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Chromatin Dynamics *in silicio*

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The packing of the genomic DNA in the living cell is essential for its biological function. While individual aspects of the genome architecture, such as DNA and nucleosome structure or the arrangement of chromosome territories are well studied, much information is missing for a unified description of cellular DNA at all its structural levels. Computer modeling can contribute to such a description. We present here Monte-Carlo and Brownian dynamics simulations of DNA and the chromatin fiber, using an elastic chain approximation for the DNA and a simple hard-core description for the histone proteins. The model allows for the prediction of possible higher-order chromatin structures and their mechanical properties. As examples, we show the unrolling of DNA from the histone core, the response of the 30 nm chromatin fiber to mechanical stretching and possible regimes of stable and unstable packing of chromatin.

Further, we show dynamics simulations of the nucleosome on the microsecond time scale, using a new coarse-grained model. Finally, describing the chromatin fiber as an elastic chain, we implement models for the transport of proteins in the cell nucleus, reproducing the anomalous subdiffusion found experimentally.

1 Introduction

One of the major challenges in modern molecular and structural biology is the structural and dynamical organization of the cell nucleus[1]. Genomic information is encoded into DNA, a long filamentous macromolecule which is compacted into chromatin by its association with histone proteins. Chromatin also forms a long flexible chain with a diameter of about 30 nm and constitutes about 5 - 10% of the total volume of the nucleus. In every human cell, 6×10^9 base pairs of DNA – that is, a total length of about 2 meters – must be packed into a more or less spheroid nuclear volume about 10 - 20 μm in diameter. This compaction must occur in such a way that the DNA molecule is still easily accessible to enzymes acting on it, such as replication, transcription and repair machineries, or regulatory factors. In addition, more and more supramolecular entities are being identified, including nucleoli, PML bodies, Cajal bodies, spliceosomes etc. that fulfill important biological roles in transcription, splicing, replication or repair mechanisms, but whose structural association with other parts of the nucleus is hardly understood. Important biological questions, such as the gene distribution inside the interphase nucleus, 'memory' of chromosome positions during cell division or the flexibility, accessibility of the folded chromatin chain, interaction of distant parts of the genome or the transport of nuclear factors to their binding site can only be understood through a detailed description of the higher order folding of the DNA molecule in the cell nucleus.

DNA organization in the cell is a phenomenon that needs to be described on many length and time scales (Fig. 1). Such a multiscale modelling problem must be approached by some adequate approximation, in which we will have to define subunits of the molecule that behave like rigid objects on the size and time scale considered. These objects inter-

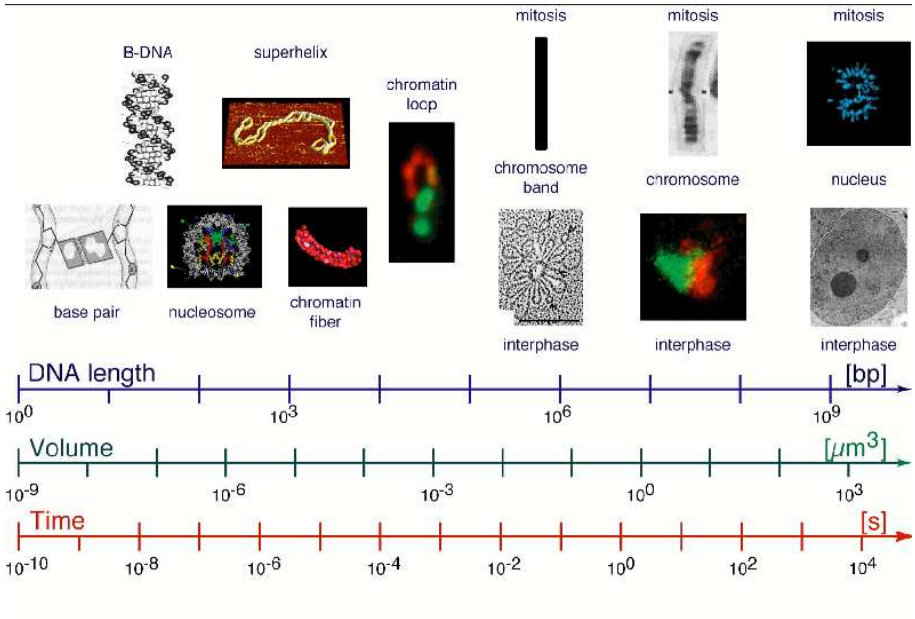

Figure 1. Overview of time and length scales relevant for genome organization.

act through potentials that may in principle be derived from the interatomic force fields; however, in practice one mostly uses potentials that have been determined experimentally.

