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.

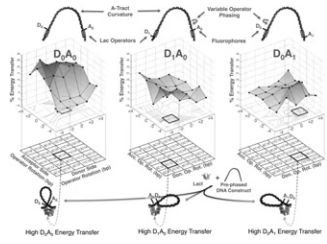
Repressor proteins (RP) regulate gene transcriptions by binding to target sequences, named operator sites, on the DNA molecule. Association rates higher than the diffusion limit were measured in several RP. These experimental data led to the facilitated diffusion model. Facilitated diffusion requires 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.

Looping efficiency depends on the length of the intervening sequence with preference for a 15 nucleotides spacer or longer between the pyrimidine-tracts. RRMs 3 and 4 bind the 5′ and the 3′ pyrimidine-tracts, respectively, in a specific directionality, and work synergistically for efficient RNA looping in vivo.

386-Pos

Single-Molecule Imaging of DNA Curtains Reveals Intrinsic Energy Landscapes For Nucleosome Deposition

Mari-Liis Visnapuu, Eric C. Greene.
Columbia University, New York, NY, USA.

We employ nanofabricated diffusion barriers to organize DNA into molecular curtains allowing us to directly image thousands of aligned molecules and determine coarse-grained intrinsic energy landscapes for nucleosome deposition on model DNA substrates. Our results reveal distributions that are correlated with recent in silico predictions, reinforcing the hypothesis that DNA contains some intrinsic positioning information. We show that cis-regulatory sequences in human DNA coincide with peaks in the intrinsic landscape, whereas valleys correspond to non-regulatory regions, and we present evidence arguing that nucleosome deposition in vertebrates is influenced by factors not accounted for by current theory. We also demonstrate that intrinsic landscapes of nucleosomes containing the centromere-specific variant CenH3 are correlated with patterns observed for canonical nucleosomes, arguing that CenH3 does not alter sequence preferences of centromeric nucleosomes. However, the non-histone protein Scm3 alters the intrinsic landscape of CenH3-containing nucleosomes, enabling them to overcome the otherwise exclusionary effects of poly(dA-dT) tracts, which are enriched in centromeric DNA. In addition, these methods establish a platform that allows direct visualization of DNA binding proteins, DNA translocases and chromatin remodelers as they interact with single fluorescent nucleosomes and denser chromatin arrays.

387-Pos

Fluctuating Forces Facilitate Protein-Mediated DNA Looping

Yih-Fan Chen, J.N. Milstein, Jens-Christian Meiners.
University of Michigan, Ann Arbor, MI, USA.

Lac repressor-mediated DNA looping has become a paradigm for long-range genetic regulation. Our earlier experimental results have shown that forces on the order of a hundred femtonewtons can drastically disrupt the formation of DNA loops. This exquisitely high sensitivity to applied force implies that tension in the DNA, two orders of magnitude smaller than typical piconewton intracellular forces, may provide a mechanical pathway for transcriptional control.

We investigate how such mechanical switching is affected by fluctuating forces instead of static forces inside a cell. Our results show that by slightly increasing the magnitude of the fluctuations, which are on the order of tens of femtonewtons, the DNA loop formation rate can be significantly increased while the magnitude of the average tension in the DNA remains the same. This result contributes to our understanding of how protein-mediated DNA looping processes, which are extremely sensitive to force, can function in a noisy *in vivo* environment.

To study the effects of force fluctuations on DNA looping, a random series of optical forces displaying the statistics of Gaussian white noise is applied to a surface-tethered DNA molecule by axial optical tweezers. The lifetimes of the looped and unlooped states are measured under fluctuating forces that have the same average magnitude but different fluctuation strengths. Our results show that, as compared to the noise free case, the lifetime of the unlooped state decreases by about a factor of two when fluctuations on the scale of tens of femtonewtons are applied while the lifetime of the looped state remains constant.

388-Pos

On the Structure, Function and Metalloregulatory Properties of the Zinc-Activated Repressor *Streptococcus Pneumoniae* AdcR

Alfredo J. Guerra[1], Hermes Reyes-Caballero[1,2], Faith E. Jacobsen[1], Uma Mahendra Kumar Koppolu[3], Robert A. Scott[3,4], Krystyna M. Kazmierczak[5], Malcolm E. Winkler[5], David P. Giedroc[1].
[1]Department of Chemistry, Indiana University, Bloomington, IN, USA, [2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA, [3]Department of Chemistry, University of Georgia, Athens, GA, USA, [4]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA, [5]Department of Biology, Indiana University, Bloomington, IN, USA.

