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

PRIVAHINI BRADOO

**A thesis submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Molecular Medicine,
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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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TABLE OF CONTENTS

ABSTRACT	I
ACKNOWLEDGEMENTS	II
TABLE OF CONTENTS	III
LIST OF FIGURES.....	VI
LIST OF TABLES.....	IX
ABBREVIATIONS	X

CHAPTER 1: PREFACE	1
--------------------------	---

CHAPTER 2: INTRODUCTION.....	5
------------------------------	---

2.1	CELLULAR MECHANISMS INVOLVED IN NEURONAL MIGRATION.....	5
2.2	NEURONAL MIGRATION IN CORTICOGENESIS	7
2.2.1	Radial migration in the cerebral cortex.....	10
2.2.1.1	‘Inside-out’ cortical formation.....	10
2.2.1.2	Radial glia in the developing cerebral cortex– a murine perspective	12
2.2.1.2.1	RG as neuronal progenitors.....	13
2.2.1.3	Modes of radial migration.....	15
2.2.1.3.1	Somal translocation.....	15
2.2.1.3.2	Glia-guided locomotion.....	15
2.2.1.3.3	Multipolar migration.....	16
2.2.1.4	Factors involved in neuron-glia association during locomotion.....	17
2.2.1.4.1	Filamin 1 in leading edge extension.....	17
2.2.1.4.2	Cdk5 and p35 in glia guided migration.....	18
2.2.1.4.3	Other molecules involved in neuron-glia interaction.....	19
2.2.1.5	Genetic influences in nucleokinesis during radial migration	20
2.2.1.5.1	The LIS1 gene.....	20
2.2.1.5.2	Doublecortin and the DCX gene.....	21
2.2.1.5.3	The role of cdk5-p35/p39.....	21
2.2.1.6	Determinants of cortical lamination.....	22
2.2.1.6.1	The ‘reeler’ mouse.....	22
2.2.1.6.2	The external limiting membrane.....	23
2.2.2	Tangential migration of cortical interneurons	24
2.2.2.1	Role of guidance cues in migration of cortical interneurons	26
2.2.2.2	Stromal Cell Derived Factor-1 in chemoattraction and neuronal regeneration.....	27
2.2.2.2.1	SDF-1 and its receptor CXCR4.....	28
2.2.2.2.2	A novel role of SDF-1/CXCR4 in neuronal migration and cortical development.....	28
2.2.2.2.3	New insights into SDF-1 involvement in neurogenesis and neuronal regeneration.....	29
2.3	DISCOVERY OF THE NOVEL NRP PROTEIN FAMILY	30
2.3.1	In vitro slice culture assays provide evidence of a migration-inducing factor	30
2.3.2	Purification of the rat NMIP fragment.....	31
2.3.3	Prediction of the <i>np</i> gene in rodents	32
2.3.4	NRP protein domain characterisation	33
2.3.5	Neuroprotection and induction of neural migration by synthetic NRPs	33
2.3.6	Induction of proliferation, neuronal differentiation and axonal outgrowth by synthetic NRPs.....	35
2.3.7	Activity profile of recombinant mouse NRP	35
2.4	AIMS OF THIS PROJECT	36

CHAPTER 3: MATERIALS AND METHODS..... 38

3.1	ANIMAL WORK AND TISSUE CULTURE METHODS	38
3.1.1	Ethical approval for the work presented in this thesis	38
3.1.2	Culturing primary astrocytes and preparing astrocyte condition media.....	39
3.1.3	Coating a 6- well plates with Poly-L-Lysine + Laminin	40
3.1.4	Dissection of cortex from embryonic mouse brains.....	40
3.1.5	Cortical cell extraction and plating in 6-well plates	41
3.1.6	Culturing neural stem cells and MEB-5 cells in 6-well plates	41
3.1.7	Obtaining neural and non-neural tissue from embryonic CD mice	42
3.1.8	Growing and culturing Neuro2A cells.....	42
3.2	MOLECULAR BIOLOGY METHODS	43
3.2.1	Total RNA isolation	43
3.2.2	Formaldehyde gels for RNA electrophoresis	44
3.2.3	DNase I treatment of RNA.....	45
3.2.4	cDNA synthesis from RNA	45
3.2.5	Polymerase Chain Reaction.....	46
3.2.6	Multiplex PCR	47
3.2.7	Gel Electrophoresis	47
3.2.8	Gel Extraction of PCR product for gene sequencing.....	47
3.3	IN SITU HYBRIDISATION AND COLOCALISATION STUDIES	48
3.3.1	Fixing and embedding mouse brains	48
3.3.2	Slicing brain sections in the cryostat	49
3.3.3	Development the sense and antisense riboprobes for in situ hybridisation and <i>in vitro</i> transcription	49
3.3.4	Determination of DIG labelling efficiency of riboprobes	50
3.3.5	In situ hybridisation on brain sections.....	51
3.3.6	Double labelling of <i>in situ</i> sections with primary mouse antibodies	53
3.3.7	Double labelling of <i>in situ</i> sections with primary rabbit antibodies.....	53

