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Recent developments in single-molecule DNA mechanics

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Over the past two decades, measurements on individual stretched and twisted DNA molecules have helped define the basic elastic properties of the double helix and enabled real-time functional assays of DNA-associated molecular machines. Recently, new magnetic tweezers approaches for simultaneously measuring freely fluctuating twist and extension have begun to shed light on the structural dynamics of large nucleoprotein complexes. Related technical advances have facilitated direct measurements of DNA torque, contributing to a better understanding of abrupt structural transitions in mechanically stressed DNA. The new measurements have also been exploited in studies that hint at a developing synergistic relationship between single-molecule manipulation and structural DNA nanotechnology.

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Introduction

DNA in cells is under constant mechanical stresses (Figure 1A): the duplex is untwisted during transcript initiation; stretched by recombination factors; tightly wrapped around histones [1]; successively bent, cleaved, and pushed through itself by topoisomerases; and forced into small spaces by packaging motors [2]. The elastic properties of the double helix influence the energetics of these local interactions as well as the global conformations of DNA in solution [3].

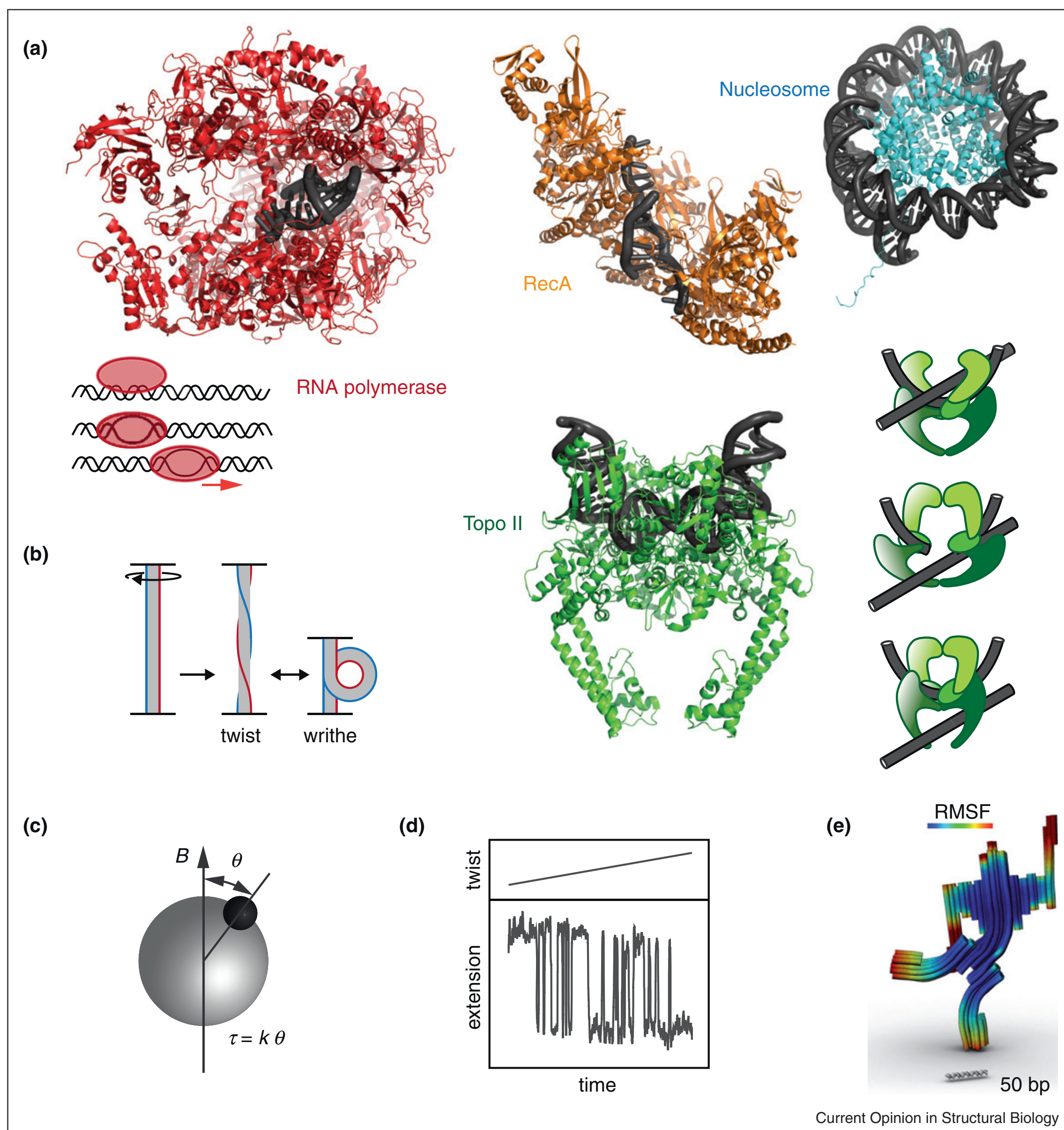
Torsion and tension can also be used to transmit information through the genome and exert sophisticated control over biological processes. In a recent striking example [4••], oscillating DNA supercoiling levels act as a global regulator of shifting transcriptional programs during the circadian rhythms of cyanobacteria: distinct promoters are simultaneously upregulated and downregulated by torsional changes, and inhibition of the supercoiling motor DNA gyrase is sufficient to induce a transcriptional response that mimics a change in the time of day.