A response to a change in transition metal ion concentration is mediated by metal-sensing transcriptional regulators that harbor metal-specific coordination sites. Zinc homeostasis in the gram positive human pathogen *Streptococcus pneumoniae* (*Spn*) is maintained by two novel zinc-regulated repressors, SczA and AdcR. *Spn* AdcR is the first putative metal-dependent member of the MarR family of transcriptional repressors. Expression profiling on BHI media under microaerobic conditions with a wild-type and isogenic Δ*adcR* strain reveals that AdcR regulates the expression of genes encoding the high affinity zinc uptake system *adcRCBA*, a group of zinc-binding pneumococcal histidine triad proteins (PhtA, PhtB, PhtD and PhtE) and an orphan AdcA homologue (AdcAII). Much of the *adcR* regulon is necessary for the virulence of *Spn*. Analytical ultracentrifugation experiments reveal that AdcR is a 32 kDa homodimer. X-ray absorption spectroscopy is consistent with a primary five-coordinate N/O regulatory site, a finding unprecedented for a zinc-sensing metalloregulatory protein. As expected, Zn(II) binding strongly activates *adc* operator DNA binding on the basis of quantitative fluorescence anisotropy assays (pH 6.0, 0.2 M NaCl, 25°C). Nearly complete backbone (H_N, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$) resonance assignments of apo-AdcR (pH 6.0, 0.05 M NaCl, 35°C) reveal a highly α-helical two-fold symmetric homodimer, and that zinc binding perturbs resonances in the C-terminal regulatory domain, as well as the N-terminal winged helical DNA binding domain. Mutagenesis of at least two His in a highly conserved histidine-rich sequence in the regulatory domain (His108, His112), significantly modulates zinc regulation in vitro and in vivo. Progress on the solution structure and residue-specific dynamics of AdcR in the apo- and zinc activated states will be reported. Supported by NIH grants GM042569 (to D.P.G.), F32 AI084445 (to F.E.J.), GM042025 (to R.A.S.) and AI060744 (to M.E.M).

389-Pos

DNA Structure Specificity of Bacillus Stearothermophilus PcrA

Karen R. Thickman, Sanford H. Leuba, Saleem A. Khan.
University of Pittsburgh, Pittsburgh, PA, USA.

Helicases are molecular motors that play critical roles in nucleic acid transactions, including replication, repair, recombination, and transcription. As observed in a number of diseases resulting from mutations in helicase genes, helicases are important for maintenance of cellular functions. A feature of these diseases is an increase in malignancies as a result of genome instability resulting from unregulated DNA recombination. Gram-positive bacteria harbor a conserved helicase, PcrA, which is involved in UV-damage DNA repair, plasmid rolling-circle replication, and regulation of DNA recombination. PcrA has been shown to inhibit RecA-mediated DNA strand exchange reaction and displace RecA from the DNA. Homologs of PcrA, including Rep and UvrD helicases, have been shown to be 3′ to 5′ helicases. However, PcrA homologs from *Staphylococcus aureus* (*S. au.*), *Bacillus anthracis*and *Streptococcus pneumoniae*also exhibit 5′ to 3′ helicase activity. In these studies, we have explored the directionality and DNA structure specificity of *Bacillus stearothermophilus* (*B. st.*) PcrA. We have demonstrated that *B. st.* PcrA does not have 5′ to 3′ directionality on standard partially duplex DNA substrates containing a 5′ oligo dT tail. However, similar to *S. au.*PcrA, *B. st.* PcrA unwound DNA substrates with a hairpin structure found at the dsDNA replication origin in the rolling-circle replication plasmid pT181 with high efficiency. These included substrates with only a 5′ single-stranded region. These results indicate that though the *S. au.* and *B. st.* PcrAs are 60% identical, they have different activities. Our future work will explore the amino acid sequence differences in these helicases that lead to their differential biochemical activities.

390-Pos

Single Molecule Studies on Hcv RNA Polymerase Activity

Pierre Karam, Colins Vasquez, Wayne Mah, Megan Powdrill, Matthias Götte, **Gonzalo Cosa**.
McGill University, Montreal, QC, Canada.

NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis de novo. However, the detailed underlying mechanism remains elusive. It is unclear how the enzyme locates the 3′-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3′-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single -donor (Cy3)/acceptor (Cy5) fluorophore labeled- RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise binding of NS5B to the template. Taken together, our single molecule studies provide direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates