

Topological constraints on the possible structures of the 30 nm chromatin fibre

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Abstract The packing of mammalian DNA into chromatin plays an important role in cell differentiation and selection of epigenetically marked genes for expression or silencing. The first level of folding, the nucleosome, is evolutionary conserved. It allows transcription, after remodeling and/or histone modifications. The second level, the transcriptionally dormant 30 nm fibre, exhibits species and tissue variations in the chromatin repeat length. Nevertheless, very similar structures of fibres have been observed in all metazoans, and therefore, have to accommodate variable linker lengths with a corresponding change of tilt of the nucleosomes, which is defined by the DNA helical periodicity. So far, none of the models for a regular fibre structure has considered this requirement nor the relationship between repeat length and orientations of nucleosomes in the fibre. Here, we present two regular structural arrangements with negatively tilted consecutive nucleosomes which can compensate for a non-integer number of bp/turn of DNA; one can accommodate a series of structures with discrete repeat lengths differing by

5 bp in the region around 200 bp and the other from around 220 to 250 bp, accommodating repeat lengths differing by 10 to 12 bp.

Introduction

The DNA is packed as chromatin in the eukaryotic nucleus at several levels. The structural and functional features of the first two levels of packing have been extensively studied. The nucleosome core particle has been reconstituted using several unique DNA sequences, crystallised and its structure resolved in detail to 1.9 Å resolution (Davey et al. 2002; Harpet et al. 2000; Luger et al. 1997). The nucleosome allows transcription, after remodeling and/or histone modifications. The second level of packing, the '30 nm chromatin fibre', represents transcriptionally dormant chromatin. Understanding the structure of the fibre and the processes which determine its folding and unfolding during proliferation and cell differentiation is a prerequisite for studying the epigenetic mechanisms that lead to transcriptionally dormant and transcriptionally poised genes Wolffe (1998).

The fibre comprises almost the entire chromatin of nucleated avian erythrocytes and is more than 85% of the chromatin in other cell types. The presence of linker histones in approximately one to one ratio to the nucleosomes is characteristic of the fibre. Chicken erythrocytes chromatin has been the most widely studied, in solution and in whole nuclei. It has been shown by small angle X-ray and neutron scattering that the fibre is a regular helix with a diameter of about 33 nm and a variable mass per unit length, which at 80 mM salt concentrations approaches 0.6 nucleosomes/nm with an 11 nm pitch. This implies that there are about six to eight nucleosomes per helical turn with their flat surfaces nearly parallel to the fibre axis. The

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unusually small cross-section radius of gyration of 9.5 nm indicates a very compact structure with close nucleosome–nucleosome contacts (Bordas et al. 1986a, b; Finch and Klug 1976; Gerchman and Ramakrishnan 1987; Greulich et al. 1987; Suau et al. 1979).

Variation effect studies have shown that gene silencing is accompanied by higher compaction, with more regularly spaced nucleosomes (Wallrath and Elgin 1995). This suggests that the nucleosomes' positions are defined by structural constraints such as nucleosome–nucleosome interactions, rather than by the DNA sequence. Long fibres of many nucleosomes, separated by some discontinuities, have been observed by electron microscopy (EM), again suggesting a very regular structure (Rattner and Hamkalo 1979). High resolution structural studies have been inhibited, as the native chromatin is a mixture of fibres with different repeat lengths.

There are several basic models for the structure of the fibre proposed in the late 1970s and early 1980s. For reviews, see van Holde (1988). They all comprise regular helices of about seven nucleosomes per turn, and thus, satisfy the experimental results from small angle X-ray and neutron scattering and low resolution EM with regard to packing of the nucleosomes. The models differ with respect to the path of the linker DNA and can be classified as follows: (1) folded linker models in which adjacent nucleosomes are brought together (Finch and Klug 1976; McGhee et al. 1983); (2) models with straight linkers parallel to the fibre axis (Woodcock et al. 1984; Worcel et al. 1981) and (3) models of single-start nonsequential helices (Staynov 1983) and two-start helices (Williams et al. 1986) with linkers criss-crossing the fibre. Alternatively, they can be classified as models: (A) which assume that the mutual orientation of the dyad axes of adjacent nucleosomes depends on the linker length (McGhee et al. 1983); (B) models in which the orientations of nucleosomes are fixed (Thoma et al. 1979; Williams et al. 1986; Worcel et al. 1981) or (C) models in which the nucleosome's axes of symmetry alternate around the radius of the fibre (Staynov 1983), discussed in van Holde (1988) and Ramakrishnan (1997). Several variants of these models have been subsequently published; for reviews see Wolffe (1998) and Ramakrishnan (1997). These models were proposed before the crystallization of the nucleosome core particle, i.e., the topological constraints which it imposes on the fibre structure were not considered. The more recently published results of nuclease digestions of whole nuclei, which give additional information on the orientations of the nucleosomes in the fibre were likewise not considered (see below).

