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Arrangement of subunits in assembled histone H4 fibers

(chromatin/electron microscopy/optical diffraction)

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ABSTRACT **The structure of assembled histone H4 fibers has been studied by analysis of electron micrographs, including optical diffraction. An individual fiber has the appearance of an 80-Å wide ribbon, twisted at intervals of about 330 Å. Thicker fibers which have been observed seem to be bundles of ribbons. In diffraction patterns from both kinds of fiber, layer lines at axial spacings of about 1/55 $Å^{-1}$, 1/37 $Å^{-1}$, and 1/27 $Å^{-1}$ were most consistently observed. The possible arrangements of molecules within the twisted ribbons have been deduced and are found to be fairly closely related. The ribbons appear to consist of two parallel, unstaggered rows of repeating units, which are probably H4 dimers.**

The similarity between the observed layer line spacings of the H4 fibers and the spacings of the maxima in x-ray diffraction patterns from whole chromatin suggests that the H4 fibers have a structure related to that of chromatin. Since homogeneous preparations of histones H2A, H2B, and H3, or any mixture of these four histones, can form similar structures, it seems likely that the basic organization of chromatin is determined by a fibrous histone core around which the DNA is wrapped.

The assembly of histone H4 into 40- to 80-Å diameter fibers under suitable *in vitro* conditions has already been described (1). Histones H2A, H2B, H3, and H4 all form similar fibers, either individually or in mixtures (2, 3), but H3 or H4, the arginine-rich histones, or mixtures of these two, do so most readily, at ionic strengths between 0.02 and 0.15. The lysine-rich histones H2A and H2B polymerize only at higher ionic strengths ($I \geq 0.5$). In solutions of pairs of histones or of all four histones, there is an equilibrium between low molecular weight complexes and fibers (1–3), the dimer being the basic unit in the assembly. The assembly pathway of pairs of histones from dimers to fibers is via tetramers, hexamers, and octomers (R. Sperling and M. Bustin, unpublished data).

This paper presents a detailed study, using the technique of optical diffraction from electron micrographs (4), of fibers of homogeneous H4, which has so far provided the best electron microscope images. On the basis of the results obtained, we discuss the possible relationship between the structure of the fibers and that of chromatin.

MATERIALS AND METHODS

Histone H4 was kindly donated by M. Bustin from the Chemical Immunology Department, Weizmann Institute of Science, Rehovot, Israel. For characterization of H4 and the preparation of assembled structures by dialysis see ref. 1. Specimens were negatively stained with 1% uranyl acetate and photographed in a Philips EM 300 electron microscope, operating at 80 kV.

Optical diffraction patterns were observed with an optical diffractometer built by R. Josephs, which is similar to that of DeRosier and Klug (5). The 23-Å spacing of tobacco mosaic virus was used for calibration. Selected micrographs were examined with an Optronix P-1000 photodensitometer, which produced an IBM-compatible magnetic tape. The Fourier transforms of the digitized images were computed by the method of DeRosier and Moore (6).

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RESULTS AND DISCUSSION

Optical Diffraction Patterns from Electron Micrographs. As already described (1), fibers assembled from purified histones are very curly structures, 40–80 Å in diameter (Fig. 1). In the clearest examples, they have the appearance of a double-stranded cable, superficially similar to F-actin (7), with intervals of 300–400 Å between crossover points [this distance was previously quoted by mistake as 150 Å (1)]. At the crossovers, the fibers appear very white and narrow, showing that here the projected protein density is relatively high, whereas the regions in between are gray and indistinct. The stretches over which the fibers are straight are not long enough to give good optical diffraction patterns. Even the best patterns (Fig. 1 *a* and *b*) are rather noisy. However, comparison of several different examples indicates that diffraction peaks occur most reproducibly along layer lines with axial spacings of about 55, 37, and 27 Å.

Under suitable conditions (see legend to Fig. 2), a small proportion of larger aggregates are formed, which we refer to as bundles. Although usually straighter than individual fibers, they are fairly heterogeneous, apparently incorporating a variable number of individual fibers. Their optical diffraction patterns are still rather noisy: the one shown in Fig. 3 has clear layer lines at 27, 37, and 55 Å, but most other examples (e.g., Fig. 4 *a* and *b*) also have peaks at many other axial spacings. The variation must be due partly to differences in the packing of the fibers and to irregularities, possibly caused by the natural tendency of the individual fibers to curl, and partly to differences in preservation and negative staining. Because of this, many different patterns were compared to determine their common features. The effect of averaging the patterns from a number of different bundles is shown in Fig. 4*c*, which shows the dominant layer lines at 55, 37, and 27 Å.

