

Sometimes even within the same subfamily, the mechanistic properties of two helicases can be very different. *S. cerevisiae* Upf1 (yUpf1) and human Smubp-2 are two helicases from Upf1-like family that belong to SF1B. In this work we show that despite having a similar helicase core, the helicase domain (HD) of these two enzymes work in a very different manner. yUpf1-HD has a strong binding affinity to ssDNA and a very high processivity, whereas Smubp-2-HD has weak binding to ssDNA and almost unrecognizable processivity. Helicase cores of Smubp2 and Upf1 have similar folds: they contain two RecA-like domains (1A and 2A) and two sub-domains, 1B and 1C, inserted in domain 1A. Rec-A like domains are believed to act as a motor in a helicase. Hence it is puzzling why the two helicases behave so differently despite having highly conserved domains 1A and 2A. We try to identify the role of sub-domains 1B and 1C in this respect. We have utilized a magnetic tweezers based single molecule assay, acting in parallel on tens of molecules at the same time, which allows detecting the unwinding of DNA hairpin substrates. Using this system, we have studied various chimeras of yUpf1-HD and Smubp-2-HD. We show that domains 1B and 1C play an equally important role in processivity of these helicases.

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Investigating the Enhancement of XPD Helicase Processivity by Single-Stranded Binding Protein RPA2

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Ferroplasma acidarmanus helicase *FacXPD* is a Superfamily 2B helicase involved in transcription initiation and nucleotide excision repair. This archaeal protein serves as a model for understanding the molecular mechanisms of yeast Rad3 and human xeroderma pigmentosum group D protein (XPD). Previous work has shown that the unwinding of double-stranded DNA by *FacXPD* is enhanced by the single-stranded DNA binding protein *FacRPA2*. However, the mechanism by which unwinding enhancement occurs remains unknown. Here, we monitored the unwinding of a DNA hairpin by XPD in the presence of RPA2 using a single-molecule optical trap assay. By loading XPD and RPA2 onto DNA in a controlled sequence and by analyzing helicase unwinding dynamics, we distinguish between different potential mechanisms of regulation. Our data point toward a model of processivity enhancement in which RPA2 transiently interacts with the XPD-DNA complex and stabilizes a faster, more processive state of the helicase.

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Probing the Nucleic Acid Binding Properties of the Single-Stranded DNA Binding Protein of Bacteriophage T4 Replication Complex at Single Nucleotide Resolution

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Previous studies have mapped the structural details and assembly properties of the single-stranded (ss)DNA binding protein (gp32) of bacteriophage T4 as it binds to various ssDNA lattices, both as isolated monomers and as cooperatively bound gp32 clusters. Building on previous studies, our work seeks to understand these binding interactions at single nucleotide resolution. We have utilized site-specifically positioned 2-aminopurine (2-AP) fluorescent base analogs of adenine incorporated into ssDNA lattices, as either monomer or dimer-pair probes, to map the detailed interactions of gp32 with ssDNA lattices of various lengths. To this end we have employed changes in the fluorescent and circular dichroism (CD) spectra of these probes in order to determine how the binding sites of the proteins interact with these site-specifically positioned probes. Our results demonstrate that gp32 binds at random at low concentrations, and then shifts to preferential binding at the 5'-end of the lattice as the proteins shift into cooperative, cluster-bound forms at higher gp32 concentrations. We have also used acrylamide quenching to monitor solvent exposure of the ssDNA bases at various lattice positions, indicating greater 'shielding' of the ssDNA bases exists at the center of the binding site under saturating conditions. These results provide new insights into the molecular mechanisms of the gp32-ssDNA interactions that are involved in controlling the functions of the T4 DNA replication complex.

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DNA Looping Mediates Nucleosome Transfer

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Proper cell function requires preservation of the spatial organization of chromatin modifications. Maintenance of this epigenetic landscape necessitates the transfer of parental nucleosomes to newly replicated DNA, a process which is stringently regulated and intrinsically linked to replication fork dynamics. The complex and dynamic environment in which nucleosome transfer occurs is a challenging setting to define the essential components that underlie the transfer mechanism. To examine the role of DNA mechanics and identify the minimum system requirements for mediating nucleosome transfer, we utilized a DNA template with a single nucleosome and incrementally added complexity by first displacing the nucleosome mechanically, then with an isolated replicative helicase, and finally with a simplified replication complex. We found that the nucleosome is passively transferred to available dsDNA as predicted by a simple physical model of DNA loop formation. These results demonstrate a fundamental role for DNA mechanics in mediating nucleosome transfer and preserving epigenetic integrity during replication.

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The Interplay between a Bacterial Chromatin Protein and DNA Polymerase

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In all domains of life the replication of DNA is carried out by functionally conserved DNA polymerase. Most DNA polymerases can perform both DNA replication and proofreading (via exonucleolysis) functions. In the cell DNA polymerase operates in a crowded environment, with multiple other proteins binding along DNA. However it is unknown how the binding of such proteins affects the activity of DNA polymerase. In this study, we use T7 DNA polymerase as model system to investigate polymerisation (pol) and exonuclease (exo), activities in the presence of DNA bound proteins as potential roadblocks. The bacterial chromatin protein HU is one of the most abundant proteins in *E. coli*. HU binds non-specifically to DNA and is involved in genome organization. We investigated the impact of this physiologically relevant roadblock protein on T7 DNA polymerase activities. We use optical tweezers with concurrent fluorescence imaging to directly observe fluorescently labeled DNA-bound HU and to determine its interplay with T7 DNA polymerase.

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Real-Time Fluorescence Assays to Monitor DNA Polymerase Activities

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We report fluorescence-based methods to study the polymerase and the exonuclease activities of thermostable DNA polymerases in real time. The methods are based on the use of a fluorescent reporter which become fluorescent upon binding to double-stranded DNA (method #1), the use of a quencher reporter strand displacement from a tripartite substrate (method #2) or the use of fluorescence fading of a nucleic acid stain concomitantly to DNA degradation (method #3). These methods are compatible with standard spectrofluorimeters, plate-readers or Real-Time PCR instruments. Here, we follow the efficiency of primer extension and degradation reactions by *Pyrococcus abyssi* thermostable family B DNA polymerases at 55°C. In addition, the size of extended products is systematically examined by gel electrophoresis followed by fluorescence visualization. These real-time methods are very sensitive, quantitative, and well suited for the screening of extension and exonuclease activity by DNA polymerases. Moreover, these assays could be used to characterize other DNA enzymes like helicases or nucleases.

Based on operational properties of different DNA pols, these results could raise specific features with possible evolutionary scenario for the origin of families DNA polymerases.

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Dynamic Proofreading in the Bacterial DNA Polymerase

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Ensuring DNA is replicated with high fidelity is important for maintaining genome stability, since the introduction of errors can lead to mutations and potentially cancer. For this reason, high fidelity polymerases contain 3' to 5' proofreading activity that can remove incorrectly inserted nucleotides from the primer terminus. DNA polymerase III (DNA Pol III) is the replicative