Systematic search for compact structures of telomeric nucleosomes

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Abstract Telomeres are structures functionally and structurally distinct from bulk chromatin. They are constituted of highly conserved 5–7 bp tandemly repeated units, organized into nucleosomes with short linkers, whereas the knowledge of the linker histone role in telomeric chromatin is still fragmentary. Experimental evidence suggests the structural organization of telomeric nucleosomes is different from that of the bulk chromatin. This work presents a systematic search of the telomeric nucleosome arrangements. A low-resolution molecular model was used to evaluate the relative nucleosome packing energy. Structures with favorable energy were found, reducing the possible telomeric chromatin conformations to two different three-dimensional folds.

Key words: Telomere; Nucleosome packing; Telomeric chromatin; Gay–Berne potential

1. Introduction

Eukaryotic cells contain from 10 to 10^4 million base pairs in a nucleus of a few micrometers in diameter. The contour length of a typical eukaryotic genome is more than 2 m, an accurate organization of the DNA inside the cellular nuclei is therefore necessary.

Packing is due to proteins, which fold DNA more than 10 000 times into a structure called chromatin [1]. Despite large research efforts, the knowledge of chromatin architecture is still fragmentary.

The structural organization of the telomeric sequences at the ends of eukaryotic chromosomes is even more puzzling. Telomeres have structures and functions distinct from bulk chromatin. They are essential for chromosome stability and are involved in many processes, from the expression of adjacent genes to the spatial arrangement of chromosomes, to cellular senescence (the last being related to telomere length shortening). In fact, only immortal cells in lower eukaryotes and germline and tumor cells in higher eukaryotes have telomeres with constant length [2].

In simpler eukaryotes the telomeric DNA tracts are very short and organized in a specific structure called the telosome [3]. In contrast, plants and animals have larger telomeres substantially less characterized. Experimental evidence suggests that most of the telomeric DNA is packed into nucleosomes (whereas the telosome structure could be still present at the very ends of the chromosomes [4]) and that the structural organization of telomeric nucleosomes is different from that of the bulk chromatin. First of all, they are constituted of highly conserved 5–7 bp tandemly repeated units, organized into arrays with short linkers, usually ~40 bp less than those in bulk chromatin [5]. Then, the role of linker histones in telomeric chromatin, whose repeat length is typically ~158 bp, is still unknown. However, gel electrophoresis experiments as well as direct isolation and analysis of nucleoproteins indicate the presence of core histone proteins and H1 in telomeric chromatin domains [6].

Therefore, the study of the telomeric-specific chromatin structure is an important task to throw light onto the role of telomeres in the biological processes they are involved in, such as chromatin stabilization and cellular senescence.

As for the bulk chromatin, there are two main proposals for the nucleosome arrangement in the ordered regions: the solenoid model [7], in which the linker DNAs are coiled between adjacent nucleosomes, and the zigzag model [8,9] in which zigzag arrays of nucleosomes form a condensed ribbon generating the 30 nm fiber. In the latter case, linker DNAs are essentially straight.

Zigzag models seem more consistent with the current experimental evidence. In addition, chromatin structure seems related to the DNA linker length, as it exhibits a preference for integral multiples of the helical repeat [10]. This evidence supports the hypothesis that DNA linker length directs the orientation of consecutive nucleosomes: if linker length differs by multiples of the helical repeat, consecutive nucleosomes are oriented in the same directions and the corresponding structures are practically equivalent.

Recently, Fajkus and Trifonov [11] presented a model for a columnar packing of the nucleosomes in the telomeric chromatin. In their model, DNA is continuously wound in a parallel manner around the stacked histone octamer and linkers are deformed in the same manner as the deformable part of the nucleosomal DNAs. The authors observed that any structure with straight linkers, which would be preferable in principle, would have the nucleosomes all separate. However, this statement is not verified for all the possible combinations of linker length, as shown hereafter.

To perform a wider conformational search, we extend the two-angle model, which has been used by several authors to describe bulk chromatin structures [8,9,12,13], and assume the dinucleosome as the repetitive unit of the fiber. The chromatin fiber is obtained as an ordered repetition of conformationally

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equivalent units, analogous to synthetic polymers, where the global conformation is the result of the local structures of the single monomers. The global chromatin structure is therefore expressed in terms of the two linker lengths (proportional to the rotation angles between consecutive nucleosomes) and the linker entry–exit angle. This model has the advantage that it describes the chromatin geometry in terms of a few parameters that characterize the single nucleosomal structure. In addition, specific sequence-dependent effects could be treated as deviations from the basic model.

To distinguish between all the possible chromatin conformations, we used a low-resolution molecular model, based on the Gay–Berne extension of the overlap potential for oblate ellipsoids [14].

