These unusually mobile particles were first predicted in 1910 by Lindemann, who conjectured that the sample would melt once atoms in a crystal could move more than some fraction of the crystal-lattice spacing (see the figure, panel C) (*7*, *8*). In the colloidal experiment, melting occurred when colloidal particles moved more than 20% of the lattice spacing, similar to what has been seen in simulations (*9*). In particular, the sample had to nucleate a small pocket of highly mobile particles, and if this pocket exceeded a critical size, this region continued to grow until the sample entirely melted. This critical size is limited by surface tension between the liquid and the crystal; Wang *et al*. measured a surface tension similar to what previous simulations of spherical particles have found. The colloidal experiment also revealed something new: If the sample was sufficiently superheated, multiple small liquid regions formed and could coalesce. Upon coalescence, surface tension caused the merged regions to adopt a spherical shape quickly. For even more extreme superheating, the colloidal crystal rapidly melted everywhere simultaneously.

Notably, what was not seen in the colloidal experiments were any defects such as dislocations, vacancies, and interstitials. Dislocations are irregularities in the crystal structure (see the figure, panel D), and vacancies are lattice sites where a particle leaves its normal position and becomes an interstitial particle, located between other particles, deforming the crystal. These defects could be caused by the additional energy added to melt the crystal, and defects have been seen as important precursors to melting in simulations (*10*, *11*). However, none of these defects were spotted in observations of dozens of melting colloidal samples. This absence is unlikely to be a limitation of colloids as a model system, because dislocations have been seen experimentally before in colloidal crystals (*12*).

Given that the experiments report phenomena similar to some simulations (*6*, *9*) and different from others (*10*, *11*), a natural concern is how to reconcile the discrepancies. Wang *et al*. compare their experiments to simulations of perfectly hard spheres (which only repel each other when they come in contact), which is a natural comparison for colloidal particles (*2*). However, there is very likely some softness in the interaction between the colloidal particles used by Wang *et al*., which allows them to expand or contract dramatically with slight changes in temperature. Also, the different simulations used varying particle interactions and heating protocols.

The simplest interpretation is that there are probably several ways that a crystal can melt from the inside out, and which behaviors are seen will depend on which crystal is melted. In this case, the most striking observations from the colloidal experiments are the good agreement with known behaviors of hard spheres (such as their crystal-liquid surface tension) and the merging of liquid regions when more strongly superheated. Given recent advances in tuning colloidal particle shapes and interactions (*13*–*15*), future colloidal experiments should be able to make even stronger connections with simulations.

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BIOCHEMISTRY

A DNA Twist Diffuses and Hops

Single-molecule techniques reveal short- and long-range dynamics of supercoiled DNA.

Maxim Y. Sheinin[1] and Michelle D. Wang[1,2]

Perhaps the reader can remember the good old days of wired phones, their cords so prone to the absent-minded twisting that eventually produced a multitude of small coiled coils. Wired phones are a thing of the past, but their cords still serve as inspiration to those interested in the coiling process of DNA. The striking beauty of DNA coiling (aptly named supercoiling) was first illustrated when Vinograd *et al*. (*1*) discovered multiple intertwined loops in their electron microscope images of a circular DNA from the polyoma virus. These loops, also called plectonemes, can play an important role in gene regulation by bringing together distant DNA elements, such as enhancers and promoters (*2*). On page 94 of this issue, van Loenhout *et al*. (*3*) use single-molecule techniques to uncover the rich dynamics of plectoneme formation and movement.

Electron microscope images of plectonemes have long captured scientists' imagination, but they provide only static snapshots of these DNA structures. Dynamic torsional manipulation of DNA was first demonstrated by Strick *et al*. in the mid-1990s using magnetic tweezers (*4*). In the experiment, a DNA molecule was torsionally constrained via multiple tags between a glass slide and a superparamagnetic bead. A pair of permanent magnets pulled the bead vertically toward the magnets until the magnetic force was balanced by the restoring elastic force from the DNA. DNA was then supercoiled through the rotation of the bead. Plectoneme growth manifested itself as a steady decrease in the DNA length as the bead was turned to add twist to the DNA.

Subsequent experiments using an angular optical trap showed that the initial plectoneme formation is abrupt, as revealed by a sudden extension drop followed by a torque plateau (*5*). However, many questions remain. How many plectonemes coexist on a single DNA molecule? Do plectonemes remain at the same locations or move about dynamically? How and where do they nucleate, grow, shrink, and disappear?

To visually locate plectonemes along a 21–kilobase pair (kbp) DNA molecule sparsely labeled with fluorophores, van Loenhout *et al*. first supercoiled the DNA with

[1]Department of Physics, Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, NY 14853, USA. [2]Howard Hughes Medical Institute, Cornell University, Ithaca, NY 14853, USA. E-mail: mwang@physics.cornell.edu

