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Higher-order structure of long repeat chromatin

Jonathan Widom[1], John T.Finch and Jean O.Thomas[2]

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, and [2]Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

[1]Present address: Departments of Chemistry and Biochemistry, University of Illinois, Urbana, IL 61801, USA

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The higher-order structure of chromatin isolated from sea urchin sperm, which has a long nucleosomal DNA repeat length (~240 bp), has been studied by electron microscopy and X-ray diffraction. Electron micrographs show that this chromatin forms 300 Å filaments which are indistinguishable from those of chicken erythrocytes (~212 bp repeat); X-ray diffraction patterns from partially oriented samples show that the edge-to-edge packing of nucleosomes in the direction of the 300 Å filament axis, and the radial disposition of nucleosomes around it, are both similar to those of the chicken erythrocyte 300 Å filament, which is described by the solenoid model. The invariance of the structure with increased linker DNA length is inconsistent with many other models proposed for the 300 Å filament and, furthermore, means that the linker DNA must be bent. The low-angle X-ray scattering in the 300– 400 Å region both *in vitro* and *in vivo* differs from that of chicken erythrocyte chromatin. The nature of the difference suggests that 300 Å filaments in sea urchin sperm *in vivo* are packed so tightly together that electron-density contrast between individual filaments is lost; this is consistent with electron micrographs of the chromatin *in vitro*.

Key words: sea urchin sperm chromatin/X-ray diffraction/electron microscopy/300 Å filaments/solenoid model

Introduction

Throughout much of the cell cycle, the nucleosomes of eukaryotic chromatin are organized in a 'higher-order structure', a filament with a diameter of ~300 Å. Finch and Klug (1976) proposed a model for the 300 Å filament in which a chain of nucleosomes is wound in a contact helix (or 'solenoid'), with ~6 nucleosomes per turn. Although several lines of evidence are consistent with the solenoid model (e.g., McGhee *et al.*, 1983; Mitra *et al.*, 1984; Suau *et al.*, 1979), support for it is not universal, and it has also been questioned (e.g., Zentgraf and Franke, 1984) whether a single higher-order structure exists for chromatin from all cell types, in which the average DNA repeat length may vary over a range of ~80 bp (Kornberg, 1977).

A recent X-ray diffraction study on partially oriented samples of chicken erythrocyte 300 Å filaments (Widom and Klug, 1985) shows diffraction patterns that are consistent with the solenoid model and inconsistent with many other models. However, these diffraction patterns only specify the packing of nucleosomes and not their connectivity. Other models can be constructed which are consistent with these patterns, yet differ in their DNA linker paths from the solenoid, in that laterally neighbouring nucleosomes come from non-consecutive locations along the DNA (e.g., Woodcock *et al.*, 1984).

Different models of higher-order structure imply different effects of a changed linker length. We have now carried out electron microscopy and X-ray diffraction experiments to study the higher-order structure of sea urchin sperm chromatin, which has the longest reported repeat length, ~240 bp (Spadafora *et al.*, 1976). By comparing the present results with those for 300 Å filaments from chicken erythrocytes (repeat length ~212 bp; Morris, 1976) described in our previous X-ray diffraction and electron microscope studies (Widom and Klug, 1985; J.Widom, in preparation), we determine the effects on higher-order structure due to the addition of 28 bp (~100 Å) of linker DNA. The results of this analysis allow us to rule out some further models for higher-order structure and to make definite statements concerning the disposition of linker DNA.

Results

Biochemical characterization of sea urchin sperm chromatin

Figure 1 shows histone compositions and micrococcal nuclease digestion patterns of sperm nuclei from the two species of sea urchin, *Psammechinus miliaris* and *Echinus esculentis*, together with those of rat liver nuclei for comparison.

The DNA repeat lengths of chromatin from the two species of sea urchin, as determined by micrococcal nuclease digestion, are identical (Figure 1B) and considerably longer than that of rat liver chromatin. The repeat length has been measured elsewhere for *E. esculentis* sperm as 240 bp (Thomas *et al.*, 1986), in good agreement with earlier values for other sea urchin species (Spadafora *et al.*, 1976; Zalenskaya *et al.*, 1981; McGhee *et al.*, 1983).

Three of the four core histones (H3, H4 and H2A) from sea urchins and rat liver have very similar electrophoretic mobilities in SDS-polyacrylamide gels (Figure 1A). Differences are, however, apparent in H2B and H1 (cf. von Holt *et al.*, 1984) even for the two species of sea urchin, despite their identical DNA repeat lengths. As is now well established, the H2B of sea urchin sperm is larger than that of somatic histones due (in *Parechinus angulosus*) to a basic N-terminal extension of ~25 amino acid residues (von Holt *et al.*, 1984 and references therein). The H2B of *P. miliaris* has two components resolved by SDS gel electrophoresis – a minor one which migrates with the H2B of *E. esculentis* and a major component which migrates slightly slower and is probably larger. The H1 histones in the two species of sea urchin, which migrate as a single component, unlike rat liver H1, are also discernibly different; in this case, that of *E. esculentis* is the larger, as judged by electrophoretic mobility (cf. *P. angulosus* H1, von Holt *et al.*, 1984). The inverse relationship between the H2B size and the H1 size apparent from a comparison of the two species may be purely coincidental.

