 3) is about 7%; the hole in the third level represents approximately 10% of the total volume of the coiled chromatid. Thus, in this model, considering these holes and assuming a hexagonal packing in the three folding levels, the final DNA concentration is about 37% lower than that corresponding to the 30−40 nm fibers from which the metaphase chromosome has been constructed.

Table 3 includes the estimates of the decrease of DNA concentration of different chromosome models. These values correspond to the minimum decrease of DNA concentration that can be expected for these models; if square packing is considered in each folding level instead of hexagonal close packing, then the DNA concentration in the final folded structure is significantly reduced. Furthermore, in the scaffold/loop model (*53*, *57*, *61*, *63*), only hexagonal packing in each level is considered because the dimensions necessary to calculate the volume of the central hole that presumably exists in the coiled chromatid are not given in the original references. The calculated DNA concentration for the irregular chromonema model (*60*, *64*) has been obtained considering three levels of folding composed of hexagonally packed fibers without holes; the actual value could be lower because probably the final chromatid is formed by folding a 200−400 nm irregular fiber not included in Table 3. The multiple helical coiling models (*52*, *56*, *58*) have relatively large holes that originate a high reduction of concentration. The values given in Table 3 can only be considered as rough estimates, but they suggest that there is a marked decrease of the DNA concentration when the 30−40 nm fiber is folded into the metaphase chromatid.

Local Concentration of DNA versus Linear Packing Ratio. Structural and Biological Implications. The observations presented in the two preceding sections indicate that the relatively high local concentration of DNA in the nucleosome (∼0.37 g/mL) is significantly reduced when these particles form the 30−40 nm chromatin fibers (Table 2) and then it is further reduced progressively when the fiber is folded into the successive higher order structures suggested in different models for metaphase chromosomes (Table 3). In apparent contradiction with this, it is well-known that the linear packing ratio increases dramatically when DNA is packaged into chromosomes (*3*−*5*). This increase occurs progressively through the folding levels. For instance, Pienta and Coffey (*59*) have calculated that the formation of nucleosomes, solenoid fibers, loops, and chromatids reduces the DNA length by a factor of 6.5-, 6.2-, 17.0-, and 17.6-fold, respectively, giving rise to an overall packing ratio of 1.2×10^4. Taken together, these observations lead to the conclusion that the consideration of the packing ratio is not enough for the study of chromatin condensation; the DNA concentration must be taken into account for the construction of models for chromatin fibers and metaphase chromosomes.

The available experimental measurements of the local concentration of DNA in metaphase chromosomes [average value: 0.17 g/mL (Table 1)] place a general constraint on any model for chromatin structure. In particular, according to the above estimates of the change of DNA concentration when the 30−40 nm fiber is folded into chromosomes [average minimum DNA concentration decrease: 40% (Table 3)], the models for folded chromatin fibers having a local DNA concentration from 0.14 to 0.20 g/mL (Table 2) will give rise to folded chromatids with a local concentration of DNA ranging from 0.08 to 0.12 g/mL. These values are significantly lower than the local concentration of DNA in metaphase chromosomes determined experimentally. The interdigitated solenoid model (*40*) has a higher local concentration of DNA [0.27 g/mL (Table 2)] than the other models and after further folding into chromatids can yield a final concentration of about 0.16 g/mL, which approaches the experimental results found for metaphase chromosomes. As discussed elsewhere (*31*, *40*), this model may generate even more compact fibers; such fibers could be necessary to construct chromatids if, as indicated above, the actual decrease of DNA concentration is higher than the average value obtained from Table 3.

Although the physical constraint resulting from the local concentration of DNA in condensed chromosomes excludes, in principle, relatively low-density structures such as the solenoid (*25*, *33*−*37*) and crossed-linker (*29*, *32*, *42*−*50*) models, the possibility that these models could be transformed into more dense structures must be considered. The formation of secondary helices by stacking of nucleosomes in the interdigitated solenoid model (*40*) gives a high density to the folded fiber. This stacking is consistent with the results of several laboratories showing that nucleosome cores have a high tendency to interact through their faces (*23*, *67*−*69*), presumably using the extended histone tails detected in core particle crystals (*22*). As pointed out previously (*40*), normal solenoids can evolve to interdigitated solenoids if they have the appropriate diameter and a nonintegral number of nucleosomes per turn in the primary helix. It remains to be demonstrated whether crossed-linker models, with the linker DNA occupying the central part of the chromatin fiber, can be so tightly packaged. Another possibility is that the extended crossed-linker fibers observed at low salt concentrations (*32*, *44*, *49*, *50*) could give rise to compact structures in the presence of Mg^{2+} by folding the linker DNA to the extent necessary to form interdigitated helices (*40*). The most

distinctive structural characteristic of the interdigitated solenoid model is the formation of parallel secondary helices (each one with a thickness equal to the nucleosome diameter, i.e, 11 nm) that occupy most of the volume of the folded fibers (*40*). It is interesting to note that in a cryo-electron microscopy study of vitrified metaphase chromosomes, McDowall et al. (*70*) have found a dominant optical diffraction ring corresponding to 11 nm. These authors have interpreted this structural feature as a consequence of the lateral association of compact filaments formed by the stacking of nucleosomes.

