**DNA Replication and Dynamics**

**1382-Pos Board B112**
Repetitive Helicase Assists DNA Polymerase in Bypassing a Lesion
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DNA polymerase must bypass DNA lesions in order to continue replication. However, lesions are known to impede DNA replication. Using single molecule techniques, we investigate the mechanism by which the helicase, RepD, assists DNA polymerase (DNAP) in a lesion bypass. We find that RepD binds to the nascent plasmid DNA and forms a complex that is resistant to DNAP degradation by a nuclease.

**1383-Pos Board B113**
DNA Unwinding by PcrA Helicase and RepD using TIRF and Magnetic Tweezers
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The bacterial plasmid of the PT181 family is replicated by an asymmetric rolling-circle mechanism. We have studied plasmid replication that is initiated when the protein, RepD, binds to the origin of replication (oriD) making a single strand nick and forming a covalent complex with the DNA. This exposure of a short length of single-stranded DNA (ssDNA) allowing PcrA helicase to bind and start unwinding the plasmid. While the DNA is being unwound, DNA Polymerase III travels behind PcrA and synthesizes a new, complementary strand. The other strand becomes decorated with single-stranded DNA binding protein (SSB) and is later replicated by a different mechanism. In earlier studies, we monitored individual PcrA unwinding reactions using TIRF microscopy to image the accumulation of fluorescently labelled SSB on the nascent ssDNA strands (Fili et al., 2010 Nucl Acids Res. 38:4448-4457). We are now using a custom-built magnetic tweezers device to observe initiation (nicking) by RepD and translocation by PcrA. Our initial studies have focussed on the effect of DNA supercoiling upon RepD DNA-nicking kinetics and on DNA unwinding by PcrA. The initiation site, oriD, consists of three inverted complementary repeats that are predicted to form secondary DNA structures (hairpins). When the circule plasmid is free of stags and breaks (e.g., no nicking) the unwinding process proceeds smoothly. However, under conditions of nicking the unwinding process is accelerated.

**1384-Pos Board B114**
Single Molecule Study of HIV-1 Reverse Transcription Polymerization Activity in the Presence of NC
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HIV-1 reverse transcriptase (RT) is a multifunctional polymerase, which synthesizes double-stranded proviral DNA from single-stranded viral RNA by catalyzing DNA- and RNA-dependent DNA polymerization and degrading RNA via its RNase H activity. Reverse transcription is an essential step in HIV-1 infection, and HIV-1 RT is the target of many anti-AIDS therapeutic drugs. HIV-1 nucleocapsid (NC) protein is a nucleic acid chaperone, which facilitates DNA duplex melting and re-annealing, and shows rapid protein-nucleic acid interaction kinetics. The effect of NC on the reverse transcription process is not fully understood. To gain insights into the polymerase activity of RT in the presence of NC protein, we use single molecule force spectroscopy to examine DNA polymerization activity of HIV-RT along long single-stranded DNA (ssDNA) templates with and without NC. Our preliminary observations show the polymerization activity of RT is dependent on the force on ssDNA templates; an increase in the force on ssDNA templates reduces the polymerization activity of HIV-RT. The observed exponential dependence of polymerization activity of RT with force on long ssDNA templates is consistent with previous single molecule studies, and NC appears to enhance the polymerization rate. Our studies will test the polymerization activity of RT in the presence of NC, allowing us to determine the biophysical mechanism by which NC enhances this activity.