As noted elsewhere (Thomas *et al.*, 1986), the solubility properties of sea urchin sperm chromatin prepared by micrococcal

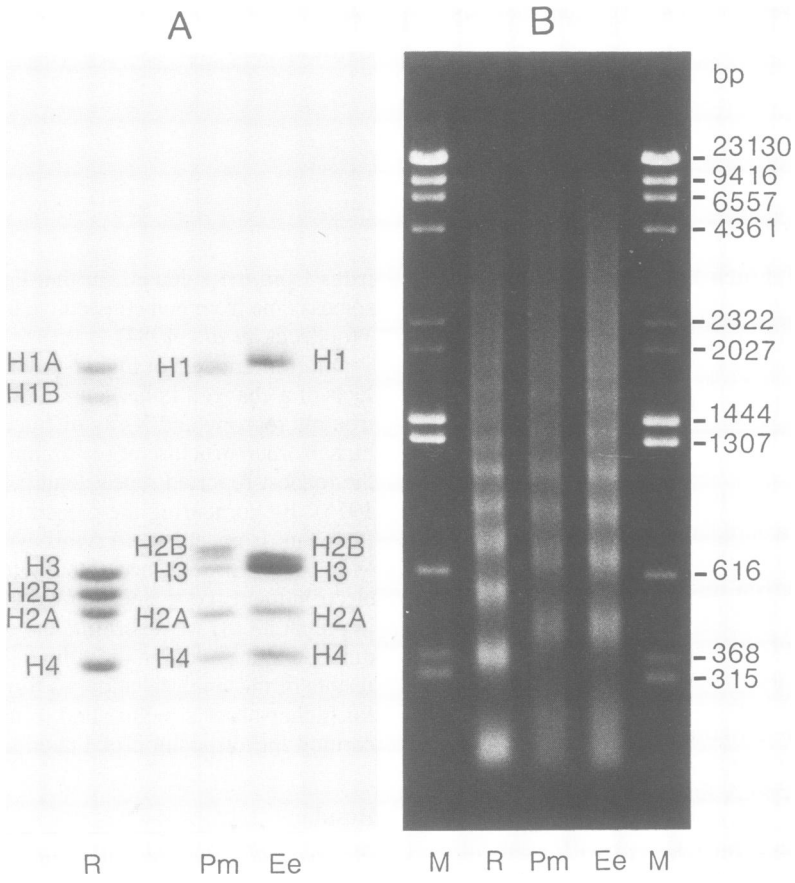

Fig. 1. Histone content and DNA repeat length of nuclei from sea urchin sperm compared with those from rat liver. **(A)** SDS/18%-polyacrylamide gel electrophoresis of histones from *P. miliaris* (Pm) and *E. esculentis* (Ee) sperm nuclei, and rat liver nuclei (R). Note the doublet H2B band in *P. miliaris*. **(B)** 1.25% agarose gel electrophoresis of DNA from micrococcal nuclease-digested sea urchin sperm nuclei (Pm and Ee) compared with that from rat liver nuclei (R). M is a mixture of *Hind*III restriction fragments of bacteriophage λ DNA and *Taq*I fragments of pBR322. Sizes are in bp.

nuclease digestion differ from those of, e.g., rat liver chromatin. Sperm chromatin becomes insoluble at ~70 mM ionic strength, even at low concentrations, whereas rat liver and chicken erythrocyte chromatin of similar length are soluble at the same concentrations. Solubility differences are also observed at much higher salt concentrations. For example, in 0.55 M NaCl rat liver chromatin is completely soluble, and the H1 dissociated, whereas sea urchin sperm chromatin is insoluble at this ionic strength and the H1 largely undissociated (data not shown).

Electron microscopy of sea urchin sperm chromatin

Chromatin prepared by digestion of nuclei with micrococcal nuclease followed by hypotonic lysis in 0.2 mM EDTA (Noll *et al.*, 1975) is released as an extended nucleosome filament (Finch and Klug, 1976) or zig-zag chain of nucleosomes (Thoma *et al.*, 1979) which can be induced to fold into 300 Å filaments by addition of Na^+ ions (Thoma *et al.*, 1979), or much lower concentrations of Mg^{2+} ions (Finch and Klug, 1976) or $Co(NH_3)_6^{3+}$ ions (J.Widom, in preparation). Many physical studies of chromatin folding by different techniques have used this approach (e.g., Butler and Thomas, 1980; Thomas and Butler, 1980; McGhee *et al.*, 1980; Bates *et al.*, 1981; Pearson *et al.*, 1984; Mitra *et al.*, 1984; Ausio *et al.*, 1984; Allan *et al.*, 1984; Widom and Klug, 1985) on chromatins with repeat lengths covering the range 165–212 bp.