2 Polymer Chain Models for DNA and Chromatin

The motif of the 'linear elastic filament' in genome organization is repeated on many length scales (Fig. 1): DNA, as well as the chromatin fiber and to some extent its higher order structures may be approximated by a flexible wormlike chain (WLC). Thus, we may develop models of DNA and the chromatin fiber based on a coarse-grained description using a linear segmented chain. Segments are assumed to behave like rigid cylinders on the time and length scale considered; they are connected by elastic joints, with bending, torsional and stretching potentials approximated by Hookean springs with spring constants that are known independently. [a]

2.1 Segmented Wormlike Chain

Fig. 2 schematizes a segmented chain geometry. A vector $\mathbf{s}_i$ defines the direction and length of each segment i, $\mathbf{f}_i$ is a unit vector normal to the segment and $\mathbf{g}_i$ is an auxiliary

[a] For DNA, the approximation of Hookean bending elasticity has recently been challenged by the finding that short DNA fragments have a cyclization probability much higher than expected for a homogeneously elastic wormlike chain[2], and atomic force microscopy observations that sharp bends occur more frequently than expected for a Gaussian distribution of bending angles[3].

vector that is used to take into account permanent bending of the DNA. The details of this chain geometry are given in[4].

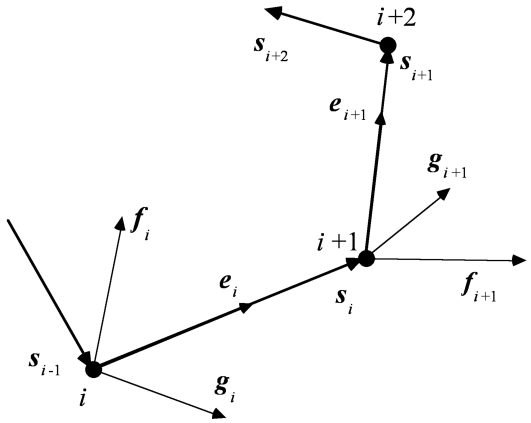

Figure 2. Section of a segmented polymer chain as used in the DNA and chromatin models described here.

2.2 Intersegment Interactions

Adjacent segments (i and $i+1$ in Fig. 2) interact with each other through bending, twisting and stretching potentials. Independent of the form of the local intersegment potential, the WLC approximation always holds for sufficiently long chains, as has recently been shown by Wiggins and Nelson[5]. Thus, if one does not consider tight local bends, the potentials between adjacent segments can be approximated by Hookean springs. Furthermore, potentials must be defined for long-range interactions between non-neighboring segments: in the case of 'naked' DNA, this interaction is the electrostatic repulsion between the negatively charged sugar-phosphate backbones and can be described by a screened Coulomb potential[4]. If the DNA is associated with proteins as in the case of chromatin, the geometry of these complexes and their specific interaction must be taken into account. In the chromatin chain model developed by Wedemann et al.[6], nucleosomes are approximated by rigid ellipsoids and their interaction by a Gay-Berne potential (an anisotropic Lennard-Jones potential[7]).

2.2.1 Bending Rigidity

In the WLC model the length of the segments should be chosen well below the *persistence length* L_p, which is a measure of the bending flexibility of the chain molecule. It is defined as the correlation length of the direction of the chain measured along its contour:

$$\langle \vec{u}\,(s)\,\vec{u}\,(s+s')\rangle = e^{-s/L_p} \tag{1}$$

Here $\vec{u}(s)$ is a unit vector in the direction of the chain ($\boldsymbol{e}_i$ in Fig. 2) and *s* resp. *s'* is the position along the chain contour, the angular brackets indicating the average over all positions and chain conformations. Molecules shorter than L_p behave approximately like a rigid rod, while longer chains show significant internal flexibility.

