FIRING ACTIVITY OF NEUROSECRETORY CELLS PRODUCING DIAPAUSE HORMONE AND ITS RELATED PEPTIDES IN THE SILKMOTH *BOMBYX MORI*

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A gene encoding a precursor polyprotein of diapause hormone (DH) and other four related peptides is expressed by three groups of neurosecretory cells in the suboesophageal ganglion of *Bombyx mori*. Chronic recordings were made from the axonal tract (NCC-3) of labial (posterior) cells and the common axonal tract (the maxillary nerve) of mandibular (anterior) and maxillary (medial) cells during the pupal period. Firing activity patterns of the labial cells differed significantly between diapause-egg (D) and non-diapause-egg (ND) producers: cells in the former were active throughout pupal period, whereas the same cells in the latter maintained an inactive state until the last quarter of pupal period. On the other hand, firing activity of mandibular and maxillary cells did not differ significantly between D and ND producers: the cells in both types of pupae were active throughout pupal period. The results suggest that pupal labial cells are responsible for the secretion of DH, while mandibular and maxillary cells are involved in the secretion of other neuropeptides. There may be different posttranslational processing of the precursor polyprotein in different neurosecretory cell groups.

IN VITRO SPERMATOGENESIS IN FISH OVARY

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Wrasse ovaries can transform into perfect testes during sex change. No apparent testicular tissues are present in their ovaries. The transformation starts with degeneration of oocytes, followed by development of testicular tissues. Here, we report that the dramatic changes in ovary can be induced in vitro, using tissue organ culture techniques. Threespot wrasse, *Halichoeres trimaculatus*, were caught from coral reefs in Okinawa, Japan. Ovaries were collected from adult females, and were dissected into small pieces. Ovarian fragments were then cultured in L-15 medium with methyltestosterone (MT). After a few weeks, these fragments were fixed for standard histological analysis. Ovaries treated with MT showed degeneration of oocytes, proliferation of presumed spermatogonia on the periphery of lamella, and formation of spermatogenic crypts. This unique system provides us an exceptional opportunity to investigate essential factors for sex differentiation of both germ and somatic cells.

FUNCTIONAL SEX REVERSAL BY THE TREATMENT OF AROMATASE INHIBITOR AFTER OVARIAN DIFFERENTIATION IN TILAPIA

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In order to clarify the bipotency of germ cells and somatic cells after sex differentiation in gonochoristic tilapia, we examined the effects of aromatase inhibitor (AI) on the overy after differentiation. AI(200 microg/g diet) were given to genetically controlled all female tilapia Oreochromis niloticus, 4 to 5 cm in length, for 2 and a half months. The ovary of fish in initial control had developed oocytes and an ovarian cavity, indicating complete ovarian differentiation. All fish treated with AI had testicular tissue with active spermatogenic germ cells in the ovary after 2 months. Fish treated with AI at 4 months after treatment had developed testes, with residual oocytes. Testicular tissue with active spermatogenesis occupied the gonads of fish treated with AI. Degenerating oocytes were distributed around the outer periphery of gonads. Sperm ducts were seen in the ovarian tissue. This fish had a territorial behavior, and mated with a normal female and finally produced offspring, indicating functional sex reversal. So far as we know, this is the first report in which a functional sex reversal has been shown after the completion of sex defferentiation in gonochoristic fish.

