Lac repressor (LacI) controls transcription of the genes involved in lactose metabolism. A key role in LacI function is played by its ability to bind simultaneously to two operators, forming a loop in the intervening DNA. Recently, several lines of evidence (both theoretical and experimental) have suggested the possibility for the LacI tetramer to adopt different structural conformations by flexing about its C-terminal tetramerization domain. At present it remains unclear to what extent different looping geometries are due to DNA binding topologies rather than distinct protein conformations. We address these questions by employing single molecule tethered particle motion on LacI mutants with intratetramer crosslinking at different positions along the cleft between the two dimers. Measurements on wild-type LacI reveal the existence of three distinct levels of effective tether length, most likely due to the presence of two different DNA looped structures. Restricting conformational flexibility with protein by cross-linking induces clear changes in the tether length distributions, indicating profound effects of tetramer opening (and its limitation due to cross-linking) on the looping conformations available to the system. Our data suggest an important role for large-scale conformational changes of LacI in the looping structures and dynamics.

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The Enfolding Arms of EcoRI Endonuclease as Probed by ESR Experiments

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Our research focuses on adducing general principles applicable to site-specific protein-DNA interactions by linking function to structural, thermodynamic and dynamic properties. We use as a model the interaction of EcoRI endonuclease with specific, miscognate (EcoRI*), and nonspecific DNA sequences. The crystal structure of the specific complex shows that the EcoRI "arms", invisible (disordered) in the structure of the apoenzyme, enfold cognate DNA upon binding. We are using four pulse Double Electron-Electron Resonance (DEER) FT-ESR experiments to map distances and distance distributions between nitroxide spin labels placed on cysteine-substituted residues in the two "arms" of the EcoRI homodimer, between Cu^{2+} ions bound near the active sites, and between nitroxide to Cu^{2+} positions. Our data show that the mean point-to-point distances between the "outer arms", between the "inner arms" and from the "outer arm" to the main domain are the same in specific, EcoRI*, and nonspecific complexes. This implies that the EcoRI arms must enfold the DNA in all three classes of complexes. However, an increase in the breadth of distance distributions is observed for noncognate complexes relative to that observed for the tightly complementary specific complex. These results are consistent with inferences from our thermodynamic analyses that the equilibrium ensemble of conformational microstates is larger for noncognate than specific complexes. Our continuous wave (CW) ESR experiments probing the dynamics of the arms support this hypothesis. Nonspecific complexes have been shown to have an important function in accelerating the location of correct recognition sites. It is striking that the EcoRI arms also embrace the DNA in the sliding nonspecific complex.

323-Pos Board B202

Nuclear RISC Originates from Cytoplasmic Loaded RISC in Human Cells

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Studies of RNA interference (RNAi) provide evidence that in addition to the well characterized cytoplasmic mechanisms, nuclear mechanisms also exist. The mechanism by which the nuclear RNA-induced silencing complex (RISC) is formed in mammalian cells, as well as the relationship between the RNA silencing pathways in nuclear and cytoplasmic compartments is still unknown. Here we show by applying fluorescence correlation and cross-correlation spectroscopy (FCS/FCCS) in vivo that two distinct RISC exist: a large ~3 MDa complex in the cytoplasm and a 20-fold smaller complex of ~158 kDa in the nucleus. We further show that nuclear RISC, consisting only of Ago2 and a short RNA, is loaded in the cytoplasm and imported into the nucleus. The loaded RISC accumulates in the nucleus depending on the presence of a target, based on an miRNA-like interaction with impaired cleavage of the cognate RNA. Together, these results suggest a new RISC shuttling mechanism between nucleus and cytoplasm ensuring concomitant gene regulation by small RNAs in both compartments.

324-Pos Board B203

A Dna Mimic Caught In The Act: 3D Electron Microscopy Shows Ecoki Methyltransferase In Complex With The T7 Antirestriction Protein Ocr.

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Type I DNA restriction-modification (R/M) systems are important agents in limiting the transmission of mobile genetic elements responsible for spreading bacterial resistance to antibiotics. EcoKI, a Type I R/M enzyme from Escherichia coli, acts by methylation- and sequence-specific recognition, leading to either methylation of the DNA target or translocation, followed by cutting at a random site, often hundreds of base pairs away. Consisting of one specificity subunit, two modification subunits, and two DNA translocase/endonuclease subunits, EcoKI is inhibited by the T7 phage antirestriction protein Ocr. Ocr mimics DNA with a pseudo-helical arrangement of charges, and is bent at a similar angle to that predicted for target DNA. We present a 3D density map generated by negative stain electron microscopy of the central core of the restriction complex, M.EcoKI M2S1 methyltransferase, bound to dimeric Ocr. Single particle analysis was carried out in IMAGIC and EMAN and resulted in a 3D reconstruction at ~18 Å resolution. An atomic model of all 5 subunits was generated by automated docking and homology modelling. This was computationally fitted into the EM density, giving excellent agreement. Ocr binds through the center of the M.EcoKI complex, spanning the two DNA recognition sites and matching the path predicted for its substrate DNA. We also present a complete atomic model of M.EcoKI in complex with its cognate DNA giving a clear picture of the overall clamp-like operation of the enzyme. The model is consistent with a large body of published experimental data on EcoKI spanning 40 years.

