MOLECULAR CLONING OF *Neuronatin*

A NOVEL BRAIN-SPECIFIC MAMMALIAN DEVELOPMENTAL GENE

RAJIV JOSEPH

Submitted for the degree of

Ph.D.

University of London
London, England

Work done at

Henry Ford Hospital & Health Sciences Center
Case Western Reserve University
Laboratory of Molecular Neuroscience
Department of Neurology
2799 West Grand Boulevard
Detroit, Michigan, 48202, U.S.A.

1996

Dedicated to the memory of my beloved Father

Professor M. P. Joseph.

ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the *Laboratory of Molecular Neuroscience* at Henry Ford Hospital, Detroit. Detailed laboratory records have been maintained before and during the entire period of registration, and provide further details of the experiments discussed in this thesis.

I would like to thank Dr. K. Michael A. Welch and Dr. Jacqueline deBelleroche for their help and support. Dr. Welch, Chairman of Neurology, Henry Ford Hospital has been a perennial source of advice and encouragement ever since I joined this Department in July 1985. I am deeply grateful to him for all the years of unfailing trust. Dr. deBelleroche is the Head of the Neuroscience Group of the Departments of Biochemistry & Neurology, Charing Cross & Westminster Medical School, London. I thank her for guiding me through this entire project, providing advice on the telephone and by facsimile, reviewing my written reports, and finally helping me put together the final version of the thesis.

In the laboratory, I wish to thank Mr. Wayne Tsang and Dr. Dexian Dou for their support. Wayne joined the laboratory in July 1992 as a technician. He carried out the casting and electrophoresis of the differential display and sequencing gels, and most of the Northern blot analyses. Dou joined the laboratory in January 1994 as a postdoctoral fellow and is now an Assistant Staff Investigator. He carried out the construction and screening of the library with the novel cDNA fragments that eventually lead to the sequencing of full length rat *neuronatin* cDNA. It is impossible for me to overstate the importance of their hard work and support for which I am grateful.

I miss not having spent more time with my sons, Arun and Rahul. I am sure someday they too will understand. Finally, my wife Shanthi has been my reassurance. Her welcoming smile and manner made homecoming the most rewarding part of each day.

ABSTRACT

Mammalian brain development and its progression to maturity and predictable culmination in senescence are governed by differential gene expression. Therefore, the genes expressed in *neonatal, adult* and *aged* rat brain were searched in order to isolate those genes that are differentially expressed in the developing brain. Brain complementary deoxyribo-nucleic acid (cDNA) samples were prepared for differential display and electrophoresed on polyacrylamide gels. Thirty five differentially expressed cDNA fragments were extracted from the gels, amplified by polymerase chain reaction and used for Northern blot analyses. Only seven cDNA fragments were confirmed to be differentially expressed. Following cloning and sequencing, five of these seven cDNA fragments had sequences without homology in the GenBank database. These five novel cDNA fragments were pooled and used to screen a rat brain cDNA library.

This lead to the isolation of *neuronatin* (neuronal-neonatal), a novel messenger ribo-nucleic acid (mRNA). *Neuronatin* mRNA had two alternatively spliced forms, α and β. *Neuronatin-*α encoded a polypeptide of 81 amino acid residues with three exons and *neuronatin-*β encoded *54* amino acids with two exons. Although, the β-form was otherwise identical to the α -form, it lacked the 81 bp middle exon. *Neuronatin* mRNA was selectively expressed in the brain (and neurons) during development. The α-form first appeared at embryonic day 7-10 (E7-10), whereas, the β-form appeared at E11-14 coinciding with the closure of the neural tube and the onset of neuroepithelial proliferation and neuroblast generation. Thereafter, the expression of both forms became more pronounced at E16-19 accompanying a surge in neurogenesis.

Neuronatin mRNA levels declined by the end of the second postnatal week. However, traces of *neuronatin* mRNA continued to be present even in the adult brain.

The cellular function of *neuronatin* mRNA was investigated in PC12 cells, an established neuronal cell line. Although, *neuronatin* mRNA was abundant in undifferentiated PC12 cells, its expression became repressed when the cells were allowed to differentiate with nerve growth factor supporting a role for *neuronatin* in neuronal growth. Other investigators have noted that the mouse homolog of *neuronatin* was first expressed in rhombomeres-3 and 5 of E8.5 hindbrain implicating this gene in the determination of hindbrain segmentation. The deduced *neuronatin* protein has a hydrophobic N-terminal and a hydrophilic C-terminal. The primary sequence and structural organization of the protein exhibited homology to *PMP1* and *phospholamban*, members of the *proteolipid* class of proteins, which function either as regulatory subunits or independent ion channels. The rat *neuronatin* cDNA sequence was used to screen human libraries leading to the identification of the human *neuronatin* mRNA isoforms and the human gene. Together these studies revealed a significant level of homology in the deduced *neuronatin* protein sequences of rat, human and mouse. The human *neuronatin* gene has also been completely sequenced and assigned to chromosome-20q11.2-12. Evaluation of the genomic organization of *neuronatin* confirmed the basis for the two alternatively spliced mRNA isoforms.

In conclusion, *neuronatin* is a novel, brain-specific and highly conserved mammalian gene involved in brain development.

