the host CRISPR locus. The small RNA products of these spacer sequences are used as guides by CRISPR proteins for silencing complementary viral sequences. Despite extensive research highlighting the maturation of the CRISPR RNA guides and the targeting step mediated by CRISPR ribonucleoprotein complexes, little is known about how spacer sequences are initially integrated into the host CRISPR locus. Recently developed in vivo integration assays revealed that Cas1 and Cas2 are the only CRISPR proteins required for this step. Here we show that Cas1 and Cas2 associate to form a protein complex. Using a combination of biochemical and structural approaches, we present the mechanism of spacer acquisition mediated by the Cas1-Cas2 complex.

3526-Pos Board B254

A Biophysical Study of the G-Quadruplex-Insulin Interaction

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The formation of guanine (G)-quadruplex structures in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) have potential effects on transcription of the insulin gene. Recent studies demonstrate that the ILPR G-quadruplexes can bind to insulin. The energetics of the binding between insulin and the G-quadruplexes formed by the most common ILPR repeat sequence have also been characterized in prior work. We have studied the proton transfer involved in the interaction between insulin and this DNA sequence by conducting isothermal titration calorimetry (ITC) experiments in various buffers and analyzing the observed enthalpy change. The transcriptional activity of a number of ILPR repeat sequences, including the consensus sequence, have been measured previously. Transcriptional activity for less common repeats is significantly lower than that of the consensus sequence but can be increased substantially by varying only one or two nucleotides. To determine the potential role of G-quadruplex formation and stability in regulating transcription, we have studied the second and third most common ILPR repeats as well as their variant sequences that exhibit increased transcriptional activity. ITC was used to characterize the energetics of the binding interaction between insulin and each of the four ILPR repeat sequences. The bulk thermodynamic measurements performed at various temperatures from 20 - 37 degrees Celsius provide insight into these biomolecular interactions.

3527-Pos Board B255

ADAR2: Towards a Structural and Kinetic Understanding of RNA Editing

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Adenosine Deaminases that Act on RNA (ADARs) are a small family of enzymes found in metazoans which edit pre-mRNAs by modifying the base adenosine to inosine. This editing results in translational mutations as inosine is interpreted as guanosine by translational machinery. Two editing events occur in humans on the Ionotropic Glutamate Receptor (GluR) pre-mRNA and are known to modify its ion permeability and resensitization kinetics. ADARs promiscuously edit perfectly complementary dsRNAs, however, in the context of complex secondary and tertiary structure, ADARs gain significant specificity for their substrates. Toward understanding the elements of ADARs which drive specificity in the context of a wild type substrate, we sought to generate a co-crystal structure of human ADAR2 with RNA and determine the structure of a flexible loop of the catalytic domain to ascertain its function. Crystals of GluR-B RNA and an ADAR2 truncation PP-R_2D have been produced in small scale screens, and work to produce larger crystals suitable for x-ray diffraction is ongoing. Several mutants of the ADAR2 catalytic domain (CD), S458G and R455A, have been solved. Both of these mutant structures give some insight into the previously undefined electron density of an unstructured loop located near the catalytic site. These structures remain incomplete, yet provide a better descriptor of the dynamicity of the loop. To understand the kinetic role of the loop we have replaced it with glycine and found that editing is strongly inhibited. Current work focuses on further mutations to the loop to determine its kinetic role. The results of these studies have shown that large complexes of ADAR2 and substrate mimics can be crystallized, which is fortuitous for further structure determination, and that the unstructured loop of the catalytic domain remains highly dynamic despite mutations to decrease mobility.

