Supercoiling biases the formation of loops involved in gene regulation

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Abstract The function of DNA as a repository of genetic information is well-known. The post-genomic effort is to understand how this information-containing filament is chaperoned to manage its compaction and topological states. Indeed, the activities of enzymes that transcribe, replicate, or repair DNA are regulated to a large degree by access. Proteins that act at a distance along the filament by binding at one site and contacting another site, perhaps as part of a bigger complex, create loops that constitute topological domains and influence regulation. DNA loops and plectonemes are not necessarily spontaneous, especially large loops under tension for which high energy is required to bring their ends together, or small loops that require accessory proteins to facilitate DNA bending. However, the torsion in stiff filaments such as DNA dramatically modulates the topology, driving it from extended and genetically accessible to more looped and compact, genetically secured forms. Furthermore, there are accessory factors that bias the response of the DNA filament to supercoiling. For example, small molecules like polyamines, which neutralize the negative charge repulsions along the phosphate backbone, enhance flexibility and promote writhe over twist in response to torsion. Such increased flexibility likely pushes the topological equilibrium from twist toward writhe at tensions thought to exist in vivo. A predictable corollary is that stiffening DNA antagonizes looping and bending. Certain sequences are known to be more or less flexible or

to exhibit curvature, and this may affect interactions with binding proteins. In vivo all of these factors operate simultaneously on DNA that is generally negatively supercoiled to some degree. Therefore, in order to better understand gene regulation that involves protein-mediated DNA loops, it is critical to understand the thermodynamics and kinetics of looping in DNA that is under tension, negatively supercoiled, and perhaps exposed to molecules that alter elasticity. Recent experiments quantitatively reveal how much negatively supercoiling DNA lowers the free energy of looping, possibly biasing the operation of genetic switches.

Keywords DNA looping · Gene regulation · Genetic switch · DNA supercoiling · DNA topology · Magnetic tweezers

Introduction

The purpose of this review is to highlight evidence that DNA supercoiling is both a product of DNA–protein interactions and a modulator of the activity of DNA-binding proteins. This mutable identity of DNA-binding proteins as producers and also regulatory targets of DNA supercoiling creates a system in which local supercoiling levels and proteomes bias the regulation of genes (Travers and Muskhelishvili 2013). Perhaps the most striking evidence of this connection arises from experiments correlating supercoiling and transcription or careful two-dimensional (2D) gel analysis of the topologies of plasmids with replication forks stalled at different stages of completion. By directly integrating a supercoiling-sensitive resolvase target at a series of positions in the *Escherichia coli* chromosome and correlating resolvase products with gyrase mutation or poisoned transcription, Higgins and colleagues found that topoisomerases must work in concert with RNA polymerases to prevent obstacles due to the accumulation of

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supercoiling as a result of transcription (Rovinskiy et al. 2012). Similarly, during replication quite complicated precatenane topologies can be imagined to occur, depending upon whether the active DNA polymerases are thought to be free to spin about the axis of the DNA template as they progress. Initial attempts to directly image the topology of partially replicated plasmids suffered from artifacts, but subsequent work showed that rotating replication forks create intertwined daughter strands and that in doing so they distribute intertwining along the length of the replicating plasmid (Peter et al. 1998). This result has been recently confirmed in studies in which stalling the twin replication forks at different stages between initiation and termination produced more or less intertwining of the daughter helices about each other and a variety of pre-catenanes on 2D gels (Cebrián et al. 2015). This emphasizes the notion that a cell must have effective tools with which to manage supercoiling, and conversely suggests that supercoiling levels should reflect cellular health (van Workum et al. 1996). While cell biological research has demonstrated the regulatory effects of supercoiling and identified the principal enzymes that modulate it, a long-term effort in biochemistry and more recent single-molecule experiments with torsion-constraining tweezers have defined the role of DNA supercoiling in a variety of DNA–protein interactions, enabling both the extraction of mechanistic details and the extrapolation of general principles.

DNA-bound proteins separated by noteworthy distances along the filament very often interact to form loops. The first evidence that supercoiling was important for loop formation in DNA originated just over 30 years ago with experiments involving the lac repressor protein. Lac repressor (LacI) binding to a series of three binding sites (operators) (Gilbert and Maxam 1973; Pfahl et al. 1979; Reznikoff et al. 1974) that had been isolated a few years before (Gilbert and Müller-Hill 1966) was known to regulate the expression of genes coding for proteins involved in the metabolism of lactose. Nearby sites with lower but above-background affinity increased the binding constant of the strongest operator, O₁, and loops secured by bivalent LacI tetramers were found to increase repression of the lac operon (Mossing and Record 1986). Experiments by Matthews and colleagues, which employed plasmids containing different combinations of operators and supercoiled to different degrees, showed that supercoiling profoundly diminished the dissociation of LacI from plasmids with multiple operators (Whitson et al. 1987). Around the same time, Muller-Hill and colleagues used a series of minicircles with LacI operators separated by 153–168 bp to show that separations for which a LacI tetramer could connect two binding sites without requiring the DNA to twist formed the most stable loops (Krämer et al. 1988). When they altered the supercoiling of the minicircles, the most stable separation lengths changed. This might have been expected, since negative torque twists the prospective binding sites along one face

of the DNA out of alignment, and with slight shifting along the helix the alignment can be restored. Using plasmids with varied spacing/phasing of operators along the DNA, repression by LacI could be modulated *in vivo* as well (Bellomy et al. 1988).

