Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Pack, unpack, bend, twist, pull, push: the physical side of gene expression

Christophe Lavelle[1,2,3,4]

Molecular motors such as polymerases produce physical constraints on DNA and chromatin. Recent techniques, in particular single-molecule micromanipulation, provide estimation of the forces and torques at stake. These biophysical approaches have improved our understanding of chromatin behaviour under physiological physical constraints and should, in conjunction with genome wide and *in vivo* studies, help to build more realistic mechanistic models of transcription in the context of chromatin. Here, we wish to provide a brief overview of our current knowledge in the field, and emphasize at the same time the importance of DNA supercoiling as a major parameter in gene regulation.

Addresses
[1] National Museum of Natural History, Paris, France
[2] CNRS UMR7196, Paris, France
[3] INSERM U1154, Paris, France
[4] Nuclear Architecture and Dynamics, CNRS GDR3536, Paris, France

Corresponding author: Lavelle, Christophe (lavelle@mnhn.fr)

Current Opinion in Genetics & Development 2014, **25**:74–84

This review comes from a themed issue on **Genome architecture and expression**

Edited by **Victor Corces** and **David L Levens**

0959-437X/$ – see front matter,

http://dx.doi.org/10.1016/j.gde.2014.01.001

Introduction

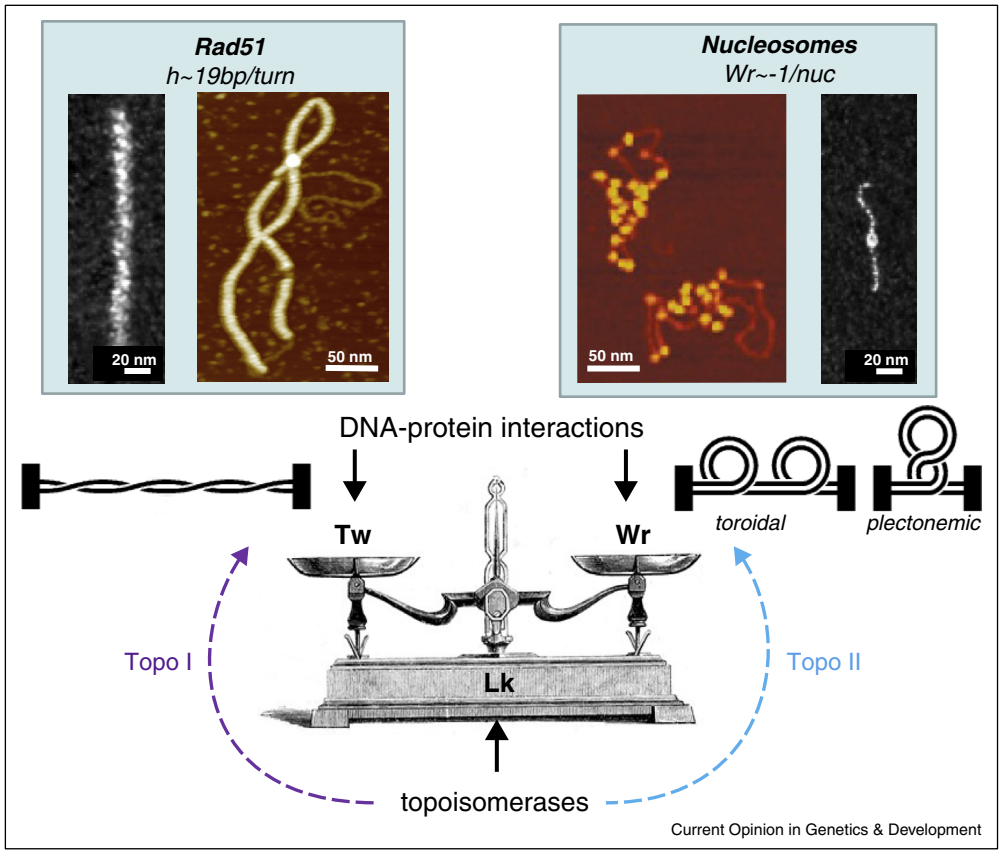
Eukaryotic genomes are divided into chromosomes, each consisting of a single molecule of several centimeters of DNA [1] compacted into a nucleoprotein substance known for 130 years as ''chromatin'' [2] for which we are still seeking for a precise characterization of its structure and properties (see [3] for an historical perspective). Chromatin structural plasticity/dynamics and 3D organization in the nucleus is thought to hold most of the keys of transcriptional regulation [4,5], hence also the secret of differential gene expression (e.g. ''epigenetics'' in its original definition, as ''the study of the causal mechanisms by which the genes of the genotypes bring about phenotypic effects''; see [6] for a recent re-edition of the original article by Waddington). Indeed, evidence has accumulated pointing out chromatin polymorphism and dynamics as critical means of control of genome accessibility in time and space, regulating the smooth progress of fundamental processes such as DNA replication, repair and transcription. Beside ubiquitous stochastic conformational changes resulting from thermal fluctuations (sometimes referred to as ''chromatin breathing''), some more persistent changes in chromatin local organization can be triggered by chemical modifications (e.g. histones post-translational modifications or DNA methylation status), protein recruitment (transcription factor, nucleosome remodelers, non-histone DNA condensing agents, etc.) or transient mechanical constraints (e.g. forces and torques produced by various proteins that drive DNA translocations such as helicases or polymerases).

