DIVISION OF REPRODUCTIVE BIOLOGY

Professor (Specially appointed)
NAGAHAMA, Yoshitaka

Professor	NAGAHAMA, Yoshitaka*
Assistant Professors	OKUBO, Kataaki
	OHNO, Kaoru
NIBB Research Fellow	SUZUKI, Aya
Postdoctoral Fellows	CUI, Jianzhou
	GUAN, Guijun
	LI, Jianzhong
	PAUL, Bindhu
	SAKAI, Fumie
	SHIBATA, Yasushi
	USAMI, Takeshi
	ZHOU, Linyan
Technical Assistants	HARA, Ikuyo
	SHIBATA, Emiko
	TAKAKI, Chikako
Secretary	SHIMADA, Yu

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 have been using medaka (*Oryzias latipes*) to investigate the molecular mechanisms of sex determination and Nile tilapia (*Oreochromis niloticus*) to investigate gonadal sex differentiation. In fish with a stable genetic XX/XY sex determining system, the sex-determining gene lies on the Y chromosome and is responsible for initiating male sex determination. We identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka, the first one in non-mammalian vertebrates. However, there is no sequence homology between the two known vertebrate sex-determining genes, *SRY/Sry* (mammals) and *DMY*. Another important difference is that *DMY* transgenic XX medaka are fully functional and fertile males, whereas *Sry* transgenic mice are sterile. 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. In medaka embryos, *gsdf* was predominantly expressed in the somatic cells in the XY gonads from the day of hatching. Conversely, expression of *gsdf* was found to be weaker in the XX gonads undergoing female sex differentiation. When the XY embryos were treated with estrogen, in order to reverse their phenotypic sex, a decline was observed in the expression of *gsdf* in those embryos. Treatment of the XX embryos with methytestosterone increased the expression of *gsdf*, proving that the expression of this gene is linked with the phenotypic sex, not the genetic sex.

In tilapia, all genetic female (XX) and male (XY) broods are available. Through cDNA subtraction between XX and XY gonads during sex differentiation and microarray hybridization followed by gene expression analyses by RT-PCR and *in situ* hybridization, we have concluded that the sex-specific expression of *Cyp19a1/Foxl2* in XX gonads and *DMRT1* in XY gonads during early gonadal differentiation (5 - 6 dph) is critical for indifferent gonads to differentiate into either the ovary or testis in the Nile tilapia. 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. It is of great interest to note that some of the sex reversed XX tilapia produced sperm with extremely high motility (Wang *et al*., unpublished).

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 swapping of the gonads is initiated through a switching in the expression of the gonadotropin hormone receptors, *FSHR* and *LHR*. These two genes act as mediators to convey the information about the change in social status to the to-be-active gonad. Most intriguingly, the sex change in these fish starts with a dramatic change in their sex-specific behavior immediately after they realize their social status. This suggests that the brain has a primary role in sexual development and plasticity and presents an interesting challenge for future researchers.

Ⅲ. Sexual plasticity in the adult gonochoristic fish

With the exception of certain hermaphroditic species, most vertebrate species are thought to have lost their sexual plasticity after differentiation of separate gonads/sexes with a single, distinct gamete type. Recently, we treated adult female tilapia with fadrozole (AI, a non-steroidal aromatase inhibitor) for two to five months to block the conversion of androgens to estrogens in order to investigate whether sexual plasticity is retained in the adult gonochoristic fish (Nakamura *et al*., unpublished). Suppression of estradiol-17β (E2) production via AI treatment caused a rapid degeneration of primary oocytes, leading to testicular germ cell differentiation in the adult ovary. Sex-changed fish show a typical male pattern of reproductive hormone levels and secondary sex characteristics, producing fertile sperms in the newly formed testes. Additionally, these fish display male-specific territorial behavior, pointing towards the changes that might have occurred to the sex-specific neuronal circuits in the brain. Conversely, co-treatment of E2 inhibited AI-induced sex reversal. Our results demonstrated for the first time in any gonochoristic species

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2008. The former title is indicated by an asterisk (*)

that tilapia retains its sexual plasticity even in the adult stage. Furthermore, this data indicates that estrogens are vital to the maintenance of female phenotype in gonochoristic species.

