INTERPARTICLE EFFECTS IN LOW-ANGLE X-RAY AND NEUTRON DIFFRACTION FROM CHROMATIN

M. SPENCER AND D. Z. STAYNOV, *Department of Biophysics, King's College, London WC2B 5RL, England*

ABSTRACT Published diffraction data are critically reviewed, and replotted in a new way to show the variation with concentration of the 8- to 25-nm diffraction maximum. Most of the early data are found to be consistent with a simple model for a liquid-type array of mutually repulsive particles, whose molecular weight is calculated to be that of a nucleosome or possibly a dimer. The data for all but the highest concentrations, where distortion due to dehydration is possible, support no particular model for the higher-order coiling of chains of nucleosomes, and cannot be used to support models for "native" chromatin. Only in the presence of excess salts or after isolation with polyamines is there aggregation in solution of nucleosomes, which then give peaks at 11 and 5.5 nm that do not change much with concentration. Recent work by the authors confirms that under some conditions nucleosomes undergo a transition to a state whose diffraction is consistent with hexagonal packing of extended DNA to which histones are still attached. This state is probably responsible for much of the strong 2.7-nm peak previously obtained from certain samples, which was in some cases assigned to nucleosome structure. Only the peak at 3.7 nm is clearly attributable to the form factor of the isolated native nucleosome.

INTRODUCTION

Previous Work

Ever since the first observations of x-ray diffraction from chromatin (1–3) there has been speculation about the origin of intensity maxima at spacings >3 nm. Luzzati and Nicolaieff (4) proposed the existence of three different phases, two of which were explained in terms of the hexagonal packing of groups of DNA molecules. Later Pardon and coworkers (5–7) developed the idea of a supercoil of nucleoprotein to explain the observed series of maxima. Subsequent workers on chromatin gels (8) and on isolated nuclei (9) accepted the general idea that all the lower angle maxima arose from some kind of superstructure.

The available evidence seemed not to support the simpler explanation of at least the lowest-angle (largest spacing) peak, which was that it arose purely from interparticle interference. In such a case the apparent Bragg spacing is determined only by the average separation of particles, which arrange themselves in an imperfect liquid-type lattice reinforced by mutual electrostatic repulsion. The peak then gives no information about the structure beyond an average value for particle weight, or mass per unit length in the case of rodlike objects. The peak is expected to disappear at low concentrations when mutual repulsion is counteracted by excess ions in the solution.

The matter rested there until the later revival of interest in chromatin structure, when

---

Dr. Staynov is on leave from the Institute of Molecular Biology of the Bulgarian Academy of Sciences.

neutron diffraction with contrast variation extended the range of useful observations and generated a flood of publications (10–21). At the same time interpretation was transformed by the discovery of nucleosomes (for reviews see references 22 and 23). The neutron diffraction data showed clearly that relatively dilute suspensions of both chromatin and isolated nucleosomes in the absence of salt gave a strong, concentration-dependent peak at spacings of 10 nm or greater (11).

It was suggested at first by Bradbury et al. (15) that the spacing of 10–11 nm observed at fairly high concentrations represented that of nucleosomes along the chromatin fiber, and further that this was maintained by histone-histone interactions even when the DNA links were broken. A distinction was made between this spacing and the larger values obtained at greater dilutions; in this case an interparticle effect not related to structure was assumed, since a concentration-dependent peak was observed even with chromatin sonicated to monomers and dimers. This peak was suppressed by the addition of salt. Interparticle interference was also suggested by Richards et al. (24) in discussing their x-ray data. Carlson and Olins (25) and Subirana and Martinéz (26) made calculations showing how such an effect would influence the diffraction, but they did not draw any firm conclusion on the spatial distribution of nucleosomes.

Later work by Carpenter et al. (13) showed that a 10-nm peak from fibers was oriented in a way consistent with a helical coil of nucleosomes, and a similar structure was suggested independently by Finch and Klug (27) from work with the electron microscope. Both groups of workers attributed the observed spacing to the pitch of a hollow supercoil or solenoid, and the variation with concentration was assumed to be a hydration effect. Other workers suggested different possible models such as flexible linear arrays (25, 28) and alternative forms of supercoil (12, 17). Thus, the idea that the lowest angle peak arose from a biologically meaningful superstructure was widely accepted, but there was disagreement about the precise model; in 1977, Pardon et al. (19) expressed the opinion that the origin of the 11.0- and 5.5-nm x-ray maxima "remains unsettled," and this is still the case.

In an attempt to clarify the situation we have reexamined all the available low-angle data, making a distinction between those obtained from solutions containing only monovalent salts and those containing ions which might induce aggregation. We have used the former data to test a simple model for random close-packing, as originally applied to protein molecules by Riley and Oster (29), and later to tRNA (30), F-actin (31), and ribosomes (32). We have also reevaluated other features of the diffraction data in the light of the conclusions reached. Comparisons with deductions from other techniques, which in some cases parallel our own conclusions, are mostly beyond the scope of this paper.

