THE DEPENDENCE OF THE LINKING NUMBER OF A CIRCULAR MINICHROMOSOME UPON THE SHAPE AND THE ORIENTATION OF ITS NUCLEOSOMES

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1. Introduction

It is now well established that a DNA molecule makes 1.5–2.0 left superhelical turns around an octamer of histones in the unit structure of eucaryotic chromatin, the nucleosome [1]. The formation of a chain of nucleosomes, a minichromosome, with histones H2A, H2B, H3 and H4 and circular, covalently closed DNA (ccDNA) in the presence of nicking-closing enzyme is coupled with the alteration of the linking number, i.e., the number of topological revolutions made by one strand about the other in the ccDNA molecule. The linking number is altered by −1 per one nucleosome [2,3] whereas the existing theory [4–6] predicts this change to be equal to the sum of turns made by DNA in each nucleosome, that is −1.7 per one nucleosome.

We have found out that the predicted change of the linking number is inherent in minichromosomes with parallel orientation of nucleosomal axes which is not consistent with real minichromosome structure. The linking number values for minichromosomes with constant and random angles between their nucleosomes are calculated here. The results obtained permit one to eliminate the contradictions between linking number theory and experiments.

2. Results and discussion

The linking number (Lk) of ccDNA is a topological invariant. It is related to the geometrical parameters of ccDNA by the following equation [4,5]:

$$Lk = Tw + Wr \quad (1)$$

where Tw (twist) represents the number of turns of DNA strands about the axis of the molecule and Wr (writhing number) depends only upon the shape of DNA axis. Their basic features have been investigated for the closed ribbon model by Fuller [4,5] and applied to nucleosomes by Crick [6].

When ccDNA is treated with nicking–closing enzyme it tends to accept the relaxed conformation with Wr = O and Lk = Tw but histones added stabilize additional topological linkings. The difference between the linking number of relaxed ccDNA and the one of nucleosomal ccDNA (ΔLk) is equal to −1 per one nucleosome [5,6]. Recent experimental data are consistent with the identity of twists for relaxed and nucleosomal ccDNAs [7–9], hence, ΔTw = O and ΔLk = ΔWr. The existing theory predicts ΔWr to be the sum of ΔWr values imposed by each nucleosome [4–6], i.e., be about $-1.7\,N$ (N is the number of nucleosomes in the minichromosome). However, the writhing number is not an additive value in minichromosomes with nonparallel orientation of nucleosomal axes. For instance, Wr of a pair of nucleosomes having 1.5 left superhelical turns of DNA in each varies from −1 to −3 depending upon the angle between them (fig.1). The angle between the ajacent nucleosomes (β) is a periodical function of the length of the internucleosomal linkers (L) owing to the helical nature of DNA:

$$\beta = 2\pi L/h^{\circ} \text{ modulo } 2\pi \quad (2)$$

Abbreviations: ccDNA, closed circular DNA; SV-40, Simian virus 40; Lk, DNA linking number; Wr, DNA writhing number; Tw, DNA twist

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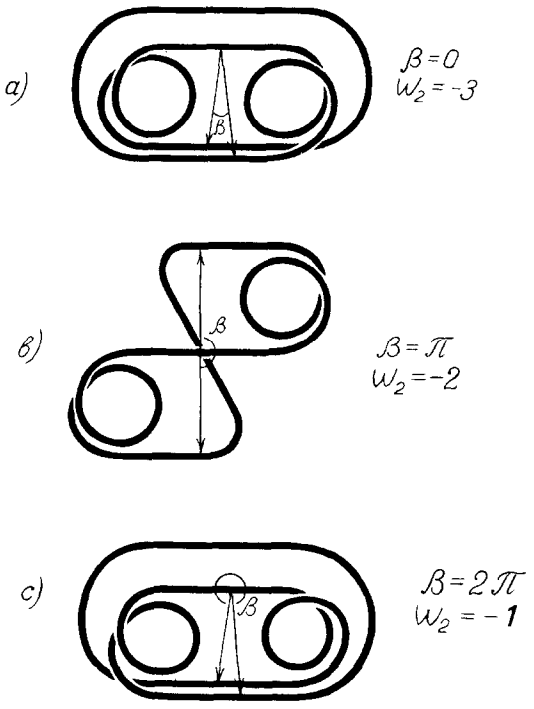