Sea urchin sperm chromatin can also be released in the unfolded state on hypotonic lysis of nuclei (albeit with rather lower yield than for the other chromatins previously studied). Here we have compared the higher-order structure of this chromatin obtained after cation-induced refolding with that of chicken erythrocyte chromatin. This study complements the recent hydrodynamic study of Thomas *et al.* (1986) on Na^+-refolded sea urchin sperm chromatin.

Parallel samples of refolded chicken erythrocyte and sea urchin sperm chromatin were prepared; for each sample, a series of micrographs was taken in which the grids were tilted at various angles from −48° to +48° about an axis so that any flattening of specimens could be assessed. The results of one such experiment are shown in Figure 2. The sea urchin sperm chromatin (Figure 2, bottom) forms filaments with a diameter of ~300 Å which are no wider than those of chicken erythrocyte chromatin under the same conditions (Figure 2, top); indeed, the two are indistinguishable, apart from a greater tendency of the sea urchin sperm filaments to aggregate. As the specimens are tilted, the filament width decreases, indicating that the specimens are slightly flattened; the extent of flattening is similar in the two samples. In other experiments (not shown) we find that many cations, including Na^+, Mg^{2+} and $Co(NH_3)_6^{3+}$, will induce the folding of sea urchin sperm chromatin into 300 Å filaments (as judged by electron microscopy), as found previously for chicken erythrocyte chromatin (J.Widom, in preparation).

It should be noted that the chromatin in Figure 2 was not fixed before negative staining. Fixation was omitted because, due to the tendency of sea urchin sperm 300 Å filaments to aggregate,

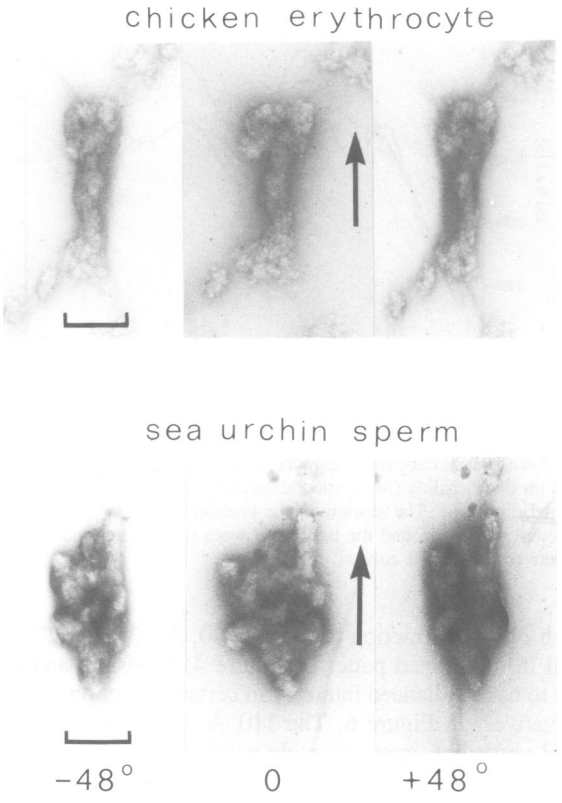

Fig. 2. Electron microscope tilt series. Chicken erythrocyte chromatin (top) and sea urchin (*E. esculentis*) sperm chromatin (bottom) in 10 mM Tris-HCl pH 7.5 + 50 μM $Co(NH_3)_6^{3+}$. Micrographs were taken at tilt angles of −48°, 0° and +48°. Bar = 1000 Å.

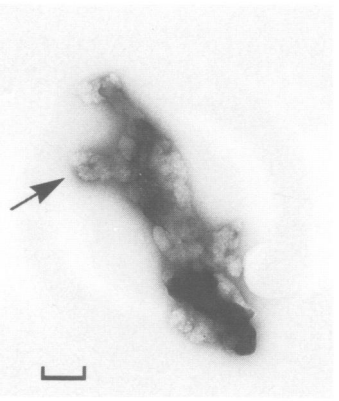

Fig. 3. Aggregate of sea urchin (*E. esculentis*) sperm chromatin showing twisted loop of 300 Å filament (arrow). Same conditions as in Figure 2. Bar = 1000 Å.

