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Review

Molecular mechanisms of sex determination and evolution of the Y-chromosome: Insights from the medakafish (*Oryzias latipes*)

Amaury Herpin[a], Manfred Schartl[b,*]

[a] *University of Würzburg, Physiological Chemistry I, Biozentrum, Am Hubland, D-97074 Würzburg, Germany*
[b] *University of Würzburg, Rudolf-Virchow-Center for Experimental Biomedicine (DFG Research Center), Verbacher Str. 9, D-97078 Würzburg, Germany*

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ABSTRACT

Fish exhibit a striking variety of sex determination mechanisms and sex chromosome structures, differing sometimes even between closely related species. Therefore fish are not only interesting objects to study the molecular mechanisms of sex determination operating in a species of interest but also provide models to better understand the evolution of this process. The review will mainly focus on one species, the medaka (*Oryzias latipes*), a small laboratory fish model species originating from freshwater biotopes of Japan and Korea, because the most advanced knowledge of genetic sex determination in a non-mammalian species has been obtained in this species. The master male sex-determining factor (*dmrt1bY*) has been isolated and identified as a duplicate of an autosomal gene that is known to function at a most downstream position of the sex-determining regulatory gene cascade from worms and flies up to mammals. In medaka, the entire male-specific region of the Y-chromosome and adjacent pseudo-autosomal regions are sequenced. The Y-chromosome is only 5–10 million years old. This allows to infer the molecular events that have shaped the medaka Y and to evaluate this against the predictions of the common theory of evolution of sex chromosomes. The molecular mechanisms how *dmrt1bY* initiates male development are only beginning to be understood, but it is apparent that the *dmrt1bY* gene functions by inhibiting male primordial germ cell proliferation at the sex-determining stage.

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* Corresponding author. Tel.: +49 931 888 4148; fax: +49 931 888 4150.
E-mail address: phch1@biozentrum.uni-wuerzburg.de (M. Schartl).

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1. Introduction

Sex determination (SD), the developmental process that determines whether the bipotential gonad anlage will differentiate towards a testes or an ovary is one of the most plastic processes in evolutionary developmental biology. The trigger for this process may be environmental (ESD) or genetic (GSD), and within each type there is a multitude of mechanisms how to spark the process of male or female development. Fish show an amazing variety of sex determination mechanisms and there is hardly anything that cannot be found in this group of organisms. Although some information is emerging about sex determination in lampreys, sharks, rays and sturgeons, by far most of our knowledge stems from studies on teleost fish. Therefore, this review will concentrate on this group of fishes, and especially one species, the medaka, where even the master sex-determining gene is known.

A considerable number of teleost species are hermaphrodites, switching either from first being males (protandrous) to become females, or the other way round (protogynous). There is even one species, *Kryptolebias marmoratus*, which is a simultaneous hermaphrodite with a well-developed ovotestes and where the eggs are fertilized inside the gonad. In the sequential hermaphrodites several mechanism have been described that initiate the switch from one sex to the other and these vary between species. Social factors, size and age are the most common ones.

The majority of teleost fish species are gonochoristic, meaning that they exist as males and females. It is generally believed that temperature-dependent sex determination (TSD) is the most primitive and ancestral mechanism of SD in vertebrates. However, confirmed examples of TSD are rare in fish and appear to be a derived state (Ospina-Alvarez and Piferrer, 2008). Other physiological mechanisms of ESD have not been described. This might point to the fact that most gonochoristic species have GSD. However, one has to confess that only a minor fraction of the approximately 25 000 species of fish has been thoroughly investigated and their sex determination mechanism unequivocally clarified (Devlin and Nagahama, 2002a).

Within the GSD species all variations of the theme have been found, ranging from male or female heterogamety to polygenic sex determination. Importantly, multiple sex chromosomes, e.g. different Y-chromosomes, or autosomal modifiers that enhance or antagonize the sex-determining genes on the gonosomes are quite frequent. This together with the fact that even between closely related fish species the sex determination mechanisms can be different (Devlin and Nagahama, 2002b; Volff and Schartl, 2001) demonstrates the high evolutionary plasticity of this fundamental process.

