Carnegie Mellon CNAST

2013 CNAST Symposium

April 27, 2013

Mellon Institute Conference Room

Time	Speaker	Title
		Session I: Synthetic Tools and Applications
8:55-9:00	John Woolford	*Welcome Remarks*
9:00-9:30	Danith Ly	*Progress and Challenges in DNA Recognition*
9:30-10:00	Jim Schneider	*Improved miRNA Detection Limits Using γPNA and DNA Nanotags in Capillary Electrophoresis*
10:00-10:30	Catalina Achim	*You CAN Teach an Organic PNA Inorganic Tricks*
10:30-10:45	Coffee Break	
10:45-11:15	Subha Das	*Backbone Branched DNA, Polymer-Nucleic Acid Hybrids and Mini-Lariat RNA: Access and Applications*
11:15-11:45	Xiaohong Tan (DSF Postdoctoral Fellow)	*Label-Free Molecular Beacons for Biomolecular Detection*
11:45-12:15	Bruce Armitage	*Guanine Heteroquadruplex Formation by PNA*
12:15-1:15	Lunch Break	
		Session II: Molecular Biology and Biochemistry
1:15-1:30	Catalina Achim & Hannah Diorio-Toth	*CNAST Outreach Activities: DNAZone*
1:30-2:00	Connor Murphy (Opresko Lab)	*Hybridization of G-Quadruplex Forming PNAs to Guanine-Rich DNA Templates Inhibits DNA Polymerase η Extension*
2:00-2:30	Anmol Grover & Marcel Bruchez	*Single Molecule Measurement of Cotranslational Folding in Eukaryotic Translation*
2:30-3:00	Kausik Chakrabarti	*Dissecting Molecular Mechanism of Telomerase RNA Function in Parasitic protozoa*
3:00-3:15	Coffee Break	
3:15-3:45	John Woolford	*Hierarchical Construction of Domains within the 60S Ribosomal Subunit--- How a Large Noncoding RNA Assembles with Proteins*
3:45-4:15	Joel McManus	*High-Throughput Sequencing Methods for RNA Structure Probing*
4:15-4:45	Yevgeniya Monisova & Deb Makin (Lopez Lab)	*Role of Alternative pre-mRNA Splicing during Aging and Oxidative Stress*

ABSTRACTS

SESSION 1: SYNTHETIC TOOLS AND APPLICATIONS

1. Danith Ly

Progress and Challenges in DNA Recognition

The presentation will highlight past accomplishments in the design of molecules for recognition of double helical DNA (our genetic material). Special emphasis will be on the development of chiral γ-peptide nucleic acid (γPNA) and its application in biology, biotechnology, and medicine. Ongoing research in the areas of molecular design and synthesis, and validation of new paradigms for treatment of genetic and infectious diseases will also be on display, with the overall goal of getting feedback and suggestions from experts in the field and establish collaboration with researchers in the Pittsburgh community and abroad.

2. Jim Schneider

Improved miRNA Detection Limits Using gPNA and DNA Nanotags in Capillary Electrophoresis

We have developed a rapid, selective, gel-free means of detecting trace DNA and RNA oligonucleotides in capillary electrophoresis based on separation of various sandwich-type complexes in the presence of interacting micelles. Here, we discuss our efforts to bring detection limits down from the picomolar range to femto- and attomole range by a combination of strategies. One is the use of isotachophoresis (ITP) to nonspecifically concentrate oligos by several orders of magnitude. A second is to carefully stain DNA nanotags with intercalating dyes to maintain uniform mobility for each component, and therefore, sharp, intense peaks. We also are designing micelle zones to provide for concentration by sample stacking while preserving elution order and resolution. We discuss our recent results on all three fronts and discuss prospects for further enhancements in detection sensitivity.

3. Catalina Achim

You CAN Teach an Organic PNA Inorganic Tricks

While the past few years have seen intense research in both DNA nanotechnology and inorganic supramolecular chemistry, the combined use of the molecular recognition properties of nucleic acids and transition metal ions to create nanoscale structures has been explored in a limited number of cases. When they are explored, the structures are made of DNA. We leverage the modular nature and relative ease of synthesis of Aeg and next generation PNA to organize a variety of metal ions in supramolecular assemblies in which metal complexes function as alternative base pairs or triplets. This presentation will describe results of structural, spectroscopic, thermodynamic and electrochemistry studies that demonstrate how non-modified and metal-containing, ligand-modified PNA can be used as easily tunable platforms for the study of fundamental chemical processes such as charge and metal transport.

4. Subha Das

Backbone Branched DNA, Polymer-Nucleic Acid Hybrids and Mini-Lariat RNA: Access and Applications

The click-chemistry (CuAAC) conditions we have optimized for nucleic acids have been applied towards straightforward access to backbone branched DNA (bbDNA), polymer nucleic acid hybrids and lariat RNA. The bbDNAs provide a novel building block for nanotechnology while the polymer-nucleic acid hybrids can be applied towards fluorescent nanotags as well as auto-transfecting siRNA. Lariat RNA can be obtained by click-ligation of the 5'-terminus of an RNA with a 2',5'-branch. These lariats are mimics of mirtrons that are processed by debranching enzyme to produce regulatory miRNAs. Synthetic access to mini-lariats allow for single-stranded RNA, rather than duplex siRNA, to be used towards RNA interference.

