The helical model of the nucleosome core

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ABSTRACT

A model of the nucleosome core is proposed based on a topologically linear array of histones attached sequentially to DNA. The linear complex folds helically forming a spring-like particle. Different variants of the particle are discussed (cylindrical springs with and without histone-histone contacts between turns of the helix, solenoidal spring). The model is consistent with known data about the nucleosome structure. Histones H3 and H4 have a special role in the model which is related also to the superstructure of chromatin.

The model of a nucleosome containing around 200 base pairs of DNA and two sets of four histones, H3, H4, H2A and H2B proposed by R.Kornberg¹ has turned out to be very fruitful and correct in its major features. The investigations of the last few years enable us to suggest a more detailed model of the nucleosome. We propose here a model of the nucleosome core in which the interacting histones attached to the DNA form a linear array (1st level of organization) and the DNA-histone complex folds helically (second level) resulting in the nucleosome core.

The reasoning leading to the model is presented below.

LINEARITY

The concept of a linear arrangement of histones in the nucleosome has been discussed earlier by D'Anna and Isenberg² and by Simpson³. It is based on investigations of different histone-histone interactions in solution^{2,4,5} and on the fact that only histones H3 and H4 can be covalently bound to the 5'-ends of DNA in the nucleosome core.³ In Table I the known association constants for different pairs of histones estimated for ionic strengths 0.003-0.2 are presented. From the table it is evident that the complexes H3-H4, H4-H2B and H2B-H2A are definitely stronger than all the other complexes. The

relative strengths of the complexes are not highly dependent on the ionic strength (see footnote to Table I). Of course interactions determined in solution are not necessarily equivalent to interactions of histones in chromatin. However, histone complexes are capable of combining with DNA to generate structures apparently identical with the nucleosome (see review⁶).

Many attempts with various crosslinking agents were made to find out which of the histones in the nucleosome are in close contact with each other. The results of these experiments with chromatin, nuclei or whole cells, are presented in Table II. Again, the poor representation of some contacts in the table does not mean that these contacts do not exist in the nucleosome. On the other hand, some of these crosslinks probably are artifacts.

The proximity of some of the histones to each other in the nucleosome has been demonstrated by other, nonchemical, data. Martinson *et al.*²⁰ have shown that the complex H2A-H2B associated with DNA is specific. It forms a UV crosslinked dimer only if the complex is preformed in solution. Sequential addition of these two histones to DNA followed by UV-irradiation does not lead to crosslinking of the histones. The proximity of H3 to H4 in the nucleosome was also demonstrated^{3,14}.

Comparison of Tables I and II evidently shows that strongest in-

Table I: Histone-Histone Interactions in Solution

H3	H4	H2B	H2A	
medial ⁷	$0.7 \times 10^{21} \text{M}^{-3}$ (tetramers ²) strong ⁷	weak ² strong ⁷	$10^5 - 10^6 \text{M}^{-1}$ (2) weak ⁷	H3
	medial ⁷ $1.5 \times 10^4 \text{M}^{-1}$ (8) $\sim 10^3 \text{M}^{-1}$ (9)	strong ⁷ 10^6M^{-1} (2) $0.6 - 3 \times 10^6 \text{M}^{-1}$ (5)	weak ⁷ $4 \times 10^4 \text{M}^{-1}$ (4)	H4
		weak ⁵	strong ⁷ $\sim 10^6 \text{M}^{-1}$ (2) $2 \times 10^6 \text{M}^{-1}$ (10)	H2B
			—	H2A

Conditions:

(2) - pH 7, I = 0.016; (4) - pH 7, I = 0.007; (5) - pH 7, I=0.003-0.24;
(7) - pH 7, I = 0.15; (8) - pH 7.4, I=0.007; (9) - pD 3.7-9.6, I=0.05
(10) - pH 7, I = 0.016

teracting pairs of histones (H3-H4, H4-H2B and H2B-H2A) are also in close proximity to each other in chromatin, as is demonstrated in each case by 5-6 crosslinking agents of different type. Therefore, it seems a reasonable assumption that just these close, strong contacts determine the inner structure of the nucleosome core. It is remarkable that the only way to arrange the four histones proceeding from the assumption that only the contacts mentioned above are essential is the linear one: H3-H4-H2B-H2A. This heterotetramer contributes one of the two sets of the four histones in the nucleosome¹⁶. Supposing that all eight histone molecules in the nucleosome are arranged in a linear sequence, we can attach the second set to the first one in the same or

