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Magnetic tweezers: a sensitive tool to study DNA and chromatin at the single-molecule level

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Abstract: The advent of single-molecule biology has allowed unprecedented insight into the dynamic behavior of biological macromolecules and their complexes. Unexpected properties, masked by the asynchronous behavior of myriads of molecules in bulk experiments, can be revealed; equally importantly, individual members of a molecular population often exhibit distinct features in their properties. Finally, the single-molecule approaches allow us to study the behavior of biological macromolecules under applied tension or torsion; understanding the mechanical properties of these molecules helps us understand how they function in the cell. In this review, we summarize the application of magnetic tweezers (MT) to the study of DNA behavior at the single-molecule level. MT can be conveniently used to stretch DNA and introduce controlled levels of superhelicity into the molecule and to follow to a high definition the action of different types of topoisomerases. Its potential for chromatin studies is also enormous, and we will briefly present our first chromatin results.

Key words: single-molecules, chromatin, topoisomerases, magnetic tweezers, force.

Résumé : L'avènement de la biologie unimoléculaire a permis de mieux comprendre le comportement dynamique des macromolécules biologiques et de leurs complexes. Des propriétés imprévues, masquées par le comportement asynchrone d'une multitude de molécules dans les expériences globales, peuvent apparaître; de plus, les propriétés de chacun des membres d'une population moléculaire ont souvent des particularités distinctives. Finalement, les approches unimoléculaires nous permettent d'étudier le comportement de macromolécules biologiques soumises à une tension ou une torsion : la compréhension des propriétés mécaniques de ces molécules nous aide à comprendre comment elles agissent dans la cellule. Dans cet article, nous faisons une revue de l'usage de pincettes magnétiques pour étudier le comportement d'une seule molécule d'ADN. Les pincettes magnétiques peuvent être utilisées de façon commode pour étirer l'ADN et introduire un taux précis de surenroulement dans la molécule et pour examiner à haute définition l'action de différents types de topoisomérases. Leur potentiel pour l'étude de la chromatine est également énorme et nous présentons brièvement nos premiers résultats concernant la chromatine.

Mots clés : molécules isolées, chromatine, topoisomérases, pincettes magnétiques, force.

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Introduction: studying biological macromolecules under applied force

Forces play an essential role in the functioning of cells and organisms and are exerted at both the extracellular and intracellular levels. Examples of intracellular forces include the forces exerted during the reversible structural transformations of chromatin and chromosomes during the cell-division cycle, chromosome movements during mitosis and meiosis, the action of molecular motors involved in mechanical movements (myosin moving along actin filaments during muscle contraction; kinesin moving along microtubules during vesicle trafficking). RNA and DNA polymerases threading template DNA through their active sites can also be viewed as molecular motors; other enzymes that act onto the DNA to change its structure (helicases, topoisomerases, etc.) also exert forces of various magnitudes. Intramolecular forces, on the other hand, create and maintain proper structuring and folding of macromolecules, properties that are of crucial importance for their functioning. Such forces hold the two strands of double-helical DNA together, fold RNA molecules into functional entities, and govern protein folding and denaturation. The magnitude of forces acting at the molecular level varies

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within several orders of magnitude, ranging from a few piconewtons to several nanonewtons (for reviews see Strick et al. 2000*a*; Zlatanova and Leuba 2002, 2003).

There is a clear need for methods that would allow these relatively small interaction forces to be measured. In addition, if we can actively apply forces to macromolecules, in vitro or in vivo, we can study their behavior in response to the applied force, thus mimicking the cellular environment and conditions in which they function. While earlier instrumentation allowed bulk measurements of such forces (e.g., the surface force apparatus, Israelachvili 1992), the past decade has witnessed a boom in the technology that allows application and measurements of forces at the single-molecule level (Leuba and Zlatanova 2001). These single-molecule methods allow unprecedented force and distance resolution; even more importantly, they uniquely allow elucidation of the heterogeneity among individual members of a molecular population. Thus, properties that remain masked in bulk biochemical and biophysical measurements (these provide measurements over the entire ensemble of molecules in the sample) can be revealed and studied.

Single-molecule manipulation techniques; the magnetic tweezers

The single-molecule approaches developed so far can be classified, on the basis of their principle of action, into two major categories: mechanical force transducers and external field manipulators (for recent reviews see Bustamante et al. 2000*a*, 2000*b*; Clausen-Schaumann et al. 2000*b*; Strick et al. 2001; Zlatanova and Leuba 2002, 2003). In the mechanical force transducers — the AFM, microneedles and optical fibers — forces are applied or sensed through bendable beams. In the external field manipulators — optical tweezers (OT), magnetic tweezers (MT), and flow fields — the molecule is acted upon from a distance, by application of external fields (photonic, magnetic, or hydrodynamic) either to the molecule itself or to an appropriate handle to which the molecule is attached. In OT the handle is a transparent polystyrene bead, in MT it is typically a Dynabead®, composed of highly crosslinked polystyrene with magnetic material (Fe_2O_3 and Fe_3O_4) evenly distributed throughout the pores of the bead. Dynabeads are superparamagnetic, i.e., they exhibit magnetic properties only when placed within a magnetic field. To manipulate single molecules with external fields, it is necessary to attach the other end of the molecule to a surface or to an additional bead (a micropipette can be used to hold this second bead with suction). In this article, we will focus on the use of MT for the study of single molecules of DNA and their interaction with DNA-manipulating enzymes; we will also briefly summarize some recent data on chromatin-fiber assembly obtained with MT.