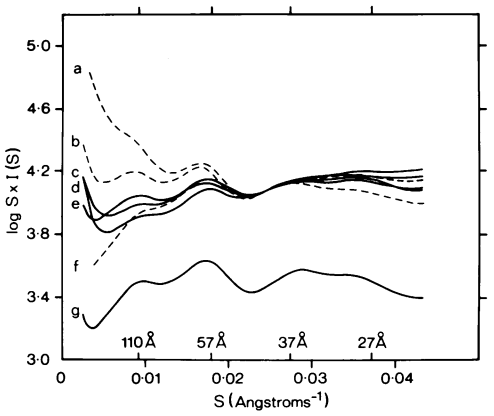

Fig. 4. Low-angle X-ray diffraction patterns of 300 Å filaments from chicken erythrocytes (dashed lines) and sea urchin sperm (solid lines). Solution conditions: **(a)** 50 μM $Co(NH_3)_6^{3+}$ (total) + 20 mM Na^+ + 5 mM Tris-HCl pH 7.5; **(b)** 50 μM $Co(NH_3)_6^{3+}$ + 20 mM Na^+ + 1 mM Tris-HCl pH 7.5; **(c)** *E. esculentis* chromatin, 50 μM $Co(NH_3)_6^{3+}$ + 20 mM Na^+ + 1 mM Tris-HCl pH 7.5; **(d)** *P. miliaris* chromatin, 50 μM $Co(NH_3)_6^{3+}$ + 25 mM Na^+ + 1 mM Tris-HCl pH 7.5; **(e)** *P. miliaris* chromatin, 50 μM $Co(NH_3)_6^{3+}$ + 35 mM Na^+ + 1 mM Tris-HCl pH 7.5; **(f)** 200 μM $Co(NH_3)_6^{3+}$ (total) + 2.5 mM Tris-HCl pH 7.5; **(g)** *P. miliaris* chromatin, 10 mM $MgCl_2$ + 1 mM Na_3EDTA + 10 mM Tris-HCl pH 7.5. X-ray films were densitometered and radially averaged (the shadow of the beamstop is masked out, and thereby omitted from the radial average). Background is low, but has not been subtracted. Curves **(a–f)** are normalized for intensity of scattering in the 37 Å region (which is due to scattering internal to nucleosome cores). Vertical placement is arbitrary.

it became impossible to see individual filaments. A consequence of omitting fixation is that 300 Å filaments occasionally unfold into 'puddles' of nucleosomes, as can be seen for the chicken erythrocyte chromatin in Figure 2 (top). This has been noticed previously by Sperling (1977) and is discussed elsewhere (J. Widom, in preparation).

The noticeably greater tendency to aggregate, which occurs regardless of which cation is used to induce folding, is also observed in a recent hydrodynamic study of the Na^+-induced folding of sea urchin sperm chromatin (Thomas *et al.*, 1986). 300 Å filaments in these aggregates often pack so tightly together that it is difficult to distinguish individual filaments. Examples of such aggregates can be seen in Figures 2 and 3. The aggregate in Figure 3 has an interesting feature (arrow) in which a long 300 Å filament appears to loop back and twist around itself. Structures of this type are characteristic of the folding of 300 Å filaments within metaphase chromosomes (Marsden and Laemmli, 1979), as pointed out for chicken erythrocyte chromatin (Widom and Klug, 1985).

X-ray diffraction from sea urchin sperm 300 Å filaments

The similarity in diameter of 300 Å filaments from sea urchin sperm and chicken erythrocytes could arise either by chance or because of similarities in nucleosome packing. To determine what effects, if any, the additional 100 Å of linker DNA would have on the basic arrangements of nucleosomes within the higher-order structure, we have carried out a series of low angle X-ray scattering experiments with sea urchin sperm chromatin, under various different conditions for which the chromatin was found by electron microscopy to be folded into 300 Å filaments. Experiments were carried out in which $Co(NH_3)_6^{3+}$ was used to induce chromatin folding in the presence of three different concentrations of Na^+, and an additional experiment was carried out in which Mg^{2+} was used to induce folding. The results are shown in Figure 4 together with a series of curves from chicken erythrocyte 300 Å filaments in a similar range of Na^+ and $Co(NH_3)_6^{3+}$ concentrations studied previously (J.Widom, in preparation).

The range of ionic conditions produces a range of X-ray patterns which are generally similar to those from chicken erythrocyte 300 Å filaments (dashed lines) but differ in certain respects. These differences are due to changes in the extent of aggregation or packing of 300 Å filaments and in the degree of intramolecular disorder; but each curve shows the bands at 110 and 57 Å which are due, respectively, to the edge-to-edge packing of nucleosomes in the direction of the 300 Å filament and the radial disposition

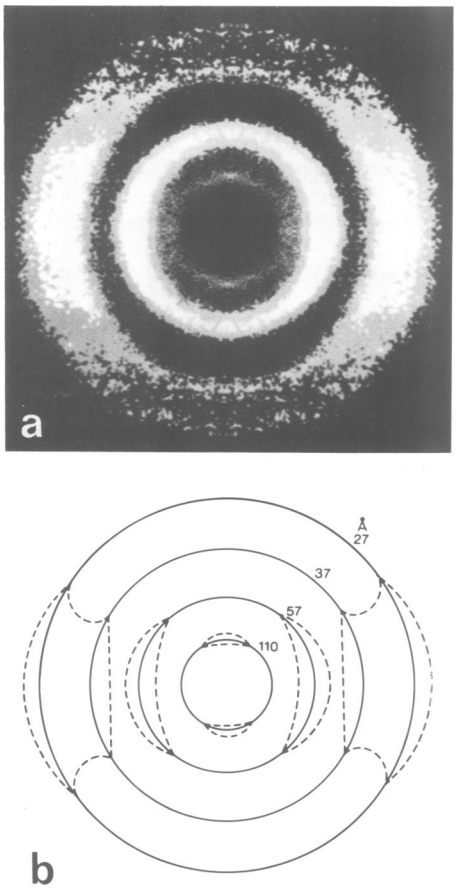

Fig. 5. Diffraction pattern from partially oriented sea urchin sperm 300 Å filaments (*P. miliaris*). The capillary direction is vertical; solution conditions as in Figure 4(g). **(a)** Computer graphics display: the X-ray film was densitometered, mirror-mirror symmetry was imposed, log SI(S) was computed for every point, and the results scaled from black to white with increasing SI(S) (S is the inverse of the Bragg spacing). **(b)** Schematic drawing of the same film, illustrating regions having enhanced intensity; scale as in **(a)**.

around it (Widom and Klug, 1985). Importantly, the curves for sea urchin sperm 300 Å filaments show no new bands between 110 and 400 Å whether the 300 Å filaments are induced with $Co(NH_3)_6^{3+}$ or Mg^{2+}.

