Platform P: Member-Organized Session: Biophysical Approaches to Study Nucleoid Compaction, Recombination & Gene Regulation

1067-Plat
Direct Visualization of Fis-DNA Interactions
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The multistep kinetics through which DNA-binding proteins bind their targets is heavily studied, but relatively little attention has been paid to mechanisms of how proteins leave the double helix. We used a single-DNA stretching and fluorescence detection approach to study the kinetics of unbinding of the *E. coli* nucleoid-associated protein Fis, which is known to have site-specific regulatory and nonspecific chromosome-compacting functions. We find that a fraction of Fis bound to DNA spontaneously dissociates into protein-free solution leaving some Fis tightly bound to the double helix on cell-cycle-long timescales. However, if Fis is present in solution, we find that a concentration-dependent exchange reaction occurs which turns over all the bound protein, with a rate of $k_{exch} = 4\text{x}10^5\ \text{M}^{-1}\text{sec}^{-1}$. Thus, solvated proteins can play a key role in facilitating removal and renewal of proteins bound to the double helix. In addition, we compare the pattern of Fis binding along the DNA to the base-pair sequence and find a greater amount of Fis bound to GC-rich regions than to AT-rich regions.

1068-Plat
DNA Shape Recognition by the Nucleoid Protein Fis and Its Role in Chromosome Compaction and DNA Recombination
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The Fis nucleoid-associated DNA bending protein dynamically binds DNA in a largely sequence-neutral manner. Fis also forms stable complexes to DNA segments that share little sequence conservation. In order to understand how Fis is forming these specific DNA complexes and how it distorts DNA structure upon binding, we have determined X-ray crystal structures of 12 Fis-DNA complexes of variable affinities and stabilities. These structures reveal that Fis selects targets primarily through indirect recognition mechanisms involving the shape of the DNA minor groove and sequence-dependent induced fits over adjacent major groove interfaces to generate overall curvatures of 60-75°. In silico DNA modeling of Fis-bound and unbound DNA molecules suggests a model in which Fis initially selects binding sites based on their intrinsic minor groove shape and then bends the DNA to generate the bound structure. The structures show how binding of Fis leads to DNA compaction, and the location of residues involved in capturing DNA loops implies protein-protein interactions are required for loop stabilization. We have shown that DNA supercoiling and torsional energy associated with the Fis-bound DNA enhancer segment controls the rotational direction and number of Hin subunit translocations that mediate DNA exchange during Hin-catalyzed site-specific DNA inversion. The Fis-DNA structures, together with supporting experimental evidence, enable us to propose a new model for the structure of the Fis-bound Hin invertasome structure.

1069-Plat
Fidelity and Target Location During RecA-Catalyzed Homologous Recombination
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Homologous recombination plays pivotal roles in DNA repair and in the generation of genetic diversity from prokaryotes to man. To locate homologous target sequences at which strand exchange can occur within a timescale that a cell's biology demands, a single-stranded DNA-recombinase complex must search among an extraordinarily large number of sequences on a genome, by forming synapses with chromosomal segments of DNA. A key element in the search is the time it takes for the two sequences of DNA to be compared, i.e. the synapse lifetime. Here we visualize for the first time fluorescently-tagged individual synapses formed by RecA, a prokaryotic recombinase protein, and measure their lifetime as a function of synapse length and differences in sequence between the participating DNAs. Surprisingly, lifetimes can be ~10 seconds long when the DNAs are fully heterologous, and much longer for partial homology. Synapse lifetime increases rapidly as the length of a region of full homology at the 3' end of the invading single-stranded DNA increases above 30 bases. Few mismatches can reduce dramatically the lifetime of synapses formed with nearly-homologous DNAs. Analogous measurements in the case of Rad51 are expected to yield even longer lifetimes. These results suggest the need for facilitated homology search mechanisms to complete successfully the location of homology within the timescales observed *in vivo*, and the implications of these findings on the eukaryotic case will be discussed.

1070-Plat
Biophysical Studies of H-NS Binding to DNA
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Heat-stable nucleoid structuring protein (H-NS) is an abundant prokaryotic protein that organizes chromosomal DNA and plays an important role in gene silencing. Here we report that H-NS can have two distinct binding modes that can be switched from one to another by changes in divalent ion concentrations. Further, we show that the switching does not require dissociation of H-NS from the DNA. Our finding resolves an important controversy in the field in which mutually exclusive observations of H-NS/DNA interaction were reported. In single-molecule manipulation experiments, one binding mode leads to stiffening of DNA backbone, while the other leads to DNA folding. AFM imaging showed that the stiffening is caused by polymerization of H-NS along DNA starting from a few nucleation sites, while the folding is caused by formation of large DNA hairpins. The transition from stiffening to bridging occurs when the magnesium or calcium concentration is around or above 5 mM, suggesting that both binding modes are likely present at physiological conditions. Furthermore, the susceptibility of the two binding modes to physiological stimuli (pH and temperature), which are known modulators of H-NS activity, are discussed.

1071-Plat
The Role of SsrB And H-NS in Transcription Activation and Silencing/ Anti-Silencing During Salmonella Pathogenesis
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In bacterial pathogenesis, virulence gene regulation is controlled by two-component regulatory systems. In Escherichia coli, the EnvZ/OmpR two-component system regulates expression of outer membrane proteins, but in Salmonella enterica, OmpR activates transcription of the SsrA/B two-component system located on pathogenicity island 2 (SPI-2). SsrB controls expression of a type three secretory system whose effectors modify the vacuolar membrane and prevent its degradation via the endocytic pathway. Vacuolar modification enables Salmonella to survive and replicate in the macrophage phagosome and disseminate to the liver and spleen to cause systemic infection. The signals that activate EnvZ and SsrA are unknown, but are related to the acidic pH of the vacuole. SsrB binds to regions of DNA that are AT rich, with poor sequence conservation (Liao et al., J Biol Chem 284: 12008-12019, 2009). Although SsrB is a major virulence regulator in Salmonella, very little is known regarding how it binds DNA and activates transcription. Pathogenicity island genes are silenced by the heat-stable protein H-NS (Lucchini et al., PLoS Pathog 2: e81, 2006; Navarre et al., Science 313: 236-238, 2006). How transcription factors counter or relieve H-NS silencing is presently a major focus of study. The sifA gene is located outside of SPI-2 and encodes a product required for maintenance of the Salmonella-containing vacuole, involving formation of Salmonella-induced filaments or Sifs (Stein et al., Mol Microbiol 20: 151-164, 1996). SsrB directly activates expression of the sifA gene and relieves H-NS silencing to activate transcription. We used atomic force microscopy and single molecule experiments to examine H-NS/DNA interactions and the effect of SsrB on H-NS/DNA binding. Our recent findings will be discussed. Supported by NIH GM-058746, VA BX000372-01 to LJK and the RCE in Mechanobiology, National University of Singapore, Singapore.

1072-Plat
Unravelling the Role of Alba in the Organization of the Archaeal Nucleoid
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Alba is one of the most abundant proteins in thermophilic and hyperthermophilic archaea and is believed to play an important role in DNA organization. It is a dimeric protein that binds DNA with no apparent sequence specificity. Earlier studies have shown that Alba is capable of bridging DNA duplexes, which may be key to its organizational role. However, a comprehensive understanding regarding the action of Alba in DNA organization is currently lacking. Using a combination of single-molecule imaging and micromanipulation techniques we now define mechanistic, structural and kinetic aspects of the Alba-DNA interaction.