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Semblanzas Ictiológicas Argentinas
Juan Ignacio Fernandino

Hugo L. López
y
Justina Ponte Gómez

Indizada en la base de datos ASFA C.S.A.
2014

El tiempo acaso no exista. Es posible que no pase y sólo pasemos nosotros.

Tulio Carella

Cinco minutos bastan para soñar toda una vida, así de relativo es el tiempo.

Mario Benedetti

Semblanzas Ictiológicas

A través de esta serie intentaremos conocer diferentes facetas personales de los integrantes de nuestra "comunidad".

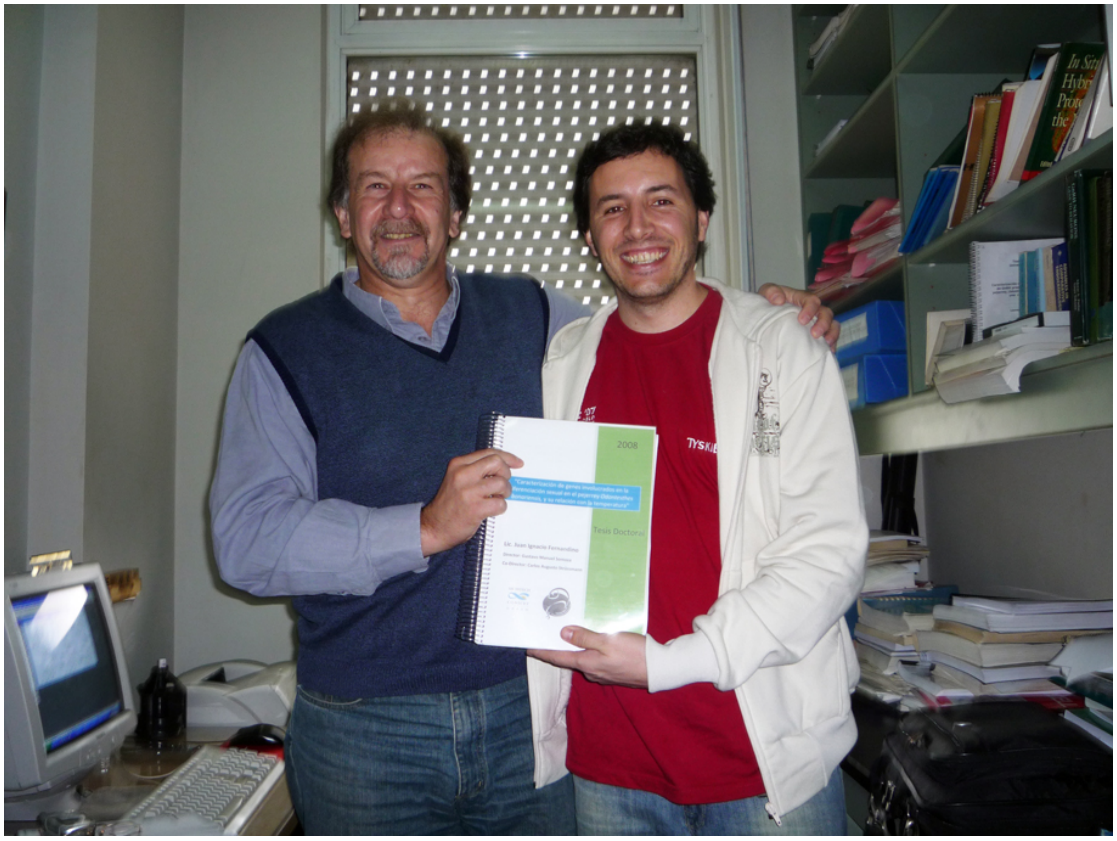
El cuestionario, además de su principal objetivo, con sus respuestas quizás nos ayude a encontrar entre nosotros puntos en común que vayan más allá de nuestros temas de trabajo y sea un aporte a futuros estudios históricos.