CHAPTER 4: DISCOVERY OF THE *MOUFSNRP* GENE..... 55

4.1	INTRODUCTION.....	55
4.2	METHODS.....	58
4.3	RESULTS.....	59
4.3.1	Verifying the existence and expression of the predicted <i>mouFSnrp</i> gene.....	59
4.3.2	Prediction of a new gene organisation structure.....	69
4.3.3	Sequencing the <i>mouFSnrp</i> gene	71
4.3.4	Identifying a part of the <i>mouFSnrp</i> 5' transcription region.....	74
4.3.5	Determining the <i>mouFSnrp</i> 3' non-translated region.....	79
4.3.6	The identified <i>mouFSnrp</i> mRNA transcript sequence	80
4.3	DISCUSSION.....	82

CHAPTER 5: TISSUE SPECIFICITY AND CELL ONTOGENY OF *MOUFSNRP* EXPRESSION..... 87

5.1	INTRODUCTION.....	87
5.2	METHODS.....	88
5.3	RESULTS.....	89
5.3.1	Non-neural expression of <i>mouFSnrp</i>	89
5.3.2	<i>mouFSnrp</i> expression in different parts of the embryonic mouse brain	90
5.3.3	<i>mouFSnrp</i> expression in neural stem cells	91
5.3.4	Effect of E15 and MEB-5 NSC differentiation towards astrocytic and neuronal lineages <i>in vitro</i> on <i>mouFSnrp</i> expression	92
5.3.5	<i>mouFSnrp</i> expression in postnatal astrocytes.....	93
5.4	DISCUSSION.....	94

CHAPTER 6: *IN SITU* HYBRIDIZATION STUDY OF *MOUFSNRP* EXPRESSION DURING MOUSE NEUROGENESIS..... 96

6.1 INTRODUCTION.....96
6.2 METHODS..... 100
6.2.1 Developing a mouFSnrp specific DIG labelled probe.....100
6.2.2 Obtaining brain sections from embryonic and postnatal mouse brains102
6.2.3 ISH on mouse brain sections.....103
6.2.4 IHC using neural molecular markers *in situ* hybridised mouse brain sections104
6.3 RESULTS..... 106
6.3.1 Spot assay to match the sense and antisense probe concentrations106
6.3.2 In situ confirmation of mouFSnrp mRNA expression in embryonic mouse brain107
6.3.3 Pattern of mouFSnrp *in situ* expression during embryonic neural development111
6.3.4 *mouFSnrp* expression continues in early postnatal mouse brain tissue119
6.3.5 Characterising the cell ontogeny of *mouFSnrp* expression in neural development.....128

CHAPTER 7: GENERAL DISCUSSION AND FUTURE PERSPECTIVES.....147

7.1 THE FRAMESHIFT STRUCTURE OF THE *MOUFSNRP* GENE: IMPLICATIONS FOR OUR UNDERSTANDING OF MAMMALIAN GENOMICS 147
7.2 *MOUFSNRP* IN NEURAL DEVELOPMENT 150
7.3 LIMITATIONS OF THE WORK PRESENTED IN THIS THESIS 155
7.4 CONCLUSIONS AND FUTURE PERSPECTIVES..... 157

