Eur. Phys. J. E (2006)
DOI 10.1140/epje/i2005-10050-6

THE EUROPEAN PHYSICAL JOURNAL E

Chromatin fiber functional organization: Some plausible models

A. Lesne[1,2,a] and J.-M. Victor[1,b]

[1] Laboratoire de Physique Théorique de la Matière Condensée, Université Pierre et Marie Curie, Case courrier 121, 4 Place Jussieu, 75252 Paris Cedex 05, France
[2] IHÉS (Institut des Hautes Études Scientifiques), Le Bois-Marie, 35 route de Chartres, 91440 Bures-sur-Yvette, France

Received 11 August 2005 /
Published online: 23 February 2006 –

Abstract. We here present a modeling study of the chromatin fiber functional organization. Multi-scale modeling is required to unravel the complex interplay between the fiber and the DNA levels. It suggests plausible scenarios, including both physical and biological aspects, for fiber condensation, its targeted decompaction, and transcription regulation. We conclude that a major role of the chromatin fiber structure might be to endow DNA with allosteric potentialities and to control DNA transactions by an epigenetic tuning of its mechanical and topological constraints.

PACS. 87.16.Sr Chromosomes, histones – 87.15.He Dynamics and conformational changes

1 Introduction: why to focus on the chromatin fiber?

1.1 The chromatin fiber: a functional organization

It has been long believed that the function of chromatin (*i.e.* the wrapping of DNA onto histone octamers) was to pack the meter-long genomic DNA inside the nucleus volume. But numerous facts point *towards an adapted functional organization of the chromatin fiber and its regulatory role in cell functions, in particular at the level of transcription*. Let us first cite the acknowledged conjunction of highly conserved features (histone sequences, nucleosome structure) and polymorphism (repeat length, nucleosome state, histone post-translational modifications) endowing the fiber with both reproducible and adaptive properties, *i.e.* with both robustness and flexibility. Indeed, histones are among the most conserved proteins, with only a few amino-acids differing from one species to another, but they might experience a wealth of post-translational modifications, occurring at well-defined, highly conserved positions and specifically achieved by dedicated enzymes [1]. The nucleosome structure is highly conserved, but the core itself might presumably experience conformational changes (*e.g.* our gaping hypothesis, Fig. 4), and the entry/exit conformation of linker DNA might arise in at least three variants (open, positive crossing or negative crossing) [2]. It is also to be underlined that chromatin is a specifity of the eukaryotic logic: it is ubiquitous in all eukaryotic cells, with no exception, while DNA packaging in prokaryotic cells, although also involving architectural proteins, follows quite different rules.

We shall thus focus on the chromatin, arguing that it is the central functional step in the stairs climbing from DNA seen at the atomic level to micron-size chromosomes (see Fig. 1). Moreover, we shall view the chromatin not only as a complex assembly of DNA, histones and other proteins but *as a hyper-structure: the so-called 30 nm fiber*. Our studies show that the fiber should be considered as an emergent entity with properties of its own, for instance elastic properties (Sect. 2) and topological invariants (Sect. 4). We shall see that our multi-scale modeling approaches give strong arguments supporting the functional role, both regulated and regulatory, of the fiber architecture.

Presumably, the fiber functional organization varies in space, along the chromosome (*e.g.* heterochromatin *vs.* euchromatin) and in time, along the cell cycle. For instance, during mitosis, it should exhibit a tight condensed structure dedicated to compaction. After mitosis, when the cell cycle resumes in each daughter cell, it should exhibit an ordered, rationalized regulatory structure to monitor gene expression, beginning with a local decompaction and followed with a targeted control of transcription initiation. We have performed several modeling studies substantiating this view and further elaborating on its mechanistic and kinematic implications. In Section 3, we propose a structural model accounting for mitotic condensation. Based mainly on topological grounds, Section 4 suggests a plausible pathway for targeted decompaction and the

[a] e-mail: lesne@lptmc.jussieu.fr
[b] e-mail: victor@lptmc.jussieu.fr, victor@lptl.jussieu.fr

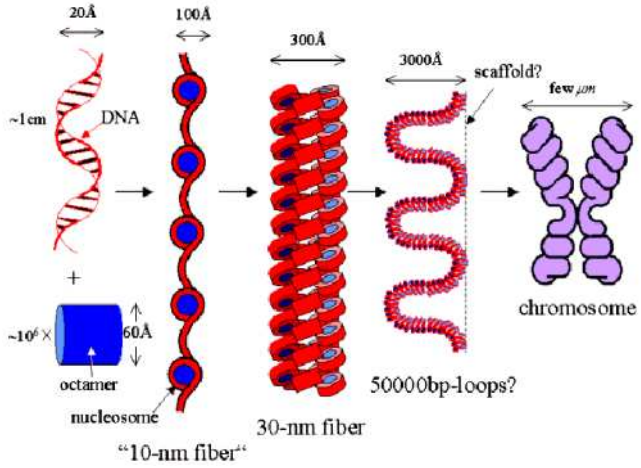

Fig. 1. From DNA to chromosome... and back. The figure presents the different levels of organization of eukaryotic genetic material, from the DNA molecule up to the chromosomes, here represented in the mitotic stage (courtesy of H. Schiessel, see also [3]). Regulation of gene expression is thus an essentially multi-scale issue, that cannot be understood either with a bottom-up or with a top-down approach: higher levels of organization are highly sensitive to the properties of their elementary parts, mainly DNA conformation and nucleosome state, but they also modify back these properties, through steric, topological, mechanical and kinetic constraints. The aim of our modeling approaches is to account for this complex, multi-scale feedback loop and to describe how it is tuned and controlled all along the cell cycle.

transition towards a functional organization of the fiber adapted to transcription; Section 5 is devoted to its regulatory potentialities. Section 6 opens onto some perspectives and novel views gained from our results: we suggest that the chromatin fiber hyper-structure induces an allosteric behavior in DNA. In any cases (Sect. 7), a non-trivial interplay between the DNA and the fiber levels of organization is at work and should be taken into account not only in the kinematic studies presented here, but also in the kinetic and dynamics studies that will extend them.

1.2 The role of physical aspects

A general claim resulting from our modeling studies and providing a guideline for our on-going investigations is the fact that physical properties of the chromatin fiber play a key role in its biological functions and their regulation all along the cell cycle. Any chromatin structural reorganization is faced to physical constraints and, arguing of some joint adaptation in the course of evolution, presumably takes advantage of these constraints for regulatory purposes, selection, recognition or switches. In using the adjective "physical", we here refer to all mechanisms beyond the biochemical scenarios. Even a tentative list of physical features possibly at work would be too long, and we shall only cite a few representative examples: steric hindrance, conformational changes at various scales, topological constraints (on DNA and the fiber), elastic properties (of DNA and the fiber), electrostatics. By contrast, what we termed "biochemical scenarios" involve rather site recognition, recruitment of a variety of specific factors, and dedicated enzymatic chemical reactions.

The challenge we are faced to is then to integrate physical links together with biochemical data and ensuing schemes, to get a complete view of the regulatory pathways involving the chromatin and to understand the coordinated sequence of their steps. In each instance, physical mechanisms possibly at work and inescapable physical constraints have thus to be carefully, and if possible, quantitatively determined. The objective is to go beyond mere correlations and suggest explicit and concrete mechanisms allowing to determine the essential ingredients and identify the control parameters. One of the goals of the present paper is to give a brief overview of this viewpoint and its benefits in understanding chromatin functions and epigenetic regulation.