Single-molecule manipulation enables direct measurements of DNA mechanics. Previous reviews have described a pattern in which fundamental studies of DNA physics have repeatedly paved the way for detailed investigations of enzyme mechanism [3]. Here, we will focus on a set of closely interconnected recent developments. New variants of magnetic tweezers assays have been used to follow sequences of structural changes in nucleoprotein complexes. A flurry of new methods have been introduced for making direct measurements of DNA torque. The rich behavior of torsionally strained DNA has continued to surprise researchers, motivating the development of theoretical models and investigations of sequence-dependent mechanics. Finally, an interesting relationship is emerging between single-molecule mechanics and structural DNA nanotechnology: mechanical properties of the double helix have been used to predict the equilibrium shapes of complex three-dimensional origami structures; magnetic tweezers have been used to test the mechanical response of DNA origami; and DNA nanotechnology promises to contribute molecular tools that enable better control and resolution in a range of single-molecule manipulation experiments.

Dissecting the structural dynamics of nucleoprotein complexes

The physical mechanisms of many cellular processes involve the formation and rearrangement of large complexes involving extensive DNA deformations. Crystal structures of these nucleoprotein complexes are static and often fragmentary, and cannot be unambiguously assigned to functional states. Although single-molecule manipulation does not provide atomically detailed structural information, it is uniquely suited to observe dynamic transitions in functional complexes. In 'DNA-centric' assays (in which the protein component is an unseen hand) at least two structural properties may in principle be measured for a nucleoprotein complex: changes in *DNA contraction* are caused by bending [5], stretching, or sequestering DNA contour length; while changes in *linking number* may be due to either trapped writhe (as in nucleosome wrapping), trapped twist deformations (such as DNA unwinding within the complex), or global linking number changes caused by topoisomerization [6].