Our knowledge of whether the repeat lengths of the fibres are a discrete set or vary continuously is limited, as micrococcal nuclease (MNase) digests rarely give a higher accuracy than ± 4 bp. Compilations of the published repeat

lengths have shown some partial preference for multiples of 10 bp, but also some pronounced intermediate lengths (van Holde 1988; Widom (1992). However, if the fibre is a compact structure with close nucleosome–nucleosome contacts, consecutive nucleosomes must have defined orientations and tilts which depend on the length of the linker DNA. Crystal structures of nucleosome core particles of two different lengths of a selected sequence indicate that the ends of the 147 bp DNA are well defined, and if there is a lack of 1 bp, this is compensated by a distortion of the DNA 20 bp inside the core particle, rather than by a change in the orientation (twist) of the end-base pairs (Davey et al. 2002; Luger et al. 1997). Whilst long circular DNA is flexible and can accommodate several topoisomers via helical twist and writhe, short DNA fragments are torsionally quite rigid (Bates and Maxwell 1989; Shore and Baldwin 1983). Due to this rigidity, stretches of 20 to 40 bp linker DNA must impose strong constraints on the mutual tilts of consecutive nucleosomes. Thus, the intrinsic structure of the fibre must allow for several different repeat lengths (differing not only by $10n$ bp, where n is an integer) and accommodate a non-integer periodicity of the DNA helix (10.5 bp/turn).

For the orientations and the tilt of the nucleosomes and their relationship with the linker length and the DNA helical periodicity and for the path of the linker DNA, we need additional information. Some of it comes from important, but often overlooked observations of digestions with bulky nuclease molecules, such as DNases I and II that cannot penetrate the fibre and cut (nick) only the exposed parts on its surface. Digestions of high-molecular weight chromatin show that:

1. DNase I produces dinucleosomal repeat patterns from chromatin of several different repeat lengths. This was originally explained by the inaccessibility of every second nucleosome to the enzyme (Burgoyne and Skinner 1981), but we have later shown that it simply suggests alternating orientations of consecutive nucleosomes along the DNA (Staynov et al. 1983; Staynov 1983);
2. Single-stranded gels of DNase I-digests show that the linkers and either sites -6 , -7 and -8 or sites $+6$, $+7$ and $+8$ (Fig. 2a) are inaccessible to DNase I (Staynov 2000; Staynov and Proykova 1998);
3. DNase II preferentially cuts nucleosomal DNA at sites ± 5 (Fig. 2a) and not the linker DNA (Horz et al. 1980; Horz and Zachau 1980).

Taken together, these results imply that the nucleosomes alternate, and the linker DNA is inside the fibre (extensively discussed in Staynov 1983; Staynov 2000; Staynov and Proykova 1998). The data unambiguously eliminate all proposed structures with identically oriented nucleosomes

such as the models of Thoma et al. (1979) and Williams et al. (1986). They are also incompatible with models having exposed linker DNA, e.g., those of Worcel and Benyajati (1977) and Woodcock et al. (1984), and also models having varying orientations of nucleosomes dependent on the repeat length (McGhee et al. 1980).

Experimental data on the path of the linkers comes from scanning transmission electron microscopy of reconstituted mononucleosomes (Hamiche et al. 1996) and from cryo-electron microscopy for repeat lengths around 200 bp (Bednar et al. 1995; Bednar et al. 1998). These show a short stem, after which the two linkers split. It is not clear whether the length of the stem is constant or if it depends on the repeat length and the type of the linker histone. Thus, geometrical considerations for the fibre should allow for some differences between the actual linker lengths and the real distances between consecutive nucleosomes.

There are some alternative structures of condensed chromatin, with or without H1 histone, interacting with specific proteins such as MeCP2 and other trans-acting factors, reviewed in Luger and Hansen (2005). Although these structures are important for the regulation of specific genes, they are not considered here.

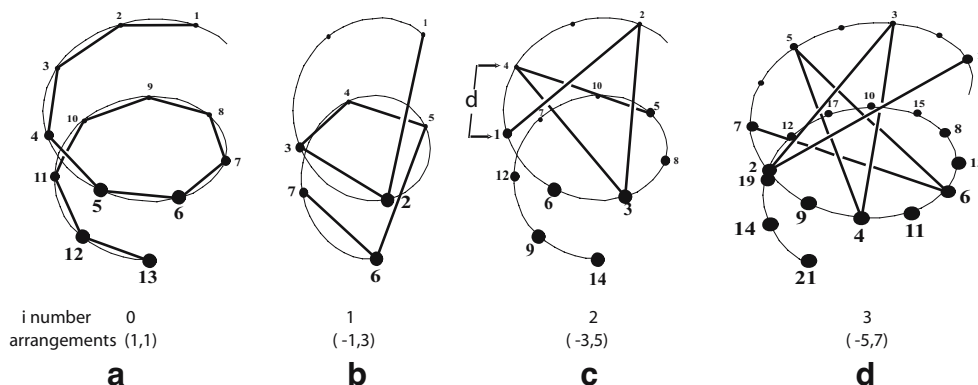
To avoid uncertainties created by the mixtures of repeat lengths in ‘native’ chromatin, several studies have been carried out with reconstituted fibres on particular DNA positioning sequences having uniform repeat lengths. Tse and Hansen (1997) showed that core histone tails are necessary for the compact structure, again suggesting close nucleosomes contacts. Dorigo et al. (2004) studied reconstituted oligonucleosome arrays containing core histones with substituted cysteine residues, both with and without linker histones, for repeat lengths of 167, 177 and 208. The EM photographs of these reconstituates show two-start flat ribbons with about five nucleosomes per 11 nm length, rather than the helical arrangements with about seven nucleosomes per 11 nm length, as observed in native chromatin (Finch and Klug 1976; Rattner and Hamkalo 1979). In a subsequent paper, Schalch et al. crystallized a tetranucleosome with a 20 bp linker and found a structure

with nucleosomes stacked perpendicular to its axis (Schalch et al. 2005). These reconstituates most probably represent a special class of structures of short repeat lengths, different from the ‘native’ fibres of higher eukaryotes.

