OIST OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY

カブトクラゲにおけるグループA bHLH転写因子の同定と機能解析

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Thesis title: Identification and Functional Analysis of Group A bHLH Transcription Factor in Ctenophore *Bolinopsis mikado*

Research aim:

Animals have acquired neurons during their early evolutionary phase. However, their evolutionary origin(s) remain unclear. An animal-specific group A bHLH transcription factor (TF) is known to be involved in the differentiation of neuron, enteroendocrine, and exocrine pancreatic cells in bilaterians. These group A bHLH TFs also play important roles in the differentiation of cnidarian neuropeptide-producing cells. However, the ancestral function of group A bHLH is still enigmatic. Therefore, in this study, I identified and analyzed the function of group A bHLH in ctenophore, the earliest branching animal with nervous systems, to elucidate the molecular basis of ctenophore neurogenesis and get new insight into the evolutionary origin of peptidergic systems in metazoans.

Material and method:

Group A bHLH genes in ctenophores were identified by molecular phylogenetic analysis using the maximum likelihood method. Among the identified Group A bHLH genes, an antibody targeting the bHLH named BmbHLH1 was generated, and its expression was visualized by immunostaining. Translation of BmbHLH1 protein was knocked down by microinjection of morpholino antisense oligos, and downstream genes were identified by transcriptome analysis. Expression patterns of the identified downstream genes were visualized by fluorescence *in situ* hybridization.

Result:

Molecular phylogenetic analysis identified six group A bHLH genes from ctenophore *Bolinopsis mikado*, and one of them, BmbHLH1 was expressed at the pharynx in the cydippid stage. Immunostaining confirmed the correct knockdown of bHLH1. Analysis of the 89 down-regulated genes revealed that many down-regulated genes are enzymes with specific functions. Fluorescence *in situ* hybridization visualized the down-regulated enzymes and revealed that they are expressed in the pharynx, as is the BmbHLH1 protein.

Conclusion:

Group A bHLH is a well-conserved transcription factor that regulates cell differentiation. However, the results of this research indicated that ctenophore group A bHLH has unexpected functions. This study established a method for analyzing the function of neurogenic transcription factors using ctenophore. Future studies are expected to analyze the functions of other transcription factors to elucidate the neurogenic transcription factors in ctenophores.