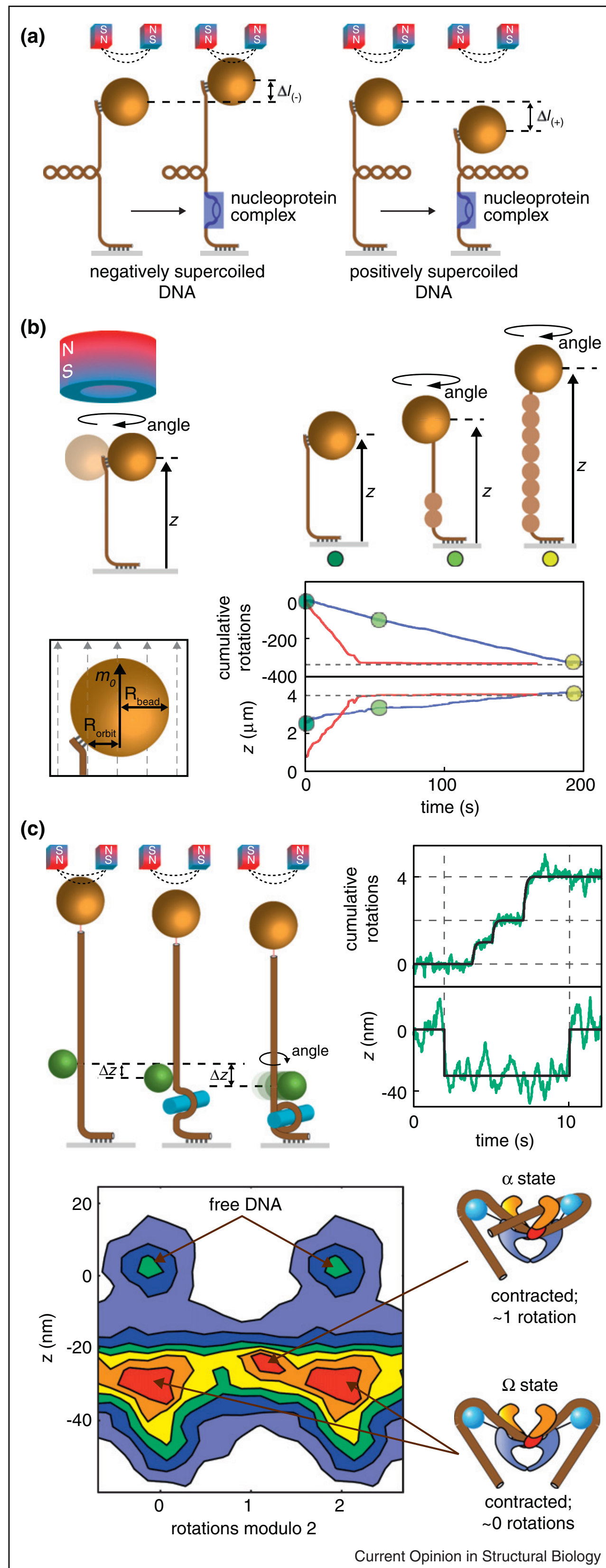
Figure 1



Overview. **(A)** Mechanical processes in DNA biology. RNA polymerase (red, PDB: 1I6H) unwinds DNA during transcript initiation; RecA (orange, PDB: 3CMT), nucleosomes (blue, PDB: 1AOI) distort their substrates. Type II topoisomerases (green, PDB: 2RGR) couple changes in DNA topology to ATP consumption, using a mechanism in which a DNA segment is passed through a transient protein-bridged gap in the duplex. **(B)** Twist (*Tw*) and writhe (*Wr*). In micromanipulation experiments, the effective linking number (*Lk*) of a DNA molecule can be controlled by rotation. Changes in *Lk* can partition into local *Tw* and global *Wr* deformations [54]. **(C)** New methods allow torque measurements in magnetic tweezers, using setups that confine beads to soft angular potentials [21•]. **(D)** DNA exhibits rich behavior under torque, such as abrupt buckling transitions [28]. **(E)** Single-molecule measurements of the DNA duplex have been used to parameterize predictions of equilibrium structures and fluctuations in DNA origami [41•,44]; heat map indicates predicted RMS fluctuations.

A notable early approach was exemplified by Strick and co-workers, who monitored structural dynamics during transcription, observing transitions from open promoter complex to initially transcribing complex to elongation complex to dissociation [7,8•]. Their method (Figure 2A) relies on what has become known as 'conventional' magnetic tweezers (MT), in which magnets apply a pulling force while imposing a fixed angle on the tethered bead. Rotating the magnets introduces supercoiling (overwinding or underwinding relative to relaxed B-form DNA), and can be used to generate a plectonemic substrate. Changes in the linking number of the nucleoprotein

