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Intercalation and buckling instability of DNA linker within locked chromatin fiber

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The chromatin fiber is a complex of DNA and specific proteins called histones forming the first structural level of organization of eukaryotic chromosomes. In tightly organized chromatin fibers, the short segments of naked DNA linking the nucleosomes are strongly end constrained. Longitudinal thermal fluctuations in these linkers allow intercalative mode of protein binding. We show that mechanical constraints generated in the first stage of the binding process induce linker DNA buckling; buckling in turn modifies the binding energies and activation barriers and creates a force of decondensation at the chromatin fiber level. The unique structure and properties of DNA thus yield a novel physical mechanism of buckling instability that might play a key role in the regulation of gene expression.

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Notwithstanding its evident biological importance, DNA is a fascinating object for physicists due to its remarkable physical properties. First, DNA is a molecular spring exhibiting stretch, twist, and bend elasticities. These elastic properties have been thoroughly studied both theoretically [1] and by single molecule experiments that led to unexpected results as for instance the structural transitions observed when a sufficiently strong pulling force is applied to the DNA molecule [2]. Second, DNA is a polyelectrolyte and is involved in numerous electrostatic effects [3]; in particular, its negative charge density is enough large to attract a sheath of counterions almost independent of the salt concentration, a phenomenon known as Manning's condensation [4]. Third, the DNA double helix undergoes a denaturation transition studied both theoretically [5,6] and experimentally [7]. We here focus on a fourth specific feature of DNA, namely, intercalation [8] that allows the binding of planar molecules between adjacent DNA base pairs (see Fig. 1).

Quite recently, theoretical [9–11] and experimental groups [12,13] started to explore the nanomechanics of chromatin since free DNA is not the relevant instance of DNA *in vivo*. In the nuclei of plant and animal cells, DNA is actually organized in a hierarchy of structural levels. The first one is the wrapping of 146 bp of DNA around histone octamers to form the nucleosomes. Nucleosomes remain connected by naked DNA segments—the linkers—of length between 10 bp and 100 bp according to the species and cell type. The nucleosome-dressed DNA molecule is further organized into a helical folding of about 30 nm in diameter that is called ''the chromatin fiber'' [14]. We recently proposed on mechanical grounds [9] that the chromatin fiber might exhibit a columnar packing of nucleosomes similar to columns observed in colloidal solutions of mononucleosomes [15]. It suggests that chromatin might be locked into a strongly organized structure, induced by interactions within stacked nucleosomes and secured by histone tails (Fig. 2). We call ''locked chromatin'' such a structure in which the ends of each linker are fixed in space due to strong three-dimensional positioning of nucleosomes within the fiber. A quite similar structure has been suggested long ago by Worcel *et al.* [16]. We claim that it provides a plausible structure for facultative heterochromatine.

In this paper, we propose and describe a different mechanical property occuring specifically within constrained DNA as encountered in locked chromatin. It consists of a buckling instability generated by longitudinal thermal fluctuations stabilized by intercalation.

Intercalative mode of binding plays an important role *in vivo*; for instance, the TBP (TATA-box-binding protein), which binds on specific sequences called the TATA boxes and plays a seemingly universal role in eukaryotic transcription initiation, is a (multiple) intercalator [17]. Intercalation is additionally involved in experimental *in vitro* or *in vivo* studies through the use of fluorescent dyes, as for instance ethidium bromide [8]. In the classical model of intercalation, the binding process is decomposed into three steps [18,19]. The first step is a thermally activated local opening of DNA, creating a binding site for an intercalating molecule. The activation barrier is of order $\Delta G^0_{conf} \approx 6.5kT$ according to Chaires [18,19]. This opening is achieved through a local stretching $\Delta l > 0$ of the interbase-pair distance and a local

