

# THERAPEUTICS AND DIAGNOSTICS

## Non-coding RNAs

### Summary of the Opportunity

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RNA-based diagnostic, prognostic and therapeutic treatments are set to revolutionize molecular diagnostics medicine. The RNomics Group at The University of Queensland's Institute for Molecular Bioscience (IMB) in Brisbane, Australia has discovered two new classes of regulatory tiny non-coding RNAs (ncRNAs). Functional characterisation of these ncRNAs is yielding insight into the epigenetics of gene regulation and the etiology of diseases such as cancer. This is a unique opportunity to commercially exploit the intellectual property and know-how associated with these early stage discoveries through the development of new therapeutics and molecular diagnostics.

### Technologies

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One of the enigmas to arise out of the initial human genome project was that protein coding genes account for only 2 percent of genomic DNA, suggesting that there must be additional elements guiding human biological complexity. Consistent with this, the RNomics group at the IMB has been part of small cluster of researchers to show that the majority of the genome is transcribed into a host of interweaving non-protein coding RNAs (ncRNAs). Indeed, a body of evidence is emerging that ncRNAs form an epigenetic regulatory network that directs gene expression during development, and is dysregulated in disease. Two classes of epigenetic-associated ncRNAs have recently been identified and characterised at the IMB:

- Transcription Initiation RNAs (tiRNAs) are 18-19 nucleotides in length and map specifically to transcription start sites on the genome; and
- Splice-site RNAs (spliRNAs) are 17-18 nucleotides in length map specifically to the 5' splice sites of gene exons.

Both tiRNAs and spliRNAs have been identified in humans, mouse, fruit fly, nematode worm and marine sponge; they are conserved across metazoa but absent from plants. They are GC-rich, located in the nucleus and are particularly associated with highly expressed genes. To date, approximately 20,000 human tiRNAs and 10,000 human spliRNAs have been sequenced and their genomic locations mapped.

### Applications

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Noncoding RNAs have been implicated in developmental processes and in a number of conditions including cancer, neurodegenerative disease and complex metabolic disorders such as diabetes. As the mechanism by which tiRNAs and spliRNAs regulate gene expression is unravelled (in both specific diseases and in healthy tissues), there is an opportunity to commercially exploit these discoveries in a broad range of applications, including:

- Molecular diagnostics and prognostics;
- Nucleic acid-based therapeutics;
- New druggable targets through antisense or small molecules; and
- Stem cell and regenerative medicines.

The integration of tiRNA and spliRNA expression profiling together with therapeutics that either specifically mimic or target tiRNAs and spliRNAs opens a novel strategy to personalised medicine. Indeed, one of the principal drawbacks of siRNA and miRNA therapeutics is that they target transcripts that have (by definition) already been transcribed. tiRNAs and spliRNAs act at the epigenetic level *before* transcription, which opens up an entirely new therapeutic option of directly silencing or activating gene loci.

## Proof of Concept

tiRNAs and spliRNAs are found just downstream of the transcription start site and precisely at the 3' ends of internal exons (i.e. the splice donor site) at thousands of genes across the genome (Figure 1). These RNA molecules have the potential to regulate early transcriptional processes and exon splicing – two processes with significant therapeutic impact.

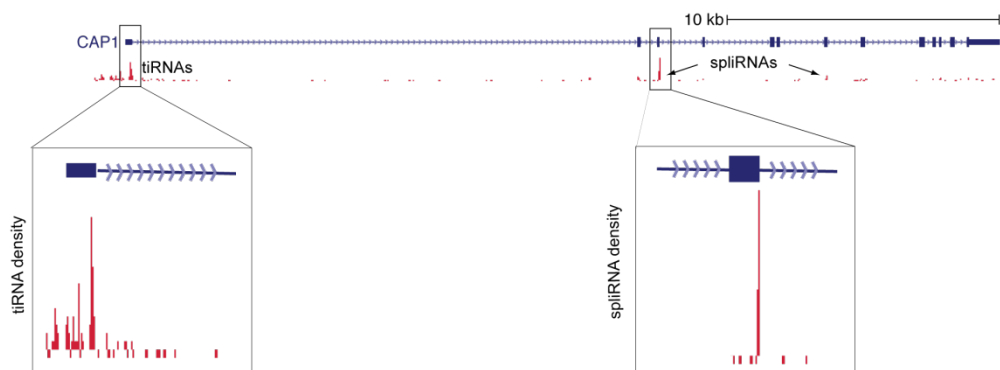


Figure 1. tiRNAs and spliRNAs in the CAP-1 gene.

Soon to be published studies by the RNomics Group demonstrate the role of tiRNAs in the modulation of gene expression. One such study involves the investigation of human cyclin-dependent kinase inhibitor 1A (p21). p21 is a tumour suppressor which acts at the G1 checkpoint to inhibit cell cycle progression. While mutations in p21 are rare, its down-regulation is a common feature of many cancers.

CCCTC-binding factor (CTCF) is an important, multifunctional transcription factor and chromatin insulator protein in human cells that has been shown to enhance expression of several tumour suppressor genes, including p21.

Inhibition of CTCF-proximal tiRNAs using antisense (sponge) constructs resulted in increased CTCF localization to p21 (Figure 2a). CTCF localization increased p21 RNA and protein levels (Figure 2b). CTCF-proximal tiRNA mimicking constructs had the opposite effect (Figure 2a).

These data therefore demonstrate that CTCF-proximal tiRNA inhibitors represent potential anticancer therapeutics. Similar results are anticipated for tiRNAs and spliRNAs at gene loci associated with other diseases.