Figure 2



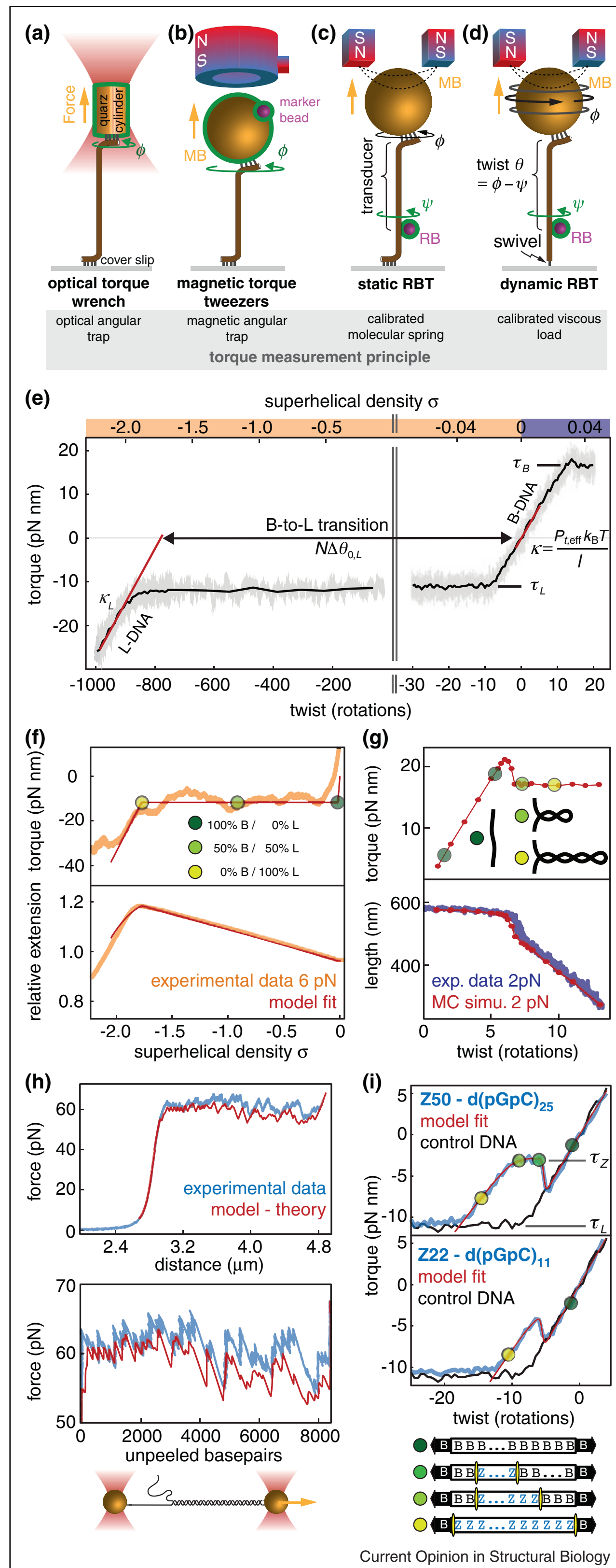
DNA-centric measurements of structural transitions in nucleoprotein complexes. **(A)** Approach based on conventional MT [7,8•]. Plectoneme formation provides a large signal reflecting linking number changes trapped in the nucleoprotein complex. Independent contributions to bead height from linking number and DNA contraction can be deconvoluted by repeating measurements on negatively and positively supercoiled DNA. **(B)** Freely orbiting magnetic tweezers (FOMT) assay [10]. A vertically oriented magnetic field allows free rotation of the bead about the DNA axis. In the presence of RecA under 6.5 pN (blue curve) or 1.5 pN (red curve) tension, the bead traces out a spiral as RecA unwinds and lengthens the duplex, reaching saturating values (dashed lines) of angle (linking number) and *z* expected for complete filament formation on the basis of crystallographic data. **(C)** Rotor bead tracking (RBT) assay. The DNA template is stretched using magnetic tweezers, and a fluorescent rotor bead (diameter ~300 nm) is imaged to obtain angle and *z* measurements reflecting interactions with protein (blue cylinder). Data are shown from a study of ATP-dependent dynamics in DNA gyrase [11••]. Changes in extension and stepwise directional introduction of supercoils can be observed during processive bursts of activity owing to individual enzymes. Structural intermediates visited by the enzyme can be mapped out on an angle-extension plane (2D histogram).

complex cause compensatory changes in the amount of writhe present in the plectonemic regions, yielding large changes in the *z* height of the magnetic bead. The power of this approach is its sensitivity: plectonemes act as potent amplifiers of small linking number changes; thus for example the unwinding of ~3 bp can be detected with a time resolution of ~1 s, and still smaller changes can be detected with longer integration times [7,8]. This sensitivity was critical to the success of the approach in confirming a 'DNA scrunching' mechanism that underlies abortive initiation. This mechanism is thought to generate an important on-pathway intermediate in promoter escape [8•,9], in which accumulated elastic energy is used to disengage the polymerase from the promoter.

