Published in final edited form as:

Biochim Biophys Acta. 2012 July ; 1819(7): 632–638. doi:10.1016/j.bbagr.2011.12.007.

The importance of being supercoiled: how DNA mechanics regulate dynamic processes

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Abstract

Through dynamic changes in structure resulting from DNA-protein interactions and constraints given by the structural features of the double helix, chromatin accommodates and regulates different DNA-dependent processes. All DNA transactions (such as transcription, DNA replication and chromosomal segregation) are necessarily linked to strong changes in the topological state of the double helix known as torsional stress or supercoiling. As virtually all DNA transactions are in turn affected by the torsional state of DNA, these changes have the potential to serve as regulatory signals detected by protein partners. This two-way relationship indicates that DNA dynamics may contribute to the regulation of many events occurring during cell life. In this review we will focus on the role of DNA supercoiling in the cellular processes, with particular emphasis on transcription. Besides giving an overview on the multiplicity of factors involved in the generation and dissipation of DNA torsional stress, we will discuss recent studies which give new insight into the way cells use DNA dynamics to perform functions otherwise not achievable.

Keywords

DNA supercoiling; DNA topology; Non-B DNA; Transcription

1. Introduction

As soon as the helical structure of DNA had been drawn, understanding how the DNA strands, which intertwine around each other, are separated during DNA replication or transcription was an open and fundamental question. This task appeared to be even more challenging after the discovery of circular DNA molecules [1]. The solution used by the cell to overcome the topological problem was revealed with the discovery of DNA topoisomerases that catalyze changes in the linkage of DNA strands and modulate DNA topology [2]. It is now certain that all DNA transactions involve alterations in the structure of DNA. The structural changes that distort the double helix through overtwisting/undertwisting and associated loop-like plectoneme structures are referred to as DNA supercoiling or DNA torsional stress (Fig 1a) [3]. *In vitro* and *in silico* studies have shown that DNA supercoiling modulates the probability of DNA melting, affects DNA-protein

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interactions, and increases the local concentration of distal DNA sites [4]. Consequently, the activities that induce DNA supercoiling may be exploited in regulatory pathways.

In bacteria, the genomic DNA is maintained in an undertwisted state which facilitates localized melting of the double helix at origins of replication or transcription initiation sites, contributes to the formation of the nucleoid structure and promotes recombination events [5,6,7]. The concerted activities of topoisomerases and gyrases (DNA supercoiling enzymes) are determinant for maintaining the supercoiling homeostasis necessary to optimize these key genetic processes [8]. Eukaryotic organisms lack enzymes such as DNA gyrase that directly introduce supercoils into DNA, but statically their genome is supercoiled to a similar degree of bacterial genome [9]. Each nucleosome of the chromatin is wrapped by DNA 1.8 times and constrains approximately one negative supercoil which cannot diffuse to remote areas until released by nucleosome removal [10,11]. Thus, as a consequence of the chromatin organization, the net of DNA supercoils is fixed in the eukaryotic genome and is known as constrained supercoils. The unconstrained supercoils must be accommodated within the linker DNA (regions separating the nucleosomes) which in average represents only 20% of the genomic DNA in higher eukaryotes and decreases up to 6% in the yeast [12,13]. Dynamic interplay between broadly distributed constrained supercoils and the local unconstrained supercoils in the eukaryotic genome complicates the assessment of the DNA torsional state in the cells [14,15,16]. Only recently the experimental approaches have advanced to the point where it is feasible to interrogate the role of DNA topology in gene regulation.

2. Origin of DNA supercoiling

Cellular processes dynamically change DNA topology. According to the “supercoiled domain model” the activities that force DNA to revolve around its axis generates a local domain of DNA supercoiling (Fig 1b). This hypothesis applies with minor modification to the movement of transcription and replication complexes as well as for some helicase and restrictase activities [17,18,19,20]. Currently, the best investigated example is transcription-generated supercoiling. Due to the overwhelming molecular mass of the RNA polymerase and given the arguments in favor of immobilization of RNA polymerase in transcription factories, the DNA template is forced to rotate around its axis as the double helix threaded through the transcriptional machinery [21,22,23,24]. The upstream DNA becomes untwisted, while the downstream DNA becomes overtwisted which is referred to as negatively and positively supercoiled, respectively. If the translocation proceeds without pauses then the RNA polymerase could generate up to 10 supercoils per second and up to 3000 supercoils for a typical 30 kbp gene [25,26]. This enormous torsional stress might be inhibitory for efficient transcription [17,27,28]. Consequently, it is relieved by DNA topoisomerases which transiently break and rejoin the backbone of DNA [19].