REFERENCES

1. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. (1994) *Molecular Biology of the Cell*, Garland Publishing, New York.
2. Lewin, B. (1997) *Genes VI*, Oxford University Press, Oxford.
3. Wolffe, A. (1998) *Chromatin: Structure and Function*, Academic Press, London.
4. DuPraw, E. J. (1970) *DNA and Chromosomes*, Holt, Rinehart and Winston, New York.
5. Wagner, R. P., Maguire, M. P., and Stallings, R. L. (1993) *Chromosomes: A Synthesis*, Wiley-Liss, New York.
6. Kellenberger, E., Carlemalm, E., Sechaud, J., Ryter, A., and de Haller, G. (1986) in *Bacterial Chromatin* (Gualerzi, C. O., and Pon, C. L., Eds.) pp 11−25, Springer-Verlag, Berlin.
7. Manuelidis, L. (1990) *Science 250*, 1533−1540.
8. Suciu, D. (1986) *J. Theor. Biol. 117*, 587−596.
9. Manuelidis, L., and Chen, T. L. (1990) *Cytometry 11*, 8−25.
10. Bennett, M. D., Heslop-Harrison, J. S., Smith, J. B., and Ward, J. P. (1983) *J. Cell Sci. 63*, 173−179.
11. Heslop-Harrison, J. S., Leitch, A. R., Schwarzacher, T., Smith, J. B., Atkinson, M. D., and Bennett, M. D. (1989) *Hum. Genet. 84*, 27−34.
12. Bohrmann, B., Haider, M., and Kellenberger, E. (1993) *Ultramicroscopy 49*, 235−251.
13. Fritzsche, W., and Henderson, E. (1996) *Scanning Microsc. 10*, 103−110.
14. Pogany, G. C., Corzett, M., Weston, S., and Balhorn, R. (1981) *Exp. Cell Res. 136*, 127−136.
15. Suzuki, M., and Wakabayashi, T. (1988) *J. Mol. Biol. 204*, 653−661.
16. Green, G. R., and Poccia, D. L. (1985) *Dev. Biol. 108*, 235−245.
17. Lepault, J., Dubochet, J., Baschong, W., and Kellenberger, E. (1987) *EMBO J. 6*, 1507−1512.
18. Strzelecka, T. E., Davidson, M. W., and Rill, R. L. (1988) *Nature 331*, 457−460.
19. Booy, F. P., Newcomb, W. W., Trus, B. L., Brown, J. C., Baker, T. S., and Steven, A. C. (1991) *Cell 64*, 1007−1015.
20. Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., and Klug, A. (1984) *Nature 311*, 532−537.
21. Arents, G., and Moudrianakis, E. N. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10489−10493.
22. Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature 389*, 251−260.
23. Leforestier, A., and Livolant, F. (1997) *Biophys. J. 73*, 1771−1776.
24. Cantor, C. R., and Schimmel, P. R. (1980) *Biophysical Chemistry*, Freeman, San Francisco.
25. Finch, J. T., and Klug, A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1897−1901.
26. Bradbury, E. M., and Baldwin, J. P. (1986) *Cell Biophys. 9*, 35−66.
27. Gerchman, S. E., and Ramakrishnan, V. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7802−7806.
28. Koch, M. H. J., Sayers, Z., Michon, A. M., Marquet, R., Houssier, C., and Willführ, J. (1988) *Eur. Biophys. J. 16*, 177−185.
29. Williams, S. P., and Langmore, J. P. (1991) *Biophys. J. 59*, 606−618.
30. Zlatanova, J., Leuba, S. H., Yang, G., Bustamante, C., and van Holde, K. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 5277−5280.
31. Bartolomé, S., Bermúdez, A., and Daban, J.-R. (1994) *J. Cell Sci. 107*, 2983−2992.
32. Bednar, J., Horowitz, R. A., Grigoryev, S. A., Carruthers, L. M., Hansen, J. C., Koster, A. J., and Woodcock, C. L. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 14173−14178.
33. McGhee, J. D., Nickol, J. M., Felsenfeld, G., and Rau, D. C. (1983) *Cell 33*, 831−841.
34. Butler, P. J. G. (1984) *EMBO J. 3*, 2599−2604.
35. Widom, J., and Klug, A. (1985) *Cell 43*, 207−213.
36. Widom, J. (1989) *Annu. Rev. Biophys. Biophys. Chem. 18*, 365−395.