Attempts to manipulate magnetic objects in solution for biologically relevant research date back more than half a century (Crick and Hughes 1949; Yagi 1960; for further historic notes see Gosse and Croquette 2002). The prototype of the instrument used in single molecule studies was built in the laboratories of D. Bensimon and V. Croquette in Paris, and used in a series of high-impact, elegant studies that will be discussed in some detail below. In MT set-up, the macromolecule is attached between a surface and a magnetic bead (Fig. 1a). Manipulation of the external magnetic field can be used to apply stretching forces onto the tethered molecule, and (or) to induce precisely known levels of superhelicity: stretching is achieved by changing the distance between the magnets and the cuvette, while superhelicity is introduced by rotating the external magnets either clockwise or counterclockwise. The forces created by permanent magnets (e.g., Smith et al. 1992; Strick et al. 1996) or by electromagnets (Ziemann et al. 1994; Gosse and Croquette 2002) are very stable and can be very small, in the femtonewton range.

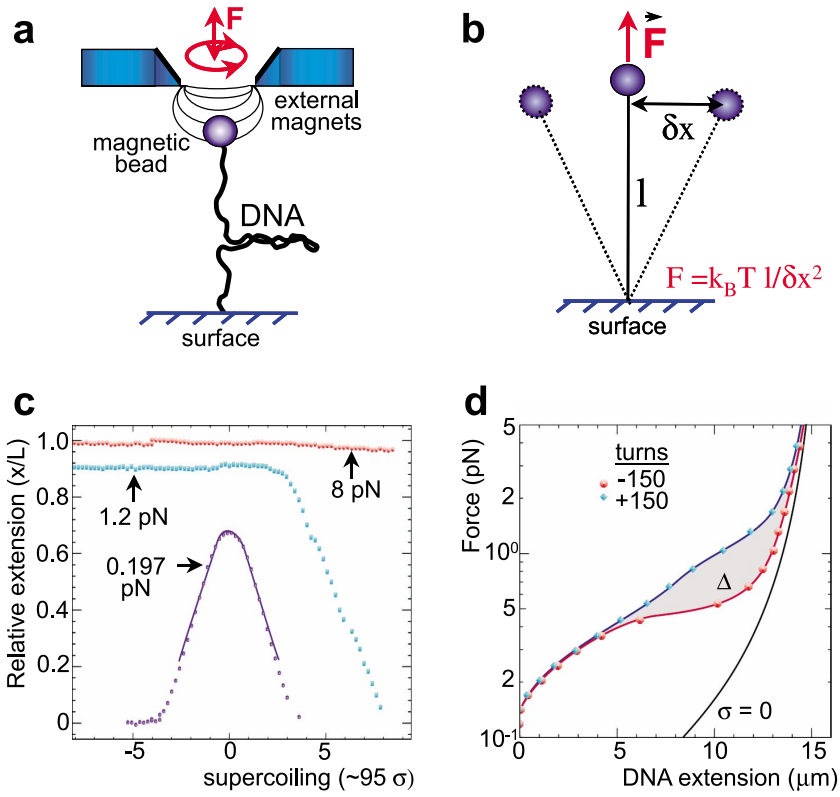
MT can also be used for following the rotational movement of a pair of molecules with respect to each other (in these experiments force is applied through one pole of the magnet only). If, in their biological interaction, two molecular partners rotate relative to one another, this rotation can be followed in real time by immobilizing one of the partners to the surface and attaching the other partner to the bead: the rotation of this second molecule with respect to the immobilized one can be followed by following the rotation of the bead. The rotation of the bead around its axis can be easily monitored by video-microscopy, if the bead is asymmetrical, or alternatively, asymmetrically decorated with smaller beads ("Mickey Mouse" ears). Such an approach has been used to visualize the rotation of a DNA template molecule with respect to the active site of an immobilized RNA polymerase molecule during transcription (Harada et al. 2001). The data were convincingly interpreted as showing that the transcribing polymerase closely follows the helical path of the DNA template, with one rotation observed for each turn of the double-helical DNA.