Along with phasing and supercoiling, the proteins securing the loops play important roles. The lac repressor can form loops of varied topology and stability as a result of the spacing of operators and the available conformations of the protein (Wong et al. 2008) which may determine the disposition of other proteins that assemble on the loop (Mehta and Kahn 1999) and has been shown to regulate access by RNA polymerase (Becker et al. 2014). In addition, accessory proteins that modify the intervening DNA by adding kinks, and/or altering flexibility, shift the looping equilibrium. Theoretical studies estimated that kinks produced by heat-unstable (HU) protein binding enhanced looping of DNA segments 75–150 bp in length to levels comparable to those measured *in vivo* (Czapla et al. 2013). In fact, HU-binding appears to generally enhance flexibility to the point that it masks the effect of intrinsic sequence-dependent flexibility on looping both *in vitro* and *in vivo* (Boedicker et al. 2013; Becker et al. 2007).

Clearly the lac repressor is a well-established model for genetic switching (Oehler 2009; Oehler et al. 1990) and continues to be useful for probing contributions to looping by the phasing of operators, the flexibility of the intervening DNA, and the binding of accessory proteins (Johnson et al. 2013; Mulligan et al. 2015). Studies of lac repressor and other loop-mediating proteins have advanced our understanding of genetic circuits and can guide attempts to devise artificial genetic switches (Amit et al. 2011).

Nonetheless, this understanding should be extended, and another active arena for exploration of the effects of supercoiling is the behavior of loops longer than the persistence length of DNA, such as that mediated by the lambda repressor. The lambda (λ) bacteriophage is a temperate phage which infects *E. coli* and propagates in a quiescent (lysogenic) manner in favorable conditions but may effectively switch to the virulent (lytic) growth mode when the host is threatened by poisoning, starvation, or irradiation (Ptashne 2004). Specific binding sites for the lambda repressor protein CI were characterized more than 40 years ago (Maniatis and Ptashne 1973). Dimers of CI bind to triplets of specific sites, *o*L1-3 and *o*R1-3, which are separated by 2317 base pairs. When CI binds to *o*L1 and *o*R1, it represses transcription from nearby promoters for expression of the lytic genes, *p*L and *p*R. Binding to *o*R2 stimulates expression of CI itself from the relatively weak *p*RM. Early work concentrated on the *o*R region where the promoter for CI and a number of lytic genes are located (Ackers et al. 1982). Meticulous studies of binding affinities revealed subtle differences in affinity as well as cooperative effects that were important for the regulatory switch

(Burz et al. 1994). Finally, 2001 Dodd et al. proposed a model based on DNA looping to accommodate the biochemical and cell biological observations on this system. These authors recognized that while adjacent dimers within the *oL* or *oR* triplet may cooperatively interact to laterally form tetramers, cooperative, head-to-head interactions between *oL* and *oR* tetramers can secure a DNA loop. Both the repression of the lytic promoters and the activation of *pRM* are increased by looping (Anderson and Yang 2008; Lewis et al. 2011; Little and Michalowski 2010). Similar to the lac repressor, the lambda repressor has been used as a module in a synthetic circuit that can be regulated by small molecules (Atsumi and Little 2006). In the following section, we review attempts to quantitatively characterize the effect of supercoiling on CI-mediated looping. New information obtained from studies of these loop-mediating proteins operating on supercoiled DNA in single-molecule formats that allow arbitrary control of torsion (Normanno et al. 2008) will be particularly relevant for deriving the mechanistic details in organisms like *E. coli* in which DNA is supercoiled.

DNA under torsion twists and then supercoils

A basic familiarity with the behavior of supercoiled DNA is best achieved through experiments conducted with a magnetic tweezer. This instrument is a relatively inexpensive, user-friendly tool which creates a magnetic gradient that attracts and aligns a bead attached at one end of a DNA molecule anchored to the surface of the glass coverslip (Finzi and Dunlap 2010; Strick et al. 1998). This allows arbitrary stretching and twisting of single DNA molecules. Although the torsional elasticity of DNA had been previously characterized, the DNA response to torsional stress applied using magnetic tweezers produced an unprecedented level of mechanical detail (Strick et al. 1999). Beyond a threshold level of torque, DNA buckles to form plectonemes that shorten the overall extension of the tether. This response is identical for over- or under-winding under moderate tensions, less than 0.5 pN. Thus, a plot of DNA extension versus positive and negative twist applied to the double helix exhibits a symmetric peak centered at zero twist (Fig. 1, green). At higher tension, as the molecule is unwound (negative or left-handed supercoiling) the extension of the tether shrinks more slowly (Fig. 1, blue). Tension changes the partitioning of the negative twist between negative writhe and left-handed helices such that the slope of the extension versus twist curve for unwound DNA decreases and eventually flattens at high tension or even increases gradually as the DNA is unwound (Sheinin et al. 2011) (Fig. 1, red). In comparison, the slope of the right side of the curve (positive supercoiling) only changes slightly as plectonemes become more tightly wrapped. This asymmetry reflects the

Fig. 1 Extension versus twist curves for DNA as a function of tension. The extension of a 4396-bp DNA tether is plotted as a function of the number of mechanically applied turns under 0.3 (green), 0.7 (blue), and 1 pN (red) of tension. At low tension the DNA extension versus twist curve is symmetric. Torsionally relaxed DNA has the maximum extension. After that, each positive or negative mechanical turn induces one additional plectonemic gyre and decreases the extension by a constant amount. At intermediate tension, for up to four positive turns, no writhe forms in the DNA but the twist increases and the end-to-end extension remains constant. The first supercoil forms at the buckling point, and more turns produce tightly wrapped plectonemes in positively supercoiled DNA giving a slightly decreased slope. In contrast, unwound DNA partitions into segments of negatively supercoiled, B-form DNA and left-handed helical forms. The slope decreases noticeably due to this lesser tendency to form plectonemes. At higher tension, plectonemes fail to form in unwound DNA, and the left side of the extension vs. twist curve is almost flat. Positively supercoiled DNA forms even tighter plectonemes producing another mild decrease in slope

chirality of the double helix and is due to conformational changes in unwound DNA under tension (Leger et al. 1999; Marko and Neukirch 2013). After extensive unwinding that converts the entire molecule to a left-handed form, the extension finally shrinks again as left-handed plectonemes form in left-handed DNA (Sheinin et al. 2011).

