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.

**322-Pos Board B201**

**The Enfolding Arms of EcoRI Endonuclease as Probed by ESR Experiments**

**Jacqueline E. Townsend**, Katherine Stone, Zhongyu Yang, Jessica Sarver, Sunil Saxena, Linda Jen-Jacobson.
University of Pittsburgh, Pittsburgh, PA, USA.

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**

**Joerg Muetze**, Thomas Ohrt, Wolfgang Staroske, Karin Crell, Petra Schwille.
TU Dresden, Dresden, Germany.

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.**

**Christopher Kennaway**[1], Agnieszka Obarska-Kosinska[2], John H. White[3], Irina Tuszynska[2], Laurie P. Cooper[3], Janusz M. Bujnicki[2], John Trinick[1], David T.F. Dryden[3].
[1]University of Leeds, Leeds, United Kingdom, [2]International Institute of Molecular and Cell Biology, Warsaw, Poland, [3]University of Edinburgh, Edinburgh, United Kingdom.

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**

**Matthias Hoeller**, Karin Fellinger, Carina Frauer, Heinrich Leonhardt, Don C. Lamb.
University of Munich, Munich, Germany.

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**

**Krystle J. Williams**, Jermaine L. Jenkins, Clara L. Kielkopf.
Department of Biochemistry and Biophysics, University of Rochester School of Medicine, Rochester, NY, USA.

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