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DNA thermodynamics shape chromosome organisation and topology

Andrew A. Travers^{1,2} and Georgi Muskhelishvili³

¹MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

²Department of Biochemistry, Tennis Court Road, Cambridge CB2 1GA, UK

³Jacobs University Bremen, School of Engineering and Science, Campus Ring 1, D-28759 Bremen, Germany

Abstract

How much information is encoded in the DNA sequence of an organism? We argue that the informational, mechanical and topological properties of DNA are interdependent and together act to specify the primary characteristics of genetic organisation and chromatin structures. Superhelicity generated *in vivo*, in part by the action of DNA translocases, can be transmitted to topologically-sensitive regions encoded by less stable DNA sequences.

DNA is now the predominant genetic material in the living world. Which properties of this long polymer favour this ubiquity? The defining property of this polymeric tape is its informational capacity encoded as a digital sequence of base-pairs. Not only does this information perform a, presumably, primary function in specifying protein sequences but also, because DNA is a heterogeneous polymer, its sequence determines the local thermodynamic and mechanical properties of the molecule. Equally fundamentally, the chirality of the oft-quoted defining characteristic - the right-handed double helix - implies that the structural transitions required for the replication, transcription and recombination of the molecule potentially generate topological changes both locally and within the molecule as a whole. In turn the nature of these changes depends on the mechanical and thermodynamic properties of the DNA. This interdependence of the informational, mechanical and topological properties of a DNA chromosome implies a sufficiency for specification of a self-replicating entity (1) and a powerful argument for the usurpation of RNA by DNA as the primary information-carrying molecule.

Within a closed domain the configuration of the DNA double-helix is principally determined by its topology. In both eukaryotes and bacteria DNA is usually negatively supercoiled, either when unconstrained, or when bound to a protein surface. The superhelicity can take the form of variation in the intrinsic twist of the molecule and/or the generation of writhe (2). In principle these topological transitions are interconvertible such that $Lk = Tw + Wr$ where Lk is a given change in linking number, equivalent to a change in superhelical density. However, whether this change in superhelicity is expressed as writhe or twist change depends on the thermodynamic and mechanical properties of the DNA sequence. While many biologically relevant twist changes, such as DNA melting, are highly localised, writhing involves an extensive stretch of DNA chain being organised into a distinct coiled

configuration and hence is, in general, much more delocalised. Whereas delocalization can readily accommodate both positive and negative superhelicity, localised changes in twist are more apparent in negatively supercoiled DNA. Thus studies of negatively supercoiled plasmid DNA show that DNA strand separation is restricted predominantly to TpA base-steps which are enriched at natural sites of DNA strand separation (3). Similarly, the propensity of DNA to writhe in a particular sense can be encoded in the DNA sequence and this preference can then be stabilized and refined by the binding of certain abundant bacterial nucleoid-associated proteins (NAPs) (4-7). For example, the sequence periodicity of the regulatory region of the *E. coli tyrT* promoter is ~10.2 bp (<10.5 bp) while that in the vicinity of the *proV* regulatory region is 10.95 bp (>10.5 bp) (AAT, unpublished observations), potentially encoding respectively left-handed and right-handed coiling. Thus while the *tyrT* regulatory region binds three FIS (Factor for Inversion stimulation) dimers likely stabilising a toroidal DNA configuration, the *proV* regulatory region binds H-NS (Histone-like Nucleoid Structuring protein) which stabilises negatively supercoiled plectonemes (Figure 1). Both these NAPs have been implicated as important players in the delimitation of topological domains in vivo (8).

A prerequisite for the formation of these different topological structures is, of course, the generation of the necessary superhelicity. In bacteria a major mechanism is the ATP-dependent generation of negative supercoils by DNA gyrase but in both bacteria and eukaryotes a fundamental process is the formation of positive and negative supercoils by the constrained motion of DNA translocases (8). In this process positive supercoils are generated ahead of the translocating enzyme while counter-balancing negative supercoils are generated behind. The most processive translocases are the replisomes and RNA polymerase. These enzyme complexes can generate significant amounts of superhelicity. Each replisome replicating at a rate of ~1000 bp/sec can potentially generate 100 positive and 100 negative superhelical turns/second, or, in total, 400 superhelical turns/chromosome. Similarly in *Escherichia coli* 2000 RNA polymerase molecules actively transcribing ~50 bp/sec could potentially generate ~10000 positive and ~10000 negative supercoils/sec. Many of the RNA polymerase dependent supercoils would be dissipated by mutual annihilation in trains of enzymes transcribing the same transcription unit. These numbers also assume that both replisomes and RNA polymerases are completely constrained within a topological domain. There is also the potential for some of the generated superhelicity to be immediately relaxed by topoisomerases. Nevertheless at least some of the generated supercoils are likely transmitted to topologically sensitive regions of the chromosome. We argue here that these supercoils generated by the universal Liu and Wang (8) effect are a major influence shaping chromosome organization and architecture in all organisms.