A limitation of the Strick method is that structural parameters must be deconvoluted (Figure 2A): changes in DNA contraction and linking number cannot be directly distinguished from each other during real-time measurements. Recent studies (Figure 2B, C) employing freely orbiting magnetic tweezers [10] (FOMT) and enhanced rotor bead tracking [11••] (RBT) overcome this limitation and also circumvent the requirement for supercoiled substrates. Both FOMT and RBT allow real-time measurements of angle and extension using 3D tracking of a bead that is attached off-center to a stretched DNA molecule. Extension is measured from the focal depth *z*, while angle is measured by tracking *xy* motion constrained to a circular orbit about the DNA axis. FOMT relies on a magnetic bead for both structural readout and applying force, while RBT segregates these functions between a magnetic force handle and a fluorescent probe.

FOMT builds on previous studies that have used magnet configurations producing fields aligned parallel to the DNA axis, permitting approximately free rotation of the magnetic bead [12–14]. Notably, Kinosita and co-workers [12] used this configuration to directly confirm

Figure 3



Torque measurements and structural transitions in mechanically stressed DNA. **(A–D)** Methods for measuring torque in single stretched DNA molecules. Tension is applied using force handles (gold) that are often but not always identical with rotational probes (highlighted in green) used to measure torque. **(A)** The optical torque wrench employs a linearly polarized optical trap and nanofabricated birefrigent cylinders [19]. **(B)** In magnetic torque tweezers [21•] (MTT) the orientation of the magnetic bead (MB) is measured using marker beads, allowing accurate determination of angular deviations proportional to the applied torque (Figure 1B). **(C)** In static rotor bead tracking (RBT) the angular position of the rotor bead (RB) may be used to infer the torque based on calibration of the upper transducer DNA segment [26••], allowing precise investigation of the response of the lower DNA segment. **(D)** In dynamic RBT, twist introduced by rotating the magnets is relaxed by free rotation of the rotor bead, but a steady-state fixed twist can be maintained using a feedback algorithm [26••]. Torque is measured from the RB angular velocity after calibrating its rotational drag. **(E)** Torque-twist diagram of a 4.6 kb DNA, showing characteristic features including the post-buckling torque τ_B, critical torque for the B–L transition τ_L, and torsional spring constant κ. l, length of the DNA; $P_{t,eff}$, effective twist persistence length; $\Delta\theta_{0,L}$, extrapolated change in twist per basepair for the B–L transition; N, number of basepairs of DNA; κ_L, torsional spring constant for L-DNA. Data are from [26••], obtained using dynamic RBT. **(F)** Torque and extension for a complete B-to-L transition measured using an optical torque wrench, and global fit to an analytical model [34]. **(G)** Torque and extension at the plectonemic buckling transition. Extension data are shown together with simulated extension and simulated torque [30]. **(H)** Sequence dependent unpeeling of dsDNA under extension [39], shown together with an equilibrium model based on known basepair stabilities. **(I)** The torsional response of GC repeats [26••] is explained by cooperative B–Z transitions. In the depicted microstates, energetically costly junctions between B-form and Z-form are shown as yellow bars.

that the DNA template rotates relative to RNA polymerase during transcription. Important new contributions of FOMT include dispensing with marker beads, incorporating simultaneous extension measurements, and introducing an alignment procedure that reduces the residual horizontal component of the field to a negligible contribution. In a demonstration of a promising application area, FOMT was used to follow DNA stretching and untwisting during assembly of a RecA filament on dsDNA. The saturating levels of extension and unwinding match predictions based on crystal structures (Figure 2B).

Building on previous work that measured angle alone [15], Basu *et al.* [11••] used RBT measurements of angle and z to characterize ATP-dependent structural transitions in the mechanochemical cycle of the supercoiling motor DNA gyrase (Figure 2C). The key directionality-determining step in the gyrase motor mechanism is the formation of a chiral DNA wrap on a similar scale to the nucleosome. Unexpectedly, formation of this structural intermediate was found to be a multistep process gated by ATP binding: the nucleoprotein complex initially sequesters extensive DNA contour length without trapping supercoils, and then requires a major ATP-accelerated conformational change to generate the chiral wrap. It will be interesting to compare this stepwise mechanism with other ATP-dependent processes – such as nucleosome

remodeling [16] – in which extensively wrapped DNA:-protein complexes are formed, rearranged, and dissolved. The angle-z RBT assay, in which changes in trapped writhe and sequestered contour length can be observed at high spatiotemporal resolution under low tension, should be directly applicable to observing the structural dynamics of diverse nucleoprotein complexes ranging from nucleosomes to preinitiation complexes.

