1478-Pos  Board B325  
Direct Observation of DNA Untangling Magic by a Type-II DNA Topoisomerase  
Katsunori Yogo, Taisaku Ogawa, Saki Obata, Gen Nakajima, Junpei Suzuki, Kazuhiko Kinoshita, Jr.  
Waseda University, Tokyo, Japan.  
Type-II DNA topoisomerases (topoII) are ubiquitous enzymes that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and untangle the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell death. TopoII untangles DNA catenanes in an ATP-dependent manner, by catalyzing the trans-catenation of DNA, which is a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience, by solving a knot of rope without touching the knot. Here, we show movies of this unlinking magic taken in real time under an optical microscope.

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Real-time Observation of Positive Supercoiling by Reverse Gyrase  
Taisaku Ogawa.  
Waseda University, Tokyo, Japan.  
The degree of DNA supercoiling in living cells is an important factor that affects diverse processes such as DNA replication, transcription, and recombination. Supercoils are under control of DNA topoisomerases which either increase or decrease the linking number (Lk) of DNA. Type I topoisomerases cut one of the two strands of DNA, rotate and reseal the nicked strand, whereas type II cuts both strands to pass another DNA segment and then reseals. Although many topoisomerases only relax DNA supercoils, DNA gyrase and reverse gyrase actively introduce negative and positive supercoils, respectively, into a substrate DNA. The reverse gyrase, found in thermophilic archaea, is unusual because positive supercoils are normally harmful. Thermophiles are thought to exploit the reverse gyrase to overwind DNA to prevent denaturation at high temperatures. Bulk studies have shown that the reverse gyrase is an ATP-dependent type I topoisomerase active only at temperatures above 50°C. Here we show the action of reverse gyrase from Sulfolobus in real time, tracking the rotation of DNA under a temperature-controlled microscope (50–70°C).

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Effect of Single-Strand Break on Holliday Junction Migration Dynamics: A Single-Molecule Fluorescence Study  
Mikhail A. Karymov, Dmytro Palets, Yuri L. Lyubchenko.  
University of Nebraska Medical Center, Omaha, NE, USA.  
Single-strand breaks (SSBs) are the most common DNA lesions in living cells. SSBs occur three orders of magnitude more frequently than double-strand breaks. Unrepaired SSBs lead to blockage or collapse of DNA replication forks, possibly causing formation of double-strand breaks. Holliday junctions (HJ) play a central role in various DNA functions including repair of lesions, replication, homologous and site-specific recombination. Branch migration, either spontaneous or protein-mediated, is among widely employed mechanisms in these functions. Therefore, elucidation of the SSB effect on spontaneous branch migration of HJs is a problem of great importance. To accomplish this task, we employed single-molecule FRET approach developed before, allowing us to follow spontaneous branch migration of one HJ at a single base pair level in real time. One SSB was incorporated in the middle of the homologous region of mobile HJ with the donor and acceptor dyes placed on its opposite arms. The data showed that branch migration does not stop at these lesions or reflects from them. Our previous results showed that branch migration is a step-wise process and one step can cover entire homology region. The analysis of the time trajectories showed that such specific sites in the genome, based on the sequence-dependent unzipping force of the underlying naked DNA sequence. We will present proof-of-principle results demonstrating the ability to match experimental data for pBR322 unzipping to the correct pBR322 sequence hidden in a library of approximately 3,000 yeast genome sequences arising from the known localizations of Xhol recognition sites. We do so via an algorithm that scores the experimental data against simulated unzipping forces from a quasi-equilibrium model (Bockelmann, Essevaz-Roulet, & Heslot, 1997). Our next step is to perform SDM on yeast genomic DNA fragments produced by ligation of Xhol-digested DNA to unzipping constructs. Enhancements of the matching algorithm, data processing, and unzipping simulation will be discussed, along with studies of the robustness of the SDM method as a function of number of sites in genome and other parameters. In addition to the impact on our goal of single-molecule mapping of chromatin from living cells, SDM may have important applications in other areas of genomics, including high-throughput structural DNA mapping and genome-wide mapping of sequence-specific DNA binding proteins.