The importance of this subject is attested to by the overwhelming number of reviews published on an almost daily basis about various elements of chromatin structure and dynamics. Although many reviews focus on biochemical and ''1D'' aspects (distribution of transcription factors, nucleosomes, chromatin remodelers, histone variants, histone modifiers, etc.; see the ENCODE project [7]), more and more papers increasingly also point towards the importance of physical and ''3D'' aspects (nuclear architecture, chromatin loop domains, physical constraints in the nucleus, etc.). Here, we wish to emphasize one of these physical characteristics of genome organization, DNA and chromatin response to mechanical constraints, in order to provide a more complete framework in which to interpret the control of gene expression. Indeed, as RNA polymerases push, pull and twist DNA, transient forces and torques develop within chromatin, with expected consequences on transcription events [8,9]. In addition to providing some basic mechanical and topological background for the general reader, we will also discuss some recent quantitative and qualitative insights into gene transcription and chromatin dynamics.

How tight is DNA in the nucleus?

Genomic DNA is often said in textbooks to undergo a ''drastic condensation'' to fit its $L_{DNA} = 6 \times 10^9$ bp $\times$ 0.34 nm = 2 m length (human cell, in G2) into a $D_{nuc} = 10$ μm large nucleus. This condensation is achieved by several factors including DNA supercoiling and DNA interactions with various proteins such as histones, which make the nucleosome (see Figures 1,2a) and other additional structural components (non-histone proteins [5] and non-coding RNAs [10]). Let's find a rough estimation of how drastic this compaction really is. From a geometrical (1D) point of view, one has a linear reduction $L_{DNA}/D_{nuc} = 2$ m/10 μm = 2×10^5. This does indeed look like an impressive compaction ratio;

Figure 1



The supercoiling balance. Lk is a topological parameter that only cuts in the DNA backbone (e.g. by topoisomerases) can change; mathematically, both topoisomerases I and II can change Lk, but physically topo I is more efficient on twist and topo II on writhe. Tw and Wr are geometrical parameters that are influenced by DNA sequence, ionic conditions or temperature (unconstrained supercoiling) and DNA/protein interactions (constrained supercoiling). Two extreme examples of constrained supercoiling are shown: (left) recombinase Rad51 is an enzyme that impact only Tw by untwisting DNA to an helical pitch of 19 bp/turn (compare to 10.5 bp/turn for relaxed DNA; see the tight plectonemic DNA protruding from the Rad51 filament, due to supercoiling compensation in the unconstrained -naked- region); (right) by wrapping DNA in a left-handed superhelix, nucleosomes produce mainly a change in Wr. TEM images (grey color, on a 350 bp linear DNA) from [109•]; AFM images (orange color, on a 5300 bp plasmid) courtesy of Olivier Piétrement.

however, this figure is not very relevant from a physical point of view, since DNA would never stretch that way in a free environment, but rather would spontaneously fold into a random coil. So for a more physical (3D) estimation of the actual compaction required to transition from a ''free'' DNA molecule (that occupies a volume V_{coil}) to the same molecule confined in a nucleus (with a volume V_{nuc}), one has to calculate the volume reduction V_{coil}/V_{nuc}. A rough approximation for the radius of gyration $R_g = L_p(N/6)^{1/2}$ of DNA taken as a Gaussian polymer (for a more rigorous calculus, see [11]), where $L_p \sim 50$ nm is the bending persistence length of DNA (see Table 1) and N the number of individual segments of length L_p in the whole genome, gives $R_g = 50\ \text{nm} \times ((2\ \text{m}/50\ \text{nm})/6)^{1/2} \sim 130\ \mu\text{m}$ and the volume occupied by this random coil is $V_{coil} = 4/3\pi R_g^3 \sim 10^7\ \mu\text{m}^3$ while the volume of the nucleus is $V_{nuc} = 4/3\pi(D_{nuc}/2)^3 \sim 500\ \mu\text{m}^3$. This makes a volume reduction of $V_{coil}/V_{nuc} \sim 2 \times 10^4$ (about ten times less than the linear reduction), which is still quite a huge volume reduction. Now, one might ask how much free space is there in the nucleus for stuff other than DNA? For this, one can calculate the excluded volume of genomic DNA: $V_{DNA} = \pi r_{DNA}^2 = \pi \times (1\ \text{nm})^2 \times 2\ \text{m} \sim 6\ \mu\text{m}^3$; so the packing fraction V_{DNA}/V_{nuc} is about 1%. DNA ''occupies'' only 1% of the nucleus interior, leaving plenty of room for other nuclear components.

In conclusion, DNA condensation is not so much a matter of packing *per se* (there is room for much more DNA in our nucleus; remember for a comparison the almost 70% packing fraction found in virus [12]). Rather, DNA condensation is a matter of *functional* organization. This means it is not just a *physical* problem (like in virus, where DNA is only supposed to be transiently packed for