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Defining the Superfamily Conserved Mechanism for Flap Endonucleases FEN1 and XPG Specificity for 5' Flap DNA and DNA Bubbles, Respectively, by Hybrids Methods of Crystallography, SAXS, EM, and Computation

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Flap endonuclease 1 (FEN1) and XPG are essential 5′ nuclease superfamily endonucleases in DNA replication and repair. FEN1 incises in the dsDNA region adjacent to 5′ flaps, while XPG incises in the dsDNA region adjacent to DNA bubbles. We have used a hybrids method analysis combining crystallography, Small Angle X-ray Scattering (SAXS), Electron Microscopy (EM), and computation to characterize FEN1 specificity and activity on double flap substrates, and in the presence of sliding clamps 9-1-1 and PCNA and XPG specificity for DNA bubbles. Our crystallographic work shows a 5′nuclease superfamily conserved mechanism for resolving aberrant DNA structures that involves both structural motifs and flexibility. Our structures of product and substrate-bound complexes suggested FEN1 resolves 5′ flaps using a dsDNA binding - ssDNA incision mechanism. The structure revealed FEN1 binding to a bent duplex DNA structure and an active site shielded by a helical gateway that would select for single-stranded DNA or RNA to reach the active site. Key structural elements mediating duplex DNA binding, substrate specificity and activity are superfamily conserved in our XPG catalytic domain crystal structure. Further computational and EM work with FEN1 complexes to sliding clamps 9-1-1 and PCNA showed distinct functionally-relevant differences in how FEN1 interacts with the sliding clamps and how the sliding clamps interact with upstream duplex DNA. We propose that the PCNA complex is more dynamic, consistent with PCNA's role in replication and that the 9-1-1 complex is more stable, consistent with 9-1-1 acting locally at the DNA damage.

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Roadblocks on the E.Coli Genome: The Workings of a Molecular Mouse Trap at the Single-Molecule Level

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During DNA replication in E.coli, replication forks are prevented from moving beyond the termination site by Tus proteins that bind to specific, asymmetric Ter DNA sites. The Tus-induced blockage is known to depend on the fork's direction of approach. It is hypothesized that strand separation on the blocking side triggers the formation of a molecular roadblock by the binding of a base to a high affinity site on the Tus protein. In this study, we use single-molecule multiplexed magnetic tweezers to investigate the origin of the asymmetry of the Tus-Ter block. We use 1kb DNA hairpins that contain a single Ter site in either the permissive or non-permissive orientation. Application of pulling forces >16pN causes mechanical unzipping of the hairpins, thereby mimicking the DNA unwinding that accompanies DNA replication. When wtTus binds to the hairpin with the permissively oriented Ter site, strand separation is transiently blocked; conversely, when wtTus is bound to the non-permissive Ter hairpin, strand separation is fully blocked. Under physiological conditions the non-permissive Tus-Ter lock was ubiquitous, independent of the loading rate, and withstood the largest forces we could apply (>70pN). Interestingly, the probability of lock formation strongly depends on the ionic strength of the solution. Our ability to distinguish simple DNA binding from the locking process and to apply this to a series of Tus mutants allows us to put together a complete picture of the Tus-Ter lock.

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Sliding, Pausing and Bridging: How Human XRCC4 and XLF Interact with DNA

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Non-Homologous End Joining (NHEJ) is an efficient mechanism to repair DNA double-strand breaks. XRCC4 and XLF are two structurally-related core NHEJ proteins. They can directly interact at the protein-protein level and interact with DNA by an unknown mechanism. Here, we use optical tweezers and fluorescence microscopy to visualize XRCC4-XLF complexes on DNA in real time. We find that the behavior of XRCC4-XLF on DNA is multi-faceted: complexes rearrange continuously and show periods of rapid diffusion along the DNA, interspersed by stationary binding. By manipulating two DNA molecules independently we study bridge formation and demonstrate that XRCC4-XLF complexes can directly bridge two independent DNA molecules. Our approach reveals the transient and dynamic aspects of the interaction of XRCC4-XLF with DNA providing new insights on how they could function during DNA double-strand break repair by NHEJ.