DNA manipulation

The mechanical properties of DNA have been studied upon stretching (e.g., Smith et al. 1992, 1996; Cluzel et al. 1996; Rief et al. 1999; Clausen-Schaumann et al. 2000*a*; reviewed in Bustamante et al. 2000*b*; Williams and Rouzina 2002; Zlatanova and Leuba 2002). The most prominent feature in the force-extension curves of naked DNA is the so-called "overstretching" transition observed at ~65 pN, where the molecule yields to the applied force by stretching to almost twice its contour length (it should be noted that the overstretching transition occurs at a much higher force, ~110 pN, in DNA molecules that do not contain nicks, Leger et al. 1999). The structural changes occurring during the overstretching transition are still under debate (see Williams and Rouzina 2002; Zlatanova and Leuba 2002). Single-molecule approaches have also been used to study DNA melting by mechanical unzipping of the double helix (Lee et al. 1994; Essevaz-Roulet et al. 1997; Noy et al. 1997; Bockelmann et al. 2002).

We will focus here on the behavior of the double helix upon twisting. If a single DNA molecule is topologically constrained between the glass surface and the magnetic bead, i.e., all four ends of the molecule are attached through multiple points to the respective surfaces so that DNA is prevented from swiveling about its anchoring points, then rotating the external magnets will pump-up superhelical tension

Fig. 1. (*a*) Principle of operation of magnetic tweezers. A single DNA molecule is tethered between a superparamagnetic bead and a surface. (*b*) Based on the equipartition theorem, force is determined by $F = k_B Tl/\delta x^2$, where l is the distance between the DNA-tethered bead and the surface, k_B is Boltzmann's constant, T is the temperature, and δx^2 is the Brownian fluctuations. (*c*) DNA supercoiling experimental data redrawn from Strick et al. (2000*a*; reprinted with permission from Elsevier). Relative extension of a DNA molecule versus the degree of supercoiling. At the low force of 0.197 pN, the relative extension of the DNA decreases symmetrically for either (+) or (–) supercoiling. At 1.2 pN, the relative extension decreases only during (+) supercoiling, and a force of 8 pN prevents changes in relative extension for either (+) or (–) supercoiling. (*d*) Force-extension curves for DNA with either 150 (–) (circles) or (+) (diamonds) turns. The difference in work of stretching the two kinds of DNA is indicated in the shaded region by Δ. Righthand curve is for DNA without supercoiling (σ = 0). The value of Δ can be used to estimate the elastic torsional persistence length of the molecule (see text).



in the molecule (Strick et al. 1996, 1998*a*, 1998*b*; Allemand et al. 1998). The value of superhelical density σ can be determined very precisely by counting the number of rotations of the magnets and knowing the original length of the tethered molecule. The force applied to the molecule is calculated using the equipartition theorem (Fig. 1*b*).

Force-extension curves were recorded at fixed values of superhelical density σ at constant forces, ranging from 6 fN to 20 pN. Extension versus σ curves revealed intriguing differences in the behavior of positively (+) vs. negatively (–) supercoiled molecules, and these differences were dependent on the degree of superhelicity (Fig. 1*c*). Below 0.4 pN, DNA responded in a symmetrical manner to (+) and (–) supercoiling, forming plectonemes, thus measurably reducing the extension of the tether. At intermediate forces (e.g., 1.2 pN), the extension of the (–)-supercoiled DNA was insensitive to changes in the molecule's linking number: the rotation of the external magnets was not accompanied by shortening of the tether. Instead, the force-driven increase in torsional stress was absorbed by local denaturation observed by the acquired ability of the stretched DNA to hybridize with a homologous single-stranded DNA probes (Strick et al. 1998*b*). As more and more (–) torsion was introduced at such intermediate forces, DNA progressively denatured, forming stable denaturation bubbles (nucleated, as expected, in AT-rich regions). Supercoils still formed for (+) coiling at these forces (Fig. 1*c*). Finally, in the high force regime (>3 pN), no plectoneme formation was observed for either (–)- or (+)-supercoiled DNA (Fig. 1*c*). The (+)-supercoiled DNA underwent a transition to a new phase called P-DNA (Pauling-DNA) (Allemand et al. 1998). Chemical reactivity experiments and numerical simulations suggest that this conformation is characterized by winding of the phosphate–sugar backbone inside the structure, with the bases exposed to the solution. A structure similar to P-DNA presumably exists in nature in the DNA of a specific virus, Pf1, where the unusual conformation is stabilized by viral coat proteins (Liu and Day 1994). These studies clearly and elegantly show that the sense of DNA supercoiling confers different structural properties to the molecules, with the differences becoming more pronounced under increasing load.

As detailed in Strick et al. (2000*a*), measuring the work done while stretching (–)- or (+)-supercoiled DNA of equal superhelical density allows an evaluation of the elastic tor-

sional persistence length of the molecule (Fig. 1*d*). The shaded area between the two curves represents the work difference Δ; plotting the square root of the work difference vs. the number of turns the molecule is over- or under-wound gives a straight line whose slope can be used for extracting the torsional constant. The estimate gave 86 ± 10 nm, in reasonable agreement with the current and very imprecise estimate of 75 ± 25 nm (the latter value is estimated only indirectly from the rapid motions of spectroscopic probes bound to DNA and from equilibrium topoisomer distributions, Hagerman 1988).

