Chromatin Topological Transitions

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DNA transaction events occurring during a cell cycle (transcription, repair, replication) are always associated with severe topological constraints on the double helix. However, since nuclear DNA is bound to various proteins (including histones) that control its accessibility and 3D organization, these topological constraints propagate or accumulate on a chromatin substrate. This paper focuses on chromatin fiber response to physiological mechanical constraints expected to occur during transcription elongation. We will show in particular how recent single molecule techniques help us to understand how chromatin conformational dynamics could manage harsh DNA supercoiling changes.

§1. Introduction

Genomic DNA in eukaryotic cells is organized in discrete chromosome territories, each consisting of a single huge supercoiled nucleosomal fiber. Through structural changes resulting from the transient modifications of its constituents (recruitment of histone variants and non-histone proteins, histone chemical modifications, nucleosome remodeling), chromatin plays a critical role in the regulation of all DNA transaction processes, such as repair, replication, recombination and transcription. Namely, since DNA is almost never naked in eukaryotic nuclei, chromatin processing necessarily precedes DNA processing. Hence, chromatin not only provides a convenient way to pack two meters of DNA into the nucleus volume, it is also a polymorphic and highly dynamic structure which regulatory role is now largely acknowledged.

Chromatin fibers are made of a repetitive unit, the nucleosome, which consists of 147 bp of DNA wrapped ∼1,7 times in a left-handed superhelix around an octamer of histones containing two copies each of the four core histones H2A, H2B, H3 and H4.[1)] This leads to both compaction and topological deformation of the DNA by one negative turn per nucleosome.[2)] Acting both as a compaction and regulatory tool, nucleosomes must be reasonably stable while keeping some dynamic properties to allow transient DNA access for, e.g. transcription factor or RNA polymerase binding to specific sequences upon transcription initiation, or, even more dramatically, chromatin clearance during transcription elongation. These seemingly contradictory

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properties raise many questions, such the way RNA polymerase progresses along chromatin template, the subsequent fate of nucleosomes, and the mechanistic role of various elongation factors and topoisomerases.[3)–6)]

What happens to nucleosomes during transcription (or, equivalently, what happens to polymerase as it encounters nucleosomes) is indeed a question that has not lost much of its mystery,[3),7)] partly due to the difficulty to obtain a clear and unifying picture from the apparently inconsistent results arising from many different experimental studies (see references in Ref. 3)) In particular, it is often hard to make the difference between direct (mechanical, through contact) or distal (topological, through DNA elastic constraints propagation) effects of polymerase tracking.

In this review, we wish to focus on the distal aspects. Single-molecule manipulations have indeed recently complemented conventional biochemical and biophysical techniques to decipher the complex mechanisms ruling chromatin dynamics. Micromanipulations with magnetic tweezers opened opportunities to handle single chromatin fibers and to measure their mechanical response to forces and torques mimicking the constraints produced by various molecular motors acting on DNA.[8)] We will show how these experiments unveiled (i) the unsuspected high torsional resilience of chromatin fiber[9)] and (ii) the nucleosome capacity to change its chirality.[10)]

§2. Dealing with supercoiling waves produced by a transcribing polymerase

Several nuclease digestion assays gave early evidences that although chromatin structure is usually altered during gene activity,[11)] nucleosomes are not completely lost.[12),13)] To what extent nucleosomes dissociate or simply unfold when polymerase gets through remains an open question, as are the partners and mechanisms involved.[14)] The different histone-DNA destabilization models rely on direct interaction with the polymerase and elongation factors potentially assisted by the propagation of topological stress ahead of the polymerase.[3)] Namely, as RNA polymerase tracks along the DNA template, it follows an helical path: transcription elongation thus requires a rotation of the transcription complex relative to the DNA template.[15)] Now, the question remains whether polymerase rotates around DNA or DNA rotates through polymerase.[16),17)] In the later case, tracking would generate positive supercoils ahead of the polymerase and negative supercoils behind it, as it was proposed by Wang and coworkers in the "twin supercoiled domain" model to describe bacterial transcription[18)] (Fig. 1(a)) and since then sustained by many experimental evidences (see Ref. 3) for a review).