1.3 An essential inter-level feedback loop

Another guiding idea of our work is that chromatin architecture exerts feedback onto elementary ingredients and their properties, through mechanical and topological constraints. In particular, it might be directly involved in regulating DNA transactions. In this context, a multi-scale viewpoint is essential: our models thus investigate the conformational and kinematic properties of the chromatin *jointly at the level of the fiber and the level of its ingredients, namely DNA and nucleosomes*. Unraveling the interplay between these two levels requires at the same time bottom-up and top-down approaches. On the one hand, we have to describe how any local modification of nucleosomes or linker DNA has repercussions on the fiber structure, its topology, its mobility and its dynamics. On the other hand, we have also to determine, quantitatively, all the constraints experienced by the DNA and nucleosomes within the fiber, their consequences on the local fiber state and, in turn, the ensuing control of accessibility and binding affinities for various enzymatic or transcription factors; these constraints obviously depend on the fiber hyper-structure, for instance its degree of compaction, further motivating on-going structural investigations of the fiber.

Although simple in its basic principle, this feedback loop between the DNA and the chromatin fiber levels accommodates a wealth of regulatory mechanisms and checkpoints; it is presumably implemented in various ways and with various actors, according to the functional context, genome location and cell cycle timing. In particular, we shall see that our multi-scale models suggest a physical role to histone post-translational modifications and other epigenetic events, beyond their implications on molecular recognition and factor recruitment. We claim that *their direct repercussions on the fiber structure and more generally, the functional organization of the fiber and its tuning, provide a novel, partly physical bridge between genomic sequences, epigenetic factors, signalling pathways and transcription regulation*. The aim of this paper is to present some case studies supporting this claim.

2 Chromatin: a tunable spring

A preliminary step in understanding the chromatin fiber function(s) is to describe the features of its own, when considered as a well-defined autonomous entity. Namely, we cast the fiber into an *effective continuous rod-like model* and investigated both its structural parameters, like its diameter or density, and its linear response to mechanical stresses, *i.e.* its elastic properties. The issue is to relate these properties of the fiber to those of the underlying ingredients, at the DNA level.

2.1 Two basic models

Relating properties at the DNA and the fiber levels implicitly means to bridge two models of chromatin. The model at the larger scale describes the fiber as a *homogeneous elastic rod*, able to bend, twist and stretch. It generalizes the worm-like–chain model currently used for semi-flexible polymers (only able to bend) and the worm-like–rod model devised for DNA (able to bend and twist but not to stretch, at least in the range of forces encountered *in vivo*). Within such a continuous rod-like model, the linear elastic response of the fiber to external stresses involves *four elastic coefficients*: the stretch modulus γ (a force), the twist-stretch coupling g, the twist persistence length $\mathcal{C}$ and the bend persistence length $\mathcal{A}$ (of the fiber, not to be confused with the twist persistence length C and bend persistence length A of the DNA, with $C \approx 75\,\mathrm{nm}$ and $A \approx 50\,\mathrm{nm}$ in physiological conditions). This model is fully determined by the local density ϵ of the elastic energy of the fiber (an energy per unit length),

$$\epsilon = \frac{k_B T \mathcal{A} \varrho^2}{2} + \frac{k_B T \mathcal{C} \Omega^2}{2} + \frac{\gamma\, u^2}{2} + k_B T g\, \Omega u\,, \qquad (1)$$

where ρ is the local curvature of the fiber (bending degree of freedom), Ω its local twist rate (torsional degree of freedom) and u its local relative extension (stretch degree of freedom). To adopt such a modeling amounts to consider that (ρ, Ω, u) fully describes the local deformations of the fiber.

At the lower scale, by contrast, the model still explicitly accounts for the inner structure of the fiber. We adopted a *two-angle model*, inspired by [4] and describing the assembly of nucleosomes by means of two angles: the entry/exit angle Φ between the linkers, *i.e.* the DNA stretches connecting successive nucleosomes, and the twist angle τ along a linker (or, equivalently, the length of the linker if it is torsionally relaxed[1]). When supplemented with the description of the DNA path onto the nucleosome, these two angles fully determine the DNA path within the chromatin fiber. Considering here the nucleosomes as identical solid bodies, it is in fact enough to describe the relative positions of the DNA and its grooves at the entry and exit of a nucleosome. The entry/exit angle Φ is an effective parameter: we did not try to describe quantitatively nor even to list all the different factors possibly affecting its value (for instance the pH, the presence of linker histone H1, non-histone proteins, histone tails post-translational modifications, ion binding), and considered it as a tunable control parameter.

[1] Φ and τ in our references [5] and [6] have been changed to α and β in [7–9].

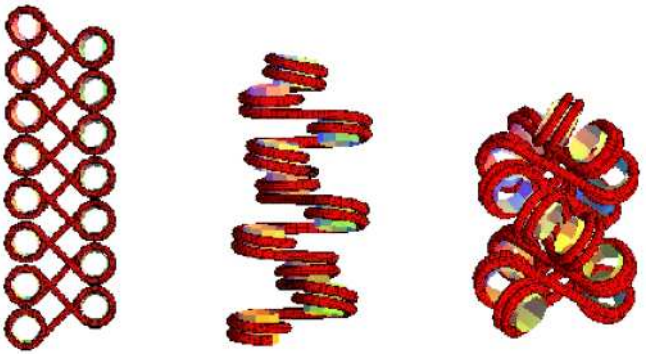

Fig. 2. Minimal geometric modeling of the chromatin fiber architecture. It evidences the large variety of fiber structures that can be obtained by varying only the repeat length (number of base pairs per nucleosome) or the entry/exit angle of the DNA linkers on a nucleosome [5,6].

2.2 A highly sensitive fiber structure

The main structural prediction of the two-angle model is, on the one hand, the *extreme sensitivity* of the fiber conformation, varying from a flat ribbon to a compact superhelical coil when either Φ or τ is varied, as illustrated in Figure 2; on the other hand, the *robustness of the diameter* value, remaining around 30 nm whatever the inner structure and compaction of the fiber might be.

Due to the utmost simplicity of the two-angle model, we cannot expect to faithfully reproduce all the structural modulations. But this very simplicity is also a strong argument towards the genericity of the results, yielding robust basic features to be further modulated and tuned by local specificities, sequence effects, and ion or factor binding to nucleosomal or linker DNA (note that more detailed models have been introduced, reviewed in [10], but they basically do not modify the above conclusions and bring no novel feature).

We underline another basic feature put forward by our model study: the structural variability following from cell species variation in linker histone (which affects the angle Φ) and repeat length (average number of base pair per nucleosome) Finally, it is to note that our simple model is able to account in an effective way for torsional stress experienced by DNA; indeed the torsional angle (along the DNA grooves) might be translated into an effective linker length reflecting the actual positioning of the nucleosome along the DNA. Also, the effective linker length value used in the model accounts for the possible modulation achieved if linker histones clamp further linker DNA, if, on the contrary, some unwrapping occurs, or if a stem-like structure is formed.

2.3 Tunable elastic properties

In this framework, we were able to derive *analytical expressions for the fiber elastic coefficients* γ, g, $\mathcal{C}$ and $\mathcal{A}$ as a function of the DNA persistence lengths C and A and fiber architectural parameters (its diameter D and the angles describing the relative position of two successive nucleosomes around the rod-like fiber axis). The derivation[2] is based on the fact that the fiber elastic energy coincides with the elastic energy stored in linker DNA (the nucleosomes being here considered as rigid bodies, experiencing no strains) [5,6]. Among the results, the values obtained for γ show that the chromatin fiber is *highly stretchable*, with γ values ranging about a few pN (by contrast to γ_{DNA} equal to a thousand pN); accordingly, the twist-stretch elasticity g coupling cannot be ignored. The fiber is also *highly flexible*, with persistence lengths varying in a range of values around 30 nm, namely of the same order of magnitude as the fiber diameter. This flexibility can be understood in analogy with a macroscopic spring, where the stretch elasticity of a spring is typically far different from the (in general weak) stretch or bend elasticity of the metal rod of which the spring is made (here the DNA). It is nevertheless to note that nucleosome stacking interactions should be taken into account in the most compact conformations, which noticeably lowers[3] their flexibility, as evidenced by simulations [13,14] and recent experimental observations in living yeast cells [12]. As a matter of fact, the expressions of the fiber elastic coefficients show that the fiber elasticity is highly sensitive to its structure, being itself controlled by any modification of the architectural parameters at the DNA level, here accounted for by means of the two angles τ and Φ. This tunable elasticity is likely to be exploited *in vivo* in any regulatory mechanism involving chromatin conformation, mobility and mechanical response to factor binding.