Fig.1. The DNA writhing number of the pair of nucleosomes depends upon their orientation.

where h° is the pitch of DNA double helix. We shall further estimate the writhing number for minichromosomes with constant and random β values. It is convenient to calculate Wr for the closed ribbon which has an axis coinciding with the axis of minichromosome DNA and, therefore, their writhing numbers are equal. We consider the ribbon to be as follows: its repeating unit is a spool which makes the same number of turns of the ribbon for the angle α as the number of superhelical turns of DNA in one nucleosome and the axis of the spool is parallel to the ribbon (fig.2a). The ribbon axis is straight between the ajacent spools but the ribbon itself has a twist equal to $\beta/2\pi$. Neither Lk of the ribbon nor its Tw correspond to those in a real minichromosome. We can easily calculate the Lk of the ribbon and then obtain the needed Wr by subtracting the known Tw value. At first we shall neglect the finite dimensions of the system, i.e., let $d \ll R \ll L$ (d is the diameter of DNA and R is the radius of the nucleosome). This assumption will be considered later.

The part of the ribbon's writhing number which originates from the number of self-intersections (M) made by the ribbon axis in the spool is additive and does not depend on the angle β. This part of the ribbon can be expelled from the whole one and its Wr (N . M) can be counted independently [5]. The angle α will consequentally change for the angle $\alpha' = \alpha - 2\pi M$, where α' varies from $-\pi$ to π. The Lk of the residuing helix is equal to the number of revolutions of the vector pair p, r along the circle on the unit sphere through the angle γ (fig.2b). The hand of the helix depends upon the angle β. One can easily obtain its linking number after simple geometrical calculations:

$$Lk = -N/2\pi \cdot 2 \arccos(\cos(\alpha'/2) \cdot \cos(\beta/2)) \qquad (3)$$

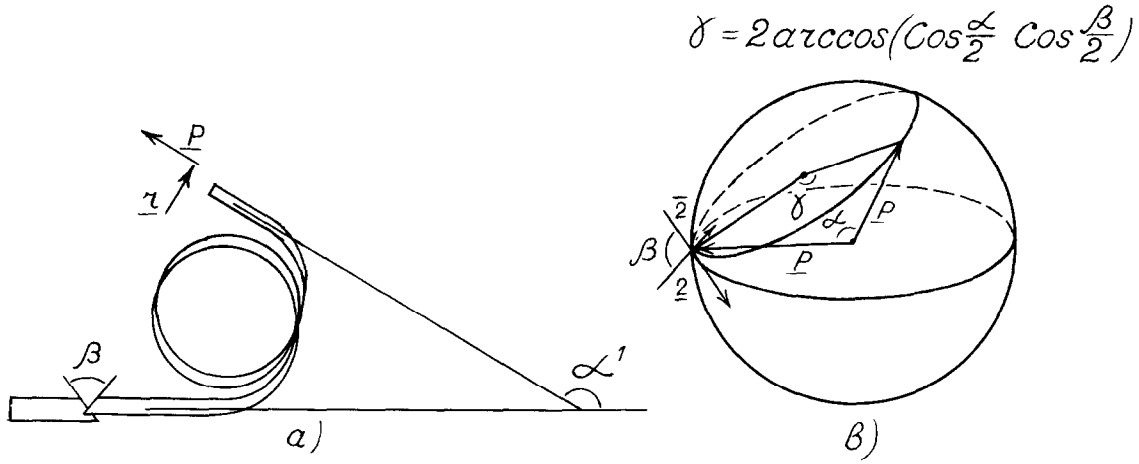

Fig.2. The repeating unit (a) and the vector interpretation (b) of the helical ribbon.