Measuring torque using magnetic tweezers

Torsion plays a key role in biological processes such as transcription and replication, and torque spectroscopy is a natural complement to widely used force spectroscopy methods [17] for dissecting the thermodynamic, structural, and kinetic properties of double-stranded nucleic acids. Direct torque measurements in optical tweezers, based on viscous drag RBT measurements [18] or angular trapping [19] (Figure 3A), have been reported for some time, but it is only recently that methods have been developed for measuring torque on stretched DNA molecules using magnetic tweezers, with advantages for low force measurements and ease of implementation on existing setups.

In conventional MT, superparamagnetic beads can be rotated because the preferred axis [20] of the bead aligns with a strong horizontal field. The torque on the bead could in principle be measured by observing the angular deviation of the bead from this alignment, but the torsional potential is too stiff [20,21•] for accurate measurements of the deviation to be practical. The challenges have thus been (i) to design magnet configurations that provide a soft angular confinement while still maintaining large axial forces for DNA stretching, and (ii) to develop accurate measurements of angular displacement. Several groups have now demonstrated solutions, based on either using cylindrical magnets (similar to FOMT) to generate steep gradients but nearly vertical fields with only a small horizontal component [21–23,24•], or using dynamic modulation of the horizontal field in an electromagnetic trap [25]. A representative method [21•] dubbed magnetic torque tweezers (MTT) is depicted in Figure 3B.

Torque resolution is limited by rotational drag. Noise in force measurements arises from Brownian fluctuations, and the integration time required to achieve a given Brownian-limited force resolution is proportional to the viscous drag of the probe [17]. As discussed [25], this consideration becomes more acute for torque measurements because the rotational drag scales with the *cube* of the probe radius. Small torque probes are thus desirable, but this must be balanced by the requirement to apply stretching forces, which also scale with the volume of the magnetic particle. To allow the application of moderate forces and to facilitate high-resolution angle measurements, Lipfert *et al.* employed 2.8 μm beads in their MTT measurements. Seidel and co-workers developed new magnet designs and new image tracking methods to extend MTT's applicability to 1 μm particles, improving the practicality of torque measurements. Oberstrass *et al.* employed methods that sidestep these compromises (Figure 3C, D) using RBT, in which tension is applied using conventional MT but torque is measured using a separate rotational probe [26••]. A recent study shows the possibility of extremely high-resolution torque measurements using angular trapping of a gold nanorod [27], but technical challenges including laser-induced heating would need to be overcome before this could be applied to DNA measurements.

Structural transitions in DNA under torque

Torque measurement techniques have contributed to recent detailed characterizations of torsionally strained DNA, which have rigorously challenged our understanding of the physical properties of the double helix. When DNA is overwound under moderate tension (Figure 3E,G), it first accumulates twist until a tension-dependent critical linking number is reached; the molecule then buckles to form plectonemic structures that absorb subsequent turns introduced into the molecule. It has only recently been observed that there is an abrupt transition in extension upon initial plectoneme formation in short stretched molecules [28]. Subsequent experiments using conventional MT [29,30], angular trapping [31], and RBT [26••] confirm the abrupt transition, which can now be understood in considerable quantitative detail [29–31,32•]. The formation of an end loop provides an energetic cost for plectoneme initiation, and suppresses configurations in which writhe is divided among multiple plectonemes [32•]. Thus for short DNA molecules, torque accumulates linearly while the extension of the DNA remains approximately constant, then the torque and the extension both drop abruptly as a single initial plectoneme is formed. Subsequently, the torque remains constant as further turns are added, extending the existing plectoneme (Figure 3G). A similar pattern of a torque overshoot followed by a plateau is seen in B–Z transitions (Figure 3I) observed in GC repeats [26••], reflecting similar underlying physics. In this case a free energy penalty at B/Z junctions [33] creates a nucleation penalty analogous to the plectonemic end loop; after a domain of Z-DNA is formed it can be extended readily at a characteristic coexistence torque.

The torsional responses of GC repeats were assayed as part of an effort to dissect sequence-specific responses to biologically relevant negative torques. B-DNA becomes unstable when underwound; this effect can be studied if moderate tensions are maintained to prevent buckling. Recent studies using optical angular tracking [34] and RBT [26••] agree on the properties of the underwound 'L-DNA' phase that forms in random sequences (Figure 3E, F). L-DNA has substantial left-handed helicity [35,3], and is now seen to be torsionally stiff in

comparison to expectations for strand-separated DNA. It may represent a mixed phase due to multiple competing structural transitions with different sequence propensities – including strand separation and Z-DNA formation [36,37] – that are close to each other in energy.