Understanding enzymes that work on DNA using MT

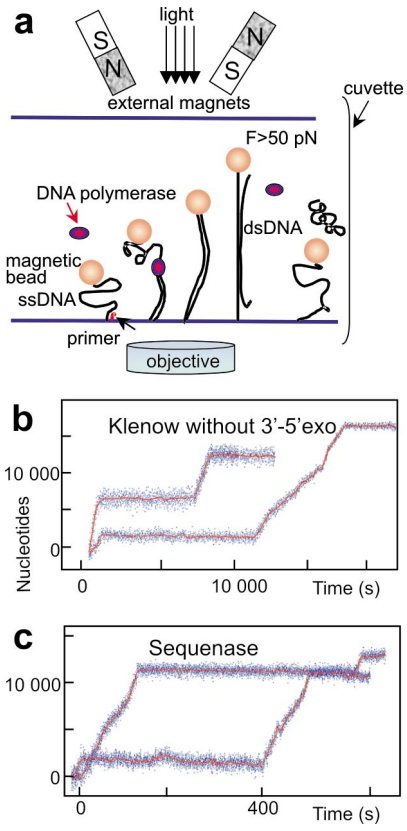
DNA polymerase

DNA polymerase action was studied in real time using the marked difference between the elastic properties of single- and double-stranded DNA (Maier et al. 2000). Two different enzymes were investigated: Sequenase, a mutant of T7 DNAP, a fast, processive enzyme that lacks exonuclease activity; and Klenow fragment of DNAP I from *Escherichia coli*, a slower, less processive polymerase. The single-strand template DNA was attached between the magnetic bead and the surface and a specific primer was attached near one end to allow the polymerase to start replication (Fig. 2*a*). The progression of the polymerase reaction was followed by the changes in the molecule extension or the rigidity of the tether. The primary data presented in Figs. 2*b* and 2*c* show that both studied polymerases pause at certain sites, without any sequence-specificity to the pausing. The force dependence of the replication rate was interpreted as suggesting that the biochemical steps limiting replication are coupled to movement. The decay of the replication rate with force was fit to an Arrhenius law, interpreted as an indication of multiple bases on the template strand involved in the rate-limiting enzymatic step: for Klenow four bases are involved, whereas for Sequenase this value is only 2. A value of 2 has been previously reported for wild-type T7 DNAP based on OT experiments (Wuite et al. 2000). Later, in an attempt to reconcile the observed dependence of the replication rate on template tension (Maier et al. 2000; Wuite et al. 2000) with the existing crystallographic data on DNA polymerases complexed with DNA and dNTP (e.g., Doublie et al. 1998), Goel et al. (2001) presented a model for the "tuning" of the replication rate by mechanical tension. This model considers the local properties of the template in the immediate vicinity of the enzyme active site, rather than those of the entire DNA chain, and concludes that only one template base is converted from single-stranded to double-stranded geometry at each polymerization cycle, consistent with the structural data.

Topoisomerases

Topoisomerases have attracted considerable attention since their discovery in the 1970s (Wang 1996; Champoux 2001, 2002). These enzymes are indispensable for the life of any cell, as they are charged with resolving the numerous and complex topological problems that the double-helical DNA molecule encounters during its transactions. The complexity

