Structural changes in single chromatin fibers induced by tension and torsion

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Chapter 1

Introduction

In this thesis, we describe the mechanical properties of chromatin, the ubiquitous DNA protein complex that organizes our genome, at the single-molecule level. This chapter briefly introduces the structural properties of DNA, nucleosomes and chromatin. We introduce the theory to describe the elastic and topological characteristics of DNA and discuss the single molecule technique, magnetic tweezers, which is used in this thesis to study the structural changes of DNA and chromatin under tension and torsion. Finally, we provide a brief overview of the contents of this thesis.

Almost 60 years have passed since the discovery of the right-handed helical structure of DNA [1]. The unique structure of DNA enables genetic and epi-genetic information to be stored and regulated in prokaryotes and eukaryotes. Prokaryotes are single-celled organism that lack a cell nucleus, and their DNA, though compacted by supercoiling and a host of DNA organizing proteins, floats freely in the cytoplasm. Eukaryotic cells, in contrast, have intracellular organelles that organize the cellular contents into compartments. In human cells, for instance, our genes consist of 3 billion base pairs (bp) of DNA that are packed in the nucleus. DNA is under constant mechanical stress and exists in various structures. This organization of DNA is highly dynamic with various enzymes stretching and twisting the helix. This dynamical structure plays a role in regulating transcription, replication and mitosis. Understanding the role of DNA compaction in gene regulation represents one of the most challenging questions in current life sciences. To address this question, we need better physical descriptions of DNA and its higher order organization, chromatin.

1.1 DNA, nucleosomes and chromatin

DNA in cells typically consists of two complementary polynucleotide chains that are interwound, forming a doublestranded helix, though other shapes, such as G-quadruplexes and Holliday junctions may also occur. Among several known conformations, B-DNA prevails under physiological conditions (Fig. 1.1A). B-DNA is a right handed helix with a pitch of 10.5 bp per helical turn (3.4 nm) and a diameter of 2.0 nm. The human genome DNA consisting of 3 billion bp is therefore about 1 meter long when stretched out. Without physical constraints, it may fold into a swollen coil occupying volume of $10^7\ \mu m^3$ [2]. In the nucleus of a typical human cell however, it is condensed into a volume of about 500 μm^3. This formidable magnitude of condensation into the nucleus is achieved by several steps of compaction involving a choice of structural proteins. The complex of DNA and those structural proteins is known as chromatin.

The basic repeating unit of chromatin is the nucleosome, consisting of 147 bp of DNA wrapped 1.7 times around a wedge-shaped histone octamer. The octamer is composed of a $(H3\text{-}H4)_2$ tetramer and two H2A-H2B dimers (Fig. 1.1B) [6]. In vitro studies revealed that the nucleosome is highly dynamic and undergoes spontaneous, re-

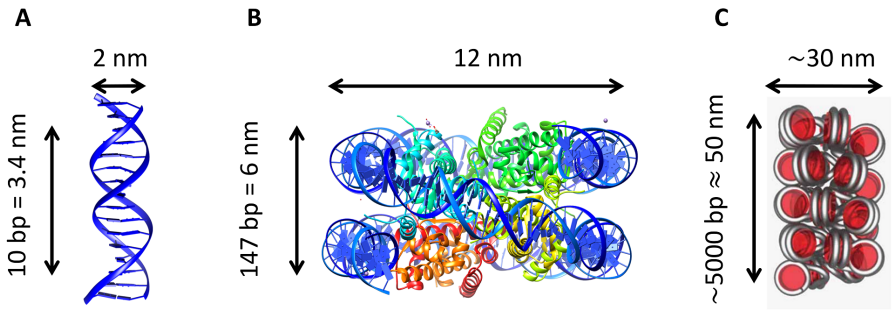

Figure 1.1: Overview of several levels of DNA organization with typical dimensions. (A) The crystal structure of B-DNA (3BSE) [3] shows a right-handed chirality with about 10 bp per helical turn. (B) The nucleosome crystal structure (1KX4) [4] shows 147 bp of DNA wrapped around a histone octamer with a left-handed chirality. (C) Schematic drawing of a folded chromatin fiber with one-start solenoid model proposed in [5]. The nucleosome arrays are connected by 30-60 bp of linker DNA.

