論文の内容の要旨

論文題目 Non-redundant function of two subtypes of octopamine receptors in food deprivation-mediated signaling in *C. elegans*

（線虫の餌非存在下で活性化されるシグナルにおける２種類のオクトパミン受容体の非重複的な機能）

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Four biogenic amines of *C. elegans* octopamine, tyramine, dopamine and serotonin function as neurotransmitters in response to environmental cues. Previously Suo et al. showed that amine neurotransmitters regulate activation of CREB (cAMP response element-binding protein) in *C. elegans* (Suo et al., EMBO J., 2009). CREB is a transcription factor that plays essential roles in a variety of biological processes. It binds to specific DNA sequences called cAMP response elements (CRE), and regulates expression of its target genes upon phosphorylation. Using a *gfp* reporter for CREB activation, it was found that CREB is activated in the cholinergic SIA neurons in the absence of food. This signaling is mediated by octopamine, which is considered to be the biological equivalent of mammalian noradrenaline. SER-3, a Gq-coupled octopamine receptor functions in the SIA neurons to induce CREB activation.

In addition to SER-3, *C. elegans* has another putative octopamine receptor, SER-6. In this

study, I analyzed functions of SER-6 in octopamine-mediated CREB activation in SIA neurons. First, I cloned cDNA of *ser-6* and compared the amino acid sequence of SER-6 to that of SER-3. As expected, SER-3 and SER-6 were highly homologous. Next, I analyzed whether SER-6 is involved in octopamine-mediated CREB activation, similar to SER-3. As seen in *ser-3* deletion mutants, octopamine- and food deprivation-mediated CREB activation were decreased in *ser-6* deletion mutants compared to wild-type animals (Fig.1), suggesting that SER-6 is required for signal transduction. Cell-specific expression of SER-6 in the SIA neurons was sufficient to restore CREB activation in the *ser-6* mutants, indicating that SER-6 functions in SIA neurons. Taken together, these results suggest that SER-3 and SER-6 function in the same cells, and that both of these receptors are required for normal signaling. Decreased CREB activation seen in *ser-3* and *ser-6* single mutants could be due to a decrease in the amount of octopamine receptors. To address this possibility, I assayed CREB activation in double heterozygous *ser-3/+;ser-6/+* animals and found that they responded slightly weaker to exogenous octopamine treatment than wild-type animals but much stronger than that of the *ser-3* or *ser-6* single mutants. The response to food deprivation was not different between *ser-3/+;ser-6/+* double heterozygous animals and wild-type animals. Furthermore, *ser-3* mutants overexpressing SER-6 responded to exogenous octopamine and food deprivation as weakly as *ser-3* mutants. In *ser-6* mutants overexpressing SER-3, some spontaneous CREB activation was observed upon exogenous octopamine treatment, suggesting that SER-3 can partially respond to exogenous octopamine without SER-6 when overexpressed. In contrast, the level of CREB activation induced by food deprivation in *ser-3*-overexpressing animals was not different from that of *ser-6* mutants. Taken together, these results suggest that both *ser-3* and *ser-6* are required for full activation of CREB regardless of their quantity and that *ser-3* but not *ser-6* can partially function by itself only when it is overexpressed.

Thus, in this study, I showed that both SER-3 and SER-6 are required for CREB activation in SIA neurons and these two similar octopamine receptors are working in the same cells, and function in a non-redundant manner *in vivo*.

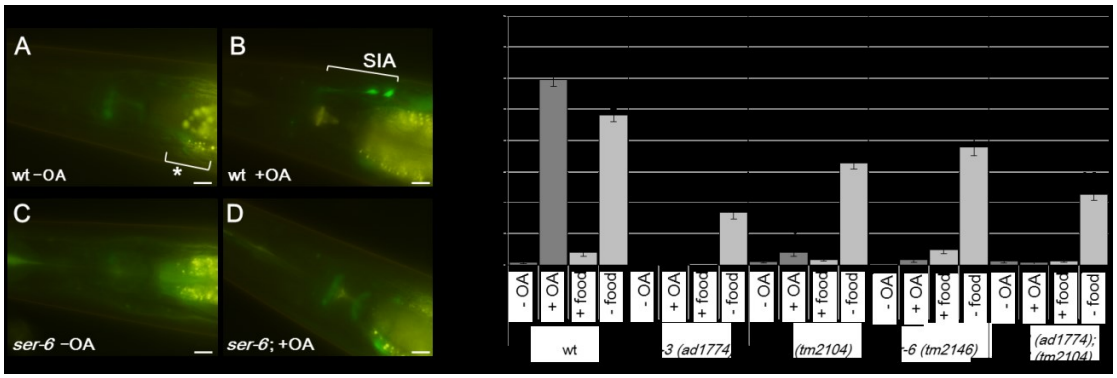

Fig. 1 Octopamine and food deprivation-induced CREB activation in the SIA neurons. The bracket marked with an asterisk indicates autofluorescence of the intestine. Each bar represents 10 μm. wild-type N2, *ser-3(ad1774)*, *ser-6(tm2104),* *ser-6(tm2146)* and *ser-3(ad1774);ser-6(tm2104)* mutants carrying *cre::gfp* were incubated on plates containing 0 or 3 mg/mL octopamine (OA) for 4 h, or on NGM plates with or without food for 6 h. The number of GFP-expressing SIA neurons per animal was then determined (E-I). Error bars indicate the standard errors of the mean. At least 53 animals were tested. Fluorescent images were obtained from wild-type N2 animals (A, B) and *ser-6(tm2104)* mutants (C, D) after 4 h of octopamine treatment. *1P<0.001 (Tukey–Kramer multiple comparison test), compared with +OA of wild-type animals. *2P<0.001, compared with -food of wild-type animals. *3P<0.001, compared with -food of *ser-6(tm2104)* mutants. *4P>0.05, compared with -food of *ser-3* mutants.