CONTENTS

J. Joseph, Dou, Tsang. *Brain Res* 1995; 690: 92-98 241

K. Dou, Joseph. *Nature Conference: Patterns of Life 1995* (abstract) 248

L. Joseph, Dou, Tsang. *Soc Neurosci Abs* 1995; *21:* 284 (abstract) 249

M. Dou, Joseph. *Soc Neurosci Abs 1995;* 21: 284 (abstract) 250

N. Joseph, Dou. *Neurology* 1996; 46: A148 (abstract) 251

0. Dou, Joseph. *Neurology* 1996; 46: A148 (abstract) 252

P. Dou, Joseph. *Genomics* 1996; 33: 292-297 253

Q. Dou, Joseph. *Brain Res* 1996; 723: 8-22 259

R. Joseph, Tsang, Dou, Nelson, Edvardsen. *Brain Res* 1996 (in press) 274

TABLES

FIGURES

CHAPTER-I: INTRODUCTION

CHAPTER-Il: METHODS

CHAPTER-III: RESULTS

CHAPTER-IV: DISCUSSION

PUBLICATIONS

PAPERS

1. Joseph R, Dou D, Tsang W. Molecular cloning of a novel mRNA *(Neuronatin)* that is highly expressed in neonatal mammalian brain. *Biochem Biophys Res Commun* 1994; 201: 1227-1234.

2. Joseph R, Dou D, Tsang W. *Neuronatin* mRNA: Alternatively spliced forms of a novel brain-specific mammalian developmental gene. *Brain Res* 1995; 690: 92-98.

3. Dou D, Joseph R. The structure and organization of the human *neuronatin* gene. *Genomics* 1996; 33: 292-297.

4. Dou D, Joseph R. Cloning of human *neuronatin* gene and its localization to chromosome-20q1 1 .2-12: The deduced protein is a novel "proteolipid" . *Brain Res* 1996; 723: 8-22.

5. Joseph R, Tsang W, Dou D, Nelson K, Edvardsen K. *Neuronatin* mRNA in PC12 cells: Downregulation by nerve growth factor. *Brain Res* 1996 (in press).

ABSTRACTS

1. Joseph R, Tsang W. Molecular cloning of differentially expressed genes in brain aging. Proceedings of the 119th meeting of the American Neurological Association, San Francisco, CA. October 1994. *Ann Neurol* 1994; 36: 311.

2. Joseph R, Dou D, Tsang W. Molecular cloning of, a novel mammalian brain developmental gene. Proceedings of the meeting of *Human Genome 1994: The Genes and Beyond,* Washington D.C. October 1994, #43, p24.

3. Dou D, Joseph R. Human Homolog of a novel mammalian brain developmental gene. Proceedings of the meeting of *Human Genome 1994: The Genes and Beyond,* Washington D.C. October 1994, #24, p20.

4. Joseph R, Dou D, Tsang W. *Neuronatin* is a novel brain specific developmental gene. Proceedings of the 24th meeting of the Society for Neuroscience, Miami, FL. November 1994. *Soc Neurosci Abs* 1994; 20: 882.

5. Joseph R, Dou D, Tsang W. Molecular cloning of *neuronatin:* Alternatively spliced forms of a novel brain-specific mammalian developmental gene. Proceedings of the 1994 Miami Bio/Technology European Symposium, Monaco. November 1994. In: Advances in Gene Technology: Molecular Biology and Human Genetic Disease. *Miami BiolTechnology Short Reports* 1994; *5:* 22; *Nuc Acids Symp* 1994; 29: *15.*

6. Joseph R, Dou D, Tsang W. *Neuronatin* is a novel mammalian gene involved in neurogenesis. Proceedings of the 47th meeting of the American Academy of Neurology, Seattle, WA. May 1995. *Neurology* 1995; 45 (Suppl 4): A355.

7. Dou D, Joseph R. Molecular cloning of human *neuronatin.* Proceedings of the 47th meeting of the American Academy of Neurology, Seattle, WA. May 1995. *Neurology* 1995; 45 (Suppl 4): A355.

8. Joseph R. Developmental genes link neuroembryogenesis and carcinogenesis. Proceedings of the 120th meeting of the American Neurological Association, Washington, DC. October 1995. *Ann Neurol* 1995; 38: 309.

9. Dou D, Joseph R. Human *neuronatin* gene and brain development. Proceedings of the *Nature* Conference, Patterns of Life: The Nature of Biological Development, Boston, MA. November 1995.

10. Joseph R, Dou D, Tsang W. Spliced forms of *neuronatin* mRNA in brain development. Proceedings of the 25th meeting of the Society for Neuroscience, San Diego, CA. November 1995. *Soc Neurosci Abs* 1995; 21: 284.

11. Dou D, Joseph R. Structure and expression of a novel human brain developmental gene. Proceedings of the 25th meeting of the Society for Neuroscience, San Diego, CA. November 1995. *Soc Neurosci Abs* 1995; 21: 284.

12. Joseph R, Dou D. *Neuronatin* is a novel "proteolipid" involved in human neurogenesis. Proceedings of the 48th meeting of the American Academy of Neurology, San Francisco, CA. March 1996. *Neurology* 1996; 46: A 148.

13. Dou D, Joseph R. Human *neuronatin* gene maps to chromosome 20q11.2-12. Proceedings of the 48th meeting of the American Academy of Neurology, San Francisco, CA. March 1996. *Neurology* 1996; 46: A 148.