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Asymmetric lateral distribution of unshielded phosphate groups in nucleosomal DNA and its role in DNA bending

(electrostatic charges/DNA flexibility)

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ABSTRACT **We suggest that an asymmetric charge neutralization of DNA phosphate groups produces part of the driving force for nucleosome folding. In nucleosome core particle DNA, many of the phosphate groups are neutralized by histones, and a lateral alignment of these histones along the core DNA has been demonstrated [Mirzabekov, A. D., Shick, V. V., Belyavsky, A. V. & Bavykin, S. G. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4184–4189]. Histones appear to shield DNA phosphates asymmetrically at one side of the surface of the DNA double helix along all its length inside the core. The external side of the DNA helix remains unneutralized. The electrostatic repulsion between negatively charged unneutralized phosphates may fold the nucleosomal DNA towards the side occupied by histones.**

The DNA in the nucleosome core particle is about 140 base pairs in length, and there are eight histones in the particle: two molecules each of histones H2A, H2B, H3, and H4. There is evidence from various studies that in the core particle the DNA makes approximately 1–1.75 superhelical turns around the outside of the histone core. These results come from neutron scattering studies (1, 2), x-ray diffraction and electron microscopy of nucleosome crystals (3), measurements of superhelical coiling of DNA after the protein is removed (4, 5), and nuclease digestion experiments (6–9).

Among the problems facing us in understanding the coiling of DNA in nucleosomes are the manner in which DNA complexed with histones is folded and the nature of the driving force for this folding. Several models have been proposed for the way in which DNA folding occurs in the nucleosome. These include bending towards the minor groove of the double helix as proposed by Crick and Klug (10), a bending towards the major groove as proposed by Sobell *et al.* (11), and, more recently, the possibility of continuous deformation of the DNA molecule giving rise to a smooth bending as suggested by Sussman and Trifonov (12) as well as Levitt (13). Camerini-Otero and Felsenfeld (14) have estimated that the energy involved in the specific interactions between pairs of histones bound to DNA in the nucleosome would be adequate to account for the folded conformation of the DNA. In considering this problem, we have been struck by the potential driving force that may accrue in a system in which there is an asymmetric neutralization of phosphate groups along one side of the helical surface of the DNA molecule, which has been found in nucleosome core particles (15). This asymmetric neutralization may be a significant component of the coiling of the DNA double helix and in its function.

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Neutralization of phosphates in double-stranded nucleic acids increases their flexibility and folds them

Electrostatic interactions play an important role in determining the conformation of double helical DNA. In solution the molecule is largely extended and maintains a rigid straightened conformation in great part because of the balanced electrostatic repulsion of the charged phosphate groups on two coiled complementary polynucleotide chains (16). Neutralization of phosphates with monovalent cations progressively increases DNA flexibility (17). Upon more effective neutralization of DNA with polycations—spermine (18), spermidine (19), polylysine (20, 21), or histones (22)—DNA collapses into a folded toroidal structure or into some other forms. Furthermore, upon concerted charge neutralization and dehydration by ethanol DNA collapses into beaded nucleosomelike fibers (23). Some experimental data and the theory of DNA flexibility induced by neutralization of its phosphates have been discussed in detail by Manning (16).

Another example is seen in the three-dimensional structure of yeast phenylalanine transfer RNA. Two spermine molecules have been identified in the lattice, and one of them fits in the major groove of the anticodon stem near the point at which it joins the dihydrouridine stem (24). The spermine molecule arches across the major groove neutralizing phosphate residues on both sides of the major groove. This asymmetric neutralization of the phosphate residues is associated with two ribose-phosphate chains on either side of the major groove coming closer together than in a normal double helix. This produces a bend in this part of the molecule. Thus, the axis of the anticodon stem is bent 25° away from the axis of the adjoining dihydrouridine stem. In this case it is likely that the asymmetric neutralization of four phosphate groups on one side of the double helical structure has given rise to a bending of the molecule associated perhaps with increased flexibility of the neutralized double helical segment.