In addition to delocalised mechanically soft DNA sequences other sequences which adopt non-B form structures at high negative superhelical densities can also serve as repositories for superhelical stress. Such sequences include those which can form, for example, cruciforms, slipped loops, triple-stranded structures, Z-DNA and G-quadruplexes (9-12). These sequences, and related toroidal protein-stabilised structures (13) often occur in the immediate vicinity of transcription start sites (10-13). It is hypothesized that these repositories of superhelicity can act as topological devices (9,14). On RNA polymerase–

mediated strand-separation stored superhelicity is released compensating, at least in part, for the constrained untwisting. With the escape of the polymerase from the promoter the superhelicity-dependent structures can reform utilising the negative superhelicity generated by the Liu and Wang process. For example, the left-handed DNA coil upstream of the *E. coli tyrT* promoter (13) stabilized by the binding of three FIS-dimers loses the central FIS dimer on interaction of the polymerase with the -10 region of the promoter (14).

Bacterial genomes

A striking characteristic of γ - and α -Proteobacterial genomes is that the gene order relative to the origin of replication is highly conserved (15). Notably those genes that are required for rapid vegetative growth are clustered close to the origin while genes that are preferentially expressed during anaerobic stress are predominantly located close to the Ter region (15). This organisation is paralleled by the average underlying physical properties of the circular genomic DNA. An analysis of 300 γ -Proteobacterial genomes revealed that, at low resolution (with sliding windows of 50 and 500 kb) when averaged to eliminate genetic noise, symmetric gradients of DNA melting energy were apparent extending from the replication origin to the terminus in both replichores with a high average melting energy in the Ori-proximal region and a low average melting energy in the Ter-proximal region (P. Sobetzko, M. Glinkowska, A. Travers, G. Muskhelishvili, unpublished). Nevertheless at higher resolution a compact region of low melting energy is apparent at the origin of replication. Such gradients, albeit less symmetric, are also observed in the individual genome of *E. coli* arguing that the general pattern is not an artifact of the averaging process. The symmetry of this pattern about the Ori-Ter axis argues strongly that the physical properties of the DNA are related to the replication process itself. Not only does the DNA itself exhibit Ori-Ter gradients but so also do the binding sites for DNA gyrase, which are preferentially located in the OriC-proximal half of the genome, as well as the genes whose expression is preferentially stimulated by high negative superhelicity (15) (Figure 2). These patterns correlate strongly with gene expression, with the genes dependent on the stress sigma factor σ^S clustering in the terminal region while those uniquely dependent on the vegetative sigma factor σ^{70} , such as the *rrn* genes are preferentially located closer to the origin (15).

A simple model explaining the functional significance of the gradients of expression and of DNA structure is that they reflect an average Ori-Ter gradient of DNA negative superhelicity from high to low in both replichores. This is consistent with the distribution of DNA gyrase sites, the preferred location of *hyp* genes (16), whose expression is associated with hypernegative superhelical densities, and the differential location-dependent response to σ^{70} and σ^S (since, unlike σ^S -containing RNA polymerase holoenzyme, σ^{70} -containing holoenzyme has a preference for initiating transcription on highly negatively supercoiled DNA templates (17)). How could such a gradient of superhelicity be generated? DNA gyrase is activated by a high ATP/ADP ratio which transiently increases on shift-up of bacteria to rich media (18). Such a mechanism could account for the initial generation of a superhelicity gradient. However, a possible potent driver of the formation and maintenance of such gradient would be the procession of the two replisomes along the replichores after the initiation of DNA replication. The replisomes would generate negative superhelicity

preferentially in the OriC-proximal half of the chromosome and positive superhelicity in the Ter-proximal half. Additionally the convergence of the two replisomes would result in a build-up of positive superhelicity around the terminus of replication. The movement of the replisomes could thus activate the *hyp* genes closer to the origin with the generation of positive superhelicity suppressing gene expression closer to the terminal region. The positive superhelicity would be absorbed by the delocalised region of low melting energy, and hence low mechanical stiffness, close to the Ter sites. This region would thus act as a topological sink where positive superhelicity, in the form of writhed braids, would be relaxed by topoisomerase IV (19). When the growing bacteria exhaust the oxygen or nutrients in the medium the initiation of DNA replication would slow or cease resulting in the collapse of the superhelicity gradient. This would relax the DNA in the OriC-proximal region of the chromosome accompanied by a concomitant switching-off of the *hyp* genes. Coinciding with the abrupt reduction in average superhelical density the σ^S -dependent genes are switched on (20).

