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Role of linker histones in extended chromatin fibre structure

Modelling and scanning force microscopy studies provide insight into the organisation of chromatin fibres

Sir — For nearly two decades, the three-dimensional structure of the chromatin fibre has been the subject of both extensive experimentation and speculation[1]. Perhaps most interesting to molecular biologists is the extended conformation of the fibre that is presumed to exist in transcribed regions. Structures with an extended conformation can be obtained under low salt conditions *in vitro*, and the fibre is especially extended in the absence of the lysine-rich 'linker' histones—H1, H5, H1°, etc. as shown by electron microscopy studies (for example refs 2, 3)

To analyze the role of the lysine-rich histones and other determinants of the extended chromatin fibre structure, we have used atomic force microscopy (SFM)[4], also known as atomic force microscopy (SFM) and molecular modelling. In the last three years, several groups have shown that SFM can be a powerful tool of structural analysis of biological materials under conditions that largely preserve their integrity (for reviews, see refs 5, 6). In particular, several SFM studies of chromatin fibres have appeared recently[7–10].

The histone octamer in itself is capable of wrapping 146 bp of DNA in approximately 1.75 left-handed superhelical turns; the lysine-rich histone, when present, appears to interact with an additional 20 bp of DNA[1]. It has been suggested by many researchers that the role of the lysine-rich histones is to bind to the DNA entering and exiting the nucleosomes (see, for example, refs 3, 11). Support for the idea that H1 interacts with the DNA cross-over at the nucleosome has come from studies of binding to superhelical DNA[12,13] and to synthetic four-way junctions[14,15].

The structure of the chromatin fibre is likely to be determined, in part, by the linker histones which may fix the angle between successive linkers and determine the length of the DNA wrapped around the octamer. Excluded volume effects, the torsional and bending rigidity of the linker DNA expected at low ionic strengths, and the fact that the DNA cannot rotate freely on the surface of the octamer due to restricted surface interactions between the octamer and its associated core-particle DNA[16], should also impose additional restrictions on the position and relative orientation of adjacent nucleosomes in the fibre[9,17]. Under