Foreseeing the consequence of such a model in a chromatin context, Lee and Garrard proposed that positive supercoiling in front of the RNA polymerase could provide a mechanism to unfold the nucleosomes and decondense the chromatin fiber in preparation for polymerase passage[19),20)] (Fig. 1(b)). In a further refinement of this model, Bancaud et al. proposed that part of the supercoiling constraints could be absorbed by changes in the crossing status of nucleosomes within chromatin fiber, hence enabling a smooth elongation[9)] (Fig. 1(c)). Indeed, from a kinetic point of view, the amount of supercoiling generated by a polymerase translocating at ∼10–

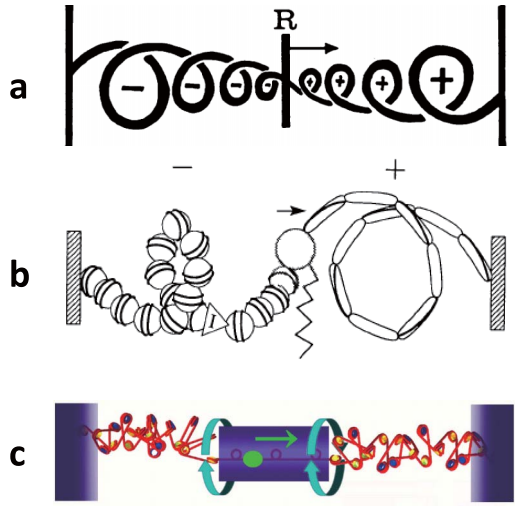

Fig. 1. The twin-supercoiled model and its transcriptional consequences. (a) Twin-supercoiled domain model: when a transcription ensemble R (include polymerase, nascent RNA, and eventually proteins bound to RNA) tracks along DNA without rotating, the DNA in front of the polymerase becomes positively supercoiled and the DNA behind becomes negatively supercoiled.[18] (b) In a nucleosomal context, positive supercoils in front of the transcription complex could unfold nucleosomes, while negative supercoils in the back would re-fold them and tightly pack the structure.[19] (c) In the three-state model of chromatin fiber, chromatin fiber in front of the polymerase shifts to a positive state (nucleosomes cross positively) whereas chromatin in the back shifts to a negative state (nucleosomes cross negatively).[9]

100 bp/s, as suggested from polymerase transcriptional velocity measurement in vitro[21] an in vivo,[22] should be roughly 1–10 supercoils/s, potentially exceeding the relaxation capability of DNA topoisomerases.[23),24]

By comparing yeast minichromosomes relaxation by topoisomerase I (topoI) and II (topoII) in vivo, Roca and co-workers showed that, in contrast to what is observed with naked DNA, topoII is much more efficient than topoI.[25] Considering that chromatin could impose barriers for DNA twist diffusion (which impair the DNA strand-rotation mechanism of topoI), whereas it favors the juxtaposition of DNA segments (which facilitates the DNA cross-inversion mechanism of topoII), transcription elongation could doubly benefit from the high torsional resilience of chromatin evidenced in Ref. 9). First, it could cushion the supercoiling waves generated by polymerases and dampen the driving torque produced by polymerase translocation. Second, it could simultaneously favor the DNA transport activity of topoII by increasing the juxtaposition probability of DNA segments and facilitate nucleosome entry/exit DNAs to shift from a crossed negative state to a crossed positive one.[3),25] Moreover, one can speculate that topoI mainly relaxes negative supercoiling produced in the wake of the polymerase and present as negative torsional constraint in linker DNA,[26] while topoII relaxes positive supercoiling produced in front of the polymerase and present as positive writhe, since no sink for torsional stress such as melting exists in this case.[27] On the other hand, in regions where the transcription rate is high, nucleosomes may be depleted due to frequent polymerases passage, and DNA-pulling

forces (see below) exerted by polymerases may hinder the formation of writhe: helical tension would then deform DNA mostly by twist, allowing topoI to be more efficient than topoII. This hypothesis still needs to be tested in vitro and in vivo.