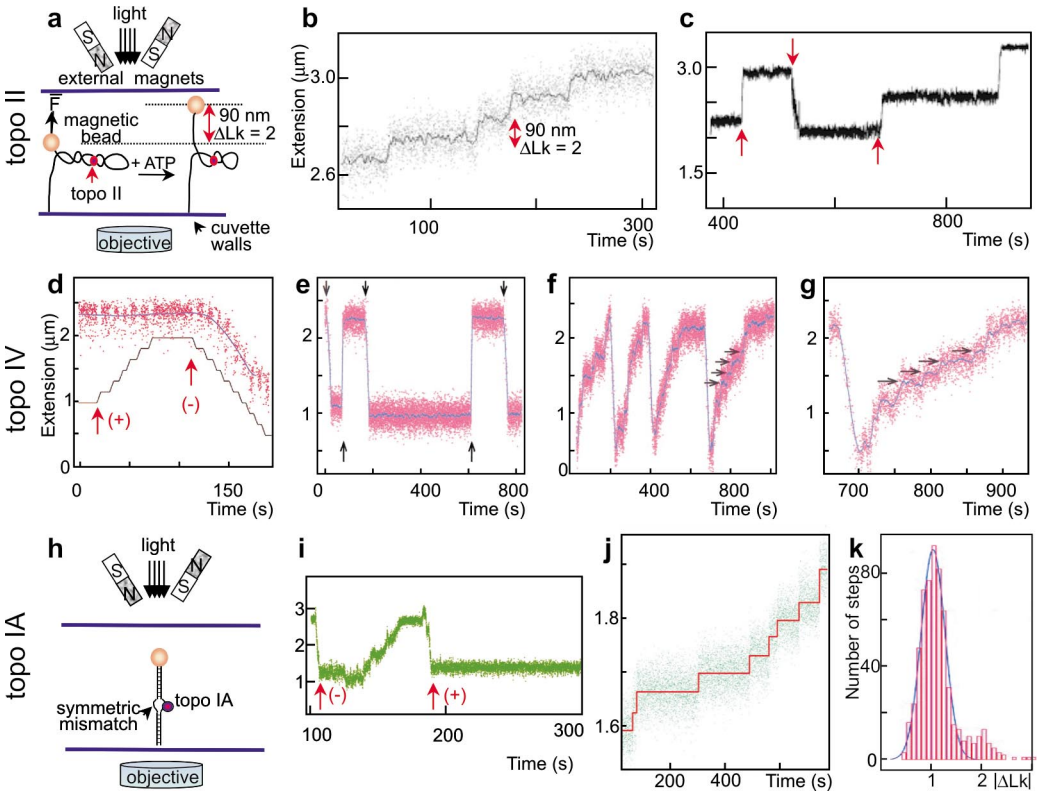
Fig. 2. Studying DNA polymerase by magnetic tweezers. (*a*) Schematic of experiment (see text). (*b*) Two example kinetic curves for replication by Klenow polymerase lacking 3′ to 5′ exonuclease activity, recorded at tension of 1 pN. (*c*) Two example kinetic curves for replication by Sequenase polymerase, recorded at 1 pN. Note the regions of long pauses (plateaus of various durations in the kinetic curves). Figure panels (*a–c*) redrawn from (Maier et al. 2000; copyright 2000 National Academy of Sciences U.S.A.).



and diversity of function has demanded the evolution of a wide array of different topoisomerases, each with its specific niche and specific mechanism of action.

The first topoisomerase to be studied by MT was topo II from *Drosophila melanogaster.* Topo II are ubiquitous ATP-dependent enzymes that untangle DNA and relax supercoils, changing the linking number of the molecule in steps of two. They do this by transporting one double-helical DNA segment through a transient double-strand break in a second DNA segment, and then resealing the break. The experimental approach used to follow topo II activity in real-time is illustrated in Fig. 3*a*; an individual time course for the relaxation of (+)-supercoiled DNA is presented in Fig. 3*b* (to slow down the enzyme turnover so that individual relaxation events can be observed as steps in the extension-time curve, the reaction was performed under suboptimal concentration of ATP, 10 μM). The size of the elongation steps was interpreted as resulting from removing two plectonemes during a single enzymatic cycle, exactly as expected on the basis of the biochemically determined properties of the enzyme. Finally, Fig. 3*c* shows as example time course at 300 μM ATP, where interruptions in relaxation (pauses) are readily

Fig. 3. (*a–c*), *Drosophila melanogaster* topo II. (*a*) Schematic of the experimental approach. (*b*) Topoisomerase II mediated relaxation of (+) supercoiled DNA at 0.7 pN. The 90 nm vertical steps correspond to relaxation of two plectonemes of supercoiled DNA. (*c*) Mechanical overwinding of DNA (down arrow) followed by topoisomerase II-mediated relaxation (up arrow) at 300 μm ATP. (*d–g*), *E. coli* topo IV. (*d*) (+) supercoils but not (–) supercoils are relaxed at low enzyme concentration. When the magnets were rotated counterclockwise (~1 min per step in lower black curve, each step being 5 turns of the magnet) to introduce (+) supercoils, the extension of the molecule did not change. However, when the magnets were rotated clockwise, (–) supercoils accumulated and the DNA extension decreased. (*e*) Relaxation of (+) supercoils by 10 ng/mL topo IV was monitored by the change in DNA extension. Down arrows indicate the start of mechanical overwind of the DNA to σ = +0.03 (30 turns), and up arrows indicate the initiation of relaxation. (*f*) Relaxation of (–) supercoils by topo IV. At time zero the DNA was (–) supercoiled to σ = –0.035, and relaxation by 200 ng/mL topo IV was followed by the change in DNA extension. Four cycles of (–) supercoiling followed by relaxation are shown. The horizontal arrows show the change in extension corresponding to ΔLk of +2, a single enzyme turnover. (*g*) Blow-up of the last relaxation event in (*f*). (*h–k*) *Escherichia coli* topo IA. (*h*) Schematic of the experimental approach. (*i*) Topo IA relaxes (–) supercoils (indicated by up arrow and (–)), but not (+) supercoils (indicated by up arrow and (+)). Substrate: fully duplex 11.5-kb DNA. (*j*) Topo IA relaxes (+)-supercoiled DNA containing a mismatch of 12 nucleotides. Steps in the data indicate single relaxation events. (*k*) Frequency distribution histogram of the step size observed in seven experiments, indicating a single step size of 1 ΔLk. (*a–c*) redrawn from Strick et al. (2000*b*) (with permission from Elsevier), (*d–g*) redrawn from Crisona et al. (2000), (*h–k*) redrawn from Dekker et al. (2002) (Copyright 2002 National Academy of Sciences, U.S.A.).