REFERENCES159

APPENDIX A.....170

APPENDIX B.....176

APPENDIX C.....178

APPENDIX D185

APPENDIX E.....191

LIST OF FIGURES

Figure 2.1: Nucleokinesis during cell migration	6
Figure 2.2: Anatomical structure of the developing mouse forebrain	8
Figure 2.3: The two major modes of migration during mouse corticogenesis.....	9
Figure 2.4: Inside-out cortical patterning via radial migration in mouse.....	11
Figure 2.5: RG cells are the predominant neuronal progenitors of the VZ	14
Figure 2.6: Somal translocation and glia-guided locomotion during radial migration	16
Figure 4.1 Initially hypothesised structure for the <i>mouFSnrp</i> gene	57
Figure 4.2 Selection of <i>mouFSnrp</i> specific F1 and R1 primers	60
Figure 4.3 Selection of <i>mouFSnrp</i> specific F2 and R2 primers	63
Figure 4.4: RNA Formadehyde gels	64
Figure 4.5: Using F1R1, F2R2 and F1R2 pimer combinations to identify <i>mouFSnrp</i> expression via RT-PCR.....	65
Figure 4.6: β -actin PCR using Eppendorf HotMaster Taq.....	67
Figure 4.7: F2R2 PCR using Eppendorf HotMaster Taq.....	67
Figure 4.8: β -actin, F1R2 and F2R2 PCRs on DNase I and RNase A treated E15 cortical cDNA	68
Figure 4.9 The new proposed structure for the <i>mouFSnrp</i> gene	70
Figure 4.10: <i>mouFSnrp</i> CDS Sequencing result.....	72
Figure 4.11 CLUSTALW gene sequence alignment of the predicted <i>mouFSnrp</i> gene with the F2R2 sequencing result	73
Figure 4.12: F3R2 PCR to identify <i>mouFSnrp</i> 5' transcription region	74
Figure 4.13: <i>mouFSnrp</i> 5' Sequencing results	75
Figure 4.14: CLUSTALW gene sequence alignment of the F3R2 sequencing result over the predicted <i>mouFSnrp</i> 5' transcription region.....	77
Figure 4.15: CLUSTALW protein sequence alignment of the putative <i>mouFSnrp</i> protein product with the RIKEN predicted hypothetical protein	79
Figure 4.16 The 1335bp confirmed <i>mouFSnrp</i> gene sequence.....	81
Figure 5.1: Expression of the <i>mouFSnrp</i> gene in non-neural tissues.....	90
Figure 5.2: <i>mouFSnrp</i> expression in neural tissues	91
Figure 5.3: <i>mouFSnrp</i> expression in primary and secondary stem cells	91
Figure 5.4: <i>mouFSnrp</i> expression in undifferentiated, and astrocytically and neuronally differentiated primary and secondary stem cells.....	92
Figure 5.5: <i>mouFSnrp</i> expression in postnatal astrocytes	93

Figure 6.1: The layers of the cerebral wall at different stages of development	97
Figure 6.2: Anatomical layers of the early postnatal rodent OB.....	98
Figure 6.3: <i>mouFSnrp</i> specific region chosen for development of sense and antisense ISH riboprobes.....	101
Figure 6.4: Spot assay comparing sense and antisense probe concentrations with control RNA	106
Figure 6.5: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E15 mouse cortex (10X, Coronal Section)	108
Figure 6.6: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E15 mouse cortex (20X, Coronal Section)	109
Figure 6.7: Sense control for <i>in situ</i> hybridisation on E15 mouse cortex (10X, Coronal Section)	110
Figure 6.8: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E13 mouse cortex (10X, Coronal Section)	112
Figure 6.9 <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E17 mouse brain (2.5X, Coronal Section)	113
Figure 6.10: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E17 mouse cortex (20X, Coronal Section)	114
Figure 6.11: Sense control for <i>in situ</i> hybridisation on E17 mouse cortex (10X, 20X, Coronal Section)	115
Figure 6.12: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E19 mouse brain (2.5X, Coronal Section)	116
Figure 6.13: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in E19 mouse cortex (10X, Coronal Section)	117
Figure 6.14: Sense control for <i>in situ</i> hybridisation on E19 mouse cortex (10X, Coronal Section)	118
Figure 6.15: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P0 mouse cortex (10X, Coronal Section)	120
Figure 6.16: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P0 mouse brain (2.5X, Sagittal Section).....	1201
Figure 6.17: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P0 mouse hippocampus (10X, Sagittal Section).....	1202
Figure 6.18: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P0 mouse Olfactory Bulb (10X, Sagittal Section)	123
Figure 6.19: Sense control for <i>in situ</i> hybridisation on P0 mouse cortex (10X, Coronal Section)	124
Figure 6.20: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P2 mouse brain (2.5X, Sagittal Section).....	1205
Figure 6.21: <i>In situ</i> expression of <i>mouFSnrp</i> mRNA in P4 mouse forebrain (2.5X, Sagittal Section).....	1206
Figure 6.22: <i>mouFSnrp in situ</i> signal in P4 mouse olfactory bulb (10X, Sagittal Section)	127
Figure 6.23: IHC using nestin on an <i>in situ</i> hybridised E13 brain section.....	129