X-ray diffraction from partially oriented specimens

X-ray patterns of sea urchin sperm 300 Å filaments often show slight but detectable orientation similar to that from chicken erythrocyte filaments; the orientation is not as good as that obtained with erythrocyte filaments, possibly because of shorter lengths of chromatin being used here, or because of a more 'twisty' character to the aggregates. However, even these poorly orientated samples provide valuable structural information.

X-ray diffraction patterns from such specimens recorded on films (not shown) consist of a set of concentric rings corresponding to each of the bands in Figure 4, which have intensities just perceptibly enhanced in certain directions. The contrast in films such as these can be brought out by computer processing to allow easier visualization of any intensity differences within diffraction bands. An example of such a pattern is shown in Figure 5a; Figure 5b indicates schematically the full set of observed diffraction bands and those regions of the films having enhanced intensities. The capillary direction (the preferred 300 Å filament axis direction) is vertical in these pictures.

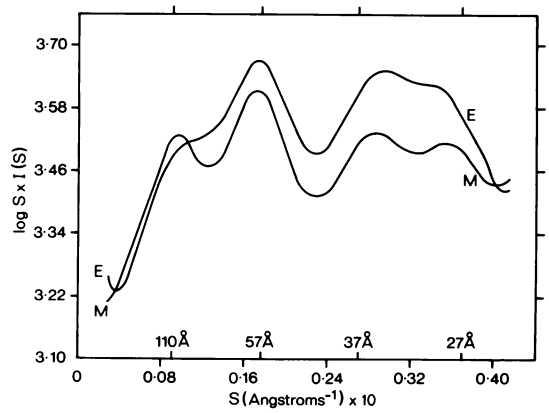

Fig. 6. Quantitative plot for oriented diffraction pattern. The X-ray film of Figure 5 was densitometered and radially averaged in 30° wide sectors centred on the meridian (M, vertical direction) or on the equator (E, horizontal direction). The shadow of the beamstop and any traces of background scatter (around the beamstop) were masked out and so do not contribute to the plotted curves.

Each of the diffraction bands (at 100, 57, 37 and 27 Å) observed in unoriented patterns (Figure 4) is visible and each is found to have enhanced intensity in certain directions, as shown quantitatively in Figure 6. The 110 Å band is meridional (enhanced vertically, approximately parallel to the capillary axis); bands at 57, 37 and 27 Å are equatorial (enhanced horizontally, approximately perpendicular to the capillary axis). Equatorially enhanced intensity extends inwards from a peak at 57 Å towards 100 Å, as found previously for chicken erythrocyte filaments (Widom and Klug, 1985). No additional low angle bands are seen on either the meridian or the equator. The orientation of the bands at 100, 57, 37 and 27 Å relative to the capillary axis is the same as found previously for chicken erythrocyte filaments, which also show a 340 Å equatorial band due to the side-by-side packing of 300 Å filaments (Widom and Klug, 1985). This means that 300 Å filaments from sea urchin sperm tend to lie with the (long) filament axis parallel to the capillary, as expected.

Discussion

Solenoid model for sea urchin sperm chromatin in vitro

We have shown by electron microscopy that sea urchin sperm chromatin will fold *in vitro* into 300 Å filaments which appear similar to those from chicken erythrocytes in the same conditions, suggesting that the packing of nucleosomes within these 300 Å filaments might be similar. This hypothesis is supported by the similarity of the oriented X-ray diffraction bands from these two chromatins, and by the absence of any new diffraction bands from sea urchin sperm 300 Å filaments which might arise from a different packing of nucleosomes. Therefore we conclude that the packing of nucleosomes within sea urchin sperm 300 Å filaments resembles that in chicken erythrocytes, and is described by the solenoid model (Finch and Klug, 1976; Widom and Klug, 1985). This is consistent with the conclusion reached previously by McGhee *et al.* (1983) on the basis of optical studies of chromatin oriented in an electric field, and with the conclusions of a recent hydrodynamic study (Thomas *et al.*, 1986).