This study illustrates one of the benefits of a multiscale modeling: to predict properties at the fiber level knowing parameter values at the DNA level (here the DNA elastic coefficients). In turn, comparing these predictions with the actually observed properties of the fiber offers a way to support or to invalidate the modeling hypotheses, *e.g.* the structural hypotheses on the fiber local architecture involved in the derivation. It also provides a way to interpret experiments at the fiber level in terms of local properties at the DNA or nucleosome level. Finally, it might explain the selection of a specific fiber architecture or a specific tuning of microscopic parameters, showing that they lead to specially well-adapted overall properties. In the present case, our predictions can be compared with values actually measured in a single-fiber micromanipulation, in a relaxation experiment where nucleosome interactions can be ignored as done in the two-angle model used for our computations (Cui and Bustamante, 2000) [15]. The predicted values for $\mathcal{A}$ and γ both coincide with the measured ones when computed for the fiber structure represented in the right panel of Figure 2, whereas they differ for other model structures, obtained with different values of the angles Φ and τ. This result gives a first, mechanical support for a helical, crossed-linker structure, against the previously acknowledged solenoid structure.

In conclusion, elastic properties of the fiber are a first manifestation of its emergent properties, similar to the qualitative differences arising between a spring and the metal thread of which it is made. The fiber is a resilient object able to store elastic energy, in the same way as a macroscopic rod. Nevertheless, at this stage, the fiber has been considered as a mere physical object made from the assembly of DNA and histones. But the *in vivo* chromatin fiber is obviously more than a spring, whatever sophisticated it may be: to go further, biological features and functions have now to enter the scene, in several ways, as we shall see in the following sections (see, in particular, the methodological discussion in Sect. 3.3).

3 The chromatin fiber condensed structure

3.1 The fiber structure(s)

The structure of the chromatin fiber has long been and remains a matter of debate (see, for instance, [16–18]). After an initial supremacy of the solenoid model proposed by Finch and Klug about thirty years ago [19], an increasing number of experimental evidences now favor a crossed-linker structure. A major difficulty encountered in this structural issue comes from the size of the fiber, in-between the range of light microscopy (scales larger than a few hundreds of nanometers) and cristallography and other experimental tools available at molecular scales (less than a few tens of nm). An additional difficulty comes from the fact that it is by no means certain that the structures observed *in vitro* for purified fibers or with reconstituted nucleosome arrays reproduce the actual functional (and possibly dynamic) structure of the fiber *in vivo*. To be faithful, information should be gained from *in vivo* imaging.

Moreover, the fiber experiences major structural reorganizations during the cell cycle. Several experimental studies recently provided a direct, visual access to mitotic

[2] Our elastic coefficients for the fiber only account for the DNA and linear contribution to its elastic response to stresses. The discussion presented for the DNA in the article by Vaillant *et al.* in this issue [11] is also relevant for the fiber. Numerous possible additional contributions might then be taken into account: internucleosome interactions, thermal fluctuations, structural disorder (linker length, entry/exit angle, local modification of the elastic coefficients of DNA) or nonlinear contributions (changes in the nucleosome structure or topology ...) to the elastic response of the fiber.

[3] Indirect measurement of the fiber flexibility has been achieved in live yeast cells, by FISH and *in vivo* imaging technique [12], yielding a value of 170–220 nm for the bend persistence length. It is nevertheless to notice that the data have been interpreted within a simple worm-like–chain model, not well suited for the chromatin fiber since it accounts for only a bend degree of freedom, hence a single elastic coefficient: its bend persistence length; twist and stretch elasticities are there ignored, without any argument supporting that they were actually negligible.

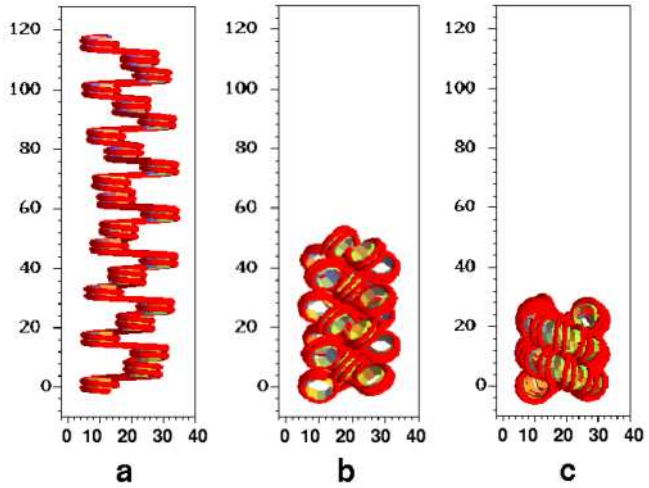

Fig. 3. Fiber condensation. The fiber compaction (a) → (b) achieved within the two-angle model by varying, *e.g.*, the entry/exit angle, is not sufficient to reach the compaction ratio observed in mitotic chromosomes. We here show that a further compaction is achieved after a nucleosome structural change ("nucleosome gaping") improving nucleosome stacking interactions [8]. Compare (b) and (c) with the tetranucleosome crystal structure, Figure 3 of [20].

condensation [21–23] to be compared to *in vivo* investigations of nuclear organization during transcription [24,10, 25]. These observations evidence that different condensed structures are to be considered according to the cell cycle stage and to the functional role of the compaction. The structure designed to achieve the tight and maximal packing required during mitosis is presumably different from the organized condensed structure framing transcription regulation. Furthermore, it has been observed that the condensed mitotic structure itself exhibits two levels of compaction (6 nucleosomes per 10 nm, and 10 nucleosomes per 10 nm) [21]. Conversely, the decompaction also occurs in two stages [22]. We refer to [9] for a further discussion of the fiber structure, references about experimental supports and structural reorganizations of the zig-zag crossed-linker structure (resembling Fig. 3b in interphase and Fig. 3c in metaphase).

The raw data presented in [12] hint at the coexistence, in the course of time or/and in a cell population, of very compact and more extended conformations for a given chromatin loop.

3.2 The gaping hypothesis

The highest compaction rate obtained within the two-angle model (Fig. 3b) is not sufficient to account for the mitotic condensed structure of the chromatin. We proposed that an additional conformational transition between two structures of the nucleosome, dubbed "nucleosome gaping", takes place to achieve the mitotic compaction ratio. This gaping hypothesis has been further investigated within the framework of molecular modeling, as shown in Figure 4 and discussed in Sect. 3.3. At this molecular scale, nucleosome gaping corresponds to the unsticking of both H2A and H2B dimers. Gaping is beneficial insofar as it improves the strength of internucleosomal stacking interactions (it is to note that recent observations of tetranucleosome crystals by the Richmond group support the key role of stacking interactions between nucleosomes [20]). The energy balance should also take into account the variation in the electrostatic repulsion of nucleosomal DNA and the change in linker twist energy upon gaping. The detailed estimation leads to the conclusion that the nucleosome gaped conformation is a metastable state, and that some active process is required to cross irreversibly the energy barrier separating it from the native state [8,9].