A different set of reconstituates with native core histones and chicken linker histone H5 were reported by Robinson et al. (2006) for discrete repeat lengths of 177 to 237 bp, i.e., differing by 10 bp. They observed fibres with helically arranged nucleosomes, similar to the fibres which diffuse out of the MNase digested chicken erythrocytes nuclei and which therefore have direct relevance to ‘native’ fibres. Robinson et al. also showed that their reconstituates comprise two different classes of structures; one with repeat lengths of up to 207 bp and a diameter of 33 nm, the other with repeat lengths of 217, 227 and 237 and a diameter of 44 nm.

In search of arrangements of closely interacting nucleosomes along the fibre which can accommodate non-integer numbers of base pairs per turn of DNA, we have found a general solution for single-start helices which relates the numbers of the nucleosomes along the fibre helix with their numbers along the DNA (Lasters and Staynov 1983; Staynov 1983). If the position number of a nucleosome on the fibre helix is v , then its number along the DNA is found by adding $(\pm 2i+1)$ in alternation where $i=0, 1, 2, \dots$. Thus, apart from the sequential arrangement of nucleosomes along the fibre ($i=0$, Fig. 1a), there is a series of possible nonsequential single helix arrangements with up to 4, 8, 12, 16, ... nucleosomes per turn. The solution for $i=1$, gives, for $v=1$, nucleosome numbers along the fibre helix as 1, 0, 3, 2, 5, 4, 7, ... where ‘0’ denotes a missing nucleosome. It is called the $(-1,3)$ arrangement, with up to 4 nucleosomes per turn (Fig. 1b). It applies to fibres with short repeat lengths. The consecutive nucleosomes form a left-handed fibre helix but the linkers, i.e. adjacent nucleosomes along the DNA, follow a right-handed helical path. The solution for $i=2$, the $(-3,5)$ arrangement, is a fibre with up to eight nucleosomes per turn, with the sequence 2, 0, 4, 1, 6, 3, 8, 5, ... for $v=2$ (Fig. 1c). If $v=1$ the sequence is 1, 0, 3, 0, 5, 2, 7, 4, ... Thus depending on whether we start

Fig. 1 Single helical arrangements: **a** $i=0$, (1,1) sequential; **b** $i=1$, $(-1,3)$ nonsequential; **c** $i=2$, $(-3,5)$ nonsequential; **d** $i=3$, $(-5,7)$ nonsequential. For clarity, not all linkers are shown. The numbers denote nucleosomes along the DNA



with $(+2i+1)$ or $(-2i+1)$ at the ends of the sequence there are discontinuities with one or two missing nucleosomes. We have shown that this arrangement can accommodate chromatin repeat lengths of up to around 220 bp (Staynov 1983). The next solution with $i=3$, the $(-5,7)$ arrangement (Fig. 1d), can accommodate longer repeats with discontinuities of two or three missing nucleosomes at each end.

Here, we relate the $(-3,5)$ arrangement for $i=2$ and the $(-5,7)$ arrangement for $i=3$ to a DNA helical periodicity of 10.5 bp per turn and show that one of them, the $(-3,5)$ arrangement, can accommodate a series of structures with discrete repeat lengths differing by 5 bp in the interval around 190–220 bp, whilst the $(-5,7)$ arrangement accommodates repeat lengths differing by 10 to 12 bp in the interval around 220 to 250 bp.

Materials and methods

Calculations for both the $(-3,5)$ and $(-5,7)$ arrangements were carried out, associating a rectangular coordinate system with each nucleosome and assuming that a standard transformation places each nucleosome at the position of the next one along the DNA (Staynov 1983). The standard transformation can be reduced to one translation between consecutive nucleosomes (L), which we will call ‘linker length’ and two rotations at Euler’s angles α and β . The distance between consecutive nucleosomes is restricted in the interval between their closest contacts, defined by their size (Fig. 2b) and a maximum separation, defined by the lengths of straight linkers (Fig. 2c). The DNA linkers do not have to be straight. They can be bent or kinked as in Fig. 2d, but if bent or kinked, all of them have to be bent or kinked in the same manner. The angle α is defined as the angle between two imaginary straight linkers from one nucleosome. The first rotation along the Z -axis at an angle $(\pi-\alpha)$, brings the ingoing linker of a nucleosome parallel to its outgoing linker. A translation of distance L brings this nucleosome to the next nucleosome along the DNA. The second rotation by angle $(-\beta)$ along the outgoing linker is defined by the relationship between the linker length and the DNA helical periodicity.