Figure 6.24: Nestin colocalisation with <i>mouFSnrp in situ</i> signal in E13 cortex (20X, Coronal Section)	130
Figure 6.25: Nestin colocalisation with <i>mouFSnrp</i> -positive cells in E15 cortex (40X, Coronal Section)	131
Figure 6.26: Vimentin colocalisation with <i>mouFSnrp</i> -positive cells in E17 cortex (20X, Coronal Section)	131
Figure 6.27: GFAP double labelling with <i>mouFSnrp</i> -positive cells in P0 cortex (40X, Coronal Section)	132
Figure 6.28: <i>mouFSnrp</i> is not expressed in neuronal precursor cells in the mouse cortex (20X, Coronal Section)	133
Figure 6.29: MAP2 colocalisation with <i>mouFSnrp in situ</i> signal in E17 cortical plate (20X, Coronal Section)	134
Figure 6.30: MAP2-positive neurons express <i>mouFSnrp</i> in P0 cortex (20X, Coronal Section)	136
Figure 6.31: Calbindin does not colocalise with <i>mouFSnrp</i> in the P0 brain (20X, Coronal Section)	137
Figure 6.32: Summary of <i>mouFSnrp</i> gene expression in the embryonic and early postnatal mouse brain	144

Figure 7.1: Summary of suggested roles of <i>mouFSnrp</i> gene during the embryonic and early postnatal mouse brain development	154
---	-----

LIST OF TABLES

Table 1:	Oxidative injury conditions used on N2A to induce <i>monFScnp</i> expression.....	62
Table 2:	Neuronal regenerative effects of SNRPs as shown by Gorba, Bradoo <i>et al.</i> , 2005	82
Table 3:	Advantages and Disadvantages of Probes used for ISH.....	191
Table 4:	Merits of Radiolabelled and other Probes	192

ABBREVIATIONS

3-NP	3-Nitropropanoic Acid
aa	amino acids
ACM	Astrocyte Conditioned Medium
AlkB	Alkylated DNA repair protein homolog in <i>Mus musculus</i>
ApoER2	Apolipoprotein E Receptor type 2
<i>Astn</i>	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
<i>Dab1</i>	Disabled 1 encoding gene
DCC	Deleted in Colorectal Cancer
<i>DCX</i>	Doublecortin
DIG	Digoxygenin
DIV	days <i>in vitro</i>
dsDNA	double stranded DNA
<i>Edr</i>	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 ₂ O ₂	Hydrogen Peroxide
IHC	Immunohistochemistry
ISH	<i>in situ</i> hybridisation
IZ	Intermediate Zone

LGE	Lateral Ganglionic Eminence
LIS1	Lissencephaly 1 protein
<i>LIS1</i>	Lissencephaly 1 gene
MEM	Minimum Essential Medium
MGE	Medial Ganglionic Eminence
<i>mouFSnrp</i>	<i>mouse frameshift nrp</i> gene
MZ	Marginal Zone
N2A	Neuro2A neuroblastoma cell line
NB	Neurobasal Medium
NCBI	National Centre for Biotechnology Information
NEP	Neuroepithelial Progenitors
NGF	Nerve Growth Factor
NMIP-1	Neuronal Migration Inducing Peptide -1
NRG	Neuregulin
NRP	Neural Regeneration Peptide
NSC	Neural Stem Cells
<i>nud</i>	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