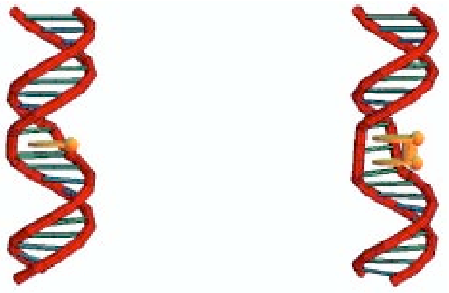

FIG. 1. (Color online) Intercalative mode of binding within DNA for a monointercalator (left) and a bisintercalator (right). One (respectively, two) domain(s) of the binding protein comes in between two successive base pairs, inducing a rise Δl and an unwinding $-\Delta\tau$. For instance, ethidium bromide is a monointercalator with $\Delta l = 2$ Å and $\Delta\tau = 26^o$.

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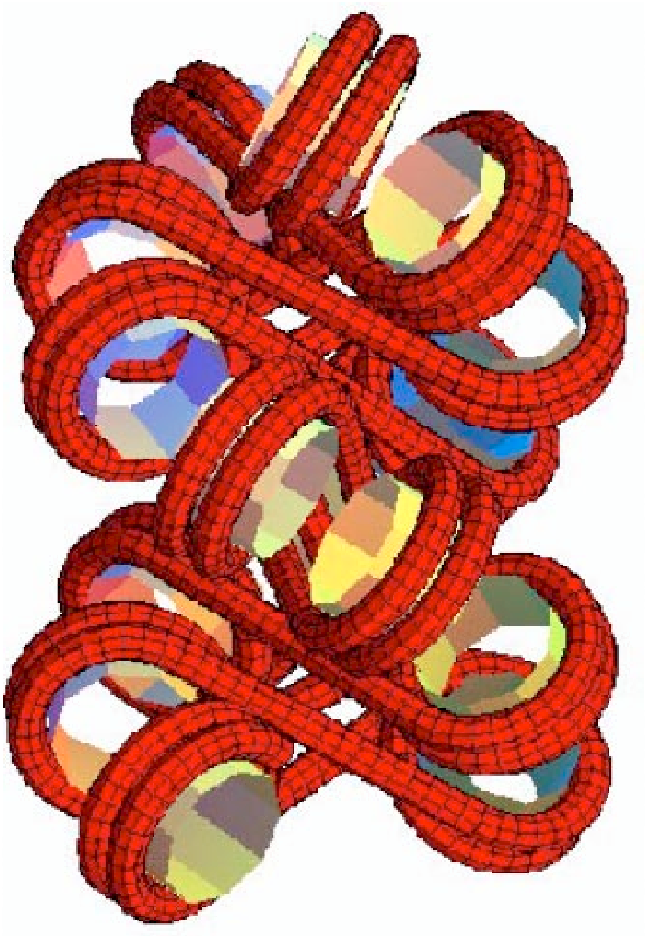

FIG. 2. (Color online) Proposed model of condensed chromatin, locked by interactions between stacked nucleosomes and presumably also by histone tails [9]. The chromatin fiber axis is here vertical.

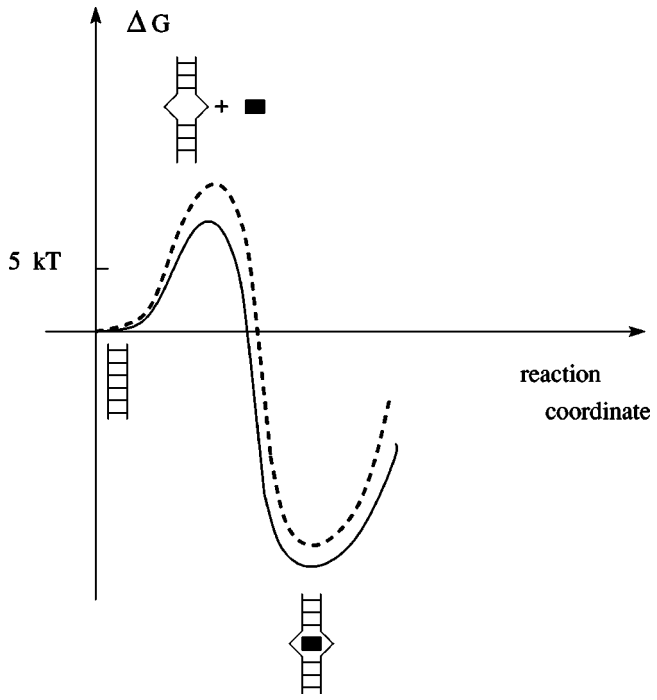

FIG. 3. Schematic drawing showing the modification of intercalation energy barrier and energy well coming from mechanical contribution $\Delta\Delta G$. The units are realistic: the barrier is of order $7kT$; the excess energy is there about $2-3kT$. The binding energy is of order $-20kT$; the excess energy is there about $1-2kT$ (the intercalated site is smaller than the ''open'' site required in the first step of intercalation, which reduces $\Delta\Delta G$ into $\Delta\Delta G_{int}$) [18,19].

unwinding $-\Delta\tau$ (Fig. 3). More precisely, it corresponds to a two-state local conformational transition of the binding site involving changes in the DNA backbone and orientation of the bases and sugars [20]. The second step is the insertion of intercalator inside the binding site (hydrophobic transfer process) followed in a third step by the formation of molecular interactions, namely, hydrogen bonds between the intercalator and surrounding base pairs. The intercalated site is then slightly smaller in size than the preintercalation site required to first accommodate the intercalator. We underline that the binding site opening is not induced by the presence of intercalator but merely by thermal fluctuations, *then* stabilized by intercalator insertion: the mechanism is not an induced fit but rather a conformational capture, in which intercalation captures an ''excited state'' reached spontaneously by thermal fluctuations [21].

Let us now consider an intercalation within a linker embedded in a locked chromatin fiber, hence with translationally and rotationally fixed ends. We describe the linker DNA within a standard continuous model (generalized wormlike chain) as an extensible rod of length l whose mechanical properties are fully described by its bending persistence length $A\approx 50$ nm, its twist persistence length $C\approx 75$ nm and its stretch modulus $\gamma\approx 1200$ pN [1]. Since the distance between linker ends is fixed, prescribed by the global architecture of the chromatin fiber, the first step of intercalation generates mechanical constraints in the remaining part of the linker, namely, a compression $-\Delta l$ and an overtwist $\Delta\tau$, from which follows an excess energy $\Delta\Delta G$. The formation of an intercalation site thus requires to overcome the energy barrier $\Delta G_{conf}=\Delta G^0_{conf}+\Delta\Delta G$, with

$$\Delta\Delta G=\gamma\frac{(\Delta l)^2}{2(l-h)}+kTC\frac{(\Delta\tau)^2}{2(l-h)}, \qquad (1)$$

where h is the interbase-pair distance. We estimate this excess energy with $h=3.4$ Å, $\Delta l=3$ Å, $\Delta\tau=30^o$ (we take these structural values slightly larger than those observed after intercalation, presuming that the preintercalation site should be larger in size). We find $\Delta\Delta G\approx 2.4kT$ for $l=30$ bp (10 nm) and $\Delta\Delta G\approx 3.5kT$ for $l=20$ bp (6.7 nm). This excess energy is of order of thermal energy provided l is not too small; it shows that thermally activated creation of an intercalation site within a constrained linker is still possible for l greater than a minimum length ≈ 20 bp. We underline that the thermal fluctuations here invoked are longitudinal fluctuations; bend fluctuations play a negligible role due to the short length of the linker compared to the bend persistence length of DNA [22]. Mechanical constraints do not significantly modify the energy terms involved in the following steps, namely the free energy cost associated with the

hydrophobic transfer step, the energy associated with modification of counterion surrounding and the energy of newly established molecular interactions between intercalator and DNA [18]. Note that after intercalation, $\Delta\Delta G$ is to be computed with final values of Δl and $\Delta\tau$, which gives a ''mechanical'' correction $\Delta\Delta G_{int}$ to ΔG that is smaller than $\Delta\Delta G$. Mechanical constraints modify both the thermodynamics of the binding (depth of the energy well) and the kinetics of the binding (height of the activation barrier): the activation barrier is increased by an amount $\Delta\Delta G$ whereas the binding energy rises by a smaller amount $\Delta\Delta G_{int}$ (see Fig. 3).

Compared to intercalation within free DNA, the difference lies also in the *stresses* generated when an intercalation site opens in an end-constrained linker. The force induced by the compression $-\Delta l$ of the linker outside the intercalation site writes

$$F_{int}=\gamma\frac{\Delta l}{l-h}. \tag{2}$$

For $l=30$ bp, i.e. $l=10$ nm, and $\Delta l=3$ Å, we obtain $F_{int}=36$ pN.

It is well known that above a critical threshold, the compression of a column shifts into bending, what is called ''buckling.'' This phenomenon persists up to nanometer scale [23]. In order to determine whether the force F_{int} will induce, or not, buckling of the linker, we compute the critical force F_c corresponding to buckling instability threshold. At this stage, the boundary conditions at the linker ends, namely, the mode of anchoring of linkers onto the nucleosomes, have to be described precisely. Standard computation of material mechanics [24] shows that boundary conditions might be taken into account through a numerical coefficient ν reflecting the degree of end fixing, e.g. $\nu=1/2$ for clamped ends and $\nu=1$ for hinged ends; any value of $\nu\geqslant 1/2$ is in fact possible for various elastic joints. The critical Euler load writes accordingly,

$$F_c=\frac{\pi^2 kTA}{(\nu l)^2}, \tag{3}$$

where A is the above-mentionned DNA-bending persistence length. Buckling takes place if $F_{int}>F_c$ or equivalently if

$$l>\frac{\pi^2 kTA}{\nu^2\gamma\Delta l}\left(1-\frac{h}{l}\right)\approx\frac{\pi^2 kTA}{\nu^2\gamma\Delta l}\equiv l_c. \tag{4}$$

This criterion could have been obtained by comparing the compression energy $\gamma(\Delta l)^2/2(l-h)$ with the critical buckling energy $lF_c^2/2\gamma$. We get

$$\begin{aligned} &F_c\approx 20 \text{ pN and } l_c\approx 16 \text{ bp} \quad \text{for} \quad \nu=1,\\ &F_c\approx 80 \text{ pN and } l_c\approx 65 \text{ bp} \quad \text{for} \quad \nu=1/2, \end{aligned} \tag{5}$$

hence intercalation induces buckling in typical linkers (30 bp) if their ends are hinged but not if they are clamped. This result shows that for linker length values between 30 and 60 bp, buckling may be selectively controlled by the anchoring of the linkers at the entry-exit points on the nucleosomes. Indeed, according to the biochemical status of the nucleosome core, either linker DNA is tightly grafted onto the core, which corresponds to clamped ends ($\nu=1/2$), either DNA might unwrap, typically by 5–10 bp on each side, at negligible energetic cost [13,25], which corresponds to hinged ends ($\nu=1$) (Fig. 4).

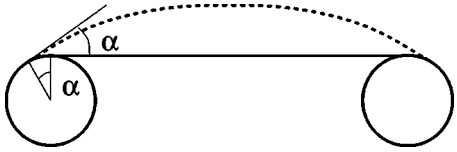

FIG. 4. Buckling of the linker is possible only when the linker ends are allowed to unwrap from the nucleosomes. Unwrapping by 5 bp on each side is enough to accommodate a buckling angle $\alpha=20°$, associated with $\Delta l/l=0.03$; within these bounds, the situation is mechanically equivalent to hinged ends ($\nu=1$).

