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The pituitary-gonadal axis plays an important role in regulating gametogenesis in vertebrates. Gonadotropins typically act through the biosynthesis of gonadal steroid hormones which in turn mediate various stages of gametogenesis. Their effects are particularly profound in teleost fishes which provide several excellent models for investigating the basic hormonal mechanisms regulating gonadal sex differentiation and gametogenesis (spermatogenesis, sperm maturation, oocyte growth and oocyte maturation). Our research focuses on (1) the identification of steroidal mediators involved in gonadal sex differentiation and gametogenesis, and (2) the mechanisms of synthesis and action of these mediators.

I. Endocrine regulation of gonadal sex differentiation and sex change

Sex determination and gonadal development vary considerably in fish. In addition to gonochorism, several types of hermaphroditism (protandry, protogyny and synchronous hermaphroditism) are found in fish. Tilapia, *Oreochromis niloticus*, is an excellent example of the precise nature of steroidogenic actions during gonadal sex differentiation. In this fish, all genetic female (XX) or male (XY) broods can be obtained by artificial fertilization of normal eggs (XX) and sex-reversed, pseudo male sperm (XX), or normal eggs (XX) and super male sperm (YY), respectively. Fertilized eggs hatch after 4 days at 26℃. On the day of hatching, primordial germ cells (PGCs), which are morphologically distinguishable from somatic cells, are located in the outer layer of the lateral plate mesoderm around the hind gut. At 3 days post-hatching, PGCs are located in the gonadal anlagen after the formation of the coelomic cavity in the lateral plate mesoderm rather than through active migration. Mitosis of germ cells begins 15-20 days post-hatching in genetic females, but can not be confirmed until after sex differentiation in testes of genetic males.

During the course of morphological sex differentiation, the behavior of somatic cells in the gonad is of often sex-specific. In these cases, the sex of the gonad is easily distinguished. In tilapia, gonadal sex is morphologically distinct at 20-25 days post hatching. Ovarian differentiation is initially marked by stromal elongations of the gonad for the formation of the ovarian cavity. Testicular differentiation is characterized by the appearance of a narrow space in the stromal tissue representing the formation of the efferent duct. Steroid-producing cells in ovaries, but not testes, at the undifferentiated and differentiating stages express all of the steroidogenic enzymes required for estradiol-17β biosynthesis from cholesterol. These results strongly suggest that endogenous estrogens act as the natural inducers of ovarian differentiation in tilapia. This hypothesis is further supported by evidence of masculinization of genetic female tilapia by inhibition of estrogen synthesis using an inhibitor of cytochrome P450 aromatase (Fig. 1). In contrast, the ability of steroid-producing cells to synthesize steroid hormones could not be confirmed in the testis during sex differntiation of testicular differentiation.

Sequential, protogynous hermaphroditism, i.e. female to male sex change, provides an ideal comparative model for studies on the endocrine regulation of sexual differentiation. Prior to sex change, the gonad of *Thalassoma duperrey* contains primordial germ cells and oogonia but not spermatogonia. To begin investigating the importance of hormonal signals on germ cell differentiation during sex change, we examined the expression of P450arom and 11β-hydroxylase mRNAs in *T. duperrey*. The ovarian form of P450arom is predominantly expressed in the ovary prior to the initiation of sex change. Shortly thereafter, expression diminished to undetectable levels suggesting ovarian P450arom/estrogen is critical for the maintenance of ovarian function and/or detrimental to testicular differentiation. In contrast, P450 11β-hydroxylase is likely to be a major factor regulating the gonadal changes. P450 11β-hydroxylase mRNA was relatively abundant in the ovary; however, it was up-regulated concomitant with the onset of testicular differentiation indicating the importance of P450 11β-hydroxylase/11-ketotestosterone (11-KT) to spermatogenesis and spermiation. Consequently, the switch from an ovary to a testis corresponds exceptionally well to a switch in the steroidogenic pathway of the gonad thus providing further evidence of the critical nature of steroid hormones in teleost sexual differentiation.

II. Endocrine regulation of spermatogenesis

Spermatogenesis is an extended process of differen-

tiation and maturation of germ cells resulting in haploid spermatozoa. The principal stimuli for vertebrate spermatogenesis are thought to be pituitary gonadotropins and androgens. However, the mechanisms of action of these hormones remain unresolved. Using an organ culture system for eel testes consisting of spermatogonia and inactive somatic cells, we have shown that the hormonal regulation of spermatogenesis in eel testes involves the gonadotropin stimulation of Leydig cells to produce 11-KT, a potent androgen in fish. In turn, 11-KT activates Sertoli cells to stimulate production of activin B. Addition of recombinant eel activin B to the culture medium induced proliferation of spermatogonia, producing late type B spermatogonia, within 15 days in the same manner as did 11-KT. cDNAs encoding two androgen receptors (eARα and eARβ) were cloned eel testes. The amino acid sequences of these two ARs share low homology. In transient transfection assays of mammalian cells, both eAR proteins showed androgen-dependent activation of transcription with 11-KT being the most effective activator. These results indicate that the cloned eAR cDNAs encode functional eel ARs, whose native ligands are 11-KT. *In situ* hybridization revealed that both eAR mRNAs are present in testes prior to HCG injection, only eARα transcripts increased during HCG-induced spermatogenesis suggesting that eARα and eARβ play different roles in spermatogenesis.