§3. Mimicking transcription elongation constraints in vitro, at the single molecule level

Several studies showed that genomic regions dynamically expand from the surface of chromosome territories upon transcription[28),29)] (Fig. 2(a)). In this scheme, transcription complexes have been suggested to be part of fixed structures called "transcription factories" that participate into the functional partitioning of the genome.[30)–33)] Polymerases are indeed among the most powerful molecular motors, potentially exerting tenth of piconewtons (pN) on DNA templates.[34)–36)] Although these measurements were made with prokaryotic enzymes, one can reasonably postulate that eukaryotic polymerases (potentially assisted by secondary molecular motors such as myosin; see Ref. 37) for discussion) have enough power to pull the chromatin template through the viscous nucleus medium. At the same time, this could unfold the so-called 30 nm fiber into a linear 10 nm nucleosomal array[38)] (Fig. 2(b)), and even disrupt nucleosomes, as shown by many single molecule studies (see Ref. 8) and references therein). Indeed, single-molecule manipulation techniques appear as convenient tools to investigate DNA or chromatin response to mechanical constraints. In our magnetic tweezers set up, a pair of external magnets is used to physically twist and pull microscopic paramagnetic beads to which a piece of chromatin is attached (Fig. 2(d)). Then, by approaching and/or rotating the magnets, we can change the tension and torsion applied to the fiber, hence mimicking the action of a translocation polymerase (Fig. 2(c)).

In a typical experiment, one follows the extension of the molecule as a function of torsion (Fig. 3(a)). The response of naked DNA (dotted red curve) displays the signature of a supercoilable (non-nicked) single duplex DNA. The central (plateau-like) part of the curve corresponds to the elastic regime, and the quasi-linear compactions on either side to the formation of positive or negative plectonemes upon introduction of positive or negative rotations.[39)] The lower compaction on the negative side is due to a force-dependent DNA melting at high negative torsions, which prevents further increase of the torque on the molecule and prevents further plectoneme formation.[39)] Compared to DNA, chromatin is shorter and its centre of rotation is shifted to negative values (blue curve). The shift is the expected consequence of the absorption of approximately one negative superhelical turn per nucleosome ($\Delta\mathrm{Lkp} \sim -1$), the observed shortening (~ -50 nm, i.e. 150 bp per nucleosome) resulting from DNA wrapping around the histone core. Compared to DNA of the same length, the fiber appears to be torsionally extremely flexible, i.e. it can absorb large amounts of torsion without much shortening.[9)] This large torsional resilience was interpreted as a reflection of the nucleosome dynamic equilibrium between the three conformational states previously identified in minicircles studies.[40)–42)] At the same time, this experiment provides a prediction of the torque exerted by chromatin fiber as a function of its torsion: this torque can go up to 3 pN.nm.rad-1 (compared to 6 pN.nm.rad-1