37. Widom, J. (1998) *Annu. Rev. Biophys. Biomol. Struct. 27*, 285−327.
38. Bartolomé, S., Bermúdez, A., and Daban, J.-R. (1995) *J. Biol. Chem. 270*, 22514−22521.
39. Bermúdez, A., Bartolomé, S., and Daban, J.-R. (1998) *J. Cell Sci. 111*, 1707−1715.
40. Daban, J.-R., and Bermúdez, A. (1998) *Biochemistry 37*, 4299−4304.
41. Woodcock, C. L. F., Frado, L.-L. Y., and Rattner, J. B. (1984) *J. Cell Biol. 99*, 42−52.
42. Makarov, V., Dimitrov, S., Smirnov, V., and Pashev, I. (1985) *FEBS Lett. 181*, 357−361.
43. Williams, S. P., Athey, B. D., Muglia, L. J., Schappe, R. S., Gough, A. H., and Langmore, J. P. (1986) *Biophys. J. 49*, 233−248.
44. Bordas, J., Perez-Grau, L., Koch, M. H. J., Vega, M. C., and Nave, C. (1986) *Eur. Biophys. J. 13*, 175−185.
45. Koch, M. H. J., Vega, M. C., Sayers, Z., and Michon, A. M.-(1987) *Eur. Biophys. J. 14*, 307−319.
46. Koch, M. H. J. (1989) in *Protein−Nucleic Acid Interaction* (Saenger, W., and Heinemann, U., Eds.) pp 163−204, Macmillan Press, London.
47. Staynov, D. Z. (1983) *Int. J. Biol. Macromol. 5*, 3−9.
48. Subirana, J. A., Muñoz-Guerra, S., Aymamí, J., Radermacher, M., and Frank, J. (1985) *Chromosoma 91*, 377−390.
49. Woodcock, C. L., Grigoryev, S. A., Horowitz, R. A., and Whitaker, N. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 9021−9025.
50. Leuba, S. H., Yang, G., Robert, C., Samori, B., van Holde, K., Zlatanova, J., and Bustamante, C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 11621−11625.
51. Langmore, J. P., and Paulson, J. R. (1983) *J. Cell Biol. 96*, 1120−1131.
52. Sedat, J., and Manuelidis, L. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 331−350.
53. Marsden, M. P. F., and Laemmli, U. K. (1979) *Cell 17*, 849−858.
54. Rattner, J. B., and Lin, C. C. (1985) *Cell 42*, 291−296.
55. Adolph, K. W., Kreisman, L. R., and Kuehn, R. L. (1986) *Biophys. J. 49*, 221−231.
56. Taniguchi, T., and Takayama, S. (1986) *Chromosoma 93*, 511−514.
57. Paulson, J. R., and Laemmli, U. K. (1977) *Cell 12*, 817−828.
58. Back, A. L., Zeuthen, J., and Crick, F. H. C. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1599−1599.
59. Pienta, K. J., and Coffey, D. S. (1984) *J. Cell Sci. Suppl. 1*, 123−135.
60. Belmont, A. S., Sedat, J. W., and Agard, D. A. (1987) *J. Cell Biol. 105*, 77−92.
61. Boy de la Tour, E., and Laemmli, U. K. (1988) *Cell 23*, 937−944.
62. Filipski, J., Leblanc, J., Youdale, T., Sikorska, M., and Walker, P. R. (1990) *EMBO J. 9*, 1319−1327.
63. Saitoh, Y., and Laemmli, U. K. (1994) *Cell 76*, 609−622.
64. Belmont, A. S., and Bruce, K. (1994) *J. Cell Biol. 127*, 287−302.
65. Schulz, G. E., and Schirmer, R. H. (1979) *Principles of Protein Structure*, Springer-Verlag, New York.
66. Lerman, L. S., Wilkerson, L. S., Venable, J. H., and Robinson, B. H. (1976) *J. Mol. Biol. 108*, 271−293.

67. Finch, J. T., Lutter, L. C., Rhodes, D., Brown, R. S., Rushton, B., Levitt, M., and Klug, A. (1977) *Nature 269*, 29−36.
68. Dubochet, J., and Noll, M. (1978) *Science 202*, 280−286.
69. Uberbacher, E. C., and Bunick, G. J. (1985) *J. Biomol. Struct. Dyn. 2*, 1033−1055.
70. McDowall, A. W., Smith, J. M., and Dubochet, J. (1986) *EMBO J. 5*, 1395−1402.
71. Morton, N. E. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 7474−7476.
72. Adolph, K. W. (1989) in *Molecular Biology of Chromosome Function* (Adolph, K. W., Ed.) pp 283−301, Springer-Verlag, New York.

BI992628W