The bending elasticity A - the energy required to bend a polymer segment of unit length through an angle of 1 radian - is related to the persistence length by $L_p = A/k_BT$, k_B being Boltzmann's constant and T the absolute temperature. Thus, the energy required to bend two segments of the chain of length *l* by an angle θ with respect to one another is:

$$E_b = \frac{k_BT}{2}\frac{L_p}{l}\theta^2 \tag{2}$$

For DNA, L_p has been determined in a number of experiments (for a compilation, see [2]). While some uncertainties remain as regards the value at very high or low salt concentrations, the existing data agree on a consensus value of L_p = 45-50 nm (132-147 bp) at intermediate ionic strengths (10-100 mM NaCl and/or 0.1-10 μM Mg^{2+}). For high values of θ, the potential may deviate from the simple harmonic form (see footnote a and ref.[3]).

2.2.2 Torsional Rigidity

The torsional rigidity C, defined as the energy required to twist a polymer segment of unit length through an angle of 1 radian, may be related in an analogous way to a *torsional persistence length* L_T, defined as the correlation of a vector normal to the chain axis and with fixed orientation relative to the molecular structure of the polymer chain. The torsional rigidity C has been measured by various techniques, including fluorescence polarization anisotropy decay[8–10] and DNA cyclization[11–13], and the published values converge on a value of L_T = 65 nm (191 bp).

2.2.3 Stretching Rigidity

The stretching elasticity of DNA has been measured by single molecule experiments[14,15] and also calculated by molecular dynamics simulations[16,17]. The stretching modulus σ of DNA is about 1500 pN, where $\sigma = F \cdot L_0/\Delta L$ (ΔL being the extension of a chain of length L_0 by the force F). The stretching energy of a segment of length l that is stretched by Δl is:

$$E_{str} = \frac{1}{2}\frac{\sigma}{l}\Delta l^2 \tag{3}$$

DNA stretching does not play a significant role in chromatin structural transitions, since much smaller forces are already causing large distortions of the 30 nm fiber (see below).

2.2.4 Intrachain Interactions

The average DNA helix diameter used in modeling applications such as the ones described here includes the diameter of the atomic-scale B-DNA structure and – approximately – the thickness of the hydration shell and ion layer closest to the double helix. Both for the calculation of the electrostatic potential and the hydrodynamic properties of DNA (i.e. the friction coefficient of the helix for viscous drag) a helix diameter of 2.4 nm describes the

chain best[18–20,4]. The choice of this parameter is supported by the results of chain knotting[21] or catenation[22], as well as light scattering[23] and neutron scattering[19] experiments.

As pointed out in[4,24] DNA intrachain electrostatic repulsion can be adequately described by a Debye-Hückel electrostatic potential between two uniformly charged non-adjacent segments (*i*, *j*) in a 1-1 salt solution:

$$E_{ij}^{(e)} = \frac{\nu^2}{D} \iint d\lambda_i d\lambda_j \frac{e^{-\kappa r_{ij}}}{r_{ij}} \tag{4}$$

Here, the integration is done along the two segments, λ_i and λ_j are the distances from the segment beginnings, r_{ij} is the distance between the current positions at the segments to which the integration parameters λ_i and λ_j correspond; κ is the inverse of the Debye length, so that $\kappa^2 = 8\pi e^2 I/k_B T D$, I is the ionic strength, e the proton charge, D the dielectric constant of water, ν the linear charge density which for DNA is equal to $\nu_{DNA} = -2e/\Delta$ where Δ = 0.34 nm is the distance between base pairs. More details as to the normalization of the linear charge density etc. have been given in our earlier paper[4].

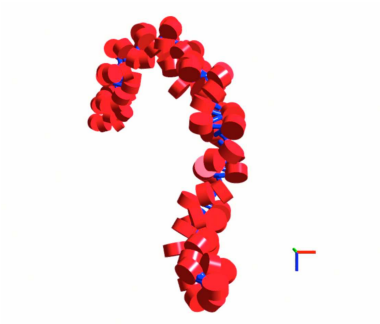

Figure 3. Example of a Monte-Carlo equilibrated structure of a chromatin fiber consisting of 100 nucleosomes (red), linker segments (blue) of repeat length l = 205 nm.