Lateral alignment of histones along DNA in the nucleosome core

Recently it has been demonstrated that histones are laterally aligned along the DNA in the nucleosome core particles (15). With techniques that involve the crosslinking of histones to DNA, the arrangement of histones along the core DNA has been determined. Fig. 1 demonstrates this arrangement. Histones were shown to be attached to DNA along regularly spaced, discrete DNA segments of about five to seven nucleotides in length. Between these discrete DNA segments covered with histones there are gaps or segments of DNA in which the sugar-phosphate chains are not crosslinked to the histones. These

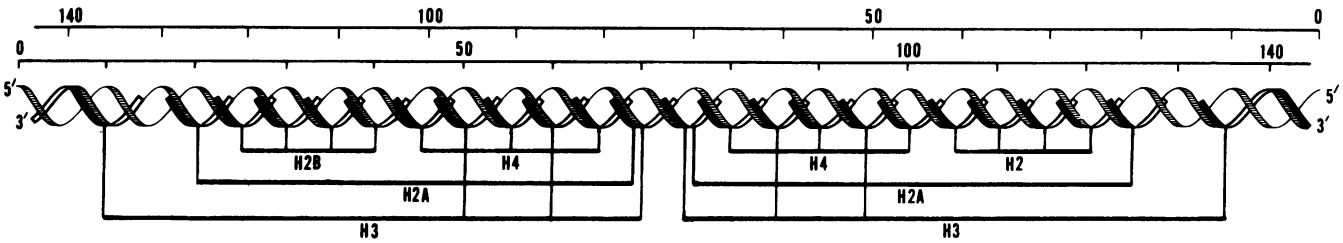

FIG. 1. A model of the arrangement of histones along double helical DNA in the nucleosome core particle (see ref. 15 for details). The solid black and white bands correspond to histones. The numbers indicate the distance in nucleotides from the 5′ end of each DNA strand. Histones H2A and H3 both interact with DNA segment 70–80. Histone H4 in DNA segment 80–90 is omitted, because H4 seems to interact simultaneously with both DNA strands there. The histones interact with DNA from the side of the major groove and neutralize DNA phosphates on the bottom side of the diagram. Histone-free gaps are all located on the top part of the DNA helix.

gaps are separated by 10 nucleotides and are located at distances of about $10 \times n$ nucleotides from the 5′ ends of the core DNA, where n is an integer. The position of the gaps corresponds to the sites on the core DNA that are susceptible to the action of various nucleases (6–9). Taken together, these data strongly suggest the existence of regions of DNA that are regularly spaced along nucleosomal DNA and cannot be crosslinked to histones but are accessible to nuclease action and are, therefore, not covered with histones. The striking feature of these crosslinking experiments is that all of the histones appear to be located on one side of the DNA double helix. On the other side of the helix there are a series of uncovered histone-free gaps of DNA that maintain their normal negative electrostatic charge. These histone-free gaps appear to face outward towards the surrounding medium, whereas the DNA complexed to histones is folded into the nucleosome.

Here we are concerned with the effect of the asymmetric distribution of these unshielded charged segments of DNA and the role it may play in the bending of DNA around the nucleosome particle. In Fig. 1 nucleosome DNA is shown schematically with attached histones represented by broad bands. The DNA segments without attached broad bands represent DNA in the gaps which maintain their normal charge. The position of histone-free gaps has been determined in the crosslinking experiments with an average precision of about ±3 nucleotides (15). The gaps could thus be shifted by a few nucleotides from the positions shown in Fig. 1. The gap lengths correspond to about three to five nucleotides. The latter value can be estimated in another way by considering the number of charged groups in the histones and in the DNA.

The stoichiometry of the balance of charged groups in nucleosomes is outlined in Table 1. In core DNA, there are 145 base pairs (15) and thus 290 negative charges on the phosphate groups. The histone octamer of calf thymus contains a total of about 224 positive charges (lysine, arginine, and NH_2-terminal residues) and 82 negative charges (glutamic acid, aspartic acid, and COOH-terminal residues) giving rise to a net positive charge of 142. The known primary structures of histones have been reviewed (25). In the crosslinking experiments it was shown that the 20 nucleotides located at each of the 5′ ends of the nucleosomal DNA are not crosslinked to histones and appear to be unshielded segments in the nucleosomal DNA. If we exclude the 20 phosphate groups at each of the 5′ ends, we have a total of 250 negative charges in the remaining segment. Since there is a net charge of +142 in the histone octamer of nucleosomes, this leaves a maximum number of 108 phosphate groups that are not neutralized by positively charged histones. This estimate is based on the simple assumption that all the positively charged groups in the histones that are not neutralized by the negatively charged carboxyl groups in the proteins are available to neutralize the phosphate groups. This has been demonstrated for lysine residues of histones in chromatin (26). Our calculation is thus an upper limit calculation. There are approximately 14 double helical turns of DNA and 24 unshielded histone-free gaps in Fig. 1. This rough approximation suggests that there may be four or five negatively charged phosphate groups in each of the DNA gaps that are not shielded by the histones. The estimate of four or five is clearly an upper limit; the number of unneutralized phosphates may be less than four or five in each group, especially if not all of the phosphate groups are neutralized in the regions of contact with histones. Therefore the net electrostatic asymmetry may be less than the average number of 4.5 obtained from this rough calculation. However, it has been demonstrated that the actual cleavage regions in the core DNA that are accessible to the action of different nucleases span 4 out of each 10 repeating nucleotides (7). If, indeed, the four or five unshielded nucleotides form a continuous region along one side of the nucleosomal DNA, then the other nucleotides of the repeating unit in the lower part of the double helix (Fig. 1) should be completely shielded and should therefore acquire increased flexibility.