Theory

Our simple packing model is based on theoretical and experimental work by Oster and Riley (29, 33, 34). Let the apparent Bragg spacing d of an interparticle interference peak be related to the mean center-to-center separation s by the approximate relation $s = kd$; for hydrated or repelling spheres in a liquid configuration $k = 1.10$, while for crystalline close packing $k = 1.22$ (29). For hexagonally packed cylinders k varies between ~ 1.0 for pairs of cylinders and 1.15 for a crystalline array (34). $k = 1.1$ fits the data for F-actin (31), so this value appears suitable for both spherical and cylindrical particles. Because of imperfections in packing and deviations from symmetry only one fairly broad peak is expected.

For particles of average separation s in spherical close packing the mass per unit volume is $\sqrt{2} \cdot M/N_0 s^3$, where M is the molecular weight and N_0 is Avogadro's number. If c is the concentration expressed as a volume ratio and ρ is the dry particle density, it may then be shown that

$$\log d = \frac{1}{3} \log A - \frac{1}{3} \log c, \tag{1}$$

where $A = \sqrt{2} \cdot M/k^3 \rho N_0$. A double-logarithmic plot of d against c will then be a straight line of slope $-\frac{1}{3}$, and M can be determined from the intercepts.

For cylindrical particles of molecular weight m per unit length the corresponding expression is:

$$\log d = \frac{1}{2} \log B - \frac{1}{2} \log c, \tag{2}$$

where $B = 2m/\sqrt{3} \cdot k^2 \rho N_0$. The slope is now $-\frac{1}{2}$.

In most published work the concentrations are given in percentages wt/wt or wt/vol, and conversion to the volume ratio is necessary. The necessary formulae are:

$$c = \left[1 + \frac{\rho}{\rho_s}\left(\frac{100}{c'} - 1\right)\right]^{-1} = \frac{c''}{100\rho}, \tag{3}$$

where c' = % wt/wt, c'' = % wt/vol or $g\ dl^{-1}$ and ρ_s = solvent density in $g\ cm^{-3}$ (1.105 for D_2O at 20°C).

RESULTS AND DISCUSSION

Concentration Dependence of the Lowest-Angle Peak

In Fig. 1 we have replotted published data for the lowest-angle peak observed with x-ray and neutron diffraction. We have plotted all known values for suspensions containing only monovalent salts, derived from histone-containing chromatin that gives the 3.7-nm peak associated with "native" material. Published concentrations were converted to volume ratios assuming that $\rho = 1.59$ for whole chromatin and 1.61 for H1-depleted material; these values were deduced for nucleosomes containing 200 base pairs of DNA and proteins of total mol wt 130,600 (including H1 of 21,000), assuming partial specific volumes of 0.54 and 0.72 $cm^3 g^{-1}$ for DNA and protein, respectively. Such assumptions lead to partial specific volumes of 0.63 for whole chromatin and 0.65 for core particles, a value close to the 0.66 determined experimentally by Olins et al. (40).

In Fig. 1 we have also plotted theoretical lines derived from Eq. 1 for nucleosome monomers and dimers in liquid-type arrays. Most of the points lie quite close to the theoretical straight line for single nucleosomes, though the predominance of points above this line could indicate either a tendency to form dimers, or attachment of nonhistone proteins. The conclusion is unavoidable that interparticle interference from liquid-type packing of single nucleosomes can explain most of the results for all concentrations, even when the nucleosomes are compressed together by removal of water. On the basis of this evidence alone there is no a priori reason for believing that the spacings give information about any such thing as nucleosome spacing along