The similarity in the diffraction patterns from images of individual fibers and bundles suggests that the bundles are indeed lateral aggregates of fibers. Both structures show layer lines that are submultiples of 110 Å, although all of these layer lines may not be apparent simultaneously in any single pattern. This result strongly suggests that the substructure of the fibers is related in some way to that of chromatin, since x-ray patterns from isolated chromatin show a series of peaks at around 110, 55, 37, 27, and 22 Å (8–13). It is therefore of considerable interest to discover how the histone molecules might be arranged in the assembled fibers.

Possible Helical Lattices. The lack of long straight regions of individual fibers and the variation in the packing of the bundles makes a full analysis of the substructure impossible at

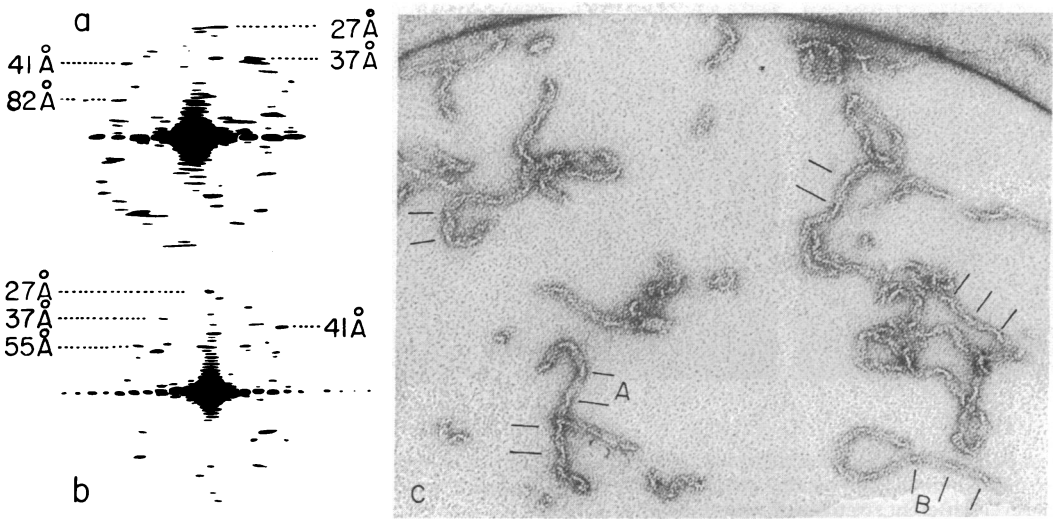

FIG. 1. Histone H4 fibers and optical diffraction patterns of short straight sections of fiber. (*a* and *b*) Patterns derived from stretches marked A and B in *c*. (*c*) Electron migrograph of H4 fibers at 1 mg/ml in 10 mM cacodylate buffer, pH 7.0, $I = 0.3$, 5°, photographed over a hole in the carbon substrate. The fibers appear to consist of two helical strands, with crossover points at intervals of about 330 Å, as indicated by lines. (×126,000.)

present. However, the data are sufficient to define the most probable arrangements of subunits.

The repeat distance observed in electron micrographs of the double-stranded cables averages about 330 Å (see Fig. 1*c*), which is much longer than the 110-Å repeat that would give rise to the system of main layer lines described above. The most reasonable explanation is that 110 Å is itself a subrepeat of 330 Å and that each interval between the 110-Å series of layer lines should be divided into three. This would account for many of the optical diffraction peaks that are found between the main layer lines (such as those which lie on lines at 41 and 82 Å in Fig. 1, for example).

Fig. 5 shows how the diffraction patterns can be tentatively indexed; a point ($n = 2$) on the first (330-Å) layer line is defined by the family of two helices that follow the two strands of the cable. (This reflection has not actually been observed in any pattern, since rather long straight lengths of fiber would be needed to provide enough 330-Å repeats to produce a peak here.) Another point in the reciprocal lattice is fixed by the fact that, wherever the 27-Å layer line is observed, the diffracted intensity appears to be meridional (i.e., $n = 0$). The positions of peaks on other layer lines, such as the 55 and 37 Å, are rather variable, which leaves the final choice of lattice undetermined, as discussed below. In spite of this, the possibilities are strictly limited. The two most likely solutions of the diffraction patterns are illustrated in Fig. 5 and the corresponding helical surface lattices in Fig. 6. The first lattice has six repeating units per 330-Å axial repeat along each of the two strands, the second lattice has only four.

