Ultrastructure of chromatin

I. Negative staining of isolated fibers

C. L. WOODCOCK*, H. WOODCOCK† and R. A. HOROWITZ
Department of Zoology, University of Massachusetts, Amherst, MA 01003, USA

*Author for correspondence
†Present address: Department of Forestry and Wildlife Management, University of Massachusetts, Amherst, MA 01003, USA

Summary

The ultrastructure of chromatin fibers isolated from erythrocyte nuclei of Necturus maculosus and contrasted with a number of negative stains is described. Long (>1000 nm) fibers are prepared under ionic conditions that promote fiber integrity, fixed with glutaraldehyde and negatively stained with aurothioglucose, ammonium molybdate, methylamine tungstate, sodium phosphotungstate, uranyl acetate and a uranyl acetate–sodium phosphotungstate sequence. All stains yield images of '30 nm' chromatin fibers, but aurothioglucose gives the most consistent diameter measurements (33 nm, s.d. 3.5 nm), and provides the clearest images of individual nucleosomes. Regions of fiber showing structural order are seen with all stains. The most commonly observed is a regular pattern of oblique cross-striations consistent with the visualization of the 'top' or 'bottom' of a helical structure. There is a significant relationship between fiber diameter and the cross-striation angle, consistent with an extensible chromatin fiber. Examination of power spectra prepared from selected ordered regions confirms the visual impressions, and indicates a striation spacing ranging from 11 nm to 18 nm, and dependent on the stain type. Fibers allowed to unfold slightly in a buffer containing 50 mM monovalent ions show evidence of a two-stranded helix-like organization. These results are discussed in terms of current models for the structure of the chromatin fiber.

Key words: chromatin, 30 nm fiber, negative stain, ultrastructure.

Introduction

In most eukaryotes, nuclear DNA is complexed with histones to form nucleosomes that, by interacting with each other, form chromatin fibers approximately 30 nm in diameter. The manner in which nucleosomes are packed into the 30 nm fiber has been difficult to determine and remains a matter of controversy (reviewed by Butler, 1983; Felsenfeld and McGhee, 1986; Pederson et al. 1986; van Holde, 1989). One widely discussed possibility is that the linear array of nucleosomes is coiled to form a simple (i.e. one-start) helix or solenoid (Finch and Klug, 1976), and a number of variations on this theme have been proposed (e.g. see Thoma et al. 1979; McGhee et al. 1980, 1983; Butler, 1984). More recently, evidence suggesting a very shallow simple helix containing few nucleosomes per turn has been presented: in forming the 30 nm fiber, nucleosomes from adjacent turns interdigitate to give a helix-like structure (Bordas et al. 1986a, b). Other investigators have suggested a second, rather different type of ordered arrangement in which the nucleosomes form a two-start helix (Worcel et al. 1981; Woodcock et al. 1984; Williams et al. 1986). Within a two-start helical arrangement, there are again a number of possible ways in which the nucleosomes and linker DNA may be positioned, each having a unique consequence for the process of folding and unfolding, and for the accessibility of the DNA prior to transcription and replication.

Other less-ordered models for the structure of the 30 nm fiber include the formation of clusters of nucleosomes or 'supranucleosomal' units that when linearly arranged form a fiber (e.g. see Stralling et al. 1978; Zentgraf et al. 1981; Zentgraf and Franke, 1984), and a layered organization in which nucleosomes are arranged in irregular discs that stack to form the fiber (Subiran et al. 1985). In addition, McDowell et al. (1986) have proposed a liquid model that predicts no long-range order.

The existence of so many model structures reflects the ambiguity of much of the biophysical and ultrastructural data on chromatin fibers. To date, electron micrographs have not provided a clear picture of the arrangement of nucleosomes in the 30 nm fiber: in fully compact fibers, individual nucleosomes are generally poorly resolved, and in unfolded fibers where single nucleosomes are clearly seen, patterns of association tend to be lost. It is possible to induce intermediate states in the folded to unfolded continuum by adjusting the ionic strength of the medium (Thoma et al. 1979) and this approach has been used to identify one distinct intermediate structure, the zig-zag ribbon. This ribbon takes the form of a double linear array of nucleosomes in which the linker DNAs form a zig-zag arrangement (Thoma et al. 1979; Worcel et al. 1981; Woodcock et al. 1984). However, the further compaction of the zig-zag ribbon to form the 30 nm fiber has been open to various interpretations.

