



A DUPLICATED, Y-LINKED COPY OF THE ANTI-MULLERIAN GENE DETERMINES TESTICULAR FORMATION IN PATAGONIAN PEJERREY *ODONTESTHES HATCHERI*

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Introduction:

In many vertebrates, gonadal sex determination follows a sequence of genetically programmed events. However, only a handful of genes have been described as the ultimate triggers of differentiation and the mechanism of genotypic sex determination still remains elusive for most vertebrates. In order to clarify the process of genotypic sex determination in the Atherinopsid fish Patagonian pejerrey (*Odontesthes hatcheri*), a species with the XX-XY sex determining system, we have conducted transcriptomic analyses of several sex-related genes during early sex differentiation. Those studies lead to the identification of a duplicated copy of the anti-Müllerian hormone (*amh*) gene which plays a critical role in sex determination of this species.

Methods:

The complete cDNA sequences of the two forms of *amh* were obtained by degenerated RT-PCR and RACE PCR using larvae and adult samples. The non-transcribed sequence of the male-specific locus was obtained by PCR of genomic DNA from an XY

individual. Fluorescence *in situ* hybridization (FISH) was performed for chromosome mapping. mRNA expression was analyzed by RT-PCR and *in situ* hybridization during gonadal sex differentiation. Functional analysis was conducted by microinjection of antisense morpholinos.

Results and Discussion:

The male-specific *amh* (termed *amhy*) gene showed 92.2% of amino acid identity in relation to the autosomal one (termed *amha*). Physical mapping of *amhy* allowed the identification of the Y chromosome in this species and its transcripts were detected from 6 days after fertilization in presumptive Sertoli cells. *Amhy* knockdown in XY embryos resulted in the development of ovaries.

Conclusion:

These results establish *amhy* as a candidate for the master sex-determining gene in *O. hatcheri* and for the first time associate a hormone-related gene, a member of the TGF- β superfamily, with such a role.