3528-Pos Board B256

Activation of PKR by Stem-Loop RNAs with Flanking SsRNA Tails

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Protein Kinase R (PKR) is a central component of the innate immunity antiviral pathway and is activated by double stranded RNA (dsRNA). PKR contains a C-terminal kinase domain and two tandem dsRNA binding motifs. In the accepted model for activation, binding of multiple PKR monomers to dsRNA enhances dimerization of the kinase domain. A minimum dsRNA length of 30 bp is required for binding two PKR monomers and eliciting strong enzymatic activation. However, short (15 bp) stem-loop RNAs containing flanking single stranded tails (ss-dsRNAs) are capable of activating PKR. Activation requires a 5'-triphosphate and the presence of both 5' and 3' ssRNA tails. The mechanism of PKR activation by ss-dsRNAs is not understood. Here, we have characterized the structural features of ss-dsRNAs that contribute to activation. We have designed a model ss-dsRNA PKR activator containing two single stranded tails of 15 nt and a 15 bp stem (5'-15-15-15-3') and made systematic truncations of the tail and stem regions. Analytical ultracentrifugation experiments combined with autophosphorylation assays were used to correlate RNA binding affinity with the ability to activate the kinase. Both tails are required for PKR activation; however, they can be truncated to produce either 10-15-5 or 5-15-10 without abolishing activation. Activity is retained upon reducing the stem to 10 bp but is lost upon further reduction to 5 bp. All of the ss-dsRNAs bind two PKR monomers in 75 mM NaCl. The loss of PKR activation is correlated with weaker PKR binding, consistent with a model where the autophosphorylation rates are proportional to the concentration of RNA species containing two PKR monomers. A 5'-triphosphate is required for activation. Although the binding affinity decreases upon removal of the 5'-triphosphate, the reduction is not sufficient to explain the loss of activation.

3529-Pos Board B257

Double-Strand RNA Binding Protein Profiling

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The double-strand RNA-binding proteins (DRBP), which are featured by the existence of evolutionarily conserved dsRNA-binding domain (DRBD), have been reported to function in a variety of significant cellular activities, involving RNA processing, RNA cleavage, RNA interference and anti-viral immunity pathways.

However, it still remains unclear about the molecular mechanism and dynamic properties underlying the interaction between DRBP and dsRNA. Recently single-molecule experiments have shown an ATP-independent diffusion/sliding activity of purified TRBP as well as its two homologs, PKR activator and R3D1-L, on double strand RNA molecules (*Koh et al., 2013*), indicating a potential generalizability of diffusion/sliding activity in dsRNA-binding proteins family. To test this hypothesis and to further characterize DRBPs in terms of substrate specificity, binding affinity, features of motion upon binding and multiple DRBD collaboration or redundancy, we examined in vivo expressed proteins with one or more DRBDs at single-molecule level.

In this research, DRBP genes have been cloned from Human Open Reading Frame Library and overexpressed in human A549 cells. Then we immobilized the protein products on quartz slide surface by *Single Molecule Pull Down (Jain et al. 2011)* and investigated their dynamic property upon biding various dsRNA substrates using *Protein Induced Fluorescence Enhancement* (*PIFE*) (*Hwang et al, 2011*). PIFE data from one of our candidates, TRBP, showed the intensity fluctuation of Cy3, the fluorescent label of dsRNA substrates, indicating that TRBP is sliding back and forth along dsRNA strand upon binding to it (Fig. 1). This result is highly consistent with the experimental observation *in vitro* (*Koh et al., 2013*).

1. Koh H. R *et al.* (2013) Proc. Natl. Acad. Sci USA 110:151-15.
2. Jain A *et al.* (2012) Nat Protoc 7:445-452.
3. Hwang H *et al.* (2011) Proc Natl Acad Sci USA 108:
7414-7418.

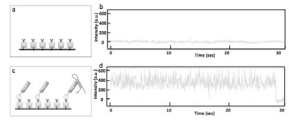

Figure 1. PIFE of TRBP bidirectional sliding along dsRNA. dsRNA concentration was 0.5nM for both control (a and b) and test (c and d). Control group was recorded without TRBP.

3530-Pos Board B258

Thermodynamic and Structural Studies of Pdx1 Binding to Elements from Natural Promoters and Near-Consensus Sites

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The production of insulin and islet amyloid polypeptide (IAPP) is limited to β-cells due to restricted expression of a set of tissue-specific transcription factors, the most well known of which is Pdx1. Naturally, insulin is expressed in much higher levels than IAPP, perhaps owing to the affinity of Pdx1 to its target sites. Like many transcription factors, Pdx1 contains regions of disorder whose function in transcription has not been adequately studied. It is well documented that the Pdx1 homeodomain binds to a core DNA recognition sequence containing the tetranucleotide TAAT, for which its consensus binding site is reported as 5′-CTCTAAT(T/G)AG-3′. Interestingly, while Pdx1 regulatory elements in the human *insulin* promoter