カタユウレイボヤにおける CCK/gastrin の機能解析

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Shiho TANIGUCHI, Nobuo SUZUKI, Toshio SEKIGUCHI：Functional analysis of CCK/gastrin in the ascidian, *Ciona intestinalis*

In vertebrates, cholecystokinin (CCK) and gastrin act as brain-gut peptides. They have a sulfated tyrosine residue and share the consensus tetrapeptide sequence (Trp-Met-Asp-Phe-NH_2) at the C-terminus (Fig.1). They also share two paralogous CCK receptors (CCKRs). These facts demonstrate that CCK/gastrin is a family peptide in vertebrates. Cionin is a CCK/gastrin family peptide that was identified from the ascidian, *Ciona intestinalis* (Johnsen and Rehfeld, 1990). It shares sequence features with vertebrate CCK/gastrin family peptides. In vertebrates, treatment with cionin elicits gallbladder contractions and gastric acid secretions, which are typical CCK and gastrin functions, respectively. Sekiguchi et al. confirmed that cionin acts two *Ciona* orthologous receptors of CCKRs (Sekiguchi et al., 2012). These findings imply that the CCK/gastrin family peptidergic system is conserved in chordates. However, the evolution of the biological role of the CCK/gastrin family remains unclear due to the lack of functional data for cionin in *C. intestinalis*.

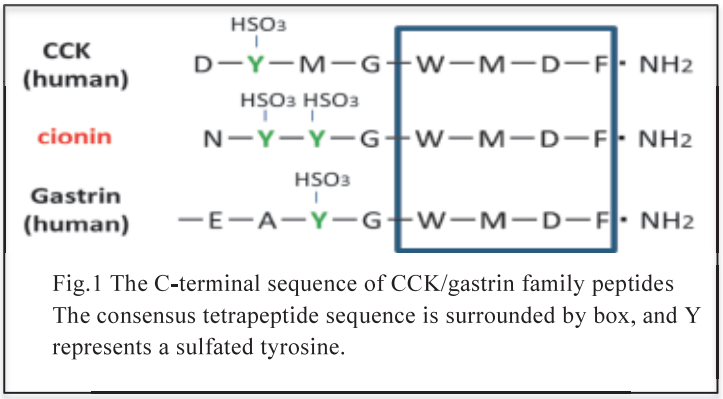

Fig.1 The C-terminal sequence of CCK/gastrin family peptides
The consensus tetrapeptide sequence is surrounded by box, and Y represents a sulfated tyrosine.

In the present study, I first analyzed the tissue distribution of cionin and CioRs mRNA using real-time PCR. Cionin mRNA was exclusively expressed in the neural complex. Moreover, the transcripts of the CioR1 and CioR2 genes were strongly expressed in the neural complex and are moderately expressed in the endostyle, stomach, intestine, and ovary. These results suggest that cionin is produced in the central nervous system similar to mammalian CCK, and is involved in physiological functions such as food intake, digestion, and reproduction in *C. intestinalis*. Interestingly, the expression of cionin in the digestive tract was relatively low, unlike that of the vertebrate CCK/gastrin family peptide gene.

Next, I evaluated the localization of cionin mRNA and peptide in the neural complex using *in situ* hybridization and immunohistochemistry, respectively. *In situ* hybridization analysis demonstrated that the localization of cionin mRNA was observed in the anterior part of the cerebral ganglion. Immunohistochemical analysis of serial sections of the neural complex revealed that the cionin peptide was expressed in the cell body of the anterior part of the cerebral ganglion and neural fibers throughout the cerebral ganglion. Furthermore, I

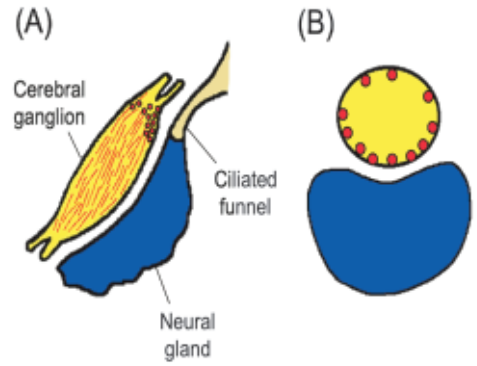

Fig. 2 Localization of cionin mRNA and cionin peptides in the adult neural complex
(A) A side view of the neural complex Both cionin mRNA and cionin peptides are distributed in cell bodies of the anterior part of the cerebral ganglion (dots). Cionin peptides were also detected in neural fibers throughout the cerebral ganglion (lines).
(B) Cross section of the anterior part of the neural complex Cionin mRNA and cionin peptides are localized in the cell bodies in the outer periphery (dots).

performed *in situ* hybridization analysis of CioR1 mRNA. A transcript of the CioR1 gene was detected in the entire cerebral ganglion.

To characterize the cioninergic neuron, I compared the localization of cionin mRNA with the Ci-vesicular acetylcholine transporter (Ci-VACHT) mRNA, which is a marker gene of cholinergic neurons. The localization of Ci-VACHT mRNA was observed mainly in the middle and posterior part of the cerebral ganglion. This expression pattern partially overlapped with that of CioR1 mRNA, suggesting that cioninergic neurons interact with cholinergic neurons.

Finally, I traced the CioR1 gene expression in various developmental stages using a fluorescent expression vector driven by a 6.8-kbp upstream sequence of the CioR1 gene. CioR1 gene expression was detected in the larval stage. Fluorescence signals were detected in the visceral ganglion and the nerve cord, which is mainly comprised of cholinergic neurons. The introduction of the CioR1 reporter construct into fertilized egg of the Ci-VACHT maker transgenic line demonstrated that the CioR1 promoter-driving fluorescence signals correspond with those of cholinergic neurons. Larval cholinergic neurons are essential for swimming behavior. In vertebrates, CCK functions as both neurotransmitter and neuromodulator. These facts suggest that cionin modulates larval swimming behavior through the cholinergic neurons.

In this study, I have clarified the localization of cionin and CioR1. These data will give us a clue to understanding the physiological function of cionin in *C. intestinalis*.

References

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