

Temporal Expressions of Neurotrimin in Differentiating Neural Cell Lineages¹

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Neurotrimin(Ntm) is a member of IgLON family which is attached to the membrane by a glycosylphosphatidylinositol (GPI)-anchor and is expressed at high levels in the nervous system. We previously reported mouse Ntm cDNA(AF282980) and established its expression patterns in the hippocampus and the cerebellum, of adult brain. To determine temporal expression patterns, cellular specificity and regulation of Ntm in neural differentiation, we analyzed newly cloned promoter of Ntm in neural differentiating P19 embryonic carcinoma stem (EC) cells or mouse N2a neuroblastoma cells. The PCR-cloned 2.5kb of the 5'-upstream region (promoter) was inserted into a vector containing a GFP/LacZ dual reporter, designated pNtmGL. The pNtmGL was used to demonstrate its promoter activity and cell-specific expression in both P19 EC and N2a cells in vitro. The results showed that simultaneous expression of GFP/LacZ and MAP2b were found in pNtmGL-transfected primary brain cells. When the transfected p19 EC cells and embryoid body were analyzed by immunocytochemistry and RT-PCR with neural cell lineage markers including nestin, GFAP and MAP2b, GFP/LacZ and either nestin or MAP2b were concurrently expressed in neurally-differentiating N2a cells. Cell-specific promoter activity and expression of NtmGL also suggest that the expression of the dual reporter gene should allow us to establish an endogenous Ntm expression patterns not only in neural differentiation but also during the developing nervous system. These results suggest that the Ntm promoter may be used as a crucial marker for neural stem cells and mature neurons.

Keywords: Ntm promoter, GFP/LacZ(GL) dual reporter, pNtmGL, P19 EC, N2a cells.

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