The ability to arbitrarily manipulate the superhelicity of a DNA fragment is a powerful tool for studies of topology-modifying enzymes and their substrates, such as topoisomerases, RNA polymerases, helicases, chromatin, and chromatin remodeling factors, as well as of the effect that supercoiling has on the interaction with accessory factors (Charvin et al. 2005; Bryant et al. 2012; Harada et al. 2001; Lee et al. 2013b; Lia et al. 2006; Nollmann et al. 2007; Recouvreur et al. 2011). Twist can also be applied to single DNA molecules using birefringent quartz cylinders and light, instead of magnetic beads. With this arrangement, Ma et al. (2013) demonstrated that supercoiling, built-up due to active elongation, stalls RNA polymerase. In the following section, we review studies conducted in our laboratory using magnetic tweezers to analyze the interplay between DNA supercoiling and transcriptionally relevant proteins.

DNA torsional and bending stiffness affect the formation and structure of supercoils

DNA torsional stiffness modulate DNA buckling and the size of plectonemic gyres, respectively

Supercoiling is a ubiquitous feature of many genomes. It not only plays a role in chromatin compaction but also in the regulation of DNA–protein interactions (Baranello et al. 2012). Given the helical structure of double-stranded DNA, it is a byproduct of transcription and replication, and proteomes contain arrays of enzymes that adjust local superhelicity. Supercoils may be topologically constrained, such as when DNA wraps around a protein or protein complex, for example the histone octamer, or may freely propagate for significant stretches of the genome (Kouzine et al. 2013; Naughton et al. 2013). The topology and actual dimensions of DNA supercoils are influenced by their electrostatic environment which modulates repulsions between phosphates to change the persistence length and the interactions of helical segments of DNA (Manning 2006). This can be seen quite easily when supercoiled plasmids are deposited on surfaces of different charge density (Fig. 2). When a higher density of poly-L-ornithine was used to produce a more highly charged mica surface, supercoiled plasmids deposited on the surface exhibited more topological nodes. This is exactly what would be expected when charge neutralization shrinks the repulsive force that extends and stiffens the polymer. Increased flexibility promotes buckling at lower levels of torque, or in this case, plasmids with identical linking number values will adopt more writhe and less twist (the buckled form) on a more highly charged surface with respect to those on a lesser charged surface.

Intracellularly, polyamines are small, multivalent, positively charged molecules which are highly regulated during the cell cycle (Wallace et al. 2003). They bind non-specifically to DNA and modify topology, as shown in our recent study (Shao et al. 2012), in which we in particular considered the effects of spermidine (three positive charges) and spermine (four positive charges). Both spermine and spermidine facilitated plectoneme formation in either wound or unwound DNA and, as expected, this effect correlated with the polyamine charge. Neither polyamine changed the persistence length of DNA in the high-salt buffers used. For either polyamine, plectoneme formation was sensitive to the handedness of the added turns and to both tension and polyamine concentration. At low tension (0.2 pN), negatively twisting DNA in the presence of polyamines produced more compact plectonemes with than without polyamines, while at medium-high tension (0.6 pN) polyamines favored the formation of more extensive plectoneme, likely by stabilizing the B-form of DNA. At relatively high tension (1 pN), spermidine produced tighter plectonemes in positively twisted DNA, and spermine caused

Fig. 2 Plasmids on highly positively charged surfaces exhibit more writhe. Supercoiled, 5052-bp plasmids were deposited on mica-coated with poly-L-ornithine. Highly supercoiled plasmids are visible in topographs recorded using scanning force microscopy. The height of each pixel is color encoded. Intersections between DNA segments, where one DNA duplex crosses over another, were easily distinguished as local maxima. **b** The number of nodes were counted for ensembles of plectonemic plasmids deposited on surfaces prepared with either 0.1 (red) or 0.01 (blue) ug/ml of 1-kDa poly-L-ornithine

the buckling transition to occur at lower levels of (+) twist. Overall, the DNA complexed by these polyamines more easily forms plectonemes that have smaller gyres. These findings shed light on the possible physiological role of polyamines in the nucleus: tuning polyamine levels could limit changes in twist density and maintain the B-form despite the action of polymerases which exert sufficient force to distort the B-form

double helix. This would maintain the binding affinity of DNA-binding proteins in critical cellular processes and avoid large shifts in the writhes–twist equilibrium.

DNA bending stiffness modulates the activity of topoisomerase type II enzymes

In fact, there are families of enzymes dedicated to governing the twist–writhe equilibrium, and dynamic management of DNA supercoiling is essential for the maintenance of chromosome structure as well as the regulation of replication and transcription. For example, the excess supercoiling generated by elongating RNA polymerases (Liu and Wang 1987) may stall transcription (Ma et al. 2013), is the cause of transcriptional bursting in bacteria (Chong et al. 2014), and eventually needs to be resolved by topoisomerases (Rovinskiy et al. 2012). Recently developed tools for mapping supercoiling on a large scale have shown gradients across the *E. coli* genome (Lal et al. 2016) and demonstrated the feedback between supercoiling and transcription in which topoisomerases play a prominent role (Rovinskiy et al. 2012). Topoisomerases alter supercoiling through a reaction called strand passage (Schoeffler and Berger 2008). Type II topoisomerases actually create a break in one double helix, pass another one through the break, and then mend the break. During this catalysis, they sharply bend DNA (Lee et al. 2013a). Bacterial gyrases, a class of type II topoisomerases, can introduce negative supercoiling by creating a right-handed wrap of DNA which strand passage reverses to left-handed.