Linker buckling in turn modifies the energetics of intercalation. When buckling occurs, excess energy is now the sum of three contributions: the compression energy *before* buckling $\gamma(\Delta l_b)^2/2(l-h)$, where $\Delta l_b=F_c(l-h)/\gamma$ is the stretching deformation required to reach the buckling threshold, the compression energy *after* buckling $F_c(\Delta l-\Delta l_b)$ and the overall twist energy $kTC(\Delta\tau)^2/2(l-h)$. It comes

$$\Delta\Delta G^{buckled}=F_c\left[\Delta l-\frac{F_c(l-h)}{2\gamma}\right]+\frac{kTC(\Delta\tau)^2}{2(l-h)}. \tag{6}$$

This expression points out that once a linker is buckled, the energy cost to stretch it further is linear in the length increase and proportional to $1/\nu^2$, hence tunable by a modification of the end status. Buckling thus facilitates the insertion of additional intercalators, and perhaps more crucially, it allows the insertion of multi-intercalators as, for instance, the above-mentionned TBP.

At the fiber level, the buckling force F_c induces stresses, that are easy to estimate within our structural modeling of the condensed chromatin fiber. When the two linker ends are anchored in the same way on the nucleosome core, the force acts along the intercalated linker; it exhibits a component $F_c\cos z$ along the chromatin fiber axis, where z is the angle between the linkers and this axis [9]. Buckling also generates a radial shear whose exact expression depends on the details of the chromatin fiber structure. In the proposed locked structure of the fiber (Fig. 2), $\cos z$ is around 0.6, close to its maximum value: this structure is thus at the same time the more easy to lock thanks to interactions between stacked nucleosomes and the more easy to open thanks to the decondensing force generated by intercalation-induced linker buckling. This two-fold property provides additional support of the biological relevance of the proposed structure. Note that once the linker is buckled, the force experienced by the linker, hence the force of decondensation does not vary significantly with the number of bound intercalator proteins.

Buckling and decondensing forces are generated by the hyperstatic structure of the locked chromatin fiber. Actually, intercalation induces not only compression but also torsional

strains within the linkers (which hence behave not only as columns but also as shafts). Twist-induced stresses are yet proportional to the number of intercalated molecules and can produce a decondensing force of several piconewtons along the chromatin fiber axis, but buckling, occuring before multiple intercalation, modifies the picture and bypasses twist effects by turning them into writhe. We checked that the resulting contribution plays a secondary role.

Taken together, the above results suggest the following scenario providing the first physical bases for the decondensation process:

(i) Thermal fluctuations allow the formation of an intercalation cavity which induces a compression force in the remaining part of the linker. According to the mode of linker anchoring onto the nucleosome core, the compression force may induce buckling of the linker.

(ii) Buckling is further stabilized by insertion of an intercalator into the cavity. Moreover, buckling allows insertion of further intercalators in the same linker so that the limiting step is indeed buckling.

(iii) The buckling force generates in turn decondensation forces at the chromatin fiber level: a stretching force along the axis of the chromatin fiber as well as shearing forces between stacked nucleosomes.

This nonlinear decondensation mechanism is irreversible; chromatin condensation will occur along another pathway, involving electrostatics to induce compaction of the fiber (currently under study).

The physical mechanism here proposed might be of biological importance in the regulation of transcription. Transcription initiation requires a step of decondensation of the chromatin fiber in order to give access to transcription machinery [26]. In this paper, we raised the point that binding of transcription factors and other DNA-binding proteins that monitor the early stages of transcription should occur within a locked fiber, hence in mechanically constrained linkers. Structural modifications of linker DNA here play a role through the forces they generate, without any ATP-consuming mechanism. This point has yet been put forward to account for the cooperativity of protein binding on naked DNA [27].

Presumably, the chromatin fiber architecture has been deviced in the course of evolution not only to efficiently pack DNA inside the nucleus, but also, as proposed here, to play a mechanically active role in gene expression regulation.

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