The role of microRNAs in neurons

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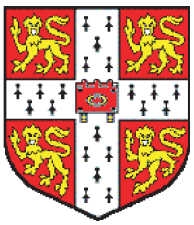

A dissertation submitted to the University of Cambridge
for the degree of Doctor of Philosophy

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June 1, 2011

To Alex, Leo, Marija,
Matias and Steve

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

This thesis does not exceed the specified length limit of 60,000 words as defined by the Biology Degree Committee.

This thesis has been typeset in 12pt font using LaTeX according to the specifications defined by the Board of Graduate Studies and the Biology Degree Committee.

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"The role of microRNAs in neurons"

Abstract

Many individual functional microRNA (miRNA) targets have been identified in neurons, and their importance for neuronal differentiation is well established. However, with over 50% of genes in a mammalian genome being computationally predicted as miRNA targets, the global significance of the role of miRNAs in neurons is not yet fully understood. Using chemical transfection, I artificially overexpressed ten miRNAs in primary neuronal cultures. For six of them I identified hundreds of putative direct targets through analysis of the differential gene expression associated with the transfection experiments. Among these six miRNAs, there were two that are naturally enriched in the adult mouse brain (miR-124 and miR-434-3p), three miRNAs that were depleted from neurites (miR-143, miR-145 and miR-25) and one non-mouse miRNA (cel-miR-67). Analysis of the miRNA mediated effects on gene expression revealed that upon overexpression both miR-124 and miR-434-3p destabilised mRNA transcripts that are seen to be induced in stress conditions. The effect of overexpression of the other four miRNAs was found to be similar to that of miR-124 and miR-434-3p, although it was less significant. The ability of miRNAs to downregulate the inducibly expressed genes, and a widespread upregulation of these genes in stress conditions, implies that miRNAs normally act to prevent changes to equilibrium in the transcriptome. The results of this thesis also demonstrate that a repertoire of miRNA targets, including that of the neuron specific miR-124, is context-dependent. Given that the context can be influenced by a stress associated with experimental treatments, this work bears direct implications for future experiments aiming to ascribe particular functions to miRNAs.

Acknowledgements

I would like to start by acknowledging people who made it possible for me to study in Cambridge University: the people behind the Darwin Trust of Edinburgh, the Wellcome Trust, and EMBL. The Darwin Trust funded my final two years of the undergraduate education in Edinburgh University and then the first three years of my postgraduate education in Cambridge University. I would especially like to thank Sir Kenneth Murray and Noreen Murray of the Darwin Trust, who I was fortunate to know personally and who will remain an inspiration for me for the rest of my life. I am also grateful to The Wellcome Trust, which provided me (in addition to the Darwin Trust) with support through the first three years, and which was the sole source of my funding in the fourth year. A big thanks to EMBL, too, which paid me a stipend during the remainder of my stay in Cambridge.

I would like to thank people who organised and ran the PhD program at the Wellcome Trust Sanger Institute, and my two principle supervisors, Seth Grant and Anton Enright. I would also like to thank Derek Stemple and Frederick Livesey for additional supervision.

Apart from the mentoring provided by the supervisors, it would be impossible for me to go through the project without learning from my colleagues in the laboratories of Anton Enright and Seth Grant. In the laboratory of Anton Enright, I would like to especially thank Cei Abreu-Goodger and Stijn van Dongen. I learned all of my bioinformatic tricks from the two of them, and they provided me with day to day guidance in my work for the duration of the project. Incredibly, Cei Abreu-Goodger even found time to review my experimental designs and protocols, for which I am very grateful. Additionally, I would like to thank other people in the laboratory of Anton Enright, and in particular Mat Davis, Harpreet Saini and Nenad Bartonicek, whose support and help was very valuable. In the laboratory of Seth Grant, I learned the skills and techniques necessary to conduct the experiments of the thesis. For that, I am grateful to all people of the team, but I would especially like to express my gratitude to Rene Frank, Andrew Morton, Eric MacLaren, Ellie Tuck, Fei Zhu, Sharifah Syura, Alex Bayes and Tomas Ryan who took their valuable

time to train me and provide me with the required materials. Also, thanks to Meng Li for providing me with a plasmid and Elena Vigorito for helpful advice with RT-PCR.

After spending some significant amount of time on an experimental project, it is very obvious that a great amount of work is undertaken outside of the lab, perhaps unnoticed, but without which the work in the lab would be impossible. I would like to thank the Wellcome Trust Sanger Institute Animal Research Facilities and also the Seth Grant's laboratory, for providing me with the animals for my experiments. Additionally, a massive amount of work has been done by the Institute's microarray facilities, and without the contribution of Peter Ellis, Naomi Hammond and Cordelia Langford my project would not be possible.

Finally, I would like to acknowledge my parents and my friends. My parents directly supported me through the duration of my project, and provided me with the shelter of their kind words and home. As for my friends, Marija Buljan, Alexandra Nica, Leopold Parts, Steve Pettitt and Matias Piipari... the margins of this thesis are too narrow to contain all my gratitude to them. What would the whole time and work in Cambridge be worth, if it was not for the friends like them?

Abbreviations and comments

Abbreviations:

Cat. no.	catalogue number
DIV	days of *in vitro* development
miRNA	microRNA
***n*-mer**	an oligomer of a length n
nt	nucleotides
ref.	reference
RT-PCR	real-time PCR
qRT-PCR	quantitative real-time PCR
P	P-value
UTR	Untranslated region

Comments:

- Very small numbers are presented using "E notation" as an alternative to the standard decimal notation. In this notation a letter e is used to represent *times ten risen to the power of*. For example, 0.000000012 in "E notation" is presented as $1.2e-8$ or $1.2e-08$.
- DNA is a polymer consisting predominantly of four types of units (nucleotides) containing the following four bases: adenine (the corresponding nucleotide is commonly denoted as A), cytosine (C), guanine (G) and thymine (T). RNA is also a polymer, which predominantly consists of nucleotides containing adenine, cytosine, guanine and uracil (the corresponding nucleotide is denoted as U) bases. In conventional Watson-Crick double stranded forms of RNA, DNA or DNA-RNA heteroduplexes, Gs form connections with Cs, while As pair with both Ts and Us. Therefore, U is RNA's equivalent of DNA's T. For purposes of consistency, sequences of DNA and RNA are frequently stored in databases as a sequence of the four letters A, G, C and T, where T is understood to be U in case of RNA sequences. In this thesis, I

preserved this notation, and both DNA and RNA nucleotide words are represented as sequences of A, T, G and C.

- The research of miRNA function that is presented in this system was conducted in an *in vitro* cell culture system derived from mice (*Mus musculus*). Conventionally, names of genes that encode miRNAs and names of miRNAs themselves are preceded by a three letter prefix, which uniquely corresponds to the species of the origin. Mouse miRNAs are preceded by three letters "mmu" (as in mmu-miR-124 or mmu-let-7c), while, human miRNAs (*Homo sapiens*) are preceded by "hsa" (as in hsa-miR-124 or hsa-let-7c). For convenience the three letter prefix of mouse miRNAs is frequently omitted, therefore names miR-124 and let-7c mean mmu-miR-124 and mmu-let-7c. Prefixes for other species are not omitted.

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