Another source of DNA supercoiling is provided by the reorganization of eukaryotic chromatin: the disassembly or assembly of nucleosomes releases or absorbs DNA superhelicity. Special protein complexes called chromatin remodelers are able to remove or slide nucleosomes in an ATP-dependent fashion [29,30]. Notably, *in vitro* experiments have shown that these chromatin remodeling activities directly generate torsional stress of DNA in the presence of nucleosomes [31]. While the remodeling of the chromatin structure is a broad phenomenon that could involve sometimes entire loci, it is very difficult to assess and measure *in vivo* the extent of generated unconstrained supercoiling due to the transient nature of this process which could be unsynchronized in a population of cells [32,33]. Consequently, direct evidence is still needed.

In addition to DNA-tracking activities and chromosome remodelers, the existence of nuclear actins and myosin in principle may allow mechanical forces to be applied directly to chromatin fibers [34,35]. Single DNA molecule experiments *in vitro* have demonstrated a dynamic coupling between twisting-untwisting of the double helix and stretching forces, a possibility which remains largely unexplored *in vivo* [36].

3. Tuning of transcription-generated DNA supercoiling

The level of supercoiling depends on two opposite processes: how fast torsional stress is introduced into the DNA, and how fast it is relaxed or diffused into remote regions of the genome. The supercoil generation in the DNA flanking RNA polymerase complexes depends on the rate of transcriptional elongation which may be relatively invariant in the absence of specific RNA polymerase pausing or stalling and on the rate of transcriptional initiation [17,37,38]. Thus low level transcription produces a pulse of torsional stress followed by DNA relaxation, while high level transcription, due to repetitive initiation, may establish stable dynamic supercoiling upstream of transcription start sites [39,40]. In the transcribed unit of highly active genes the DNA regions between RNA polymerases transcribing in tandem contain supercoils of opposite polarity that could annihilate each other. Other important parameters include the distribution of promoters which, in divergent orientation, could reinforce DNA supercoiling upstream transcription start sites by untwisting the double helix as well as by inducing directly plectonemes [41], and the presence or absence of barriers to diffusion of torsional stress [42]. The dynamics of supercoil diffusion should depend on the behavior of chromatin fibers: in principle, the position of individual nucleosomes, the interactions between them, the linker binding proteins and the nucleosome modifications will govern supercoil propagation. We still do not know much about these important properties of chromatin, but single nucleosome array experiments *in vitro* reveal high torsional flexibility of chromatin compared to naked DNA [15,43]. Successively, It has been found that chromatin fiber behaves qualitatively similar to the nucleosome arrays, probably due to the conformational flexibility of nucleosomes [44]. If the same observation will be confirmed *in vivo*, then the chromatin might acts as a buffer which transiently absorbs torsional stress to keep the chromatin environment comfortable for DNA-tracking complexes [43,45]. Comparison of the expression profiles of cells wild type or mutant for different topoisomerase, revealed that these enzymes play an important role during transcription [19,46,47]. According to their capability to cut and reseal one or two DNA strands, topoisomerases are divided broadly into two families: type I enzymes transiently break one DNA strand; type II topoisomerases cleave and rejoin both strands [46]. The ability of the two types of enzyme to efficiently remove both positive and negative supercoiling in eukaryotes reflects a mechanical and functional redundancy between different topoisomerases [19,46]. Since supercoils generated in front of the transcribing RNA polymerase have a different effect on transcription and reside in a different molecular environment compared to those generated behind it, different solutions of topological problems and specialized roles of topoisomerases may occur in each circumstance. Indeed, in yeast, positive torsional stress in front of the RNA polymerase I is largely resolved by topoisomerase II (Topo II), while topoisomerase I (Topo I) is responsible for the removal of the negative torsional stress behind the polymerase [48]. Topo II is the main relaxase on chromatin fibers *in vitro* but it binds primarily to the nucleosome-free regions *in vivo* [49,50]. Notably, under the same experimental conditions, naked DNA was relaxed by Topo I much faster than by Topo II [50]. This finding suggests that Topo I is a more processive and “rapid” enzyme which probably works near the regions stripped of nucleosomes with a high demand for relaxation, i.e., close to RNA polymerase. In support of this idea, magnetic tweezers experiments also revealed Topo I to be a torque-sensitive enzyme as the mean number of relaxed supercoils increases with the torque stored in the DNA [51].