325-Pos Board B204

Investigation of Dnmt1-DNA Interaction using Fluorescence Fluctuation Spectroscopy

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DNA methyltransferase 1 (dnmt1) is an important factor in the epigenetic process of DNA methylation. It is responsible for the regulation of tissue-specific patterns of methylated cytosine residues. Pathological changes in these methylation patterns are connected with various diseases, for example certain types of cancer.

We investigated the functional nature of the interaction between dnmt1 and DNA. A construct was formed, consisting of a synthetic DNA strand, labeled with a synthetic fluorescent dye, and dnmt1, labeled with Green Fluorescent Protein (GFP).

To determine whether the functional form of dnmt1 is monomeric, dimeric or consists of even larger complexes, we measured the ratio of GFP to synthetic dye molecules using Fluorescence Fluctuation methods such as Fluorescence Cross Correlation spectroscopy (FCCS), stoichiometry determination from a Burst Analysis experiment as well as Photon Counting Histograms (PCH) analysis.

326-Pos Board B205

Thermodynamic Characteristics of pre-mRNA Splice Site Recognition

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Here, we reveal that an unusually large enthalpy-entropy compensation underlies recognition of polypyrimidine (Py) splice site signals. Competitive binding to Py tract splice site signals represents a prevalent means for alternative pre-mRNA splicing. The thermodynamic forces driving association of splicing factors with single-stranded (ss) pre-mRNAs represents a gap in the current understanding of splice site selection. We compared Py tract interactions among three splicing factors: (1) $U2AF^{65}$, an essential pre-mRNA splicing factor that recognizes constitutive 3′ splice site signals; (2) Sex-Lethal, a prototypical alternative splicing factor that antagonizes $U2AF^{65}$; and (3) TIA-1, an alternative splicing factor that promotes use of specific 5′ splice sites. All three proteins bound polyuridine (U_{20}) sequences with comparable or higher affinity than natural splice site sequences in fluorescence anisotropy assays. Consistent with the ability of Sex-Lethal to outcompete $U2AF^{65}$ during splice site selection, $U2AF^{65}$ displayed the lowest and Sex-Lethal the highest affinities for the RNA sites.

The enthalpic and entropic contributions were investigated in detail using ITC, initially using the homogeneous U_{20} site to avoid sequence-dependent complications of the binding isotherms. All three splicing factors exhibited an unusually large enthalpy-entropy compensation underlying U_{20} binding, with magnitudes ~10-fold greater than those of typical protein-protein or protein-ligand complexes. Given that full thermodynamic characterizations of protein association with single-stranded RNAs are rare, this raised the question of the source of this unusual thermodynamic signature: Is a large enthalpy-entropy

compensation a general characteristic of ssRNA binding, an inherent property of Py tracts, or a signature of sequence-specific ssRNA recognition? These possibilities are clarified by thermodynamic comparison of purine-tract association by these Py tract splicing factors, contrasted with the purine-specific protein PAB.

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The DNA Bridging Protein H-NS and the SsrB Transcription Factor Counteract One Another to Silence and Activate Pathogenicity Island Genes in Salmonella

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The transcription factor SsrB activates transcription of genes located on Pathogenicity Island 2 (SPI-2) in *Salmonella enterica*. These gene products are responsible for forming a type-three secretion system that secretes effectors that modify the host macrophage vacuole, enabling *Salmonella* to replicate and then disseminate to the liver and spleen. Recent studies have reported that pathogenicity island genes are silenced by the nucleoid-like protein H-NS[1,2]. How transcription factors counter or relieve H-NS silencing is 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, providing an intracellular niche conducive to *Salmonella* replication and survival. Part of this process involves formation of *Salmonella*-induced filaments (Sifs). We set out to determine whether SsrB directly activates expression of the *sifA* gene, and whether H-NS could counter or prevent this interaction. In vitro transcription assays indicate that SsrB directly activates *sifA* transcription and this stimulation is prevented in the presence of H-NS. SsrB activation requires super-coiled templates; in the presence of linear DNA, no *sifA* transcripts are observed. Using atomic force microscopy, we show that H-NS forms multiple bridging complexes on super-coiled DNA. Additional experiments are underway to examine the effect of SsrB on these complexes. Supported by NIH GM-58746 and NSF MCB-0613014 to LJK and NUS R144000171712 to Y.J.