The patterns for chicken erythrocyte and sea urchin sperm 300 Å filaments differ in just one important respect: unlike chicken erythrocyte 300 Å filaments, those from sea urchin sperm lack the equatorial diffraction band at 340 Å (which in chicken ery-

throcyte chromatin is due to the side-by-side packing of 300 Å filaments) and generally have very low scattering intensity in the 300−400 Å region. This difference is observed for these same chromatins *in vivo* (see below; Langmore and Paulson, 1983). An explanation is suggested by our electron micrographs (Figures 2 and 3) which show that sea urchin sperm 300 Å filaments form tight aggregates in which contrast between individual 300 Å filaments is reduced or lost. The lack of electron density contrast on a 300−400 Å size scale, as judged by electron microscopy, is expected to reduce the X-ray scattering intensity in the corresponding region, so that no peak will be visible. Similar behaviour can also be induced with chicken erythrocyte chromatin by addition of sufficient amounts of Mg^{2+} or $Co(NH_3)_6^{3+}$; this causes individual filaments to pack tightly together so that the 340 Å X-ray band disappears (J.Widom, in preparation).

Relation to the structure in vivo

It is important to ask whether the model we arrive at for the structure of sea urchin sperm chromatin *in vitro* also applies to the structure *in vivo*. The chromatin we prepare is initially unfolded, and it is possible that the higher-order structure formed after refolding might differ from that *in vivo*, e.g., as claimed by Zentgraf and Franke (1984). However, the similarity between the present X-ray diffraction patterns from sea urchin sperm chromatin *in vitro* and those for the same chromatin *in vivo* (Langmore and Paulson, 1983) suggests that the structures may be the same, and this hypothesis is reinforced by the similarity of the structure of sea urchin sperm 300 Å filaments to those from chicken erythrocytes, as judged by the oriented X-ray diffraction patterns and electron microscopy.

The general question of whether unfolding and subsequent refolding has any effect on the higher-order structure has been answered in earlier studies with rat liver and chicken erythrocyte chromatins. Sedimentation analysis showed no difference in the folding behaviour of rat liver chromatin released in the folded state by lysis of nuclei at ~65 mM ionic strength and chromatin released in the extended state by hypotonic lysis (Butler and Thomas, 1980); similarly, electron microscopy showed no difference in the appearance of 300 Å filaments released in folded form in the presence of Mg^{2+} and those refolded with Mg^{2+} from an extended state (D.Rhodes and J.T.Finch, unpublished data). For chicken erythrocyte 300 Å filaments the X-ray diffraction pattern of the refolded chromatin *in vitro* (Widom and Klug, 1985) is consistent with that obtained from whole chicken erythrocytes (i.e., *in vivo*) or from isolated nuclei (Langmore and Schutt, 1980; Langmore and Paulson, 1983). These patterns are interpreted in terms of the side-by-side packing of solenoids of nucleosomes, which is consistent with electron micrographs of thin sections through chicken erythrocyte nuclei showing densely staining objects with a centre-to-centre distance of 280 Å (Walmsley and Davies, 1975).

We therefore conclude that for rat liver and chicken erythrocyte chromatins, and now also for sea urchin sperm chromatin, the higher-order structure formed by refolding chromatin *in vitro* resembles that which exists *in vivo*, and this is likely to hold for other chromatins as well. This is inconsistent with a previous interpretation of electron micrographs of thin sections cut through sea urchin sperm heads in which densely staining objects with a diameter of 370 Å were observed (Zentgraf and Franke, 1984). These were interpreted as individual filaments and, taking into account the estimated shrinkage due to preparation for microscopy, the filament diameter *in vivo* was estimated as 460 Å, significantly greater than the 300 Å that we observe.

An alternative interpretation of these electron micrographs is suggested by the X-ray diffraction patterns from sea urchin sperm chromatin *in vitro* and *in vivo*, which show no band at 340 Å and have generally reduced 300−400 Å scattering intensity relative to chicken erythrocyte chromatin. This suggests that 300 Å filaments in sea urchin sperm heads *in vivo* do not exist as individual filaments but may be tightly packed in bundles of two or more, perhaps twisted around each other as seen for the chromatin *in vitro* (Figure 3). The failure to resolve substructure in the thicker filaments observed in thin sections is not surprising, given that X-rays indicate little or no electron-density contrast between 300 Å filaments *in vivo*, and that these tightly aggregated filaments are further compacted by a factor of 1.5 in cross-sectional area during preparation for microscopy [calculated from the shrinkage reported by Zentgraf and Franke (1984)].

Several alternative models have been proposed for the structure of the 300 Å filament, including one in which the chromatin is proposed to consist of a linear assembly of globular clusters of nucleosomes, termed 'superbeads' (e.g., Zentgraf and Franke, 1984, and references therein). Superbeads isolated from different cell types are reported to have significantly different diameters, which are broadly related to the different filament diameters deduced from thin sections of nuclei, and to contain significantly different numbers of nucleosomes; for chicken erythrocyte chromatin the particles have a diameter of 350 Å and contain an average of 20 nucleosomes, whereas for sea urchin sperm the particles are 480 Å in diameter and contain an average of 48 nucleosomes (Zentgraf and Franke, 1984).