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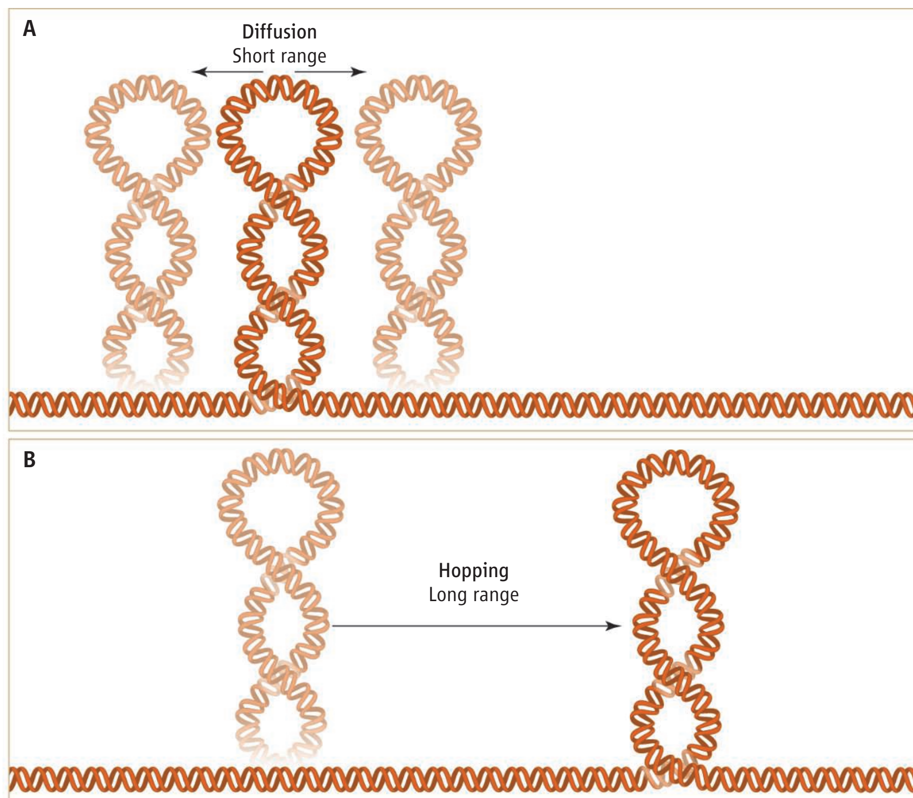

Dynamics of DNA plectonemes. Plectonemes can undergo short-range diffusive motion along DNA (**A**). Sequence preferences—rather than hydrodynamic drag on a plectoneme, which is small—slow down the rate of such excursions. Plectonemes can also display hopping behavior (**B**), disappearing from one location and reappearing thousands of base pairs away within tens of milliseconds.

a pair of permanent magnets, as described above, and then used a side magnet to pull the supercoiled DNA tether sideways. The DNA molecule was visualized in this configuration using fluorescence. In this supercoiled DNA, bright fluorescent spots were detected. These spots reflected high local DNA density, indicative of the existence of plectonemes.

Under certain conditions, the researchers observed multiple plectonemes along the DNA. Their number varied from a single plectoneme at high force and salt concentration (3.2 pN, 300 mM NaCl) to about three at low force and salt concentration (0.4 pN, 20 mM NaCl). The authors attribute this trend to an interplay between the entropic gain provided by multiple plectoneme domains and the enthalpic cost due to the formation of initial plectonemic loops. The experimental results are in good qualitative agreement with two recent theoretical models (*6*, *7*), underscoring the substantial progress that has been made in the quantitative understanding of DNA mechanics.

Plectoneme visualization was only the first step: Several seconds of imaging time permitted the authors to observe the long-coveted plectoneme dynamics. They found that each plectoneme diffused along the DNA. However, the diffusion constant of an average plectoneme was much smaller than expected from a simple hydrodynamic model. This discrepancy is explained by the observation that the plectonemes were not evenly distributed along the DNA, but instead preferentially localized near certain positions. This suggests that diffusion takes place on a rugged energy landscape, modulated by the intrinsic curvature and bendability of the underlying DNA sequence. The authors found that roughness on the order of 1 to 2 $k_B T$ (where k_B is Boltzmann's constant and T is temperature) was sufficient to explain their diffusion data. This energy landscape roughness is of the same order of magnitude as has been estimated for the analogous energy barrier of plectoneme formation in DNA with intrinsic bends (*8*).

However, plectoneme dynamics turns out not to be limited to diffusion. Van Loenhout *et al*. report that plectonemes can hop, suddenly disappearing and rapidly reappearing thousands of base pairs away from their original locations. This unusual behavior appears to be one of the defining features of plectoneme dynamics, given that the mean lifetime of a plectoneme under most experimental conditions was less than 1 s. Hopping could transplant the plectoneme several thousand base pairs within tens of milliseconds, whereas diffusional motion during that time was limited to a few hundred base pairs.

The visualization of plectonemes is a substantial experimental achievement. However, questions remain for further research. For example, van Loenhout *et al*. could detect plectonemes with a minimum size of ~0.5 kb, which is larger than the size of an initial plectonemic loop. Recent theoretical work (*6*) suggests that single loops can coexist with plectonemes; a more sensitive detection method is needed to test this hypothesis.

It would also be of interest to further investigate the hopping process. Van Loenhout *et al*. focus on hopping as formation of a new plectoneme concurrent with the disappearance of an existing one, but their data also hint at the redistribution of length among existing plectonemes. Further work will be required to determine if hopping is indeed a special case of length exchange among plectonemes.

The findings of van Loenhout *et al*. have important implications for processes that take place over DNA. The observation of preferential plectoneme localization suggests new ways in which DNA sequence can regulate genetic transactions. Indeed, certain DNA sequences may be designed to "pin down" plectonemes and thus bring neighboring regulatory DNA elements into close proximity. More research is required to identify the sequences that can localize plectonemes and analyze their distribution genome-wide. Plectoneme hopping, a dramatic long-range rearrangement of the DNA conformation on a millisecond time scale, could permit fast searching during DNA recombination or enhancer-activated gene expression. The next challenge will be to identify instances of plectoneme hopping in vivo and relate them to specific biological functions.

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