Despite the large accessible conformational space, only few structures have non-overlapping nucleosomes, showing that it is possible to have compact structures even with very short straight DNA linkers.

2. Materials and methods

The initial structures were obtained by repetitions of the fundamental nucleosome–linker 1–nucleosome–linker 2 unit. The orientation of the consecutive nucleosomes could be varied either by changing the base pair number of the linkers or by changing the DNA helical periodicity. The latter case should require an additional twisting energy cost; however, real DNAs show some sequence-dependent variability of the intrinsic helical periodicity.

Consequently, it is convenient to translate the content of information relative to the actual linker length, l_{act}, and its periodicity, ν_{act}, into a virtual number of base pairs, l_{vir}, namely the virtual number of base pairs with an average periodicity (10.4 bp/turn) that ensures the same nucleosome orientation as the actual linker. It is easily calculated as $l_{vir} = (10.4/\nu_{act})l_{act}$. Therefore, since ν_{act} is not restricted to be an integer as l_{act} is, continuous changes of the rotation angles can be considered.

Nucleosomes were generated imposing the correct curvature on 145 bp DNA tracts, which form a superhelix with a 41.8 Å radius and a 23.9 Å pitch, in agreement with the crystallographic data [15,16]. Analogously, the nucleosomal DNA periodicity was set equal to 10.2 bp/turn. The entry–exit linker angle was modulated from 40° to 60°, vanishing the DNA curvature at both ends of the nucleosomal DNA tract, analogously to the crystal structure.

Interactions between nucleosomes were parameterized using the Gay–Berne potential for oblate ellipsoids [14]. This potential was previously adopted by Wedemann and Langowski [17] and by Schiessel and coworkers (personal communication) to simulate nucleosomes:

$$V(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}}) = \varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}}) \left\{ \left[\frac{1}{r-\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})+1} \right]^{12} - \left[\frac{1}{r-\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})+1} \right]^{6} \right\} \quad (1)$$

where $\hat{\mathbf{u}}_1$ and $\hat{\mathbf{u}}_2$ are unit vectors specifying the axes of the two ellipsoids, and $\mathbf{r}$ is the vector distance between their centers. As for $\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})$:

$$\sigma(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}}) = \sigma_0 \left(1 - \frac{\chi}{2} \left\{ \frac{(\hat{\mathbf{r}}\cdot\hat{\mathbf{u}}_1+\hat{\mathbf{r}}\cdot\hat{\mathbf{u}}_2)^2}{1+\chi(\hat{\mathbf{u}}_1\cdot\hat{\mathbf{u}}_2)} + \frac{(\hat{\mathbf{r}}\cdot\hat{\mathbf{u}}_1-\hat{\mathbf{r}}\cdot\hat{\mathbf{u}}_2)^2}{1-\chi(\hat{\mathbf{u}}_1\cdot\hat{\mathbf{u}}_2)} \right\} \right)^{-1/2} \quad (2)$$

where the anisotropy parameter, $\chi = (\sigma_\parallel^2 - \sigma_\perp^2)/(\sigma_\parallel^2 + \sigma_\perp^2)$, σ_0, $\sigma_\parallel$ and $\sigma_\perp$ are chosen to set the positions of potential minima for the longitudinal and lateral orientation of the nucleosomes. We assumed that these correspond to the distances found in the dense phases formed by nucleosome core particles. Very recently, X-ray diffraction experiments in a range of salt concentrations typical of the living cell have shown a certain variability of the orientations of the nucleosomes and of the equilibrium distances, depending on the ionic force [18]. Although these variations are confined within a few Å, the crystal parameters are slightly smaller than the previous proposals [19,20]. Therefore we set the positions of potential minima for the longitudinal and lateral orientation of the nucleosomes to 11.6 nm and 6.0 nm respectively, corresponding to an intermediate salt concentration.

$\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})$ takes into account the anisotropy of the potential depth of the internucleosomal interaction. As in principle the experimental data in the literature do not make it possible to directly evaluate the interaction energy in the different configurations, we considered no anisotropy in the shape of the energy well. Therefore $\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2,\hat{\mathbf{r}})$ reduces to $\varepsilon(\hat{\mathbf{u}}_1,\hat{\mathbf{u}}_2) = \varepsilon_0[1-\chi^2(\hat{\mathbf{u}}_1\cdot\hat{\mathbf{u}}_2)^2]^{-1/2}$.