The failure of electron microscopy to provide unequivo-
cal results may be attributed a number of factors including preparation-induced effects, poor contrasting and inherent disorder. Some disorder in vivo is expected from forms of microheterogeneity such as linker DNA length variations, histone variants, histone modifications and an irregular complement of non-histone proteins (van Holde, 1989). However, it is probable that the fiber arises from and is stabilized by equivalent interactions between neighboring nucleosomes that will tend to produce a structurally disorder. Some disorder in vivo preparation-induced effects, poor contrasting and inherent disorder may be attributed a number of factors including preparation-induced effects, poor contrasting and inherent disorder.

However, it is probable that the fiber arises from and is stabilized by equivalent interactions between neighboring nucleosomes that will tend to produce a structurally disorder. Some disorder in vivo preparation-induced effects, poor contrasting and inherent disorder may be attributed a number of factors including preparation-induced effects, poor contrasting and inherent disorder. It has been our rationale for persisting with efforts to refine the preparative and staining techniques for chromatin. It has been our rationale for persisting with efforts to refine the preparative and staining techniques for chromatin. It has been our rationale for persisting with efforts to refine the preparative and staining techniques for chromatin. It has been our rationale for persisting with efforts to refine the preparative and staining techniques for chromatin.

In this report, we discuss the isolation of chromatin fibers and their structure after negative staining. Following the observations of Williams et al. (1986), that chromatin from erythrocyte nuclei of Necturus maculatus was relatively well-ordered, we have explored strategies for optimizing the release of long fibers from these nuclei. Negative staining of these fibers using a variety of staining agents confirms the frequent occurrence of non-random nucleosome packing. In many cases, the arrangement of nucleosomes appears to be helical, but diffraction patterns reveal considerable variability. In the accompanying paper (Woodcock et al. 1991), the results of tomographic reconstructions of selected regions are presented.

Materials and methods

Isolation of nuclei

Nuclei were isolated from Necturus maculatus erythrocytes by the method of Williams et al. (1986) with slight modifications: fresh blood was collected and washed three times by centrifugation in 130 mM NaCl, 5 mM KCl, 2 mM MgCl2, 10 mM Hepes, pH 7.3, and then once with MB (60 mM NaCl, 15 mM KCl, 2 mM MgCl2, 0.1 mM PMSF, 15 mM Hepes, pH 7.3). After resuspending in a volume of MB equal to 10 times the starting blood volume, the erythrocytes were stirred gently on ice and MB containing 0.1% NP-40 was added slowly while monitoring the lysis process. Lysis was usually judged complete when the final concentration of NP-40 reached about 0.05%; further addition of detergent beyond this point did not result in lysis with MB containing 0.1% NP-40 was added slowly while monitoring the lysis process. Lysis was usually judged complete when the final concentration of NP-40 reached about 0.05%; further addition of detergent beyond this point did not result in lysis with MB containing 0.1% NP-40 was added slowly while monitoring the lysis process. Lysis was usually judged complete when the final concentration of NP-40 reached about 0.05%; further addition of detergent beyond this point did not result in lysis with MB containing 0.1% NP-40 was added slowly while monitoring the lysis process. Lysis was usually judged complete when the final concentration of NP-40 reached about 0.05%; further addition of detergent beyond this point did not result in lysis with MB containing 0.1% NP-40 was added slowly while monitoring the lysis process. Lysis was usually judged complete when the final concentration of NP-40 reached about 0.05%; further addition of detergent beyond this point did not result in lysis.