5. Xiaohong Tan

Label-Free Molecular Beacons for Biomolecular Detection

In this project we developed novel probes using label free molecular beacons for biomolecular detection. The probe consists of two single-stranded DNA (ss-DNA) parts: one is the human telomeric G-quadruplex (HTG) sequence and the other is the recognition sequence. HTG can specifically bind to Thioflavin T (ThT) and generate fluorescence signal. In the absence of an analyte such as a nucleic acid or a protein, the HTG sequence is caged in the form of a hairpin structure, preventing the formation of a G-quadruplex. Because the interaction between ThT and double-stranded DNA (dsDNA) or ssDNA is very weak, the background fluorescence of this molecular beacon (MB) is low. Upon addition of the target, through the binding between the target and the loop part of the MB, the stem part will be dissociated and HTG sequence element can refold into a G-quadruplex. As a result, this HTG G-quadruplex can bind ThT and generate enhanced fluorescence signals. We have demonstrated the usage of these probes for the specific detection of an RNA sequence (with a limit of detection LOD = 5.7 nM) and PDGF-BB protein (with a LOD = 12.6 nM). Our method offers several advantages. First, the cost of probe synthesis can be reduced by more than 95% since it is quite expensive to add the fluorophore and quencher label to traditional MBs. Second, because they are label-free, these probes can provide very clean background even after degradation. Finally, new applications can be derived from this new MB structure through the formation of the aptamer–substrate complex.

6. Bruce Armitage

Guanine Heteroquadruplex Formation by PNA

Guanine-rich DNA and RNA is known to fold into G-quadruplex secondary structures, which have been proposed to regulate numerous aspects of gene expression. Targeting G-quadruplexes with synthetic molecules represents a promising approach to manipulate gene expression. Prior work demonstrated that guanine-rich PNA hybridizes with high affinity to homologous DNA or RNA targets to form heteroquadruplexes. Recent research has shown that homologous PNA is much more effective than complementary (i.e. heteroduplex-forming) PNA at binding to certain quadruplex targets. In addition,

preliminary experiments with a luciferase reporter system indicate that quadruplex-forming PNA can significantly inhibit mRNA translation. Future work will focus on improving the potency and selectivity of quadruplex-forming PNAs, developing transcriptional reporter assays and assessing the cellular distribution and activities of this new class of nucleic acid targeting agent.

SESSION II: MOLECULAR BIOLOGY AND BIOCHEMISTRY

7. Connor Murphy

Hybridization of G-Quadruplex Forming Peptide Nucleic Acids to Guanine-Rich DNA Templates Inhibits DNA Polymerase η Extension

The guanine quadruplex (G-quadruplex) is a highly stable secondary structure that forms in G-rich repeats of DNA and RNA. Evidence suggests that G-quadruplex formation can interfere with DNA metabolism including DNA replication and transcription. Previous work shows that short guanine rich Peptide Nucleic Acids (PNA) can form highly stable hybrid quadruplexes with DNA. We hypothesize that such structures will provide a stronger block to polymerase extension on G-rich templates than a native DNA/DNA G-quadruplex due to the greater thermal stability of the PNA/DNA hybrid structures. For this project we analyzed the extension activity of polymerase η, a translesion polymerase implicated in synthesis past G-quadruplex blocks, on DNA templates containing guanine repeats. We observed a PNA concentration dependent decrease in polymerase η primer extension to the end of the template and an increase in polymerase η stalling at the sequence prior to the G-rich repeats. No DNA synthesis was detected past the G-rich repeats at 500nM of PNA. In contrast, the addition of a complementary C-rich PNA that hybridizes to the G-rich repeats by Watson-Crick base pairing led to a decrease in stalling and an increase in full-length polymerase extension products. Furthermore, we observed that the G-quadruplex forming PNAs inhibited polymerase progression to a similar extent as a well-established DNA G-quadruplex stabilizing ligand BRACO-19. Our results indicate that homologous PNA targeting of G-quadruplex sequences creates stable PNA/DNA hybrid G-quadruplexes that inhibit polymerase η extension more effectively than a DNA/DNA G-quadrupex or a PNA/DNA duplex. This is, to our knowledge, the first reported instance of a PNA/DNA G-quadruplex having an effect on a biological enzyme. The implications of these results for the potential use of targeted PNA/DNA G-quadruplexes as therapeutics to arrest DNA replication of proliferating cancer cells will be discussed.

8. Anmol Grover/Marcel Bruchez

Single Molecule Measurement of Cotranslation Folding in Eukaryotic Translation

While single ribosomes studies have been used in bacterial translation systems to address a range of mechanistic questions, it has been difficult to perform similar studies in eukaryotic systems due to the increased structural and functional complexities and a lack of genetic tools for simple labeling of the ribosomes. We have used fluorescent Peptide Nucleic Acids (PNAs) to label native saccharomyces cerevisiae ribosomes, both purified and in translation competent lysates. Using this approach we are developing a single molecule biophysical set up to investigate the relation between the timescales of

translation elongation rates and nascent chain folding. These studies will provide new insights about the role of the degenerate genetic code in specifying protein function during the translation process and potentially open the door to new therapeutic approaches that manipulate the translation process in a sequence-directed manner.