Also in this case, recent studies by osmometry and by electrophoretic mobility measurements show a certain variability of the interactions between isolated nucleosomes depending on the salt concentrations [21]. In addition, the authors proposed a mechanism of bridging of the histone tails as the main factor responsible for the attraction at intermediate and high salt concentrations. Taking into account these results, ε_0 was chosen to have an energy well depth equal to 10 $k_B T$, a value of the same order of magnitude as in other proposals [22], characteristic of the intermediate salt concentrations. However, it should be noted that anisotropic variations of the energy well depth can in principle change the absolute energy of the fiber structures, but do not change their relative stability. Therefore, the entity of the internucleosomal interactions is not a critical parameter for the search for the compact structures of the nucleosomes, at least in the framework of this model.

Interactions between linker DNAs and between linker DNAs and nucleosomes were considered barely repulsive.

3. Results and discussion

Fig. 1 shows the diagram of the chromatin packing energy for different values of the linker entry–exit angle as a function of the two nucleosomal repeat lengths. These were varied within a range of 11 bp, from 154 to 165 bp, since if linker length differs by multiples of the helical repeat, the corresponding structures are practically equivalent, differing only in the distance between nucleosome cores.

As shown in the maps, most of the linker length combinations correspond to not favorable conformations, the nucleosomes being too close together (dark red zones) or too far from each other (orange zones and the central dark red zone in the maps). However, all the maps show very narrow minima in equivalent positions corresponding to stable compact structures (see Table 1). Changing the entry–exit angle has very few effects on the global shape of the structures, even though a better nucleosome packing is found at lower angles. Therefore, we identified two different conformations which correspond to the most stable structures in terms of linker length and entry–exit angle.

The most stable (Fig. 2A) has consecutive nucleosomes in almost *cis* conformations, equal linker lengths (corresponding to a nucleosomal repeat length of 163.5 bp) and an entry–exit angle of 40°. It very closely resembles the proposed structure for bulk chromatin [8,9]. The calculated fiber diameter is 25 nm and the linear density is rather high, equal to 5.8 nucleosomes per 11 nm of fiber, proving that it is possible to have compact structures even with very short straight linkers.

The second structure (Fig. 2B) has consecutive nucleosomes in almost *trans* conformations, two different linker lengths (corresponding to an average nucleosomal repeat length of 158.1 bp) and an entry–exit angle of 40°. It is substantially a flat ribbon of nucleosomes in zigzag arrangement, which winds up to form a tube. Also this structure is rather dense (6.4 nucleosomes per 11 nm of fiber) and the calculated fiber diameter is 30 nm.

At first sight, it could be surprising that nucleosomes as-

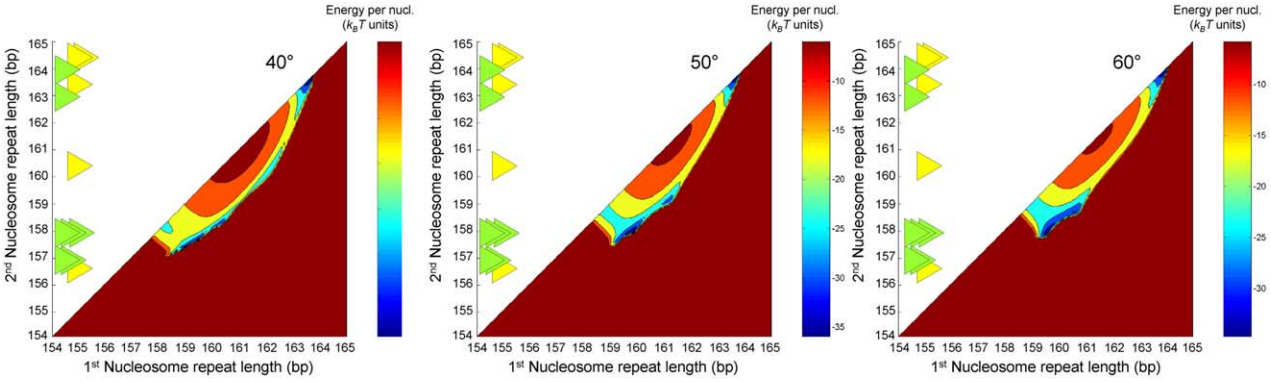

Fig. 1. Diagram of the chromatin packing energy per nucleosome as a function of the two nucleosomal repeat lengths for different values of the linker entry–exit angle. Positive energy values have been cut off as they correspond to not favored conformations. Arrowheads represent experimental values of the telomeric nucleosome repeat length [11]. Green arrowheads refer to the actual experimental values whereas yellow arrowheads refer to the actual values scaled by ± 10.4 bp to report them into the map range. Map diagrams were generated using Matlab 5.2 [23].

sume a non-standard arrangement, as their top and bottom faces point toward the outside of the fiber. Certainly, the high stability of this kind of structure depends on the assumed anisotropy of the internucleosomal potential. However, even if lower interactions are assumed for the side-by-side arrangement, these structures still remain possible, corresponding to local energy minima. In addition, nucleosome organization resembles that found in liquid crystal solutions [20] and crystal structures of nucleosomes [25].