Nuclease digestion and release of chromatin

Several methods for nuclease digestion were tested, including the buffer A procedure of Ruiz-Carrillo et al. (1980) and the modification used by Woodcock et al. (1984). However, the highest yields of long chromatin fibers were obtained with the following method. Nuclei were resuspended gently to 2.0 A260 units ml-1 in digestion buffer consisting of 60 mM KCl, 15 mM NaCl, 0.5 mM CaCl2, 10 mM PMSF, 15 mM Hepes, pH 8.0. Micrococcal nuclease ( Worthington) was added to 0.1 enzyme unit/A260 unit and the mixture incubated on ice for 1 h. After this time, a small sample was removed and centrifuged, and the absorbance of the supernatant was measured to estimate the extent of digestion. If less than 10% of the starting material had been released, the digestion mixture was allowed to come to room temperature, and incubation was continued for a further 10 min or until the release exceeded 10%. EDTA was then added to 10 mM, and the nuclei gently pelleted. To the pellet, release buffer consisting of 60 mM KCl, 15 mM NaCl, 1 mM EDTA, 10 mM PMSF, 5 mM Hepes, pH 8.0, was added, and the suspension held at 4°C overnight without stirring. In a typical experiment, 15% of the starting material was released during the digestion process, and a further 15% into the release buffer.

In some experiments, the salt concentration of the soluble chromatin was adjusted to alter the degree of compaction of the fibers (Thoma et al. 1979). To chromatin in digestion buffer with EDTA or in release buffer, 5 mM Hepes, pH 8.0, was added to bring the final concentration of monovalent cations to 50 mM, 20 mM or 10 mM. In cases where maximal compaction was desired, MgCl2 was added to a final concentration of 2 mM, and Pipes buffer added to give a final monovalent cation concentration of 15 mM, and final pH of 6.0. Fixation of samples was carried out by adding glutaraldehyde to a final concentration of 0.1% for 12 h at 4°C (Thoma et al. 1979).

Staining and electron microscopy

Fixed chromatin solutions were diluted as necessary with 60 mM NaCl (Woodcock et al. 1981), and droplets placed on freshly glow-discharged carbon films. After a 1 min adhesion time, the grid was washed with 5 drops of 60 mM NaCl, then 5 drops of stain, after which the excess stain was removed with absorbent paper. The stains used were: 2% uranyl acetate, unbuffered (UA); 1.5% sodium phosphotungstate, pH 7.0, containing 0.015% glucose (PTA); 1.5% methylamine tungstate, pH 7.0 (MET); 4% ammonium molybdate, pH 8.5 (AM); and 2% aurothioglucone, unbuffered (ATG). An alternative method of applying aurothioglucone was to mix the chromatin with ATG to a final concentration of 2% stain, and place the mixture directly on the grid. An effective stain combination was to add UA, then wash with water before applying PTA as a negative stain. This combination is designated UA-PTA. Grids were examined in a Philips EM420, Siemens 102A or JEOL 1200EX electron microscope and photographs taken at nominal magnifications of ×30 000. For ATG-stained specimens, low-dose techniques were used. Measurements were taken from enlarged prints (×100 000 final magnification) using an ocular micrometer with 0.1 mm graduations and an ocular protractor with 0.5° graduations.

Image processing

Electron-microscope negatives were digitized with a TV camera (Hamamatsu model C2400, Newvicon tube) and Nikon 105 mm f2.8 lens connected to a Megavision 1024X4M image acquisition and display system, effective pixel size 0.55 nm, and analyzed using the SPIDER image processing system (Frank et al. 1981).

Results

Preparation of long chromatin fibers

The first prerequisite for structural studies of isolated chromatin fibers is to develop a reliable method for producing long fibers that have been exposed to as little chemical and physical stress as possible. At all stages of preparation, stirring and vortexing were avoided, and the ionic strength maintained at 80 mM monovalent ions or above. The preparative conditions were divided into three separate phases, nuclear isolation, micrococcal nuclease digestion and chromatin fiber release, and each phase was investigated independently as far as was possible. The effects of different ionic and pH conditions on the released chromatin were also explored. The results, summarized in the following, indicated that specific conditions in each of the phases were critical for obtaining fibers sufficiently long and uniform for ultrastructural studies.