Wr of the whole ribbon can be obtained by the subtracting Tw = $\beta/2\pi$ and adding the result to the Wr imposed by self-intersections (N . M):

$$Wr = LK - Tw = -N \,.\, M \pm N/2\pi \,.\, (2\ \arccos\text{-}$$

$$(\cos(\alpha'/2) \,.\, \cos(\beta/2) - \beta) \qquad (4)$$

Here arccos varies from O to π. The sign in the eq. (4) depends on the angle α'. If $\alpha' > O$ it is minus and if $\alpha' < O$ it is plus.

We can now consider the finite dimensions of the nucleosomes. Let us take into account the diameter of DNA (d). It will change the Wr values from M to $M(1 - d^2/4R^2)$ which cannot be detected experimentally. The dimensions of the nucleosome radius (R) and the linker (L) are more important. They lead to the creation of an additional self-intersection near the nucleosome when α' exceeds limiting values α'_1 and α'_2 and not when α' exceeds π as in the ideal ($L \ll R$) structure. It is easy to calculate the α'_1 and α'_2 values knowing the radius of the nucleosome and its linker size.

$$\alpha'_1 = 2\ \arctan(R/L) + \pi \qquad (5)$$

$$\alpha'_2 = 2\ \arctan(2R/L) + \pi \qquad (6)$$

The number of self-intersections can be determined now. We must expect this number to be between 1 and 2 from the known nucleosome geometry. When $\alpha' < \alpha'_1$ there is one self-intersection of DNA axis per one nucleosome and $M = 1$. When $\alpha'_1 < \alpha' < \alpha'_2$, two extra self-intersections appear. They have different signs and $M = 1$ when $0 < \beta < \pi$ and identical signs and $M = 2$ when $\pi < \beta < 2\pi$. When $\alpha' > \alpha'_2$ two self-intersections with identical signs appear and $M = 2$ just as in the case when $\alpha' < \pi$ in the ideal model. The estimated M values permit one to obtain the writhing number of a minichromosome with constant linkers from eq.(4).

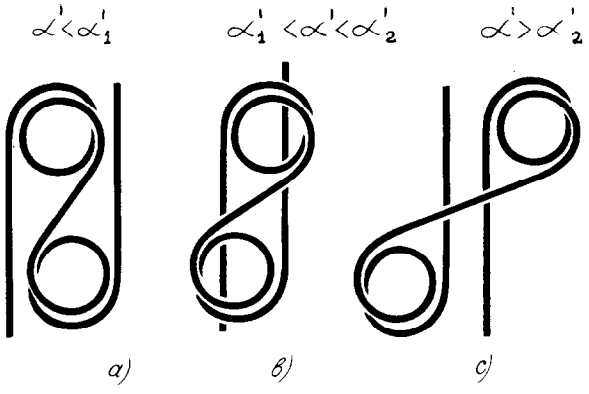

Fig.3. The number of self-intersections of DNA axis in the nucleosome depends upon the angle α'.

Now consider a minichromosome with random values of the angle β. The structure obtained after the subtraction of the self-intersections from it contain equal number of helical turns of both hands. The mean Lk and Wr values in this structure are equal to zero, while their mean square values are in proportion to $\sqrt{N}$ as in any diffusion process. Hence, the total ΔWr and ΔLk values in the minichromosome with random β are distributed near $N \cdot M$ with standard deviation proportional to $\sqrt{N}$. This is consistent with the mode of ΔLk distribution [10] which the previous model failed to explain.