This poses several questions. At first we would like to know if the underlying molecular mechanisms and the major sex determination genes are always the same but just switch their chromosomal location or dosage dependent activity or acquire different responsiveness to external cues. Alternatively the molecular mechanisms may be different and evolving independently in different species. In several studies it was described that many genes that were previously identified in man, mouse or chicken to be involved in sex determination have orthologues in fish and show expression patterns consistent with a comparable role in fish (Devlin and Nagahama, 2002a). It should be noted, however, that the master sex-determining gene of mammals, *Sry*, has not been found outside the eutherians (see Wilhelm et al., 2007 for review). A more general question asks for the reason of this plasticity in fish, which is in stark contrast to the situation in birds and mammals where almost the entire class has a genetic sex determination of the ZZ/WZ or XX/XY type, respectively. There is also some plasticity with respect to Sry in mammals (lack or multiple copies in some rodents), this is far from the variability observed in teleost fish. Hence, fish are an attractive group of organisms to study the evolution of sex determination in general and of sex chromosomes in particular.

A gene family involved in sex differentiation in organisms as phylogenetically divergent as *Caenorhabditis elegans*, *Drosophila*, corals, fish, frogs, reptiles, birds and mammals, are the DM gene orthologs (Hodgkin, 2002). Members of this family belong to a known putative transcription factor clade sharing a conserved DNA-binding motif, the so-called DM (*D*oublesex and *M*ab-3 homologous) domain. In humans, mice, chickens, alligators, and turtles, expression of the *dmrt1* gene was detected in the developing gonads, and at higher levels in testes compared with ovaries, usually in the late sex-determining or early testis-differentiation period (Winkler et al., 2004; Raymond et al., 1999a; Smith et al., 1999; Kettlewell et al., 2000; Marchand et al., 2000; Kobayashi et al., 2004; Aoyama et al., 2003). Interestingly, it was shown that the DM intertwined zinc finger-like DNA binding module domains from nematodes, fly or mammals select very similar DNA sequences (Murphy et al., 2007).

In *Drosophila* and *C. elegans* the orthologous protein products regulate in a sex specific manner the transcription of genes involved in establishing the male and female phenotype (An and Wensink, 1995; Yi and Zarkower, 1999). Much less is known for the orthologous *dmrt1* genes from vertebrates. Their importance, however, for sex determination becomes apparent not only from their male development associated expression pattern but also from the facts that in birds *dmrt1* is present on the Z, but absent from the W (Shetty et al., 2002; Nanda et al., 1999), that in humans haploinsufficiency for *dmrt1* is connected to male to female sex reversal (Raymond et al., 1999b), that in *Xenopus laevis* a truncated but functional duplicate is encoded by the W-chromosome (Yoshimoto et al., 2008) and last not least from findings in medaka, which will be outlined in detail in this review.

2. Sex determination in medaka

The medaka, *Oryzias latipes*, is a small freshwater fish species that lives in the small rivers and rice fields of East Asia. During the last decade it became a widely used laboratory fish for developmental and biomedical research, comparable to the well-known zebrafish with which the medaka shares many advantages and characteristics (Wittbrodt et al., 2002). With respect to sex determination, the medaka is certainly the best understood fish species. It has a XX/XY sex determination system with undifferentiated (homomorphic) sex chromosomes (Matsuda et al., 2002; Nanda et al., 2002). Already in the twenties of the last century, the medaka was the first vertebrate, where sex chromosomal inheritance and sex chromosomal crossovers were described (Aida, 1921). More recently, the male sex-determining gene was identified (Matsuda et al., 2002; Nanda et al., 2002), making the medaka so far the only vertebrate species outside mammals, where such a gene has been cloned and shown to be necessary and sufficient for directing testes development.

The medaka male sex-determining gene is a duplicated version of the *dmrt1* gene. The duplicated copy was designated *dmrt1bY* or *dmY*. The ancestral copy, which is located on an autosome (linkage groups), consequently is named *dmrt1a*. The duplicated fragment, consisting of dmrt1 and the three neighbouring genes, was inserted into another chromosome, which became the Y-chromosome, while its homologue became the X (Fig. 1). The duplication event occurred approximately 5–10 million years ago in the lineage leading to the medaka and a sister species, *O. curvinotus* (Matsuda et al., 2002; Kondo et al., 2004). Compared to the human Y, which is more than 200 million years old, the medaka Y is very young and allows to study the initial events of Y-chromosome evolution.