1446-Pos Board B216
Combining Magnetic Tweezers and Fluorescence to Study DNA Mismatch Repair by MutS, MutL and MutH
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Magnetic Tweezers are a powerful, highly parallelizable, single-molecule technique that allows the conformational manipulation of individual, double-strand DNA molecules. It allows the user to measure mechanical or enzymatic work done on DNA by proteins present in the solution. Total-Internal Reflection Fluorescence (TIRF) Microscopy is another prolific single-molecule technique which allows the user to detect and localize individual proteins in real-time. We have constructed an apparatus which combines the two techniques, allowing the user to simultaneously detect both the localization of proteins on DNA and the subsequent action of these proteins on the DNA. We apply this novel technique to study properties of the mismatch repair system embodied by the MutS, MutL, and MutH proteins. During genome replication, single-nucleotide errors are inadvertently created in the daughter-strand sequence. The detection and elimination of these errors is essential in maintaining an organism's genomic stability. In E. coli, MutS, MutL and MutH are the three proteins responsible for detecting error-induced mismatches in the double-strand DNA and then initiating the excision process that will remove the mismatch. MutS recognizes the mismatch, MutL links MutS to MutH and MutH cuts the error-containing daughter strand, initiating the mismatch's removal by downstream agents. Although the roles of these proteins are crucial to quenching replication mutations, the process by which they assemble onto the DNA remains unknown. Using the combined Magnetic Tweezers and TIRF techniques we are able to probe the recruitment of these proteins and measure the kinetics of their assembly onto DNA, their movement along it, and their eventual nicking of one of the strands.

1447-Pos Board B217
In Vivo Investigation of DNA Replication in Escherichia Coli using Single-Molecule Fluorescence Microscopy
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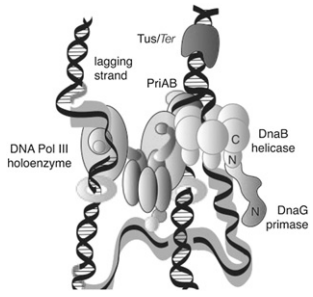
Our research involves investigating the process of DNA replication in living cells. More specifically the dynamics of the replisome components in living Escherichia coli cells during the replication termination process. Our current knowledge of the replisome dynamics, and the termination process, has been obtained mainly from in vitro experiments that were not done on the single molecule level. However, the natural environment of the cell is considerably different from that of in vitro solutions. These differences can have a significant influence on how certain proteins function in a cell. Interestingly, it is now possible to monitor single-molecule processes using fluorescence microscopy inside genetically modified living cells, by tagging their native proteins with fluorescent probes. In our research we employ single-molecule fluorescence microscopy to track individual replisomes with other replication-related proteins, and investigate their dynamics during the process of DNA replication in living bacterial cells. The single-molecule sensitivity can add to our understanding by providing further insights into processes that are hidden by ensemble-averaging techniques. Here we describe the microscopy techniques, the micro-fluidic devices and the various gene manipulation techniques utilized in researching the above mentioned molecules.



1448-Pos Board B218
Computational Analysis of Electron Tunneling Pathways of Blue-Light Photoreceptors
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DNA phtolyases and cryptochrome DASH (cry-DASH) enzymes in the blue-light photoreceptor family repair UV-damaged DNA by photo-induced electron transfer reaction. Recently (Miyazawa et al., 2008) we analyzed the electron tunneling pathways in a class I CPD photolyase derived from A. nidulans and identified a key residue, Met-353, which is perfectly conserved in the class I CPD photolyases. Interestingly, this site is switched to histidine in 6-4 photolyases, and to glutamine in cry-DASH enzymes. For instance, Met-353 is replaced to Gln-395 in cry-DASH. It is likely that the amino acid residue at the site controls the enzymatic functions of the blue-light photoreceptor family. Until now, the electron transfer pathways in cry-DASH enzymes remain unclear, while those of CPD photolyases are studied well.
To characterize the roles of the amino acid residue at the site, we performed molecular dynamics simulation and electronic state calculations of the CPD photolyase of A. nidulans and the cry-DASH. We analyzed the electron tunneling pathways in the forward and backward electron transfer reactions between FADH- and CPD of these two enzymes. As a result, we observed busy trafficking of electron-tunneling current at both Met-353 of the CPD photolyase and Gln-395 of the cry-DASH.

Transcription

1449-Pos Board B219
Dissecting the Nucleosomal Barrier to Transcription
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The nucleosome, which represents the fundamental unit of chromatin and an intrinsic regulator of transcription, consists of a histone octamer and ~150 base pairs of DNA. The histone proteins in turn contain two functional regions: the histone-fold domains, which make strong contacts with the DNA and organize it into the superhelical structure specific to the nucleosome, and the histone tails, which are highly positively charged and can stabilize the nucleosome further. Both histone regions, but especially the tails, are the target of many post-translational modifications associated with gene expression. The sequence of the DNA wrapped around the histones, in addition to facilitating the positioning of the nucleosome, can promote RNA polymerase II (Pol II) pausing, thus modulating the nucleosomal barrier.
We will describe how each nucleosomal component - the histone tails, the specific histone-DNA contacts, and the DNA sequence - contributes to the barrier that the nucleosome imposes on transcription elongation by Pol II. Removal of the histone tails favors progression of Pol II into the entry region of the nucleosome by locally increasing the wrapping-unwrapping fluctuations of the DNA from the histones. In contrast, point mutations in the histone-fold domains that affect histone-DNA contacts at the nucleosome dyad abolish the barrier to transcription in the central region by decreasing the local rewrapping rate. Additionally, the nucleosome amplifies sequence-dependent transcriptional pausing, an effect mediated through the secondary structure of the nascent RNA. Each of these elements of the nucleosome barrier controls transcriptional elongation by affecting in a distinct manner the density and duration of pauses, and thus provides alternative mechanisms for the regulation of gene expression by chromatin remodeling and other transcription factors.

1450-Pos Board B220
Freeing the Promoter Site: Mechanistic Insights into the Interaction of Mot1 with TBP using spFRET
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Gene transcription is a central process of life and is highly regulated with a multitude of transcription factors. Recently, we could show that dynamics of the Tata-box Binding Protein (TBP) on the promoter site also plays a role in gene regulation. Using single-pair Förster Resonance Energy Transfer (spFRET) experiments, we have monitored the conformation of TBP bound to the H2B promoter site in the presence and absence of Mot1. Upon the binding of Mot1 to the TBP-DNA complex, a high FRET state between TBP and labeled DNA is formed. Contrary to what was expected, Mot1 bound to TBP-DNA complexes was unable to dissociate TBP from the DNA upon addition of ATP. Instead, the TBP-DNA complex returned to its original conformation even though Mot1 remain bound to the complex. Only when Mot1 was also present at nM concentrations in solution was dissociation of TBP from the promoter site observed upon addition of ATP. This suggests that a single Mot1 is either insufficient or inefficient at dissociating TBP alone.
We also used spFRET to monitor the DNA conformation during the interaction of Mot1 with TBP-DNA. TBP-bound DNA was observed to fluctuate between three conformations. The FRET values of the conformation varied depending on the orientation of TBP on the promoter. Fluctuations between the same conformations were observed when Mot1 was bound to the TBP-DNA complex but