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Dynamics of DNA Supercoil Relaxation by Type II Topoisomerases
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Dynamics of DNA supercoil relaxation by type II topoisomerases
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Type II topoisomerases are some of the main targets of anti-cancer drugs, since they catalyze DNA decatenation and unwinding which is crucial for cell division. A recent crystal structure shows that, during the catalytic cycle, a yeast type II topoisomerase can bend a 34 base pair DNA segment by up to 150 degrees. Bacterial gyrase, another type II topoisomerase, can wrap an approximately 100 bp DNA segment into a tight 180 degree turn. By substituting diaminopurine (DAP) deoxyribonucleotides for dATP in PCR reactions, completely triply hydrogen-bonded DNA fragments have been produced and found to be stiffer than normal DNA. These and normal DNA fragments were used as substrates for observations of topoisomerase II-mediated relaxation of plectonemes introduced in single molecules using magnetic tweezers. Observations at several ATP concentrations revealed bursts of stepwise events separated by pauses. Michaelis-Menten fitting of the data for both recombinant human topoisomerase II alpha and *E. coli* gyrase showed that Vmax and Km both decrease in DAP-substituted with respect to normal DNA. However, while the characteristic pause interval increased for human topoisomerase II alpha operating on DAP-substituted with respect to normal DNA, it was unchanged for *E. coli* gyrase. These dynamic measurements not only support the hypothesis that the strand passage reaction involves DNA bending but also suggest that DNA bending and subsequent steps in the catalytic cycle, perhaps involving ATP hydrolysis, are more efficiently coupled in gyrase than in human topoisomerase II alpha.

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Complex Kinetics of Apobec3g Interaction with Single-Stranded Nucleic Acids
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Human APOBEC3G (A3G) is a host cell cytidine deaminase capable of restricting replication of retroviruses by deaminating single-stranded (ss) viral DNA, and also by directly inhibiting RT-catalyzed polymerization reactions. We have studied the kinetics of A3G interaction with model 10-, 20-, 40- and 60-nt ssDNAs as well as with long polymeric DNA by three independent approaches: (i) surface plasmon resonance (SPR) analysis, (ii) fluorescence polarization spectroscopy (FP), and (iii) single-molecule (SM) DNA stretching. Our results suggest the following: (1) Binding kinetics of A3G protein to all ssDNA oligonucleotides are multi-step with the faster, weaker binding gradually converting to more stable binding with much slower on/off rates; (2) The typical A3G-ssDNA on/off times measured in our experiments range between ~10 and 10,000 sec, and the corresponding K_d values range between 0.1 and 10 μM; (3) Higher concentrations of A3G, longer A3G/ssDNA incubation times, as well as ssDNA length were found to correlate with slower ssDNA on/off kinetics, suggesting that A3G/A3G interactions in the bound state are responsible for slow protein kinetics.Taken together, these observations suggest that multimerization of A3G on ssDNA leads to a gradual decrease in its on/off kinetics, which in turn enhances this protein's ability to stall reverse transcription via formation of a ''roadblock'' for reverse transcriptase.

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Single-Molecule Studies of the Effects of Small Compounds on the Activity of Translation Initiation Factor eIF4A
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The translation initiation factor eIF4A is the prototypical DEAD-box RNA helicase as a subunit of eIF4F. It facilitates the binding and scanning of the ribosome via unwinding secondary structures in the 5' UTR of mRNAs during translation initiation. Here single-molecule Fluorescence Resonance Energy Transfer (sm-FRET) assay for both structured and unstructured substrates is performed and it shows that eIF4A unwinds the substrates in a discrete step manner although it is a highly nonprocessive motor. It also shows that eIF4A is a bidirectional helicase and the step size is about 6 base pair. Pateamine A and silvestrol are small-molecule modulators of eIF4A activity and they are identified as potent inhibitors of translation. It was demonstrated that Pateamine A and silvestrol act as a chemical inducer of dimerization and promote the interaction between eIF4A and RNA, however the molecular mechanisms by which they regulate eIF4A activities still remain elusive. Our sm-FRET assay shows that both pateamine A and silvestrol stimulate the helicase activity of eIF4A. Understanding how the processivity of eIF4A is influenced by these molecules will help regulating the translation at the initiation step.

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Single Molecule Studies Reveal Sliding Dynamics of Hcv NS5B in Complex with RNA
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NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis de novo. However, the detailed underlying mechanism remains elusive. It is unclear how the enzyme locates the 3′-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3′-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single -donor (Cy3)/acceptor (Cy5) fluorophore labeled- RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise binding of NS5B to the template. Taken together, our single molecule studies provide direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block de novo initiation of RNA synthesis.

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Protein Sliding and Hopping Kinetics on DNA
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DNA-binding proteins' alternating diffusion kinetics on and off nonspecific DNA (also called sliding and hopping respectively) are important for quantifying their target binding mechanisms. Using Monte-Carlo simulations, we deconvolved the sliding and hopping kinetics of GFP-LacI proteins on elongated DNA from their experimentally observed seconds-long diffusion trajectories. Our simulation results suggest the following: (1) In each diffusion trajectory, a protein makes on average hundreds of alternating slides and hops with a mean sliding time of several tens of ms; (2) sliding dominates the root mean square displacement of fast diffusion trajectories, whereas hopping dominates slow ones; (3) flow and variations in salt concentration have limited effects on hopping kinetics, while in vivo DNA configuration is not expected to influence sliding kinetics; furthermore, (4) the rate of occurrence for hops longer than 200 nm agrees with experimental data for EcoRV proteins. Experimental investigations of sliding proteins on DNA using SIMA [1] measurements on the timescale of milliseconds will be presented.
[1]. DeCenzo, S. H., M. C. DeSantis, and Y. M. Wang. 2010 Optics Express 18(16):16628-39.

1057-Plat
Chromatin Distribution Guides Cell Division in *E. Coli*
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We recently discovered that *Escherichia coli* bacteria that are confined to narrow nanofabricated channels still divide unimpededly, despite the large size and irregular cell shapes that they adopt [1]. Previously, it was shown that