In contrast to this, we find that the two chromatins are strikingly similar: both give long filaments which have a similar diameter (~300 Å), and both 300 Å filaments have a similar internal arrangement of nucleosomes, which is described by the solenoid model. We suspect that superbeads may not be *bona fide* units of higher-order structure. There are several possible reasons for their possible existence in micrococcal nuclease digests of nuclei (see also review by Butler, 1983). For example, they might simply be fragments of solenoid, the fragment size being proportional to the stability of the interactions between turns of the solenoid as suggested elsewhere (Thomas *et al.*, 1986). Alternatively, they might result from a redistribution of H1 (or H5 in the case of chicken erythrocyte nuclei) during micrococcal nuclease digestion, a process known to give bead-like aggregates in some cases (Strätling and Klingholz, 1981; Thomas *et al.*, 1985).

Bent linker DNA

There is no hard evidence in the literature bearing on the question of whether linker DNA is straight or bent. This question is of interest because bent linker DNA is implicit in many models for 300 Å filament structure − e.g., in the solenoid model − (cf. Butler, 1984). The studies that have provided the most information to date on linker DNA conformation are electric and photochemical dichroism studies (McGhee *et al.*, 1980, 1983; Yabuki *et al.*, 1982; Mitra *et al.*, 1984) which place constraints on possible linker DNA trajectories but cannot specify if the linker is straight or bent.

Our electron micrographs show that the additional 100 Å of linker DNA in sea urchin sperm chromatin, as compared with chicken erythrocyte chromatin, has little or no effect on the diameter of the 300 Å filament. This is consistent with the similarity of the equatorial X-ray diffraction, from the peak at 57 Å inwards towards 100 Å, for sea urchin and chicken erythrocyte 300 Å filaments, which implies that the two higher-

order structures have a similar radial disposition of nucleosomes. Furthermore, the conservation of the meridional 110 Å diffraction peak and the absence of other low-angle bands (on or off the meridian) in patterns from sea urchin sperm 300 Å filaments implies that the two chromatins have a similar edge-to-edge packing of nucleosomes in the direction of the 300 Å filament axis.

These studies, taken together, monitor all of the possible directions in which nucleosomes might move within 300 Å filaments to accommodate an additional 100 Å of straight linker DNA, yet no changes are observed. Thus the linker DNA is not contributing significantly as a structural component to the 300 Å filament, and must be bent and looped clear of the contacts between nucleosomes. The mechanism of this bending is not known; it is possible that bending is facilitated by interaction with H1 and/or one or more core histone domains.

These results rule out many previously proposed models for the structure of the 300 Å filament. For example, models based on a zig-zag chain of nucleosomes proposed by Worcel *et al.* (1981) and Woodcock *et al.* (1984) are incompatible with our results because they predict that the diameter and the low angle (110–300 Å) meridional X-ray diffraction should change significantly as the linker length is increased, contrary to observation.

Materials and methods

Isolation of nuclei and chromatin

Both species of sea urchin were obtained from the University Marine Biological Station, Millport, Isle of Cumbrie, UK. Spermatozoa were collected and stored, and nuclei prepared from them exactly as described elsewhere (Thomas *et al.*, 1986).

Sperm nuclei in the 0.34 M sucrose buffer A of Hewish and Burgoyne (1973) were adjusted to $A_{260}=100$ (read in 1 M NaOH); it was necessary to incubate the nuclei with a small amount of micrococcal nuclease and 1 mM $CaCl_2$ before taking this reading, since a turbid solution was otherwise obtained, either because the undigested chromatin is insoluble or because the undigested nuclei do not lyse. To obtain long chromatin (weight-average size in the range 55–80 nucleosomes), the nuclei were digested with micrococcal nuclease (15 U/ml) in the presence of 1 mM $CaCl_2$ for 2 min at 37°C, and the pelleted nuclei allowed to lyse for at least 2 h at 4°C in 10 mM Tris-HCl, 0.2 mM Na_2EDTA, 0.25 mM phenylmethylsulphonyl fluoride, pH 7.5, to give soluble chromatin. The integrity of the histones was checked by electrophoresis in SDS/18%-polyacrylamide gels (Thomas and Kornberg, 1978, with modifications described by Thomas *et al.*, 1986).

Determination of nucleosomal DNA repeat length

This was carried out exactly as described elsewhere for *E. esculentis* sperm nuclei (Thomas *et al.*, 1986).

X-ray diffraction

Chromatin from either species of sea urchin was diluted to 100 μg/ml and dialyzed into buffers containing various concentrations of Na^+, $Co(NH_3)_6^{3+}$ and Mg^{2+}. Chromatin from chicken erythrocytes (prepared as described elsewhere, J.Widom, in preparation) at 100 μg/ml was dialyzed as for sea urchin chromatin, except for some cases in which Na^+ and $Co(NH_3)_6^{3+}$ were added directly. For samples prepared by the latter method, the activity of $Co(NH_3)_6^{3+}$ may be slightly less than the total concentration due to binding to the chromatin; in this case, the total (i.e., not the 'free') concentration is given, and this is stated. For all samples, the chromatin formed 300 Å filaments, which subsequently aggregated (cf. J. Widom, in preparation) over a period of minutes to hours. These aggregates were collected by low-speed centrifugation and loaded into thin glass capillaries for X-ray diffraction. The X-ray camera is described in detail elsewhere (J.Widom, in preparation). It combines good resolution (800 Å horizontally and 500 Å vertically) with a very low background.