Table II: Close Contacts of Histones in Nucleosome

H3	H4	H2B	H2A	
MMB ¹¹ Sub ¹² MMPI ¹³	MMB ¹¹ Sub ¹² MMPI ¹³ CH ₂ O ¹⁴ CD ¹⁵	MMB ¹¹	MMB ¹¹	H3
	MMB ¹¹	MMB ¹¹ Sub ¹² MMPI ¹³ CH ₂ O ^{16,17} TNM ^{18,19} GA ¹⁷	MMB ¹¹ Sub ¹² MMPI ¹³	H4
		MMB ¹¹	MMB ¹¹ Sub ¹² MMPI ¹³ CH ₂ O ^{16,17} GA ¹⁷ UV ^{19,20}	H2B
			MMB ¹¹	H2A

MMB - Methyl-4-mercaptobutyrimidate; Sub - Dimethyl suberimidate;
MMPI - Methyl-3-mercaptopropionimidate; CH₂O - Formaldehyde;
TNM - Tetranitromethane; CD - 1-Ethyl-3-(3-dimethylamino-propyl)
carbodiimide; GA - Glutaraldehyde; UV - Ultraviolet light.

in reverse order. As has been demonstrated by Simpson,³ only histones H3 and H4 (not H2A or H2B) are positioned close to the ends of nucleosomal DNA. Thus a reasonable way to combine two linear tetramers together is H3-H4-H2B-H2A-H2A-H2B-H4-H3, that is their symmetrical head-to-head combination.

There is experimental evidence supporting the linear scheme. Martinson and McCarthy¹⁹ found that after treatment of cells by UV-light and by tetranitromethane, the crosslinked trimer H4-H2B-H2A is formed having two different sites of attachment of H2B to H4 and to H2A. Chalkley showed that after reaction of chromatin with dimethyl-suberimide²¹ practically only dimers and oligomers of H2B and H2A are formed without measurable crosslinking of H3 and H4. It means that histones H3 and H4 and histones H2A and H2B are arranged in the nucleosome as spatially separated groups. This is in accordance with the proposed linear arrangement. After salt extraction of the histones H2A and H2B from chromatin the complex is left, DNA-H3-H4, which yields only dimers H3-H4, after treatment with formaldehyde at low ionic strength.¹⁵ At higher ionic strength, the treatment leads to formation of higher oligomers of the histones. That is at low ionic strength histones H3 and H4 are probably combined with DNA as a dimer separated by stretches of DNA previously occupied by histones H2A and H2B. Treatment of chromatin by trypsin leads to preferential hydrolysis of histones H3²²⁻²⁴ and then, tandemly, H4.²⁴ The longest micrococcal nuclease limit-digest fragments of DNA from trypsinized chromatin disappear simultaneously.²⁴ Since histone-histone contacts offer protection against trypsinization of histones,²⁴ the order of hydrolysis (H3, H4 and then H2B, H2A) also supports the proposed linear scheme.

As most of the histones in the nucleosome (if not all of them) are associated with DNA,^{20,25,26} a natural way to make a complex between the linear array of histones and DNA is again the linear one, i.e. a parallel complex with DNA attached alongside the chain of histones.