For the $(-3,5)$ arrangement, if the transformation is carried out three times, the fourth nucleosome will be adjacent to the first at a distance d , defining the nucleosomes separation along the fibre helix (Fig. 1c). If it is carried out five times, the sixth nucleosome will be positioned symmetrically with respect to the fourth on the other side of the first, at the same distance d (Fig. 1c). This gives two equations with four variables, L , d , α and β , which can be solved parametrically for the angles α and β as functions of the ratio of the linker length L and nucleosome separation along the fibre helix, d (L/d). For

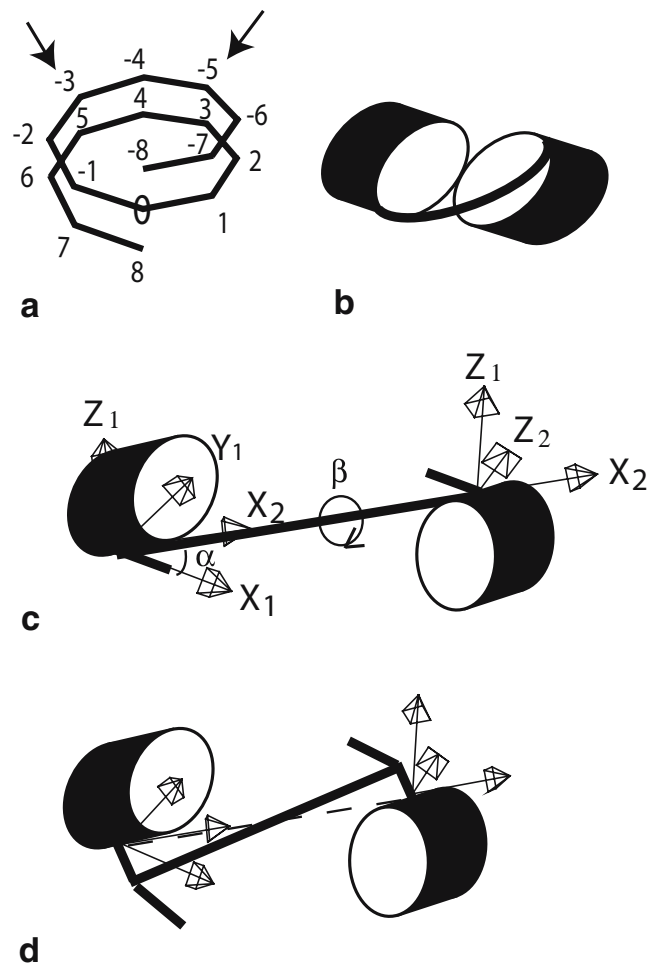


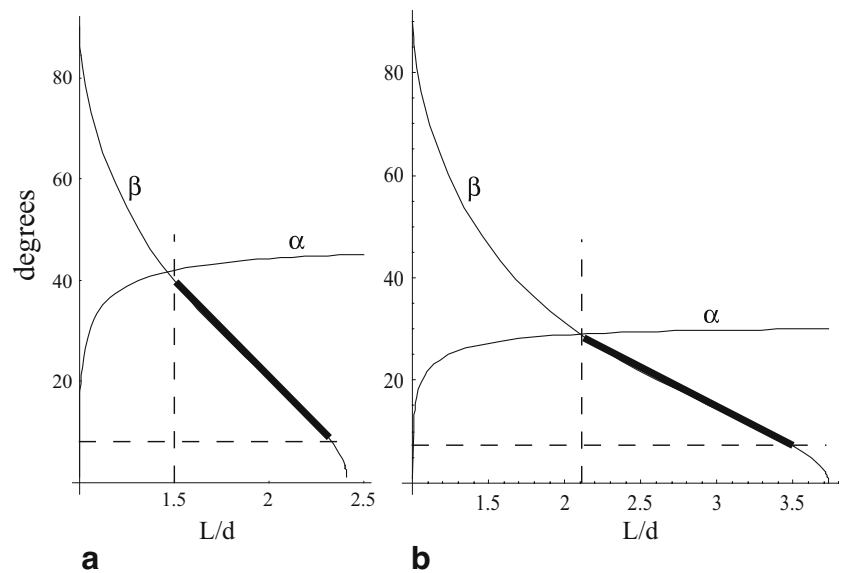
Fig. 2 Schematic representation of a dinucleosome. **a** Numbering of the DNase cutting sites from the dyad axis of the nucleosome. Arrows indicate positions of DNase II cuts when nucleosomes are the 30 nm fibre; **b** two nucleosomes in close contact; **c** two nucleosomes at maximum distance allowed by a straight linker. The angle α between ingoing and outgoing linkers of the first nucleosome lies in the X, Y plane. A rotation around Z_1 at an angle $(\pi-\alpha)$ establishes two new axes Y_1' and X_2 . A translation is carried out along X_2 for a distance L . A second rotation around X_2 by an angle $(-\beta)$ makes Z_1 coincide with Z_2 ; **d** as **c**, but the two nucleosomes are separated by a shorter distance as the linkers start with a stem

the $(-5,7)$ arrangement with up to 12 nucleosomes per turn the equations can be solved in the same fashion but assuming that the seventh and the ninth nucleosomes are symmetrically adjacent to the second nucleosome at a distance d (Fig. 1d) (For further details, see [Electronic Supplementary Material](#)).