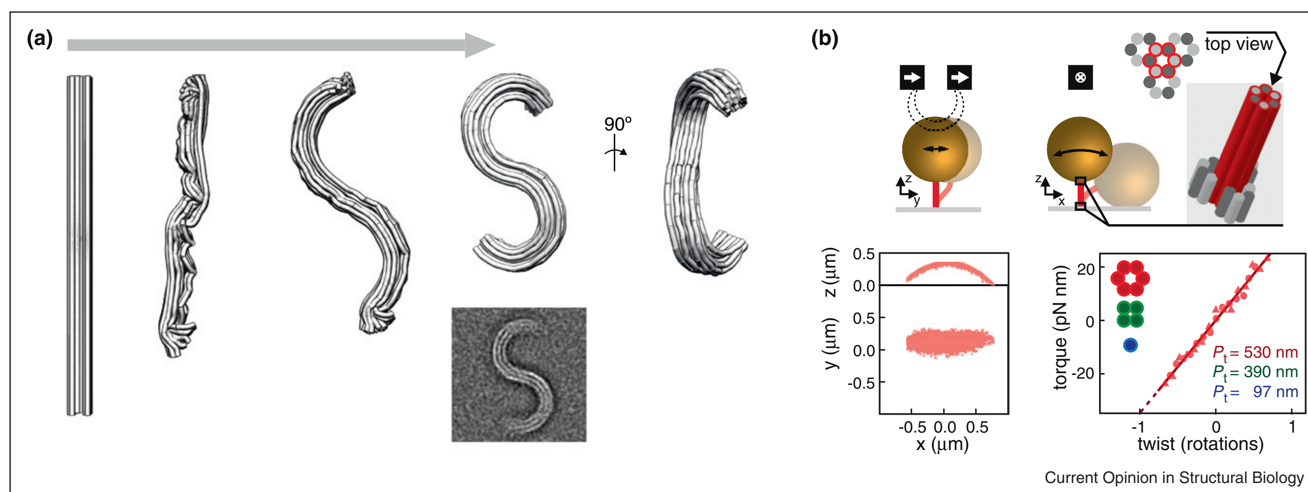
Torque spectroscopy of specific sequences has only just become practical, and more studies of defined sequences will be needed to build predictive models for the full structural landscape of underwound DNA. Meanwhile, in the more mature field of force spectroscopy, nearest-neighbor basepair free energy models have been used and improved in high-resolution DNA unzipping [38] and unpeeling [39] (Figure 3H) studies. The latter measurements were made during high-force overstretching, which does not obligatorily require unpeeling [18,40] but is associated with unpeeling under some conditions.

Mechanics for DNA nanotechnology (and vice versa)

Single-molecule mechanics have begun to play a role in the recent explosion of capabilities and applications in structural DNA nanotechnology. In scaffolded DNA origami [41•], the folding of a long scaffold strand is programmed by designing many short 'staple strands' that hybridize to bring together distant parts of the scaffold sequence, ultimately generating complex shapes composed of DNA helices connected by crossover motifs. Many 3D origami structures have now been designed on a hexagonal 'honeycomb' lattice [42], relying on knowledge of the geometry of B-form DNA: any two adjacent helices are connected by crossovers spaced once every 21 bp (2 helical repeats), in an overall pattern in which each helix is connected by crossovers spaced every 7 bp to one of three adjacent helices in the lattice. Within this context, insertions and deletions of basepairs between crossovers can force deviations from B-form DNA [43]. For example, if deletions are made uniformly across the cross-section of a multihelix beam, then maintaining the regularized lattice would require overwinding the helices. Some of this local torsional strain can be relaxed if the beam takes on a compensatory global superhelical twist. Similarly, graded insertions and deletions across a beam can be used to generate programmed bends in multihelix structures. Researchers immediately recognized that accurate design of curved and twisted structures could require accounting for the mechanical properties of DNA [43]: the shapes of equilibrium structures are influenced by tradeoffs between bending, stretching, and twisting.

