DEVELOPMENTAL TRACING OF LH BETA GENE EXPRESSION USING A STABLE LINE OF TRANSGENIC MEDAKA REVEALS PUTATIVE DEVELOPMENTAL FUNCTION AND PITUITARY DISTRIBUTION

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Background:

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are key regulators of vertebrate reproduction. The differential regulation of these hormones by pituitary gonadotrope cells, however, is still poorly understood. Fish, in contrast to mammals, have two distinct gonadotrope cell types, producing LH and FSH, respectively, making teleosts a good model to study the differential regulation of these hormones. Japanese medaka (*Oryzias latipes*) is a powerful model for fish reproductive physiology research, combining a wealth of advanced biotechnological tools, genomic resources, short generation time, a close phylogenetic relationship to many important aquaculture species and a well-defined sexual development. We have thus chosen to use the medaka model for our research to characterize the pituitary gonadotrope cells that produce and secrete LH and FSH.

Methods:

To this aim we have developed a stable transgenic line of medaka with the endogenous LH beta gene (*lhb*) promotor driving green fluorescent protein (*gfp*) expression by microinjection of a BAC construct with hrGFP inserted downstream of the *lhb* promotor. This line has been used to trace the developmental expression of LH beta using fluorescent light and confocal microscopy in whole larva and histological sections. Additionally, qPCR and *in situ* hybridization have been used to measure developmental and tissue specific gene expression.

Results and Discussion:

We demonstrate by *in situ* hybridization that *lhb* mRNA and Gfp protein are co-localized in pituitary cells of sexually mature medaka, and we have characterized the three dimensional distribution of LH gonadotrope cells in the pituitary. qPCR analysis reveals that the *lhb* gene is expressed early during development, already after 24 hours post-fertilization (hpf), and Gfp protein can first be detected by 32 hpf, approximately stage 20. Gfp expression starts posterior of the eyes as paired lateral clusters which then extend to the midline and posteriorly. By 48 hpf, Gfp producing cells are dispersed along the ventral surface of the larva, extending from behind the otic vesicle to the metencephalon – myelencephalon boundary in the developing hindbrain. By 72 hpf, dispersed Gfp producing cells extend to the mesencephalon – metencephalon boundary and a few distinct cells are located at the posterior margin of the eye. *In situ* hybridization of pituitary marker genes show that Gfp producing cells are initially localized outside the primordial pituitary and Gfp is first detected in the developing pituitary by 2 weeks post-fertilization. Confirmation of embryonic *lhb* expression in extra-pituitary Gfp-producing cells is currently underway to validate a potentially novel developmental function for LH.

Conclusion:

We have established a powerful model for characterizing the developmental and reproductive physiological regulation of LH gonadotropes. These data lay the framework for future research into the function of LH during early pituitary developmental studies. This transgenic line also provides the technological basis for further characterization of gonadotropes by cell specific gene expression analysis, electrophysiological and calcium imaging experiments (see abstract by Stransdabo et al, this meeting).