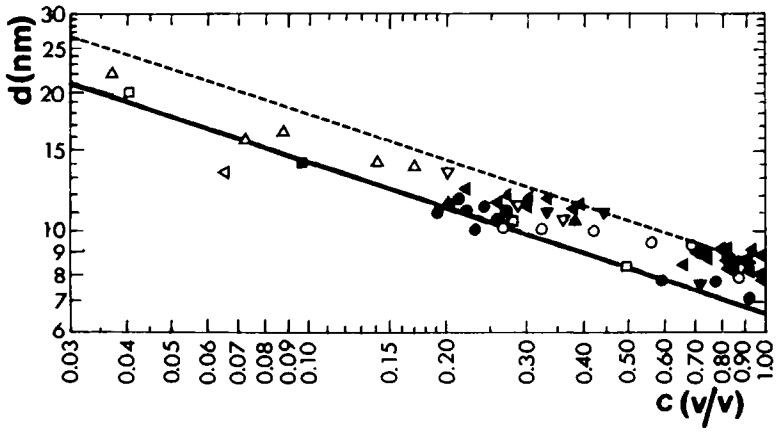

FIGURE 1. Double-logarithmic plot of spacing against concentration. (——) theoretical line for mutually repulsive nucleosomes; (— —) nucleosome dimers. Experimental data, x-ray diffraction: (●) chicken erythrocyte chromatin, reference 4, Fig. 1, ω and χ phases; (■) erythrocyte nucleosomes, reference 24, Fig. 1; (▲) H1-depleted calf thymus chromatin, reference 26, Table I; (▼) oriented gels of sheared thymus chromatin, reference 35, Fig. 3 and text; (◀) chromatin from frog, carp, and goldfish sperm, reference 36, Fig. 8; (▶) erythrocyte nucleosomes, reference 37, Fig. 10. Experimental data, neutron diffraction: (○) thymus chromatin (39) in H_2O and D_2O, reference 11, Figs. 4 and 5; (□) sheared thymus chromatin in H_2O and D_2O, reference 14, Figs. 1 and 4; (△) sonicated thymus chromatin in D_2O, reference 15, Fig. 7; (▽) H1-depleted thymus chromatin (39) in D_2O, reference 38, Fig. 4; (◁) thymus chromatin (39) in H_2O, reference 20, Fig. 3. In some cases values have been estimated from data published in graph form.

the DNA, or about any superstructure that changes in scale with the concentration (the evidence for preferred orientation is discussed later).

It is not unreasonable to accept the simple interpretation even when, as in many cases, the material consists of whole chromatin. The effect of coiling DNA into nucleosomes, even though some of its charge is neutralized by histones, will be to produce concentrations of ionized groups which we would expect to dominate the distribution of particles. In the absence of factors causing aggregation of nucleosomes we would then expect the particles to arrange themselves in a liquid-type lattice with linker DNA running between one nucleosome and the next. The model is entirely consistent with the deduction from contrast variation studies (11) that the "11-nm" neutron diffraction peak arises largely from the protein cores.

This interpretation is not inconsistent with the low-angle scattering studies of Sperling and Tardieu (41), who concluded that their nucleosomes were packed together in columns; although the ionic strength of their final medium was low, the preparation used (42, 43) was one in which polyamines are added to stabilize the nuclei, and no steps are taken (other than resuspension once in lysis medium) to remove bound polyamines (24). Such factors might, therefore, have inhibited a full transition of the type observed at greater dilutions in the electron microscope (44–46) between "condensed" and "beaded" forms of chromatin. Alternatively, it may be true that any method using mild digestion without shear gives a greater compaction of nucleosomes; there would then be an upper limit to the lowest-angle spacing of less than the 25–30 nm expected for fully extended linker DNA. There is some evidence that this is so from neutron scattering of nucleosome oligomers (R. I. Cotter, J. F. Pardon, D. M. J. Lilley, B. M. Richards, and D. L. Worcester, personal communication).

However, most of the diffraction data discussed here were obtained with material subjected to shear and to repeated washes in buffers containing low concentrations of monovalent cations (39, 47). The very large spacings observed by Bram et al. (10, 20) (not plotted in Fig. 1) follow the same trend as the other data, but to fit them to our model it would be necessary to assume that degradation to mononucleosomes or dimers had occurred.

Diffraction Evidence for Helical Arrays

Let us now consider the evidence (11, 13, 26, 48) for meridional or off-meridional orientation of the lowest-angle peak. The best orientation was demonstrated with neutron diffraction (13) under quite dry conditions (32% relative humidity [RH]), and with stretched films at 98% RH (11, 13, 49). In a salt-free specimen there can be some loss of bound water at any humidity below 100%; estimates of the concentration to which chromatin equilibrates at 98% RH vary from 45% (49) to 60% (14). The latter value corresponds to a volume ratio of ~0.5.

It may be calculated from the data of Finch et al. (50, 51) that the volume ratio in crystals of nucleosome cores is only ~0.4. All data from dried material at higher concentrations than this must therefore be treated with reservation; removal of a hydration layer (26) during preliminary drying may well induce distortion and nonphysiological aggregation, from which the material does not recover until fully rehydrated. Thus, although dehydration induces the particles to pack together in a way that gives information about their external shape, it does not follow that the dehydrated structure exists in vivo.

In support of this view (20) it may be noted that other x-ray diffraction maxima change considerably at very high concentrations (2, 4, 35, 36, 52). The problem is highlighted when we note from Fig. 1 that many of the data relate to volume ratios even greater than 0.74, the value for close-packed spheres. The use of dehydrated material followed the tradition established in work with DNA, but even there it is now suggested that the structure in solution may be slightly different from that in fibers (50, 53).

