

replication and facilitating stalled replication fork rescue. It is becomingly increasingly clear that SSB plays a critical role in the function of RecG, but the mechanisms of its interaction with SSB remain unclear. Here we use the atomic force microscope (AFM) to image the structure of RecG with a model fork substrate in the presence or absence of the single strand DNA binding protein (SSB). The DNA substrate has a 3'-end 69nt single stranded DNA (ssDNA) segment inserted between the two DNA duplexes. This design mimics a stalled fork with an ssDNA gap in the leading strand. The SSB proteins bind very specifically to the ssDNA segment with a yield of ~90%. RecG protein also binds to the fork but the yield was ~10%. However, the RecG loading on the same substrate increases three-fold in the presence of SSB suggesting that SSB facilitates RecG loading onto the fork. Moreover, in the presence of SSB, RecG becomes capable of translocating along the DNA duplex in an ATP hydrolysis-independent manner. No such mobility of RecG was observed in the absence of SSB. The preferable translocation direction is moving of RecG along parental arm which implies RecG could clear obstacles bound ahead of the fork. This novel activity of SSB requires the SSB C-terminus as a truncated SSB mutant does not substitute for wild type. SSB loading of RecG and subsequent translocation are unaffected by ATP, ADP or the non-hydrolyzable analog ATP- γ -S. Overall the results obtained reveal novel properties of RecG and highlight a new chaperone-type role of SSB in the DNA repair process.

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Interplay MutS with β Clamp on Mismatched DNA

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MutS, a mismatch repair initiation protein, forms a transient clamp to search for mismatched nucleotides by rotational-coupled translational diffusion while in continuous but nonspecific contact with the helical duplex DNA. When MutS encounters a mismatch, ATP binding by MutS results in an extremely stable sliding clamp that slides off the mismatch and moves along the adjacent duplex DNA driven simply by 1D thermal diffusion. The DNA mismatch repair is a timing process to be operated after DNA is newly synthesized. The β -clamp protein, a subunit of the DNA polymerase III holoenzyme, encircles duplex DNA and stably diffuses on the DNA after a lagging strand is synthesized. The long dwell time of the sliding β -clamp on DNA results in its accumulation on the lagging strand, which may obstruct the way of the translocation of MutS to find a mismatch and to transmit its finding to a downstream site. We will present how the sliding β -clamp interacts with MutS on mismatched DNA. The single-molecule studies using FRET and a single particle tracking suggest that the β -clamp increases the searching time of MutS required to locate a mismatch site through the physical interaction with MutS and is separated from MutS bound to ATP at the mismatch.

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Influence of DNA Conformation and Repair Enzyme on Guanine and 8-Oxoguanine Base Flipping

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8-oxoguanine (8oxoG) is an abundant product of oxidative damage in DNA and if not repaired can result in mutations upon DNA replication. It is removed by repair glycosylases (e.g. MutM-glycosylase in bacteria) after flipping out the damaged base towards an extra-helical conformation into the repair enzyme active site. The exact mechanism how the repair enzyme identifies a damaged site within a large surplus of undamaged DNA is not fully understood. Binding of a repair enzyme results also in significant DNA deformation such as bending and minor groove opening. Looping out nucleotides from an intra-helical base paired conformation is energetically costly and it is not clear how the presence of a repair enzyme or the deformation of DNA may facilitate the looping out process. In this study we use Molecular Dynamics free energy simulations to evaluate the effects of DNA deformation and enzyme binding on the DNA base flipping process. The simulations indicate distinct free energy profiles for flipping 8oxoG or guanine and resulted in an overall calculated free energy for the flipping process in accordance with experimental imino proton exchange from Nuclear Magnetic Resonance spectroscopy. Distortion of the DNA towards a conformation as observed in complex with the repair enzyme lowered the free energy barrier and penalty for the flipping process. The result indicates that the DNA deformation induced by the repair enzyme binding has a significant influence on the flipping process. The additional effect of protein-DNA contacts on the calculated free energy for the flipping process will also be presented.

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Elasticity-Driven Single Stranded Gap Creation Mechanism by an Exonuclease III/AP Endonuclease

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DNA damage occurs as many as 1 million individual molecular lesions per cell per day in human. One of the most frequently occurring DNA lesions is base damage, which are quickly processed into AP (apurinic/aprimidinic) sites, resulting in "missing base" in DNA, so-called AP site. AP endonuclease catalyzes them for the Base Excision Repair (BER) process. Exonuclease III (exoIII) in E.coli functions as not only an AP endonuclease but also a 3'->5' exonuclease. We used single molecule FRET to examine how the multifunctional enzyme plays the dual role during the NER process. When the substrate is a blunt-ended DNA, the enzyme works as a distributive exonuclease, performing multiple rounds of binding and dissociation events during the cleavage reaction. However, in the presence of an AP site, exoIII cuts the AP site as an AP endonuclease, and then transforms into a processive exonuclease, performing a continuous digestion by strongly anchoring to the AP site. The strong binding affinity onto the AP site is the origin of the transformation mechanism, allowing the enzyme to digest a series of nucleotides without dissociation from the substrate. The close examination of the enzyme behavior reveals an elasticity-based mechanism where exoIII creates a gap by forming a single stranded intermediate loop during BER process. The size of the gap robustly relies on the physiological salt concentration due to the extent of the repulsive property in the negative charged single stranded loop.

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How Does the Replication Machinery Deal with Roadblocks: A Single-Molecule Investigation

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DNA replication is a fundamental process in life. Decades of study have elucidated the most important mechanistic principles underlying the duplication of DNA, but most often these processes are studied and represented as if they occur on naked DNA. We set out to employ single-molecule techniques to study the effect of intracellular crowding on the process of replication. We develop methods that allow us to mechanically stretch DNA molecules and use fluorescence microscopy to visualize replication at the single-molecule level. In particular, we reconstitute in vitro the E. coli replication reaction and observe how common DNA-binding proteins such as the histone-like proteins H-NS and HU affect the replication kinetics.

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Real-Time RecA Filament Disassembly in the Presence of RecX Monitored using Single-Molecule Manipulation by Optical Tweezers

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Homologous recombination in the bacteria cell is regulated at many levels. The central enzyme of homologous recombination RecA and its regulation has been a subject of intense studies by single molecule techniques during the last decade. The RecX protein of Escherichia coli is known to be a negative regulator of RecA. Here we combined optical trapping and microfluidics in order to address the dynamics of RecA-dsDNA filament in the presence of RecX at the single molecule level. Our preliminary results show that RecX blocks elongation of RecA filament and facilitates RecA disassembly from dsDNA in the concentration-dependent manner.

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Toward Adding Complexity in Single Molecule FRET Studies of DNA Mismatch Repair

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Mistakes made during DNA replication are targeted for post-replicative repair by the DNA mismatch repair system. Purified reconstitutions of DNA mismatch repair require multiple protein interactions, and in vivo studies of DNA mismatch repair function have identified layers of spatial and temporal regulation. We have previously developed single molecule FRET experiments