The ability of gyrases to wrap DNA into a tight loop was recently studied in our laboratory. Magnetic tweezer experiments showed that DNA in which 2, 6-diaminopurine substituted for adenine formed larger plectonemic gyres upon twisting than does normal DNA. This completely triply hydrogen-bonded DNA is stiffer (Peters et al. 2014; Virstedt et al. 2004), and although they are not thought to tightly loop the DNA during the relaxation of positive supercoils, both *E. coli* gyrase and human topoisomerases type II α were slightly slower on 2,3-diaminopropionic acid (DAP)-substituted DNA than on normal DNA (Fernández-Sierra et al. 2015). However, in the presence of ATP, gyrases can introduce negatively supercoiled DNA under low tension (Fig. 3). In this reaction a tight loop of DNA must be formed (Basu et al. 2012), and *E. coli* gyrase was found to introduce negative supercoils in DAP-substituted DNA at a much lower frequency. The enzyme struggled to wrap DAP-substituted DNA, and the wrapped state had a much shorter lifetime in this DNA than in normal DNA. DNA stiffness clearly modulated the activity of type II topoisomerases and is likely to affect many DNA processing enzymes, since many catalyze bending or twisting of the double helix.

DNA supercoiling is generated by chromatin remodeling factors

DNA supercoiling can be modified not only by topoisomerases, polymerases, or enzymes with helicase activity, but also by many chromatin remodeling factors that spool DNA in an ATP-dependent fashion. One example is the RSC complex. RSC is a large, multi-subunit protein that catalyzes the sliding of nucleosomes along the DNA (Harada et al. 2016). RSC alone spooled bare DNA into and out of looped conformations (Lia et al. 2006). Loop formation also generated negative DNA supercoils. This is what would be expected from a translocase that tracks the helical groove of DNA while clinging to an initial anchor point, although the enzyme seems to lose its grip occasionally during translocation and hence does not create a predictable amount of unwinding per base pairs traversed. Nonetheless chromatin remodeling proteins such as those in the Snf2 family of proteins can act as specialized DNA translocases that catalyze DNA unwinding (Havas et al. 2000), which is associated with catalyzing the movement of DNA across the surface of the nucleosome (Deindl et al. 2013).

DNA unwinding (negative supercoiling) is a key regulator of loop-based genetic switches

The gal repressor system

The gal repressor, GalR, regulates transcription of the genes responsible for the metabolism of galactose in *E. coli*. Dimers of GalR bind to the O_I and O_E sites separated by 113 bp which encompass the partially overlapping P_{G1} and P_{G2} promoters (Irani et al. 1983). Looping by GalR was hypothesized to explain the molecular mechanism that defined the dependence of P_{G1} and P_{G2} promoter activity from O_I and O_E . However, repression of P_{G1} and P_{G2} is not achieved in the absence of the HU protein and of negative DNA supercoiling (Aki and Adhya 1997).

Thus, magnetic tweezers were used to test the looping hypothesis and the roles of HU protein and DNA supercoiling (Lia et al. 2003). GalR was added to single DNA tethers that were unwound by different amounts. Under a tension of 0.9 pN, at least 3 % unwinding was necessary to observe GalR-induced loop formation, which was observed to dynamically occur in time traces of DNA tether extension. The average lifetimes of the unlooped and the looped DNA conformations were measured as a function of tension from a series of traces. Interestingly, while the lifetime of the unlooped state dramatically increased with tension, that of the looped state was not greatly affected.

A Boltzman model was then used to extrapolate from these conditions to estimate the average lifetimes of the two

Fig. 3 *Escherichia coli* gyrase inserts negative supercoils into DNA under low tension. A 3055-bp DNA tether was held under 0.3 pN of tension in the presence of 50 nM *E. coli* gyrase and 1 mM ATP. The extension was monitored as a function of time (light-blue points data points, dark-blue line moving average), and mechanically introduced supercoils were varied, as indicated by the red line plotted on the axis on the right. Beginning at about 650 s into the recording, the unwound tether was extended to just above 0.8 μm . Just before 700 s gyrase began to negatively supercoil the molecule processively for about 40 s (shaded

area denoted by *a*). Two sets of five positive supercoils introduced mechanically were quickly annihilated by gyrase activity which returned the tether extension to near zero (shaded areas denoted by *b* and *c*). Ten additional positive supercoils extended the tether briefly before gyrase also annihilated those as well (shaded area denoted by *d*). Mechanically introducing about 17 negative supercoils did not significantly change the near-zero extension, and no further gyrase activity was observed when 27 positive supercoils were introduced

conformations in the absence of tension. These lifetimes were used to calculate the free energy of GalR-mediated looping in the absence of tension, -12 kT, which is quite significant. This is considerably more than the free energy for the lac repressor-mediated loop in an unwound DNA template, -7 kT, which was estimated from lifetime data obtained in the absence of tension using tethered particle microscopy (Finzi and Gelles 1995) and from unlooping equilibrium constants derived from filter binding assays (Hsieh et al. 1987; Whitson et al. 1987)—and suggested a thermodynamic contribution by HU. Considering that (1) LacI has a higher affinity for its operator site, O_1 , than GalR has for O_I and O_E , (2) LacI is a stable bidentate tetramer, whereas GalR dimers interact weakly, and (3) the 305-bp, LacI-mediated DNA loop is more flexible than the 113-bp, GalR-mediated loop, HU seems to dramatically stabilize looping in the *gal* system. The picture that emerged from these results and control experiments with the single-stranded binding protein, SSB, was that DNA unwinding is necessary to denature the AT-rich HU binding site at the apex of the *gal* loop. This facilitates HU binding and bending of the DNA, which in turn, stabilizes GalR dimer–dimer interactions, looping, and the repression of P_{G1} and P_{G2} .