In situ hybridization shows that activin type I and II receptors are localized in spermatogonia. Activin B acts via these receptors on spermatogonia to induce *de novo* synthesis of G1/S cyclins (cyclins A, D, and E) and Cdks (cdc2, cdk2, and cdk4), leading to the initiation of mitosis (spermatogonial proliferation). Despite the above-mentioned progress in the study of hormonal regulation of spermatogenesis (mitosis), our attempts to induce meiosis *in vitro* with recombinant eel activin B have been unsuccessful. One possible explanation is that another factor(s), perhaps a meiosis-initiating substance produced in the testis in response to gonadotropins or 11-KT, may be responsible for the mitosis-meiosis transition. Interestingly, cyclin A1 transcripts were first detected in primary spermatocytes during HCG-induced spermatogenesis in eel testes suggesting an important role of cyclin A1 in the progression to meiosis of male germ cells. Further studies on the transcriptional regulation of cyclin A1 expression may therefore answer some of the basic questions related to the initiation and progression towards meiosis during spermatogenesis.

III. Endocrine regulation of oocyte maturation

Meiotic maturation of fish oocytes is induced by the action of maturation-inducing hormone (MIH). 17α, 20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) was identified as the MIHs of several fish species. The interaction of two ovarian follilce cell layers, the thecal and granulosa cell layers, is required for the synthesis of 17α,20β-DP. The theal layer produces 17α-hydroxyprogesterone that is converted to 17α,20β-DP in granulosa cells by the action of 20β-hydroxysteroid dehydrogenase (20β-HSD). The preovulatory surge of LH-like gonadotropin is responsible for the rapid expression of 20β-HSD mRNA transcripts in granulosa cells during oocyte maturation. 17α,20β-DP induces oocyte maturation by acting on a pertussis toxin-sensitive G-protein-coupled membrane receptor. The early steps of l7α,20β-DP action involve the formation of downstream mediator of this steroid, the maturation-promoting factor or metaphase-promoting factor (MPF) consisting of cdc2 kinase and cyclin B. 17α,20β-DP induces oocytes to synthesize cyclin B which activates a preexisting 35-kDa cdc2 kinase via phosphorylation of its threonine 161 by a threonine kinase (MO15), thus producing the 34 kDa active cdc2. A 54-kDa Y box protein and polyadenylation of cyclin B mRNA are thought to be involved in 17α,20β-DP-induced initiation of cyclin B mRNA translation. Upon egg activation, MPF is inactivated by degradation of cyclin B. It was demonstrated that the 26S proteasome initiates cyclin B degradation through the first cut of its NH_2 terminus at lysine 57.

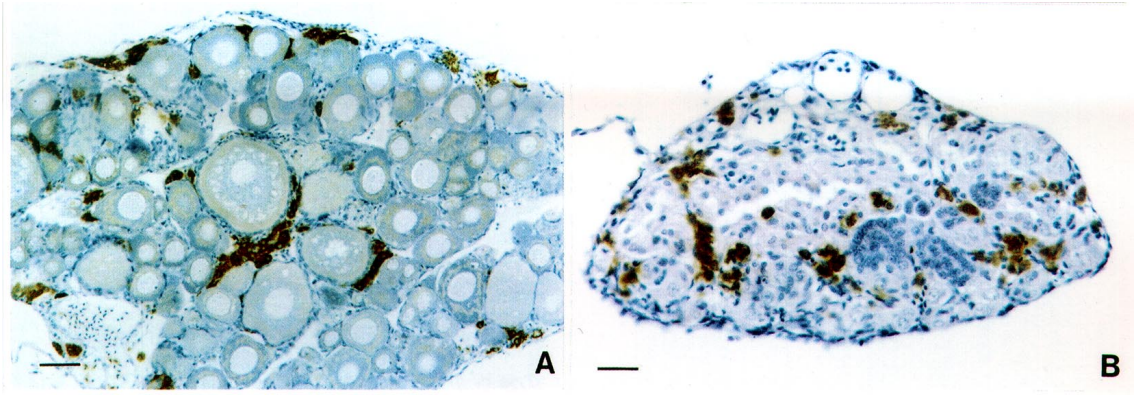

Fig. 1. Masculinization of genetic female (XX) tilapia by inhibition of estrogen synthesis using an inhibitor of cytochrome P450 aromatase (P450arom), fadrozole (Fig. 1). Fadrozole was given to fry at 8 days after hatching for 22 days. A, Control (Female, ovary); B, Fadrozole (Sex reversed male, testis). Gonads were stained with an antibody against P450arom (A) or an antibody against 3β-hydroxysteroid dehydrogenase (3β-HSD) (B).

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