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67**Retinoic Acid Metabolic Genes,
Responsiveness and Meiosis in Medaka**Mateus Contar Adolff¹, Amaury Herpin¹, Joshua S. Waxman² and Manfred Scharf¹

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Sex determination is a complex developmental process regulated and fine-tuned by cascades or networks of genes, involving the cellular fate of the undifferentiated soma and the primordial germ cell (PGC). One of the first recognizable differences between male and female gonad development is the onset of meiosis. This is regulated by retinoic acid (RA) in mammals. The retinoic acid receptors (RARs) bind to DNA at retinoic acid response elements (RAREs) in the absence of their ligand. RARs, through RAR heteromeric binding with a retinoid X receptor (RXR) on RAREs control the expression of RA-responsive genes, repressing the expression in the absence of RA and releasing the expression in its presence. Since little information is known of the involvement of RA in the entry of meiosis and sex development in fish, we studied sex determination in the model fish species medaka to elucidate it. We generated transgenic lines for the main RA-metabolic enzymes (Aldh1a2, Cyp26a1 and Cyp26b1) that report the expression of those genes during the development. Expression of those genes was also followed by whole amount *in situ* hybridization. We also generated a RA-responsive reporter transgene containing 12 copies of the RARE together with eGFP (12XRARE). We observed different expression domain for the RA-synthesis gene and the RA-degrading genes, which can create a RA gradient during A-P axis formation. We found that the metabolic genes expressions are regulated by exogenous all-trans-retinoic acid treatments. We also demonstrate that the presence of Aldh1a2 gene expression does not reflect that RA is acting in those same cells. The responsiveness to RA starts in the very early stages of the development, and treatments with exogenous all-trans-retinoic acid and DEAB (Raldh inhibitor) show that the RA-responsive reporter is RA inducible and dependent. There is a clear sex specific expression of the Aldh1a2 gene in the testis, and of the Cyp26a1 gene in ovary, demonstrating an important sex-bias of the RA production in the gonads. In summary, we hypothesize that RA is involved in the onset of meiosis in adult medaka, acting directly in the germ cells in females and through the Sertoli cells in male, complementary to what was shown in zebrafish.

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68**Development of the galaninergic system in the
brain of an elasmobranch, the dogfish
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Galanin is a 29 (30 in human)-amino acid neuropeptide widely distributed in the central and peripheral nervous system in vertebrates. In fish, the organization of galaninergic systems is well known. However, in contrast with the numerous studies carried out on the brain of adult fish, including elasmobranchs [Vallarino et al., 1991. *Peptides* 12:351-357], little investigation has been made of the expression of galanin during development. To extend the evolutionary analysis of galaninergic systems we have studied the development of galaninergic cell groups and fibers in the brain of embryos, juveniles and adults of the shark *Scyliorhinus canicula* using immunohistochemistry to galanin (GAL). Immunoreactive (GALir) cells appeared along the ventral telencephalon (including preoptic area) and hypothalamus of very early embryos, when the characteristic walls layering become evident. The earliest GALir cells appeared in the preoptic region of stage 26 (S26) and in the subpallium (primordia of Interstitial nucleus of the anterior commissure and area superficialis basalis) and hypothalamus of S27. At S28, GALir cells occupied the vascular organ of the terminal lamina. All the galaninergic cell groups observed in juveniles were already recognized at S34 (prehatching). Most of the GALir cells in preoptic and hypothalamic periventricular regions were cerebrospinal fluid (CSF)-contacting neurons. The distribution of GALir cells in adults was similar to that previously observed [Vallarino et al., 1991]. Along development, GALir fibers innervated different regions of the telencephalon, hypothalamus, mesencephalon and medulla oblongata being especially abundant in the median eminence, indicating that GAL may be related with hypophysis function. The developmental sequence of GALir cell groups in the dogfish is similar to that described for teleosts, apart from the abundance of galaninergic CSF-contacting cells which appear to be a primitive characteristic of elasmobranchs. The early expression of GAL may indicate a crucial role in development probably related to the development of nerve centers involved in food intake and growth.

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