NEWS AND VIEWS

# JW Jenkinson Memorial Lecture

30th January 2006 - Professor Stephen Cohen (EMBL, Heidelberg) delivered the JW Jenkinson Memorial Lecture at the University of Oxford, entitled "*Recent insights into the functions of miRNAs in animal development*". The JW Jenkinson Memorial Lectures, established in memory of the pioneering British experimental embryologist, annually hear the latest developments in developmental embryology. Professor Cohen's lecture was the first to address the increasingly prominent role of small RNAs in animal development.

Professor Cohen's experimental interest in small RNAs stem from the unexpected identification of the *bantam* miRNA of *Drosophila,* identified in a gain-of-function screen for genes that affect tissue growth (Hipfner et al, 2002). The bantam locus does not express a protein of known identity, but a ~90nt 3' region of extensive nucleotide homology to related *Anopheles gambiae* gene exists and this sequence is predicted to form a stable RNA hairpin structure.

Overexpression via inducible transposable elements inserted at the *bantam* locus lead to tissue overgrowth due to an increase in cell number, whereas flies homozygous for *bantam* deletion show poor growth and die as early pupae. Heterozygous for a *bantam* deletion survived and were morphologically normal but smaller than normal flies, indicating that *bantam* may have anti-apoptotic properties. Further work in rescued homozygous bantam deletion mutants led to the identification of the target sites of the bantam miRNA in the 3'UTR of the pro-apoptotic gene *hid.*

Computer aided prediction of functional miRNAs and their targets in plants has been very successful, but remains less so in animals where functional duplexes can be more variable in structure and mismatch toleration. By creating a simple *in vivo* assay in the *Drosophila* wing imaginal disc using eGFP conjugated target and the *miR-7* miRNA, Cohen and colleagues showed that 7-8nt complementarity to the miRNA 5′ end is sufficient for target site function *in vivo (*Brennecke et al, 2005). G:U base pairs where generally detrimental to function, although may be tolerated at certain positions more than others. Professor Cohen provided evidence for the existence of distinct structural sub-groups of miRNAs delineated by their 5' and/or 3' base pairing, and suggested that the differences in these miRNAs might reflect their role in family specific gene silencing, or the level of expression of less complementary miRNAs required for a functional effect.

Professor Cohen briefly surveyed the predicted and experimentally validated miRNAs to date. With miRNAs predicted to encode 1-5% of animal genes, and potentially each control >100 genes, a greatly complicated network of small RNA regulation seems likely to exist. Small RNAs are relatively newcomers to research into cancer and other diseases however, and thought potentially previously ignored candidates, this might equally be taken to indicate that their roles are less crucial than their protein counterparts, else there is much redundancy of function between them.

The final third of the lecture focused on the developmental importance of miRNAs, and a hypothesis for their prime biological role. Moving away from previous notions of switch-like regulation of just a few genes, Cohen described a extensive analysis of 3'UTR sequences conserved between related *Drosophila* species. Using prediction rules determined by systematic experimental analysis in vivo (Brennecke et al, 2005), and further supported by luciferase activity assays in vitro, 3125 predicted miRNA targets and 5129 "antitargets" (those 3'UTRs lacking target sites) were identified. Two important trends stood out in this analysis. First, that cooccurrence of different miRNA target sites in the same 3'UTR was common, indicting that single target switch-like miRNA regulation is a relatively rare method of posttranscriptional gene regulation. Second, and perhaps more fundamentally informative, was that discernible selection between functional catergories of genes is apparent for genes

regulated by miRNAs. Housekeeping genes and those involved in general cell processes are under-represented as miRNA targets relative to developmentally expressed gene – which are themselves over-represented. Indeed, antitargets circumvent miRNA-mediated regulation by limiting 3′UTR length and by selective avoidance of miRNA sites. Target genes have longer 3′UTRs that are enriched in evolutionarily conserved sites. This relationship takes a further interesting twist when considering differentiation of cell lines; miRNAs and their targets are expressed in a largely nonoverlapping manner, whereas miRNAs and antitargets tend to be coexpressed and miRNAs preferentially target genes expressed in adjacent tissues. Such *mutual exclusion* of miRNAs/target expression may have evolved to prevent aberrant expression of target transcripts in differentiating cells derived from common progenitors. This fine regulation might dampen "leaky" transcription that might impede cell differentiation.

Professor Cohen concluded the lecture by suggesting that miRNAs provide robustness to gene expression. *Dicer* mutants do not show gross patterning or organogenesis, suggesting that miRNAs do not act as master switches in gene regulation, but probably act as a fine control for specific sets of differentially expressed genes Implicit from his findings is, of course, that miRNAs targeting genes expressed in neurogenesis are not likely to be expressed in the CNS. Mutations in miRNA genes may lead to very subtle alterations in gene expression, and no appreciable phenotype, yet this almost indiscernible evolutionary drift might equally facilitate gradual rather than punctuated evolution of new traits.

Clearly communicated, and well received, the lecture stimulated varied interest from those present – such as how miRNA might be involved in chromatin remodelling, or the potential for a link between small ribonucleoproteins involved in RNA transport, e.g., the SMN protein complex, and miRNA based post-transcriptional silencing. The identification of a specific human disorder of miRNA dysregulation would bring a timely boost in recognition for this growing field.

The expansion in miRNA sequence databases is mirrored by the increase in the number of miRNA related publications. Computer aided prediction of miRNAs and their targets, coupled with better understanding the roles that miRNAs play in cells, looks certain to continue this trend – the Journal of RNAi and Gene silencing looks forward to reviewing many such manuscripts in the near future.

## REFERENCES

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