Table 1. Charged groups in protein and DNA of nucleosome core particle

Molecule	Number of charges
DNA (145 base pairs)	−290
Histone octamer:	
Total number of positive charges	+244
Total number of negative charges	−82
Net charge	+142
Histone-free regions in the first 20 nucleotides at each 5′ DNA end	−40
DNA in 24 histone-free gaps (excluding 20 nucleotides at each 5′ end)	−108
Average histone-free gap in DNA	≈4.5

DNA folding in the nucleosome

The asymmetric neutralization of phosphate groups in the core DNA caused by the lateral alignment of histones must change the balance of electrostatic repulsions responsible for DNA rigidity and should induce DNA bending. Fig. 2 illustrates such a bending of nucleosomal DNA and shows a short segment of the DNA complexed with histones. Histones are attached and neutralize the phosphates of the DNA helix along its bottom side. The histone-free gaps are tentatively arranged equally around the uncomplexed nucleotides located on the top of the helix. The unneutralized phosphate groups are shown as circled minus signs, which stand for four or five negative charges.

The lower part of the DNA helix covered with histones acquires flexibility as a result of charge neutralization as discussed above (16). The electrostatic repulsion of unneutralized phosphate groups located on the other, upper side of the DNA in Fig.

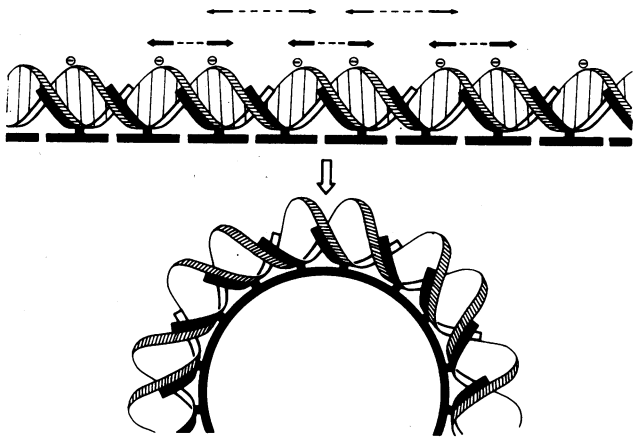

FIG. 2. Schematic diagram of bending of nucleosome core DNA induced by the asymmetric neutralization of DNA phosphates by histones. The bending is a result of the electrostatic repulsion between unneutralized DNA phosphate groups. The circled negative charges stand for four or five phosphate groups. The repulsion between unneutralized phosphates located on the complementary DNA strands is shown across the minor groove (⟶) and the major groove (→). This introduces a bending towards the major and the minor grooves, respectively, at these sites.

2, tends to bring about bending of the helix towards the opposite side covered by histones. The manner, curvature, and location of the bending would depend on many factors, in particular on the arrangement of unshielded phosphate groups. The repulsion between charged unneutralized phosphates located on the top side of two DNA strands and separated by the minor DNA groove (the heavier arrows in Fig. 2) will tend to induce folding into the major groove. The repulsion between the charges located across the major groove (lighter arrows) would cause the bending into the minor groove. The repulsion between DNA charges located in other DNA regions could, in addition, induce smooth bending.

If there are 1.75 superhelical turns of DNA around a nucleosome (3), then any model of DNA coiling should account for a total of 630° of bending. It has been suggested that this bending may be discontinuous by folding into the minor groove (10) or into the major groove (11), or it may also have a form of continuous deformation (12, 13).

It is, of course, impossible at present to estimate the relative amount of these different types of bending since it depends on the detailed pattern of charge neutralization, the organization of the histones, and the exact positioning of the unneutralized negative charges. However, if one were to make the assumption that the effectiveness of electrostatic repulsion across both DNA grooves is roughly comparable, this would lead to a model of segmental DNA kinking in which bending occurs into both the major and minor groove. As mentioned above, there are 24 histone-free gaps in the DNA giving rise to 25 positions where bending can occur into the major and minor groove. If the segmental bending of this type is equally distributed, then each bend will have a magnitude of about 25° in order to produce the total bending of 630°. It is interesting that this figure is very close to the 25° bend that is found in the tRNA molecule in which charges on both sides of the deep groove of the RNA double helix are neutralized by a spermine molecule (24).