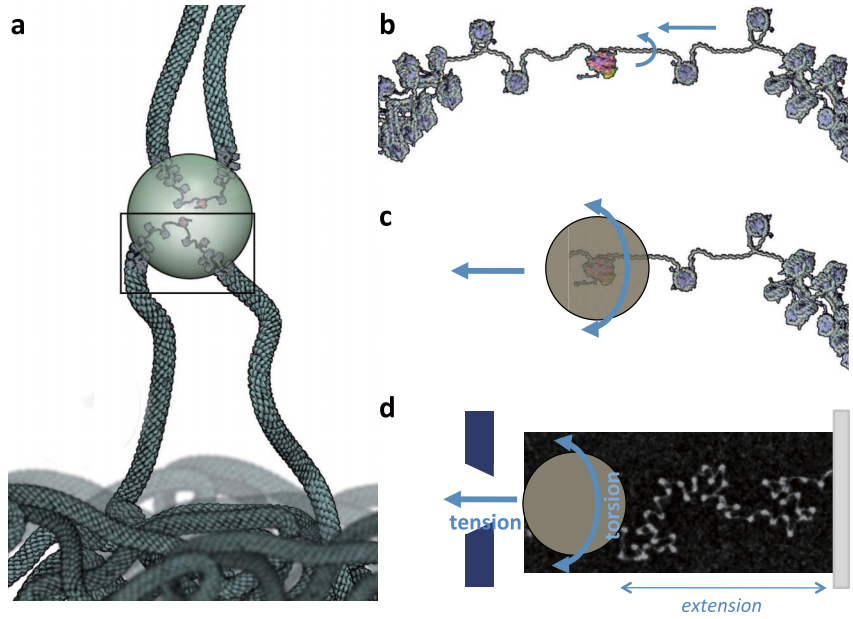

Fig. 2. The transcription factory model and its mechanical consequences assessed in vitro (a) Upon transcription, a chromatin loop expands out of a chromosome territory and polymerase pulls chromatin through the factory (potentially shared by other transcribed genes from adjacent chromosome territories). (b) Inside the factory, chromatin is pumped and screwed through the polymerase, undergoing both tension and torsion. (c,d) Both of these constraints can be reproduced in vitro by attaching a chromatin fiber between a glass slide and a paramagnetic bead which rotation and displacement is controlled by a pair of magnets. Reconstituted chromatin fibers from purified components (DNA and histones) are used for these studies, and usually checked by transmission electron microscopy (see picture in (d)) before use.

for naked DNA), that is, in any way, smaller than that exerted by advancing polymerase (> 5 pN nm.rad-1),[15] further suggesting that chromatin torsional resilience may facilitate transcription elongation.[9]

Now, since a sufficient amount of tension was shown to disrupt nucleosomes,[9] we set out to test the Lee and Garrard scenario,[19] and measure the amount of torsion necessary to destabilize nucleosomes. To our surprise, virtually any positive or negative supercoiling could be applied to chromatin fibers without loss of nucleosomes.[10] Even more surprisingly, these chromatin fibers, after extensive positive supercoiling, display a hysteretic behavior (Fig. 3(a); blue and green curves). This hysteresis was interpreted as a consequence of the trapping of positive turns in individual nucleosomes through their transition to an altered "chirally reversed" form, called reversome (for reverse nucleosome), to which the next section is dedicated.

§4. Chiral issues

Nucleosomes depicted with right-handed chirality are usually the result of inadvertent infographics errors, either because artists who draw these pictures are