Results

Graphs of the solutions for α and β as functions of L/d for the $(-3,5)$ and $(-5,7)$ arrangements are shown in Fig. 3a and b. These solutions will have structural significance only if they give nucleosomes with a constant tilt, as this permits

Fig. 3 Parametric solutions for angles α and β as functions of the ratio L/d . **a** for the $(-3,5)$ arrangement; **b** for the $(-5,7)$ arrangement. Vertical dashed lines mark the closest contact between adjacent nucleosomes. Horizontal dashed lines mark the minimum angle, β , at which linkers do not clash. Bold lines represent the linear parts of the β vs L/d curves



their face-to-face interactions along the fibre helix. We will consider the $(-3,5)$ arrangement first. The numerical solution shows that the equations for α and β are compatible for $1.0 < L/d < 2.4$. The relations of these equations with the real geometrical parameters of the fibre are not straightforward, as we do not know the actual linker path. Assuming that consecutive nucleosomes along the helix are cylinders with a radius/height ratio = 0.85, (5.5 nm/6.5 nm, see below and Fig 4), these cylinders will clash when $L/d < 1.5$ (vertical dashed line in Fig. 3a). Similarly, assuming straight linkers with diameters of 2 nm, they will clash in the middle of the fibre if the angle $\beta < 8^\circ$ (horizontal dashed line). Thus, this structure is possible in the interval $1.5 < L/d < 2.3$. To be applicable to real linker lengths, i.e., chromatin repeat lengths, we have to make some assumptions. If we consider the two linkers to start from one point on the dyad axis at the edge of the chromosome (Fig. 2c) and assume very tight packing with $d = 6.5$ nm, we obtain a possible range of linker lengths of 20 bp and repeats between 194 and 214 bp; for $d = 7.5$ nm, this interval will be between 200 and 220 bp. For alternative linker paths, as shown in Fig. 2d, this interval can increase up to about 25 bp.

The dependences of the angles α and β on the ratio L/d in the geometrically allowed interval are very interesting. The angle α of the standard transformation, which we assume to be defined by the linker histone, is nearly constant.

The angle β in the allowed interval (the thick line in Fig. 3a) is a linear function of L/d with a negative slope, and thus, it can compensate for a non-integer excess number of base pairs per turn of the DNA helix in the

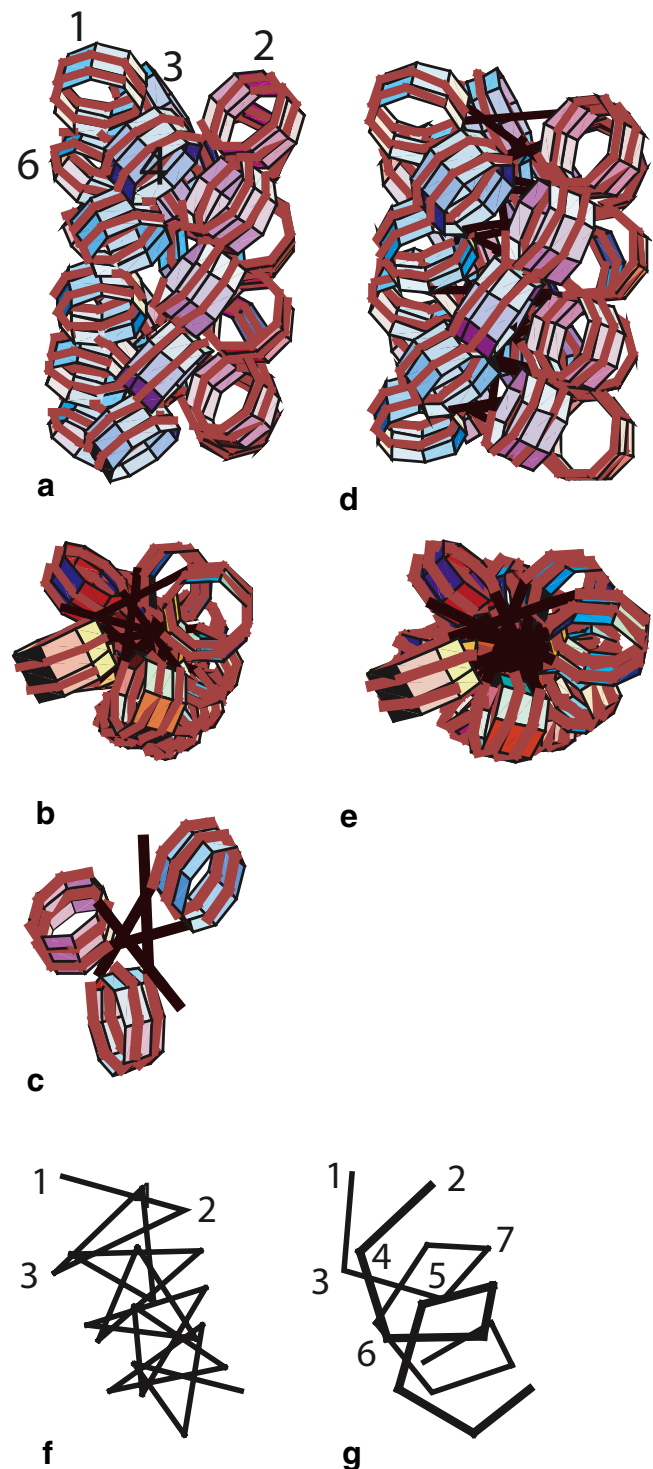
linker and bring consecutive nucleosomes face-to-face. It can be expressed as

$$\beta^\circ = \text{constant} - 36.4^\circ \left(\frac{L}{d} \right) \quad (1)$$

Thus, a change of the dimensionless ratio L/d requires a corresponding change in the mutual tilt of consecutive nucleosomes along the DNA (negative tilt). However, the tilt of consecutive nucleosomes is defined by the helical periodicity of the DNA, $\phi \sim 10.5$ bp/turn. We can then ask the question: are these two requirements compatible for any increment of the linker length, or do they allow only a discrete set of repeat lengths? An elongation by 1 bp (0.34 nm), which is accompanied by a very small change of L/d , causes a very small change in the tilt according to Eq. 1. However, the DNA periodicity requires a considerable change in the tilt (by 34.28°). Thus, these two requirements are incompatible for a difference in the repeat length by 1 bp. Examination of different increments has shown that there is a set of increments differing by 5 bp ($\Delta L = 5 \text{ bp} = 1.7 \text{ nm}$), for which the two requirements are compatible. Assuming very close contacts between adjacent nucleosomes along the fibre ($d = 6.5$ nm), then according to Eq. 1, $\Delta\beta = -(36.4 \times 1.7)/6.5 = -9.5^\circ$. Thus, for a nucleosome with a twofold axis of symmetry, it is short by 9.5° from the symmetrical structure with 180° tilt, or for elongation of 5 bp, this is $34.1^\circ/\text{bp}$, giving DNA periodicity $\phi = 10.56$ bp/turn. If we assume relatively distant nucleosomes at $d = 8$ nm, we find for the periodicity of DNA

$$\phi = 10.45 \text{ bp/turn.}$$

Fig. 4 Schematic representations of the $(-3,5)$ arrangement for two linker lengths differing by 5 bp. **(a, b and c)** for $L/d=1.54$; **(d and e)** for $L/d=1.78$ using Wolfram's Mathematica®. The straight linkers are drawn starting from the ends of the core particle-DNA. **a** and **d** side view; **b** and **e** view along the fiber axis; **c** a trinucleosome. The nucleosomal DNA (red) is drawn with half of its real diameter and the linkers (black) are drawn one quarter of their real diameters. The histones are represented as colored hollow octagons. **f** Straight lines connecting the midpoints of the two linkers exiting each nucleosome to illustrate their alternating orientations with respect to the fiber axis; **(g)** connecting lines between the odd and even nucleosomes to illustrate that they follow two different helices, shifted from each other at one-half of the pitch. For clarity **f** and **g** are shown in different projections



Thus, the assumption that the distances between the nucleosomes along the fibre differ by more than 20%, gives DNA periodicities differing by only 1%. This implies that if we assume the linkers do not start from one point, this will slightly shift the possible repeat lengths interval but will not change the overall results. Thus, the $(-3,5)$ arrangement covers most of the metazoan chromatin repeat lengths and the magic number of 10.5 bp/turn of the DNA helix persists.

We can ask this question in reverse: what must the distance d between adjacent nucleosomes be in the fibre for the structure to be possible for linker lengths L_1 , and for L_1+5 bp, assuming a DNA periodicity of $\phi=10.5$ bp/turn? We find that the next nucleosome will be rotated by 171.40° , i.e., 8.6° short of 180° . Thus, we obtain $d=7.2$ nm, a very realistic value for the distance between consecutive nucleosomes along the fibre. From liquid crystals of nucleosome particles, it has been found that the mean distance between the core particles with their flat surfaces parallel to each other is 7.16 ± 0.65 nm (Leforestier and Livolant 1997). These results show that an increment of 5 bp is possible. Thus, if the structure is possible for a repeat length of 194 bp, it has to be also possible for repeat lengths of 199, 204, 209 and 214 bp.

The linkers of nucleosome 3 (assumed straight) are connected to two nucleosomes (2 and 4), up the helix, whilst nucleosome 4 is connected to two nucleosomes (3 and 5) down the helix (Fig. 1c). The same is seen in Fig. 4f (see below). According to the original assumption, the two linkers exit symmetrically with respect to the dyad axis of the nucleosome. This also implies that the dyad axes of consecutive nucleosomes along the fibre helix and along the DNA are alternating.

The $(-3,5)$ arrangement, however, supports only a discrete set of repeat lengths, differing by 5 bp and does not support a continuum of repeat lengths. For the possible interval $1.54 < L/d < 2.06$, the diameter of the fibre changes from 31.3 to 35.5 nm. As the angle β decreases with increase in L/d , the mean step of nucleosomes along the fibre decreases with increase in L/d from 2.4 to 1.64 nm.

Thus, the number of nucleosomes per 11 nm length changes from 9.2 to 13.4 nucleosomes, and the tilt of the nucleosomes decreases from 18.6° to 9.3° .

Schematics of the geometrical solutions of $(-3,5)$ arrangement for two linker lengths differing by 5 bp ($L/d=1.54$ and 1.78) are shown in Fig. 4a–g. The consecutive nucleosomes along the fibre helix have alternating dyad axes around the

radius of the fibre (Fig. 4f). The centres of the nucleosomes also alternate up and down away from a smooth helix (Fig. 4g). For $L/d=1.54$, the dyads alternate $\pm 26^\circ$. Such alternation, which causes exposure of different parts of the consecutive nucleosomes on the surface of the fibre, can explain the ‘dinucleosomal’ repeats in the DNase I digests (Staynov 1983 and references therein). Furthermore, Fig. 4 shows that adjacent nucleosomes from consecutive turns of the fibre intercalate between each other at one-half of the pitch (Fig. 4g). As the nucleosomes are wider at the linker entry/exit sites, this arrangement allows them to be packed closer to each other than if they were all positioned with their dyad axes radial to the fibre.

Fibres with longer than 220 bp repeat lengths are described by the next solution of the series, the $(-5,7)$ arrangement with 12 nucleosomes per turn. The solution for the $(-5,7)$ arrangement shows a relationship between β and L/d similar to Eq. 1 but the slope is shallower, -14.5° (Fig. 3b), and the possible interval is $2.22 < L/d < 2.78$. The average step is from 1.53 to 1.23 nm or 14.4 to 19.6 nucleosomes per 11 nm. For a 5 bp difference in the repeat length, we obtain an unacceptable periodicity of DNA, $\phi=10.2$ bp/turn. However, this arrangement tolerates differences in the 10 bp register. For the two values of $d=6.5$ and 8 nm, the DNA periodicity is 10.4 bp/turn, and for an 11 bp as well, a 12 bp linker difference $\phi=10.4$ and 10.5 bp/turn, respectively. For such long linker lengths, up to 70 bp, it is not clear, however, whether the DNA might be torsionally more flexible, and thereby, allow all three repeat length differences for face-to-face nucleosomal contacts.

Discussion

The aim of this paper is to find topologically possible structures in real geometrical space, which allow for different repeat lengths, rather than to build a precise structure of the 30-nm fibre; neither do we consider its rigidity or thermodynamics. The results show that a single nucleosome arrangement cannot cover the whole interval of repeat lengths over which fibres have been observed, and there must be at least two different structures. The $(-3,5)$ and $(-5,7)$ arrangements presented here were arrived at only on the assumption that consecutive nucleosomes along the fibre are equidistant from each other (at distance d) and that a single geometrical transformation superimposes each nucleosome on the next nucleosome along the DNA (at distance L). Both arrangements allow only discrete sets of helices for repeat lengths differing by 5 and by 10–12 bp, respectively. The conditions giving rise to these arrangements did not imply that they have to be straight helices. However, the discrete solutions shown here result in straight helices with closely spaced nucleosomes. The two

structures differing by 5 bp repeat length, shown in Fig. 4, were built with linkers starting from the ends of the core particle DNA. This confirms that the initial simplification, with linkers starting from one point at the edge of the chromatosomes is not critical. At this approximation, it is not clear whether there is a gap of several forbidden repeat lengths between the possible intervals of the $(-3,5)$ and $(-5,7)$ arrangements, or they overlap.

For the $(-3,5)$ arrangement, the diameter of the fibre (31.4 to 35.5 nm), is within the experimental error of the EM measurements. The number of nucleosomes per 11 nm increases from 9.2 to 13.4 in the model, and the tilt decreases from 18.6° to 9.3° . The number of nucleosomes per turn differs from the early X-ray scattering estimates, as at that time, intercalated nucleosomes between adjacent turns of the helix were not envisaged. Comparison of our values for the nucleosome tilt with those calculated from electric dichroism measurements is not straightforward, as the latter depends on assumptions for the contributions of the linker. However, it is widely accepted that the nucleosome faces are close to parallel to the fibre axis, and most of the estimates give tilts around 30° for ~ 200 bp repeats and around 20° for the long-repeat sea urchin sperm; (discussed in van Holde 1988). Thus, although our tilt interval is shifted by about 10° , there is a similar inverse relationship between the tilt and the repeat length.

There are contradicting reports for the diameter of the fibre of sea urchin sperm chromatin that has a repeat length of about 240 bp. Widom et al. (1985) did not find a significant difference between its diameter and the diameter of chicken erythrocyte fibres (repeat ~ 212 bp), but Williams et al. (1986) reported 12 nucleosomes per turn. X-ray scattering also supports the much larger diameter of about 45 nm for echinoderm sperm chromatin, with a cross-section radius of gyration of 16 nm (Koch et al. 1988). This agrees with our predictions for the $(-5,7)$ arrangement: up to 45 nm diameter, a mean step along the axis of the fibre of between 1.53 and 1.23 nm, and correspondingly, the number of nucleosomes per 11 nm being between 14.4 and 19.6.

The fact that the $(-3,5)$ arrangement allows repeat lengths differing by 5 bp is very important. We do not have direct evidence as to whether the repeat lengths of native fibres are continuous or only certain repeats exist. One can get some insight into this from the 10 nt repeat of the DNase I digests beyond the intranucleosomal bands. In yeast, the so-called ‘ $10n \pm 5$ nt shift’ of the internucleosomal bands suggests regularly positioned nucleosomes in a considerable part of the genome (Lohr and van Holde 1979). These bands are very faint in higher eukaryotes and are superimposed on a high, continuous background (Staynov 2000). However, these results do not indicate whether the background comes from randomly positioned

nucleosomes, or it is caused by a superposition of two different repeats, with the faint bands representing the excess of one over the other. The superposition of digestion patterns of equal amounts of two repeats differing by 5 bp would completely mask the discrete internucleosomal bands that are observed in yeast (Staynov 2000). Thus even in the dormant chromatin of chicken erythrocytes, there are at least two repeat lengths in comparable abundance which differ by (5 ± 1) bp.

Intercalated nucleosomes were first proposed by Staynov (1983) and later also by Bordas et al. (1986a, b) for non-sequential arrangements and by Daban and Bermudez (1998) for a sequential arrangement. Daban and Bermudez have found nucleosome tilts very similar to the tilts of the nucleosomes calculated for the $(-3,5)$ arrangement. However, they disregarded the DNase I digestion results and built their models on the assumption that the nucleosomes dyad axes are orthogonal to the fibre axis. Moreover, as the nucleosomes are wider at the linker entry/exit sides, Daban and Bermudez have assumed a 36 nm fibre diameter for all repeat length models. This assumption places adjacent nucleosomes at larger distances and prevents their close face-to-face contacts, or alternatively, it imposes special constraints on the path of the linkers. The authors have explained this discrepancy by suggesting that if there are some unforeseen distortions and interpenetrations of adjacent nucleosomes and DNA linkers, the structure would be more compact.

