1013 Control of transcriptional termination, poising and bidirectionality by the non-coding snRNA *7SK*

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Lineage-specification genes in stem/progenitor cells are poised for transcription, via a mechanism involving the bivalent tri-methylation Histone H3 at lysine 27 (H3K27me3) and at lysine 4 (H3K4me3) at their promoters. Other mechanisms such as RNA Polymerase II pausing might be implicated in this process. We identified the non-coding RNA *7SK* as a multifaceted regulator of transcription in embryonic stem (ES) cells. We found that *7SK* represses a specific cohort of genes with bivalent and active chromatin marks in ES cells. Genome-wide analysis shows that *7SK* also prevents failed transcriptional termination and suppresses divergent upstream antisense transcription, including of many divergent long non-coding RNAs, implicating 7SK in the control of transcriptional bidirectionality. We are currently investigating the mechanisms by which 7SK snRNP complex is recruited to specific loci and identified the transcription factor Sox2 as a major candidate for this role.

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1014 lncRNA-IL1a: a natural antisense transcript modulates the expression of interleukin-1 alpha mRNA in murine macrophages

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Advances in sequencing and array technologies have led to the discovery of thousands of long non-coding RNA (lncRNA) transcripts in mammalian genomes. When a lncRNA is complementary to a protein-coding gene, it can be classified as an antisense lncRNA or natural antisense transcript (NAT). We identified a NAT that was induced in mice infected with the gram positive bacterial pathogen *Listeria monocytogenes* that has partial complementarity to the corresponding sense transcript of the proinflammatory cytokine, interleukin-1 alpha (IL-1a). Ribosome association studies indicated that unlike IL-1a, lncRNA-IL-1a was unlikely to encode for protein. IL-1a functions as a danger signal released from damaged or dying cells. Although IL-1a is vital for activating the immune response, its regulation is poorly understood. Macrophages express very low levels of IL-1a or lncRNA-IL1a, but transcription of both are upregulated when cells are exposed to microbial components such as lipopolysaccharide (LPS) or bacterial lipopeptides (Pam3CSK4) indicating that this NAT is only transcribed during an active infection. To study the relationship between lncRNA-IL-1a and IL-1a we generated macrophage cell lines in which expression of lncRNA-IL-1a was targeted by shRNA. In these cell lines, the inducible expression of IL-1a mRNA and protein expression was severely attenuated, indicating a positive correlation between IL-1a expression with lncRNA-IL1a. We also demonstrate that lncRNA-IL1a resides in the cytosol, suggesting possible post-transcriptional mechanisms involved in lncRNA-IL1a mediated control of IL-1a levels. Ongoing studies are focused on elucidating the molecular relationship between these transcripts. Together, our study demonstrates that NATs contribute to gene regulation in the innate immune system.

1015 Long Noncoding RNA CR933609 Acts as a Decoy to Protect INO80 Complex Subunit D in A549 Cell Line

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Long noncoding RNAs (lncRNAs) have impact on biological functions and development through diverse regulatory mechanisms. INO80 chromatin remodeling complex plays an important role in DNA transcription, replication and double-strand break repair that have crucial influence on tumorigenesis. Therefore, we are interested in the relationship between lncRNAs and INO80 gene in oncogenesis.

We utilize bioinformatic methods to find one lncRNA (accession ID: CR933609) with sequence length about 8811bp, which is homolog as one part of INO80 complex subunit D (INO80D) mRNA. We use real-time quantitative reverse transcriptase PCR to analyze the expression of the lncRNA in various cancer tissues, their non-cancer parts and associated cancer cell lines. We find that gene expression of the lncRNA is significantly lower in 68 non-small cell lung cancer tissues than non-cancer parts.

In A549 cell line, both lncRNA and INO80D are down regulation after RNA interference at their common regions. Only INO80D is down regulation after RNA interference at INO80D rather than lncRNA. Endogenous miRNA5096, which targets at the same regions of lncRNA and INO80D, can knock down them simultaneously. But, INO80D is not down regulation by miRNA5096 in A549 cell line with lncRNA overexpression. In addition, we prove that lncRNA and INO80D have different promoter activity in different regions by luciferase reporter assay.

In conclusions, we demonstrate that over-expressed lncRNA CR933609 in A549 cell line can protect INO80D from gene knockdown by miRNA5096. This phenomenon reveals that lncRNA CR933609 acts as a decoy to protect INO80D to maintain its vital functions.

1016 Towards intracellular structural characterization of sRNAs from extremophiles

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RNA structure has been extensively studied using a variety of *in vitro* and *in silico* techniques. However, to date, only a few RNA structural characterization techniques have been used within an intracellular environment. In addition, although *in vitro* and *in vivo* RNA folding share some basic features, is still unclear how RNA folding *in vitro* compares to *in vivo*. In this study, we exploit molecular interactions that trigger activation of GFP to engineer a controllable riboswitch-inspired system capable of detecting structural perturbations in RNAs that result from sequence mutations. In this synthetic reporter system, expression of GFP is tuned by controlling local structural changes in an RBS and a cis-blocking element (cb) containing-loop. We explore different riboswitch conformations in the context of their interactions with the *tetrahymena* ribozyme Group I Intron (GI). Flow cytometry data indicates the potential of this method to detect differences between RNA variants and RNA conformers. We are now extending application of this reporter system to the dynamic characterization of (shorter) regulatory bacterial sRNAs that we have uncovered in extremophiles. We will discuss various design features of the system, and design algorithms that we have now established for optimal sensitivity.