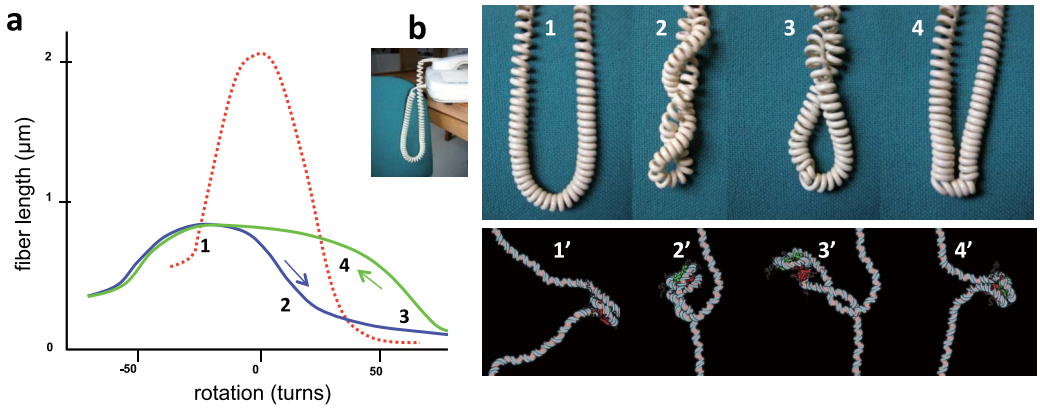

Fig. 3. (color online) The reversome hypothesis. (a) Length vs rotation response of a saturated reconstituted nucleosomal array. Hysteresis is observed between the onward (blue) and backward (green) curves when a high positive torsion is applied. The zero-turn rotation reference corresponds to the relaxed state of naked DNA (red dotted curve). The shortening, shifting and flattening of the blue and green curves are interpreted as the consequence of nucleosome reconstitution (each nucleosome wraps 50 nm of DNA in one negative superhelical turn) and conformational flexibility (three-state model),[9] while the hysteresis between both curves is interpreted as the consequence of a transient chiral transition of nucleosomes upon high positive torsional stress to an altered right-handed form called "reversome".[10] (b) The nucleosome-reversome transition has been modeled:[47] starting from a canonical nucleosome [1], constraints accumulate in DNA (forming plectonemes) [2] and trigger the unfolding of the nucleosome [3] that refold in a right-handed reversome, relaxing at the same time the plectonemes [4]. The whole scenario can be conveniently experimented at desk by using an old XXth century phone (inset): starting from a relaxed cord [1′], one can pick up the phone and apply a few turns (opposite to the cord chirality) before putting the telephone down; the constraint first accumulate as plectonemes within the cord [2′], but is eventually relaxed by the spontaneous chiral inversion of some cord gyres [3′,4′].

not aware of chirality matters, or if canonical nucleosome pictures are accidentally mirrored upon publication.[43] DNA is another victim of neglected chirality, which results in journal covers and commercial advertisements full of unintentional left-handed DNA. Amazingly, nucleosome chirality must have evolved as a consequence of DNA chirality. Indeed, since a canonical nucleosome constrains one negative turn of DNA,[2] the removal of a nucleosome to gain access to a particular sequence, such as a promoter, simultaneously favours the melting of DNA, which is a necessary step for initiating transcription. In other words, a gene with right-handed nucleosomes would be condemned to silence: each time a nucleosome is removed, the DNA helix would tighten and thus restrict its unwinding. However, several papers have challenged the idea of unalterable cylindrical left-handed nucleosomes and have accumulated evidence for the existence of alternative histone/DNA assemblies, some of which being potentially topologically reversed (right-handed) (reviewed in Ref. 44)).

Based on topological assays of particles reconstituted on DNA minicircles, Prunell and colleagues provided the first in vitro evidence for the existence of right-handed

(H3-H4)2 tetrasomes.[45),46)] We then showed that nucleosome arrays submitted to large positive torsional stress transiently trap approximately one positive turn of DNA topological deformation per particle[10)] (Fig. 3). We suggested this to reflect the chiral transition of nucleosomes, resulting into metastable octameric particles built on the right-handed tetrasome, that we called reversomes. We were then able to model the transition steps and to propose an atomic structure for the reversome[47)] (Fig. 3(b)). The role of such a transition during transcription elongation is easily visualized by looking at a phone cord (Fig. 3(b), the most difficult part nowadays being to find such a phone apparatus...). By repetitive picking up/putting down cycles at each phone call, supercoiling constraints potentially accumulate within the cord,[48)] which first triggers plectonemes formation within the cord, eventually subsequently relaxed by chiral inversion of some cord gyres. This potential cooperative ("domino-like") transition of successive gyres has been proposed to facilitate transcription elongation, thanks to the high relaxation efficiency of a so-called "reversome wave" in front of the polymerase.[49)] Note however that, in order to truly facilitate transcription, these left-to-right chiral transitions of nucleosomes triggered by the propagation of the supercoiling wave are expected to occur far in front of the polymerase, acting as a topological buffer to absorb excess of positive supercoiling. On the opposite, the presence of stable right-handed reversomes in the path of the polymerase would rather create a block for transcription, since these positively constrained structures would either be further stabilized by the positive supercoiling wave pushed ahead of the polymerase (therefore causing quite intractable steric hindrance) or prevent local DNA strand separation in case of reversome destabilization (see former discussion on the co-evolution of DNA and nucleosome chirality). Now, one should keep in mind that this model mainly applies to intense transcription events, where local loss and exchange of all core histones during passage of the polymerase are observed, while moderately transcribed genes show no nucleosome displacement (see Refs. 3),50),51) and references therein). In that case, nucleosome as well as reversome, even if present in the vicinity of the polymerase, are not expected to represent a strong constraint for the elongating enzyme coming into contact of (and turning around) these particles. To sum up, we propose a model in which, in the vicinity of the polymerase, elongation factors (such as FACT; see Refs. 3),7) for reviews) help to destabilize nucleosome (potentially benefiting from the nucleosome unfolding during the course of the chiral transition to catch histones "on the run"), while complete reversome transition could happen farther in the front (in absence of elongation factors, outside the transcribed sequence, therefore on a place polymerase would never reach) to alleviate excess positive supercoiling. Indeed, the reversome transition may take place in uncondensed (non-transcribed) regions, as supported by our recent data showing that this transition happens even in the presence of linker histones.[52)]