The height of this energy barrier is highly sensitive to the local ionic strength and could be tuned by any local supply of charges: this is a first hint towards the possible role of ionic exchanges in fiber conformational changes and associated regulatory mechanisms (see also Sect. 5.3). The control parameters involved in this study and the determination of the condensed structure, Figure 3c, are the geometric properties of the assembly and its ingredients. This model is now to be supplemented with kinetic features, *e.g.* hypercycles involving post-translational modifications of the histones [26] in order to describe chromatin conformational dynamics.

3.3 Requirements for a functional approach

Chromatin modeling, even in what concerns its structural or mechanical properties, cannot be reduced to a "soft matter problem", whatever care is taken to include all the different ingredients. A qualitative step has to be achieved, to take into account a specific biological feature, namely that we are today faced to the result of million years of evolution. It *a posteriori* supplements the plain physico-chemical laws with additional principles of natural selection, adaptation and some kind of optimization when several designs are equally possible or plausible[4]. In practice, it implies that the guiding rule in devising a model *should be the biological functional scenario*; this biological backbone is to be articulated and further substantiated with physico-chemical ingredients (interactions,

[4] Almost each term of this assertion is to be defined with care, in concrete contexts. For instance, "equally possible" is in most cases a rather subjective appreciation; invoking natural selection requires to precise the selection level and mechanisms; and so on: the topic would deserve a debate on its own, which is evidently beyond the goal of the present paper. We here only mean to stress that the involvement of natural selection, whatever its precise course has been, is enough to change the rules between physical and biological systems. Typically, an accumulation of fine tunings, highly specific ingredients, low-probability and marginally stable events is the rule in biological systems, whereas only generic and robust behaviors are observed in natural physical systems. To assume adaptation and optimal performances might be in some cases a way to account for evolution and natural selection in modeling the biological systems of today.

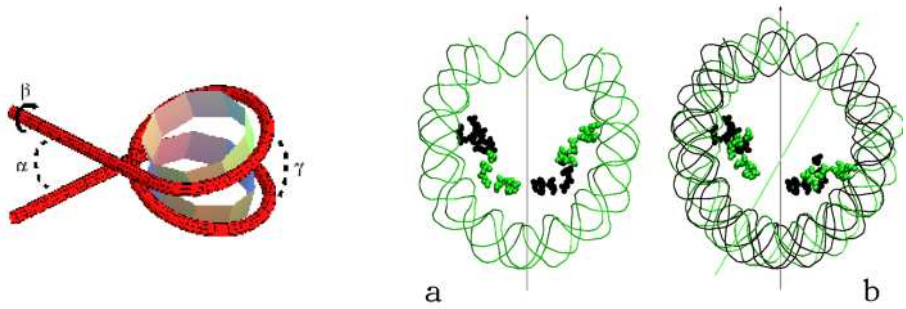

Fig. 4. Molecular modeling of nucleosome gaping. Left: nucleosome gaping introduces an additional degree of freedom turning the geometric modeling of Figures 2 and 3 into a three-angle model. Right: at a finer scale, molecular modeling shows that gaping is supported by the spatial coincidence of two specific residues, stabilizing the open, gaped structure of the nucleosome [8].

water, ions and electrostatics, thermal fluctuations) and constraints (forces, energies, time scales, dissipation, entropic cost). Moreover, joint evolution and adaptation of the different levels of organization typically led to situations where all levels are consistently coupled and *cannot be investigated separately*: upper levels regulate and even shape the lower levels, and conversely.

Following this functional logic led us to look for gaping within a molecular modeling study, because it "should" occur: a thorough blind scan of all possible metastable states of the nucleosome would have obviously been out of reach. On the contrary, we here investigated whether the gaping hypothesis, that would be functionally beneficial, could find any support in the molecular structure of the nucleosome and its degrees of freedom (Fig. 4). We have thus performed a molecular modeling *guided by the function*, insofar as the function here suggested test movements, observables and mechanisms to be further investigated and validated.

4 Topological properties of the chromatin fiber

4.1 Topological constraints at the fiber level

In Sect. 2, we have exploited a rod-like model for the chromatin, quite similar to the model currently used for DNA but now at the fiber level. In the same way as elastic energy can be expressed directly at the fiber level, topological constraints associated with architectural stiffness of the nucleosome assembly can also be expressed at the fiber level: it is possible *to define a linking number* $L_k^{\rm fiber}$, *a writhe* $W_r^{\rm fiber}$ *and a twist* $T_w^{\rm fiber}$ *for the chromatin fiber.* The writhe $W_r^{\rm fiber}$ is merely the writhe of the fiber axis seen as a continuous elastic rod; its twist $T_w^{\rm fiber}$ is related to the helical path of the nucleosome array around this axis. Note that it is the mere transposition at the fiber level of what is currently done for DNA in modeling the double-helix chiral and stiff structure by an elastic rod. In particular, DNA twist is a smoothed effective variable reflecting the step-wise rotation of each base pair with respect to the previous one. Similarly, the DNA writhe is a feature of the continuous curve interpolating the double-helix local axis and providing the very backbone of the rod-like description of DNA. At the fiber level, we analogously consider a smooth fiber axis, thus having a well-defined writhe, and a continuous twist angle interpolating the step-wise rotation angle around the fiber axis required to pass from a nucleosome to the following one. A straightforward analysis shows that the corresponding Fuller theorem holds true at the level of the fiber: $L_k^{\rm fiber} = W_r^{\rm fiber} + T_w^{\rm fiber}$. The description of topological features at the fiber level is another manifestation of its emergent nature. This topological analysis gives rise to a conservation law, namely *the conservation of the linking number* of a closed chromatin loop, or equivalently a loop with fixed ends. This conservation of the linking number $L_k^{\rm fiber}$ is a global conservation law, at the level of a chromatin loop, with no local formulation. As in any macroscopic rod, it generates topological constraints: any deformation of a loop with fixed ends should accommodate this conservation law. This requirement induces couplings and correlations at a global scale (that of a loop) and the system should be considered as a whole at this scale: it would be totally irrelevant to consider isolatedly a sub-region, *e.g.* a stretch of a few nucleosomes within a loop. The central step of this multi-scale analysis is to relate the fiber level and the DNA level, which is expressed through the simple relation $L_k^{\rm DNA} = L_k^{\rm fiber} + {\rm cte}$, where the constant is proportional to the overall DNA length [7] (note that it gives an additional proof of the conservation of the linking number of the fiber). In view of the topological constraints associated with the conservation of $L_k^{\rm fiber}$ in-between any two fixed regions of the fiber (typically between two boundaries or anchoring points on the MAR, involving about 500 nucleosomes, *i.e.* 200 kb), a relevant question is to determine what are the 30 nm fiber compact structures which are kinematically consistent with the condensation/decondensation process.

4.2 Topologically induced conformational change of the fiber

It is possible to investigate the consequence of nucleosome gaping on the fiber twist and the consequence of the fiber

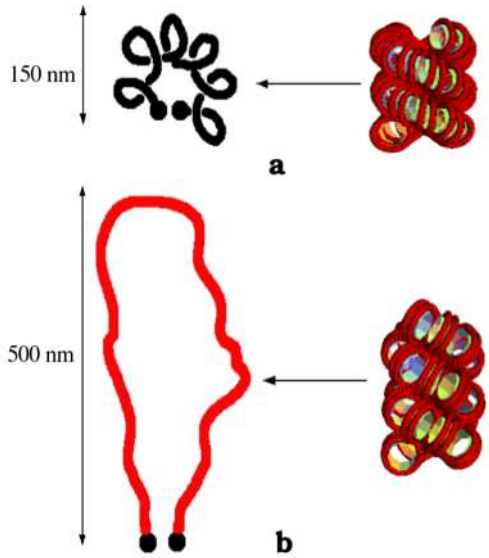

Fig. 5. Topological constraints at work in condensation/decondensation. A chromatin loop with fixed ends possesses a topological invariant, its linking number L_k^{fiber} [7]. Our scenario for condensation/decondensation of such a loop is based on the compensatory writhe variation $\Delta W_r^{\text{fiber}}$ that should balance the twist variation $\Delta T_w^{\text{fiber}}$ following from nucleosome gaping (b) $\rightarrow$ (a) or ungaping (a) $\rightarrow$ (b) according to the Fuller theorem $\Delta L_k^{\text{fiber}} = \Delta W_r^{\text{fiber}} + \Delta T_w^{\text{fiber}} = 0$. Such a writhe variation drives the conformational change of the chromatin fiber between a coiled or plectonemic conformation (a) and an extended loop (b) [9].