**1385-Pos Board B115**
A Novel Function of the Bacterial Replication Initiator Protein DnaA
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Proper cell division requires multiple molecular machineries to function in exquisite synchrony with each other. In bacteria, unlike eukaryotes, chromosome replication and chromosome segregation take place concurrently during cell division. Very importantly, however, chromosome segregation does not initiate before chromosome replication gets underway. How these two machineries communicate with each other during the initiation of replication and segregation are not well understood. To try to understand the mechanisms involved in this communication, we analyzed the effects on chromosome segregation, in Caulobacter crescentus, by varying the concentrations of the replication initiator protein, DnaA. Our data show that C. crescentus cells with limited levels of DnaA have aberrant cell division. These mutant cells release multiple mini-cells with stalks (e.g., no single strand breaks). This mechanism relies on genetic elements termed integrons. They allow the sections - coding for antibiotic resistances or other adaptive traits - between integrons to rearrange, in cis or trans, between bacterial chromosomes. This mechanism enhances the spread of antibiotic resistances, which mediates recombination between a double stranded integron recombination site (attI site) and a single-stranded cassette recombination site (attC site). This integration involves an enzyme, integrase, which mediates recombination between a double stranded DNA (dsDNA) allowing PcrA helicase to bind and start unwinding the plasmid. While the DNA is being unwound, DNA Polymerase III travels behind PcrA and synthesizes a new, complementary strand. The other strand becomes decorated with single-stranded DNA binding protein (SSB) and is later replicated by a different mechanism. In earlier studies, we monitored individual PcrA unwinding reactions using TIRF microscopy to image the accumulation of fluorescently labelled SSB on the nascent ssDNA strands (Fili et al., 2010 Nucl Acids Res. 38:4448-4457). We are now using a custom-built magnetic tweezers device to observe initiation (nicking) by RepD and translocation by PcrA. Our initial studies have focussed on the effect of DNA supercoiling upon RepD DNA-nicking kinetics and on DNA unwinding by PcrA. The initiation site, oriD, consists of three inverted complementary repeats that are predicted to form secondary DNA structures (hairpins). When the circule plasmid is free of stags and breaks (e.g., no nicking) the unwinding process proceeds smoothly. However, under conditions of nicking the unwinding process is accelerated. These low concentrations of DnaA, although not sufficient to initiate replication, were sufficient to-directly or indirectly-trigger the initiation of chromosome segregation. In other words, these mutant cells had their single chromosome copy translocated to the opposite cellular pole in the absence of chromosome replication. Using DnaA mutants, we showed that this effect is not due to DnaA acting as a transcription factor.

**1386-Pos Board B116**
Dynamics of dsDNA Break, Organization of the Nucleus Based on Polymer Dynamics
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To study DNA and chromatin dynamics in the nucleus, we develop a novel polymer model. We estimate the mean time for a DNA molecule to loop inside chromosomal territories, their distribution and statistics. We obtain precise physical laws for the mean time a DNA locus finds a small target. Finally, we extract properties of a DNA locus from live cell images in Yeast, before and after a dsDNA break. We obtain novel biophysical properties beyond the classical Brownian dynamics, we relate the anomalous exponent to the physical properties of the DNA. By applying novel mathematical analysis, we find the confinement domain of a single locus from chromosomal Capture data.

**1387-Pos Board B117**
DNA Secondary Structure Formation in Bacterial Gene Capture Systems at Single-Molecule Resolution
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Bacteria with multiple antibiotic resistances are a threat to human health. These resistances spread faster than could be expected from mutations alone. It was shown that bacteria exhibit a mechanism of exchanging and collecting genetic sections - coding for antibiotic resistances or other adaptive traits - between individuals or even across species boundaries. This mechanism relies on genetic elements termed integrons. They allow the incorporation and expression of exogenous gene cassettes through a site-specific recombination process. The process involves an enzyme, integrase, which mediates recombination between a double stranded integron recombination site (att site) and a single-stranded cassette recombination site (attC site). The attC site is supposed to be recognized by integrase through specificity to the secondary structure of the DNA hairpin formed by the single-stranded attC site (Svoboda et al. 2008). This poses the question of how the DNA hairpin forms inside the living cell. It was shown in vivo that negative superhelicily promotes integron recombination, most likely through cruciform extrusion from double-stranded DNA (2). Here, we use single-molecule FRET and magnetic tweezers to study the formation of the postulated DNA hairpin in the presence of various proteins. We present data on the competition between SSB and integrase and on the extrusion of...