SEX IN SALMONIDS: FROM GONADAL DIFFERENTIATION TO GENETIC SEX DETERMINATION

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Introduction:

Members of the salmonid family are present worldwide and many of them are species of major importance for aquaculture, wild stock fisheries or recreational sport fisheries. Sex differentiation and sex determination in salmonid species have been studied since the early twenties [1, 2] and, from that moment on, a lot of data has been gathered on this topic both because of its importance for fisheries and aquaculture and because salmonids can be excellent research models [3]. This communication will review some of the available published knowledge on sex differentiation and sex determination in salmonid species along with some results obtained from genomic, genetic and expression screens studies that have been carried out recently in our laboratory.

All salmonid species in which sex determination has been studied in details thus far belong to the Salmoninae subfamily and they possess a male heterogametic sex determination system (XY/XX) (reviewed in [4]). This strict genetic system has been used to produce genetic all-male and all-female populations (all XX or all XY individuals) that are now one major experimental feature allowing to work on male and female sex differentiation at very early developmental stages when the gonad is not yet histologically differentiated. We extensively used these monosex populations to compare the early steps of rainbow trout (*Oncorhynchus mykiss*) male and female gonadal differentiation using microarrays, *in situ* hybridization screens and next generation sequencing (NGS).

Results and Discussion:

Our recent results first demonstrated that gonadal differentiation starts prior to any histological differentiation and much earlier than initially thought. The first sexually dimorphic gene expression patterns were detected around hatching (30 days post fertilization = 30 dpf). Among these early actors, the estrogen synthesis potentiality of the differentiating ovary was confirmed to be crucial [5], with an early and female-specific expression of ovarian aromatase (*cyp19a1a*). We also found that follistatin (*fst*) is the earliest gene co-expressed with *cyp19a1a* in the differentiating ovary suggesting that *fst* is also a key player in ovarian differentiation and a potential regulator of *cyp19a1a* expression (Nicol et al., this meeting). *In situ* hybridization screens revealed an unexpected complexity of the early differentiating gonads but also that these gonads are already well structured. For instance, we identified that transforming growth factor beta 2 (*tgfb2*) expression pattern clearly delimits the ventral epithelium of the differentiating gonads. A lot of these new actors of the gonadal sex differentiation cascade were identified from microarray screens, but we recently switched to NGS to identify novel genes that would be specifically expressed in embryonic male or female gonads. Using Roche's 454 Titanium sequencing technology we compared cDNA libraries made from male and female embryonic gonads sampled a few days after hatching. We were thus able to characterize a novel gene that could be a prime candidate as a rainbow trout master sex determining gene. This gene is expressed only during testicular differentiation and tightly linked to the Y chromosome (Yano et al., this meeting). Furthermore, even though rainbow trout sex determination is mainly genetic, some recent studies also report that temperature can influence gonadal sex differentiation leading to skewed sex ratio [6, 7]. Using a genetic all-female rainbow trout population carrying a masculinizing mutation [8] we demonstrated that rearing larvae at high temperature can increase the masculinization rate of this population (Valdivia et al., this meeting) and that *cyp19a1a* was down-regulated during gonadal differentiation in these mutated populations.

Conclusion:

By combining various genomics approaches we now have a better understanding of the early steps of gonadal sex differentiation in rainbow trout including the first sex determination switch acting at the top of the gene cascade.

References:

[1]MRSIC W. 1923. Die spatbefruchtung und deren Einfluss auf Entwicklung und Geschlechtsbildung. Arch. Micr. Anat., 98: 129-209.

[2]MRSIC W. 1930. Uber die Eireifung bei der Forelle und deren Bedeutung fur die ubliche Methode der

kunstlichen Laichgewinnung. Arch. Hidrobiol., 21: 649-678.

[3]THORGAARD G.H., BAILEY G.S., WILLIAMS D., BUHLER D.R., KAATTARI S.L., RISTOW S.S., HANSEN J.D., WINTON J.R., BARTHOLOMEW J.L., NAGLER J.J., et al. 2002. Status and opportunities for genomics research with rainbow trout. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 133: 609-646.

[4]DAVIDSON W.S., HUANG T.K., FUJIKI K., VON SCHALBURG K.R., KOOP B.F. 2009. The sex determining loci and sex chromosomes in the family salmonidae. Sex. Dev., 3: 78-87.

[5]GUIGUEN Y., FOSTIER A., PIFERRER F., CHANG C.F. 2010. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. Gen. Comp. Endocrinol., 165: 352-366.

[6]MAGERHANS A., HORSTGEN-SCHWARK G. 2010. Selection experiments to alter the sex ratio in rainbow trout (*Oncorhynchus mykiss*) by means of temperature treatment. Aquaculture, 306: 63-67.

[7]MAGERHANS A., MULLER-BELECKE A., HORSTGEN-SCHWARK G. 2009. Effect of rearing temperatures post hatching on sex ratios of rainbow trout (*Oncorhynchus mykiss*) populations. Aquaculture, 294: 25-29.

[8]QUILLET E., AUBARD G. , QUEAU I. 2002. Mutation in a sex-determining gene in rainbow trout: Detection and genetic analysis. J. Heredity, 93: 91-99.