outlooping on its writhe (Fig. 5). We found that each nucleosome gaping induces a twist change $\Delta T_w^{\text{fiber}} = 0.007$. When gaping occurs in a chromatin loop with fixed ends, composed of N nucleosomes, a corresponding writhe variation of $\Delta W_r^{\text{fiber}} = -0.007\,N$ should thus occur according to the topological constraint $\Delta L_k^{\text{fiber}} = \Delta W_r^{\text{fiber}} + \Delta T_w^{\text{fiber}} = 0$. This compensatory writhe change corresponds to the transition towards a coiled or plectonemic compact conformation for the chromatin loop (transition (b) $\rightarrow$ (a) in Fig. 5), hence appearing to be *topologically driven*. Conversely, ungaping should be accompanied by a change $\Delta W_r^{\text{fiber}} = 0.007\,N$ enforcing the outlooping of the compact fiber loop (transition (a) $\rightarrow$ (b) in Fig. 5); such a decondensation of a chromatin loop, at fixed ends, will occur without generating topological constraints, altough a general deformation of the fiber conformation typically does so, both at the level of the fiber and at the DNA level. Decompaction at constant linking number L_k^{fiber} is thus a strong natural selection rule: topological constraints select one and only one unfolding path [7].

The exact cancellation between twist and writhe variations invoked here should not be seen as a highly improbable event (to be rejected for this very reason of non-genericity if it were concerning a physical system). It should rather be considered as the selected consequence of joint adaptation of the fiber basic ingredients and architecture (co-evolution). Such a fine tuning, that would indeed be an improbable miracle from a pure physical viewpoint, is rather the rule in biological functions. In the same spirit, let us also underline the remarkable efficiency of *in vivo* structures, here as regards the compaction ratio; it points at a specific adaptation of the elementary units towards the overall structural requirements in the course of evolution, and indirectly supports our multi-scale condensation/decondensation scenario and gaping hypothesis.

In conclusion, our investigations based on the two-angle modeling of the fiber architecture show its emergent nature. It can actually be seen as an elastic rod in what concerns its geometric, topological and energetic properties. Nucleosome gaping is not only required to improve nucleosome stacking, thus achieving a higher compaction rate and a stronger locking of the fiber; it is also essential in providing the adapted compensatory change in the fiber compaction, when it occurs in a loop experiencing the topological constraint $L_k^{\text{fiber}} = \text{cte}$ [9].

4.3 Active condensation vs. spontaneous decompaction

From the kinematic viewpoint, the previous scenario can be traveled both ways, in a uniquely determined fashion. The loop decondensation can be thus reverted (once transcription has been fulfilled), provided some energy is supplied to achieve nucleosome gaping, as we shall discuss in this section. Our scenario is thus in agreement with the *in vivo* observation of a compact chromatin fiber in interphase (actually a coexistence of very compact structure and a more extended one) and the conjecture of a rapid refolding after the passage of the RNA-polymerase (FISH experiments in budding yeast [12])

We even claim that a compact conformation of the fiber is a prerequisite for a *controlled* and *targeted* opening of the fiber around selected genes to be transcribed; a monitored decondensation requires a highly organized and tightly structured fiber. It allows an alternation of unfolding and refolding after the passage of/in the transcription machinery. Note that linker histones are not necessary for refolding nor even for obtaining the most compact structure: presumably, the role of linker histones is to stabilize these compact conformations, turning them into locked conformations, and to slow down the turnover of unfolding/refolding.

As yet noticed in Section 3, nucleosome gaping corresponds to a *metastable* configuration and the transition from the native ungaped state to a gaped state should be driven by some free energy supply. It is actually acknowledged that condensation is an active process. By contrast, the transition back to the ungaped native state can occur spontaneously, all the more than it is *cooperative*: ungaping implies unstacking and destabilizes the neighboring nucleosomes, thus propagating the local decondensation of the fiber. Moreover, such a decondensation scenario takes advantage of the stored energy, namely the elastic and gaping energy stored in the condensed gaped structure of

the fiber. It is thus apt to generate a rapid process, in contrast with the active condensation process (during which energy has been accumulated) limited in particular by the enzymatic rates. It is enough to trigger decondensation for it to occur rapidly, in analogy with the rapid relaxation of a coiled spring (or any other resilient mechanical device) when some clamp locking the constrained state is released [9]. Many plausible mechanisms might be invoked for this clamping/triggering switch, among which histone tail post-translational modifications [27]. Experimental observation also evidenced the major role of the histone H3 N-tail phosphorylation/dephosphorylation during the condensation/decondensation accompanying mitosis [28].

5 Transcription regulation within the chromatin fiber

5.1 Mechanical control of DNA/protein interactions

Embedding of DNA within the highly organized and constrained chromatin structure *strongly modifies its affinity for any ligand, compared to free DNA*. Actually, any way of controlling the mechanical constraints experienced by linker DNA would in turn control its affinity for any protein whose binding is accompanied by some distorsion of DNA (bend or twist), hence associated with an elastic energy cost in an end-constrained linker DNA. Experimental values of the compaction ratio in live yeast cells, by FISH and *in vivo* imaging technique are available [12]. They show that the fiber is surprisingly compact in interphase (7 to 10 nucleosomes per 11 nm turn): this suggests that *mechanical and topological constraints are actually engendered by the fiber structure even in interphase*, hence all our proposed scenarios, relying on the presence of such constraints (on the constraining effect of the fiber hyperstructure) are yet relevant in interphase, hence are likely to be involved in transcription regulation.

In a locked chromatin fiber, the strength of the constraints is controlled by the linker DNA anchoring onto fixed nucleosomes; any modification of this anchoring or any weakening of the tight three-dimensional positioning of the nucleosomes would relax these constraints and lower the associated energy barrier. Carrying on the argument allows *to connect the degree of chromatin organization and the nature and rate of DNA transactions*. This provides a mechanistic link between the variations of gene expression pattern and the variations of chromatin compaction with the cell type and along the cell cycle. It thus hints at a direct interplay between chromatin fiber conformation (at different scales), cell cycle and cell differentiation. It is to note that recent experiments indicate that the sequence accessibility is not much affected by the chromatin fiber compaction [29]; this observation indirectly brings further support to the essential role in transcription regulation of mechanical constraints induced at the DNA level by the chromatin condensed architecture.

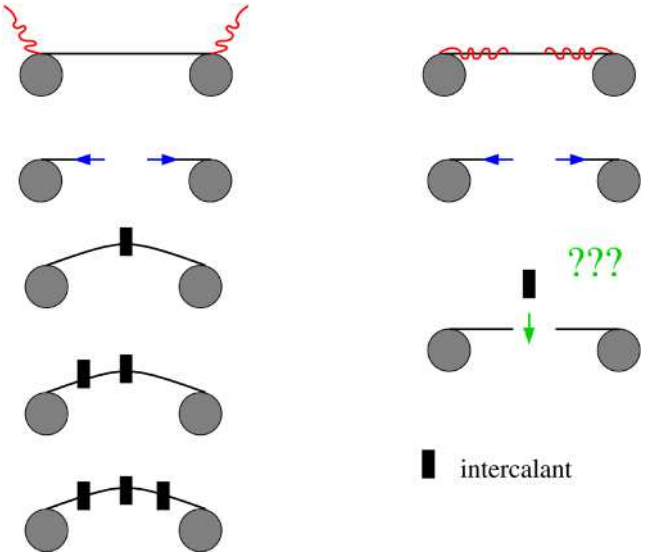

Fig. 6. A possible scenario for physical reading of chromatin epigenetic information. When DNA is strongly anchored on nucleosomes, within a compact, locked chromatin fiber (right), mechanical constraints might prevent the binding of transcription factors to DNA. By contrast, when histone post-translational modification loosen the anchoring of DNA onto the nucleosomes, the first-occurring intercalation might induce an allosteric conformational change of DNA ("buckling") lowering the energy barrier for further intercalations and promoting a cooperative binding of proteins in DNA [30].