Esperamos que esta iniciativa pueda ser otro nexo entre los ictiólogos de la región, ya que consideramos que el resultado general trascendería nuestras fronteras.

Hugo L. López

Semblanzas Ictiológicas Argentinas

Juan Ignacio Fernandino



Impresión de Tesis Doctoral, 2008
Juan Ignacio Fernandino (derecha) junto al director de su tesis, Dr. Gustavo Somoza

Hugo L. López y Justina Ponte Gómez

ProBiota
División Zoología Vertebrados
Museo de La Plata
FCNyM, UNLP

Abril, 2014

Imagen de Tapa
Pescando en Tateyama Station, 2009

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Cuestionario

-**Unlibro**: *Lord of the Rings*., J. R. R. Tolkien
-**Unapelícula**: *The Matrix*; *The Wachowski Brothers*
-**Un CD**: *And Justice for All*, Metallica
-**Un artista**: Gustavo Dudamel
-**Un deporte**: sailing
-**Un color**: verde
-**Una comida**: sashimi
-**Un animal**: perro, tamaño mediano a grande
-**Una palabra**: optimista
-**Un número**: 6
-**Una imagen**: el nacimiento de mi primer hijo
-**Un lugar**: mi ciudad
-**Una estación del año**: primavera
-**Un nombre**: Sara
-**Un hombre**: Raúl Alfonsín
-**Una mujer**: Eva Duarte
-**Un personaje de ficción**: Jerry Seinfeld
-**Un superhéroe**: Spiderman

Chiba, bahía de Tokyo, 2008
Juan Fernandino con su mujer Valeria, su hijo Augusto y el Dr.Carlos Strüssmann Codirector de su Tesis Doctoral

Con el Dr. Ricardo Hattori y Augusto en Tokio, 2009

The Cortisol and Androgen Pathways Cross Talk in High Temperature-Induced Masculinization: The 11β-Hydroxysteroid Dehydrogenase as a Key Enzyme

Juan Ignacio Fernandino, Ricardo Shohei Hattori, Ai Kishii, Carlos Augusto Strüssmann, and Gustavo Manuel Somoza

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In many ectotherm species the gonadal fate is modulated by temperature early in life [temperature-dependent sex determination (TSD)] but the transducer mechanism between temperature and gonadal differentiation is still elusive. We have recently shown that cortisol, the glucocorticoid stress-related hormone in vertebrates, is involved in the TSD process of pejerrey, *Odontesthes bonariensis*. Particularly, all larvae exposed to a male-producing temperature (MPT, 29 C) after hatching showed increased whole-body cortisol and 11-ketotestosterone (11-KT; the main bioactive androgen in fish) levels and developed as males. Moreover, cortisol administration at an intermediate, mixed sex-producing temperature (MixPT, 24 C) caused increases in 11-KT and in the frequency of males, suggesting a relation between this glucocorticoid and androgens during the masculinization process. In order to clarify the link between stress and masculinization, the expression of hydroxysteroid dehydrogenase *(hsd)11b2*, glucocorticoid receptors *gr1* and *gr2*, and androgen receptors *ar1* and *ar2* was analyzed by quantitative real time PCR and *in situ* hybridization in larvae reared at MPT, MixPT, and female-producing temperature (FPT, 17 C) during the sex determination period. We also analyzed the effects of cortisol treatment in larvae reared at MixPT and in adult testicular explants incubated *in vitro*. MPT and cortisol treatment produced significant increases in *hsd11b2* mRNA expression. Also, gonadal explants incubated in the presence of cortisol showed increases of 11-KT levels in the medium. Taken together these results suggest that cortisol promotes 11-KT production during high temperature-induced masculinization by modulation of *hsd11b2* expression and thus drives the morphogenesis of the testes. **(*Endocrinology* 153: 6003–6011, 2012)**

