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ABSTRACTS

HGM 2008 regulatory variation and non-coding DNA sequences symposium abstracts

005: microRNAs and their targets

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I will summarize our recent efforts to identify as well as quantify known and novel microRNAs by next-generation sequencing of small RNA samples. I will further describe proteomics techniques that allowed us to quantify the impact of miRNAs on protein synthesis of thousands of human proteins. These data were also used to systematically compare existing miRNA target prediction methods. I will finally present some data highlighting the importance of assaying the proteome, and not only the transcriptome, in miRNA biology.

006: Identification and molecular characterization of novel protein partners of piwi in Drosophila

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RNA interference is more than a just protective response against exogenous genetic material. It regulates a variety of phenomena including gene function, epigenetic regulation, genome control and stability etc. both in post-transcriptional and transcriptional level. In Drosophila piwi that encodes a nuclear protein of Argonaute family, functions as a linker between TGS and PTGS transgene silencing. PIWI associated small RNA (piRNA) is required for heterochromatin and telomeric silencing. A wide range of piRNA (nearly 13,000) in fly genome that are derived from the antisense strand of RNA by the cleavage of a yet to be identified RNA nuclease, plays central role in different epigenetic phenomena in the nuclei. But no information has been documented regarding piRNA and PIWI associated proteins. Here we isolate and purify Piwi associated transcriptional silencing complex termed PITS (piwi induced transcriptional Silencing complex) that is required for heterochromatin and transgene silencing. The PITS complex contains known RNAi factors including Ago-1 and R2D2 and several novel proteins including lamindm0 regulating nuclear transport, heterochromatin protein 1, Polycomb etc. Piwi is also directly associated with dre-4, that controls larval development, sf2 (splicing factor 2) RNA helicase for regulating RNA splicing. These results demonstrated that PITS complex controls a wide variety of biochemical phenomena in both cell fractions. Interestingly direct association of Ago-1 and piwi may provide certain role in cytoplasmic RNAi machinery that is corroborated with piwi localization in the cytoplasm of the somatic cells.

007: Exon evolution and human genomic diversity

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Alternative splicing enhances transcriptomic diversity and presumably leads to speciation and higher organism complexity, especially in mammals. Alternative splicing also contributes to human phenotypic diversity. At least 74% of human genes produce more than one type of mRNA transcript through alternative splicing; however, it is unclear which of these products are biologically functional and which are non-functional products of inaccurate splicing. Thus, understanding the changes in the genome that dictate fixation of beneficial alternative splicing events or deleterious events is of great interest. There are three known origins of alternatively spliced exons: (1) exon shuffling, which is a form of gene duplication; (2) exonization of intronic sequences; and (3) change in the mode of splicing from constitutive to alternative splicing during evolution. I will talk about the evolution of alternative splicing, and more specifically about the contribution of the primate-specific retrotransposons called Alu to human genomic complexity, and how the extensive RNA editing (A-to-I) in the human transcriptome lead to human-specific transcriptomic diversity.

008: MicroRNAs and non-micro-short RNAs (nmsRNAs) in cell quiescence and cancer

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We are using three approaches to discover microRNAs that regulate cell proliferation. In the first approach, we focused on miRNAs

induced during differentiation of C2C12 myoblasts into myotubes in vitro. One of the microRNAs induced during differentiation, miR-206, suppresses cell proliferation. By surveying mRNAs that are down-regulated by miR-206 for genes predicted to be targets of the microRNA, we discovered the mRNA of DNA polymerase alpha p180 is a direct target that is destabilized upon miR-206 transfection. We now report that additional microRNAs induced during muscle differentiation target other cell-cycle regulators. After noting that targeted mRNAs are often destabilized by microRNAs, in the second approach, we tested whether global downregulation of microRNAs by knock-down of Dicer or Drosha can help identify microRNA-repressed mRNAs. The principle was proved by the discovery that HMGA2 oncogene is repressed by the growth-suppressive let-7 microRNA. Indeed, chromosomal translocations in lipomas and leiomyomas de-repress HMGA2 by deleting the 3′UTR that is normally repressed by let-7. Conversely, downregulation of let-7 has been noted in lung cancers and large leiomyomas and is correlated with over-expression of the HMGA2 oncogene. In the third approach we have cloned short RNAs from androgen-dependent prostate cancer cells grown in the presence or absence of androgens and subjected them to ultra-high-throughput sequencing. The frequency of a number of clones present in the libraries is changed by androgens and we have identified several microRNAs that change upon androgen depletion. Over 30–40% of the short RNAs cloned, however, do not correspond to known microRNAs and are produced by cleavage of known mRNAs and noncoding RNAs. The diversity and abundance of the non-micro-short RNAs (nmsRNAs) contrast with how little is known of their function and suggest that much remains to be done before we understand the biological functions of many short RNAs present in the cell.

009: Unusual Non-B DNA structure-mediated gene regulation

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DNA sequence as regulatory elements is well-established; however, DNA structure is relatively unexplored in this context. Using G-quadruplex or G4 DNA (structural motifs commonly found within eukaryote telomeres) as a model we have explored the role of DNA structure in transcription. Recent findings demonstrating a transcription factor that may target DNA structure for gene activation will be presented. This portends an interesting model that involves cross-talk between local DNA structure and global chromatin architecture in a regulatory context.