versible unwrapping of part of the DNA from the histone core [7]. Although there are no sequence-specific interactions between histones and DNA bases, certain DNA sequences have a strong preference to form a nucleosome. The chromatin fibers studied in this thesis are reconstituted with tandem arrays of the Widom 601 sequence [8, 9]. This DNA sequence was selected from a random DNA pool to bind with high affinity to the histone octamer. Even though 601 sequence is widely used in vitro research, only recently was the mechanism of its strong affinity explained by a statistical mechanics model [10].

Nucleosomes are connected by short DNA segments (linker DNA) into nucleosome arrays, which fold into chromatin fibers by interactions with neighboring nucleosomes. Although the structure of chromatin fibers has been heavily debated for 30 years, in vitro it is commonly found that these fibers form condensed 30 nm fibers under physiological conditions (Fig. 1.1C). Several parameters determine the conformation and the degree of compaction of the 30 nm fiber, such as the linker DNA length, salt concentration and histone post transcriptional modifications. The linker DNA varies in nature between 10 and 90 bp [11]. Two groups of competing models, the "one start" solenoid model and the "two start" zigzag model have been proposed to explain the chromatin structure [12]. In the solenoid model, consecutive nucleosomes interact with each other

and follow a helical trajectory. This model is supported by the Electron Microscopy experiments on reconstituted chromatin fibers with 30-60 bp of linker DNA [13]. In the zigzag model, two rows of nucleosomes twist into a double helix, so that alternate nucleosomes become interacting partners. This model is supported by the crystal structure of a tetranucleosome array with 20 bp of linker DNA [14].

1.2 DNA supercoiling

Overwinding (positive) or underwinding (negative) the DNA double helix results in DNA supercoiling, which is an expression of the torsional strain on the helix. DNA supercoiling plays an important role in DNA compaction such as the formation of nucleosomes and the higher order chromatin structures. The DNA wraps around the histone core in a left-handed helix, therefore it is negatively supercoiled. Additionally, DNA supercoiling acts as an important feature during replication and transcription. During transcription, the double helix is underwound, gets melted and forms a transcription bubble [15]. DNA is also thought to experience the torsional stress created by RNA polymerase II [16], when RNA polymerase II is bound to the nuclear matrix and processes through a torsionally constrained DNA domain. However, how chromatin fibers respond to the torsional stress is not well understood. To further answer this question, we need physical and mathematical descriptions of DNA supercoiling.

The term topology is used in mathematical studies of shapes, to quantify the structural properties that are conserved under continuous deformations, including stretching and bending. It is commonly used to describe the conformation change of supercoiled DNA. A torsionally constrained DNA segment with no free rotation of its ends, is called a topological domain. An example of a topological domain is circular DNA.

In our studies, we create a topological domain from a linear DNA fragment, using multiple anchor points to stick the DNA to a glass slide and a bead. A topological domain is characterized by its linking number (Lk). The linking number is defined as the number of times that the two DNA strands are interwound, independent of the exact conformation. The linking number of a relaxed B-DNA molecule with N bp is