Careful inspection of the images of the fibers (Figs. 1 and 7) shows that the apparent number of subunits between crossover points is about ten. This would be consistent with either 12 or 8 subunits per strand (Fig. 6 *a* and *b* or *c* and *d*), that is, twice as many "subunits" as "repeating units." Thus, in either case, it seems to be necessary to postulate some difference in alternate subunits along each strand. In the first case (Fig. 6 *a* and *b*), the first contribution to the 55-Å layer line would otherwise lie too far ($n = 12$) from the meridian to explain the observed intensity distribution. In the second case (Fig. 6 *c* and *d*), a grouping in pairs or alternation in subunit position is required to explain the 27-Å meridional, which would then occur as the third order of an 82-Å (=330 Å/4) axial periodicity. The dominance of the 27-Å repeat over the expected meridionals at 82 and 41 Å would

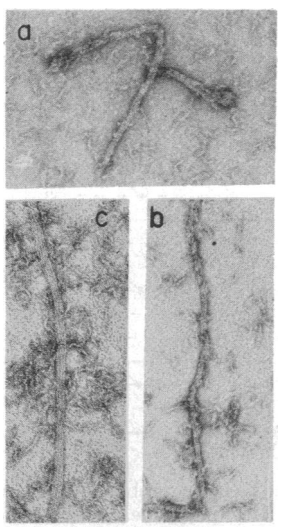

FIG. 2. Electron micrographs of histone H4 bundles, formed at 2 mg/ml of histone concentration. (*a* and *b*) In 5 mM phosphate buffer, pH 6.4, $I = 0.15$, 5°. (*c*) In 10 mM cacodylate buffer, pH 6.4, $I = 0.3$, 5°. (×81,400.)

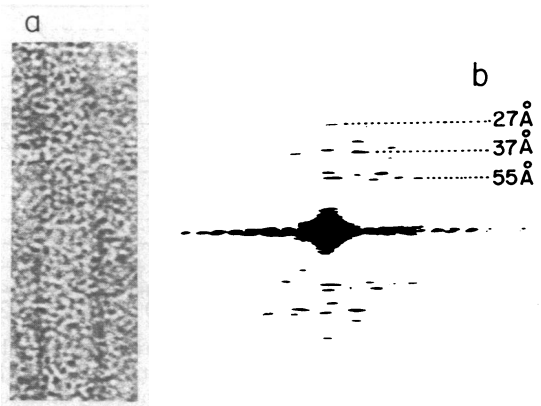

FIG. 3. Optical diffraction from a histone H4 bundle. (*a*) Enlarged straight region of the bundle shown in Fig. 2*c*. (×368,500.) (*b*) Optical diffraction pattern of part of *a*.

