region and so allows the helicase and polymerase to bind and begin unwinding and replication, respectively. After travelling all around the plasmid probably as a single protein-antibody complex the protein then reach the newly synthesised origin of replication, which provides the signal for termination. RepD then does a series of strand exchanges to close the two plasmid circles.

We are using a combination of measurements with whole plasmids and oligonucleotides to elucidate the series of events at each stage of the replication. In particular, by following individual processes in real time, we are able to describe the order of biochemical steps that enable this process to occur.

363-Pos Board B143
Cooperative Activity of SARS Coronavirus Nsp13 Helicase Characterized by Single Molecule FRET

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SARS was epidemic in 2003 worldwide. SARS-CoV helicase plays critical roles in viral replication, and has been proposed to be a potential candidate for anti-SARS therapy. We use single molecule fluorescence resonance energy transfer to examine the unwinding and re-winding mechanism of nsp13 helicase on partial DNA duplexes as a function of protein, ATP concentration, and tail length. Our results reveal that the tail length of the substrates determines the total amount of DNA unwound by increasing the number of proteins loaded. In contrast, unwinding rate and step size increase as a function of the protein and ATP concentration for the partial duplex with a long tail (45nts long), but independent of protein concentration for the short tail (30nts long). We also observed a repetitive unwinding displaying multiple rounds of re-unwinding and re-winding events where re-unwinding becomes dominant, resulting in a higher protein concentration. We also found that the relative extent of constitutive unwinding and repetitive fluctuation is defined by the modality of DNA-Protein complex in the presence or absence of ATP concentration. The ratio between them determines the processivity of the cooperative helicases in tandem. In general, our results identify the important cellular parameters, governing the cooperative unwinding and repetitive re-winding behavior of helicase. This is a new attempt to understand the complicated behavior of unwinding motor coherents at the single molecule resolution.

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Measuring the Kinetics of Restriction Endonucleases with Single Molecule Resolution

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We have developed a simple assay for observing the cleavage of DNAs with single molecule sensitivity. DNAs are attached to a surface at one end using a dsDNA-covalent couple link and to a magnetic micro bead at the other end via a biotin-streptavidin link. The DNAs are stretched by applying fluid drag and magnetic forces. The exact time of cleavage of individual DNAs is recorded with video microscopy by observing the time of disappearance of each bead. We are using our technique to measure the kinetics of two type II restriction endonucleases, EcoRI and NdeI. With our kinetic data, we hope to elucidate the target site search mechanisms of these enzymes.

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Dynamic Control of Processivity during DNA Degradation by a Ring-Shaped Nuclease

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DNA exo-nucleases catalyze numerous essential biological processes such as DNA replication, recombination, and repair. λ exo-nuclease (λ exo) is composed of three active sites and forms a ring structure for processive degradation. The detail molecular basis governing the alostrophy of trimers has not been well understood yet. Here we used single molecule fluorescence resonance energy transfer (FRET) to examine how the three enzymatic sites of λ exo are coordinated. We find that only one of three active sites is utilized and the ring of λ exo is rotated along DNA helix during degradation. We further examine how the previous motion of λ exo-nuclease influences on the following enzymatic activity, and found that the continuous cleavage activity guides the enzyme to competently position, making it tilted around 45° to the DNA's helical axis. This coordinated comprehensive motion is required for efficient and processive degradation, suggesting a hierarchy nature for processivity. We also find that the tendency of backtracking on ssDNA increases when the degradation rate slows down.