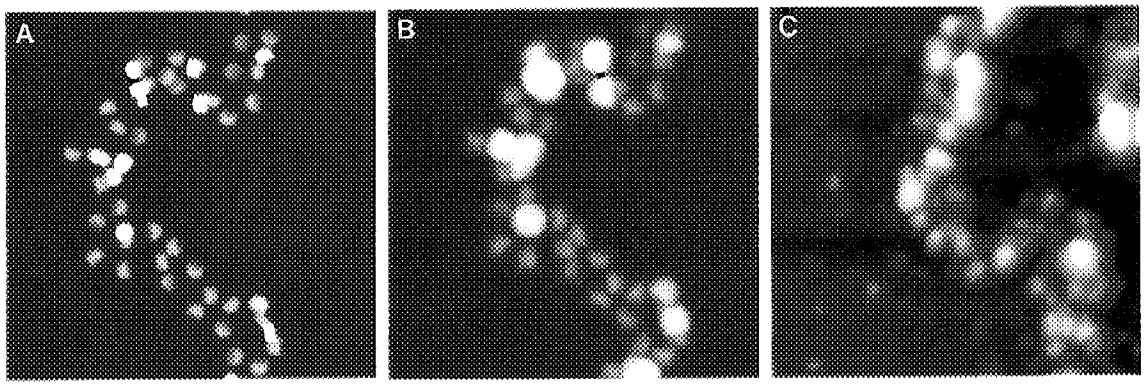

Fig. 1 Simulated and experimental SFM images of native chromatin fibres. *a*, The computer-generated image of a model chromatin fibre. The nucleosome core is modelled as a disc of 5.5 nm high and 11 nm in diameter, which includes 1.75 turns of DNA wrapped around a histone octamer in a left-handed fashion with a pitch of 28.6 Å. The exit angle of the DNA is determined by the tangent at the point it leaves the nucleosome. The linker DNA is assumed to be straight between nucleosomes. The length of the linker DNA varies between 51 and 73 bp, which is determined using a uniform deviate random-number algorithm. The volume exclusion effects are taken into account by assuming a spherical hard-core potential with a radius of 10.2 nm around each nucleosome. A smaller potential sphere leads to non-fibre-like structures. A helical repeat length of 10.15 bp perturn is used for the DNA wrapped around the histone octamer, whereas 10.40 bp per turn is used for the linker. To generate the image, each nucleosome in the model fibre is projected onto a plane without changing its orientation. The plane is chosen such that the sum of its distances to all the nucleosomes in the fibre is a minimum. *b*, Simulated SFM image of the model chromatin fibre in '*a*'. To simulate the imaging process in SFM, the model was scanned and partially flattened by a parabolic tip with a radius of curvature of 10 nm. *c*, Experimental SFM image of a glutaraldehyde-fixed chromatin fibre deposited on mica in 5 mM TEA-HCl, pH 7.0 [4]. The measured height of the nucleosomes is around 3 nm, which determines the degree of flattening for the model fibre in *b*. All images are 250 nm x 250 nm in size.

these constraints, a regular linker length will prescribe a well defined helical structure[17,18]. However, linkers are rarely uniform, even locally, therefore most chromatin should adopt irregular, distorted helical structures at low ionic strength[8,9,17].

The kind of structures obtained by computer modelling based on the above-mentioned constraints and variable-length linkers are shown in Fig. 1*a*. Even a narrow distribution of linker lengths leads to irregular structures (not shown); increasing the breadth of the distribution to values closer to those measured experimentally (for example, ref. 19) enhances the irregularity and produces more compact structures (Fig. 1*a*).

Fig. 1*b* depicts a simulated SFM image corresponding to the fibre in Fig. 1*a*. This image is to be compared with experimental images obtained in tapping-mode SFM studies of fixed chicken erythrocyte chromatin (Fig. 1*c*). As described in detail elsewhere[8,9], the SFM experiments demonstrate that at low ionic strength, chromatin fibres do not exist as extended beads-on-a-string, nor as flattened zig-zags, but as three-dimensional, irregular helices.

What happens if the lysine-rich histones are removed? According to the assumption that the linker histones fix the DNA entry/exit angle (θ), removal of these histones should relax the constraints on θ and change the wrapping of the DNA about the histone core. With 1.75 turns, θ has the value of 90°. An angle of 180° would result from either one or two complete turns around the octamer: relaxing to one turn would lead to an increase in linker length of about 60 bp over that found for 1.75 turns, whereas formation of two complete turns would lead to a decrease of about 20 bp in linker length.

The computer modelling of H1-depleted fibres assumes that on removal of this histone, the DNA wrapped around the octamer can vary continuously between the extremes of one and two turns. Two turns is probably the longest DNA stretch that can contact the helical ramp of the histone octamer, whereas one turn would be expected if binding to H2A and H2B is weaker than binding to the $(H3.H4)_2$ tetramer[16]. The structure predicted on the basis of this assumption is illustrated by a computer-generated model of an H1-depleted chromatin fibre (Fig. 2*a*) and its simulated SFM image (Fig. 2*b*). For comparison, Fig. 2*c* shows experimental SFM images of chicken erythrocyte chromatin, depleted of histones H1 and H5 by a method expected to produce negligible nucleosome sliding[20]. Both the simulated and the experimental SFM image exhibit the typical 'beads-on-a-string' structure characteristic of stripped chromatin[1,3].

The distribution of internucleosome (centre-to-centre) distances has been used to quantitatively estimate the length of DNA wrapped around the histone core in the absence of the linker histones for both the model and the experimental data. The insets in Figs 2*b* and 2*c* show a remarkable similarity between the distributions in the model (mean 26.4 nm) and the experimental data (mean 31.4 nm). Compared with the mean value of ~22 nm characteristic of native chicken erythrocyte chromatin (62 bp linkers), these results indicate that H1 removal leads to a release of the DNA from the histone cores and the formation of longer length linkers. There is no evidence for closer packing of nucleosomes, as would be expected if two turns are occupied on successive nucleosomes, or if extensive nucleosomal sliding occurred. The data support the idea that the linker histones fix the entry-exit angle of the DNA at the nucleosome and indicate that, in the absence of linker histones, as much as one turn of DNA can unwrap from the octamer.