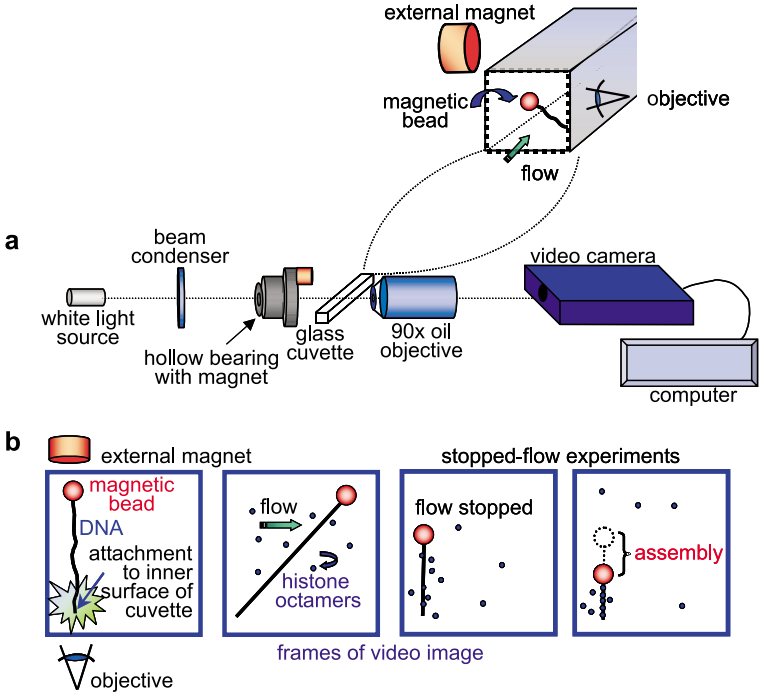


observed as lines parallel to the *x* axis at intermediate extensions of the DNA. Interestingly, raising the force led to a decrease in the velocity (number of enzymatic cycles per second) that suggested that the rate-limiting step of the reaction might be the closure of the cleaved DNA segment.

Equally fascinating was the report on the preferential relaxation of (+)-supercoiled DNA by *E. coli* topo IV (Crisona et al. 2000). The signature activity of topo IV in vitro is decatenation, but there is evidence that the enzyme can also remove the (+) supercoils generated during transcription in front of the polymerase (in vitro, the enzyme is capable of removing both (–) and (+) supercoils, but with high preference for the latter).

Indeed, the MT experiments convincingly confirmed the preference of topo IV for (+) supercoils. The experiment was performed at low enzyme concentration, starting with relaxed DNA. Counterclockwise rotation of the external magnets introduced (+) supercoiling of the DNA tether, but there was no change in the extension of the molecule: the enzyme relaxed the supercoils as fast as the magnets could add them, a Sisyphean effect, as termed by the authors by analogy with the endless and difficult labor of Sisyphus, the Greek mythology hero (Fig. 3*d*). If, at a certain point, the direction of rotation of the magnets was changed to clockwise, (–) supercoils quickly accumulated, with no evidence for relaxation. The strong preference for (+) supercoils can also be seen in the kinds of experiments presented in Figs. 3*e* and 3*f*; (+) supercoiling introduced by mechanical overwinding was quickly relaxed even at a low enzyme concentration (10 ng/mL, calculated rate 180 passages/min), whereas (–)

Fig. 4. (*a*) Schematic of the experimental approach used in our chromatin assembly experiments (Leuba et al. 2003; copyright 2003 National Academy of Sciences, U.S.A.). The box above is an enlargement of the cross-section of glass cuvette with the DNA-tethered superparamagnetic bead and the external permanent magnet. (*b*) Schematic of stopped-flow chromatin assembly experiments (see text).



supercoiling introduced by mechanical underwinding was relaxed much more slowly (calculated rate 8 passages/min), even at 200 ng enzyme/mL. Finally, Fig. 3*g* shows a blow-up of the last relaxation event in Fig. 3*f*, with a clear distinction of the unit change of extension, corresponding to a ΔLk of +2.