The ΔLk values have been previously estimated for native SV-40 minichromosomes which have nonconstant linkers [11] and for reconstituted circular chains of nucleosomes with random linker sizes [12]. The electron microscopy data reveal [12,13] that there is only one self-intersection of DNA axis per one histone H1 lacking nucleosome. Therefore, the chain of this nucleosome must have ΔLk value equal to $-N$ which is in agreement with the observed one.

Minichromosomes with $M = 1$ must have angle $\alpha' < \alpha'_1$, i.e., $\alpha' < 200°$ for the SV-40 minichromosome. This angle α' corresponds to 1.56 turns of DNA in one nucleosome and 93 basepairs of DNA/turn when 146 basepairs of DNA make a smooth turn about histone core. It also seems very probable that there are only 80 basepairs/turn (as shown for nucleosome core crystals [14]) but the ends of the nucleosomal DNA (~10 basepairs) do not follow the path of the superhelix and emerge from the nucleosome with the angle α' equal to 180–200°.

The contradictions between the predicted and the estimated ΔLk values have led to the assumption that the twist of DNA in nucleosomes is not equal to the one in relaxed DNA and the pattern of nucleosomal DNA cleaved by DNase I which is consistent with the identity of the twists appears owing to the interference of the ajacent turns of DNA within the nucleosome [8]. We must point out, that this assumption is inconsistent with the finding that the DNase I pattern is stable when the ajacent turns of DNA unfold in the presence of ethidium bromide [15].

A nucleosomal chain with antiparallel axes of ajacent nucleosomes ($\beta = \pi$) has $\Delta Lk = -N$ [16]. This is consistent with the nucleosomes having <1.5 turns of DNA. However, this model demands constant linkers having $L = h^{\circ}(n + 0.5)$, where n is 1,2,3 etc., and, therefore, does not match the minichromosomes taken during the ΔLk measurements.

The results obtained here allow to consider the minichromosome model with only one self-intersection of DNA per nucleosome and random orientation of the nucleosomes to be the only structure that matches the previously obtained experimental data. We hope that by eliminating the contradictions between the known geometry of DNA in the nucleosome and the linking number we can help to establish the real structure of eucaryotic chromatin.

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References

[1] McGhee, J. D. and Felsenfeld, G. (1980) Annu. Rev. Biochem. 49, 1115–1156.
[2] Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Proc. Natl. Acad. Sci. USA 72, 1843–1847.
[3] Keller, W. (1975) Proc. Natl. Acad. Sci. USA 72, 4876–4880.
[4] Fuller, F. B. (1971) Proc. Natl. Acad. Sci. USA 68, 815–819.
[5] Fuller, F. B. (1978) Proc. Natl. Acad. Sci. USA 75, 3557–3561.
[6] Crick, F. H. C. (1976) Proc. Natl. Acad. Sci. USA 73, 2639–2643.
[7] Lutter, L. C. (1979) Nucleic Acids Res. 6, 41–56.
[8] Prunell, A., Kornberg, R. D., Lutter, L. C., Klug, A., Levitt, M. and Crick, F. H. C. (1979) Science 204, 855–858.
[9] Wang, J. C. (1979) Proc. Natl. Acad. Sci. USA 76, 200–203.
[10] Shure, M., Pulleybank, D. E. and Vinograd, J. (1977) Nucleic Acids Res. 4, 1183–1205.
[11] Shelton, E. R., Wassarman, P. M. and De Pamphilis, M. (1980) J. Biol. Chem. 255, 771–782.
[12] Noll, M., Zimmer, S., Engel, A. and Dubochet, D. (1980) Nucleic Acids Res. 8, 21–42.
[13] Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[14] Finch, J. T., Lutter, L. C., Rhodes, D., Brown, R. S., Rushton, B., Levitt, M. and Klug, A. (1977) Nature 269, 29–36.
[15] Wu, H. M., Dattagupta, N., Hogan, M. and Crothers, D. M. (1980) Biochemistry 19, 626–634.
[16] Stein, A. (1980) Nucleic Acids Res. 8, 4803–4820.