5.2 DNA buckling scenario

In case of intercalating proteins, we proposed a complete scenario describing the mechanical control of their DNA binding within a compact chromatin fiber and how it might couple with histone post-translational modifications [30]. The main points are sketched in Figure 6.

The binding of intercalating proteins is accompanied with a noticeable increase of the distance between the base pairs flanking the binding sites, to accommodate the sizeable intercalating residues. This is of no consequence in free DNA (the DNA ends will move accordingly) but not if the DNA stretch is anchored onto fixed nucleosomes, as is the case for linker DNA within a condensed chromatin fiber. Considering the DNA as an elastic rod (see the discussion in Sects. 2.1 and 4.1), we suggested that the compression force generated by the opening of an intercalation site might induce a buckling of the linker. This force is indeed quite high due to the large value of the DNA stretch modulus γ_{DNA} (more than 1000 pN). A precise estimate of this force, compared to the buckling instability threshold, shows that the occurrence of buckling is controlled by the anchoring of the linker onto the nucleosomes: it is prohibited if the linker is tightly clamped, as is the case when the histone tails are deacetylated (Fig. 6, right panel); it is allowed if the linker is more loosely anchored, somehow hinged, as is the case upon acetylation of the histone tails, mainly H3 and H4 tails (Fig. 6, left panel). Energy estimates show that thermal fluctuations are sufficient for achieving transiently the opening of an intercalation site

and, if allowed, the ensuing buckling. This transient DNA conformational change is stabilized upon binding of the intercalating protein. The alternative is then the following. If the linker cannot buckle, the energy cost for opening a second intercalation site in a yet constrained DNA is too high; indeed, the elastic energy increases in a quadratic fashion as a function of the strain. On the contrary, we have shown that if the linker is buckled, the binding energy barrier is the same for each additional intercalating protein, and multiple intercalations are thus possible (even easier than the initial one). It is to be underlined that the mechanism here described is very similar to the conformational capture of the "R form" (the catalytic active form) of the enzyme in the classical allosteric scheme for enzymatic catalysis [31]. This led us to introduce a notion of DNA allostery, as discussed below, Section 6.2.

5.3 Transcription initiation: a somehow ignored puzzle

A currently ignored issue concerns *the very first step of transcription*, at the stage when the DNA is close-packed into a condensed chromatin fiber. The puzzle lies in the fact that the condensed structure of the fiber prevents the binding of transcription factors, for at least two reasons: the binding sites are buried into the chromatin fiber, hence are presumably not accessible; even if they are yet accessible [29], mechanical constraints exerted on DNA by the locked fiber architecture (*e.g.* DNA tight anchoring on stacked nucleosomes) put a strong barrier on protein binding, as detailed in Section 5.1.

One of our working hypotheses is that *ion exchange* is involved. Indeed, on the one hand, many transcription factors exhibit a high affinity for divalent cations, *e.g.* calcium, and in some cases an allosteric behavior with ions as effectors [32]. On the other hand, localized binding of highly charged ions is known to influence DNA conformation. An ion-induced DNA conformational change is then relayed and amplified up to the level of chromatin fiber and reflects in a destabilization of the condensed fiber architecture. This preliminary loosening of the condensed structure now allows the binding of transcription factors and a more specific control of the transcription initiation to take place. In other words, it endows the fiber with transcriptional competence, allowing specific activation mechanisms to enter the scene.

Among the cations possibly endowed with such a regulatory role, polyamines are specially relevant candidates due to the strength (three positive charges for spermidine) and the spatially extended nature of their charge. Although the importance of polyamines in signalling and regulatory pathways has been acknowledged for long, their possible involvement in chromatin functions has been ignored in most studies. Some experimental works nevertheless assessed a coordinated interplay between phosphorylation/dephosphorylation of the H3 N-tail, polyamine binding to linker DNA and chromatin fiber compaction [28,33].

5.4 Epigenetic regulation

Regulatory mechanisms involving the chromatin structure offer numerous ways of epigenetic control of transcription, beyond the schemes based only on genomic sequences. Let us cite some examples.

The chromatin fiber structure is sensitive to the intrinsic variability of mechanical properties of the linker DNA associated with its sequence (natural curvature, local increase of flexibility). In this way, it might amplify direct "physical by-products" of the genomic sequence, giving a physical meaning to the DNA sequence, beyond the genetic code and its protein translation.

There is an ever growing number of experimental evidences of the interplay between the chromatin fiber structure and the histone post-translational modifications. It suggests physical ways of reading these modifications, beyond the biochemical ones (*e.g.* molecular recognition, recruitment of factors and co-activators). A first way is the electrostatic landscape modification due to the lowering of the histone tail positive charge associated with some histone post-translational modifications, as phosphorylation and acetylation. Histone post-translational modifications tune histone tail affinities for DNA, hence control the entry/exit angle Φ made by the linkers; they also affect the stacking interactions between nucleosomes. In consequence, they indirectly control the fiber architecture and the local mechanical constraints on DNA [27]. The histone tail status finally tunes DNA anchoring onto nucleosomes; it thus controls DNA conformational changes and indirectly the consequences of these conformational changes on the fiber structure and on the DNA binding affinities.

The central regulatory role of the chromatin fiber, supported by our modeling studies, and the previous remarks lead to the notion of an *epigenetic information imprinted in the features of the fiber*. It is mediated by a huge variety of elementary features: DNA methylation, nucleosome gaping, linker histones (H1, H5), histone-like proteins (HMG), and all the histone tail post-translational modifications. Speaking of information requires to specify how it is read and interpreted, *i.e.* how it gets a meaning. Actually several meanings superimpose: the reading of epigenetic information, say, histone tail modifications, might be either chemical (molecular recognition and factor recruitment), physical (mechanical constraints) or kinetic (involved in hypercycles [26,34,35]). We here recover the requirement, yet underlined in the introduction (Sect. 1.2) for hybrid and multi-scale approaches, where physical, chemical and biological arguments intermingle. The involvement of mechanisms of different nature is likely to play a key role in regulation pathways: for instance, physical mechanisms do not compete on the same footing with plain chemical reactions, and might be the limiting, controlling step in the kinetic control of a pathway. A noticeable feature of these physical links, in particular mechanical and topological constraints, is their long-range nature, connecting distant regions of the genome and propagating constraints, *i.e.* information, over a large number of base pairs. This suggests that transcription regulation relies jointly on mechanisms, mainly physical, controlling a

global transcriptional potentiation, and specific biochemical pathways, locally controlling a targeted activation. Accordingly, a general working hypothesis following from our results is that transcriptional regulatory networks involve also mechanical links and topological links, alternating with more conventional biochemical links involving factor binding.