The replication of the bacterial chromosome also results in a relative increase of gene copy number in the Ori-proximal region. This, in principle, has the effect of further enhancing the expression of genes located in this region. However, by itself, increase in copy-number is not sufficient to explain the concentration of active *hyp* genes in the same region. Rather increased negative superhelicity and increased copy number should be viewed as complementing mechanisms that maximise expression of those genes required for rapid growth.

Control of the distribution of superhelicity throughout the chromosome thus could be a crucial component of the regulation of gene expression. Although the stacking and melting energies of DNA provide an energy landscape that is a major expression determinant it is likely that other components, notably NAPs, refine and stabilize particular superhelical structures (4-7). One candidate for such a role is the ubiquitous HU protein. In the absence of both functional subunits of this protein, expression in the OriC-proximal half of the genome is increased relative to the wild-type while that in the Ter-proximal half is decreased (21). Put another way, in the mutant the gradient of expression from OriC to Ter is potentially enhanced. HU constrains negative superhelicity both by untwisting DNA and by left-handed wrapping (22,23) and thus it is plausible that its absence results in an increased superhelicity gradient. HU could thus act globally to modulate the gradient generated by the processing replisomes in a manner analogous to the Boulton-Watt steam-engine governor (24).

Other NAPs that constrain negative superhelicity include FIS and the paralogues StpA and H-NS (4-7). FIS stabilises more open supercoiled structures and is most abundant during periods of rapid growth (25) while the latter form protein-DNA fibres in which the supercoils are writhed in right-handed plectonemes which are generally repressive (26,27). Such structures would be sensitive to general levels of superhelical density and likely less stable on DNA relaxation, resulting in derepression.

We have argued that the underlying physical properties of the bacterial chromosome reflect primarily a means for regulating gene expression by the superhelicity generated by DNA

replication. Essentially the higher energy availability driving rapid growth is harnessed by increasing the intrinsic temperature of DNA in the form of higher levels of negative superhelicity characteristic of that growth phase (20). This in turn selectively facilitates the activation of promoters with higher activation temperatures (28) that are located in general in regions where the superhelical density is most negative. Indeed both in vitro and in vivo such promoters require higher superhelical densities for activation (29). Conversely supercoiling of extended delocalized A/T-rich regions in a plectonemic configuration facilitates H-NS-mediated repression of genes (30-33).

The yeast genome

Topological signatures in DNA similar to those observed in prokaryotes are also characteristic of eukaryotic organization. Analysis of the physical properties of yeast genomic DNA at much higher resolution (sliding windows of 51 and 125 bp) reveal a pattern that is common to many genes (Figure 3a) (34; B. Adryan, C. Vaillant, H. Cole, D. J. Clark, A. Arneodo, A. Travers, unpublished). In general open reading frames (ORFs) have a slightly higher average stacking/melting energy than the average value for the entire genome, whereas at both the 5' and 3' ends of genes the stacking and melting energies are significantly lower than the average values. This low stacking/melting energy is most notable in the 3' flanking regions of genes and indeed the two lowest stacking/melting energy regions occur at 3'-3' intergenic boundaries of transcriptionally convergent genes. These stretches of less stable DNA are typically ~100 bp in extent and highly enriched in pyrimidine-purine base-steps, especially TpA (B. Adryan, C. Vaillant, H. Cole, D. J. Clark, A. Arneodo, A. Travers, unpublished). They often contain the sequence TATATA, identified as the optimal binding site for topoisomerase II (35). Formally these elements are similar to the Ter region of the bacterial chromosome. Both occur at the convergence point of active translocation, both contain delocalised regions of relatively unstable DNA and both are potential sites for topoisomerase activity. Both also can potentially act as anchor points for the chromosome either via topoisomerase II in eukaryotes (36) or via ParS sites (a pair of short contiguous sequence elements known to be involved in chromosome segregation) in bacteria (37). They are thus candidates for defining topological domain separation and could act in both cases to prevent invasion of positive supercoils across the convergence point. In eukaryotes such invasion would have the potential to destabilise the negative wrapping of DNA around nucleosomes.