For nuclear isolation, media contained (in addition to approximately 40 mM monovalent ions) the minimal amount of Mg2+ to keep the nuclei intact, and the polyamines spermine and spermidine were avoided. Exposure of nuclei to Mg2+ levels above 2 mM or to
polymamines at the concentrations used by Ruiz-Carrillo et al. (1980) prevented release of digested material even when the nuclei were washed prior to digestion. For nuclease digestion, the buffer contained no Mg²⁺ and was held at pH 8.0 to promote enzyme efficiency (Cuatrecasas et al. 1967) and chromatin relaxation (Labbhart et al. 1981). Preincubation of nuclei at 0°C with enzyme, followed by warming briefly to room temperature, provided a higher yield of long fibers than when the preincubation was omitted. After digestion was stopped with EDTA, release was promoted by holding the nuclei overnight in the cold without agitation. With N. maculosus nuclei, these conditions gave an acceptable yield (~30%) of soluble chromatin containing some very long (>1000 nm) fibers of uniform width, and all micrographs shown here were made from this material.

When chicken erythrocyte nuclei were prepared and processed similarly, it was not possible to release compact fibers longer than about 300 nm (200 nucleosomes) at 80 mM monovalent ionic strength. Attempts to produce longer fibers by reducing the nuclease digestion time, temperature or enzyme concentration resulted only in a decrease in yield, without a concomitant increase in fiber length. It is possible that at 80 mM monovalent ions, sufficient inter-fiber interactions are retained within these nuclei to prevent release of fibers greater than a certain size.

**Fixation and staining**

Released fibers were adjusted to the desired ionic strength to relax or compact the chromatin (see below), and fixed with 0.1% glutaraldehyde overnight on ice (Thoma et al. 1979; Woodcock et al. 1984). In order to identify as far as possible any stain-specific ultrastructural features, a number of negative stains were used (see Materials and methods). All proved satisfactory for fixed chromatin fibers (Fig. 1), but there were differences in the degree to which individual nucleosomes were resolved. The general appearance of the material is similar for all the stains except uranyl acetate (UA), which appears to produce a breakdown from the ends (Fig. 1I). With the other stains, the longer fibers contain irregularities that may be actual breaks, or places where the fiber has been disrupted, and the fragments connected by linker DNA. The sharp bends and folds probably arise during deposition on the carbon film, and suggest that the fixed fibers in solution are very flexible. In a few cases, e.g. Fig. 1B, a ‘branch’ effect, presumably resulting from aggregation, is seen, but these events are rare, and there is no evidence for the side-to-side adhesion or intertwining that has been suggested as the origin of double-helical fibers (Widom and Klug, 1985).

Of all the stains used, aurothioglucose (ATG) gives the clearest visualization of nucleosomes (Fig. 1A,B), and also the most consistent diameter measurements. Since it is a non-ionic sugar derivative, it is likely to have the least impact on the structure of the fibers and, because of this, most measurements were taken from ATG-stained fibers. With the other stains, nucleosomes are seen less frequently. Both methylamine tungstate (MCT) (Fig. 1D, 2B–E) and the uranyl acetate–phosphate tungsate combination (UA–PTA) (Fig. 1C) result in fibers that often appear to be constructed of strands, rather than nucleosomes (Fig. 1B,C, Fig. 2B–E). The strands are interpreted as arrays of nucleosomes within which penetration of stain is restricted. Ammonium molybdate (AM) (Fig. 1E,F) gives the clearest images where the stain layer is quite thin. The contrast change at the edges of fibers, giving the appearance that they are substantially thinner than with the other stains. However, measurements indicate that AM produces only a very small decrease in mean diameter, compared with the other stains. Neutral sodium phosphotungstate (PTA) (Fig. 1G,H; Fig. 2A) yields images with nucleosomes visible in some regions, and strands in others. Its relative insensitivity to beam damage made it the choice for the first tomographic reconstructions (Woodcock and McEwen, 1988a,b; Woodcock and Baumeister, 1990).