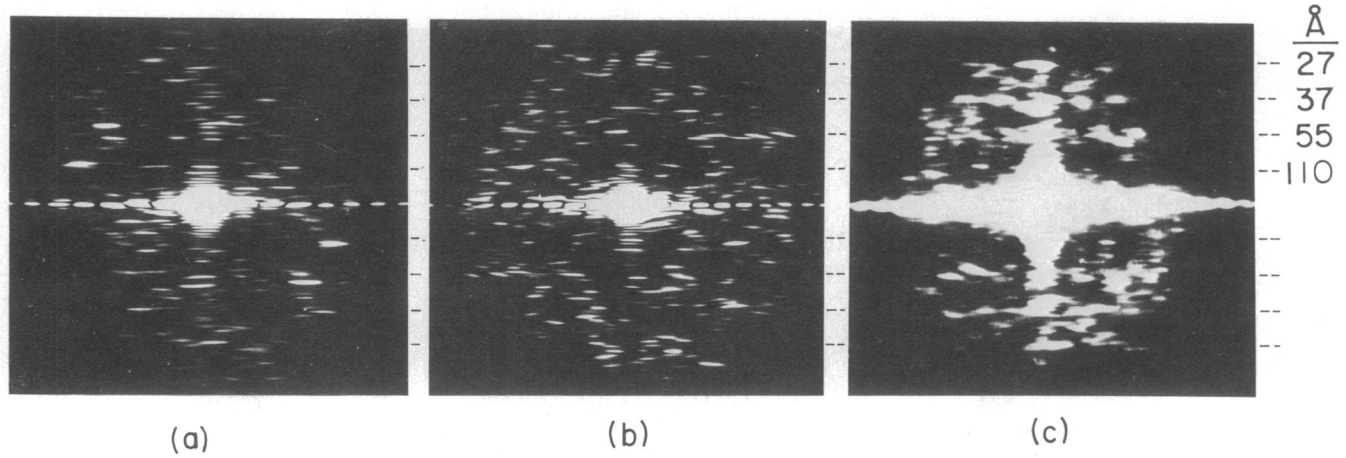

FIG. 4. Optical diffraction patterns from histone H4 bundles. The four layer lines most frequently observed are labeled. (*a* and *b*) Typical patterns, from two different individual bundles similar to those shown in Fig. 2 *a* and *b*. (*c*) Composite diffraction pattern obtained by averaging the patterns from seven different stretches of various bundles.

have to be explained in terms of some feature of the internal structure of the 82-Å double unit.

Both of these two possibilities require that subunits in the two geometrical strands of the twisted ribbons be in phase, rather than staggered as for F-actin (7). In other words, only even orders of n should contribute to the diffraction patterns. This has been checked by digitizing a number of images, both of individual fibers and the bundle in Fig. 3, and computing their Fourier transforms (6). Phases of the amplitude peaks on the three main layer lines, 55, 37, and 27 Å, were investigated. Where corresponding peaks on a particular layer line could be identified on either side of the meridian, the differences in their phases were always closer to 0 than to π, suggesting a completely even-handed structure (see ref. 15), at least at this resolution.

The choice between the two lattices depends largely on identifying the contributions to the 55 and 37-Å layer lines. Although in a few examples, such as the bundle in Fig. 3, meridional reflections are found at 55 and 37 Å the major peaks on both layer lines are usually off-meridional, in positions which, for a 40- to 80-Å diameter fiber, would be consistent with orders of n between 2 and 8. This would appear to favor the second lattice (Fig. 5 *right*). However, in the case of the bundles, the intensity distribution on the layer lines must be affected by the way in which the fibers are packed together. Also, the distribution of matter within the fibers themselves is rather unusual; they appear to be rather more ribbon-like than F-actin, for example. Contrast with the negative stain therefore occurs over a relatively large range of radii, giving rise to a complex distribution of diffracted intensity on each layer line. So it is possible that an $n = 0$ contribution could give rise to an off-meridional peak. However, the order in the specimens as shown by the diffraction patterns is not really good enough to decide the issue at present.

Relationship between Observed Subunits and Histone Molecules. Assuming that histones have a similar density to that of other well-known proteins, for a molecular weight of about 11,000 each molecule should have a volume approximately equivalent to a 25- to 30-Å diameter sphere. This estimate is quite consistent with the interpretation of the subunits observed in the electron micrographs as histone monomers, which would mean either 12 or 8 histone monomers per strand per 330-Å repeat, depending on which (if either) of the above two possible lattices is correct. However, previous estimates of molecular volumes of negatively stained particles have been found to be in error by more than a factor of two (see, e.g., ref. 16), so this interpretation must be regarded as merely tentative.

Relationship to the Structure of Chromatin. It has been suggested in recent models of chromatin that the DNA is wrapped around a core of histones (1, 17, 18). In the model proposed by Kornberg (17), the chromatin is organized in units of about 200 base pairs of DNA associated with two molecules