Finite element models [41•,44] have now been used to aid the design of 3D origami (Figure 4A), using parameters (twisting, bending, and stretching moduli) derived from single-molecule mechanics. Although many factors (such as electrostatic repulsion) are ignored, the models succeed in predicting the superhelical pitch of the twisted beam designs [41•] and also the out-of-plane twisting and bending of planar origami tiles [45]. The models have been extended to include approximations of the entropic elasticity of single stranded DNA (used in tensegrity structures [46]) and of the mechanical response of nicks [44]. As these methods mature, they may be

Figure 4



Mechanics and DNA nanotechnology. **(A)** Snapshots of DNA origami 'S'-structures during deformation and relaxation in finite element calculations [44] parameterized using single-molecule measurements. In-plane features are reproduced in an averaged TEM image; additional out-of-plane deformations are predictions awaiting confirmation. **(B)** Characterization of DNA origami structures using magnetic tweezers. Oriented rigid attachment of multihelix bundles is achieved using wide plinth structures with several attachment points. The MB position thermally samples an arc in the *xz* plane, as expected for oriented attachment of a rigid beam. Torsional persistence lengths of the origami structures can be extracted from slopes in torque-twist plots (right lower panel contains data for the six-helix bundle and summarizes results) and show moderate stiffening in comparison to a single duplex [24].

useful for validating and refining mechanical models of DNA.

It is also desirable to predict the flexibility of nanostructures; the finite element methods have been used to predict flexible regions (Figure 1E), and the mechanical response of DNA origami beams has now been characterized using magnetic tweezers [24•], including direct torque measurements (Figure 4B). The measurements rely on specialized nanostructures to produce oriented anchors, a technique that may be generally useful in single-molecule manipulation. The data are consistent with very high bending rigidities for multihelix bundles (close to what would be expected from simply scaling B-DNA stiffness by the increase in the area moment of inertia), and moderately higher twist rigidities than an individual helix, which may be approximately explained using simple models when crossovers are treated as discrete linkages [24•].

Conclusion

The study of DNA mechanics continues to benefit from single-molecule technology development. Recent key advances involve the development of new magnetic tweezers modalities. For example, Dekker and co-workers have demonstrated a versatile approach in which the same instrument can be used for manipulating DNA with the twist either fixed (MT), confined to a torsional potential for torque measurement [21•] (MTT), or free to fluctuate (FOMT) [10]; and RBT has been extended to make torque measurement [26••] easier and more versatile than previous incarnations [18], and to allow simultaneous high-resolution low-force measurements of angle and extension [11••]. In the coming years, DNA nanotechnology may also produce new tools for manipulation and measurement of nucleic acids and proteins. DNA duplex handles have already become ubiquitous features of single-molecule measurements – even those involving manipulation of proteins [47] for unfolding [48] or cytoskeletal motor [49] studies – because of their ease of synthesis and well-defined mechanical properties. This review includes an example (static RBT, Figure 3C) of employing DNA as a calibrated molecular spring [26••]. 3D origami can improve control by allowing oriented attachment [24•] and can also provide a large range of stiffnesses for handles and calibrated springs, including stiff transducers that may be useful for pushing the limits of Brownian-limited noise in single-molecule measurements. Ultimately, even actuators constructed using dynamic DNA nanotechnology designs [50] may be used in manipulation experiments.

Through new measurements and theoretical studies, researchers are developing a detailed mechanical picture of idealized DNA under tension and torque [32•] but have only scratched the surface of sequence-dependent properties of the double helix critical to biology. Local inhomogeneities can have strong effects on processes described in this review, such as localizing and pinning [29] plectoneme formation; our understanding of multiple competing structural transitions is insufficient to predict the response of arbitrary sequences to torque; and we lack satisfactory physical models for biological responses to stresses such as the sequence-dependent response of promoters to changes in supercoiling [4••,51].

Much of DNA biology involves large nucleoprotein complexes in which DNA is bent, wrapped, stretched, or unwound – but the dynamics of these processes have only begun to be described, and direct dynamic measurements can produce surprises even in systems that have been subject to decades of biochemical and structural investigation [11••]. The evolution of methods such as FOMT and RBT will help dissect these processes, particularly if the DNA-centric twist and extension measurements can be complemented with measurements of internal degrees of freedom based on fluorescence [37,52,53]. Discoveries based on single-molecule DNA manipulation will accelerate in the coming years as methods mature and become more widely used by DNA researchers outside of specialist laboratories.

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