**Ionic strength effects**

As previously shown (Thoma et al. 1979; Woodcock et al. 1984), it is possible to produce all degrees of fiber compaction by adjusting the ionic strength at which fixation is carried out. When chromatin released from N. maculosus erythrocyte nuclei was fixed at the ionic strength of the digestion buffer (90 mM monovalent ions) or release buffer (80 mM monovalent ions), fibers of uniform width were observed, and these provided the most informative micrographs (Fig. 1). The level of fiber compaction could be increased by reducing the pH to 6.5 (Labbhart et al. 1981), or by adding Mg²⁺ to 2 mM. These fibers were not penetrated well by the stains, and the treatment was often accompanied by precipitation (Widom, 1986; Koch et al. 1988). Chromatin fibers could also be unfolded to produce randomly arranged strings of nucleosomes with little indication of specific nucleosome–nucleosome interactions by reducing the monovalent salts to 25 mM, or below (not shown). At 50 mM monovalent ions, the chromatin is in the form of partially relaxed fibers that include short regions showing evidence of a two-stranded organization (Fig. 2A–C), especially with MET. Stereo views of these fibers suggest that the strands form a double-helical arrangement. The two lower stereo-pairs (Fig. 2D,E) show MET-stained fibers fixed at 80 mM monovalent ions, at which point the double-stranded nature of the construction is not as evident.

**Occurrence and structure of ordered regions**

Regions of fiber 100 nm to 200 nm in length showing an ordered arrangement of nucleosomes or strands are common in these preparations (Figs 1,2). The more common form of order in compact fibers is a series of oblique cross-striations, clear examples of which are seen in Fig. 1E,F. No evidence for a preferential handedness of the striations was observed in the micrographs, or, as discussed below, in diffraction patterns derived from them. This type of image would be expected of a helical structure in which only the 'top' or 'bottom' were visible. Less commonly seen are fibers in which the nucleosomes (or strands) appear to be arranged in a lattice (Fig. 1B,C, Fig. 2D,E). To quantitate these features, measurements were taken of fiber diameter, and the angle between a striation and a normal to the fiber axis (pitch angle). In cases where a lattice structure was present, the more prominent arrays were chosen as the 'striations'. For example, in Fig. 1B the striations in the region denoted by arrowheads were considered to run from lower left to upper right in the fibers. For ATG-stained fibers, the mean diameter was 33 nm (s.d. 3.5 nm, N=106), and the mean pitch angle 32° (s.d. 8.7°, N=60). The pitch angle is very similar to the 28° (s.d. 9.7°) value obtained from a study of shadowed fibers isolated from chicken erythrocyte nuclei (Woodcock et al. 1984), and the diameter in close agreement with the

**Negative staining of chromatin**

101
values of 30.0 nm (s.d. 3.13 nm) reported by Williams et al. (1986) for UA-stained *N. maculosus* fibers and 32.0 nm (s.d. 3.0 nm) obtained by Athey et al. (1990) for frozen hydrated fibers from the same source.

The standard deviations of fiber diameter and pitch angle indicate a considerable variation in both these parameters. If the chromatin fiber were constructed as an extensible helix, then a relationship between fiber diameter (*D*) and pitch angle (*α*) would be predicted, wider fibers having smaller pitch angles and *vice versa*. As discussed by Woodcock et al. (1984; Appendix), *D* should be proportional to *cos(α)*. This prediction was tested on ATG-stained fibers by measuring pitch angles and fiber diameters at the same sites, and using the paired data

---

**Fig. 1.** Images of negatively stained chromatin fibers from *N. maculosus* erythrocyte nuclei obtained with different negative stains. (A,B) ATG; (C) UA-PTA; (D) MET; (E,F) AM; (G,H) PTA; (I) UA. Opposed arrowheads denote regions where structural order appears to be present. Bar, 50 nm.

102  C. L. Woodcock et al.
(N=54) in a linear regression model. The relationship obtained:

\[ D = 19.5 \text{ nm} + 17.1 \cos(\alpha) \quad (p=0.003, r^2=0.16) \]

shows that there is a significant correlation between fiber diameter and pitch angle, with wider fibers tending to have smaller pitch angles. However, only 16% of the total variation in fiber diameter is explained by the model. Interestingly, the regression analysis predicts a diameter of 19.5 nm when the fiber is fully extended, and no longer a helix (pitch angle 90°). This value is approximately equivalent to two nucleosome diameters.