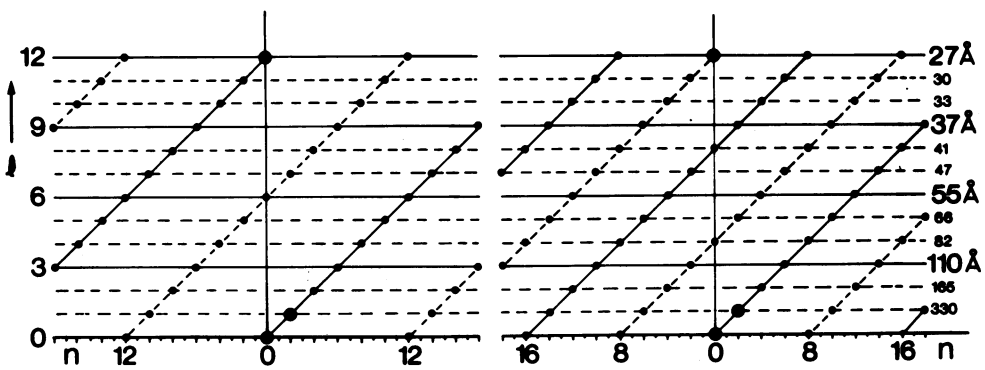

FIG. 5. Plots (n,l) (14) for the diffraction patterns of individual histone H4 fibers, showing the two most probable indexing schemes. Assuming a 330-Å axial repeat, a value of $n = 2$ on the first (330-Å) layer line and a meridional on the 27-Å layer line, the possible lattices are limited. Reciprocal lattice lines must cross the meridian obliquely at the origin and at 27 Å. The number of intermediate oblique parallel lines is variable. *Left* and *Right* have 1 and 2 intermediate lines, respectively. Reciprocal lattice points along just the solid lines would correspond to either 12 (*Left*) or 8 (*Right*) repeating units per strand, per 330-Å axial repeat (see Fig. 6); additional reciprocal lattice points along the dashed lines would be produced only if the number of repeating units were reduced to 6 (*Left*) or 4 (*Right*).

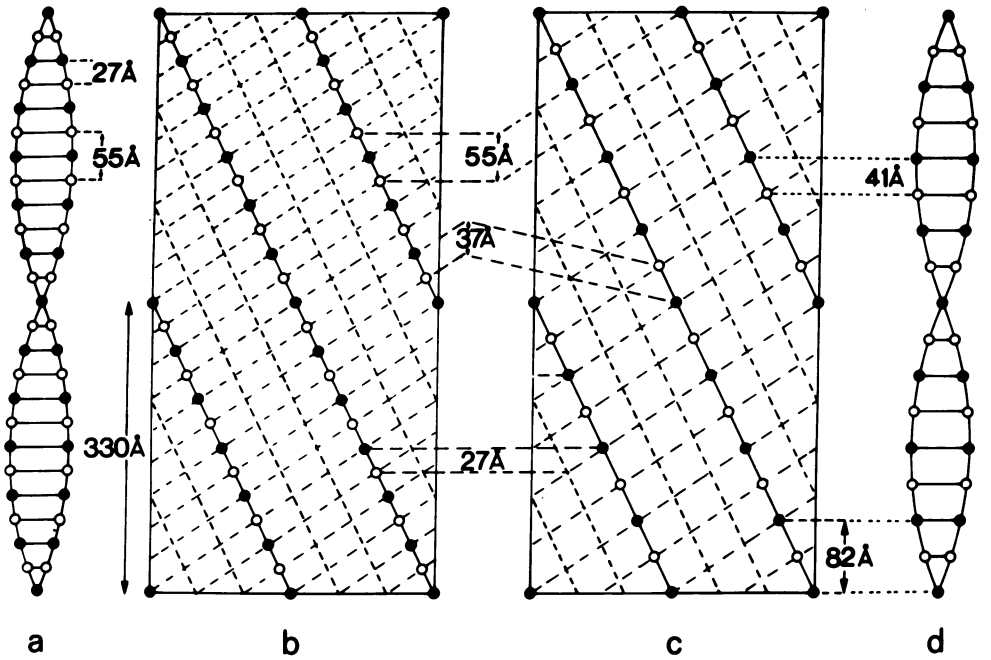

FIG. 6. (*a* and *d*) Diagrams of the two possible models of the double-stranded cables. (*b* and *c*) Opened-out versions of the helical surface lattices which would give rise to the two reciprocal lattices shown in Fig. 5. In each case, the full 330-Å repeating super-lattice is shown as solid lines superimposed on a finer lattice (dashed lines) which would give reflections only on the 110-Å series of layer lines (solid horizontal lines in Fig. 5). Since the "intermediate" layer lines (dashed horizontal lines in Fig. 5) tend to be absent from the diffraction patterns of well-ordered bundles (e.g., Fig. 3), the individual fibers may tend to pack in the bundles so as to form a structure with the finer lattice. The most likely spacings of histone subunits along the two strands of the individual fibers are indicated by the small circles. Alternate circles are shaded: in either model a difference in position or orientation between alternate subunits along each strand is required to explain the diffraction patterns (see Fig. 5 and *text*). Thus, *a* and *b* have six repeating units per strand per axial repeat; *c* and *d* have four repeating units per strand per axial repeat.