Sex steroids have been considered to play critical roles during sex determination in nonmammalian vertebrates (1). Although they are not considered as initiators of gonadal sex differentiation, their timely appearance and maintenance are fundamental for the subsequent development of ovaries and testes. In teleost fishes, for example, larvae are highly sensitive to sex steroids, and the administration of estrogens or androgens during the critical period of gonadal sex differentiation often leads to a functional sex inversion (2–4). It is currently believed that whereas estrogens are essential for female sex differentiation, androgens, on the other hand, are the product or a consequence of differentiation along the male pathway (2, 5–8). In contrast, high levels of 11-oxygenated androgens or the expression of enzymes involved in their synthesis during the critical period of sex determination/differentiation have been detected in some teleosts (9–11). These differences make the involvement of 11-oxygenated an-

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Abbreviations: cyp11β, 11β-Hydroxylase; EIA, enzyme immunoassay; FPT, female-producing temperature; GC, glucocorticoid; GR, GC receptor; HSD, hydroxysteroid dehydrogenase; 11-KT, 11-ketotestosterone; MixPT, mixed-sex producing temperature; MPT, male-producing temperature; 11β-OHT, 11β-hydroxytestosterone; RT-qPCR, quantitative real time PCR; T, testosterone; TSD, temperature-dependent sex determination; wah, weeks after hatching.

A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination

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Edited by Patricia K. Donahoe, Massachusetts General Hospital and Harvard Medical School, Boston, MA, and approved January 17, 2012 (received for review December 9, 2010)

Gonadal sex determination in vertebrates generally follows a sequence of genetically programmed events. In what is seemingly becoming a pattern, all confirmed or current candidate "master" sex-determining genes reported in this group, e.g., *SRY* in eutherian mammals, *DMY/dmrt1bY* in medaka, *DM-W* in the African clawed frog, and *DMRT1* in chicken encode transcription factors. In contrast, here we show that a male-specific, duplicated copy of the anti-Müllerian hormone (*amh*) is implicated in testicular development of the teleost fish Patagonian pejerrey (*Odontesthes hatcheri*). The gene, termed *amhy* because it is found in a single metacentric/submetacentric chromosome of XY individuals, is expressed much earlier than the autosomal *amh* (6 d after fertilization vs. 12 wk after fertilization) and is localized to presumptive Sertoli cells of XY males during testicular differentiation. Moreover, *amhy* knockdown in XY embryos resulted in the up-regulation of *foxl2* and *cyp19a1a* mRNAs and the development of ovaries. These results are evidence of a functional *amh* duplication in vertebrates and suggest that *amhy* may be the master sex-determining gene in this species. If confirmed, this would be a unique instance of a hormone-related gene, a member of the TGF-β superfamily, in such a role.

The sexual fate of the differentiating gonads in vertebrates is under the control of specific genes that initiate and direct the developmental pathway. A few genes have been already identified as master sex determiners, and they all encode transcription factors, e.g., *SRY* in eutherian mammals (1), *DMY/dmrt1bY* in medaka (2, 3), *DM-W* in the African clawed frog (4), and *DMRT1* in chicken (5). These findings might be construed as evidence that transcription factors always trigger gonadal sex determination in vertebrates. However, the molecular pathway of sex determination has been studied in relatively few non-mammalian species, and most of the details of this process remain elusive.

We have recently identified a sex-linked locus in *Odontesthes hatcheri* (Atherinopsidae), a South American gonochoristic fish with an XX-XY sex determination system (6, 7). The existence of a sex-linked single nucleotide polymorphism (SNP) marker associated with this locus has allowed us to profile the expression of a series of genes involved in early sex differentiation of putative females (XX genotype) and males (XY genotype). Analyses performed during early stages of embryonic and larval development revealed a comparatively early mRNA expression of an anti-Müllerian hormone homolog [(*amh*); also known as Müllerian inhibitory substance/factor, or *mis/mif* (8)] in relation to other teleosts (9, 10) and showed that this unique feature was due to the up-regulation of a duplicated copy of this gene. AMH, a member of the TGF-β superfamily, is secreted by Sertoli cells and is responsible for the regression of Müllerian ducts during male fetal development in mammals, birds, and reptiles (11–13). Fish have *amh* even though they lack Müllerian ducts. However, as with mammals and birds, fish *amh* is generally considered to be autosomal and is placed with other hormones or steroidogenic enzymes downstream from the molecular cascade of sex differentiation in relation to transcription factors (14, 15).

