Roles of Post-Transcriptional Gene Silencing in the Functional Regulation of Neuronal Gene Expression and Plasticity

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DECLARATION

Statement of Originality
This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications.

Thesis by Publication
I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

________________________  ____________________
Belinda J Goldie          Date
ABSTRACT

The phenomenon of synaptic plasticity in neurons is poorly understood, but is known to rely on appropriate temporo-spatial availability of mRNA. The complexity of neuronal cytoarchitecture necessitates an exquisite regulatory matrix that begins with the establishment of subcellular compartments during differentiation, however the molecular mechanisms that support trafficking and translational control are not well defined. The class of short, non-coding RNA molecules known as microRNA (miRNA) have well-established roles in neuronal differentiation and development, and growing evidence suggests that miRNA-mediated post-transcriptional gene silencing (PTGS) may be an important mediator of synaptic plasticity. To investigate this in a human genetic context, techniques were established for isolating distinct subcellular fractions of the SH-SY5Y neuroblastoma cell line and examining genome-wide miRNA and mRNA responses to neuronal cues such as differentiation and depolarisation. These studies identified a pattern of activity-associated miRNA expression changes unique to the neurites that was revealed to be connected to the release of exosomes from this compartment. Interestingly, some miRNA were found to be preferentially enriched in the nucleus. A motif detected within these sequences lead to the unexpected identification of putative transcription factor binding elements within their precursors, showing support for novel roles of miRNA outside PTGS. Connecting these findings was the unanticipated contribution of primate-specific miRNA, resulting in significant ontological enrichment of neuronal functionality. This demonstrates the importance and relevance of these cells as a vehicle for explicating the mechanisms underlying higher brain functions. Ultimately, substantial evidence was obtained to support a role for miRNA and the components of PTGS in the functional compartmentalisation of neurons and the response to activity, though further methodological developments are required to elaborate the novel mechanisms of miRNA function and investigate the direct contribution of miRNA-mediated PTGS to enabling real-time, activity-driven synaptic modification.
ACKNOWLEDGEMENTS

I am very proud of what I have achieved in the (just over) 3 years of my candidature, but I could not have accomplished so much without the help and support of some very important people and for which I am incredibly grateful.

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LIST OF ABBREVIATIONS

3' UTR  3' Untranslated Region
AChE  acetylcholinesterase
AEBSF  4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
Ago  argonaute
ALS  amyotrophic lateral sclerosis
ANOVA  Analysis of Variance
ATRA  all-trans retinoic acid
BDNF  brain-derived neurotrophic factor
Ca2+  Calcium
cAMP  cyclic adenosine mono-phosphate
CNS  central nervous system
co-IP  co-immunoprecipitation
CRM1  exportin-1 (XPO1)
CV  coefficient of variability
DABG  detection above background
DAVID  Database for annotation, visualization and integrated discovery
DCt  change in cycle threshold value (delta Ct)
DE  differential expression
DGCR8  DiGeorge syndrome critical region 8
DLPFC  dorso-lateral pre-frontal cortex
DMEM  Dulbecco’s modified eagle medium
DTT  dithiothreitol
EDTA  ethylenediaminetetraacetic acid
eIF4b  elongation initiation factor 4b
ES  enrichment score
FAC  functional annotation clustering
FCS  fetal calf serum
FDR  false discovery rate
FOS  FBJ murine osteosarcoma viral oncogene homolog
GAP43  growth-associated protein 43
GATHER  gene annotation tool to help explain relationships
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>GPCR</td>
<td>g-protein coupled receptor</td>
</tr>
<tr>
<td>GRIA3/4</td>
<td>glutamate receptor, ionotopic, AMPA 3/4</td>
</tr>
<tr>
<td>GUSB</td>
<td>glucuronidase, beta</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity pathway analysis</td>
</tr>
<tr>
<td>iPSC</td>
<td>induced pluripotent stem cell</td>
</tr>
<tr>
<td>K+</td>
<td>Potassium</td>
</tr>
<tr>
<td>kDa</td>
<td>kiloDaltons</td>
</tr>
<tr>
<td>LAMP1</td>
<td>lysosome-associated membrane protein 1</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LDCV</td>
<td>large dense core vesicle</td>
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<tr>
<td>LE</td>
<td>localisation element</td>
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<tr>
<td>LTD</td>
<td>long-term depression</td>
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<tr>
<td>LTP</td>
<td>long-term potentiation</td>
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<tr>
<td>MAP1b</td>
<td>microtubule-associated protein 1b</td>
</tr>
<tr>
<td>MASCOT</td>
<td>Matrix Software program for protein identification from peptide mass</td>
</tr>
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<td>MAZ</td>
<td>myc-associated zinc finger protein</td>
</tr>
<tr>
<td>MEME</td>
<td>multiple EM for motif elicitation</td>
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<tr>
<td>miRNA</td>
<td>microRNA</td>
</tr>
<tr>
<td>MRE</td>
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<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>NGF</td>
<td>nerve growth factor</td>
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<td>NPC</td>
<td>neural progenitor cell</td>
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<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>NTRK2</td>
<td>neurotrophic tyrosine kinase, receptor, type 2</td>
</tr>
<tr>
<td>p</td>
<td>p-value</td>
</tr>
<tr>
<td>P-body</td>
<td>processing body</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCIAA</td>
<td>phenol chloroform isoamyl alcohol</td>
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<tr>
<td>pre-miRNA</td>
<td>precursor miRNA</td>
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<tr>
<td>pri-miRNA</td>
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<tr>
<td>PSD</td>
<td>post-synaptic density</td>
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<tr>
<td>PTGS</td>
<td>post-transcriptional gene silencing</td>
</tr>
<tr>
<td>Term</td>
<td>Description</td>
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<td>-------------------</td>
<td>--------------------------------------------------------------</td>
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<tr>
<td>qPCR/qRT-PCR/RT-PCR</td>
<td>quantitative real-time PCR</td>
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<tr>
<td>RAR</td>
<td>retinoic acid receptor</td>
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<td>retinoic acid response element</td>
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<td>RBM4/10</td>
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<td>RIN</td>
<td>RNA integrity number</td>
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<tr>
<td>RISC</td>
<td>RNA-induced silencing complex</td>
</tr>
<tr>
<td>RMA</td>
<td>robust multichip algorithm</td>
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<td>RNAi</td>
<td>RNA interference</td>
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<tr>
<td>RNAPII</td>
<td>RNA polymerase II</td>
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<td>mRNA next-generation sequencing</td>
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<td>RNP</td>
<td>ribonucleoprotein</td>
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<td>ROBO1/2</td>
<td>roundabout, axon guidance receptor, homolog 1/2</td>
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<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
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<td>SDS-PAGE</td>
<td>sodium dodecylsulfate polyacrylamide gel electrophoresis</td>
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<tr>
<td>snoRNA</td>
<td>small nucleolar RNA</td>
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<td>STG</td>
<td>superior temporal gyrus</td>
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<td>SYP</td>
<td>synaptophysin</td>
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<tr>
<td>TPA</td>
<td>phorbolester</td>
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<tr>
<td>TRBP/TARBP</td>
<td>trans-activation-responsive region RNA-binding protein</td>
</tr>
<tr>
<td>trkB</td>
<td>tyrosine receptor kinase B, encoded by NTRK2 gene</td>
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