The important role that DNA supercoiling may play in the regulation of several loop-based genetic switches is underscored by a characterization of the looping probabilities of two possible antiparallel, GalR-mediated loop trajectories, A1 and A2 (Semsey et al. 2004, 2005; Geanakopoulos et al. 2001). A1, but not A2, represses the *gal* operon in vitro;

however, no difference was found in the probability or energetics of A1 versus A2 loop formation in vitro in DNA molecules unwound by 3 % (Lia et al. 2008). Thus, the probabilities of different DNA-looped trajectories alone do not predict modulations of transcription in the *gal* system. It may also be important to determine how excursions of DNA supercoiling away from the estimated genomic average of 3 % affect looping probabilities. Furthermore, one must carefully consider how the actual topology may affect proteins associated with a given trajectory. Perhaps in the A2 geometry, HU binds at a slightly different position in the loop and is less of an obstacle for RNA polymerase (Lia et al. 2008).

The lambda repressor system

Our work both with single molecules and in bulk showed that unwinding the DNA favors looping by CI, that the CI complex effectively separates the loop by the rest of the DNA, creating topologically distinct domains, and that these features may directly affect survival of the host bacterium.

When CI, purified from a transformed, CI-expressing *E. coli* strain, was incubated with supercoiled plasmids, purified from transformed *E. coli*, a CI-mediated loop split the plasmid into two topological domains. Time-resolved observation following the nicking of one domain with a large quantity of restriction enzyme revealed a relatively slow rate at which supercoils escaped from the intact domain through the protein-mediated loop closure and into the nicked domain,

where they instantaneously relax by twisting. Thus, CI is an effective barrier to the diffusion of supercoiling (Ding et al. 2014).

Negative supercoiling of DNA under tension enhanced the probability of loop formation and the stability of the loop. Tension opposes juxtaposition of the closure sites, and loops larger than 400 bp do not form spontaneously in torsionally relaxed DNA (Ding et al. 2014). Using magnetic tweezers to unwind DNA tethers lowered the free energy of loop formation until it became spontaneous. In addition, as the tension was raised from 0.3 to 0.8 pN, the DNA tether had to be unwound further to achieve the same degree of looping spontaneity. This emphasizes the importance of negatively supercoiled DNA *in vivo*, since such tension is likely to exist as a consequence of chromatin-anchoring loci and polymerase activity (Wang et al. 1998).

Perhaps the most surprising finding was that spontaneously formed CI-mediated loops in DNA supercoiled using magnetic tweezers reproducibly trapped a high degree of negative supercoiling (Fig. 4; figs. 2 and S5 and table 1 in Ding et al. 2014) and resisted very high levels of torque. Quite frequently the linking number of the looped segment corresponded to the linking number change mechanically imposed on the entire

DNA tether. In part, this is a reflection of the difficulty of plectonemes to form in DNA under tension, and therefore a single one is likely to absorb all of the mechanical twist imposed (Brutzer et al. 2010). However, some of the imposed twist should modify the natural twist of the entire DNA tether before buckling and writhing, so instances in which the imposed twist was completely captured in the loop segment are remarkable.

Such highly supercoiled and well-isolated DNA segments could significantly impact gene expression; for example, it could influence the *rexA* and *rexB* genes that reside within the DNA loop between the *OL* and *OR* operator sites. The efficiency of transcription is known to depend on the supercoiling of the template, and these genes are involved in phage exclusion and triggered cell death. Thus, in addition to creating steric features that regulate the CI promoter, the CI-mediated loop may maintain negative superhelical density, and therefore a basal level of transcription, at the *rexA* and *rexB* promoters to ensure the availability of RexA and RexB to counteract further infection of the lysogenic bacterium. This might also protect the lysogen from cell death in conditions of nutrient starvation by preserving supercoiling (transcription) at the *rexB* gene to

Fig. 4 Lambda repressor-mediated loops that form in highly supercoiled DNA trap various levels of supercoiling. At relatively low tension, 0.4 pN in this case, the extension of a 4948-bp DNA tether in a magnetic tweezer reduces when plectonemes form with the mechanical introduction of either positive or negative supercoils (*black data and spline curve*). As the winding of the DNA tether is mechanically reduced from an extreme when lambda repressor (CI) is present, a shifted curve with a reduced height is frequently observed (*red data and spline curve*). The shift

indicates the amount of supercoiling trapped within the CI-mediated loop and the height reveals the length of DNA topologically constrained by the CI loop. At 0.4 pN, a 1051-bp loop would be expected to reduce the extension by 0.27 μm , which is approximately the change shown. However, the entire linking number change that was mechanically introduced, 10 % in this case, became trapped in the loop segment that was topologically constrained

maintain levels of RexB that antagonize mazEF-triggered cell death (Engelberg-Kulka et al. 1998).

These experiments by Ding et al., in which mechanical untwisting catalyzed DNA looping into topologically distinct domains, suggested that negative superhelicity may toggle the lytic versus lysogenic switch when the λ bacteriophage infects an *E. coli* bacterium. At the establishment of lysogeny, the λ repressor binds to operators separated by 2317 bp, causing a loop that represses lytic genes, but also the transcription of CI to avoid over-repression which may interfere with efficient switching to virulence. This DNA loop, which circumscribes a few promoters, is stabilized by and can effectively constrain negative superhelical density for an average of 44 s. Together these data suggest that the phage genome may have evolved to facilitate quiescent propagation through lysogeny regulated by efficient, negative supercoiling-enhanced looping during favorable growth conditions for the bacteria. It is well known that significant UV damage triggers recombinase A-catalyzed CI degradation that trips the switch toward lysis. However, if conditions only mildly deteriorate or limited UV damage occurs, diminished superhelical density might only destabilize looping enough to increase CI levels slightly without tripping the switch to lysis in cells that are likely to recover and remain viable hosts (Baek et al. 2003).