$$Lk_o = N/10.5\,. \tag{1.1}$$

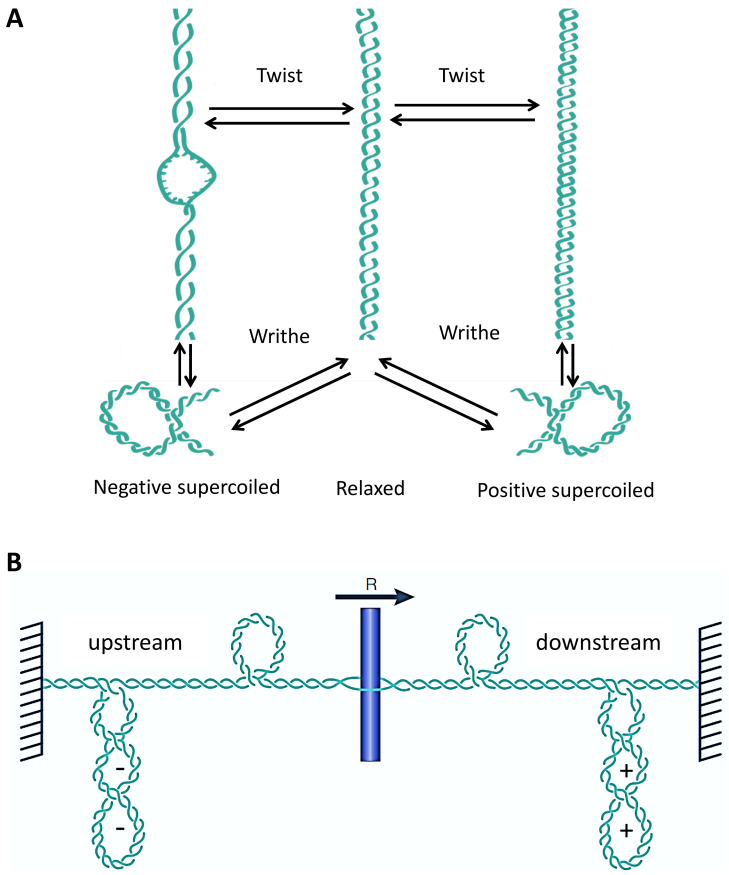

Figure 1.2: The topological properties of DNA. (A) DNA can exist as a relaxed molecule (center) having a right-handed chirality without any supercoiling as schematically depicted in the center. Overwinding (right) DNA can over-twist the DNA or form a plectonemic structure. Underwinding DNA may undertwist DNA, induce DNA denaturation to form DNA melting bubble or form a plectonemic structure. (B) A graphical illustration of the "twin supercoil domain" based on [17]. R is RNA polymerase II. If R is moving from left to right, the DNA in font of the polymerase (downstream) becomes overwound, or positively supercoiled; the DNA behind the polymerase (upstream) becomes underwound, or negatively supercoiled.

Two parameters that describe changes in DNA conformation are twist (Tw) and writhe (Wr). Twist refers to the helical winding of the DNA strands around each other in a helical conformation, whereas writhe is a measure of the looping of the double helix, quantified by the number of times it crosses itself. Without supercoiling, Lk_o equals Tw_o. Over or under-winding the DNA molecule will change Lk. This change in the linking number can be distributed into the two ways (shown in Fig. 1.2A),

$$\Delta Lk = Lk - Lk_o = Tw - Tw_o + Wr = \Delta Tw + Wr \tag{1.2}$$

The response to positive and negative supercoiling is not symmetric. Because of the chirality of DNA, it may be denatured by negative supercoiling to form a melted bubble, which has no intrinsic twist.

In eukaryotic cells, many activities that require DNA to be unwound (and rewound) involve the process of supercoiling. During transcription, for example, a RNA polymerase II can generate about seven DNA supercoils per second in a topological domain [18]. A popular model to describe the mechanism of RNA polymerase II is the "twin supercoiled domain" model [19, 20] (Fig. 1.2B). In this model, the RNA polymerase II moves along the DNA helix, and generates positive supercoiling ahead and negative supercoiling behind. However, the natural substrate for RNA polymerase II is a chromatin fiber consisting of several tens of nucleosomes (a typical length of a gene) rather than naked DNA. The effects of supercoiling therefore need to be considered in the context of chromatin, but unfortunately, we know very little about its mechanical properties. The idea that the supercoiling may be transmitted along chromatin was raised twenty-five years ago but how the chromatin fiber responds to supercoiling has nonetheless remained unclear [21, 22]. One hypothesis based on the "twin supercoiled domain" model is that positive supercoiling ahead of the RNA polymerase II will destabilize nucleosomes and negative supercoiling behind will promote reassembly of nucleosomes [23, 24]. Manipulating chromatin by single-molecule techniques can provide insight into this fundamental question.