The complexity of the processes involved in the twist diffusion through the chromatin and their transient nature, as well as the absence of a clear explanation as to how topoisomerases are recruited to active genes have made it very difficult to predict the extent of supercoiling at each particular genomic locus. Our understanding of this multifactor mechanism is still rudimentary and requires extensive experimental efforts.

4. Methods to assess the DNA supercoiling

The first techniques to study the torsional state of DNA relied on DNA supercoiling mediated changes in the compaction and the geometry of DNA (Fig 1a) observable by equilibrium and velocity sedimentation, by electron microscopy and by electrophoretic separation [52,53,54]. Currently these methods are mostly used for determining supercoiling in populations of circular DNA, i.e., plasmids. These techniques report the average behavior of many DNA molecules and do not characterize the dynamics of structural transitions. During the last one and one-half decades, controlled mechanical manipulation of single DNA molecules or chromatin fibers has been developed to study supercoil-diffusion, the behavior of nucleosome arrays under torsional stress and the active removal of supercoils by topoisomerases [55,56]. These *in vitro* methods have improved our understanding of DNA mechanics but do not allow monitoring the mechanics and dynamics of the response of DNA to torsional stress in an *in vivo* context.

The degree of supercoiling in intracellular DNA has been estimated most often using a strategy that relies on the binding of various psoralen derivatives to DNA (Fig 2a). The psoralens are cell membrane-permeable molecules with a planar, aromatic structure that allows them to intercalate into B-DNA. The extent of psoralen intercalation is linearly related to the level of negative superhelicity and provides a measure of DNA topology *in vivo* [57,58]. Such experiments have revealed that although the bulk of genomic DNA is relaxed, supercoiled DNA does exist at a few loci of mammalian cells [59,60]. In *Drosophila* polytene chromosomes, the pattern of psoralen binding has been used to directly visualize torsionally stressed DNA which appeared to localize at active genes [61]. In a recent modification of the psoralen-based technique, binding of the compound to the yeast genome *in vivo* was examined genome-wide using DNA arrays [62]. It was shown that large chromosomal compartments have different levels of DNA superhelicity but the experiment failed to detect transcription-induced supercoiling, probably due to the high density of genes in yeast and very short linker DNA which together require a method with a higher resolution.

The first direct measure of transcription-generated supercoiling *in vivo* in human cells was made by using a site-specific Cre-recombinase to excise a chromatin fragment upstream of an inducible promoter [40]. Recombinase-mediated circularization of the fragment enabled the trapping of negative supercoils that were diffusing through the chromatin (Fig 2b). This experiment showed that DNA supercoiling dynamically elicits the relaxation potential of topoisomerases [40]. The transmission of negative supercoils upstream of the actively transcribed regions has been demonstrated to occur even on linear DNA *in vitro*, showing that the generation of supercoiling is much faster than the free DNA twist diffusion [39]. In addition, since many promoters are sensitive to DNA supercoiling, indirect studies have been used to monitor the pattern of transcriptional activity to obtain information about DNA topology [28,63,64]. DNA topoisomerases also provide a valuable tool to investigate the topology of DNA and could function as *in vivo* probes to measure the level of torsional stress. Given their specialized functions, the mapping of the exact position of topoisomerases along the genome should enable an *in vivo* assessment of the supercoils distribution [48,49,65,66].

5. DNA supercoiling in regulatory pathways

In a eukaryotic cell, basal chromatin organization not only prevents access of the RNA polymerase to promoters but also restricts transcription elongation along the DNA. Because of the strong binding energy between nucleosomes and DNA, transcription requires chromatin remodelers to disrupt or to slide nucleosomes, providing a means for transcription regulation. There is substantial evidence from *in vivo* experiments to indicate that nucleosome disruption is needed for proper elongation; importantly, this disruption propagated along the gene faster than the rate of RNA polymerase II translocation [67]. Positive DNA supercoiling promotes unwrapping of DNA from the histones and modifies nucleosome structure *in vitro*; in contrast nucleosomes rapidly form on negatively supercoiled DNA [16]. Consequently, it was suggested that at each round of transcription, the positive supercoiling is pushed ahead of RNA polymerase. Accumulated positive torsional stress induces structural modification of nucleosomes and creates conditions in which polymerase efficiently elongates through the nucleosomal array [44,68]. Negative stress in the wake of the transcription machinery facilitates rapid re-formation of nucleosomes behind the elongating complex. Thus, by variation in intensity and polarity, supercoiling may directly modulate the conformation of chromatin to satisfy the demand of transcription in real-time (Fig 3a). Indeed, it was shown that treatment of cells with a Topo II inhibitor results in perturbation of chromatin structure, which seems to indicate that DNA supercoiling mediates chromatin rearrangement [69].

