DNA Replication, Recombination, and Repair

1423-Pos Board B193

Substrate Specificity and Dynamic Instability of RAD51-Filament Assembly on Single- and Double-Stranded DNA

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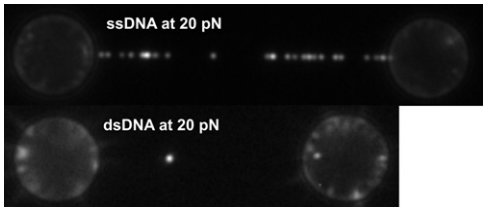
Homologous recombination (HR) is an essential DNA-repair strategy present in all life forms. The core machinery catalyzing this transaction in human cells is the recombinase protein RAD51, bound as a helical filament on single-stranded DNA (ssDNA). Here we use a combination of single-molecule fluorescence microscopy, optical tweezers and micro-fluidics to directly visualize the assembly and disassembly of RAD51 filaments on ssDNA with single monomer resolution. This approach allows us to quantify rates of nucleation and growth as well as the size of the nucleation unit. Our findings show that RAD51 can select with high specificity between ssDNA and double-stranded DNA by sensing their different mechanical properties (see figure). We also observed that RAD51 nuclei are characterized by dynamic instabilities, whose occurrence depends on the actual size of the nascent RAD51 filament. Our findings provide the basis for a molecular description of the RAD51-ssDNA assembly mechanism, which is essential to understand the role of accessory proteins, such as BRCA2 that mediate and regulate RAD51 filament formation in vivo.



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Single Molecule Studies of Bacterial Transcription Coupled Repair

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Transcription coupled repair (TCR), a sub-pathway of the nucleotide excision repair mechanism, is activated when a RNA polymerase (RNAP) is arrested during transcription by DNA damage. TCR is a ubiquitous cellular response important for maintenance of DNA integrity. Some human genetic disorders are associated with defect on TCR, like the Cockayne syndrome for instance. In the bacterium Escherichia coli, TRCF, the product of the mfd gene, is the DNA translocase that couples transcription and DNA repair: it recognizes a stalled ternary elongation complex, dissociates it, and recruits the UvrABC repair machinery.

We used a single molecule approach, by means of magnetic tweezers, to study the initiation of TCR and monitored the dissociation of individual RNAPs by individual TRCFs in real time. We have identified that TRCF acts on stalled RNAP by remodeling the transcription bubble in two successive ATP-dependent steps separated by a novel intermediate denoted RD*.

Statistical analysis of the time required to dissociate the stalled RNAP at different concentrations of TRCF has shown that the displacement of the ternary elongation complex is a three limiting-steps process: (i) recruitment of TRCF to the stalled RNAP, (ii) activation of TRCF and initiation of the dissociation of RNAP by rewinding of 2/3 of the transcription bubble, and (iii) complete dissociation of the elongation complex. The intermediate complex RD* formed after the first step of rewinding is characterized by a long lifetime, which suggests that it would behave as a temporally reliable marker, enabling the recruitment of the UvrABC repair machinery to the damaged site.

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Mechanism of D-Loop Disruption by the Human Bloom's Syndrome Helicase

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The most toxic form of DNA damage is the double-stranded DNA break (DSB). To avoid the harmful consequences of DSBs, cells use homologous recombination (HR)-based error-free DNA repair mechanisms. HR processes must be highly organized and regulated in order to take place in the right context, because illegitimate HR and incorrect DNA repair may cause severe genetic abnormalities that can lead to cell death or different types of cancer. Human Bloom's syndrome DNA helicase (BLM), a member of RecQ family, plays crucial roles in HR progression and regulation. In the early steps of HR a three-stranded complex DNA structure, a displacement loop (D-loop), is generated by the strand exchange activity of Rad51 recombinase. The formation of D-loops is essential for HR progression. BLM is able to perform quality control of HR by disrupting D-loops. To investigate the mechanism of D-loop disruption we generated a series of truncated mutants of BLM and set up a gel based fluorimetric assay to monitor the dissolution kinetics under single round conditions. Interestingly we found that all of the investigated constructs are able to disrupt D-loops. Moreover, our quantitative analysis revealed that the different constructs use distinct processing mechanisms. Our results demonstrate how BLM is capable of regulating HR by the dissolution of D-loops and how the different domains present in BLM regulate D-loop processing.