The type of ordered structure seen in the fibers was analyzed further by computing Fourier transforms and

![Fig. 2. Stereo pairs (angular separation 15°) of selected fibers stained with PTA (A), and MET (B–E). The fibers in A–C were fixed in 50 mM monovalent ions, resulting in partial relaxation. Under these conditions, the fibers appear to be composed of two strands in some regions, forming a double-helical organization (arrowheads). In the more compact fibers in D and E, the stranded effect is still seen, but is less prominent. Bar, 50 nm.](image)

*Negative staining of chromatin* 103
power spectra of selected straight regions. Fig. 3 illustrates the types of pattern obtained from the different stains, with the digitized fiber segments shown as insets. Because of the small number of repeats, and evident disorder in these, consistent features are seen only in the most prominent reflections. Most frequently observed is a one-sided pattern that would be expected from an array of striations (Fig. 3A, D, F). Less common is the 'X' pattern characteristic of a helix, and where this is observed the intensity on one side of the meridian is greater than on the other side (Fig. 3B). Williams et al. (1986) obtained similar patterns with UA-stained N. maculosus fibers, and suggested that they arose from helices in which one side was stained either weakly or not at all. Related, but more complex, patterns are shown in Fig. 3C, E.

The apparent layer lines on which the strongest reflections are located are equivalent to real-space distances of 11 nm to 18 nm (Fig. 3). Examination of the original micrographs shows that these values correspond to the center-to-center distances between striations. The variation in striation spacing was to some extent related to the stain used. AM and UA-PTA tended to give shorter spacings in the 11 nm to 14 nm range, while ATG and MET produced the longer spacings (Fig. 3). These values are similar to the spacings of 12.6 nm and 16.9 nm reported by Williams et al. (1986) for UA-stained fibers from N. maculosus. The angles of the strongest reflections with respect to the meridian ranged from 25° to 40°, in accordance with the spread of pitch angles, and the findings of Williams et al. (1986).

Discussion

In order to study the ultrastructure of chromatin fibers it is important to obtain specimens long enough to examine long-range structural properties and minimize end effects. In previous work using chicken erythrocyte chromatin, isolated fibers had a maximal length of approximately 300 nm when compact (Woodcock et al. 1984), and efforts to increase this by manipulating the isolation conditions have not been successful. The observation by Williams et al. (1986) that fibers from N. maculosus erythrocytes were well-ordered, stimulated us to investigate this source in some detail. We find that an additional advantage of this material is that long chromatin fibers may be readily obtained without exposure to destabilizing ionic conditions (Fig. 1).

Like their well-studied avian counterparts, the nucleated erythrocytes of Necturus maculosus are terminally differentiated cells with quiescent nuclei. In thin sections, the nuclei are seen to be uniformly packed with 30 nm chromatin fibers (not shown), and examination of the protein content of the nuclei (P.J. Giannasca and C.L. Woodcock, unpublished) reveals a low content of non-histone proteins, also comparable to the situation in chicken erythrocyte nuclei (LaFond and Woodcock, 1983). However, N. maculosus erythrocyte nuclei contain a single very lysine-rich histone H1, in contrast to the three (H1A, H1B, H5) found in chicken erythrocyte nuclei, and it is this simplicity that may account for the more ordered state of the chromatin. When electrophoresed in polyacrylamide gels containing sodium dodecyl sulfate, the single H1 of N. maculosus migrates between the H1A and H1B of the chicken erythrocyte (not shown). N. maculosus has a 49 bp (base-pair) DNA linker length (Williams et al. 1986), compared with the 66 bp value for the chicken erythrocyte.

Structure of ordered regions

Regions of fiber showing an ordered ultrastructure (Figs 1, 2) all have motifs consistent with an underlying helical organization. When partially relaxed in 50 mM monovalent ions, a two-stranded, two-start helical arrangement is suggested, and the depth information provided by stereo-pairs further supports this interpretation (Fig. 2A–C). In the more compact fibers, a two-start arrangement may be inferred from measurements of pitch angle and the spacing between striations. As discussed by Woodcock et al. (1984), a simple solenoidal arrangement of nucleosomes would require a pitch angle of about 16°, but a two-start helix necessitates a larger pitch angle, consistent with the 32° observed using ATG.

