DIVISION OF REPRODUCTIVE BIOLOGY †

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Fish exhibit a range of gonadal forms from gonochorism to several types of hermaphroditism, thus providing an excellent animal model to study the molecular mechanisms of sex determination, gonadal sex differentiation and gametogenesis in vertebrates. Our research focuses on (1) the identification of regulators involved in sex determination, gonadal sex differentiation, sexual plasticity, and gametogenesis (oocyte maturation and ovulation), and (2) the mechanisms of synthesis and action of these regulators.

Molecular mechanisms of sex determination, gonadal sex differentiation and sex change

We previously identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka (*Oryzias latipes*), the first in non-mammalian vertebrates. Recently, we developed a gene-specific transgenic RNA interference (RNAi) technology for the analysis of loss-of-function phenotypes that develop over long periods of time, and used it to knock down the *dmy* gene in genetically male (XY) fish. Knockdown of *dmy* strongly downregulated the expression of the only other male-associated genes (*gsdf*, *sox9a2* and *dmrt1*), and upregulated the expression of female-associated genes (*foxl2* and *Rspo1*) in XY gonads during the early stages of sexual differentiation. This shift in the gene expression pattern resulted in a complete male-to-female sex-reversal with a typical female pattern of secondary sex characteristics, producing fertile eggs. Importantly, we were able to continue a trans-generational knockdown effect on *dmy* until at least the F3 generation. In order to rescue the effect of *dmy* knockdown, we singularly injected or co-injected *sox9a2* (marked in cyan) and *gsdf* (marked with cherry) into olvas vasa-DMY-knockdown embryos of the F3 generation. Although singular injections failed to complete suppression of meiosis and proliferative mitosis but co-injection re-established the male phenotype in the XY gonad leading to complete formation of the testis, producing fertile sperm. This confirms that *gsdf* and *sox9a2* are genes downstream of *dmy* which, can regulate the sexual identity of medaka even in a DMY-independent manner. We conclude that in medaka *dmy* directly or indirectly upregulates the male sex-determining pathway by activating *gsdf* and *sox9a2* expression.

†: This laboratory was closed on 31 March, 2011.

Publication List

〔Original papers〕

- Charkraborty, T., Shibata, Y., Zhou, L.Y., Katsu, Y., Iguchi, T., and Nagahama, Y. (2011). Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Mol. Cell. Endocrinol. *333*, 47-54.
- Charkraborty, T., Katsu, Y., Zhou, L.Y., Miyagawa, S., Nagahama, Y., and Iguchi, T. (2011). Estrogen receptors in medaka (*Oryzias latipes)* and estrogenic environmental contaminants: An in vitro-in vivo correlation. J. Steroid Biochem. Mol. Biol. *123*, 115-121.
- Fernandino, J.I., Popesku, J.T., Paul-Prasanth, B., Xiong, H., Hattori, R.S., Oura, M., Strussmann, C.A., Somoza, G.M., Matsuda, M., Nagahama, Y., and Trudeau, V.L. (2011). Analysis of sexually dimorphic expression of genes at early gonadogenesis of *Pejerrey Odontesthes bonariensis* uing a heterologous microarray. Sex. Dev. *5*, 89-101.
- Mita, M., Yamamoto, K., and Nagahama, Y. (2011). Interaction of relaxin-like gonad-stimulating substance with ovarian follicle cells of the starfish *Asterina pectinifera*. Zool. Sci. *28*, 764-769.
- Mita, M., Yamamoto, K., Nakamura, M., and Nagahama, Y. (2011). Hormonal action of relaxin-like gonad-stimulating substance (GSS) on starfish ovaries in growing and fully grown states. Gen. Comp. Endocrinol. *172*, 85-89
- Ngamniyom, A., Magtoon, W., Nagahama, Y., and Sasayama, Y. (2011). Expression levels of bone morphogenetic protein 2b in fins of adult Japanese medaka (*Oryzias latipes*) exposed to sex steroid hormones. J. Fish. Aquat. Sci. *6*, 119-129.
- Okubo, K., Takeuchi, A., Chaube, R., Paul-Prasanth, B., Kanda, S., Oka, Y., and Nagahama, Y. (2011). Sex differences in aromatase gene expression in the medaka brain. J. Neuroendocrinology *23*, 412-423.
- Paul-Prasanth, B., Shibata, Y., Horiguchi, R., and Nagahama, Y. (2011). Exposure to diethylstilbesterol during embryonic and larval stages of medaka fish (*Oryzias latipes*) leads to sex reversal in genetic males and reduced gonad weight in genetic females. Endocrinology *152*, 707-717.
- Raghuveer, K., Sudhakumari, C.C., Senthilkumaran, B., Kagawa, H., Dutta-Gupta, A., and Nagahama, Y. (2011). Gender differences in tryptophan hydroxylase-2 mRNA, serotonin, and 5-hydroxytryptophan levels in the brain of catfish, *Clarias gariepinus*, during sex differentiation. Gen. Comp. Endocrinol. *171*, 94-104.

〔Review Article〕

- Shibata, N., Nakamoto, M., Shibata, Y., and Nagahama, Y. (2011). Endocrine regulation of oogenesis in the medaka, *Oryzias latipes*. In Medaka: A Model for Organogenesis, Human Disease and Evolution, H. Takeda et al. eds. (Springer), pp. 267-283.