Even if it is accepted that helical aggregation occurs in undistorted chromatin, it is clear from recent electron microscopy than nucleosome cores can also aggregate in a variety of arclike or helical arrays (50, 54); here, too, it has been suggested that large conformational changes might be involved in assembly (54). An oriented diffraction peak at 11 nm could then indeed indicate the lateral aggregation of piles of stacked nucleosomes into some form of higher-order array, but the solenoid (27) may not be the only structure adopted (55–58). What is quite clear from our analysis is that it gives no direct support to the idea, based on light-scattering studies (59) and earlier neutron work (12), that a helical array of nucleosomes persists in dilute solutions free of polyvalent ions; the diffraction data at lower concentrations can be explained entirely in terms of interparticle interference between nucleosomes. In support of this it has been pointed out that in relatively dilute suspensions the peak disappears as expected when salt is added (15, 16).

Diffraction from Aggregates and Native Chromatin

Data from solutions containing other than monovalent ions do not in general follow the behavior described above. Garrett (8) equilibrated chromatin gels against a variety of salt-containing solutions. At a chromatin concentration of 15% he observed no peaks at 11 and 5.5 nm in x-ray diffraction from gels containing only sodium phosphate buffer. However, the addition of divalent cations induced precipitation and an appearance of such peaks, and

similar peaks were obtained from H1-depleted material in 0.6 M NaCl. In the latter case there were no apparent changes in spacings over the range 17–50% wt/vol (c = 0.11–0.32); this appears to be the only report of an absence of concentration-dependence in samples containing only monovalent salt. Under some conditions (salt-free gels at high concentration, and 15% gels at Mg^{++} concentrations below 1 mM) Garrett (8) observed no 11-nm peak, but there was one at 5.5–6.0 nm.

This, in conjunction with more recent evidence referred to above, suggests that there may be an initial stage of aggregation into some higher-order structure in which nucleosomes stack in columns parallel to their short axes (giving a 5.5-nm peak), followed by side-to-side aggregation of columns giving a peak at 11 nm. Thus both peaks seem under these conditions to represent interparticle contact distances, unrelated to internal structure (60). Such aggregation, whether into "solenoids," other helical arrays, or structures such as "superbeads" (55, 56), could precede the appearance of gross precipitate. Pooley et al. (61) also observed x-ray diffraction peaks at ~11 and 5.5 nm in the presence of divalent cations, and suggested that this held also in their absence; however, their data from concentrations of chromatin below 35% wt/wt were too limited by background scatter for a clear deduction.

Sperling and Klug (52) observed that a 20% wt/wt gel of "native" chromatin (c = 0.14 vol/vol) in 0.2 mM $MgCl_2$ gave a peak at 11.0–11.5 nm, whereas at this concentration sonicated material prepared by others using another method (15) gave one at 14.2 nm. Sperling and Klug used the method referred to earlier (42, 43) in which isolated nuclei are stabilized by polyamines, which might well persist in the final material and preserve the native structure. Treatment with small amounts of EDTA could then merely remove competing metal cations and enhance any stable interparticle contacts favored by polyamine binding. This could perhaps explain why these authors observed an unexpected precipitation of concentrated chromatin on mixing with 0.2 mM EDTA. At the same time, extensive dilution with sodium EDTA solutions for electron microscopy could have removed the polyamines to give the extended structures observed after such treatment.

A similar argument could explain why Olins and Olins (9) observed a persistent "11-nm" peak in x-ray diffraction from isolated eukaryotic nuclei, even when they had been swollen at low ionic strength to cover the range of DNA concentrations 35–650 mg/ml; this is equivalent to a range of chromatin concentrations for which c = 0.046–0.85 vol/vol. It seems reasonable to postulate that here, too, nucleosome contacts were maintained by the binding of polyvalent ions, including perhaps proteins, which may not have been removed from the nuclei by the procedures used. The observed swelling could then have arisen from the unfolding of only the higher orders of coiling or aggregation.

Nucleosome Unfolding

Luzzati and Nicolaieff (3, 4) obtained some x-ray diffraction patterns from chromatin gels which showed no strong peaks except one near 2.7 nm, and they proposed the existence of a distinct phase containing parallel DNA molecules interspersed with histones and water. Since then there have been several reports of the appearance of such a phase, though not under the same conditions as those used by Luzzati and Nicolaieff. In more x-ray work Pardon et al. (5, 35) observed on stretching a chromatin fiber a reversible loss of low-angle maxima and the appearance of a strong equatorial peak near 3 nm, while Bradbury et al. (62) reported the

appearance of a "DNA phase" on stretching H1-depleted material. Garrett (63) induced a phase change by raising the salt concentration above 0.9 M NaCl or by raising the temperature, though in these cases the change was attributed to the dissociation of histones from DNA.