Diffraction patterns consistent with a partially stained helix are commonly obtained from ordered regions of fiber (Fig. 3), although there is considerable between- and within-stain variability. In the light of tomographic reconstructions of negatively stained fibers (Woodcock et al. 1991), differential staining, probably related to the adhesion process, plays a large role in the final image. As seen in Fig. 1, ordered regions of fiber were generally

Fig. 3. Computed power spectra obtained from selected areas of chromatin fiber stained with: (A) ATG; (B) UA-PTA (from Fig. 1C); (C) MET (from Fig. 2E); (D, E) AM (from Fig. 1E); (F) PTA. Apparent layer lines containing the strongest reflections are denoted by arrowheads, and the adjacent numbers refer to the corresponding distance in real space. The insets show the digitized fiber images. The full width of each inset corresponds to 40 nm (72 pixels).

104  C. L. Woodcock et al.
Effect of different stains between nucleosomes (Fig. 1; Fig. 2B-D). Our results with MET differ from those of Lawrence consistently takes the form of oblique cross-striations. We ultrastructural studies (Woodcock preservation method. The opposite argument would be that fibers are fundamentally disordered, but that ordered regions will occur at random. Our data support the former interpretation. As the preparative conditions are altered to minimize the exposure of chromatin to potentially disruptive conditions, so ordered regions are observed in greater abundance. Also, the regular internal structure consistently takes the form of oblique cross-striations. We infer that this motif is derived from the tendency of nucleosomes to be arranged in the 30 nm fiber in a specific way.

Effect of different stains

The different stains used in this study each produced a characteristic fiber image. ATG, which has not previously been employed for chromatin, gave the most consistent fiber diameter and clearest visualization of individual nucleosomes (Fig. 1A,B). Its use, however, is restricted by its extreme beam sensitivity. UA also allowed individual nucleosomes to be seen (Fig. 1I), but in our hands was clearly disruptive; a more thorough crosslinking of the chromatin with glutaraldehyde may be necessary. MET, developed as a stain with both hydrophilic and hydrophobic components (Faberge and Oliver, 1974), had the interesting property of emphasizing arrays of nucleosomes, perhaps being excluded from the contact region between nucleosomes (Fig. 1D; Fig. 2B-D). Our results with MET differ from those of Lawrence et al. (1989) with formaldehyde-fixed sea-urchin (Parechinus angulosus) sperm chromatin, deposited on Alcian Blue-treated carbon films. Their images obtained with MET are most similar in terms of texture and nucleosome visibility to those obtained with PTA (Fig. 1G,H) in this study. With AM (Fig. 1E,F), long fibers were well preserved, and oblique striations prominent in ordered regions.

Origin of ordered structures

Three lines of evidence point to a helical organization in the ordered regions of chromatin fibers: the stereo micrographs of partially relaxed fibers (Fig. 2A-C), the consistent measurements of pitch angle on isolated fibers both from N. macroclusus and chicken erythrocyte nuclei (Woodcock et al. 1984), and from diffraction patterns (Fig. 3; Williams et al. 1986; Atthey et al. 1987). Thus, one interpretation of the data presented here, and in previous ultrastructural studies (Woodcock et al. 1984; Williams et al. 1986), is that the cross-striations and strands represent helical gyres composed of nucleosomes in a face-to-face arrangement. This is consistent with data on fibers in solution indicating that the nucleosomes are oriented obliquely with respect to the fiber axis with the planes of the nucleosomal discs radially arranged (McGhee et al. 1980, 1983; Mitra et al. 1984; Sen et al. 1986).

The weight of evidence from this and similar ultrastructural studies (Woodcock et al. 1984; Williams et al. 1986) favors a two-start helical organization for the chromatin fiber. However, as demonstrated by Woodcock and Baumgeister (1990), the extrapolation of two-dimensional projection images to the third dimension must be done with caution. This principle is exemplified by the results of tomographic reconstructions carried out on selected fibers, and described in the accompanying article (Woodcock et al. 1991). While the tomographic results do not exclude the interpretation discussed above, they do demonstrate that the negative staining procedure itself has a powerful influence on the observed structure of chromatin fibers.

We thank Dr Wolfgang Baumeister for the generous use of his laboratory facilities for part of this study. This work was supported by grants NSF DCD 85-13388, BBS 8714235 and NIH GM 43786.

References


(Received 8 October 1990 — Accepted, in revised form, 28 January 1991)