Remarkably, a totally unrelated study provided two years later the first in vivo evidence for constitutively right-handed supercoiled chromatin particles.[53)] Henikoff and co-workers previously identified half-nucleosomes in interphasic Drosophila centromeres.[54)] These tetrameric particles, further called hemisomes,[44)] were then proposed to wrap DNA with an opposite helicity in comparison to "canonical" nucleo-

somes, as observed from in vivo investigation of yeast centromeres topology.[53] This raised intriguing new questions, such as how centromeric histone variants may be assembled in a right-handed particle, and how/why chromatin would retain negative supercoiling in chromosome arms but positive supercoiling in centromeres. Obviously, from the discussion above, stable particles with positive supercoiling would provide a natural block to transcription, suggesting an evolutionary reason for the presence of constitutive positive supercoiling constraints in centromeres. Now, because histone composition was not clearly determined in Henikoff's studies, the question remains as to whether these centromeric particles are truly made of right-handed hemisomes, or rather right-handed tetrasomes, hexasomes (a tetramer plus one H2A-H2B dimer), nucleosome or even other kind of histone/DNA assemblies.[47),55),56] Indeed, we showed that chromatin fibers reconstituted with CENP-A-containing (octameric) nucleosomes undergo the reversome transition under positive torsional constraints (unpublished data). These results indicate that the right-handed centromeric particles could, in principle, be made of reversomes. Furthermore, right-handedness is not expected to be an intrinsic property of CENH3-containing particles alone. Many in vitro studies showed that centromeric nucleosomes are canonical nucleosomes.[57)–61)] although their conformational dynamics is somewhat different from that of H3-containing nucleosomes.[58)] Now, to reconcile these data, it could be possible that other factors (such as centromeric histone chaperones) modulate nucleosome topology in vivo, potentially in a cell-cycle dependent manner.[56),62)] Further investigations are required to unravel this exciting chiral issue.

§5. Conclusion and perspectives

Chromatin is constantly changing its structure to dynamically and functionally accommodate DNA transcription. How transcription elongation takes place on a nucleosomal substrate remains a puzzling question, as is the precise role and/or fate of topological constraints generated by the tracking polymerase. While tension and positive supercoiling in front of the polymerase may help destabilize nucleosomes, negative supercoiling in its rear may help reassemble nucleosomes, and potentially induce some regulatory DNA secondary structures.[26),27)] Using magnetic tweezers to manipulate single nucleosomes arrays, we showed that chromatin is not inert for these torsional constraints, as the existence of a dynamic equilibrium between different crossing statuses of the entry/exit DNAs of each nucleosomes enabled chromatin fibers to reversibly accommodate large amount of supercoiling.[9)] Moreover, we showed that the application of large positive torsional stress — mimicking the supercoiling wave produced in front of a polymerase — can potentially trigger nucleosomes chiral transition to a metastable right-handed form.[10)]

Remarkably, nucleosomes are far from being "tuna cans",[63)] but are instead highly polymorphic and dynamic entities. This polymorphic nature has been extensively documented by molecular biology techniques, showing that post-translational modifications or histone variant substitutions play key roles in the regulation of genome transactions. However, the conformational dynamics of nucleosomes have remained disregarded because it could easily be sensed. Yet, our view that nucleosomes

continuously fluctuate between crossed and uncrossed states and can transiently form subparticles or other altered forms to appropriately respond to surrounding constraints[37),44),64)] adds a new line to the already rich palette of chromatin properties, and will deserve future work to understand how chromatin interacting proteins modulate these properties.

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