Subirana et al. (36) obtained variable amounts of what they termed a "fibrous component," whose proportion varied in an unexplained manner between preparations, even from the same source. A similar variability in the amount of a 2.7-nm component had been noted earlier by Baldwin et al. (64), when reconstituting heterologous histone/DNA complexes. Subirana et al. (36) obtained solely a fibrous component in attempts at reconstitution. They concluded that the normal (nucleosome) structure could be denatured to an extended form, and speculated that this might have some biological significance, but they could not define the conditions under which it appeared. In work with chromatin isolated by mild digestion of polyamine-stabilized nuclei, Sperling and Klug (52) reported that they never observed a 2.7-nm peak on its own, but there was an increasingly strong component with this spacing as the concentration increased. These authors, too, favored the idea that nucleosome unfolding was responsible. Neutron diffraction has also shown a 2.7-nm peak at high concentrations which can in some samples be very strong (e.g., reference 61, Fig. 7). Finally, our own recent work (65) has shown that a reversible transition can be induced by change of pH, to a state giving only a 2.7-nm peak in place of those at larger spacings.

Despite a general awareness of the possible origin of at least part of the 2.7-nm peak, many authors have assumed that most of it is due to some feature such as the pitch of the DNA superhelix (50), and models have been devised to account for a peak at least as strong as that at 3.7 nm (18). We believe that the unfolded phase is probably more prevalent than generally assumed, and also that it deserves more study because of its possible relevance to the problems of how gene expression is controlled and transcription effected.

Existing data can be compared with calculations from Eq. 2 (see Introduction), on the assumption that our "model nucleosomes" are unwound to give hexagonally packed threads of DNA in the B form (0.34-nm translation/base-pair), with histones distributed along the DNA. This gives a mass per unit length of ~3,860 daltons nm^{-1} for whole chromatin and 3,550 for H1-depleted material. A model of this type is supported by neutron diffraction, which indicates that the 2.7-nm peak originates from DNA (15, 66). The data for the "Ψ phase" of Luzzati and Nicolaieff (Fig. 7 of reference 4) fit this model well, though the range is insufficient to determine whether this spacing varies with concentration as expected for mutually repulsive cylinders.

Our own work (65) suggests that there is a tendency for unfolded material to form insoluble aggregates, so it is possible that such aggregates could persist even in dilute solutions, and contribute a misleading peak to the internal structure function (67). Nucleoprotamine also forms stable aggregates (4, 52), and we believe that the resemblance may be more than superficial; in both cases, for instance, interactions between exposed hydrophobic regions could stabilize the lattice.

CONCLUSION

Our analysis of diffraction data is intended to clear the ground for a better understanding of any patterns obtained in future. Past data are not all strictly comparable with each other

because of changes in preparative techniques, but when allowance is made for this they are remarkably self-consistent despite the widely varying interpretations offered in the past. It has become apparent that only the peak at 3.7 nm, which persists even in the most dilute suspensions (15, 18, 67), is assignable solely to internal structure. Peaks at larger spacings are explicable in terms of interparticle effects, either from contact between nucleosomes or from mutual repulsion in an expanded liquid-type array.

The concentration-dependent "11-nm" peak obtained with earlier procedures cannot therefore be invoked to distinguish between models for higher-order structure in native chromatin, though the existence of some such structure is indicated by other data; there is, for instance, much evidence from electron microscopy for fibers of ~25-nm Diam (56). In the samples used for most diffraction studies, such higher-order structure may well have been destroyed, but more recent neutron scattering work (68, 69) gives clear indications of structures in the size range 30–40 nm. On the other hand, recent electron microscopy (70) suggests that nucleosomes do not adopt a unique packing pattern in higher-order arrays.

Finally, the peak at ~2.7 nm may in some preparations be due largely to unfolded material, which has an intrinsic interest because it is possible that there is an analogous process (mediated by factors not yet identified) in the living cell.

We thank Doctors H. J. Gould and W. B. Gratzer for useful discussions; Prof. J. A. Subirana, Doctors J. P. Baldwin and J. F. Pardon for sending manuscripts before publication: Prof. M. H. F. Wilkins for encouragement and facilities, and the Science Research Council and the Nuffield Foundation for support to D. Z. Staynov.

This work was supported in part by the Medical Research Council.

Received for publication 27 July 1979 and in revised form 26 November 1979.