Electron microscopy

Samples of chromatin at 5–10 μg/ml were rapidly adjusted to various ionic conditions and adsorbed unfixed to carbon grids, then negatively stained with uranyl acetate (1% w/v). This procedure minimized the aggregation which was found to be particularly troublesome with sea urchin sperm chromatin. As a consequence of not fixing the chromatin, 300 Å filaments would occasionally unfold on the grid into 'puddles' of nucleosomes, as seen previously (J.Widom, in preparation; Sperling, 1977) (see text). When tilt series were required, fields were chosen in which 300 Å filaments lay parallel to the tilt axis of the goniometer.

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References

Allan,J., Rau,D.C., Harborne,N. and Gould,H. (1984) *J. Cell Biol.*, **98**, 1320-1327.
Ausio,J., Borochov,N., Seger,D. and Eisenberg,H. (1984) *J. Mol. Biol.*, **177**, 373-398.
Bates,D.L., Butler,P.J.G., Pearson,E.C. and Thomas,J.O. (1981) *Eur. J. Biochem.*, **119**, 469-476.
Butler,P.J.G. (1983) *CRC Crit. Rev. Biochem.*, **15**, 57-91.
Butler,P.J.G. (1984) *EMBO J.*, **3**, 2599-2604.
Butler,P.J.G. and Thomas,J.O. (1980) *J. Mol. Biol.*, **140**, 505-529.
Finch,J.T. and Klug,A. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 1897-1901.
Hewish,D.R. and Burgoyne,L.A. (1973) *Biochem. Biophys. Res. Commun.*, **52**, 504-510.
Kornberg,R.D. (1977) *Annu. Rev. Biochem.*, **46**, 931-954.
Langmore,J.P. and Schutt,C.E. (1980) *Nature*, **288**, 620-622.
Langmore,J.P. and Paulson,J.R. (1983) *J. Cell Biol.*, **96**, 1120-1131.
Marsden,M.P.F. and Laemmli,U.K. (1979) *Cell*, **17**, 849-858.
McGhee,J.D., Rau,D.C., Charney,E. and Felsenfeld,G. (1980) *Cell*, **22**, 87-96.
McGhee,J.D., Nickol,J.M., Felsenfeld,G. and Rau,D.C. (1983) *Cell*, **33**, 831-841.
Mitra,S., Sen,D. and Crothers,D.M. (1984) *Nature*, **308**, 247-250.
Morris,N.R. (1976) *Cell*, **9**, 627-632.
Noll,M., Thomas,J.O. and Kornberg,R. (1975) *Science (Wash.)*, **187**, 1203-1206.
Pearson,E.C., Butler,P.J.G. and Thomas,J.O. (1983) *EMBO J.*, **2**, 1367-1372.
Spadafora,C., Bellard,M., Compton,J.L. and Chambon,P. (1976) *FEBS Lett.*, **69**, 281-285.
Sperling,L. (1977) Ph.D. Thesis, University of Cambridge, Cambridge, UK.
Strätling,W.H. and Klingholz,R. (1981) *Biochemistry (Wash.)*, **20**, 1386-1392.
Suau,P., Bradbury,E.M. and Baldwin,J.P. (1979) *Eur. J. Biochem.*, **97**, 593-602.
Thoma,F., Koller,Th. and Klug,A. (1979) *J. Cell Biol.*, **83**, 403-427.
Thomas,J.O. and Kornberg,R.D. (1978) *Meth. Cell Biol.*, **18**, 429-440.
Thomas,J.O. and Butler,P.J.G. (1980) *J. Mol. Biol.*, **144**, 89-93.
Thomas,J.O., Rees,C. and Pearson,E.C. (1985) *Eur. J. Biochem.*, **147**, 143-151.
Thomas,J.O., Rees,C. and Butler,P.J.G. (1986) *Eur. J. Biochem.*, in press.
von Holt,C., De Groot,P., Schwager,S. and Brandt,W.F. (1984) in Stein,G.S., Stein,J.L. and Marzluff,W.F. (eds.), *Histone Genes*, Wiley, NY, pp. 65-105.
Walmsley,M.E. and Davies,H.G. (1975) *J. Cell Sci.*, **17**, 113-139.
Widom,J. and Klug,A. (1985) *Cell*, in press.
Woodcock,C.L.F., Frado,L.-L.Y. and Rattner,J.B. (1984) *J. Cell Biol.*, **99**, 42-52.
Worcel,A., Strogatz,S. and Riley,D. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1461-1465.
Yabuki,H., Dattagupta,N. and Crothers,D.M. (1982) *Biochemistry (Wash.)*, **21**, 5015-5020.
Zalenskaya,I.A., Pospelov,V.A., Zalensky,A.O. and Vorob'ev,V.I. (1981) *Nucleic Acids Res.*, **9**, 473-487.
Zentgraf,H. and Franke,W.W. (1984) *J. Cell Biol.*, **99**, 272-286.

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