Structural changes in single chromatin fibers induced by tension and torsion

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Summary

Since the discovery of the right-handed helical structure of DNA, 61 years have passed. The DNA molecule, which encodes genetic information, is also found twisted into coils. This extra twist of the helical structure, called supercoiling, plays important roles in both DNA compaction and gene regulation.

The DNA in eukaryotic cells is packaged into chromatin, with the basic unit called nucleosome. The nucleosome core is a protein-DNA complex, with 147 base pairs (bp) of DNA wrapped 1.7 turns in a left-handed helix around the histone octamer proteins. The strong electrostatic interactions between histones and DNA make the nucleosome one of the most stable protein-DNA complexes under physiological conditions. Because of this, it is well suited for its packaging function. It is generally accepted that arrays of nucleosomes can coil into a 30-nm-diameter helix to form the higher order structure of chromatin.

Chromatin is not a static structure, but it is highly dynamic to allow for vital processes such as replication and transcription. The two chains of DNA in the chromatin must get untwisted and separated during their activity. Many enzymes, acting on DNA, such as topoisomerases and RNA polymerases are constantly stretching and twisting the DNA helix. The effects of tension and torsion on bare DNA have been well studied by single-molecule techniques in the last 20 years. However, those effects on chromatin are still poorly understood. Understanding of this interplay has potential clinical implications, since some widely used anti-cancer drugs are known to interfere with the regulation of supercoiling. In this thesis, I give insights into chromatin fiber response to tension and torsion. With the results presented in this thesis, I aim to connect to the knowledge obtained from molecular biology and genomics on understanding the role of chromatin structure in maintaining our genes.

In order to quantify the effects of tension and torsion on a chromatin fiber, I first

studied the mechanical properties of supercoiled DNA (**Chapter 2**). I report the coexistence of twisted, plectonemic and melted states of DNA in a small topological domain. An extended experimental data set obtained with magnetic tweezers indicates the coexistence of these three states at sub-picoNewton force and linking number densities of about -0.06. A broadening of the transitions between the three states is found when the size of a topological domain reduces to several kilobasepairs. I presented a statistical mechanics model for such DNA domains by calculating the full partition function. Real-time analysis of short DNA tethers at constant force and torque shows discrete levels of extension, representing discontinuous changes in the size of the melted DNA. This new insight may have implications for the working mechanism of proteins that interact with DNA in topological domains, such as topoisomerases, transcription factors, histones and DNA based molecular motors, like DNA and RNA polymerases and chromatin remodellers.

Next, I quantitatively described the force spectroscopy of torsionally unconstrained regular chromatin fibers reconstituted with DNA containing Widom 601 sequence repeats (**Chapter 3**). Aiming to disentangle unfolding transitions in chromatin fibers, I presented new experimental data as well as a novel quantitative model for all aspects of force-induced unwrapping of the chromatin fiber. With this statistical mechanics model, I compared pulling traces of a mononucleosome with those of fully folded fibers. Despite using arrays of Widom 601 sequences and careful titration of the reconstitution dialysis, I found it necessary to include some heterogeneity of the chromatin fibers in terms of nucleosome composition into the model. When these heterogeneities were accounted for, I was able to determine consistent values for the DNA unwrapping free energy and the extension of each nucleosome conformation. A novel intermediate conformation was found, existing between 2.5 and 7 pN. Moreover, the qualitative difference in rupture behavior between chromatin fibers with 197 bp and 167 bp nucleosome repeat length indicated a different folding topology of the two repeat lengths. My results have implications for accessibility of DNA in fully-folded and partially unwrapped chromatin fibers and are essential for understanding force-unfolding experiments on nucleosome arrays.

Finally, based on the knowledge summarized above, I studied the stability of single supercoiled chromatin fibers (**Chapter 4**). By applying tension and torsion with magnetic tweezers, I found that the fiber has a strong asymmetric response to supercoiling. Negative supercoiling stabilizes the fiber against unfolding. Positive supercoiling is ab-

sorbed by the fiber. This anisotropy of the fiber reflects the chirality of a left-handed helix. When the force exceeds 2.5 pN, the fiber unfolds, and unwraps one turn of DNA. The level of unfolding depends on the degree of supercoiling. Interestingly, positive supercoiling facilitates the unfolding, but superfluous positive supercoiling refolds the fiber. An equilibrium statistical mechanics model based on chromatin topology and elasticity was presented, which captures the full complexity of chromatin folding and unfolding at different degrees of supercoiling. These results revealed for the first time the effects of torque on a folded chromatin fiber and present a new quantitative model of chromatin supercoiling.

In conclusion, using single-molecule force spectroscopy, I resolved force/torque-induced structural changes of DNA and chromatin fibers. I showed that the structural changes of chromatin fibers can be described by four conformations. I showed for the first time the folding and unfolding of a chromatin fiber under torsion. The anisotropic response of chromatin fibers to supercoiling reflects its left-handed chirality. These findings give a detailed structural insight of a supercoiled chromatin fiber, yielding a better understanding of the response of chromatin during transcription.