REFERENCES

1. WILKINS M. H. F. 1956. Physical studies of the molecular structure of deoxyribose nucleic acid and nucleoprotein. *Cold Spring Harbor Symp. Quant. Biol.* **21**:75–90.
2. WILKINS, M. H. F., G. ZUBAY, and H. R. WILSON. 1959. X-ray diffraction studies of the molecular structure of nucleohistone and chromosomes. *J. Mol. Biol.* **1**:179–185.
3. LUZZATI, V., and A. NICOLAIEFF. 1959. Etude par diffusion des rayons X aux petits angles des gels d'acide désoxyribonucléique et de nucléoproteines: (note préliminaire). *J. Mol. Biol.* **1**:127–133.
4. LUZZATI, V., and A. NICOLAIEFF. 1963. The structure of nucleohistone and nucleoprotamines. *J. Mol. Biol.* **7**:142–163.
5. PARDON, J. F., M. H. F. WILKINS, and B. M. RICHARDS. 1967. Super-helical model for nucleohistones. *Nature (Lond.).* **215**:508–509.
6. PARDON, J. F. 1967. A modified theory for the Fourier transform for a coiled-coil. *Acta Cryst.* **23**:937–939.
7. PARDON, J. F., and M. H. F. WILKINS, 1972. A super-coil model for nucleohistone. *J. Mol. Biol.* **68**:115–124.
8. GARRETT, R. A. 1970. Low angle X-ray diffraction from dilute nucleohistone gels. *Biochim. Biophys. Acta.* **246**:553–560.
9. OLINS, D. E., and A. L. OLINS. 1972. Physical studies of isolated eucaryotic nuclei. *J. Cell Biol.* **53**:715–736.
10. BRAM, S., P. BAUDY, G. BUTLER-BROWNE, and K. IBEL. 1974. Chromatin small angle neutron scattering: the proposal of a new coiled coil model. *Biochimie.* **56**:1339–1341.
11. BALDWIN, J. P., P. G. BOSELEY, and E. M. BRADBURY. 1975. The subunit structure of the eukaryotic chromosome. *Nature (Lond.).* **253**:245–249.
12. BRAM, S., G. BUTLER-BROWNE, P. BAUDY, and K. IBEL. 1975. Quaternary structure of chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **72**:1043–1045.
13. CARPENTER, B. G., J. P. BALDWIN, E. M. BRADBURY, and K. IBEL. 1976. Organisation of subunits in chromatin. *Nucleic Acids Res.* **3**:1739–1746.
14. PARDON, J. F., D. L. WORCESTER, B. M. RICHARDS, and J. C. WOOLEY. 1976. Neutron scattering from chromatin fibres, gels and solutions. Harwell Report MPD/NBS/29.