3 Monte-Carlo Model of the Chromatin Chain

As an example of the application of a polymer chain model to genome structure, we describe here the simulation of nanomechanical properties of the chromatin fiber by a Monte-Carlo model. The flexibility of the chromatin fiber has been measured in a set of experiments, either by relating the spatial distance of markers on the DNA to their genomic distance[25–27] or by direct measurements of cyclization probabilities[28,29]. The persistence obtained cover a large range from unrealistically low values of about 30 nm[28,29] to values of up to 200 nm[26]. In our recent work[30], we show that depending on the local structure of the DNA on the nucleosome, the nucleosome repeat and the presence or absence of linker histone H1, this wide range of persistence lengths may be reproduced.

In the model the chromatin fiber is approximated as a flexible polymer chain consisting of rigid ellipsoidal disks, 11 nm in diameter and 5.5 nm in height. These disks are connected by linker DNA, represented by two cylindrical segments. Incoming and outgoing

linker DNA are set 3.1 nm apart of each other. This geometry used is essentially the "two angle" model developed earlier by Woodcock et al.[31].

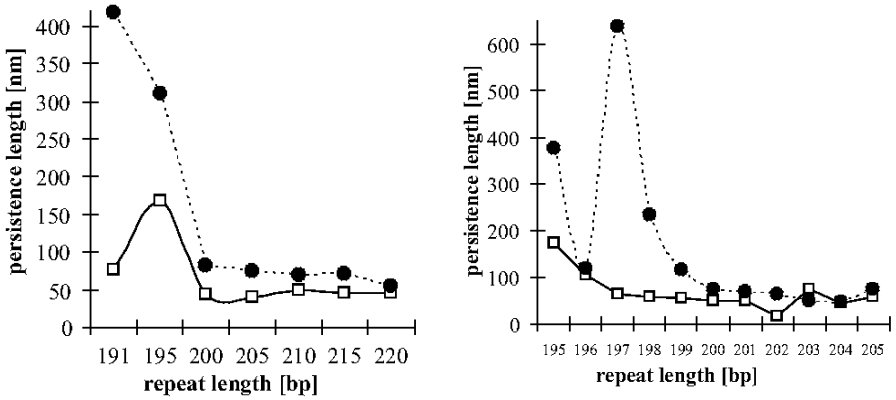

Figure 4. Persistence length of modelled 30 nm chromatin fibers with different nucleosomal repeats in the presence and absence of linker histone H1. The twisting angle between adjacent nucleosomes is adjusted to the canonical value of 360° per 10.5 bp. The persistence lengths of fibers with linker histone (closed symbols, dashed lines) are higher than for fibers without linker histone (open symbols, solid lines). This effect is stronger for short repeats and weakens with increasing repeat length. The peaks show that the twisting angle strongly influences the stiffness of the fiber, leading to a non-monotonous variation of L_p with nucleosome repeat.

A typical conformation of a 100 nucleosome chain after 3.10^6 MC steps is shown in Fig. 3. Simulations were done with either a condensed fiber as a starting conformation or an initial conformation where all segments are ordered in a straight line.

The bending and stretching rigidities of the modelled chromatin fiber are then computed over the trajectory from the fluctuations in the bending angle or the fluctuation in the overall fiber length. The results show that the bending and the stretching stiffness of the chromatin fiber strongly depend on the local geometry of the nucleosome. Both the persistence length L_p, characterizing the bending stiffness of the fiber, and the stretching modulus ϵ, which describes the stretching stiffness of the fiber, decrease if either the linker lengths or the opening angle are increased, or the twisting angle is reduced. This behavior is independent of the presence of the linker histone H1. The latter decreases the opening angle α between the entry and exit of the linker DNA and as a result leads to a more condensed fiber structure for high salt concentrations[32]. This is in agreement with our simulations, since the presence of the linker histone-induced stem motif yields higher persistence lengths thus stiffer fibers (Fig. 4).

The other major result of the simulation comes from comparing the persistence length of the modelled fibers to that of a hypothetical rod from a isotropic elastic material having the same stretching rigidity as the chromatin fiber. Such a rod would have a bending rigidity 4-10 times higher than that actually measured, or simulated here. Thus, the chromatin fiber is less resistant to bending than to stretching. This property of the chromatin fiber is important for its ability to condense and decondense, for example to prevent or allow transcriptional access. Chromatin fibers thus seem to be packed more easily via dense

loops than by a linear compression. The formation of such dense loops of hairpin structures of interdigitated chromatin arrays has been recently suggested[33], and some hairpin conformations could also be seen in our simulations (data not shown).

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