D119 Characterization of Flectin-like gene in *C.elegans*

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Flectin is a new extracellular matrix protein which was identified from eye extract of chick embryo. Homologous proteins were found in many other vertebrates suggesting that flectin may be an evolutionary conserved protein. A flectin-like gene (ZK783.4) was found in *C. elegans* genome data base, showing approximately 40% similarity (20% identity) to chick flectin. In order to examine the localization of this gene, reporter gene fused with this gene were expressed in germ-line transformed transgenic animals. Green Fluorescent protein was expressed in various neurons, hypodermis and distal tip cells from early embryonic stage throughout larval and adult stages. Interestingly, strong expression patterns were observed in neuronal cells and hypodermal cells which compose the ectodermal tissue during early embryogenesis. This observation suggest that flectin-like protein may be important for the development of hypodermis and neurons. In addition, majority of cells expressing reporter gene are migrating cells during development. We confirmed this expression pattern by immunostaining with rat polyclonal antibodies. As a first step to identify the neuronal cells, we used lipophilic dye, DiI which labels six pairs of amphid neurons, ASK,ADL,AWB,ASH,ASJ,ASI. And we concluded the neurons which express GFP are not one of the amphid neurons. By northern we detected two bands, one is 4.9kb expected for ZK783.4 mRNA and the other is 3.3kb. Now we are doing functional study using RNAi(RNA –mediated interference).

D120 Regulation of Neuronal differentiation by NeuroD/BETA2

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NeuroD/BETA2 is a member of the basic helix-loop-helix transcription factor family. The role of NeuroD/BETA2 in development of the nervous system has been reported using null mice. We previously found that NeuroD/BETA2 was expressed in both mitotic and postmitotic neuronal cells of developing mouse brain, suggesting an important function of NeuroD/BETA2 in neuronal cell differentiation. However, the precise role of NeuroD/BETA2 in neuronal cell has not been established yet. Thus, we investigated the cellular role of NeuroD/BETA2 using F11 cells and zebrafish. To induce or block the NeuroD/BETA2, expression vectors for the full length and a truncated mutant were generated. DNA binding activity of the truncated mutant was tested by electrophoretic mobility shift assay and anti-BETA2 antibody. The loss of transactivation domain of the mutant was proven by CAT assay. The truncated mutant repressed the transactivation of insulin promoter by the NeuroD/BETA2 and behaved as a dominant negative mutant. In F11 cells, which could be differentiated by cAMP, the full length NeuroD/BETA2 containing both DNA binding domain and transactivation domains induced neurite outgrowth in the absence of cAMP. On the contrary, transfection of the truncate mutant inhibited neurite outgrowth even in the presence of cAMP. Ectopic overexpression of the same mutant also inhibited the formation of trigeminal ganglionic neurons during zebrafish embryogenesis. Taken these data together, i) NeuroD/BETA2 can induce neurite outgrowth in F11 cells, ii) the truncated form containing only bHLH can function as a dominant negative mutant for neuronal differentiation of F11 as well as neurogenesis of zebrafish, iii) NeuroD/BETA2 is essential for neuronal cell differentiation.