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Swi5-Sfr1 Stabilizes Formation of RAD51 Nucleoprotein Filaments

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In mammalian cells, Swi5-Sfr1 is involved in the Rad51-mediated homologous recombinational repair of damaged DNA. Swi5-Sfr1minus cells showed a reduced level of homologous recombination events upon the challenge of BRC peptides. This observation suggests possible regulatory roles of Swi5-Sfr1 on the formation of Rad51 nucleoprotein filaments. We used a single-molecule optical tweezers method to directly measure the length of Rad51 nucleoprotein filaments. In the presence of Swi5-Sfr1, DNA substrates are found to be extended by 50 $\pm$ 7%, whereas Rad51 alone nucleoprotein filaments are only 20 $\pm$ 13% extended. The rather large length distributions in the latter case imply Rad51 binding dynamics in ATP condition. Consistently, time-course studies showed that Swi5-Sfr1/Rad51 nucleoprotein filaments are dynamically stable with 1.61 $\pm$ 0.07 fold extension in DNA length compared to B-form duplex DNA. Rad51 alone nucleoprotein filaments had 1.22 fold extension and showed a relative large fluctuation 0.14 fold in DNA length. This observation indicates Swi5-Sfr1/ Rad51 nucleoprotein filaments are not only more extended but also dynamically more stable. However, upon raising ATP concentration or using non-hydrolyzed analog of ATP, Rad51 alone nucleoprotein filaments showed stable dynamic behaviors. Together, these results suggest a nucleoprotein stabilization role of Swi5-Sfr1 upon the challenge of low ATP concentration. This function of Swi5-Sfr1 may form a mechanistic basis to elucidate homologous recombination enhancing activities observed in vivo.

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Single-Molecule Study of RecG Activity at a 3-way DNA Junction

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In *E. coli*, replicative DNA polymerases are often stalled for various reasons: head-on collision of DNA polymerases, blockage by frozen DNA-protein complexes or DNA damage by UV and oxidative stress. In particular, DNA damages causing polymerase stalling should be properly repaired by DNA repair pathways such as NER and HR. In order to avoid the impasse due to polymerase stalling and repair the DNA damages eventually, the stalled fork (3-way junction) is regressed for a proper repair on the damaged site. In the process of fork regression, RecG is thought to play a pivotal role: at the back of the fork, it may pump DNA back in order to convert the 3-way junction to a 4-way chicken-foot structure whereby the damaged spot becomes rewound and prepared for repair process.

In our study, we examined the activity of RecG at a 3-way junction at the single-molecule level. Using single-molecule FRET technique, we clarified how RecG unwinds newly-synthesized DNA strands and induces the conformational transition of branched DNA structures. We also investigated the effects of monovalent cation, ATP, and ATP analogs on RecG activity and measured the rate of unwinding of DNA by RecG.

From our study, we found in detail how RecG functions: it binds to a 3-way junction resembling a stalled replication fork and unwinds the junction to a 4-way junction in an ATP-dependent manner.

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Single Molecule DNA Interactions Between the E. Coli DNA Polymerase III α Subunit and the Polymerase Manager Protein UmuD

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The *E. coli* replicative DNA polymerase III (Pol III) α stalls upon encountering DNA damage. Specialized translesion synthesis (TLS) DNA polymerases are required for efficient bypass of damaged DNA. Although the mechanism for exchanging Pol III α for a TLS polymerase is not yet well understood, the protein UmuD is extensively involved in modulating cellular responses to DNA damage. UmuD binds both the polymerase α and the β processivity clamp,