DISCOVERY OF THE NOVEL *MOUFSNRP* GENE AND THE CHARACTERISATION OF ITS *IN SITU* EXPRESSION PROFILE DURING MOUSE NEUROGENESIS

PRIVAHINI BRADOO

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This thesis is dedicated
to my mom, Mrs. Nalini Bradoo,
for always believing in the scientist in me.

ABSTRACT

Recently, a novel protein family, named as neural regeneration peptides (NRPs), was predicted across the rat, human and mouse genomes by one of my supervisors, Dr. Sieg. Synthetic forms of these proteins have been previously shown to act as potent neuronal chemoattractants and have a major role in neural regeneration. In light of these properties, these peptides are key candidates for drug development against an array of neurodegenerative disorders.

The aim of this PhD project was to provide confirmation of the existence of a member of the NRP coding gene family, annotated in the mouse genome. This gene, called *mouse frameshift nrp* (*mouFSnrp*), was hypothesised exist as a -1bp frameshift to another predicted gene *AlkB*. This project involved the identification of the *mouFSnrp* gene, and the characterisation of its expression pattern and ontogeny during mouse neural development. Through the work described in this thesis, the *mouFSnrp* gene was identified in mouse embryonic cortical cultures and its protein coding gene sequence was verified. *mouFSnrp* expression was shown to be present in neural as well as non-neural tissues, via RT-PCR. Using non-radioactive *in situ* hybridisation and immunohistochemical colocalisation studies, interesting insights into the lineage and ontogeny of *mouFSnrp* expression during brain development were revealed. These results indicate that *mouFSnrp* expression originates in neural stem cells of the developing cortex, and appears to be preferentially continued via the radial glial lineage. *mouFSnrp* expression is carried forward via the neurogenic radial glia into their daughter neuronal progeny as well as postnatal astrocyte. In the postnatal brain, *mouFSnrp* gene transcripts were also observed in the olfactory bulb and the hippocampus, both of which are known to have high neurogenic potential. In general, the radial glial related nature of *mouFSnrp* expression appears to be a hallmark of the *mouFSnrp* expression pattern throughout neural development.

This thesis provides the first confirmation of the existence of a completely novel gene, *mouFSnrp*, and its putative -1 translational frameshifting structure. Further, preliminary data presented in this thesis regarding the *mouFSnrp in situ* expression pattern during mouse brain development may suggest a key role of the gene in neuronal migration and neurogenesis in mice.

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ABBREVIATIONS

3-NP	3-Nitropropanoic Acid
aa	amino acids
ACM	Astrocyte Conditioned Medium
AlkB	Alkylated DNA repair protein homolog in *Mus musculus*
ApoER2	Apolipoprotein E Receptor type 2
Astn	Astrotactin encoding gene
BDNF	Brain Derived Neurotrophic Factor
bFGF	basic Fibroblast Growth Factor
bp	base pairs
BrdU	Bromodeoxyuridine
Cdk5	Cyclin-dependent kinase 5
CDS	Protein Coding Sequence
CGE	Caudal Ganglionic Eminences
CNS	Central Nervous System
CNTF	Ciliary Neurotrophic Factor
CP	Cortical Plate
CXCR4	CXC Class Chemokine Receptor 4
Dab1	Disabled 1 encoding gene
DCC	Deleted in Colorectal Cancer
DCX	Doublecortin
DIG	Digoxygenin
DIV	days *in vitro*
dsDNA	double stranded DNA
Edr	Embryonal Carcinoma Differentiation Regulated gene
EGFR	Epidermal Growth Factor Receptor
EMBL	European Molecular Biology Laboratories
ERK	Extracellular Signal Regulated Kinase
EtBr	Ethidium Bromide
FACS	Flourescence Activated Cell Sorting
FBS	Fetal Bovine Serum
FILIP	Filamin 1 - Interacting Protein
GAPDH	Glyceraldehye-3-Phosphate Dehydrogenase
GDNF	Glial Derived Neurotrophic Factor
GE	Ganglionic Eminences
GFAP	Glial Fibrillary Acidic Protein
H_2O_2	Hydrogen Peroxide
IHC	Immunohistochemistry
ISH	*in situ* hybridisation
IZ	Intermediate Zone

LGE	Lateral Ganglionic Eminence
LIS1	Lissencephaly 1 protein
LIS1	Lissencephaly 1 gene
MEM	Minimum Essential Medium
MGE	Medial Ganglionic Eminence
mouFSnrp	*mouse frameshift nrp* gene
MZ	Marginal Zone
N2A	Neuro2A neuroblastoma cell line
NB	Neurobasal Medium
NCBI	National Centre for Biotechnology Information
NEP	Neuroepithilial Progenitors
NGF	Nerve Growth Factor
NMIP-1	Neuronal Migration Inducing Peptide -1
NRG	Neuregulin
NRP	Neural Regeneration Peptide
NSC	Neural Stem Cells
nud	nuclear distribution mutant gene
NUDEL	NUDE-like
ODC	Ornithine Decarboxylase
ORF	Open Reading Frame
OTC	Organotypic Tissue Culture
PAFAH1B1	Platelet-Activating Factor Acetylhydrolase Isoform 1b
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PFA	Paraformaldehyde
PLL	Poly-L-Lysine
POA	Anterior Preoptic Area
PP	Preplate
PS	Pen/Strep
RC1	Radial Cell 1
RC2	Radial Cell 2
RF2	Peptide Release Factor 2
RG	Radial glia
RMS	Rostral Migratory Stream
RT	Room Temperature
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SDF-1	Stromal Cell-Derived Factor -1
SDSC	San Diego Supercomputer
Sema-3A	Semaphorin 3A
Sema-3F	Semaphorin 3F
siRNA	Small Interfering RNA
SNRPs	Synthetic NRP peptides
SP	Subplate
sscRNA	single stranded complementary RNA

ssDNA	single stranded DNA
SVZ	Subventricular Zone
SVZa	Anterior Subventricular Zone
T_a	Annealing Temperature
TAG-1	Contactin 2
TGFβ	Transforming Growth Factor-β
T_m	Melting Temperature
VLDLR	Very Low Density Lipoprotein Receptor
VZ	Ventricular Zone
WM	White Matter