5.5 From kinematics to kinetics

Up to now, we focused on kinematic steps: what are the possible motions, deformations, conformational transitions, taking into account all constraints and estimating energy barriers encountered along a given path. This provides basic knowledge, to be supplemented with kinetics studies (determining the actual rates and timing) and dynamics studies (determining the process providing the free energy to actually travel the path). Our multi-scale modeling allows to determine quantitatively constraints of various natures: steric (accessibility), geometric (three-dimensional shape), mechanical (elastic energy), electrostatic and topological (linking number conservation). It thus gives a *full account of the fiber kinematics*, which is obviously a *prerequisite to describe conformational dynamics*. Moreover, our modeling studies give account of physical ways of controlling the binding affinities of various factors to DNA. In doing so, they *open onto kinetics studies* and offer a bridge with signalling pathways and intracellular rhythms (see the paper by A. Benecke in this issue, [35], and [26,34]). Kinematics and kinetics have now to be bridged into consistent regulatory schemes articulated at the chromatin fiber level.

6 An allosteric behavior of DNA within the fiber

6.1 The creative role of constraints

The model chromatin structure with gaped nucleosome presented in Sect. 3.2, whatever close to the actual condensed fiber it might be, illustrates a far more general and unquestionable fact: the 30 nm fiber compact conformations are accompanied with topological (conserved linking number), mechanical (elastic energies) and geometrical (three-dimensional distance and positioning) constraints. For instance, nucleosome stacking or fiber looping and anchoring prevent linker DNA and nucleosomes from being both, and everywhere along the fiber, in their mechanically relaxed state (one speaks of "frustration" in such a situation when elements cannot be all simultaneously in their ground state) and in any cases, prevent them from any free motion or deformation. In other words, constraints and frustration generated by the fiber architecture shape the energy landscape at the level of DNA and nucleosome. In this way, they might *give rise to metastable states*, either for DNA (*e.g.* linker buckling, Sect. 5.2) or for the nucleosome (*e.g.* nucleosome gaping, Sect. 3.2). These metastable states are absent in the relaxed assembly, *e.g.* the decondensed beads-on-string chromatin. They can be seen as additional mesoscopic degrees of freedom, specific of the fiber structure and productive of new potentialities, *e.g.* switch mechanisms. At the DNA level, we have seen that mechanical constraints modify DNA binding affinities. DNA conformational changes, in modifying these mechanical constraints, will thus also affect affinities (hence kinetic constants). In consequence, they might indirectly shift binding equilibria, in turn modifying back the overall architecture. Let us elaborate further on this point.

6.2 DNA allostery

The dependence of DNA/protein binding affinities with respect to the mechanical constraints experienced by the linker DNA, themselves modulated by the DNA conformation, can be seen as a *generalized allosteric mechanism*, in which the substrate is the protein, the allosteric unit is the DNA, the active site their binding site, and the effector is identified with any mechanism affecting the mechanical constraints experienced by the DNA (which indeed modulates the binding rate at the active site). The control of the very allosteric nature of a DNA stretch induced and controlled by its nesting in the highly organized chromatin superstructure is reminiscent of the nested allosteric interactions described in [36] in the context of protein complexes. A related effect is also described in [37] noticing that a hyper-structure might induce a localized allosteric switch. This scheme encompasses the general features of allostery currently encountered in enzymes [38], namely:

- *the presence of two different sites*: an effector (here the tuning of mechanical constraints experienced by the linker DNA, typically through a modification of its anchoring onto nucleosomes by histone tails post-translational modifications) and an active site (the protein binding site onto DNA). We here introduce a third class of allostery, supplementing the acknowledged homotropic and heterotropic ones, and that could be termed *"mechano-chemical allostery"*;
- *a long-range coupling between the effector and the active site*: the distance between the binding site and the linker anchoring points (where mechanical constraints originates) can be here as large as tens of base pairs (several nanometers);
- *no direct natural, causal relation between the active site and the effector*: the modifications improving the action of the effector are of no effect if directly applied to the active site; the coupling observed today is the result of an adaptive conjunction between the effector and the active site;
- *a possible cooperativity* [31]. The simplest, straightforward case is a cooperative inhibition, since each protein binding strenghtens the constraints and impairs further binding. We might also consider a more complex scenario in which the first protein binding captures and stabilizes a DNA conformational change more amenable to multiple bindings (in the example presented above, Sect. 5.2, we have shown that DNA buckling turns the quadratic increase of binding energy with respect to the number of bound proteins into a

linear dependence with respect to this number). Coregulators might be involved in DNA allostery, insofar as their binding with histone tails has repercussions on the mechanical constraints experienced by the DNA.

The allosteric entity is here the linker DNA (the DNA here involved is not a gene but rather a regulatory region flanking a gene). DNA is generally thought to be "only" the substrate of genetic information, but here it has the first role in the very mechanism of gene expression regulation: *DNA conformational changes might regulate any DNA/protein interaction*, *e.g.* the binding of transcriptional factors. It is not enough in eukaryotic cells to have allosteric enzymes; indeed, due to the packing of DNA and its regulatory sequences inside a compact chromatin fiber, the first steps of transcription have instead to be regulated by an *allosteric substrate*.

6.3 A multi-scale and physical approach of chromatin functions

The various scenarios presented in the previous sections, culminating with the DNA allosteric behavior elicited by its constraining and frustrating nesting within the chromatin fiber, underline the need and benefits of a multi-scale approach for understanding chromatin fiber functions. A consistent framework *bridging scales and levels of organization from DNA up to chromosome* is obviously required to get an integrated understanding of transcription. Moreover, a multi-scale functional modeling is essential to articulate the experimental knowledge obtained at different levels. We here mainly investigated the articulation between the DNA level and the fiber architecture. In fact, *further levels of organization* take place within the chromosome, besides still more debated than the fiber structure itself. Our multi-scale approach could be extended to include these upper levels and associated physical constraints, possibly generated by the anchoring of the fiber onto MAR (the counterpart of linker DNA anchoring onto the nucleosomes) or the involvement of architectural proteins [23].

The multi-scale approach should be at the same time bottom-up (as implemented to determine the elastic properties of chromatin fiber) and top-down (as in the investigations to validate a molecular gaping mechanism, that would achieve an efficient fiber condensation). But neither a bottom-up nor a top-down approach is enough to account for the strong interplay between the different levels of organization, due mainly to feedback from higher scales onto smaller ones, settled during the course of evolution. Hence only *"up-and-down"* procedures and integrated models can account for the full complexity and adapted behavior of the chromatin fiber during gene expression regulation. Such an inter-level, up-and-down approach is in particular essential to substantiate in concrete situations the notion of DNA allostery introduced above, Section 6.2.

A side remark is that a constrained structure exhibits less degrees of freedom, hence a more determined behavior; motions are channeled and fewer pathways can be traveled. This favors adaptive evolution and joint tuning of the elementary features towards an improvement of functional efficiency: co-evolution converges more rapidly when it is faced to some stable behavior to act upon.

Let us open here a methodological parenthesis. Physical modeling more or less relies on the existence of generic principles, mechanisms or architecture, whereas biological experiments often put forward the essential role of highly specific components. A possible way to reconcile these seemingly opposite viewpoints is to consider at the same time the existence of general structural regulatory principles, feedback loops and multi-scale organization (the core of the present paper) and the highly specific features of their actual implementation, *e.g.* using different molecules in different contexts for implementing the same general regulatory scheme. This idea of *specific implementation of general principles* can be carried further: it is precisely because the same few generic principles are at work in different instances that the molecules and the other ingredients involved need to be different, in order to adapt to the specific instance, cell or organism and ensure the robustness of biological functions.

7 The chromatin fiber, an emergent hyper-structure

A first conclusion of our modeling studies is that the different levels sketched on Figure 1 cannot be separated functionally. Our kinematic investigations of the chromosome structural reorganization during the cell cycle at different levels (nucleosome, DNA, fiber, chromatin loops) suggest how these levels might be bridged. In particular, we underline that upper levels control back smaller-scale ones. This fact might be underestimated since it is not necessarily reproducible *in vitro*: the consistent couplings at work *in vivo* between the different levels of organization, at the center of our modeling investigations, might be lost in *in vitro* experiments, irrespective of the exquisite inter-level tuning and adaptation.