THE FOLDING OF LINEAR DNA-PROTEIN COMPLEX

We suppose that in the nucleosome core the DNA-histone complex described above is folded helically, forming a spring-like particle. It is drawn schematically in Fig.1. The structure is a cylindrical spring with ~1.5 turns of the helix corresponding to 140 base pairs of nucleosome core DNA with its axis wrapped around a cylinder with

Fig.1: Nucleosome core as a cylindrical spring (scheme). The thick gray thread corresponds to DNA. Histones in the linear sequence H3-H4-H2B-H2A-H2A-H2B-H4-H3 are attached to DNA from inside and form a helix with the same pitch as DNA. Dimensions of different histone molecules are taken equivalent for simplicity. A - original helical model. B - cylindrical spring model in compressed form.

a 100 Å diameter. The geometry of the deformed DNA is chosen to be the same as in a model of DNA smoothly packed in the nucleosome.²⁷ It is also close to the helical kinked model for packing DNA.^{28,29} Both are acceptable for the proposed model of the nucleosome core. A topologically linear sequence of histones become helical, possibly because of the interactions between neighboring histone molecules. The average distance between positions of histones on DNA in the linear array is ~ 60 Å (1/8 of the length of 140 base pairs of DNA). But the diameter of a globular histone molecule with the average molecular weight taken equal to 13500 and specific volume 0.73 ml/g³⁰ is around 30 Å. Probably just bringing the molecules into close contact leads to bending of the DNA molecule attached to them.

An important feature of the spring shown in Fig.1A is that two molecules of histone H4 opposite to each other can be brought into contact by a deformation (bending) of the cylindrical spring, conserving the curvature of the DNA axis and overall dimensions of the model. As a result a tetramer H3-H4-H4-H3 could be formed (Fig.2). A tetramer with such a composition, but of unknown structure, plays a dominant role in the reconstruction of the nucleosome (see review⁶). A similar spring model with slightly changed geometry can provide an even more close association between opposite dimers H3-H4 and H4-H3

Fig.2: The nucleosome core as a solenoidal spring (scheme). The thick gray thread corresponds to DNA. A and B - two different views of the same structure.

with two histone-histone interactions. There are some experimental data, however, indicating that the two dimers in the tetramer $H_3H_4_2$ are kept together via one contact rather than two. It was shown that the association constant for the two dimers H_3 - H_4 in the tetramer is $\sim 10^5 M^{-1(7,13)}$ which is the same order of magnitude as the constant of aggregation of histone H_4 ($1.5 \times 10^4 M^{-1(8)}$). On the other hand, crosslinking experiments did not until now definitely reveal the contact $H_4 \cdot H_4$ and that favors the two-contact tetramer. In both cases, with one contact or with two contacts between dimers, a resulting tetrameric structure is far from spherical as it should be^{16,20}. The two contacting dimers H_3 - H_4 with the ends of the nucleosome core DNA attached to them probably maintain the helically bent molecule in the same or close configuration as in the nucleosome, without the other 4 histone molecules of H_2A and H_2B ³² (see also³³).

There is also another interesting feature of the proposed structure related to superstructure of chromatin. As was demonstrated by Sperling and Amos³⁴ the histone H_4 forms in solution helical fibers seen in the electron microscope. Optical diffraction patterns from the micrographs of the fibers have spacings typical for chromatin. Such helical histone fibers in chromatin, as suggested³⁴, may be important for the formation of the 300Å-diameter solenoids described by Finch

and Klug³⁵. The model particle shown in Fig.2 with an extra ~50 base pairs added to two full turns of DNA (~190 base pairs) seems to be a good candidate for an elementary repeating unit, forming the regular superstructure of chromatin. H4 histone molecules of each nucleosome in this case supposedly occupy the inner surface of the 300Å-diameter solenoid. As a result, the continuous helix of histones H4 could be formed similar to the one described³⁴. Histones H2B and H3 (see Fig.2) possibly also form heterologous continuous helices similar to known fibers of the histones.⁷

As estimated by Kornberg⁶ the DNA and histones in the nucleosome make up only approximately 1/3 of the space occupied by the whole nucleosome. Taking into account the volume which corresponds to a supposed central hole of the particle^{33,36-38} and considering histones as typical globular proteins, the distance between adjacent histone layers in the cylindrical spring model can be estimated as ~25 Å. It gives enough space (~50%) to compress the spring-like particle to ~1/2 of its height (Fig.1B). Possibly in some conditions of crystallization or preparation for electron microscopy³³ such compression takes place. This compact form of the nucleosome can be stabilized by interlayer histone-histone interactions. Half-emptiness of the nucleosome in solution was confirmed recently by Voordouw and Eisenberg³⁹ who showed that approximately the same quantity of protein as was present can be packed in the nucleosome, apparently without changing its overall dimensions.

