

MOLECULAR CLONING OF *Neuronatin*

A NOVEL BRAIN-SPECIFIC MAMMALIAN DEVELOPMENTAL GENE

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Dedicated to the memory of my beloved Father

Professor M. P. Joseph.

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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 led to the isolation of *neuronatin* (neuronal-neonatal), a novel messenger ribonucleic 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.

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