Finally, Dekker et al. (2002) reported an innovative study of the mechanism of action of two type IA topoisomerases from prokaryotic origin (*E. coli* and *Thermotoga maritima*). Type I topoisomerases introduce a nick in one strand of the DNA, transport the intact strand through the nick, and reseal it, thus changing the linking number of the molecule by one. The two families of topo I (type IA and type IB) differ in several aspects: polarity of attachment of the enzyme to the nicked strand of DNA, requirement for the presence of short single-stranded region in the substrate to allow binding, and the actual molecular mechanism of action. The experimental approach is illustrated in Fig. 1*h*.

The difficulty in studying type IA topoisomerases under load comes from the fact that the signature activity of this enzyme is the removal of (–), but not (+) supercoils. Indeed, this specificity is easily revealed under low loads (Fig. 2*i*, 0.69 pN). As already discussed above, if the load increases beyond a certain intermediate level, the (–)-supercoiled DNA starts absorbing the torsion by denaturation, not by plectoneme formation, thus compromising the option of following the process through changes in DNA extension. The authors found an ingenious way to overcome this difficulty: instead of using (–)-supercoiled DNA, they used (+)-supercoiled DNA, which contained a single-stranded bubble to provide the binding site for the enzyme. Previous work has shown that such a substrate, albeit being (+), is effectively relaxed by topo IA (Kirkegaard and Wang 1985). The necessity to work with (+)-supercoiled DNA required the engineering of special substrates containing either a symmetric short 12 bp mismatch region or an asymmetric 25 bp long bulge. The rate of relaxation of the symmetric bubble turned out to be force dependent, while that of the asymmetric bulge was not, the explanation being that with the mismatch, the region at which the enzyme binds is under tension, while in the bulge it is not (for further discussion of this intriguing point, see Dekker et al. 2002).

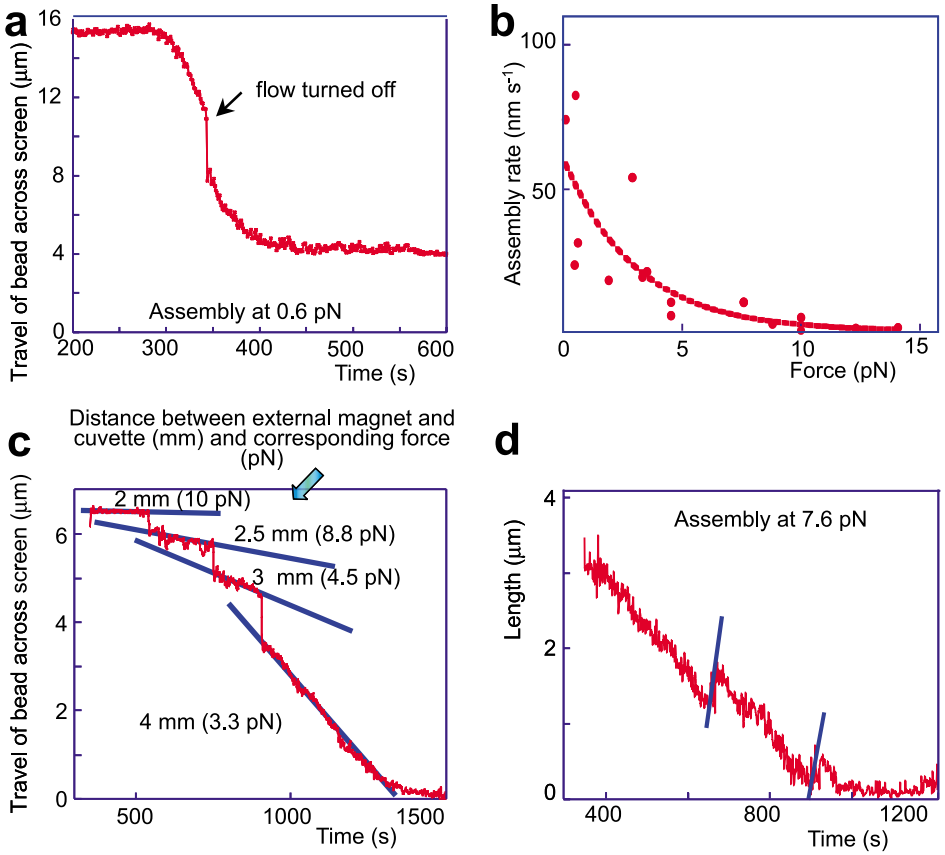
Now that appropriate substrate was available, the last hurdle to overcome was to slow down the enzymatic activity to a degree that would allow visualizing steps in the extension vs. time curve. This was achieved by using higher stretching forces and suboptimal Mg^{2+} concentration (Fig. 3*j*). The probability distribution of the step sizes (Fig. 3*k*) convincingly gave a linking number change of 1. This value confirmed the previously suggested enzyme-bridging mechanism of action of topo IA enzymes (see Fig. 1 in Dekker et al. 2002, and the accompanying commentary by Champoux 2002).

The studies of the mechanism of action of the various topoisomerases are an excellent illustration of the power of the MT single-molecule approach.