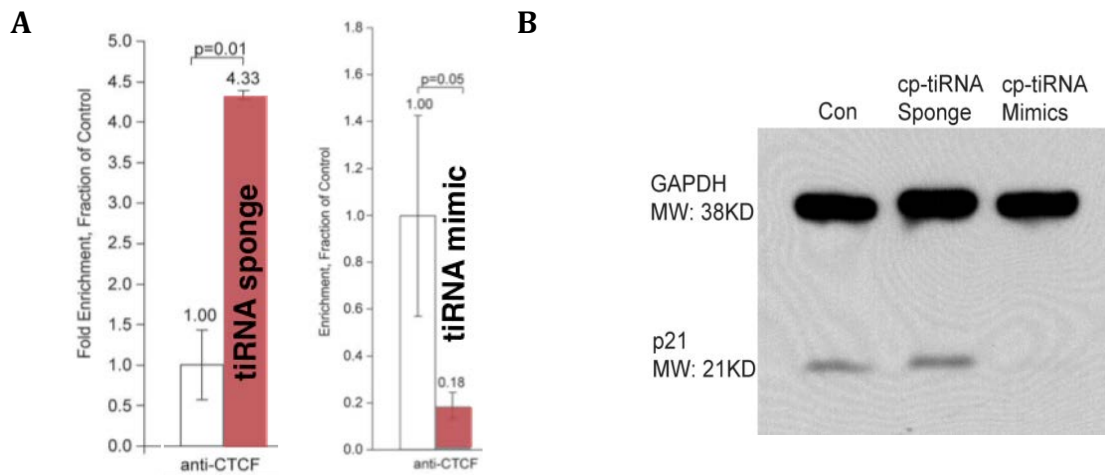


Figure 2. tiRNA sponge and mimic effects on CTCF and p21.

## Research Leaders

**John Mattick AO** is currently the Professor of Molecular Biology and Australian Research Council Federation Fellow at the IMB. He was the Foundation Director of the Australian Genome Research Facility and the IMB. Professor Mattick has been awarded the Biotechnology Medal by the Australian Biochemical Society, an Honorary Fellowship of the Royal College of Pathologists of Australasia, the Centenary Medal of the Australian Government, the CSIRO Eureka Prize for Leadership in Science, the inaugural Gutenberg Professorship of the University of Strasbourg, and the Julian Wells Medal of the Lorne Genome Society. He was elected an Associate Member of the European Molecular Biology Organisation in 2007 and a Fellow of the Australian Academy of Science in 2008. He was appointed an Officer in the Order of Australia in 2001. His research interest is RNA regulation and non-coding DNA in complex organisms, and has published articles on this topic in *Science*, *Nature Genetics*, *Nature Reviews Genetics*, *Genome Research*, *PNAS*, *Human Molecular Genetics* and *Scientific American*, among others.



**Ryan Taft PhD** is currently a University of Queensland and Australian Research Council supported Postdoctoral Research Fellow at the Institute for Molecular Bioscience. His work using high-throughput small RNA sequencing to discover three novel classes of small regulatory RNAs has been published in *Nature Genetics*, *Nature Structural and Molecular Biology*, *RNA* and *Cell Cycle*, among others. Dr Taft has had a distinguished academic career, recognized with a University of California Regent's Scholarship as an undergraduate, a US National Science Foundation Graduate Research Fellowship during his graduate studies with Professor Mattick, and recently as a finalist in two categories of the Australia Museum Eureka Prizes for excellence in science. Dr Taft is dedicated to bringing RNomics to the clinic.



## The RNomics Group

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The central hypothesis/heuristic of the RNomics group is that the majority of the genome of complex organisms is devoted to an RNA regulatory system, and that this was the enabling platform for the evolution and development of complex multicellular organisms.

The group uses bioinformatics and molecular genetics approaches to identify non-coding regions of the human genome that are under evolutionary and functional selection, and to map RNA regulatory networks. They use mathematical and computational approaches to modelling and understanding complex regulatory networks, as well as a variety of molecular biological approaches to identify higher order nucleic acid structures and their associated proteins. The group are also developing new databases, microarray chips and bioinformatics analysis tools to examine the expression of non-coding RNAs in humans and mice during development and in different disease states.

## IMBcom

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IMBcom is The University of Queensland's commercialisation company for the Institute for Molecular Bioscience (IMB). IMBcom have extensive experience in identifying and protecting intellectual property, devising development and business strategies, raising capital, licensing and founding start-up companies. Several of IMBcom technologies have progressed to Phase II.

## Intellectual Property

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A PCT application was filed in April 2009 to protect Transcription Initiation RNAs (tiRNAs) and sequences thereof.

A PCT application was filed in April 2011 to protect Splice-site RNAs (spliRNAs) and sequences thereof.

## IMBcom Contact Details

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## Key Publications

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- Hawkins and Taft et al. The relationship between tiRNAs and CTCF localization. Manuscript submitted (May 2011).
- Taft et al. Nuclear-localized tiny RNAs are associated with transcription initiation and splice sites in metazoans. *Nature Structural and Molecular Biology* (2010). E-pub ahead of print (11 July 2010)
- Taft et al. Non-coding RNAs: regulators of disease. *The Journal of Pathology* (2010) vol. 220 (2) pp. 126-39
- Taft et al. Evolution, biogenesis and function of promoter-associated RNAs. *Cell cycle* (2009) vol. 8 (15) pp. 1-7
- Taft et al. Tiny RNAs associated with transcription start sites in animals. *Nature Genetics* (2009) vol. 41 (5) pp. 572-8