Our results hint towards two different chromatin fiber organizations (at least): a mitotic structure, ensuring a tight overall condensation and whose dominant ordering rule is nucleosome stacking; a functional organization of the fiber inducing and monitoring constraints on DNA, in turn allowing to control in a specific fashion transcription initiation and DNA/protein interactions. In any cases, mechanical constraints generated at the DNA level by the chromatin hyper-structure play a key (though currently ignored) role in DNA/proteins interactions: the affinity of any factor for DNA is modified if the DNA stretch containing the binding site is anchored at its ends, due to extra binding energy associated with DNA compression, bend and twist upon protein binding. This fact suggests general mechanisms of epigenetic control of DNA/protein interactions, for instance through the influence of histone post-translational modifications on the fiber structural, topological and mechanical properties. Furthermore, the constraints generated by the fiber hyper-structure might

induce conformational switches at the DNA level, in turn affecting its binding affinities. This led us to propose a mechano-chemical allostery of linker DNA controlled by epigenetic modifications of mechanical constraints, *e.g.* through the tuning of the linker DNA anchoring onto nucleosomes by means of histone post-translational modification.

The feedback loop between the DNA and the chromatin fiber levels can be seen as the result of adaptive evolution: both levels have evolved jointly and *consistently*, in a way increasing their initial fit and interplay (so as to better achieve biological functions), up to the closed regulatory loop observed today. In doing so, evolution gradually turned physico-chemical properties into biological functions. Now it is no longer possible to isolate linear causal relations or to investigate separately one level: only a functional, self-consistent approach could provide a relevant picture, and joint adaptation then provides a guide in elaborating integrated models.

In conclusion, considering the chromatin fiber as an emergent entity is essential to properly account, in a tractable way, for the mechanical constraints encountered during its conformational changes and structural reorganization along the cell cycle. ADN is constrained by the fiber structure, on both mechanical and topological grounds. It is thus of the utmost importance to unravel the fiber structure and to understand what determines its conformational changes. Energy and topology arguments set strong rules, determining in an almost unique way the kinematics of the fiber. This view provides a common general backbone, lending itself to fine tuning, possibly local and specific, by additional biological factors controlling the various ingredients and basic mechanisms articulated in the above scenario in a unmodulated, homogeneous fashion.

We are very grateful to Maria Barbi, Eli Ben Haim, Hua Huong, Christophe Lavelle and Julien Mozziconacci: this paper is based on common published work. We warmly acknowledge Arndt Benecke for sharing with us his insights and results before publication.

References

1. A.P. Wolffe, *Chromatin: Structure and Function* (Academic Press, New York, 1998).
2. A. Sivolob, C. Lavelle, A. Prunell, J. Mol. Biol. **326**, 49 (2003).
3. H. Schiessel, J. Phys. Condens. Matter **15**, R699 (2003).
4. C.L. Woodcock, S.A. Grigoryev, R.A. Horowitz, N.A. Whitaker, Proc. Natl. Acad. Sci. U.S.A. **90**, 9021 (1993).
5. E. Ben Haim, A. Lesne, J.M. Victor, Phys. Rev. E **64**, 051921 (2001).
6. E. Ben Haim, A. Lesne, J.M. Victor, Physica A **314**, 592 (2002).
7. M. Barbi, J. Mozziconacci, J.M. Victor, Phys. Rev. E **71**, 031910 (2005).
8. J. Mozziconacci, J.M. Victor, J. Struct. Biol. **143**, 72 (2003).
9. J. Mozziconacci, C. Lavelle, M. Barbi, A. Lesne, J.M. Victor, FEBS Lett. **580**, 368 (2006).
10. J.C. Hansen, Annu. Rev. Biophys. Biomol. Struct. **31**, 361 (2002).
11. C. Vaillant, B. Audit, C. Thermes, A. Arnéodo, *Formation and positioning of nucleosomes: Effect of sequence-dependent long-range correlated structural disorder*, this issue.
12. K. Bystricky, P. Heun, L. Gehlen, J. Langowski, S. Gasser, Proc. Natl. Acad. Sci. U.S.A. **101**, 16495 (2004).
13. G. Wedemann, J. Langowski, Biophys. J. **82**, 2847 (2002).
14. B. Mergell, R. Everaers, H. Schiessel, Phys. Rev. E **70**, 011915 (2004).
15. Y. Cui, C. Bustamante, Proc. Natl. Acad. Sci. U.S.A. **97**, 127 (2000).
16. K. Van Holde, J. Zlatanova, J. Biol. Chem. **270**, 8373 (1995).
17. B. Rydberg, W.R. Holley, S. Mian, A. Chatterjee, J. Mol. Biol. **284**, 71 (1998).
18. B. Dorigo, T. Schalch, A. Kulangara, S. Duda, R.R. Schroeder, T.J. Richmond, Science **306**, 1571 (2004).
19. J.T. Finch, A. Klug, Proc. Natl. Acad. Sci. U.S.A. **73**, 1897 (1976).
20. T. Schalch, S. Duda, D.F. Sargent, T.J. Richmond, Nature **436**, 138 (2005).
21. K. Maeshima, U.K. Laemmli, Dev. Cell **4**, 467 (2003).
22. E.M.M. Manders, A.E. Visser, A. Koppen, W.C. de Leeuw, R. van Liere, G.J. Brakenhoff, R. van Driel, Chromosome Res. **11**, 537 (2003).
23. R. Gassmann, P. Vagnarelli, D. Hudson, W.C. Eamshaw, Exp. Cell Res. **296**, 35 (2004).
24. T. Cremer, C. Cremer, Nat. Rev. Genet. **2**, 292 (2001).
25. D.L. Spector, Annu. Rev. Biochem. **72**, 573 (2003).
26. A. Benecke, in *Proceedings of the Dieppe Spring School on Modelling and Simulation of Biological Processes in the Context of Genomics*, edited by P. Amar, F. Kepes, V. Norris, P. Tracqui (Platypus Press, 2003) pp. 31-40.
27. A. Eberharter, P.B. Becker, EMBO Rep. **3**, 224 (2002).
28. D.M. Sauvé, H.J. Anderson, J.M. Ray, W.M. James, M. Roberge, J. Cell Biol. **145**, 225 (1999).
29. M.G. Poirier, J. Widom, Poster at the conference *"International Biophysics Congress", August 27th-September 1st 2005, Montpellier, France*, `http://worldbiophysics2005.sfbiophys.org/`.
30. J.M. Victor, E. Ben Haim, A. Lesne, Phys. Rev. E **66**, 060901 (2002).
31. J. Monod, J. Wyman, J.P. Changeux, J. Mol. Biol. **12**, 88 (1965).
32. B. Mellström, J.R. Naranjo, Curr. Opin. Neurobiol. **11**, 312 (2001).
33. J. Parello, private communication. See also J. Parello, *Polyamines and chromatin: A tale of cations and histone N-tails*, in the *Proceedings of the conference "Chromosome Organization, Dynamics and Function", Saclay, 2005, March 23-25.*
34. A. Benecke, ComPlexUs **1**, 65 (2003). See also the published comment: M. Buchanan, ComPlexUs **1**, 65.
35. A. Benecke, *Chromatin code, local non-equilibrium dynamics and the emergence of transcription regulatory programs*, this issue.
36. C.H. Robert, H. Decker, B. Richey, S.J. Gill, J. Wyman, Proc. Natl. Acad. Sci. U.S.A. **84**, 1891 (1987).
37. K.E. Van Holde, K.I. Miller, E. Van Olden, Biophys. Chem. **86**, 165 (2000).
38. A. Cornish-Bowden, *Fundamentals of Enzyme Kinetics*, 3rd edition (Portland Press, 2004).