15. BRADBURY, E. M., R. P. HJELM, B. G. CARPENTER, J. P. BALDWIN, G. G. KNEALE, and R. HANCOCK. 1977. *In* The Molecular Biology of the Mammalian Genetic Apparatus. P. O. P. Ts'o, editor. Elsevier-North Holland, Amsterdam. **1**:53–70.
16. HJELM, R. P., G. G. KNEALE, P. SUAU, J. P. BALDWIN, and E. M. BRADBURY. 1977. Small angle neutron scattering studies of chromatin subunits in solution. *Cell.* **10**:139–151.
17. BRAM, S., P. BAUDY, J. LEPAULT, and D. HERMANN. 1977. Chromatin very small angle neutron scattering: further evidence for a 30 nm diameter super coil in dilute solutions. *Nucleic Acids Res.* **4**:2275–2282.
18. PARDON, J. F., D. L. WORCESTER, J. C. WOOLEY, R. I. COTTER, D. M. J. LILLEY, and B. M. RICHARDS. 1977. The structure of the chromatin core particle in solution. *Nucleic Acids Res.* **4**:3199–3214.
19. PARDON, J. F., R. I. COTTER, D. M. J. LILLEY, D. L. WORCESTER, A. M. CAMPBELL, J. C. WOOLEY, and B. M. RICHARDS. 1977. Scattering studies of chromatin subunits. *Cold Spring Harbor Symp. Quant. Biol.* **42**:11–22.
20. BRAM, S., S. KOUPRACH, and P. BAUDY. 1977. DNA structure in chromatin and in solution studied by electron microscopy and neutron and X-ray scattering. *Cold Spring Harbor Symp. Quant. Biol.* **42**:23–29.
21. BAUDY, P., and S. BRAM. 1978. Chromatin fiber dimensions and nucleosome orientation: a neutron scattering investigation. *Nucleic Acids Res.* **5**:3697–3714.
22. KORNBERG, R. D. 1977. Structure of chromatin. *Ann. Rev. Biochem.* **46**:931–954.
23. FELSENFELD, G. 1978. Chromatin. *Nature. (Lond.).* **271**:115–122.
24. RICHARDS, B. M., R. I. COTTER, D. M. J. LILLEY, J. F. PARDON, J. C. WOOLEY, and D. L. WORCESTER. 1976. *In* Current Chromosome Research. K. Jones and P. E. Brandham, editors. Elsevier-North Holland, Amsterdam. 7–16.
25. CARLSON, R. D., and D. E. OLINS. 1976. Chromatin model calculations: arrays of spherical ν bodies. *Nucleic Acids Res.* **3**:89–100.
26. SUBIRANA, J. A., and A. B. MARTINÉZ. 1976. Model studies of chromatin structure based on X-ray diffraction data. *Nucleic Acids Res.* **3**:3025–3042.
27. FINCH, J. T., and A. KLUG. 1976. Solenoidal model for superstructure in chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **73**:1897–1901.
28. OLINS, A. L., J. P. BREILLATT, R. D. CARLSON, M. B. SENIOR, E. B. WRIGHT, and D. E. OLINS. 1977. *In* The Molecular Biology of the Mammalian Genetic Apparatus. P. O. P. Ts'o, editor. Elsevier-North Holland, Amsterdam. **1**:211–237.
29. RILEY, D. P., and G. OSTER. 1951. Some theoretical and experimental studies of X-ray and light scattering by colloidal and macromolecular systems. *Disc. Faraday Soc.* **11**:107–116.
30. VAINSHTEIN, B. K., and L. A. FEIGIN. 1965. The ordered arrangement of molecules of "soluble" ribonucleic acid in aqueous solutions. *Dokl. Akad. Nauk. USSR.* **161**:1444–1447 (English translation, pp. 49–52).
31. SPENCER, M. 1969. Low-angle X-ray diffraction from concentrated sols of F-actin. *Nature (Lond.).* **223**:1361–1362.
32. VENABLE, J. H., M. SPENCER, and E. WARD. 1970. Low-angle X-ray diffraction maxima from ribosomes. *Biochim. Biophys. Acta.* **209**:493–500.
33. OSTER, G., and D. P. RILEY. Scattering from isotropic colloidal and macromolecular systems. *Acta Cryst.* **5**:1–6.
34. OSTER, G., and D. P. RILEY. 1952. Scattering from cylindrically symmetric systems. *Acta Cryst.* **5**:272–276.
35. PARDON, J. F., B. M. RICHARDS, and R. I. COTTER. 1973. X-ray diffraction studies on oriented nucleohistone gels. *Cold Spring Harbor Symp. Quant. Biol.* **38**:75–81.
36. SUBIRANA, J. A., F. AZORÍN, J. ROCA, J. LLOVERAS, R. LLOPIS, and J .CORTADAS. 1977. *In* The Molecular Biology of the Mammalian Genetic Apparatus. P. O. P. T'so, editor. Elsevier-North Holland, Amsterdam. **1**:71–92.
37. PARDON, J. F. 1979. *In* Chromatin Structure and Function. C. Nicolini, editor. Plenum Press, New York. 187–216.
38. BRAM, S., G. BUTLER-BROWNE, E. M. BRADBURY, J. BALDWIN, C. REISS, and K. IBEL. 1974. Chromatin neutron and X-ray diffraction studies and high resolution melting of DNA-histone complexes. *Biochimie.* **56**:987–994.
39. PANYIM, S., D. BILEK, and R. CHALKLEY. 1971. An electrophoretic comparison of vertebrate histones. *J. Biol. Chem.* **246**:4206–4215.
40. OLINS, D. E., P. N. BRYAN, R. E. HARRINGTON, W. E. HILL, and A. L. OLINS. 1977. Conformational states of chromatin ν bodies induced by urea. *Nucleic Acids Res.* **4**:1911–1931.
41. SPERLING, L., and A. TARDIEU. 1976. The mass per unit length of chromatin by low-angle X-ray scattering. *FEBS Lett.* **64**:89–91.