1.3 Magnetic tweezers

In this thesis, we used magnetic tweezers as a tool to manipulate single DNA molecules or chromatin fibers and study their response to torsional stress. Magnetic and optical

tweezers have been developed in the past twenty years. Force spectroscopy studies on DNA or DNA-protein complexes have given lots of structural insights on the single molecule level. In 1996, Strick et al. measured the response of a single supercoiled DNA to a stretching force [25]. A single DNA molecule was anchored on a glass cover slip, and at the other end to a superparamagnetic bead (Fig. 1.3A). A magnet, placed above the flow cell, exerted a force on the bead and hence on the DNA molecule. The magnetic force exerted on the bead depends on the gradient of the magnetic field [26, 27]:

$$F = \frac{1}{2}\nabla(\overrightarrow{m(B)} \cdot \overrightarrow{B}) \tag{1.3}$$

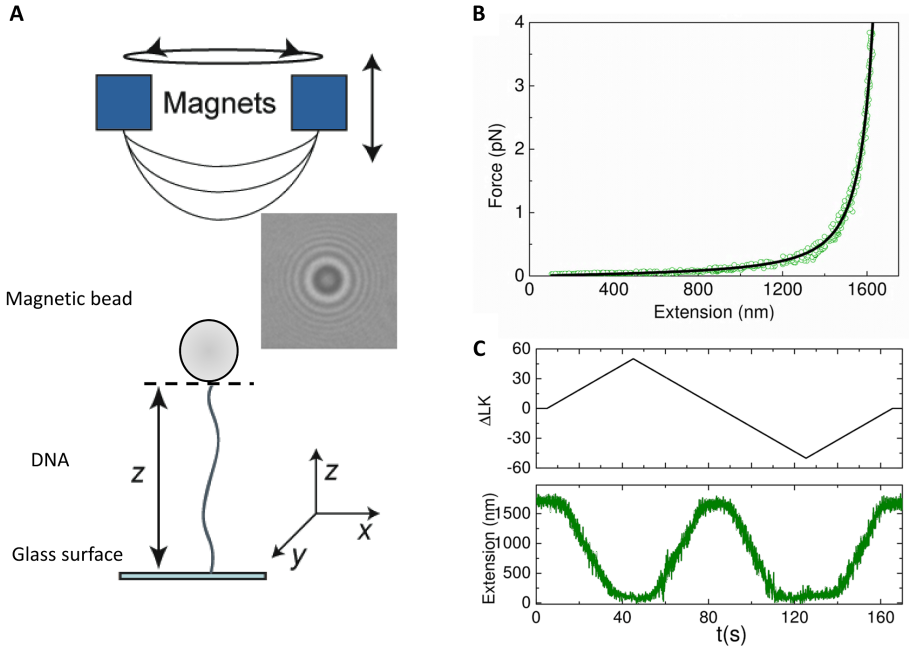

Figure 1.3: Magnetic tweezers set up and the mechanical properties of DNA. (A) A schematic drawing of the setup with an image of the bead. (B) A force-extension curve of a bare DNA molecule with 5.2 kbp. A fit by worm like chain model gives the contour length of 1750 nm, persistence length of 50 nm. (C) The real time measurements of DNA extension versus excess linking number.

where $\overrightarrow{B}$ is the external magnetic field and $\overrightarrow{m(B)}$ is the magnetic moment induced in the paramagnetic bead. The micrometer-sized bead can be tracked by ordinary light

microscopy. The position of the bead in three dimensions is determined by image analysis: the position in the x and y directions is obtained from the center of the diffraction rings with nanometer precision. The position in the z direction can also be extracted from the diffraction pattern by comparison with reference images, which measured the diffraction pattern of the bead at known distances from the focal plane.

