surface for large RNA substrates that spans both RecA-like domains and the RBD. The RNA-binding sites of the helicase cores face each other, possibly enabling subunit communication. The plasticity of the dimerization motif allows for drastic changes in the juxtaposition of the helicase cores within the dimer. Simultaneous action of the Hera subunits in the dimer on the same large RNA molecule may be important for efficient remodeling of *in vivo* RNA substrates.

381-Pos

Dna Looping By Lactose Repressor Requires Tetramer Opening

Danielis Rutkauskas[1], Hongli Zhan[2], Kathleen S. Matthews[2], Francesco S. Pavone[1], **Francesco Vanzi[1]**.
[1]LENS - University of Florence, Sesto Fiorentino (FI), Italy, [2]Rice University, Houston, TX, USA.

Transcription of the bacterial genes involved in lactose metabolism is controlled by lactose repressor protein (LacI). LacI can bind simultaneously to two operators, forming a loop on the intervening DNA. Looping is essential for efficient repression, as demonstrated by the effects of deletion of the auxiliary operators. The protein is a dimer of dimers: in the crystal structure of LacI, the two dimers are arranged in a V-shape, and each dimer binds a DNA operator. Recently, theoretical and experimental lines of evidence have suggested various possible loop structures associated with different LacI tetramer conformations (adopted by varying the inter-dimer angle through flexion at the C-terminal tetramerization domain). Different DNA binding topologies can also contribute to the complexity of available protein/DNA conformations. We employed the single-molecule tethered particle motion (TPM) method, in combination with chemical crosslinking of LacI protein mutants, to specifically address the role of tetramer opening in loop formation. Measurements on the wild-type and mutant LacI variants, with native cysteines removed and single cysteines placed at selected sites, confirmed previous observations of two distinct levels of short tether length, associated with two different DNA looping structures. Restricting conformational flexibility of the protein to various degrees by chemical crosslinking of the introduced cysteines with reagents of different spacer-arm lengths induces pronounced effects. Crosslinking the dimers at residue 36 (in the N-terminal DNA binding domain) completely suppresses looping (with no effect on binding to 40 bp operator DNA). Crosslinking at position 231 (near the C-terminal tetramerization domain) changes the looping geometry as detected by TPM. These observations lead to the conclusion that tetramer opening plays a definite role in at least a subset of LacI/DNA loop conformations in which the protein clearly must adopt a structure very different from the classic crystallographic V-shape.

382-Pos

Nucleic Acid Interaction Kinetics of APOBEC3G Investigated Using Ensemble and Single Molecule Methods

Ioulia Rouzina[1], Dominic Qualley[2], Tiyun Wu[3], Yasumasa Iwatani[4], Hylkje Geertsema[5], Denise Chan[6], Amber Hertz[3], Mark C. Williams[5], Judith Levin[3], Karin Musier-Forsyth[2].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Ohio State University, Columbus, OH, USA, [3]National Institutes of Health, Bethesda, MD, USA, [4]National Hospital Organization Nagoya Medical Center, Nagoya, Japan, [5]Northeastern University, Boston, MA, USA, [6]University of Pittsburgh Medical School, Pittsburgh, PA, USA.

Human APOBEC3G (A3G) is a host cell cytidine deaminase capable of restricting replication of retroviruses by deaminating ss viral DNA and also by directly inhibiting reverse transcriptase (RT)-catalyzed polymerization reactions. Only about 7 A3G molecules are packaged per HIV virion. Deamination by A3G may only happen during the short period that viral (-) strand DNA is available, necessitating rapid on/off nucleic acid binding kinetics. In contrast, in order for just a few A3G molecules to inhibit DNA polymerization by RT, they have to form a ''roadblock'', requiring very slow protein dissociation from DNA. Here, we use SPR and single molecule DNA stretching to investigate the DNA/A3G interaction kinetics. Our results suggest that: (i) A3G binds ssDNA with moderate cooperativity (Hill constant ~1.5), a binding site size of ~15 nt, and a K_d of ~60 nM; (ii) The on/off kinetics of A3G/ssDNA is unusually slow and multi-rate; the dominant ''on'' component has a bimolecular rate constant of ~10^5 $M^{-1}s^{-1}$; (iii) Dissociation of A3G from ssDNA has a fast and a slow component. The fraction of the slow component and the off times increase with longer incubation over ~100 s**.** Taken together, our data are consistent with the existence of both ''fast'' and ''slow'' A3G/DNA binding modes. We hypothesize that the fast mode is a feature of protein dimers, whereas the slow mode is characteristic of multimeric A3G, with protein multimerization on ssDNA occurring over an ~100 s time period.

