ペヘレイの性決定機構期における脳の性分化に関する神経解剖学・分子生物学的研究

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

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論文題目 Title	**Neuroanatomical and molecular studies on brain sex differentiation in relation to gonadal sex determination of Pejerrey *Odontesthes bonariensis*** （ペヘレイの性決定機構における脳の性分化に関する 神経解剖学・分子生物学的研究）		

The pejerrey *Odontesthes bonariensis* (Atheriniformes, Atherinopsidae) is a species native from Argentina, South America. This fish supports an artisanal fishery in many south American countries. Pejerrey also constitutes an incipient aquaculture in Japan. The pejerrey is a unique species because it has both genetic and environmental sex determination. Exposure of larvae to high (Male Promoting Temperature, MPT) and low (Female Promoting Temperature, FPT) temperatures during the critical period of sex determination (CPSD) induce masculinization (testis formation) and feminization (ovary formation), respectively. However, at intermediate temperatures the sex is determined by the Y-linked antimüllerian hormone gene, so XY individuals develop as males and XX as females. In fish, recent research has shown that not only the gonads are bipotential but also the brain is sexually labile and able to adjust its sex with those of the gonads in order to avoid functional mismatches. Moreover, recent evidence in fish has shown that the sexual fate of the brain is determined by an interaction between steroid hormones and the genotype of the cells. In fish, this issue is even more complex because environmental factors such as temperature can also influence the sexualization of the brain during early life stages. In pejerrey, some studies have suggested that the brain can influence the sex differentiation of the gonads by inducing the activation of the reproductive axis and the secretion of cortisol. However, the mechanisms by which these regulatory differences are established during early and larval development remain unknown. Therefore, the study of brain sexualization can provide valuable information about sex determination and its role during sex reversal.

In the first chapter I explored the effect of the genotype and temperature and their interactions on the brain during the process of temperature-induced masculinization and feminization in relation to the process of gonadal sex differentiation. Because temperature has been found to control the sex differentiation of pejerrey, I carried out an experiment to expose 2-6 weeks-old (wah) larvae to high (29°C, MPT) and low temperature (17°C, FPT) and used the heads and the trunks to measure the gene expression in brains and gonads, respectively. I also sampled fish for gene localization by in situ hybridization. The remaining fish were sampled at the end of the experiment (20 wah) to determine the phenotypic sex ratios by histology. Then, I conducted gene expression analysis (RT-qPCR) of the Crh (Corticotropin Releasing Hormone) family and their associated carrier protein, receptors, and other stress-related genes in response to temperature during the CPSD to investigate the potential roles of the CRH system in the sexualization of the central nervous system and those of the hypothalamus-pituitary-interrenal (HPI) axis in the sex

determination of this species. The Crh family genes *crhb*, *uts1*, *ucn3*, the receptor *crhr1* and the stress-related genes *gr1*, *gr2, nr3c2* were transiently upregulated in the heads of pejerrey larvae during the CPSD by high temperature alone or in combination with other factors. Only *crhr2* transcript abundance was not influenced by temperature but independently by time and genotype. In most cases, mRNA abundance was higher in the XX heads compared to that of XY individuals. In order to infer the functions of the upregulated genes based on their neuroanatomical location, I localized the mRNAs with *in situ* hybridization. Several of the genes were found to be expressed in the tuberal hypothalamus, neighboring the pituitary gland, which supports their neuroendocrine importance during sex determination. In addition, I measured the levels of cortisol using EIA analysis to identify potential genotype differences in the cortisol titers at MPT. Interestingly XX larvae also showed higher whole-body cortisol titers than the XY, downregulation *of cyp19a1a* and upregulation of the testis-related genes *amhy*/*amha* in trunks (gonads) and were 100% masculinized by high temperature. In contrast, at FPT, *crhbp* and *avt* were upregulated in the heads, particularly the former in XY larvae. *cyp19a1a* and *amhy*/*amha* were up- and downregulated, respectively, in the gonads, and fish were 100% feminized. Signaling via the HPI axis was observed simultaneously with the first molecular signs of ongoing sex determination/differentiation in the gonads. Overall, the results strongly suggest a temperature-dependent, genotype-specific regulatory action of the brain involving the Crh family of stress-related genes on the process of environmental sex determination of pejerrey.

In the second chapter, based on the insights obtained in the first experiment, I explored further the effect of temperature and genotype in the brain global gene expression of pejerrey larvae during the sex determination period using Next Generation Sequencing technology, to understand the transcriptomic changes occurring in the brain during sex reversal. I also aimed to identify potential new candidate genes involved in the processes of brain sexualization with a potential link to sex determination. With this aim, a De Novo Assembly of the brain transcriptome and a differential expression analysis with volcano plots followed by validation with RT-qPCR was performed. Genes found to be differentially expressed were enriched with Gene Ontology and Reactome. Twenty-nine genes were found to be upregulated (in XX brains) and 36 genes downregulated at MPT. On the other hand, 46 genes were upregulated (in XX brains) and 27 downregulated at FPT. These differences in neurochemistry may reflect genotype-specific differences controlled by the genotype in response to low temperature, which may have an influence on the process of gonadal sex differentiation via neuroendocrine mechanisms.

In summary, the present results show that the brain is involved in the process of sex determination in pejerrey. I provide evidence that thermal stress induces not only the masculinization of the gonads but has also central effects in the brain via glucocorticoid receptors and may be involved in the brain reprogramming of XX pejerrey larvae at MPT during sex reversal. At FPT on the other hand, *avt* and *crhbp* might be necessary for the induction of brain changes during low temperature-induced feminization. Moreover, I showed for the first time a genotype-biased response to thermal stress, which was confirmed further with the analysis of the transcriptome with Next Generation Sequencing. This is a novel finding that could have profound implications for the understanding of the neurological processes underlying sex determination in fish and the transduction of environmental cues.