The force can be calculated from the amplitude of the Brownian fluctuations. The Brownian motion of the bead is restricted by the DNA tether and varies with the applied force. The force follows from equipartition theorem [25]:

$$F = \frac{k_B T}{< \triangle x^2 >} < z > . \tag{1.4}$$

$k_B T$ is the product of the Boltzmann constant, k_B, and the temperature, T. At room temperature, $k_B T$ = 4.1 pN nm. $< \triangle x^2 >$ is the variance of the bead excursions in the x-direction and $< z >$ is the extension of the DNA tether. The forces range from subpiconewton to tens of piconewtons. The spatial resolution is several nanometers and temporal resolution is in the order of tens of milliseconds [28].

One should notice that due to the limited frame rate of the camera, $< \triangle x^2 >$ can be underestimated at large forces or with short tethers. Therefore, rather than using Equation 1.4 directly, the fluctuations in position are transferred into the frequency domain, and the power spectrum of the motion is further analyzed. More details about force calibration can be found in [29, 30]. Here we calibrated the force as a function of the magnet position, and use this relation to calculate the force during an experiment [31]. A major advantage of this method is that one does not need long (> minutes) calibration times, and long exposures to large forces, which is detrimental to our chromatin fibers.

During a magnetic tweezers experiment, the height of the bead that we measured is relative to the focal plane of the microscope. We subtract the relative position of the bead at a certain force with the position of the bead at the lowest force (< 0.03 pN) to obtain the extension of the tether. However, this extension is sometimes shorter than what we expected, because the tether may not always be attached to the bottom of the bead. When it is attached at the side of the bead, this leads an apparent extension shorter than the exact value [32, 33]. We correct this by adding an offset correction according to the expected contour length of the tether, using the well-documented worm-like chain properties of DNA (Fig. 1.3B, and described in the next section).

An advantage of the magnetic tweezers with respect to (traditional) optical tweezers is its ability to control the excess linking number of a single tether. Rotating the pair of magnets rotates the bead, and induces either over or under-winding of the DNA. The extension of the tether response to supercoiling can be detected in real time (Fig. 1.3C).

1.4 Mechanical properties of DNA and nucleosomes

Knowing the elastic properties of double stranded DNA is the first step to understand the mechanical properties of a chromatin fiber. The elasticity of double stranded DNA has been investigated extensively using magnetic and optical tweezers [34, 35]. A DNA molecule in solution bends locally as a result of thermal fluctuations. Such fluctuations shorten the end-to-end distance of the molecule and is called entropic elasticity. A model named the Worm like chain (WLC) is commonly used to describe this entropic elasticity. The WLC model envisions an isotropic rod that is continuously flexible, as opposed to other polymer models, like the freely jointed chain, which has discrete rigid sections that are linked by flexible hinges. The correlation of the rod's local direction reduces with distance s along the curve following an exponential decay $e^{-|s|/A}$. The decay length, A, is called the persistence length of the rod and defines its flexibility. The persistence length of double-stranded DNA is around 50 nm under physiological conditions [26].

An analytical solution of the force-extension relation for WLC is not known, but an interpolation formula to the numerical solution, given by Marko and Siggia [36] is commonly used:

$$F = \frac{k_B T}{A}\left(\frac{1}{4(1-\frac{z}{L_o})^2} - \frac{1}{4} + \frac{z}{L_o}\right) \tag{1.5}$$

where z is the end-to-end distance of the tether and L_o is the contour length. For B-DNA the contour length is the number of base pairs times 0.34 nm. A typical force-extension curve of DNA with 5200 bp is shown in Fig. 1.3B.

The mechanical properties of nucleosomes and chromatin have been studied for fifteen years, but there is far less consensus about their interpretation [37]. Brower-Toland et al. [38] first showed that the nucleosome unwraps in two steps. Two 25 nm

steps occur at forces around 5 pN and 15 pN. These stepsizes have been suggested to correspond to the outer turn and inner turn unwrapping of DNA. A DNA spool model proposed by Kulic and Schiessel [39] was able to explain nucleosome unwrapping under tension. Recently, a study of nucleosome unwrapping under torsion was presented [40]. Interestingly, torque has only a moderate influence on nucleosome unwrapping: positive torque slightly increases the unwrapping force for the outer turn, but decreases the unwrapping force for the inner turn. The structure and the mechanical properties of a chromatin fiber in which nucleosomes interact are still poorly understood, and this will be the main subject of this thesis.