383-Pos

A Multiscale Model To Analyze the Sliding Movement of Repressor Proteins on DNA

Simone Furini[1], Carmen Domene[2], Silvio Cavalcanti[3].
[1]University of Siena, Siena, Italy, [2]University of Oxford, Oxford, United Kingdom, [3]University of Bologna, Bologna, Italy.

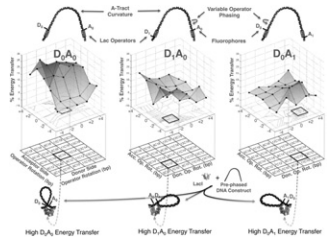
Repressor proteins (RP) regulate gene transcriptions by binding to target sequences, named operator sites, on the DNA molecule. Association rates higher than the diffusion limit were measured in several RP. These experimental data led to the facilitated diffusion model. Facilitated diffusion requires nonspecific binding of the RP to the DNA. Then, the searching for the target sequence proceeds in a reduced search space. In agreement with this model, a structure of the RP LacI bound to nonspecific DNA was revealed by NMR, and one-dimensional movements of the same protein along DNA were observed by single molecule imaging. Single molecule imaging cannot provide a molecular description of how the movement occurs at the molecular level, and two hypotheses were formulated: i) sliding of the RP, in continuous contact with the DNA major grove; ii) hopping of the RP between adjacent binding sites. The continuous contact between the protein and the DNA major grove can result only from a helical trajectory of the RP around the DNA molecule. We simulated the sliding motion of the LacI protein along this helical trajectory by a multiscale model than integrates data from molecular dynamics (MD) simulations in stochastic dynamics. The multiscale approach was necessary to extend the timescale accessible by brute-force MD, and simulate dynamics on the millisecond timescale. MD simulations were used to compute the local diffusion coefficient and the potential of mean force for the sliding movement. These data were then used in the stochastic simulations, to simulate the dynamics on a millisecond time scale, and identify the characteristics of the hypothetic sliding motion. Since the parameters of the stochastic equation were computed by MD simulations, the multiscale model is strictly based on the microscopic characteristics of the molecular system.

384-Pos

A LacI-DNA Looping Landscape and Allosteric Effects on the Loop Shapes

Aaron R. Haeusler, Kathy Goodson, Jason D. Kahn.
Univ. Maryland College Park, College Park, MD, USA.

The lac operon and its repressor (LacI) are the prototype model for gene regulation. LacI negatively regulates lacZYA by binding a primary DNA operator site overlapping the promoter, and repression is enhanced by secondary operators that deliver LacI via looping intervening DNA. LacI's ability to form stable loops with a variety of DNA lengths has been attributed to protein flexibility and/or to multiple loop topologies. Previously developed DNA constructs in which looping is hyperstabilized by an A-tract bend placed between two operators provide different loop shapes depending on the operator/bend helical phasing. Here, FRET is used to characterize the sequence/structure landscape of a set of related constructs with systematically varied operator/bend phasings. Donor and acceptor fluorophores positioned on either side of the operator provide multiple distance constraints on the orientations of the LacI-DNA loop. The results suggest that LacI can form many different looped states whose relative energetics can be measured. Also, IPTG addition demonstrates that inducer-bound LacI still forms stable loops, probably with different geometries relative to the repressed state. This comprehensive looping landscape should allow determination of whether protein flexibility is necessary to explain the results.



385-Pos

RNA Looping By PTB: Evidence Using Fret and NMR Spectroscopy and For a Role in Splicing Repression

Rajan Lamichhane[1], Gerrit M. Daubner[2], Judith Thomas-Crusells[2], Sigrid D. Auweter[2], Cristina Manatchal[2], Keyunna S. Austin[1], Oksana Valniuk[1], Frédéric H.-T. Allain[2], David Rueda[1].
[1]Wayne State University, Detroit, MI, USA, [2]Institute for Molecular Biology and Biophysics, ETH Zurich, Switzerland.

Polypyrimidine Tract Binding protein (PTB) is a key alternative splicing factor involved in exon repression. It has been proposed that PTB acts by looping out exons flanked by pyrimidine-tracts. We present fluorescence, NMR and in vivo splicing data that directly support this mechanism. We show that the RNA recognition domains (RRM) 3 and 4 of PTB can bind two distant pyrimidine-tracts and bring their 5′ and 3′ ends in close proximity, thus looping the RNA.