These experiments demonstrate that an archetypical regulatory loop in prokaryotic transcriptional regulation effectively divides a DNA molecule into loops of distinct superhelical densities and that loop formation is catalyzed by negative supercoiling. Another archetypical loop in prokaryotic transcriptional regulation catalyzed by the *lac* repressor has recently been shown to be similar (Fulcrand et al. 2016). Such features indicate that superhelical density could be the most dynamic of all epigenetic “marks” that affect transcription. In the future, correlations of transcription and DNA supercoiling within topological domains, such as that produced by bacterial repressors, would greatly enhance our understanding of the dynamics of transcriptional regulation.

Outlook

In vitro, single molecule measurements using magnetic tweezers, combined with bulk biochemical techniques, have proven very useful to understand: (1) the torsional characteristics of DNA, (2) how the torsional stiffness of DNA alters the activity of specific ATP-dependent motor enzymes, (3) the supercoiling activity of chromatin-remodeling factors, and (4) the regulatory bias of loop-based genetic switches established by genomic supercoiling. The ability to arbitrarily control the level of twist and writhe of a DNA molecule is a powerful tool for advancing our understanding of the interplay between DNA supercoiling and protein activity along the

double helix. Many questions remain about the temporal distribution and dynamics of supercoiling in prokaryotic and eukaryotic genomes and about supercoiling dynamics among the different topological domains in a genome. The resolution of these questions will require bottom-up approaches relying on magnetic tweezers measurements and top-down approaches relying on in vivo methods to detect supercoiling.

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Compliance with ethical standards

Conflict of interest Laura Finzi declares that she has no conflicts of interest.

David Dunlap declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Ackers GK, Johnson AD, Shea MA (1982) Quantitative model for gene regulation by lambda phage repressor. *Proc Natl Acad Sci USA* 79: 1129–1133
- Aki T, Adhya S (1997) Repressor induced site-specific binding of HU for transcriptional regulation. *EMBO J* 16:3666–3674
- Amit R, Garcia HG, Phillips R, Fraser SE (2011) Building enhancers from the ground up: a synthetic biology approach. *Cell* 146:105–118
- Anderson LM, Yang H (2008) DNA looping can enhance lysogenic CI transcription in phage lambda. *Proc Natl Acad Sci USA* 105:5827–5832
- Atsumi S, Little JW (2006) A synthetic phage lambda regulatory circuit. *Proc Natl Acad Sci USA* 103:19045–19050
- Baek K, Svenningsen S, Eisen H, Sneppen K, Brown S (2003) Single-cell analysis of lambda immunity regulation. *J Mol Biol* 334:363–372
- Baranello L, Levens D, Gupta A, Kouzine F (2012) The importance of being supercoiled: how DNA mechanics regulate dynamic processes. *Biochim Biophys Acta* 1819:632–638
- Basu A, Schoeffler AJ, Berger JM, Bryant Z (2012) ATP binding controls distinct structural transitions of *Escherichia coli* DNA gyrase in complex with DNA. *Nat Struct Mol Biol* 19:538–546
- Becker NA, Kahn JD, Maher LJ (2007) Effects of nucleoid proteins on DNA repression loop formation in *Escherichia coli*. *Nucleic Acids Res* 35:3988–4000
- Becker NA, Greiner AM, Peters JP, Maher LJ (2014) Bacterial promoter repression by DNA looping without protein–protein binding competition. *Nucleic Acids Res* 42:5495–5504
- Bellomy GR, Mossing MC, Record MT Jr (1988) Physical properties of DNA in vivo as probed by the length dependence of the lac operator looping process. *Biochemistry* 27:3900–3906

