DNA Replication and Dynamics

1382-Pos Board B112
Replicative Helicase Assists DNA Polymerase in Bypassing a Lesion
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Unrepaired DNA lesions can inhibit replication fork progression, leading to cell death or genomic instability. Cells and viruses have evolved to possess mechanisms whereby DNA lesions could be tolerated and bypassed. A lesion may be bypassed via replication fork reversal or recruitment of a translesion polymerase. However, less is known about whether and how a replisome directly bypasses a lesion. Here, using single-molecule methods, we investigate the effect of a cis-syn TT dimer lesion in the leading strand of a DNA template. We found that T7 DNA polymerase (DNAP) on its own was incapable of lesion bypass, consistent with findings from previous biochemical studies. Surprisingly, in the presence of T7 helicase, DNAP bypassed the lesion after a transient pause during which the helicase also paused its unwinding activity. Upon lesion bypass, DNAP and the helicase concurrently resumed their activities. Our findings suggest a new lesion-bypass mechanism that is mediated via DNAP-helicase interactions.

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DNA Unwinding by PcrA Helicase and RepD using TIRF and Magnetic Tweezers
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Bacterial plasmids of the pT181 family are 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 exposes 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 circular plasmid is damage-free (e.g. no single strand breaks) DNA gyrase converts the relaxed open circle into a compact, negatively supercoiled, form which favors extrusion of secondary structure motifs. Using magnetic tweezers to artificially supercoil a length of DNA containing the OriD sequence, we found that RepD nicking is highly sensitive to DNA supercoiling and negative supercoiling serves as a gate-keeper to ensure plasmids are damage-free before DNA replication is initiated.

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Single Molecule Study of HIV-1 Reverse Transcriptase 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 RNA- and DNA-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.

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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 under way. How these two machineries communicate with each other during the initiation of replication and segregation are not well understood. To try to understand the mechanism involved in this communication, we analyzed the effects on chromosome segregation, in *Caulobacter cresecentus,* 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 due to a flawed timing in chromosome segregation. 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.

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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 (attI site) and a single-stranded cassette recombination site (attC site). The attC site is supposably recognized by integrase through specificity to the secondary structure of the DNA hairpin formed by the single-stranded attC site (1).
This poses the question of how the DNA hairpin forms inside the living cell. It was shown in vivo that negative superhelicity 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