The double helix which is the predominant B-form, could adopt, depending on the sequence composition, a variety of alternative structures [70]. A prerequisite for the formation of these structures is duplex destabilization sponsored by high level of negative supercoiling [71]. In fact, dynamic supercoiling was indirectly measured through the identification of non-B DNA structures in susceptible sequences upstream to active promoters both *in vitro* and *in vivo* [39,40]. Non-B DNAs bind a diversity of DNA conformation-sensitive proteins some of which have regulatory function, suggesting that these unusual DNA structures are more than mere by-products of genetic activity [70,72]. Accordingly, *in silico* analyses showed an enrichment of supercoil-sensitive sequences at regulatory loci [73,74]. To date, the most complete investigation showing the important role of non-B DNA in gene regulation was conducted on the human *c-myc* proto-oncogene. Upstream of the main promoter of MYC it is located a supercoil-sensitive sequence called FUSE. During the transition from the basal level of expression to the full expression in response to activating signals, FUSE starts to melt due to increasing levels of negative supercoiling [75]. Partly melted FUSE binds the transcription activator FUSE-binding protein (FBP), which increases the promoter activity by interacting with the general transcription factor TFIID and drives the transcription of MYC to peak output. FBP-interacting repressor (FIR) binds FBP and FUSE which is fully melted due to high level of DNA supercoiling. The binding of FIR abolishes the effect of FBP, and the gene transcription is restored to basal levels. Thus, cooperation between supercoil-induced non-B DNA and DNA conformation-sensitive proteins provides a real-time feedback mechanism for controlling gene expression (Fig 3b).

Another important conformationally plastic sequence involved in *c-myc* regulation is the CT-element (also known as NHE III1) located 250 bases upstream of the main promoter [76,77]. It was observed that this element adopts non-B DNA structures in supercoiled DNA *in vitro* as well as in its endogenous location *in vivo* [78,79]. In normal B-DNA structure, the CT-element is bound by the transcriptional factor Sp1 which activates transcription. It was suggested, that as a result of supercoil accumulation due to activated transcription, the element flips into the single-stranded conformation and the transcription factors hnRNPK and CNBP bind the purine-rich and pyrimidine-rich strands, respectively, to maintain the active state [77,80,81]. Besides the single-stranded conformation, CT-element can adopt

stable non-B DNA structures, a G-quadruplex on the purine-rich strand and an i-motif on the pyrimidine-rich strand [82]. These globular structures sequester the transcription factor binding sites and consequently silence transcription. Different sets of binding proteins associate with different conformations of CT-element; consequently, gene specific responses could be achieved using ubiquitous transcriptional factors. Thus the local flipping between different DNA conformations induced by torsional stress plays as a switch in selecting which transcriptional factor to employ according to the physiological demands on the cell (Fig 3c).

One more sequence 1.8 kb upstream of the *c-myc* promoter has been predicted to assume a left-handed double helical structure called Z-DNA. The region is recognized *in vitro* by anti-Z-DNA antibodies in permeabilized cells under conditions of active transcription [83]. The function of this sequence in *c-myc* transcription is currently unknown, although proteins able to specifically interact with Z-DNA have been described [84]. Besides serving as targets for binding, supercoil-induced non-B DNA structures could modify chromatin structure by exclusion of nucleosomes [85,86,87]. It was shown that activation of the CSF1 gene by chromatin remodeling activities, results in formation of Z-DNA at the sequence located within the promoter which, in turn, stabilizes the open chromatin structure in the area critical for efficient transcription (Fig 3d). The elastic properties of non-B DNA are different from those of B-DNA. Double helix is a stiff polymer and cells should overcome its rigidity to facilitate DNA-protein-DNA interactions which are playing an important role in many cellular processes [88]. Non-B conformations expose flexible single-stranded segments that together with plectoneme formation may facilitate DNA transaction between flanking sequences (Fig 3e) [89,90].

