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Studies on the molecular mechanism of sex determination in the cobaltcap silverside Hypoatherina tsurugae

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博士学位論文内容要旨
Abstract

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論文題目 Title	**Studies on the molecular mechanism of sex determination in the cobaltcap silverside *Hypoatherina tsurugae*** （ギンイソイワシ *Hypoatherina tsurugae* の性決定の分子機構に関する研究）		

The sex-determining gene *amhy* (Y chromosome-linked Anti-Müllerian hormone) has been confirmed in Atherinopsid species of the genus *Odontesthes* (*O. hatcheri* and *O. bonariensis*) which occur naturally in inland waters of Argentina, Brazil and Uruguay. The presence of *amhy* in other families of Atheriniforms besides Atherinopsidae is unknown. In order to examine the evolution of *amhy* in this order, I selected the cobaltacap silverside *Hypoatherina tsurugae*, which inhabits the coastal waters of Japan and the Korean peninsula, as a model. The cobaltcap silverside belongs to the family Atherinidae, which is phylogenetically and geographically the most distant family from the Atherinopsidae where *amhy* has been discovered. In addition to the insight on the evolution of *amhy*, this study aims to understand the genetic mechanism of sex determination in *H. tsurugae*, in particular to clarify the role(s) of *amhy* in gonadal sex determination/differentiation and its relation to other sex related key genes (*sox9*, *cyp19a1a*, etc) in these processes.

In the 1st chapter, I searched the genome of *H. tsurugae* for the presence of *amhy* gene. The complete gene structure of *amhy* and its somatic homologue *amha* were obtained and compared. The *amha* gene is composed of 2,015 nucleotide bases and seven exons. The TGF-β domain is present in Exon 7 as in other species. The *amhy* gene is composed of 1,838 nucleotide bases and has only 4 exons. Exons 2 and 3 are completely lacking in the *amhy* gene structure. A specific insertion of 195 nucleotide bases is present at the place of exons 2 and 3. The exon 5 sequence is found in genomic sequence but it is not translated. The nucleotide identity between exons of *amha* and *amhy* was more than 80%. The deduced amino acid sequence of Amha (511 aa) and Amhy (340 aa) shared 91% identity. Exons 1, 4, 6 and 7 of *amhy* showed identity to those of *amha* as follows: 71%, 99%, 95% and 81% respectively. The exon 7, which contains the TGF-β domain, shared 93% amino acid identity with the similar domain of *amha* and contains 7 canonical cysteine residues that form disulfide bonds to make cysteine knots during dimer formation.

In the 2nd chapter, the role of *amha* and *amhy* gene in gonadal sex differentiation of *H. tsurugae* was examined. The linkage between *amhy*+ genotype and sex phenotype was analyzed in wild samples as well as in laboratory

reared fish that were raised from hatching at the average temperature of the spawning season of *H. tsurugae*. The PCR analysis yielded a linkage with male sex in 95% and 85% of the wild adults and the laboratory-reared progeny, respectively. The temporal expression of *amha* and *amhy* gene was studied by qRT-PCR. In *amhy*+ fish, the *amhy* gene is highly expressed during early sex differentiation period while the *amha* gene expression is very low until the early juvenile stage. The spatial expression of *amha* and *amhy* was studied by ISH. This analysis showed that at 4 wah (undifferentiated period), *amhy* is expressed in somatic cells surrounding germ cell. In contrast, signals of *amha* could not be detected at this time. These results indicate that the *amhy* gene in *H. tsurugae* is tightly linked to the phenotypic sex and that it is highly expressed in the gonads of *amhy*+ individuals during the early developmentof the gonads. Thus, *amhy* can be considered as a strong candidate for sex determining gene in this species.

In the 3rd chapter, the objective was to study the gene expression profile of sex-related genes that could be adjuvants to *amhy* gene in sex determination/differentiation of *H. tsurugae*. The expression of six key sex differentiation genes (*sox9, dmrt1, gsdf, foxl2, cyp19a1a* and *scp3*) in *amhy*+ and *amhy*- individuals of *H. tsurugae* were studied during the early stages of gonadal development. Most of the genes showed a dimorphic expression related to sex genotype (*amhy*+ / *amhy*-) with exception of *sox9*. The reason for the lack of sex dimorphism in *sox9* expression during this period may be that this gene is necessary for proliferation of germ cells in both sexes, as shown for other species.

In conclusion, I successfully isolated, cloned and sequenced the *amhy* gene in *H. tsurugae*. This gene is tightly linked with the male phenotype and highly expressed during early gonadal sex determination /differentiation. The sex related key genes *dmrt1, foxl2, cyp19a1a* and *scp3* showed dimorphic expression and an apparent synchronization with *amhy* gene expression. Future studies should look in more detail about their relations with as well as their regulation by *amhy* in order to corroborate the status of sex determining gene for *amhy* in *H. tsurugae*.