Fig. 1 also reports the experimental values of the telomeric nucleosome repeat length [11] (values outside the map range were scaled by ± 10.4 bp). It is interesting that almost all values cluster in two different zones, which are practically coincident with the minima in the maps. This raises the interesting question of what could be the actual telomeric chromatin structure.

First of all, it should be pointed out that approximations in the Gay–Berne potential as well as the uncertainty about the role of H1 and the small energy differences between stable structures reported in Table 1 (within the range of $\sim 6\ k_BT$ per nucleosome), do not make it possible to answer just on the basis of energy considerations.

In addition, stabilization is highly cooperative in all the possible structures (the interaction energy per nucleosome is ~ 4 times higher than the most favored nucleosome pair interaction). Therefore, it is possible that some twisting deformations could occur to linker DNAs during chromatin formation, which could be compensated by the lowering of the interaction energy between nucleosomes. However, the twisting change should be distributed on the very short DNA linkers, and considering the accepted value of the DNA twisting force constant, it is difficult to hypothesize a change in the twisting corresponding to more than two base pairs [26].

A similar explanation could justify why competitive reconstitution experiments on telomeres show that single nucleosomes prefer positions spaced by the telomeric sequence repeat [27], while the distribution of the nucleosomal repeats in telomeric chromatin in general does not.

Therefore, the interesting question could be raised if different species (or tissues) organize telomeric nucleosomes in different arrangements, or if they can coexist in different zones of the telomeric domains. It is worth noting that the two structures in Fig. 2 seem to respond to different requirements, the first being more compact, and the second having a central hole that could be filled by linker histones or specific telomere-binding proteins.

Finally, conformational transitions within the nucleosome core particles have been recently proposed to occur in the 30 nm fiber formation [13]. The authors extended the two-angle model proposing that the possible hinge opening of the nucleosome cores could result in more dense structures and perfect geometric stacking between neighboring nucleosomes. However, the general framework of Woodcock's model still remains valid and, although nucleosome transitions can modify the 30 nm fiber structures and shift the positions of the energy minima in the maps, these effects, if they occur in vivo, could be considered secondary at a first approximation.

This work is the first attempt to perform a systematic search of all the possible conformations of telomeric nucleosome arrays. Despite the very short straight DNA linkers, a few structures with favorable energy were found, practically reducing the set of the possible telomeric chromatin conformations to two different three-dimensional folds. This represents a sound basis for future research about the telomere structure, which, however, should not exclude a deeper knowledge of the role of H1 or the other telomere-binding

Table 1
Geometric parameters and interaction energy for the chromatin structures corresponding to the minima of the conformational maps in Fig. 1

Entry–exit angle (°)	Repeat 1 (bp)	Repeat 2 (bp)	Linear density (nucl./11 nm)	Fiber diameter (nm)	Energy per nucl. (k_BT units)
40	158.9	157.3	6.4	30	−38.1
	163.5	163.5	5.8	25	−39.1
50	159.8	157.9	4.4	24	−36.0
	163.6	163.6	5.2	24	−34.4
60	159.5	157.8	4.8	24	−33.4
	163.8	163.8	4.8	24	−34.8

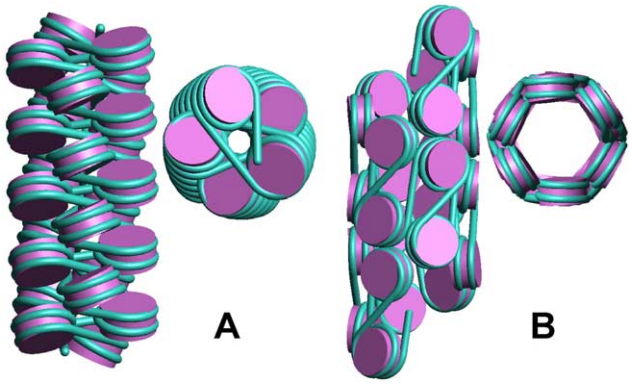

Fig. 2. Visualization of the two molecules with the lowest energy, corresponding to the two different minimum zones in the maps reported in Fig. 1. A: Structure corresponding to repeat 1 = repeat 2 = 163.5 bp and an entry–exit angle of 40°. B: Structure corresponding to repeat 1 = 158.9 bp, repeat 2 = 157.3 bp and an entry–exit angle of 40°. Pictures were realized using MOLMOL [24].

proteins, as well as the development of better potential functions.

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