is catalysed by AddAB helicase-nuclease complexes; motor proteins that unwind the DNA duplex and degrade the nascent single-strands in a manner regulated by specific single-stranded DNA sequences called Chi recombination hotspots (Yeeles and Dillingham, 2007; Yeeles et al., 2011). We have used Magnetic Tweezers to investigate the real-time dynamics of AddAB translocation on dsDNA and the effect of recombination hotspot recognition on this process. AddAB translocation traces showed a complex appearance with variable velocities between 200-400 bp/s at room temperature. We found that AddAB was prone to stochastic pausing in areas which contained many Chi-like sequences. Experiments using an AddAB mutant that is unable to recognize Chi strongly suggest that this pausing is due to transient recognition of Chi-like sequences and highlight the antagonistic nature of DNA translocation and sequence specific DNA recognition activities. Experiments using substrates containing bona fide Chi sequences showed that AddAB also pauses at Chi, but these events are longer and not exponentially distributed, suggesting a multistep process. We propose a model for the recognition of Chi and Chi-like sequences to explain the origins of this pausing behavior during failed or successful hotspot recognition.

Yeeles, J. T., and Dillingham, M. S. (2007). A dual-nuclease mechanism for DNA break processing by AddAB-type helicase-nucleases. J Mol Biol 371, 66-78.

Yeeles, J. T., van Aelst, K., Dillingham, M. S., and Moreno-Herrero, F. (2011). Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Molecular Cell 42, 806-816.

2777-Plat

Single-Molecule Studies of Nucleosome Translocation by the ACF Chromatin Remodeling Complex

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The packaging of eukaryotic genomes as chromatin restricts access to the DNA for critical processes such as replication, transcription, recombination, and repair. Thus, eukaryotic cells depend on a dynamic balance between genome compaction and access facilitated in part by chromatin remodeling enzymes (remodelers). Remodelers read epigenetic marks such as histone modifications and use the energy of ATP hydrolysis to assemble, disassemble, reposition, and modify the composition of nucleosomes. Single-molecule techniques enable characterization of transient intermediates formed during the remodeling reaction and can therefore uncover previously unobtainable insights into the mechanisms of chromatin remodeling.

In this study, we developed a single-molecule fluorescence resonance energy transfer assay to study the dynamics of chromatin remodeling by human ACF, a prototypical member of the ISWI family of remodelers. ISWI remodelers are endowed with the ability to create regularly-spaced nucleosome arrays characteristic of transcriptionally silent heterochromatin.

With the addition of ACF and ATP, nucleosomes exhibit gradual translocation along the DNA interrupted by kinetic pauses after approximately seven or three base pairs (bp) of translocation, thereby dividing the remodeling process into alternating translocation phases and pause phases. Moreover, we found that ACF is a highly processive and bidirectional nucleosome translocase capable of sliding a nucleosome back-and-forth for an average of 200 bp before dissociating.

The nucleosome spacing activity of ISWI remodelers is regulated by two substrate features: (1) length of linker DNA, and (2) histone H4 N-terminal tail. Shortening the linker DNA or altering the H4 tail decrease the catalytic activity of ISWI remodelers with little effect on binding affinity. We discovered that the catalytic defects associated with these substrate modifications are attributed solely to changes in the pause phases and not the translocation phases, providing new insights into how nucleosomal features regulate chromatin remodelers.

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Spoiiie Mechanism of Directional Translocation Involves Target Search Coupled to Allosteric Motor Activation

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SpoIIIE/FtsK are membrane-anchored, ATP-fueled, directional motors responsible for chromosomal segregation in bacteria. Directionality in these motors is governed by interactions between specialized sequence-recognition modules (SpoIIIE-γ/FtsK-γ) and highly-skewed chromosomal sequences (SRS/KOPS). using a novel combination of bulk and single-molecule methods we dissect the series of steps required for SRS localization and motor activation. First, we demonstrate that SpoIIIE/DNA association kinetics are sequence-independent with binding specificity being uniquely determined by dissociation. Next, we show by single-molecule and modeling methods that hexameric SpoIIIE binds DNA non-specifically and finds SRS by an ATP-independent target search mechanism, with ensuing oligomerization and binding of SpoIIIE-γ to SRS triggering allosteric motor activation. We propose a new model that provides an entirely novel interpretation of previous observations for the origin of SRS/KOPS-directed translocation by SpoIIIE/FtsK.

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E. Coli RNA Polymerase Searches for Promoters through 3D Diffusion

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Gene expression, DNA replication, and genome maintenance all start with site-specific DNA binding proteins, which must recognize specific targets from among a vast excess of nonspecific DNA. For example, to initiate transcription, E. coli RNA polymerase (RNAP) must locate promoter sequences, which comprise <2% of the bacterial genome. This promoter search problem remains one of the least understood aspects of gene expression, largely due to the transient nature of intermediates involved in the search process. Here we use single-molecule microscopy to visualize RNAP in real time as it searches for promoters, and we develop a theoretical framework that allows us to analyze target searches at the submicroscopic scale based on single-molecule promoter association rates. Contrary to long-held assumptions, we demonstrate that the promoter search by E. coli RNAP is dominated entirely by 3D diffusion, which has direct implications for understanding how E. coli RNAP and other proteins locate their targets within physiological settings.

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The ATP Hydrolysis Cycle and the Corresponding Motion of RecA Filament on Single-Stranded DNA

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RecA is involved in DNA repair mechanism in bacteria by catalyzing homologous strand exchange reaction. RecA forms a filament on a single-stranded DNA made at the double-strand breaks. RecA adopts either a stretched (active) structure with ATP as a cofactor, or a compressed (inactive) structure with ADP. At the filament end, a monomer is allowed to dissociate after ATP hydrolysis and to rebind. We developed a single-molecule fluorescence assay to investigate how the ATP hydrolysis is coupled with the dynamics of the RecA filament. We dissected the intermediate steps of ATP hydrolysis and discovered that a monomer at the filament end dissociated upon the hydrolysis of ATP and not after the release of Pi. Interconversion of the structure between stretched- and compressed forms was achieved via cooperative structural change of a group of neighboring monomers. Owing to this cooperativity, an internal RecA monomer continuously consumes and rapidly refreshes ATP molecule without changing its stretched conformation. Based on our observation, we suggest a model of ATP hydrolysis cycle of the RecA in the filament.

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How DNA-Binding Proteins find their Target Sites in Human Cells

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Precise and timely expression of specific genes is fundamental for the viability of any organism. Specific DNA-binding proteins (transcription factors, TFs) transiently associate to promoter sequences on the genome to activate or repress transcription of specific genes. The way TFs explore the nuclear space, find, and bind to their target sequences is the key to decipher transcriptional control but is still subject of debate.

We exploit a live-cell model system to identify what factors primarily orchestrate TFs target search. Our assay is based on human cells containing, at a single locus, specific DNA binding sites ('target') for an exogenous DNA-binding protein (TetR, 'searcher'). First, single-particle-tracking experiments revealed that individual TetR proteins move in a very composite way. They transiently interact with nonspecific DNA sequences, via their DNA-binding-domain, with residence times ranging from hundreds of millisecond up to few seconds. Furthermore, a subset of proteins shows confinement within micron-sized regions