This report describes a unique case of an *amh* paralogue in vertebrates. More importantly, this study shows that this gene is restricted to the male genome and that it is required for testis determination in *O. hatcheri*. These findings establish a hormone-related gene in such a role and an alternative mechanism for transcriptional control of sex determination in vertebrates.

Results

Males Carry a Duplicated Copy of the Anti-Müllerian Hormone Gene. To clarify the reason for the unusual expression profile of *amh* in *O. hatcheri*, extensive sequencing was conducted with mRNAs expressed in larval and adult males. Such analysis revealed the presence of two different *amh* transcripts originated from two different loci. We also determined that one of these loci was present only in the male genome and was responsible for the early transcription of *amh* in XY gonads; this copy was therefore named *amhy* (Y chromosome-specific *amh*). RACE PCR was performed, and full cDNA sequence (2,059 bp) was obtained from mRNA of a 3-wah (weeks after hatching) XY larva. The nucleotide identity values between corresponding exons of *amhy* and the autosomal *amh* (*amha*) ranged from 89.1% to 100% (Fig. 1*A*). The deduced protein comprised 514 amino acids, which includes the characteristic TGF-β domain (amino acids 421–514) with seven canonical cysteine residues. Amino acid identity values of Amhy in relation to Amha were 92.2% for the entire protein and 91.4% for the TGF-β domain. Intron sequences were also characterized by PCR using the respective genomic DNA and revealing a 557-bp *amhy*-specific insertion in the third intron as the main structural difference within untranscribed intragenic regions (Fig. 1*A*). Primers flanking this intron were then designed and used for PCR-based sex genotyping. The comparison of genotypic sex with the histological sex of the gonads in 112 individuals derived from four crosses resulted in 100% matching (Fig. 1*B*) whereas sex genotyping using the previously reported sex-linked SNP marker (6) showed disagreement in two animals. From these results, the genetic distance between *amhy* and the SNP marker was estimated as 1.78 cM. The presence of *amhy* was also confirmed in the genomes of males from another cultivated stock from Japan (Kanagawa; $n = 24$) and natural populations from Argentina (Piedra del Aguila and Mari Menuco; $n = 12$ for each location). The 5′ flanking region of *amhy* was amplified by genome walking, and, together with the *amhy* gene sequence, was used as a probe (7.3 kb) for physical mapping on metaphase chromosomes. Signals were detected in a pair of acrocentric/telocentric chro-

Author contributions: R.S.H. and C.A.S. designed research; R.S.H., Y.M., M.O., S.M., S.K.M., and J.I.F. performed research; R.S.H., T.S., J.I.F., G.M.S., and M.Y. analyzed data; and R.S.H., J.I.F., G.M.S., and C.A.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: Data have been deposited at the National Center for Biotechnology Information under accession codes HM_153803 (*amhy* cDNA) and DQ_441594 (*amha* cDNA).

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Juan Fernandino (primero a la derecha) con miembros del Laboratorio de Ictiofisiología y Acuicultura, 2003

Costillar en mi casa
Agasajo a la Dra. Valerie Langlois segunda a la izquierda; Juan Fernandino, primero a la derecha, 2012

Juan Fernandino (segundo desde la izquierda) con el Dr. Pablo Strobl (centro) y otros miembros del Laboratorio de Biología del Desarrollo, 2013

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