1.5 Statistical mechanics

In this thesis we use statistical mechanics to describe the mechanical properties of DNA and chromatin. We define different states of DNA and the chromatin fiber that each have a distinct force and linking number dependent extension and free energy. Statistical mechanics is used to describe the properties of this system in thermodynamic equilibrium.

We consider a system at a constant temperature T, with a fixed number of constituent particles. Each state that the system can occupy, is defined by a specific set of conformations of the system and is marked as i, $(i = 1, 2, 3...)$ with the total energy E_i in each state. The partition function is defined as

$$Z = \sum_i exp(-\frac{E_i}{k_B T}) \tag{1.6}$$

The probability P_i that the system occupies state i is

$$P_i = \frac{1}{Z} exp(-\frac{E_i}{k_B T}) \tag{1.7}$$

In single-molecule studies, it is more convenient for us to calculate the probability P_i of the molecule occurring as a partially folded state when the temperature (T) and the force (F) are specified for a system at 1 atm [41].

$$P_i(T, F) = \frac{1}{Z} exp(-\frac{\Delta G_i(T, z_i) - F z_i}{k_B T}) \tag{1.8}$$

The $\Delta G_i(T, z_i)$ are the free energies at constant T and average extensionz_i of state

i. The partition function ensures that the probabilities sum up to one, i.e. $\sum P_i = 1$. Using the partition function, we can calculate the statistical average value of any physical quantity in the system, for instance, for a quantity marked as x, the mean value of x is

$$< x >= \sum_i x_i P_i \tag{1.9}$$

1.6 Outline of this thesis

This thesis reports experimental work on the mechanical properties of the chromatin fiber under tension and torsion. It also contains the theoretical modeling to quantitatively understand the data and enables us to interpret the data in terms of the structural changes of the chromatin fiber at the single molecule level.

Chapter 2 reports the coexistence of twisted, plectonemic and melted states of DNA in a small topological domain. Magnetic tweezers measurements indicate the coexistence of these three states at sub-picoNewton force and linking number densities about −0.06. A broadening of the transitions between the three states is found when the size of a topological domain is limited to several kilobasepairs. We present 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 melting bubble, which should reflect the underlying DNA sequence. Our results provide a comprehensive picture of the structure of underwound DNA at low force and torque and could have important consequences for various biological processes, in particular those that depend on local DNA melting, such as the initiation of replication and transcription.

Chapter 3 describes force spectroscopy of torsionally unconstrained chromatin fibers reconstituted with 601 sequence repeats. We show that the experimental data has variations between different fibers that can be attributed to the heterogeneity in the composition of individual fibers. Comparison to force-extension curves of single nucleosome and chromatin fibers of 20 bp and 50 bp linker DNA reveals distinct physical properties of chromatin (un)folding. We propose that the unfolding of a chromatin fiber goes through four different nucleosome conformations and introduce a statistical mechanics model that quantitatively describes the extension of individual fibers in response to forces up to 30 pN. This quantitative analysis allows for a structural interpretation of all physical processes that define chromatin folding.

Chapter 4 reports the elasticity of torsionally constrained chromatin fibers. By applying tension and torsion with magnetic tweezers, we find that the fiber has a strong asymmetric response to supercoiling. Negative supercoiling stabilizes the fiber against unfolding. Positive supercoiling on the other hand can be absorbed by the fiber. When the force exceeds ~2.0 pN, the fiber unfolds and unwraps one turn of DNA. The amount of unfolding depends on the extend of supercoiling. The anisotropic response reflects the chirality of a left-handed helix. A statistical mechanics model is presented, which captures the full complexity of chromatin folding and unfolding at different degree of supercoiling. These results reveal for the first time the topology of a folded chromatin fiber and present a new description of DNA and chromatin under torsional stress.

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