# 学 位 論 文 要 旨
# Dissertation Abstract

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学位論文題目：
Title of Dissertation

Evolutionary, molecular, and expression studies on YGHL gene family
(YGHL遺伝子ファミリーの分子構造進化、発現、および機能に関する研究)

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The Yellowtail Growth Hormone Like-1 gene (*YGHL1*) and *YGHL2* were identified by our research group in 1996, while screening the yellowtail (*Seriola quinqueradiata*) pituitary cDNA library with salmon GH antibody. Thereon, these genes have been scrutinized for their functional significance in embryogenesis, growth, reproduction, and environmental responses; also they have been intensively studied in their evolutionary aspect. The yellowtail *YGHL1* is composed of one 5' non-coding exon and 3 coding exons, with putative transcription factor binding sites present in upstream of the transcription site. The yellowtail *YGHL1* was highly expressed in the brain, gill, heart, and the kidney, while no expression was detected in the liver and the skeletal muscle. The orthologs of yellowtail *YGHL1* are found in other vertebrates as well. This study showed the phylogeny and evolution of the vertebrate orthologs of *YGHL1*, by comparing their sequences and syntenic context. Based on the genomic organization and extensive conservation of the nucleotide and the protein sequences orthologous to the yellowtail *YGHL1*, we proposed to name the *HIG1* gene family as *YGHL1/HIG1* family. The deduced peptide sequence alignment, showed the "YGHL1/HIG1 exon 3 domain" peptide to be well conserved in the *YGHL1/HIG1* family. The "YGHL1/HIG1 exon 3 domain" is the characteristic domain of the YGHL1 protein, encoded by the second coding exon of the *YGHL1*. Our data also suggested that an ancestral locus similar to *YGHL1* in *Ciona intestinalis* underwent duplications to create orthologous loci of the *YGHL1/HIG1* family in the vertebrates. The regions encoding the *YGHL1/HIG1* paralogs in human and mouse were close to the regions where some homeostatically important genes are clustered. These clusters are prominent in human 3p25-22 and 17q11-12 and their orthologous region in mouse 6D1-6E3 and 11D, showing a coordinated evolution between them. *Mettl7a2-Yghl1-4* is a chimeric transcript formed by an

intergenic splicing event between *Mettl7a2* and *Yghl1-4* present in tandem position on the mouse chromosome 15F1 at a distance of ~3kb. The *Mettl7a2* is S-adenosylmethionine-dependent methyltransferase domain containing protein encoding gene and *Yghl1-4* is a member of *YGH1/HIG1* family in mouse with YGHL/HIG1 exon 3 domain. The chimeric transcript is formed with exon 1 of *Mettl7a2* fused to exons 2-4 of *Yghl1-4* using the canonical splice sites from both genes. The expression of *Mettl7a2*, *Yghl1-4* and *Mettl7a2-Yghl1-4* were found exclusive to the kidney tissues only. Further, tissue localization by non radioactive *in situ* hybridization indicated restricted expression in the epithelium of proximal tubules and adrenal cortex. The *Mettl7a2-Yghl1-4* formation was found only in mouse genome but not in human. The genome specificity of *Mettl7a2-Yghl1-4* chimeric transcript and its peptide composition with the methyltransferase and the YGHL/HIG1 exon 3 domains, hypothesize it to have functional implications in mouse and especially in the kidney, rather than merely generating genetic diversity. *YGHL2* was found along with the *YGHL1*, which, later was identified as Makorin ring finger protein 2 gene *(YGHL2/MKRN2)* encoding a protein with a characteristic array of zinc finger domains similar to its ancestral gene, *MKRN1*. *MKRN1* is a highly transcribed, intron-containing source of *MKRN* family. *YGHL2/MKRN2* overlaps and is antisense to the *RAF1* in human, mouse and zebrafish. In this study, we reported the spatio-temporal expression pattern of zebrafish *YGHL2/MKRN2* and *MKRN1* in the developing zebrafish embryos using non radioactive whole mount *in situ* hybridization technique. The *YGHL2/MKRN2* and *MKRN1* were maternally expressed in the early developmental stages. Later, they were expressed in the optic and brain primordia, neural tube, otic vesicles, forebrain, eyes, and the cerebellum by 24hpf. From 36 to 72hpf, *YGHL2/MKRN2* and *MKRN1* expression was detected in the cephalic regions, otic vesicles, condensed jaw cartilages and the pectoral fin buds. Interestingly, the *YGHL2/MKRN2* was expressed in the rhombomeres and *MKRN1* did not express there. Altogether, both *YGHL2/MKRN2* and *MKRN1* suggest a role in development of the central nervous system. Besides the reported functional data for *YGHL2/MKRN2* and *MKRN1*, our study paves way for further functional analysis of these genes in cell differentiation and proliferation during CNS development. Retinoic acid response elements and metal response elements were found in the promoter region of zebrafish *YGHL2/MKRN2* and *MKRN1* which will pave way for developing an aquatic pollutant assay model using these genes as natural biomarkers. This study is a step towards prospective applications employing the yellowtail *YGHL1*, zebrafish *YGHL2/MKRN2* and *MKRN1* in aquaculture production and environmental pollution assessment.

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