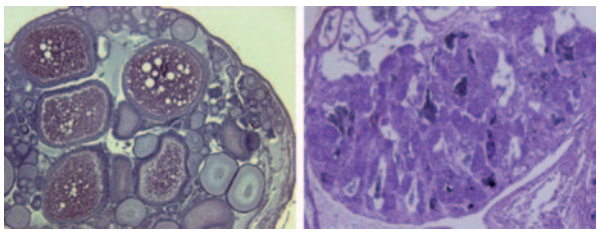

Figure 1. Aromatase inhibitor (AI)-induced female-to-male sex reversal of adult tilapia females. Left, Vehicle-treated gonad. Right, AI-treated gonad.

Ⅳ. Endocrine regulation of oocyte maturation and ovulation

A period of oocyte growth is followed by a process called oocyte maturation (the resumption of meiosis) which occurs prior to ovulation 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 (M. Mita, M. Yoshikuni *et al*., unpublished). Based on its cysteine motif, the purified GSS was classified as a member of the insulin/insulin-like growth factor (IGF)/relaxin superfamily. 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. *In situ* hybridization showed the transcription of GSS to occur in the periphery of radial nerves at the side of tube-feet. Thus, the current study represents the first evidence of a relaxin system in invertebrates and points towards a novel reproductive role for this peptide in starfish.

17α, 20β-DP has been shown to be involved in both oocyte maturation and ovulation (follicle rupture). Interestingly, these actions of 17α, 20β-DP are mediated through two different progestin receptors, the membrane (mPR) and nuclear (nPR) progestin receptors expressed in the oocyte surface and follicular granulosa cells, respectively. nPR transiently expresses after the timing of LH release. The expression of a protease, membrane-type matrix metalloproteinase 2 involved in follicle rupture rose after nPR expression by gonadotropin treatment. These results demonstrate that nPR induced by gonadotropin in granulosa cells may regulate the expression of factors involved in follicle rupture with 17α, 20β-DP as a ligand (Shibata *et al*., unpublished).

Publication List

〔Original papers〕

- Ijiri, S., Kaneko, H., Kobayashi, T., Wang, D.S., Sakai, F., Paul-Prasanth, B., Nakamura, M., and Nagahama, Y. (2008). Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol. Reprod. *78*, 333-341.
- Kobayashi, T., Kajiura-Kobayashi, H., Guan, G., and Nagahama, Y. (2008). Sexual dimorphic expression of DMRT1 and Sox9 during gonadal differentiation and hormone-induced sex reversal in the teleost fish Nile tilapia (*Oreochromis niloticus*). Dev. Dyn. *237*, 297-306.
- Matsuoka, Y., Kobayashi, T., Kihara, K., and Nagahama, Y. (2008). Molecular cloning of *Plk1* and *Nek2* and their expression in mature gonads of the teleost fish Nile tilapia (*Oreochromis Niloticus*). Mol. Reprod. Develop. *75*, 989-1001.
- Mita, M., Ito, C., Nagahama, Y., and Shibata, Y. (2008). Expression and distribution of gonad-stimulating substance in various organs of the starfish, *Asterina pectinifera*. Ann. N.Y. Acad. Sci. in press.
- Sakai, F., Kobayashi, T., Matsuda, M., and Nagahama, Y. (2008). Stability in aromatase immunoreactivity of steroid-producing cells during early development of XX gonads of the Nile tilapia, *Oreochromis niloticus*: An organ culture study. Zool. Sci. *25*, 344-348.
- Swapna, I., Sudhakumari, C.C., Sakai, F., Sreenivasulu, G., Kobayashi, T., Kagawa, H., Nagahama, Y., and Senthilkumaran, B. (2008). Seabream GnRH immunoreactivity in brain and pituitary of XX and XY Nile tilapia, *Oreochroomis niloticus* during early development. J. Exp. Zool. Part A, *309*, 419-426.

〔Review articles〕

- Okubo, K., and Nagahama, Y. (2008). Structural and functional evolution of gonadotropin-releasing hormone in vertebrates. Acta Physiol. *193*, 3-15.
- Nagahama, Y., and Yamashita, M. (2008). Regulation of oocyte maturation in fish. Dev. Growth Differ. *50 Suppl 1*, S195-219.
- Paul-Prasanth, B., Matsuda, M., Kobayashi, T., Suzuki, A., and Nagahama, Y. (2008). Functional analysis of the medaka sex determining gene, DMY - A minireview. Cybium *32*, 72-73.