- Boedicker JQ, Garcia HG, Johnson S, Phillips R (2013) DNA sequence-dependent mechanics and protein-assisted bending in repressor-mediated loop formation. *Phys Biol* 10:066005
- Brutzer H, Luzzietti N, Klaue D, Seidel R (2010) Energetics at the DNA supercoiling transition. *Biophys J* 98:1267–1276
- Bryant Z, Oberstrass FC, Basu A (2012) Recent developments in single-molecule DNA mechanics. *Curr Opin Struct Biol* 22:304–312
- Burz DS, Beckett D, Benson N, Ackers GK (1994) Self-assembly of bacteriophage lambda cI repressor: effects of single-site mutations on the monomer–dimer equilibrium. *Biochemistry* 33:8399–8405
- Cebrián J, Castán A, Martínez V, Kadomatsu-Hermosa MJ, Parra C, Fernández-Nestosa MJ, Schaerer C, Hernández P, Krimer DB, Schvartzman JB (2015) Direct evidence for the formation of precatenanes during DNA replication. *J Biol Chem* 290:13725–13735
- Charvin G, Strick TR, Bensimon D, Croquette V (2005) Tracking topoisomerase activity at the single-molecule level. *Annu Rev Biophys Biomol Struct* 34:201–219
- Chong S, Chen C, Ge H, Xie XS (2014) Mechanism of transcriptional bursting in bacteria. *Cell* 158:314–326
- Czapla L, Grosner MA, Swigon D, Olson WK (2013) Interplay of protein and DNA structure revealed in simulations of the *lac* operon. *PLoS ONE* 8, e56548
- Deindl S, Hwang WL, Hota SK, Blosser TR, Prasad P, Bartholomew B, Zhuang X (2013) ISWI remodelers slide nucleosomes with coordinated multi-base-pair entry steps and single-base-pair exit steps. *Cell* 152:442–452
- Ding Y, Manzo C, Fulcrand G, Leng F, Dunlap D, Finzi L (2014) DNA supercoiling: a regulatory signal for the λ repressor. *Proc Natl Acad Sci USA* 111:15402–15407
- Dodd IB, Perkins AJ, Tsemitsidis D, Egan JB (2001) Octamerization of lambda CI repressor is needed for effective repression of P-RM and efficient switching from lysogeny. *Genes Dev* 15:3013–3022
- Engelberg-Kulka H, Rechtes M, Narasimhan S, Schoulaker-Schwarz R, Klemes Y, Aizenman E, Glaser G (1998) rexB of bacteriophage lambda is an anti-cell death gene. *Proc Natl Acad Sci USA* 95:15481–15486
- Fernández-Sierra M, Shao Q, Fountain C, Finzi L, Dunlap D (2015) *E. coli* gyrase fails to negatively supercoil diaminopurine-substituted DNA. *J Mol Biol* 427:2305–2318
- Finzi L, Dunlap DD (2010) Single-molecule approaches to structure, kinetics and thermodynamics of transcriptional regulatory nucleoprotein complexes. *J Biol Chem* 285:18973–18978
- Finzi L, Gelles J (1995) Measurement of lactose repressor-mediated loop formation and breakdown in single DNA molecules. *Science* 267:378–380
- Fulcrand G, Dages S, Zhi X, Chapagain P, Gerstman BS, Dunlap D, Leng F (2016) DNA supercoiling, a critical signal regulating the basal expression of the *lac* operon in *Escherichia coli*. *Sci Rep* 6:19243
- Geanacopoulos M, Vasmatzis G, Zhurkin VB, Adhya S (2001) Gal repressosome contains an antiparallel DNA loop. *Nat Struct Mol Biol* 8:432–436
- Gilbert W, Maxam A (1973) The nucleotide sequence of the *lac* operator. *Proc Natl Acad Sci USA* 70:3581–3584
- Gilbert W, Müller-Hill B (1966) Isolation of the *lac* repressor. *Proc Natl Acad Sci USA* 56:1891–1898
- Harada BT, Hwang WL, Deindl S, Chatterjee N, Bartholomew B, Zhuang X (2016) Stepwise nucleosome translocation by RSC remodeling complexes. *eLife* 5, e10051
- Harada Y, Ohara O, Takatsuki A, Itoh H, Shimamoto N, Kinoshita K (2001) Direct observation of DNA rotation during transcription by *Escherichia coli* RNA polymerase. *Nature* 409:113–115
- Havas K, Flaus A, Phelan M, Kingston R, Wade PA, Lilley DMJ, Owen-Hughes T (2000) Generation of superhelical torsion by ATP-dependent chromatin remodeling activities. *Cell* 103:1133–1142
- Hsieh WT, Whitson PA, Matthews KS, Wells RD (1987) Influence of sequence and distance between two operators on interaction with the *lac* repressor. *J Biol Chem* 262:14583–14591
- Irani MH, Orosz L, Adhya S (1983) A control element within a structural gene: the *gal* operon of *Escherichia coli*. *Cell* 32:783–788
- Johnson S, Chen YJ, Phillips R (2013) Poly(dA:dT)-Rich DNAs are highly flexible in the context of DNA looping. *PLoS One* 8, e75799
- Kouzine F, Gupta A, Baranello L, Wojtowicz D, Ben-Aissa K, Liu J, Przytycka TM, Levens D (2013) Transcription-dependent dynamic supercoiling is a short-range genomic force. *Nat Struct Mol Biol* 20:396–403
- Krämer H, Amouyal M, Nordheim A, Müller-Hill B (1988) DNA supercoiling changes the spacing requirement of two *lac* operators for DNA loop formation with *lac* repressor. *EMBO J* 7:547–556
- Lal A, Dhar A, Trostel A, Kouzine F, Seshasayee ASN, Adhya S (2016) Genome scale patterns of supercoiling in a bacterial chromosome. *Nat Commun* 7:11055
- Lee I, Dong KC, Berger JM (2013a) The role of DNA bending in type IIA topoisomerase function. *Nucleic Acids Res* 41:5444–5456
- Lee M, Lipfert J, Sanchez H, Wyman C, Dekker NH (2013b) Structural and torsional properties of the RAD51-dsDNA nucleoprotein filament. *Nucleic Acids Res* 41:7023–7030
- Leger JF, Romano G, Sarkar A, Robert J, Bourdieu L, Chatenay D, Marko JF (1999) Structural transitions of a twisted and stretched DNA molecule. *Phys Rev Lett* 83:1066–1069
- Lewis D, Le P, Zurla C, Finzi L, Adhya S (2011) Multilevel autoregulation of {lambda} repressor protein CI by DNA looping in vitro. *Proc Natl Acad Sci USA* 108(36):14807–14812
- Lia G, Bensimon D, Croquette V, Allemand JF, Dunlap D, Lewis DEA, Adhya SC, Finzi L (2003) Supercoiling and denaturation in Gal repressor/heat unstable nucleoid protein (HU)-mediated DNA looping. *Proc Natl Acad Sci USA* 100:11373–11377
- Lia G, Praly E, Ferreira H, Stockdale C, Tse-Dinh YC, Dunlap D, Croquette V, Bensimon D, Owen-Hughes T (2006) Direct observation of DNA distortion by the RSC complex. *Mol Cell* 21:417–425
- Lia G, Semsey S, Lewis DAE, Adhya S, Bensimon D, Dunlap D, Finzi L (2008) The antiparallel loops in gal DNA. *Nucleic Acids Res* 36(12):4204–4210. doi:10.1093/nar/gkn389
- Little JW, Michalowski CB (2010) Stability and instability in the lysogenic state of phage lambda. *J Bacteriol* 192:6064–6076
- Liu LF, Wang JC (1987) Supercoiling of the DNA template during transcription. *Proc Natl Acad Sci USA* 84:7024–7027
- Ma J, Bai L, Wang MD (2013) Transcription under torsion. *Science* 340:1580–1583
- Maniatis T, Ptashne M (1973) Multiple repressor binding at operators in bacteriophage-lambda-(nuclease protection polynucleotide sizing pyrimidine tracts supercoils *E-coli*). *Proc Natl Acad Sci USA* 70:1531–1535
- Manning GS (2006) The persistence length of DNA is reached from the persistence length of its null isomer through an internal electrostatic stretching force. *Biophys J* 91:3607–3616
- Marko JF, Neukirch S (2013) Global force-torque phase diagram for the DNA double helix: structural transitions, triple points, and collapsed plectonemes. *Phys Rev E* 88:062722
- Mehta RA, Kahn JD (1999) Designed hyperstable *lac* repressor center dot DNA loop topologies suggest alternative loop geometries. *J Mol Biol* 294:67–77
- Mossing MC, Record MT Jr (1986) Upstream operators enhance repression of the *lac* promoter. *Science* 233:889–892
- Mulligan PJ, Chen Y-J, Phillips R, Spakowitz AJ (2015) Interplay of protein binding interactions, DNA mechanics, and entropy in DNA looping kinetics. *Biophys J* 109:618–629
- Naughton C, Avlonitis N, Corless S, Prendergast JG, Mati IK, Eijk PP, Cockcroft SL, Bradley M, Ylstra B, Gilbert N (2013) Transcription forms and remodels supercoiling domains unfolding large-scale chromatin structures. *Nat Struct Mol Biol* 20:387–395