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328-Pos Board B207

TBP Carries Out Specific DNA Binding Involving Information From Both Grooves

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The TATA binding protein (TBP) is a basal transcription factor that binds specifically to the minor groove of TATA boxes. TBP is required for efficient recruitment of the transcription machinery, as it bends DNA, generating binding sites for TFIIB and RNA polymerase II. Only ~10% of human protein coding genes possess a TATA box, and TBP is required for the transcription of the more common TATA-less genes also. We explored the energy contributions to TBP binding of a collection of 16 repeating DNA sequences, in a productive bent conformation. Binding energy is dominated by the cost of deforming DNA from the straight, B-DNA reference, to the conformation found in the complex. Interestingly, there is a positive correlation between the deformation energy of DNA and the interaction energy of DNA with TBP: poor interaction energies, derived mainly from avoided clashes with guanine amino groups and hydrophobic TBP sidechains, lead to greater deformation energies, due to clashes between exocyclic groups at the major groove of DNA. In order to uncouple deformation and interaction, we substituted guanines with inosines, eliminating putative clashes at the minor groove while keeping the major groove chemistry. Improving the interaction also ameliorated the deformation cost. Large deformation costs are incurred because we kept the complex structure fixed to a productive conformation. As high affinity binding has been shown not to be correlated always to transcription efficiency, we carried out molecular dynamics simulations of three TBP-DNA complexes, with alternating TA, CG and CI sequences. Preliminary results suggest that a large deformation cost is relieved by relaxing both TBP and DNA structures, generating increasingly unproductive complex structures.

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329-Pos Board B208

Recognition and Signaling in DNA Mismatch Repair: MD Studies of MutS Complexes with DNA and ATP

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The MutS family of DNA binding proteins has been reported to play a critical role in mismatch repair (MMR). Crystal structures of MutS (*Escherichia coli* and *Thermus aquaticus*) as well MSH homologs including human MutSα reveal intricate and complex multi-domain protein structures comprised of greater than 1,500 residues. The DNA binding domain of these proteins recognizes mispaired or unpaired bases. It has been proposed that this recognition event results in the release of a signal that travels from the DNA binding domain to the ATPase site. While much has been learned from previous binding studies of MutS, the contribution of the protein dynamics on MutS complex formation and intraprotein communication events are not fully resolved at the atomic level. In this study, state-of-the-art molecular dynamics (MD) simulations are used to investigate the dynamical processes that occur during the interactions with DNA and ATP substrates. In particular, we are interested in how the DNA mismatch recognition/binding event is signaled, triggering the initiation of DNA repair. The computational challenge represented by the size and complexity of MutS-DNA complexes provides an opportunity to develop MD approaches for large multi-component biological systems.

330-Pos Board B209

In Silico Study Of Nonspecific DNA-protein Encounter Complexes

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In search of its specific targets, a DNA-binding protein associates with nonspecific DNA and subsequently diffuses along the DNA. Structural characterization of the nonspecific DNA-protein encounter complexes is of great interest. Due to weak interactions between the protein and nonspecific DNA, however, such characterization is experimentally challenging. Here, we describe the first comprehensive computational study on the encounter complexes of 44 specific DNA-binding proteins with nonspecific canonical B-DNA. In the analysis of these encounter complex models, we found that the recognition sites for specific DNA are usually favorable interaction sites for the nonspecific DNA probe and that nonspecific DNA-protein interaction modes exhibit some similarity to specific DNA-protein binding modes. These results led us to a novel method that predicts DNA-binding sites and binding-modes for a DNA-binding protein without knowing its specific DNA target sequence. In benchmark tests, the method achieves significantly better performance than three previously established methods, which are based on sophisticated machine-learning techniques. We further apply our method to protein structures predicted through modeling and demonstrate that our method performs satisfactorily on protein models up to 5 Å from their native structures.

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Understanding DNA- and RNA-binding Proteins Using Sequence and Structural Features

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The complex interactions between nucleic acid molecules and NA-binding proteins are an integral component of the gene regulation processes. Understanding which residues on these proteins bind nucleic acids is necessary in order to characterize these interactions. Due to the complicated nature of this problem, machine learning is often used to predict which residues are involved in the act of binding. The prediction of NA-binding residues can provide assistance in the functional annotation of NA-binding proteins. These predictions can also be used to expedite mutatgenesis experiments for the study of NA-binding proteins, guiding researchers to the correct binding residues in these proteins. In this work we focus on three goals. First, we use SVM and various ensemble methods based on the C4.5 decision tree algorithm to predict DNA- and RNA-binding residues within proteins with high, balanced accuracy by analyzing sequence and structural characteristics. Secondly, we show that our classifiers can achieve similar results on several data sets which were used in previous works to identify DNA- and RNA-binding residues. Thirdly, we show that we are able to distinguish DNA-binding residues from RNA-binding residues using structure- + sequence-based features and sequence-based features only.