These results show that at low

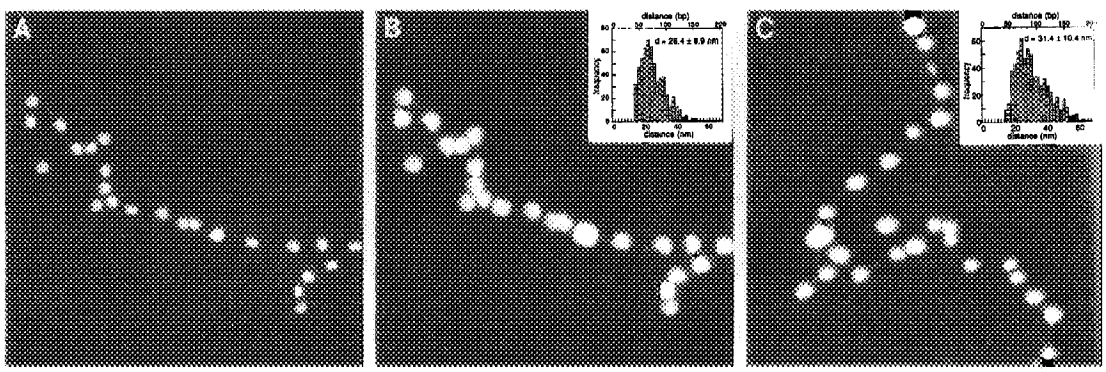

Fig. 2 SFM images of linker histone-depleted chromatin fibres. *a*, A computer-generated model of a linker histone-depleted chromatin fibre. The simulation is the same as in Fig. 1, except that the number of turns of DNA around the octamer is allowed to vary randomly between 1 and 2. *b*, Simulated SFM image of the fibre in *a* after it has been scanned and partially flattened by a parabolic tip with a radius of curvature of 10 nm. *c*, Experimental SFM image of a glutaraldehyde-fixed, H1/H5 depleted chromatin fibre deposited on mica in 5 mM TEA-HCl, pH 7.0[4]. All images are 400 nm x 400 nm in size. The insets show the distributions of centre-to-centre distances of adjacent nucleosomes along the DNA path for the kinds of fibres as shown in *b* and *c*, respectively. About 700 measurements were made for each histogram. The maxima of the distributions are similar (23 nm for the calculated and 25 nm for the experimental data). Comparing with the experimental data, the simulated fibres have a shorter mean internucleosome distance. This is probably a result of the simple projection method used here to simulate the deposition of the chromatin fibres onto the mica surface. The mean inter-nucleosome distance of the model fibres before their projection onto the plane (29.9 nm) is very close to the experimental result.

ionic strengths chromatin fibres exist as irregular, three-dimensional structures. These structures are determined by the natural variability in linker lengths, a number of conformational constraints in the DNA, and the presence of histone H1. Removal of one or more of these constraints leads to significant structural transitions in the fibre. H1-depletion changes the fibre from an irregular three-dimensional helix to a completely extended 'beads-on-a-string' structure. Similarly, factors that reduce the nucleosome-nucleosome repulsion and/or the stiffness of the linker DNA will lead to increasingly compacted structures.

In vivo, the transition into extended structures may occur in transcriptionally-active chromatin fibres which must be more extended than bulk, inactive chromatin, to allow the access of regulatory factors and enzymes to the underlying DNA template. This transition may be obtained by mechanisms involving H1 removal (reviewed in ref. 21), histone modifications such as acetylation, phosphorylation or methylation[1], and alterations in the non-histone protein complement (for example ref. 22). The *in vitro* studies described here using low-ionic strength can be viewed as a simplified model of these structural transitions. Furthermore, the three-dimensional extended structures observed in these studies suggest a simple compaction mechanism by which the fibre may attain its next level of organization.

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