- Nollmann M, Stone MD, Bryant Z, Gore J, Crisona NJ, Hong SC, Mittelheiser S, Maxwell A, Bustamante C, Cozzarelli NR (2007) Multiple modes of *Escherichia coli* DNA gyrase activity revealed by force and torque. *Nat Struct Mol Biol* 14:264–271
- Normanno D, Vanzi F, Pavone FS (2008) Single-molecule manipulation reveals supercoiling-dependent modulation of lac repressor-mediated DNA looping. *Nucleic Acids Res* 36:2505–2513
- Oehler S (2009) Feedback regulation of lac repressor expression in *Escherichia coli*. *J Bacteriol* 191:5301–5303
- Oehler S, Eismann ER, Krämer H, Müller-Hill B (1990) The three operators of the lac operon cooperate in repression. *EMBO J* 9:973–979
- Peter BJ, Ullsperger C, Hiasa H, Marians KJ, Cozzarelli NR (1998) The structure of supercoiled intermediates in DNA replication. *Cell* 94:819–827
- Peters JP, Mogil LS, McCauley MJ, Williams MC, Maher LJ 3rd (2014) Mechanical properties of base-modified DNA are not strictly determined by base stacking or electrostatic interactions. *Biophys J* 107:448–459
- Pfahl M, Gulde V, Bourgeois S (1979) “Second” and “third operator” of the lac operon: an investigation of their role in the regulatory mechanism. *J Mol Biol* 127:339–344
- Ptashne M (2004) A genetic switch: Phage lambda revisited. Cold Spring Harbor Laboratory Press, Cambridge
- Recouvreur P, Lavelle C, Barbi M, Conde e Silva N, Le Cam E, Victor J-M, Viovy J-L (2011) Linker histones incorporation maintains chromatin fiber plasticity. *Biophys J* 100:2726–2735
- Reznikoff WS, Winter RB, Hurley CK (1974) The location of the repressor binding sites in the lac operon. *Proc Natl Acad Sci USA* 71:2314–2318
- Rovinskiy N, Agbleke AA, Chesnokova O, Pang Z, Higgins NP (2012) Rates of gyrase supercoiling and transcription elongation control supercoil density in a bacterial chromosome. *PLoS Genet* 8:e1002845
- Schoeffler AJ, Berger JM (2008) DNA topoisomerases: harnessing and constraining energy to govern chromosome topology. *Q Rev Biophys* 41:41–101
- Semsey S, Tolstorukov MY, Virnik K, Zhurkin VB, Adhya S (2004) DNA trajectory in the Gal repressosome. *Genes Dev* 18:1898–1907
- Semsey S, Virnik K, Adhya S (2005) A gamut of loops: meandering DNA. *Trends Biochem Sci* 30:334–341
- Shao Q, Goyal S, Finzi L, Dunlap D (2012) Physiological levels of salt and polyamines favor writhe and limit twist in DNA. *Macromolecules* 45:3188–3196
- Sheinin MY, Forth S, Marko JF, Wang MD (2011) Underwound DNA under tension: structure, elasticity, and sequence-dependent behaviors. *Phys Rev Lett* 107:108102
- Strick TR, Allemand JF, Bensimon D, Croquette V (1998) Behavior of supercoiled DNA. *Biophys J* 74:2016–2028
- Strick TR, Bensimon D, Croquette V (1999) Micro-mechanical measurement of the torsional modulus of DNA. *Genetica* 106:57–62
- Travers AA, Muskhelishvili G (2013) DNA thermodynamics shape chromosome organization and topology. *Biochem Soc Trans* 41:548–553
- Van Workum M, Van Dooren SJ, Oldenburg N, Molenaar D, Jensen PR, Snoep JL, Westerhoff HV (1996) DNA supercoiling depends on the phosphorylation potential in *Escherichia coli*. *Mol Microbiol* 20:351–360
- Virstedt J, Berge T, Henderson RM, Waring MJ, Travers AA (2004) The influence of DNA stiffness upon nucleosome formation. *J Struct Biol* 148:66–85
- Wallace HM, Fraser AV, Hughes A (2003) A perspective of polyamine metabolism. *Biochem J* 376:1–14
- Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, Block SM (1998) Force and velocity measured for single molecules of RNA polymerase. *Science* 282:902–907
- Whitson PA, Hsieh WT, Wells RD, Matthews KS (1987) Supercoiling facilitates lac operator-repressor-pseudooperator interactions. *J Biol Chem* 262:4943–4946
- Wong OK, Guthold M, Erie DA, Gelles J (2008) Interconvertible lac repressor-DNA loops revealed by single-molecule experiments. *PLoS Biol* 6, e232