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gyrase forms a double stranded break in DNA and passes a second strand of DNA through the resulting break, a process that is linked to ATP hydrolysis. To elucidate the conformational changes necessary for this action and to obtain a comprehensive picture of the mechanism of gyrase, we will utilize a novel single molecule technique, which combines both magnetic tweezers and TIRF microscopy to simultaneously observe protein and DNA movement during the DNA supercoiling process. Incorporating information from both dynamic single molecule and static structural studies promises to provide a more comprehensive picture of the mechanism used by this molecular machine to alter DNA topology.

391-Pos Board B146

Requirements for Site-Specific Recombination in the Tyrosine-Family Recombinase Active Site

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Tyrosine recombinase (YR) shares the same active site and catalyzes the DNA rearrangement with identical phosphoryl transfer chemistry to topoisomerases IB (TopIBs). In order to find out the function of each residue within conserved catalytic center and what factor dictates the strikingly different kinetic characteristics, in cis for Cre and λ Int and in trans for Flp, the whole reaction process of tyrosine family recombinase-mediated site-specific recombinastion has been studied in detailed by using single-molecule TPM. For both systems, we found that the conserved His/Trp residue is strictly important to maintain the DNA binding capability and maintain the interaction in the protein-protein interface. The two conserved arginine residues are essential for the formation of catalytic synaptic complexes. Histidine residue participates in the strand cleavage for the Flp-FRT system but affect the synapsis for Cre-loxP system.

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Distributive and Processive Exonucleases Characterized by Single Molecule FRET

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DNA exonucleases catalyze numerous essential biological processes such as DNA replication, recombination, and repair. Their catalytic behaviors can be classified into either distributive or processive. However, the molecular basis governing the different activities has not been well understood yet. Here we used single molecule fluorescence resonance energy transfer (FRET) to examine the characteristics of distributive and processive enzymes at the single molecule level. The activities of exo-nuclease III and λ exo-nuclease are investigated and dissected: initiation and degradation. The initiation of exo-nuclease III is independent of protein concentration, but its degradation is protein-concentration dependent. The concentration-independence of initiation suggests that the functional catalytic form is monomeric whereas the concentration-dependence of degradation is an evidence of a distributive behavior. In contrast, the initiation of λ exo-nuclease shows a concentration-dependence, suggesting the functional form is an oligomer. We also examine how the three enzymatic sites of λ exo-nuclease are coordinated during degradation and find that they work one active site at a time. We further examine how the previous motion of λ exo-nuclease influences on the following enzymatic activity, and found that the coordinated rotational and translocation motion is required for efficient degradation. We also find that the tendency of backtracking on ssDNA increases when the degradation rate slows down.

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Human Replication Protein a (RPA) Can Diffuse Along Single Stranded DNA

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Replication Protein A (RPA) is a hetero-trimeric protein that plays critical roles in many cellular processes. The main function of RPA is to bind to single stranded (ss) DNA and to regulate its availability during DNA metabolic processes. RPA is also known to interact with an array of other proteins in DNA replication, repair and recombination processes in eukaryotic organisms. RPA binds to the transient ssDNA that forms during nearly all aspects of DNA metabolism and protects ssDNA from nucleases. Although RPA binds to ssDNA with a very high affinity, it must be dissociated from or redistributed along ssDNA during DNA replication. To probe this rearrangement of RPA along ssDNA, ensemble and single molecule studies have been used as complementary techniques to investigate human RPA diffusion along fluorescently labeled ssDNA oligomers. The dynamics of human RPA (hRPA) along fluorescently labeled DNA oligomers was also studied with fluorophore - labeled hRPA. These experiments illustrate that hRPA can spontaneously re-arrange along ssDNA by diffusing while remaining tightly bound. In addition, hRPA can also transiently melt DNA hairpin structures by diffusing in from ssDNA that is adjacent to the DNA hairpin. This ability of hRPA to diffuse along ssDNA means that directional DNA motor proteins such as polymerases or translocases can push RPA and re-organize it along ssDNA. This diffusion property of RPA is shared with the bacterial analogue of RPA, the *E. coli* SSB protein that also has previously been shown to diffuse along ssDNA. (GM030498 (TML), GM098509 (RG), GM044721 (MSW)).

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Human ORF1p - DNA Interactions Characterized by Single Molecule DNA Stretching

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Retrotransposons are mobile genetic elements that possess the ability to amplify themselves in the genome via a process called retrotransposition. LINE-1 is a retrotransposon that comprises about 17% of the human genome, which is still active in most modern mammalian genomes. It is a significant source of interindividual genetic variations, defects and rearrangements. LINE-1 encodes two proteins: ORF1p and ORF2p, which are essential in retrotransposition. The role of ORF2p in retrotransposition as an endonuclease and as a reverse transcriptase has been demonstrated. However, the role of ORF1p is largely unknown, making the overall molecular mechanism of retrotransposition unclear. Studies on mouse ORF1p have revealed its nucleic acid chaperone activity, while human ORF1p (hORF1p) exhibits more complex nucleic acid interactions. Recent studies conducted in bulk solution conditions have shown that hORF1p preferentially binds to single-stranded DNA (ssDNA) and RNA relative to double-stranded DNA (dsDNA), but binds mismatched dsDNA with the same affinity as ssDNA, whereupon it stabilizes the mismatched duplex from dissociation. This property would enhance the production of productive primer-template interactions, a crucial step in the LINE-1 replication process. Here we develop a method to quantitatively characterize the mechanism of hORF1p-DNA interactions using single molecule techniques with optical tweezers. Because hORF1p binds strongly to both double- and single-stranded DNA, we first overstretch dsDNA, providing a lattice of ssDNA binding sites for hORF1p binding. We then flow in protein and incubate the stretched ssDNA for fixed times, followed by releasing the DNA and allowing it to anneal. We find that the amount of ssDNA bound by protein increases with incubation time over timescales of tens of minutes. These results suggest a very slow protein oligomerization process on ssDNA, which likely plays an important role in the mechanism of retrotransposition.

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Interactions Between the SMC-Complex, Spo0J and DNA

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In bacteria the length of DNA found within the cell vastly exceeds the length of the cell itself. To overcome this problem bacteria compact their DNA into structures called nucleoids. Although how nucleoids are organised remains poorly understood several different classes of protein have been implicated in their formation, including the Structural Maintenance of Chromosome (SMC) proteins. Although these proteins are known to have DNA condensation and cohesion activity, the mechanism by which they carry out these reactions is unknown. Structural studies have shown they form large, ring-like the opening and closing of which are thought to be linked to the enzyme's ATPase activity. Additionally, a number of accessory proteins for the complex have been identified. In Bacillus subtilis SMC is known to interact with three smaller proteins: ScpA, ScpB and Spo0J. ScpA and ScpB have been shown to interact directly with the complex whereas Spo0J is thought to load the SMC complex onto DNA. Spo0J is a dimeric protein which binds to a pseudo-symmetrical binding site (parS). Spo0J has been shown interact with SMC in vitro and in vivo, but previous in vitro experiments were carried out in the absence of the ScpA and ScpB accessory proteins. Here we demonstrate the formation of a novel complex involving ScpA, ScpB, Spo0J, DNA and a transition state ATPase mutant of SMC. The formation of this complex is dependent on the presence of all four proteins, ATP and DNA, but does not require the presence of a parS site. It is possible this complex represents an