9. Kausik Chakrabarti

Dissecting Molecular Mechanism of Telomerase RNA Function in Parasitic protozoa

Telomeres are nucleoprotein complexes at the ends of linear chromosomes. Telomere DNA often consists of simple repetitive TG-rich sequences and terminates in a single-stranded G-rich 3' overhang structure. Conventional DNA polymerases cannot replicate linear DNA ends completely, and telomeres shorten after each round of DNA replication. In most eukaryotes, telomerase, a specialized reverse transcriptase, can synthesize the G-rich strand of telomeric DNA *de novo*, using its internal RNA template sequence, thereby solving this "end replication problem". The assembly, maintenance, and disassembly of functionally active telomerase holoenzyme presumably require interaction with other proteins. Since telomerase is slowly metabolized, with a half-life of more than 24 h, telomerase activity may also be subject to modulation by the holoenzyme changing in its conformation by direct protein–protein or RNA-protein interactions. Therefore, understanding the domain interactions of the RNA moiety (TR) with catalytic protein component of telomerase (TERT) and telomeres is necessary to define the significance of TR in telomerase stability, recruitment and activity in proliferating cells. *P.falciparum* and *T. brucei* are highly proliferative parasitic protozoa that cause human diseases, which are fatal without treatment. Inside the mammalian host, both parasites regularly switch their major surface antigen to evade the host immune responses. We have recently used genetically amenable features of *T.brucei* to dissect the function of parasite TR. We observed that *T. brucei* telomerase is only remotely homologous to mammalian telomerase and there are unique RNA-protein interactions between telomerese RNA and telomere proteins that may help maintain parasite virulence genes at the subtelomeric ends. Therefore we propose to dissect these molecular mechanisms to use unique features of parasite TR as targets for developing anti-parasite drugs.

10. John Woolford

Hierarchical construction of domains within the 60S ribosomal subunit--- how a large noncoding RNA assembles with proteins

Abstract: The assembly of ribosomes requires the complex interplay among folding of nascent rRNA, binding of ribosomal proteins and assembly factors to the RNA, and removal of spacer sequences from the RNA. We will report on the results of a systematic survey of the roles of ribosomal proteins in these steps. This is particularly strategic, since we know the locations of each of these r-proteins in ribosomes, from the ribosome crystal structures. These experiments have enabled us to build a model for hierarchical construction of ribonucleoprotein neighborhoods, and have revealed important principles for folding of long RNA molecules. What's next? The most striking result from determination of the crystal structure of eukaryotic ribosomes is the prevalence of eukaryotic-specific extensions of the amino- or carboxyl termini of many ribosomal proteins. These extensions thread along the surface of the ribosome,

often interacting with eukaryotic-specific expansion segments of rRNA or other ribosomal proteins. Interestingly, many of these extended sequences are predicted to be intrinsically disordered. We will briefly discuss experiments underway to explore functions of these "tails".

11. Joel McManus

High-Throughput Sequencing Methods for RNA Structure Probing

RNA is unique among biomolecules in that it can both transmit information and catalyze biochemical reactions. Both of these functions depend on RNA structure, however traditional approaches to determine RNA structure are tedious, challenging, and generally low-throughput. The recent advent of deep sequencing has created opportunities for high-throughput RNA structure probing. I will present three high-throughput sequencing methods developed in my lab to analyze RNA from one end to the other, by identifying transcription start sites, measuring mRNA poly-A tail lengths, and probing in vivo RNA structure. Our lab plans to use these approaches to compare RNA structure and function in human and yeast cells.

12. Yevgenia Monisova and Deborah Makin

Role of Alternative pre-mRNA Splicing during Aging and Oxidative Stress

Limited studies in human brain and blood cells have suggested that a large number of alternative splicing events may differ between chronologically "young" and "old" individuals. Whether these are truly age-related splicing changes, and if so, whether they are programmed changes that contribute to aging, pathological effects of the aging process, or adaptations to changing physiology and stress is unknown and difficult to assess. We are addressing these questions using the model system Drosophila melanogaster, which shares many similar aging processes with humans. Using standard RNAseq data and a novel method for detailed time course-analysis of alternative splicing by multiplex targeted RNAseq, we have identified hundreds of age-related alternative splicing changes in genes with obvious relationship to aging processes, oxidative stress, energy metabolism and lipid homeostasis. We have also found that a subset of these also respond to oxidative stress, and we starting to identify the functional consequences as well as the regulatory factors and pathways. These studies have been at the level of whole animals and thus probably represent the tip of an iceberg. In addition to identifying changes in less-abundant tissue-specific RNAs, an important question is to identify the range of organs, tissues or cell types in which the known changes take place. For this purpose a sensitive method for quantitation of alternative splicing ratios by in situ hybridization to whole mounts or sections (e.g. via multi-color FRET) would be very useful.