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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/differentiation and gametogenesis in vertebrates. Our research, which uses several types of teleost fish, focuses on (1) the identification of regulators and steroidal mediators 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 mediators.

I. Molecular mechanisms of sex determination and gonadal sex differentiation

We identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka (*Oryzias latipes*), the first one in non-mammalian vertebrates. Recently, we have developed a simple, cost effective and gene-specific transgenic RNAi technology for understanding the roles of the zygotic gene products in medaka. Knockdown of DMY in XY gonads resulted in a complete male-to-female sex-reversal in medaka (Figure 1). Importantly, we were able to continue a trans-generational knockdown effect of DMY until at least the F2 generation. Since the RNAi effect is long lasting and inheritable, this will provide a powerful tool for the analysis of not only embryos, but also phenotypic consequences that develop over longer periods of time.

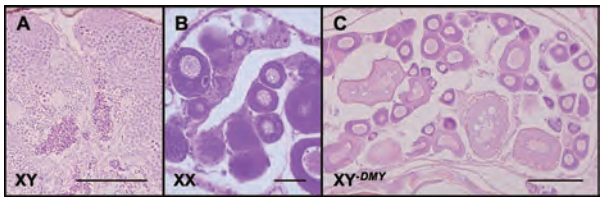

Figure 1. Knockdown of DMY in XY gonads leads to a complete male-to-female sex reversal (C). Gonads from an XY male (A) and an XX female (B). H&E staining was performed on gonads from 50 days after hatching. Bars indicate 50μm.

A search for the target genes of DMY led to the identification of gonadal soma derived factor (GSDF), a member of the transforming growth factor-beta superfamily. An XY-specific up-regulation was detected in the expression levels of *GSDF* in the whole embryos of medaka at 6 days post fertilization, coincident with the initiation of *DMY* expression in XY gonads. Conversely, the expression of *GSDF* was found to be very weak in XX gonads during embryogenesis. Importantly, *GSDF* and *DMY* were found to be co-localized in the same cell type in XY gonads. When the XY embryos were treated with estradiol-17β, in order to reverse their phenotypic sex, a decline was observed in the expression of *GSDF*. These results suggest that GSDF plays an important role in testis differentiation in medaka, probably down stream of DMY.

The molecular control of ovarian development in medaka is less understood. We examined whether R-Spondin 1 (RSPO-1), a novel regulator of the Wnt/β-catenin signaling pathway, was involved in ovarian differentiation in medaka. *RSPO-1* is expressed in XX gonads from as early as 0 day after hatching to the adult stage, while the expression was barely detected in XY gonads. Knockdown of RSPO-1 in XX gonads induced female-to-male sex-reversal, while overexpression of RSPO-1 in XY gonads induced male-to-female sex-reversal. Both loss and gain of function evidence indicates that RSPO-1 is critical to initiate the ovary pathway in medaka.

In the Nile tilapia (*Oreochromis niloticus*), we identified that *Cyp19a1*/*Foxl2* in XX gonads and *GSDF*/*DMRT1* in XY gonads during early gonadal differentiation are critical for indifferent gonads to differentiate into either the ovary or testis. The critical role of Foxl2 in ovarian differentiation was confirmed by male sex reversal of XX transgenic tilapia carrying a dominant-negative mutant of *Foxl2*. XX tilapia carrying extra copies of tilapia *DMRT1* as a transgene induced various degrees of gonadal changes including complete sex change to testis, indicating that DMRT1 plays an important role in testicular differentiation.

II. Molecular mechanisms of sex change

The sex-changing fish *Trimma okinawae* can change its sex back and forth from male to female and then back to male serially, dependent on social status in the harem. The gonad corresponding to the sexual status of the fish remains functional while the other is regressed. The expression of gonadotropin receptors (*GtHR*) was found to be confined to the active gonad of the corresponding sexual phase. The swapping of the gonads is initiated through a switching in the expression of the GtHR, *FSHR* and *LHR*. Changing of the gonads starts with switching of *GtHR* expression discernible within 8-12 h of the visual cue. These two GtHR genes act as mediators to convey the information about the change in social status to the to-be-active gonad.

III. Sexual plasticity in the adult gonochoristic fish

With the exception of certain hermaphroditic species, most vertebrates are thought to lose sexual plasticity after the differentiation of separate gonads/sexes with a single, distinct

gamete type (gonochorism). We treated females of two species of teleost, the Nile tilapia and medaka, with aromatase inhibitors (AI) for up to five months to block the conversion of androgens to estrogens in order to investigate whether sexual plasticity is retained in gonochoristic fish. In both species, suppression of estradiol-17β production via AI treatment caused a rapid degeneration of ovarian tissues, leading to the differentiation and development of testicular tissues. The reduced expression of aromatase (*P450c19a*) with a rise in the expression of *GSDF*, indicates the differentiation of testicular-type somatic cells in the AI-treated gonads. Sex-changed fish show a typical male pattern of estrogen and androgen levels, secondary sex characteristics, producing fertile sperm in the newly formed testes. Our results indicate that gonochoristic fish maintain their sexual plasticity to adulthood and that estrogens play a critical role in maintaining the female phenotype.

IV. Endocrine regulation of oocyte maturation and ovulation

A period of oocyte growth is followed by a process called oocyte maturation and is a prerequisite for successful fertilization. Our studies using vertebrate (fish) and invertebrate (starfish) models have revealed that the basic mechanisms involved in oocyte maturation are the same in these two species despite the differing chemical nature of the hormonal agents involved. In both species, three major mediators have been shown to be involved (*Three step model*): a gonad-stimulating substance (GSS), 1-methyladenine (maturation-inducing hormone, MIH), and a maturation-promoting factor (MPF) in starfish, and gonadotropin (LH), 17α, 20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) (MIH), and MPF in fish.

We recently purified GSS from the radial nerves of starfish (*Asterina pectinifera*) and the complete amino acid sequence was determined. Phylogenetic analyses revealed that starfish GSS was a relaxin-like peptide. Chemically synthesized GSS induced not only oocyte maturation and ovulation in isolated ovarian fragments, but also unique spawning behavior followed by the release of gametes shortly after injection. Thus, this study represents the first evidence of a relaxin system in invertebrates and points towards a novel reproductive role for this peptide in starfish. This work was done in collaboration with Drs. M. Mita, Tokyo Gakugei University and M. Yoshikuni, Kyushu University.

Publication List

〔Original papers〕

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〔Review article〕

- Kobayashi, T., and Nagahama, Y. (2009). Molecular aspects of gonadal differentiation in a teleost fish, the Nile tilapia. Sex. Dev. *3*, 108-117.