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

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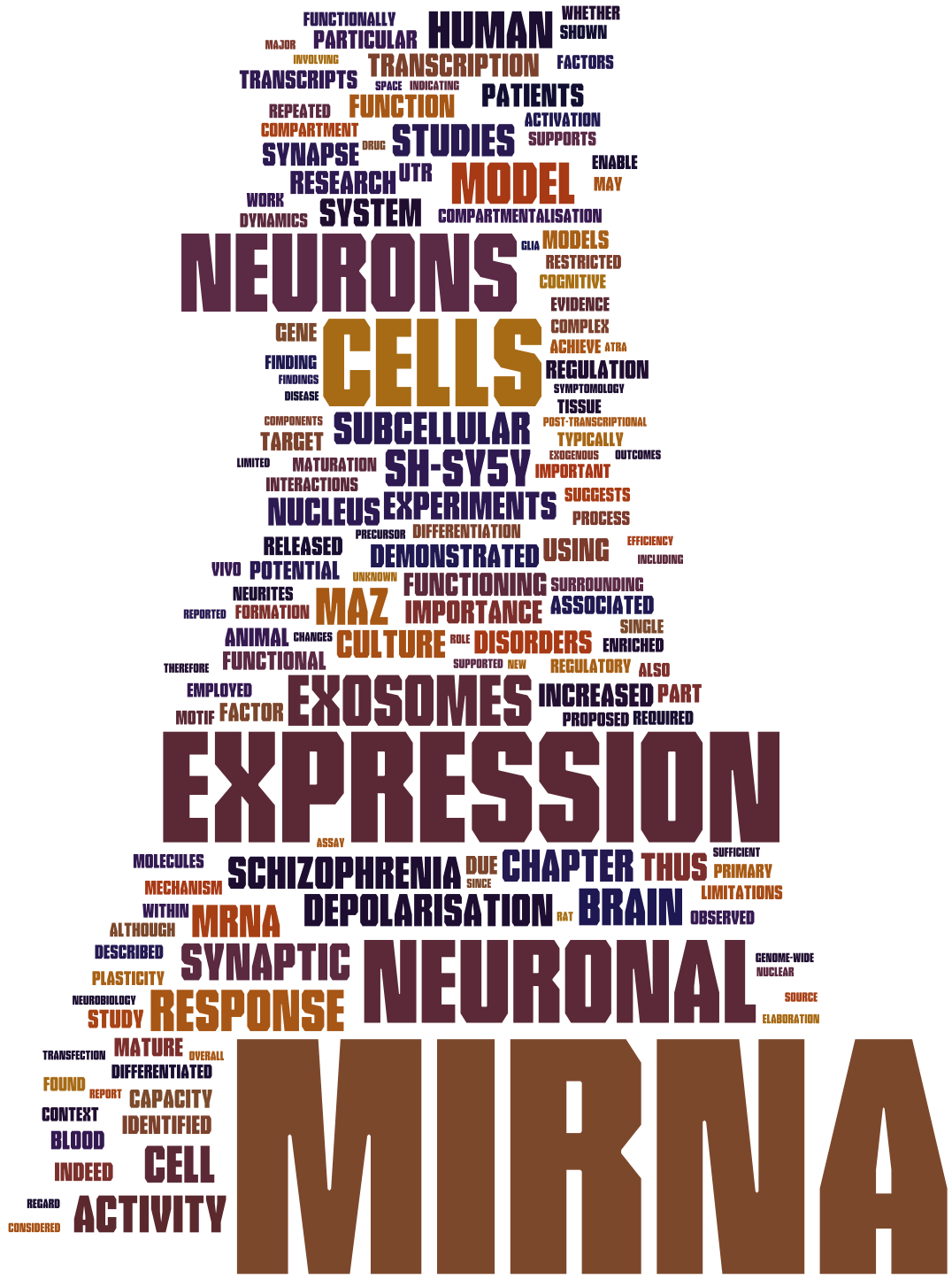
MIRNA
NEURONAL
EXPRESSION
NEURONS
CELLS
EXOSOMES
MODEL
HUMAN
SYNAPTIC
RESPONSE
ACTIVITY
CELL
BRAIN
MRNA
DEPOLARISATION
SCHIZOPHRENIA
CHAPTER
SUBCELLULAR
SH-SY5Y
MAZ
CULTURE
DISORDERS
SYSTEM
STUDIES
TRANSCRIPTION
FUNCTION
PATIENTS
RESEARCH
NUCLEUS
EXPERIMENTS
DEMONSTRATED
FUNCTIONING
IMPORTANCE
MATURE
DIFFERENTIATED
CAPACITY
IDENTIFIED
FUNCTIONALLY
PARTICULAR
WHETHER
SHOWN
FACTORS
REPEATED
ACTIVATION
SUPPORTS
COMPARTMENT
SYNAPSE
ENABLE
MAY
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DYNAMICS
COMPARTMENTALISATION
GLIA
MODELS
RESTRICTED
COGNITIVE
EVIDENCE
GENE
COMPLEX
ACHIEVE
ATRA
FINDING
REGULATION
SYMPTOMOLOGY
DISEASE
TISSUE
COMPONENTS
POST-TRANSCRIPTIONAL
TARGET
TYPICALLY
LIMITED
MATURATION
EXOGENOUS
OUTCOMES
IMPORTANT
INTERACTIONS
SUGGESTS
PROCESS
PRECURSOR
DIFFERENTIATION
RELEASED
EFFICIENCY
INCLUDING
VIVO
POTENTIAL
USING
UNKNOWN
SURROUNDING
NEURITES
ASSOCIATED
REPORTED
FORMATION
SINGLE
ANIMAL
CHANGES
ROLE
ENRICHED
FUNCTIONAL
SUPPORTED
NEW
REGULATORY
ALSO
THEREFORE
EMPLOYED
INCREASED
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PROPOSED
REQUIRED
ASSAY
SUFFICIENT
MOLECULES
DUE
THUS
PRIMARY
MECHANISM
SINCE
LIMITATIONS
WITHIN
RAT
OBSERVED
ALTHOUGH
DESCRIBED
GENOME-WIDE
NUCLEAR
PLASTICITY
NEUROBIOLOGY
SOURCE
STUDY
ELABORATION
TRANSFECTION
OVERALL
FOUND
REPORT
CONTEXT
BLOOD
INDEED
REGARD
CONSIDERED
MAJOR
INVOLVING
TRANSCRIPTS
SPACE
INDICATING
DRUG
WORK