MYC deregulation is just one of several crucial hallmarks of cancer. It was suggested that cancer genotypes are set up by eight essential alterations in single cells that dictate malignancy: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and evading immune destruction [91]. Each of these physiologic changes is manifested by alterations in expression of key genes, with many of them containing supercoil-sensitive sequences in the core or proximal promoter. Given the importance of these genes, including MYC, KRAS, RB1, BCL2, VEGFA, TERT and PDGFA, additional layers of tight regulation may be imposed at their promoters. The response of CT- and FUSE-like elements to transcription-generated supercoiling reflects the intensity of ongoing transcription, and DNA conformation-sensitive proteins close the real-time feedback loop to provide regulatory adjustment necessary to synchronize the output of gene expression within the population of cells [72,77].

6. Conclusion

In the early days much effort was expended to understand the interplay between the genetic code and chromatin structure: DNA primary structure was found to contain signals that participate in the regulation of DNA metabolism [92,93]. In the recent years there is a growing body of experimental evidence supporting the idea that DNA mechanics are responsible for a variety of regulatory functions: DNA supercoiling modulates the dynamic rearrangement of chromatin to control the final output of the specific DNA processes [40,72,75,77].

The assembly of multi-protein complexes allows a precise spatio-temporal control of DNA metabolism and particularly of gene expression. By representing the targets of transcriptional factors, *cis*-regulatory modules provide the essential instructions to coordinate genetic processes. The constellation of factors, both activators and repressors,

bound to each module sequence depends on their expression levels. Thus, the variation in the local concentration of transcriptional factors determines the transcriptional outcome, which is a common way to regulate transcription. At the same time, the delay imposed by multiple events necessary to change the relative concentration of the factors (transcription, translation, protein modification, etc) results in the danger of low synchronization between the physiological requirement and the acute response of important genes such as proto-oncogenes. In contrast, propagation of torsional stress on the DNA is fast and may serve as an efficient long-range signal. The signal could restrict or promote the enrollment of DNA conformation-sensitive proteins at the regulatory module, or could favor the proper arrangement of protein-DNA interaction over long distances. The same regulatory outputs could be reached by adjustment in transcription factor synthesis, but only DNA supercoiling has the capacity to govern the specific transaction moment-to-moment, according to the demands of a DNA-dependent processes.

Our understanding of this phenomenon is still elusive. Although chromatin biology has been gaining much more interest, the associated torsional state of DNA remains neglected since it is less amenable to analysis. Exploring the phenomenon requires the aggressive development of new techniques for measuring of DNA torsional stress with high sequence resolution and preferably at the single-cell level.

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Highlights

It has been speculated that DNA topology might play a regulatory role in chromosome biology.

The review focuses on the most recent approaches developed to evaluate this possibility.

Multiple factors contribute to dynamic changes in DNA topology.

These changes are involved in the moment-to-moment guidance of crucial processes.

This real-time regulation is necessary for rapid cellular responses to physiological stimuli.

Figure 1. Basics of DNA topology and its relevance to DNA transaction

The DNA topology is described quantitatively by the twist of double helix and by the number of times the helix crosses over on itself (plectoneme). Plectonemic structures are typically formed by bacterial plasmids. B) A graphical illustration showing the generation of supercoiling during transcription and replication. If polymerases are moving without rotation, then due to its helical structure, the DNA must be screwed through the protein complexes. In this case, the templates rotate around its axis as indicated by curved arrows.

Figure 2. Strategies to assess the DNA topology inside of the cells

A) Psoralen intercalates preferentially into undertwisted DNA and, upon exposure to UV-light, crosslinks its strands. DNA supercoiling *in vivo* can be monitored through the extent of photo-crosslinking between different loci in the cell. B) Dynamic torsional stress propagating from an activated promoter between the loxP sites is trapped in the DNA circle excised by Cre-recombinase. Two-dimensional electrophoresis of the circles gives an accurate accounting of DNA supercoiling generated during transcription.

Figure 3. Long range regulatory events due to transcription-generated DNA supercoiling

A) Torsional stress modulates the conformation of chromatin, promoting unwrapping of DNA from the histones ahead of RNA polymerase (RNAP) and rewinding behind it. B) During transcription of *c-myc* gene the melting of the supercoil-sensitive sequence FUSE promotes the recruitment of factors that enhance (FBP) or repress (FIR) the transcription. C) According to the level of torsional stress, the CT-element located upstream of the *c-myc* promoter can flip between different conformations (double-stranded, single-stranded and G-quadruplex/ i-motif) which dictate the binding of specific transcription factors. D) The chromatin remodeling in the promoter of CSF1 favors the formation of Z-DNA which stabilizes the open chromatin structure. (-) means negative supercoils, (+) means positive supercoils. E) Single-stranded structures in supercoiled region provide the flexibility needed to juxtapose distal elements.