of each of H2A, H2B, H3, and H4. These units have been identified as 80- to 120-Å diameter beads by electron microscopy (19–21). Chromatin has also been observed in the electron microscope as 100-Å and 300-Å diameter fibers (22–25). The 100-Å fibers, which probably consist of continuously associated beads, are characteristic of unsheared, H1-depleted chromatin, in media containing very low concentrations of magnesium ions. The 300-Å fibers, which are formed when histone H1 and magnesium ions are present, appear to be supercoils (solenoids) of the 100-Å fibers (25).

The similarity between the layer line spacings found in the optical diffraction patterns of H4 fibers and the spacings found in x-ray diffraction patterns of chromatin (8–13) raises the possibility that the structure of the assembled histone fibers is closely related to the substructure of chromatin, most probably to the continuous 100-Å fibers. The fibers described here consist of H4 only, but similar fibers are formed by other single histones and by mixtures of histones either in pairs or all four together (2, 3). Therefore, this pattern of linear aggregation seems to be a common property of histones (1–3). It is likely that H2A, H2B, H3, and H4 are all homologous with respect to fiber formation, but that differences among them are necessary for other functions, for example, for differentiating the continuous 100-Å fibers into bead-like nucleosomes, or for the formation of higher order structures such as the 300-Å diameter solenoids described by Finch and Klug (25).

H3 and H4 polymerize most readily, so the fibers analyzed here may be analogous to a polymer of H3·H4 tetramers, forming the inner core of the 100-Å chromatin fiber. Strands of H2A·H2B dimers could be incorporated in the grooves between the two H3·H4 strands, producing a four-stranded polymer. Alternatively, the core might simply consist of a two-stranded heteropolymer of all four histones. Winding DNA around either type of histone core would produce a fiber with a maximum diameter of 80–120 Å.

Conclusion. Because of the difficulty in obtaining sufficiently well-ordered specimens, it is not yet possible to translate the results of the present analysis reliably into molecular arrangements. Nevertheless, the data do suggest that histone–histone interactions are a major factor in determining the structure of chromatin, and it is hoped that the structure of these

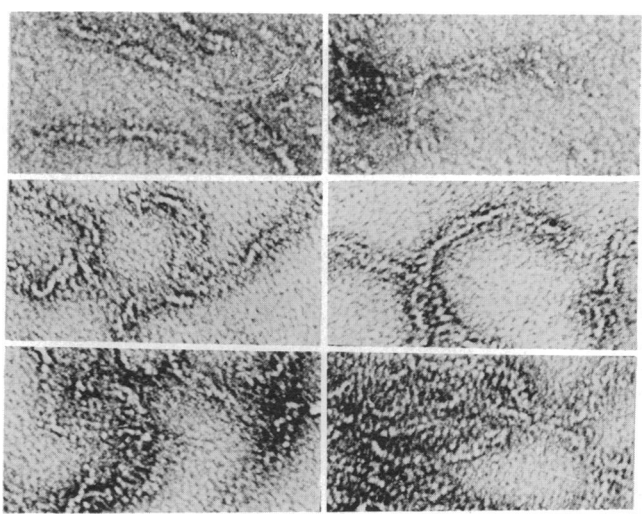

FIG. 7. Enlargements from electron micrographs of individual H4 fibers, showing the two-stranded appearance. The number of subunits per strand between crossovers seems to be between 8 and 12 (obtained by dividing crossover distances by the average center-to-center distance of dots, as measured from electron micrographs on the Nikon microcomparator). (×250,000.)

histone fibers will provide a useful insight into such interactions, once the structure of chromatin itself is understood in more detail.

Note added in proof. The structure of crystals of nucleosome core particles, consisting of about 140 base pairs of DNA plus 8 histone molecules, has recently been studied by electron microscopy and x-ray diffraction (26). Assuming the histone fibers studied here are structurally related to the nucleosome core particles, the first model (Fig. 6 *a* and *b*), which divides readily into nominal 55-Å-thick segments, is more consistent with the crystal data and therefore is more likely to be correct for the histone core of the nucleosome than the alternative shown in (Fig. 6 *c* and *d*).

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