FUNCTIONAL SEX REVERSAL AFTER OVARIAN DIFFERENTIATION IN THE GONOCHORISTIC FISH, NILE TILAPIA: ENDOCRINE CHANGES

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We have recently found that gonochoristic tilapia show sexual bipotency far beyond the period of gonadal sex differentiation. Treatments with aromatase inhibitor (AI) caused sex reversal of genetical females into functional males, but not with methyltestosterone (MT) (see Nakamura et al, this issue). We, therefore, examined changes in steroidogenic enzymes mainly P450scc, 3-beta-HSD and aromatase (arom) immunoreactive (ir) cells in the gonads and correlative changes in plasma levels of sex steroid hormones. The ovary of control females showed strong signals of P450scc, 3-beta-HSD and arom-ir cells, whereas in the gonads of AI-administered fish, arom-ir cells appeared only around oocytes, but not around the area of spermatogenic germ cells proliferation. In contrast, immunoreactive cells to all steroidogenic enzymes were absent in the gonads of MT-administered fish. Plasma levels of E2 were significantly decreased in the AI-administered fish, while the levels of all steroid hormones were decreased in the MT-administered fish. Thus, present results suggest that inhibition of estrogen synthesis by AI causes oocyte degeneration and induces testicular differentiation in the ovary.

SEX STEROID SECRETION OF GONADS IN THE MANGROVE KILLIFISH *RIVULUS MARMORATUS*

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Mangrove killifish is the only known self-fertilizing vertebrate. However, there is no study on the reproductive physiology of this species. Thus, we investigated the hormonal expression in gonads of this species. Gonads were dissected and removed from 9 hermaphrodites and 5 males. The folliculated oocytes from hermaphrodites and the testes from males were cultured in L-15 with the different concentrations of human chorionic gonadotropin (HCG), 17α-hydroxyprogesterone (OHP) or testosterone (T). After 24 hours culture, concentrations of T, estradiol-17β(E_2), 11-ketotestosterone (11KT) and 17α,20β-dihydroxy-4-pregnen-3-one (DHP) in the culture medium were measured by ELISA. Both ovary and testis secreted T, E_2, 11KT and DHP synchronously. T was identified as the precursor of $E_2$and 11KT, and OHP as the precursor of T and DHP, respectively.

MOLECULAR INTERACTION BETWEEN PROGESTOGEN AND ANDROGENS VIA PROGESTOGEN RECEPTOR IN JAPANESE EEL (*ANGUILLA JAPONICA*)

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We previously isolated and characterized a cDNA encoding a progestogen receptor (PR) for a final maturation inducing hormone, 17α,20β-dihydroxy-4-pregnen-3-one (DHP) from an eel testis cDNA library. It was found that the PR was bound to testosterone, but the androgen did not induce transcriptional activity via PR. In the present study, in order to demonstrate that androgens have antagonist activity for DHP via PR, transactivation analyses were performed using transgenic cell lines which stably express PRs and the PR-responsive reporter genes. Testosterone, 11-ketotestosterone and androstenedione inhibited the transactivity for DHP in a dose-dependent manner. Therefore, it is suggested that androgens inhibit DHP-regulated genes through PR, as well as they regulate their down-stream genes through AR.

EXPRESSION OF AD4BP/SF-1 IN RED-SPOTED DWARF GOBY, *TRIMMA OKINAWAE*, DURING MULTI-SEX CHANGE

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Red-spoted dwarf goby, *Trimma okinawae* is a teleost fish that exhibits multi-sex change in response to social cues. It is an excellent animal model to elucidate the mechanisms of sex change. In this fish, cytochrome P-450 aromatase (P450arom) plays key roles in the organization of sex-typical behavior and gonadal transition. However, schematic analysis of transcriptional regulation activity of P450arom is limited. Ad4BP/SF-1 is a transcriptional regulator for P450arom expression and activity. In the present study, we isolated and characterized Ad4BP/SF-1 cDNA from goby. Subsequently, changes in Ad4BP/SF-1 expression were examined in ovary during sex change using real-time PCR analysis. A cDNA encoding Ad4BP/SF-1 was cloned from ovarian follicles. Phylogenic tree analysis of goby Ad4BP/SF-1 indicated high homology to other teleost. RT-PCR showed that Ad4BP/SF-1 was expressed in gonadal tissues, brain and kidney. Real-time PCR analysis revealed that expression of Ad4BP/SF-1 declined sharply from female-to-male change and increased in male-to-female change. These results indicate that Ad4BP/SF-1 might play important role in sex change and oocyte development process.