Even though we have used an average figure of 25° for the bending into both the major and the minor groove, it is quite clear that there are many reasons for believing that such segmental bending is not likely to be equal into the major and the minor grooves. There are significant differences in the geometry of the grooves, and the distance between the repelling phosphate groups is not equal. According to Coulomb's law, the electrostatic repulsion is inversely proportional to the squared distance between charges. Since the distance between phosphates in double helical DNA on both DNA strands is shortest just across the minor groove, one might expect that the repulsion between them would dominate and could cause prevalent bending or kinking towards the major groove. Furthermore, the histones are found bound to the sugar-phosphate backbone of DNA on the side of the major groove, but not the minor groove (27). This suggests that segmental bending of this type is not likely to be equal throughout. It is also possible that the bending or kinking does not occur in a discontinuous fashion at one base pair but rather may extend over two or three successive base pairs. This would tend to introduce even a smaller discontinuity than the 25° cited above.

The thrust of our analysis is to stress the fact that the asymmetric neutralization of negatively charged phosphate groups along one side of the DNA double helix may provide a significant driving force for producing the bending of nucleosomal DNA. In the absence of additional information, it is difficult to make a selection among the various bending models. However, the detailed nature of the bending should eventually become apparent with further analysis.

One has to note that the resultant force of the repulsion between unneutralized phosphates in the core DNA might not coincide with the axis of the double helix but deviate from it. Such a deviation, if it exists, could direct the formation of the left-handed superhelix observed in the nucleosome.

The asymmetric electrostatic repulsion arising from the lateral neutralization of DNA phosphates by histones, together with specific histone–histone interactions, could be the main component of the delicate balance of forces involved in the folding of the nucleosome core DNA. Disturbance of this balance leading to nucleosome unfolding can be induced *in vitro* by low (28) and high (29) ionic strengths as well as by urea (30–31).

Functional implications

Nucleosomes appear to represent a first level in the compact organization of eukaryotic DNA. During transcription and replication of DNA some changes in nucleosome conformation are likely to occur. For example, no nucleosomal beads could be detected by electron microscopy in actively transcribed ribosomal genes in a number of organisms (32–35). The DNA in these genes is also slightly less extended than in normal, protein-free DNA. The disappearance of nucleosomes is likely to be due to their unfolding since this ribosomal DNA is still covered by basic proteins (32, 35). Inactivation of ribosomal genes is associated with the reappearance of nucleosomes (35, 36). These dynamic transitions between folded and unfolded conformations of nucleosomes during chromatin activation are likely to have a structural basis.

The nucleosome itself may be in a metastatic equilibrium between states in which the DNA associated with histones is folded into a nucleosome structure or opened up. We suggest that an important component of this equilibrium balance is represented by the asymmetric shielding of DNA phosphates, which will tend to produce the bending of the molecule. Elimination of the asymmetric neutralization would have an opposite effect. Thus, if additional unneutralized phosphates become distributed more or less randomly or symmetrically around the surface of the DNA helix, then the repulsion between these phosphates will have a tendency to straighten the nucleosomal DNA. Thus, a dynamic transition from asymmetric to random or symmetric neutralization of DNA by histones might direct the folding–unfolding processes in nucleo-

somes. This transition could be reached in many ways without significant rearrangement of histones or changes in histone–DNA interactions. It could occur by shifting histones along the DNA strands towards the histone-free gaps by only a few nucleotides, or by neutralization of basic residues in histones through modification (acetylation, phosphorylation). It could also occur by binding acidic nonhistone chromosomal proteins, RNA polymerase, low molecular weight chromosomal RNA, etc. Some of these processes appear to be important in regulating the chromatin activity. Finally, nucleosome unfolding might break the chromatin superstructure and thus lead to the dispersion of chromatin.

Specific histone–histone interactions could also participate in the nucleosome folding (14). In this case, disturbance of asymmetric neutralization of DNA might cause a nucleosome structure to be more susceptible to unfolding. For example, Weintraub *et al.* (37) have suggested a model of nucleosome unfolding which is based on specific histone–histone interactions between half-nucleosomes. The unfolding is induced by unpairing of half-nucleosomes which were believed to constitute a full nucleosome.

Further analysis should enable us to interpret more fully the role of asymmetric phosphate neutralization in nucleosomes.

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