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主　論　文　の　要　旨

論文題目　Evolutional features and biological significance of the CMP-sialic acid synthetase
(CMP-シアル酸合成酵素の進化的特徴と生物学的重要性)

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論　文　内　容　の　要　旨

Sialic acid (Sia) is a family of nine-carbon, carboxylated sugars, consisting of *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), deaminoneuraminic acid (KDN), and their modified forms. The CMP-sialic acid synthetase (CSS) catalyzes the synthesis of CMP-sialic acid (CMP-Sia), an only donor substrate of sialyltransferases, from Sia and CTP. CSS is widely distributed in various organisms from bacteria to vertebrates as an essential enzyme for the *de novo* synthesis of Sia-glycoconjugates. Numerous studies have shown that CSS had unique structural and functional features, compared with enzymes providing other nucleotide sugars. The first unique feature that this thesis focuses on is that the vertebrate CSS is localized in nucleus, while the nucleotide-sugar pyrophosphorylases that provide most other nucleotide sugars are localized in cytosol. It has been already shown that the nuclear localization signal (NLS) in the vertebrate CSS molecules determines the nuclear localization. However, in insect, DmCSS is reported to be localized in the Golgi, but not in nucleus. It is thus suggested that subcellular localization of CSS appears to be diverse depending on animal species, and why such a difference happens is an interesting open question on CSS. I thought that more insight into the subcellular localization should be gained from an evolutional point of view. The second feature that this thesis focuses on is functional roles of CSS at animal level. It is well known that a CSS deficiency never results in lethality at cellular level. For example, one of the lectin-resistant mutant CHO cells, LEC29Lec32, is immortal despite of CSS deficiency. By sharp contrast,

CSS-deficient mice are lethal at an embryonic stage. These facts clearly indicate the critical importance of CSS exists at animal level, but not at cellular level. However, no or very few studies have been done for understanding of CSS during early development and morphogenesis. Therefore, I decided to elucidate functional importance of CSS at animal level using medaka, *Oryzias latipes*, as a model animal. My final goal is to solve the above mentioned long-term and mysterious problems about CSS. Thus, objectives of this thesis are two-fold: (1) To characterize the insect CSSs for their enzyme activity and unique subcellular localization (Chapter 2); (2) To understand impacts of impairments of CSS molecules on early development of medaka (Chapter 3).

(1) Characterization and evolutional feature of the insect CSS for their enzyme activity and subcellular localization: I focused on not only *Drosophila melanogaster* (fruitfly), but also *Aedes aegypti* (mosquito) and *Tribolium castaneum* (beetle) for the subcellular localization study of CSSs. (i) I cloned the CSS genes from *A. aegypti* and *T. castaneum* and compared their structure and subcellular localization with those of *D. melanogaster*. The insect CSSs were shown to share high sequence identities with the vertebrate CSSs in the catalytic domain, which contained evolutionarily conserved motifs. While the C-terminal domain typical to mammalian CSSs is absent in insect CSSs; (ii) The enzymatic activities of each insect CSS were characterized in different host cells. Like DmCSS, AaCSS and TcCSS showed *in vivo* and *in vitro* activities when expressed in mammalian and insect cells. When bacterially expressed, they did not show any activity until the N-terminal hydrophobic region was removed; (iii) Subcellular localization of these insect CSSs was tested in both mammalian cells and insect cells. In both CHO cells and *Drosophila* S2 cells, AaCSS and TcCSS were predominantly localized in ER, but not in Golgi. Surprisingly, DmCSS was mainly secreted into the medium, although partially detected in the Golgi. Consistent with these results, it was shown that the N-terminal hydrophobic regions of AaCSS and TcCSS worked as signal peptide to make them soluble in the ER, while that of DmCSS worked as membrane-spanning region of type II transmembrane protein whose cytosolic KLK sequence functioned as an ER export signal. Thus, the destination of insect CSSs is more complicated and diverse than previously recognized. The elusiveness of the insect CSS destination might tells some interesting feature of sialic acid evolution.

(2) Impacts of impairments of CSS on early development of medaka: To understand how significant the developmental stage- and organ-specific expression of Sia at animal level, I generated medaka fish with point-mutations of the *CSS* gene based on the TILLING method. After the TILLING library was screened for gametes with mutations in the exons of the CSS gene, I230N and L303Q mutants, whose CSS contain a point-mutation in the N- and C-terminal domains, respectively, were obtained. These two mutant CSSs I230N and L303Q CSS had low *in vitro* activity to Neu5Ac, indicating the importance of the particular mutations in the enzyme activity. I230N CSS showed less enzyme activity than L303Q CSS. Interestingly, significant abnormalities and subsequent early death were observed in pre-hatching embryos of I230N, suggesting essential roles of CMP-Sia in early development of medaka. The L303Q could not survive beyond two months and died as young fry. Considering that L303Q contain a mutation in the C-terminal domain, it is suggested that the C-terminal domain of CSS is also functionally important at animal level. Collectively, these results indicate that CSS is an essential enzyme for early development, and that provision of critical amounts of CMP-Sia may be necessary at each developmental stage.