42. HEWISH, D. R., and L. A. BURGOYNE. 1973. Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. *Biochem. Biophys. Res. Commun.* **52**:504–510.
43. NOLL, M., J. O. THOMAS, and R. D. KORNBERG. 1975. Preparation of native chromatin and damage caused by shearing. *Science (Wash. D. C.)* **187**:1203–1206.
44. GRIFFITH, J. D. 1975. Chromatin structure: deduced from a minichromosome. *Science (Wash. D. C.).* **187**:1202–1203.
45. TSANEV, R., and P. PETROV. 1976. The substructure of chromatin and its variations as revealed by electron microscopy. *J. Microsc. Biol. Cell.* **27**:11–18.
46. OUDET, P., C. SPADAFORA, and P. CHAMBON. 1977. Nucleosome structure. II. Structure of the SV40 minochromosome and electron microscopic evidence for reversible transitions of the nucleosome structure. *Cold Spring Harbor Symp. Quant. Biol.* **42**:301–312.
47. CLARK, R. J., and G. FELSENFELD. 1971. Structure of chromatin. *Nat. New Biol.* **229**:101–106.
48. RICHARDS, B. M., and J. F. PARDON. 1970. The molecular structure of nucleohistone (DNH). *Exp. Cell Res.* **62**:184–196.
49. BALDWIN, J. P., B. G. CARPENTER, H. CRESPI, R. HANCOCK, R. M. STEPHENS, J. K. SIMPSON, and E. M. BRADBURY. 1978. Neutron scattering from chromatin in relation to higher-order structure. *J. Appl. Cryst.* **11**:484–486.
50. FINCH, J. T., L. C. LUTTER, D. RHODES, R. S. BROWN, B. RUSHTON, M. LEVITT, and A. KLUG. 1977. Structure of nucleosome core particles of chromatin. *Nature (Lond.).* **269**:29–36.
51. FINCH, J. T., and A. KLUG. 1977. X-ray and electron microscope analyses of crystals of nucleosome cores. *Cold Spring Harbor Symp. Quant. Biol.* **42**:1–9.
52. SPERLING, L., and A. KLUG. 1977. X-ray studies on "native" chromatin. *J. Mol. Biol.* **112**:253–263.
53. LEVITT, M. 1978. How many base-pairs per turn does DNA have in solution and in chromatin? Some theoretical calculations. *Proc. Natl. Acad. Sci. U.S.A.* **75**:640–644.
54. DUBOCHET, J., and M. NOLL. 1978. Nucleosome arcs and helices. *Science (Wash. D. C.).* **202**:280–286.
55. RENZ, M., P. NEHLS, and J. HOZIER. 1977. Histone H1 involvement in the structure of the chromatin fiber. *Cold Spring Harbor Symp. Quant. Biol.* **42**:245–252.
56. OLINS, D. E., and A. L. OLINS. 1978. Nucleosomes: the structural quantum in chromosomes. *Am. Sci.* **66**:704–711.
57. RATTLE, H. W. E., G. C. KNEALE, J. P. BALDWIN, H. R. MATTHEWS, C. CRANE-ROBINSON, P. D. CARY, B. G. CARPENTER, P. SUAU, and E. M. BRADBURY. 1979. *In* Chromatin Structure and Function. C. Nicolini, editor. Plenum Press, New York. 451–513.
58. BALDWIN, J. P., E. BOULTER, G. W. BRADDOCK, E. M. BRADBURY, P. D. CARY, B. G. CARPENTER, G. G. KNEALE, and P. SUAU. 1979. *In* Gene Function. S. Rosenthal et al., editors. Pergamon Press, Oxford. 171–191.
59. CAMPBELL, A. M., R. I. COTTER, and J. F. PARDON. 1978. Light scattering measurements supporting helical structures for chromatin in solution. *Nucleic Acids Res.* **5**:1571–1580.
60. PARDON, J. F., and B. M. RICHARDS. 1979. *In* The Cell Nucleus. H. Busch, editor. Academic Press, Inc., New York. Vol. VII, Pt. D. 371–411.
61. POOLEY, A. S., J. F. PARDON, and B. M. RICHARDS. 1974. The relation between the unit thread of chromosomes and isolated nucleohistone. *J. Mol. Biol.* **85**:533–549.
62. BRADBURY, E. M., H. V. MOLGAARD, R. M. STEPHENS, L. A. BOLUND, and E. W. JOHNS. 1972. X-ray studies of nucleoproteins depleted of lysine-rich histone. *Eur. J. Biochem.* **31**:474–482.
63. GARRETT, R. A. 1971. A physical study of the stability of native nucleohistone conformation to salt dissociation and heating. *Biochemistry.* **10**:2227–2230.
64. BALDWIN, J. P., E. M. BRADBURY, G. S. BUTLER-BROWNE, and R. M. STEPHENS. 1973. Histone interactions with prokaryote DNA. *FEBS Lett.* **34**:133–136.
65. STAYNOV, D. Z., M. SPENCER, J. ALLAN, and H. J. GOULD. 1979. A reversible conformational transition in chromatin. *Nature (Lond.).* **279**:263–265.
66. BRADBURY E. M., J. P. BALDWIN, B. G. CARPENTER, R. P. HJELM, R. HANCOCK, and K. IBEL. 1975. Neutron-scattering studies of chromatin. *Brookhaven Symp. Biol.* No. 27 **4**:97–117.
67. SUAU, P., G. G. KNEALE, G. W. BRADDOCK, J. P. BALDWIN, and E. M. BRADBURY. 1977. A low resolution model for the chromatin core particle by neutron scattering. *Nucleic Acids Res.* **4**:3769–3786.
68. BAUDY, P., and S. BRAM. 1979. Neutron scattering on nuclei. *Nucleic Acids Res.* **6**:1721–1729.
69. SUAU, P., E. M. BRADBURY, and J. P. BALDWIN. 1979. Higher-order structures of chromatin in solution. *Eur. J. Biochem.* **97**:593–602.
70. RATTNER, J. B., and B. A. HAMKALO. 1979. Nucleosome packing in interphase chromatin. *J. Cell Biol.* **81**:453–457.