Recently Finch et al.³³ made a thorough and revealing analysis by X-ray diffraction and electron microscopy of crystals of nucleosome cores. They calculated the first electron density map of the nucleosome core, and certainly any new model of the nucleosome should be compared with their data. Unfortunately, the supposed wedge shaped nucleosome core seen on the low resolution map³³ does not give sufficient grounds for any clear judgement on the helicity of the nucleosome. Close examination of the electron density map, however, shows that the maxima of electron density are situated at approximately regular distances along parallel lines. The lines are apparently arranged pairwise and shifted relative to each other by about half the intermaxima distance. Such a pattern is characteristic for density projections of helical structures and that indicates a possibility of alternative interpretation of the data presented in Ref.33 (in preparation).

The helical packing not only of DNA but also of histones has been mentioned in Ref.33 as one possibility (see also Refs. 34, 40 and 41).

Three models of the nucleosome core described above (cylindrical spring, Fig.1A; compressed cylindrical spring, Fig.1B; solenoidal spring, Fig.2), present different states of the same helical structure. Probably nucleosomes also exist in different states depending on ionic strength, compaction of chromatin and interactions with macromolecules. The structural mobility is also an essential feature of the proposed helical model of the nucleosome core.

Both cylindrical and solenoidal models have an axial symmetry, as also predicted by Weintraub *et al.*⁴²

OTHER RELEVANT FEATURES AND SOME PROBLEMS

Electron microscope investigation of the unfolding of chromatin at high concentrations of NaCl³⁷ shows circular structures which possibly correspond to the intermediate stage of unfolding of the helical nucleosome into a linear complex. A similar unfolding in urea was also reported.⁴³ Such unfolding can be realized probably, by just mechanical stretching of the spring-like structure. As was demonstrated by Pardon *et al.*⁴⁰ such mechanical stretching of chromatin leads to the disappearance of its low angle X-ray diffraction pattern and it is restored after taking the stress away.

A simple organization of the DNA-histone complex in the nucleosome as described above provides both compact folding of the DNA and histones and the linearization of chromatin, possibly important for the processes of replication and transcription. In the unfolded nucleosome core model of Weintraub *et al.*⁴², the histones are attached to DNA as tetramers. Such tetramers are possibly bound to DNA via several attachment sites that could prevent unwinding of DNA during its replication or transcription. The proposed topologically linear model in its extended form is free of hindrances of this kind. Possibly in the unfolded nucleosome each of the separated histones has one site of attachment to DNA and they stay attached to it during the processes.

There are, of course, several unpleasant questions to be answered. Does the comparatively strong complex H3-H2A exist *in vivo* (see Table I) and if yes, how can it be explained on the basis of the model? Contacts H3-H3 (see Table II) may correspond to internucleosomal interactions but how can the contact H4-H2A be explained if it is not an artifact? Why do the pairs H4-H4 and H2A-H2A which the model predicts prac-

tically never crosslink?¹¹ The last pair corresponds to the contact between two heterotetramers¹⁶ composing the nucleosome and does not necessarily have to be strong, if the heterotetramers are to be dissociated under some conditions or during some in vivo process⁴². It is possible also that two molecules of H2A in the nucleosome are separated by a small region of DNA not bound to the histones or by non-histone protein.²⁶ Further investigation will shed light on these questions.

Some of the features of the proposed model (but not their combination as described above) are characteristic also of many models of the nucleosome proposed earlier.^{16,26,34,36,40-42,44,45} The present new helical model of the nucleosome core appears as a logically consistent superposition of these models and an attempt to extend Kornberg's model.¹

We hope that the proposed structure will presently serve as a reasonable working hypothesis.

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