The observation of a discontinuity in the relationship between the sedimentation coefficient and the ionic concentration between penta- and hexa-nucleosome samples has been explained as a result of the sixth nucleosome interacting with the first (Butler and Thomas 1980; Thomas and Butler 1980). This implies that the fibre has between five and six nucleosomes per turn, and the sixth nucleosome interacts very strongly with the first and locks one turn of the helix. An alternative explanation is suggested by the $(-3,5)$ arrangement. As there are always one or two protruding (flexible) nucleosomes at each end of the fibre, in up to pentamers, there are none or only one pair of adjacent nucleosomes. In hexanucleosomes, however, four nucleosomes, those with numbers 4, 1, 6 and 3 are adjacent, hence, interacting, and the structure is rigid (Fig. 1c).

The predicted $(-3,5)$ and $(-5,7)$ arrangements are in striking agreement with recently published results by Robinson et al. (2006) for reconstituted fibres on positioned nucleosomal DNA repeats differing by 10 bp in the range of 177–237 bp repeat lengths. They have found that indeed, there are two distinct structures: one with a diameter of about 33 nm for up to 207 bp repeat lengths, and a second with a diameter of about 44 nm for 217 to 237 bp repeat lengths. There are some further similarities between our proposed structures and those observed by Robinson et al.

(2006). The nonsequential arrangements in our model can easily explain their observation that the fibres polymerize by end-to-end self-association instead of side-by-side. At each end of nonsequentially arranged fibres, there are one or two missing and protruding nucleosomes: the protruding nucleosomes of one fibre can thus intercalate into the positions of the missing nucleosomes of a second fibre (Fig. 1c).

Thus, although there are no discrepancies between our analytical solutions and the experimental results from the reconstitutes of Robinson et al., there are differences between our analytical solution, and the model they propose with regard to the orientation and the tilt of the nucleosomes. They assume that the nucleosomes are uniformly oriented with respect to the fibre radius, although this is incompatible with the DNase I digestion results. They assume a tilt of 17° , independent of the repeat length, a value in good agreement with our calculation of 18.6° for the shortest repeat $L/d=1.54$. Robinson et al. also assume that the linker histones define different paths of the linker DNA inside fibres for different repeat lengths. As they have used only one kind of linker histone for all different repeat length reconstitutes up to repeat lengths of 207, such an assumption is unnecessary. The diameter of their reconstitutes having the sea urchin sperm repeat is unchanged, whether H5 or sea urchin sperm H1 histones are used. Furthermore, this suggests that the main factor defining the shape of the fibre is the nucleosome–nucleosome interaction and not the type of linker histone. Our models show that as the angle α is nearly constant in the allowed geometrical space, one type linker histone can support fibres with different repeat lengths, but the nucleosomes in the fibre would have different tilts. The two structures proposed by Robinson et al. conform, however, to the observed dimensions of the fibres in both repeat length regions, and although the path of the linker DNA and the linker histones are not seen, such reconstitutes are a good start for further high-resolution studies.

In situ pyrimidine dimer formation has shown that the DNA linkers in the fibre are straight (Pehrson 1995). A simulation of fibre dynamics for di- and trinucleosomes has been carried out by Beard and Schlick (2001) who also showed that straight linkers are energetically more favourable. The rationale originally proposed in Staynov (1983) has been also used by several other groups. Woodcock et al. (1993) carried out similar model calculations. However, they assumed random linker lengths and did not look for regular structures or for a relationship between linker length and regularity.

An elegant geometrical treatment of a fibre with consecutive nucleosomes by Ben-Haim et al. (2001) has examined possible structures with two fixed angles between the consecutive linkers. They took into consideration that the DNA is a double helix with a non-integer number of

base pairs per turn. They showed that all structural parameters vary with a pseudo-periodicity of around 10 bp, and in the considered interval, there is only one linker length which produces a straight flexible fibre with regularly positioned nucleosomes. In a subsequent paper, they have tried to reconcile this finding with the fact that fibres with different repeat lengths exist, by suggesting that a change of the linker length can impose conformational changes on the core particle (Mozziconacci and Victor 2003). However, there is no experimental evidence for alternative core particle conformations.

Conclusions

The arrangements proposed here are, to date, the only ones that do not contradict the available experimental data from native chromatin fibres. They predict that there must be two different structures for short and long repeat length chromatin, as observed in native and in reconstituted fibres and that one linker histone type can support fibres built from several different repeat lengths. These structures suggest alternating nucleosomes, and therefore, explain the dinucleosomal repeat in the DNase I digestion patterns of fibres with different repeat lengths. The (−3,5) arrangement is the only proposed structure which can accommodate different repeat lengths outside the ‘10n’ bp register. To unequivocally decide between models of the fibre, further experimental work needs to be carried out to ascertain the consecutivity of the nucleosomes and the path of the linker DNA. This is crucial for addressing the problems of interactions of the fibre with transacting factors that might cause its unfolding for transcriptional competence.

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