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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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.

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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

GPCR	g-protein coupled receptor
GRIA3/4	glutamate receptor, ionotropic, AMPA 3/4
GUSB	glucuronidase, beta
IPA	Ingenuity pathway analysis
iPSC	induced pluripotent stem cell
K+	Potassium
kDa	kiloDaltons
LAMP1	lysosome-associated membrane protein 1
LC-MS/MS	liquid chromatography-mass spectrometry
LDCV	large dense core vesicle
LE	localisation element
LTD	long-term depression
LTP	long-term potentiation
MAP1b	microtubule-associated protein 1b
MASCOT	Matrix Software program for protein identification from peptide mass
MAZ	myc-associated zinc finger protein
MEME	multiple EM for motif elicitation
miRNA	microRNA
MRE	miRNA recognition element
mRNA	messenger RNA
NGF	nerve growth factor
NPC	neural progenitor cell
NPY	neuropeptide Y
NTRK2	neurotrophic tyrosine kinase, receptor, type 2
p	p-value
P-body	processing body
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCIAA	phenol chloroform isoamyl alcohol
PFC	pre-frontal cortex
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA
PSD	post-synaptic density
PTGS	post-transcriptional gene silencing

qPCR/qRT-PCR/RT-PCR	quantitative real-time PCR
RAR	retinoic acid receptor
RARE	retinoic acid response element
RBM4/10	RNA binding motif protein 4/10
RIN	RNA integrity number
RIP	RNA co-immunoprecipitation
RIP-seq	RNA co-immunoprecipitation followed by RNAseq
RISC	RNA-induced silencing complex
RMA	robust multichip algorithm
RNAi	RNA interference
RNAPII	RNA polymerase II
RNAseq	mRNA next-generation sequencing
RNP	ribonucleoprotein
ROBO1/2	roundabout, axon guidance receptor, homolog 1/2
RRM	RNA recognition motif
rRNA	ribosomal RNA
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
snoRNA	small nucleolar RNA
STG	superior temporal gyrus
SYP	synaptophysin
TPA	phorbolester
TRBP/TARBP	trans-activation-responsive region RNA-binding protein
trkB	tyrosine receptor kinase B, encoded by NTRK2 gene