The lower average stacking/melting energy in the 5' flanking regions is often coincident with the presence of a TATA box and presumably in part, with the facilitation of DNA bending and untwisting by TATA-binding protein. This region is also enriched in stretches of oligo(dA)(dT) (38) which also have a lower than average melting, although not stacking, energy. Not only do sequences associated with transcription initiation have a lower melting temperature, so also, as in bacteria, do most, 87%, of the autonomously replicating sequences defining the initiation of DNA replication (B. Adryan, C. Vaillant, H. Cole, D. J. Clark, A. Arneodo, A. Travers, unpublished).

The picture emerging from this analysis is that, on average, ORFs are significantly enriched in DNA of higher stacking/melting energy while the flanking regulatory regions contain less

stable DNA. The thermodynamics of the DNA sequence thus correlate strongly with the genetic organization. Moreover, since both *in vitro* and *in vivo* nucleosome occupancy directly correlates with DNA stacking and melting energy the average occupancy of ORF DNA is substantially higher than that of the flanking 3' and 5' regions. DNA thermodynamics thus also correlate with chromatin stability. This correlation is stronger for chromatin assembled *in vitro* than that assembled *in vivo* and thus implies that the raw energy landscape of the DNA specifying histone-DNA interactions is refined *in vivo* by additional factors – and hence additional encoded information in the genome - including chromatin-remodelling assemblies, histone variants, bound RNA polymerase and packaging considerations (39-44). Together these generate the characteristic patterns of *in vivo* chromatin (45-47).

The instability of the 3' flanking regions, as well as that of 5' flanking regions, imply a significant influence of Liu and Wang generated superhelicity. This superhelicity could also facilitate the progression of both replisomes and RNA polymerases through chromatin (48). The positive superhelicity preceding the advancing translocase would destabilise downstream nucleosomes while the negative superhelicity behind would facilitate the reformation of upstream nucleosomes (49). Further since the displacement of the histone octamer to a different site during, at least, transcription, is essentially a one-dimensional process, the thermodynamics of the process would differ from those of histone deposition on DNA when both molecules are free in solution. In particular the balance between the entropic and enthalpic components would be altered such that the enthalpic component becomes more dominant. One consequence of this is that the sequence preferences of the histone octamer under conditions of one-dimensional placement are different from those where three-dimensional placement dominates.

Topological specification of genome organization

We have argued that the essential chiral characteristic of the DNA molecule is a major influence on chromosomal organisation. Regions associated with DNA melting – and hence a change in the partition between twist and writhe – contain often highly localized less stable DNA sequences. Other regions, notably those at the convergence points of DNA translocases, are characterized by more delocalised topologically sensitive sequences, which can potentially act as topological sinks by writhing in response to either positive or negative superhelicity (Figures 3b and 3c). Such sequences are also characteristic of regions that are repressed by proteins stabilising writhe. In both bacteria and eukaryotes the Liu and Wang process is a major mechanism for generating the superhelicity reflected in genomic organisation. Ultimately this reflects the dynamism of chromosomes.

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A

B

Figure 1.

AFM visualisation of (A) 3 FIS dimers bound to the *tyrT* upstream activating region and of (B) fibres formed by H-NS binding to two *proV* regulatory regions cloned into pUC18. The scale bars represent 5 and 50 nm for A and B respectively. Figures courtesy of S. Maurer and A. Japaridze respectively.

Rings from outside

Figure 2.

Relation of DNA gyrase binding sites and σ^{70} and σ^S RNA polymerase factor target sites to melting energy profile of the *E. coli* chromosome. High melting energy corresponds to higher negative ΔG values. For reference macrodomain extents are indicated external to each data wheel. All distributions were calculated across sliding windows of 50 kb. Original figure produced with permission from P. Sobetzko.

Figure 3.

A) Stacking energy profile of the ADH2 gene. Stacking energy is calculated by summing the stacking energy (G_{st}) of individual base-steps (bs) across a 51 base-pair sliding window. The diagram shows low average stacking energy in the 5' and 3' flanking regions relative to the ORF. Although individual genes differ in detail this pattern is typical of *Saccharomyces cerevisiae* genes (35,36).

B) Cartoon illustrating relationship of domain transversed by DNA translocases such as DNA and RNA polymerases to topologically sensitive regions (topological sinks). The elongating translocase generates a gradient of superhelicity, negative upstream and positive downstream. Non-dissipated superhelicity acts on topologically sensitive regions (TSRs) to facilitate strand separation upstream at promoters or replication origins and writhing downstream. The TSRs are generally regions of low average stacking and melting energy.

C) Model for the function of topological sinks downstream of transcription units. The cartoon shows polymerases actively transcribing convergent genes to generate positive superhelicity which induces positive writhing in the downstream TATATA-enriched regions. It is assumed that active transcription of only one gene would suffice to generate such downstream structures.