Chromatin fiber assembly under applied force

Our long term interest in chromatin structure and dynamics led us to investigate the dependence of chromatin assembly on the force applied to the DNA tether (Leuba et al. 2003). This is an important issue to study, since it may give us an insight of how fast, and under what conditions, nucleosomes form in the wake of passing polymerases in

Fig. 5. Example experimental data on chromatin assembly with magnetic tweezers (Leuba et al. 2003; reprinted with permission from National Academy of Sciences, U.S.A., copyright 2003). (*a*) Raw assembly curve recorded at 0.6 pN. (*b*) Data from 18 individual assembly experiments performed at different forces: each point represents the initial assembly rate at a specified force. The dotted curve is an exponential fit to the data. (*c*) A rheostat experiment, in which step-wise changes of the distance between the external magnet and the cuvette cause step-wise changes in the force applied to the magnetic bead. (*d*) An assembly curve recorded at 7.6 pN. The upward steps on the otherwise descending curve appear as a result of the dynamic equilibrium between nucleosome assembly and disassembly (for further details, see text, and Leuba et al. 2003).



vivo. At least some polymerases seem to displace, temporarily, the nucleosomes out of their way; thus, nucleosomes need to reform on the already transcribed portion of the DNA to restore chromatin function in DNA packaging and regulation of gene expression. The DNA molecule exiting the polymerase is still under tension, as the enzyme pulls on the DNA to thread it through its active center, and would thus also push the already reformed double helix following transcription, out of its territory. The dependence of nucleosome formation on the tension in substrate DNA molecules has been approached by alternative single-molecule techniques: flow (Ladoux et al. 2000) and optical trapping/flow (Bennink et al. 2001), with qualitatively similar results (for further discussion, see Zlatanova and Leuba 2003).

Figure 4*a* is a schematic of our instrumental set up, with a blow-up of the cross-section of the cuvette to help understand the way the experiments were done. Figure 4*b* illustrates the experimental approach. We first attached a single λ-DNA molecule between the inner wall of the cuvette and the magnetic bead. We then introduced the buffer solution containing preformed histone octamers and a recombinant nucleosome assembly factor (NAP-1); as soon as we saw initial chromatin formation (shortening of the distance between the wall and the bead), we stopped the flow and allowed assembly to take place against the magnetic force only. The initial assembly rate at each given force was measured from the linear portion of the respective assembly curve (an example curve is presented in Fig. 5*a*). Plotting the calculated initial assembly rate as a function of the tension applied to the DNA tether revealed a strong force dependence of the assembly rate on the force applied to the DNA tether (Fig. 5*b*).

The instrumental set-up allowed us to perform some more sophisticated experiments, in which we changed the applied force rheostatically, in the course of a single assembly experiment (see Fig. 5*c*). The data revealed a very interesting, and potentially important, characteristic feature of the assembly process: the assembly rate changed instantaneously upon changing the tension applied to the DNA tether. This may mean that in vivo the rate of chromatin reformation in the wake of the passing polymerase is dictated, to a large degree, by the DNA tension, which in turn is a function of the rate of transcription.

Finally, Fig. 5*d* illustrates another interesting characteristic of the assembly process. The monotonically decreasing assembly curve (change of the length of the DNA tether with time) is occasionally interrupted by upward-going stretches.

As detailed in Leuba et al. (2003), these abrupt changes in the direction of the assembly curve reflect the dynamic equilibrium between nucleosome assembly and disassembly. This is the first real-time demonstration of this phenomenon that has previously been observed in mononucleosome bulk experiments (Yager and van Holde 1984), and theoretically predicted (Widom 1999).

The next step in studying chromatin assembly under force will involve the use of topologically constrained DNA tethers attached at the four ends of the DNA double-helix to the bead and the surface. Such an attachment will preclude the swivel of the tether around the attachment points, and will thus mimic more closely the situation in vivo, where DNA is anchored to insoluble components of the nuclear matrix in a topologically constrained manner (e.g., Zlatanova and van Holde 1992).

Concluding remarks

The brief overview of the single-molecule work performed with MT reveals the power of the new methodology in allowing both tension and torsion to be applied to individual DNA molecules. The controlled introduction of superhelical tension into DNA molecules, easily achievable by this type of single-molecule instrumentation, has allowed exciting new data to be obtained. The properties of both positively and negatively supercoiled DNA molecules under load have been assessed, with rather unexpected and intriguing structural transitions being recognized. Detailed analysis of DNA polymerase action allowed the identification of the rate-limiting step in the reaction and the simultaneous involvement of multiple bases on the template strand in the polymerization process. The investigation of the mechanism of action of different topoisomerases was especially fruitful, albeit not always easy. Finally, the recent successful application of MT to the study of chromatin opens new venues for looking into important issues concerning chromatin structure, dynamics, and function. MT should be put to good use in the chromatin field.

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