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Single DNA Glycosylase Molecules Diffuse One-Dimensionally and use a Wedge Residue to Probe for Oxidatively Damaged Bases

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DNA glycosylases are enzymes that maintain genomic stability by identifying and excising damaged bases from DNA. Structural and biochemical studies implicate a key "wedge residue" in the recognition of oxidized bases. To define how glycosylases locate damaged bases, we tracked and characterized the motion of single, Qdot-labeled bacterial glycosylases (Fpg, Nei, and Nth) and their alanine-substituted wedge variants on normal and oxidatively damaged lambda DNA tightropes. All three wildtype glycosylases diffused along undamaged DNA with an effective 1D diffusion coefficient of $0.004 \pm 0.001 \mu m^2/s$ and binding lifetime of $1.2 \pm 0.2s$. In contrast, the wedge variants diffused 10-fold faster with no change in their bound lifetime, suggesting that the wedge residue is involved in base interrogation. On damaged DNA, wildtype glycosylases bound 2.5 times longer and diffused 80% slower due to the emergence of stationary periods during a trajectory. To explain these data, we developed a multistate chemo-mechanical simulation in which glycosylases diffuse along DNA, punctuated with momentary pauses during base interrogation by the wedge residue. Upon recognition, a damaged base is excised, leading to a prolonged pause as observed experimentally with damaged DNA. Although they diffuse faster than their wildtype counterparts, the wedge variants also exhibited 56-98% slower diffusion in response to DNA damage. This can be explained by a 4-fold reduction in the rate at which these mutant enzymes interrogate the DNA. These results combined with structural data in the literature suggest that damaged base detection requires glycosylases to scan long stretches of DNA, utilizing only thermal energy while interrogating bases by insertion of the wedge residue into the DNA at a frequency sufficient to detect damage and to successfully excise the damaged base in the first step of base excision repair.

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One ORC with Many Faces

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The eukaryotic chromosomal Origin Recognition Complex (ORC) is a six-protein ATPase complex that not only binds to origin DNA, but together with Cdc6, also loads the Mcm2-7 helicase onto DNA. We have visualized by cryo-EM how the yeast ORC alters its structure as it interacts with and responds to its partners. The yeast ORC is a bi-lobed structure with Orc1, 4, and 5 in one lobe and Orc2, 3, and 6 in the other. ORC alone is flexible and binding to DNA rigidifies the structure. The replication initiator Cdc6 profoundly changes the ORC structure. Cdc6 completes a ring by bridging the bi-lobed structure from the side, and in doing so, nearly doubles the DNase I footprint. Cdc6 also causes Orc1 to rotate against Orc4. These changes lie at the heart of the molecular switch that transforms the ORC from a passive DNA binder to an active helicase loader. Using cryo-EM, we have caught ORC in the act of loading the first MCM2-7 hexamer. More precisely, we have captured a 1.1-MDa 14-protein complex comprised of ORC-Cdc6-Cdt1-Mcm2-7 in the presence of ATP-γS. ORC is transformed into a spiral and has partially loaded the Mcm2-7 hexamer onto dsDNA, as DNA density is visible going through the ORC-Cdc6 spiral and into the Mcm2-7 central chamber. This loading mechanism bears striking similarity to the loading of PCNA ring by the RFC ATPase spiral. ORC will eventually recruit a second MCM2-7 hexamer and deposit a Mcm2-7 double hexamer on dsDNA before letting go of the latter. We have established the architecture of the double hexamer, providing insights into Dbf4-dependent Cdc7 kinase regulation.

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Interactions Between the *E. Coli* Sos Response Protein Umud and DNA Polymerase III Alpha Subunit Have Implications for Regulating Replication in Response to DNA Damage

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Faithful and efficient DNA replication is critical for the survival of all organisms; however, DNA is constantly subject to damage from endogenous and exogenous sources. Attempts to replicate damaged DNA can result in harmful