Aquatic Toxicology 191 (2017) 141–163

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aqtox

Review

Zebrafish sex differentiation and gonad development: A review on the impact of environmental factors

Dércia Santos[1], Ana Luzio*[,1], Ana M. Coimbra*

Centre for the Research and Technology of Agro-Environmental and Biological Sciences, CITAB, Life Sciences and Environment School, University of Trás-os-Montes and Alto Douro, Departamento de Biologia e Ambiente (DeBA), University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

ARTICLE INFO

Keywords:
Genetic sex determination
Environmental sex determination
EDCs
Temperature
17α-ethinylestradiol

ABSTRACT

Zebrafish (*Danio rerio*) is extensively used in research; however the mechanisms that control this species sex determination are still poorly understood. In the latest decades, it has been established that zebrafish sex is determined by genetic factors on a polygenic basis, as various candidate genes with sex dimorphic expression, as well sex-linked loci have been identified in different zebrafish strains. However, it has been evidenced that sex determination in this species is also influenced by environmental factors. For instance, temperature can have a crucial role in zebrafish sex determination. Likewise, the exposure to endocrine disrupting chemicals (EDCs), the most studied zebrafish sex changing factor, can strongly influence the course of sex differentiation and unbalance the sex ratio of zebrafish populations. Despite this, so far the influence of environmental factors is still less understood and only few studies have addressed this topic.

Therefore, this review intends to gather current knowledge on the environmental factors involved in sex determination of zebrafish and identify important gaps in this research area. Briefly, the current understanding on zebrafish sex related genetics is also addressed.

1. Introduction

Vertebrates exhibit a multitude of sex determination mechanisms, which are key events in the differentiation of gonads into testis or ovary (Devlin and Nagahama, 2002; Wilhelm et al., 2007). These mechanisms range from systems under strict genetic control – genetic sex determination (GSD) – to systems that are dependent on environmental factors – environmental sex determination (ESD) (Budd et al., 2015; Bull, 1985). The GSD can either have a monogenic or a polygenic basis, while in ESD environmental factors play a crucial role (Devlin and Nagahama, 2002; Godwin, 2003).

In mammals, sex is determined genetically through the XX/XY system, being males the heterogametic sex (Wilhelm et al., 2007); while in birds, it is through the ZZ/ZW system, being females the heterogametic sex (Chue and Smith, 2011). Reptiles may display either genetic or temperature-dependent sex determination (Modi and Crews, 2005). But, among vertebrates, fish are the ones that show the higher variety of sex determination mechanisms, including GSD (XX/XY, ZZ/ZW, polygenic or autosomal), ESD (temperature, pH, population density) or even GSD-ESD interactions (genotype/environment interactions) (reviewed in Baroiller et al., 1999; Devlin and Nagahama, 2002). Moreover, fish show several patterns of sexual differentiation, ranging from hermaphroditic to differentiated or undifferentiated gonochoristic species (reviewed in Devlin and Nagahama, 2002; Yamamoto, 1969). This multiplicity of mechanisms suggests that fish sexual development is a labile process and that the evolution of sex determination mechanisms between fish and higher vertebrates are substantially different (Devlin and Nagahama, 2002).

Zebrafish (*Danio rerio*; family Cyprinidae), a small freshwater teleost, is a widely used animal model in development biology, ecotoxicology, cancer and neuroscience research (Chew et al., 2014; Hortopan and Baraban, 2011; Huang et al., 2015; Kimmel et al., 2001; Levin et al., 2007; Scholz et al., 2008). As a model organism, zebrafish has several advantages, such as a short generation time, small size, high fecundity, *ex utero* and transparent embryonic development and a high genetic homology to humans (Hill et al., 2005; Segner, 2009). It is also a cost-effective species from the technical and methodological points of view (Segner, 2009). Although zebrafish has many advantages as a model, the mechanism controlling its sex differentiation and determination remains very complex, which does not allow the exploitation of the full potential of this species as a model organism.

Given zebrafish importance to research, the present work overviews

---

* Corresponding authors.

E-mail addresses: aluzio@utad.pt (A. Luzio), acoimbra@utad.pt (A.M. Coimbra).

[1] Dércia Santos and Ana Luzio contributed equally to this work.

http://dx.doi.org/10.1016/j.aquatox.2017.08.005
Received 10 August 2016; Received in revised form 26 July 2017; Accepted 7 August 2017

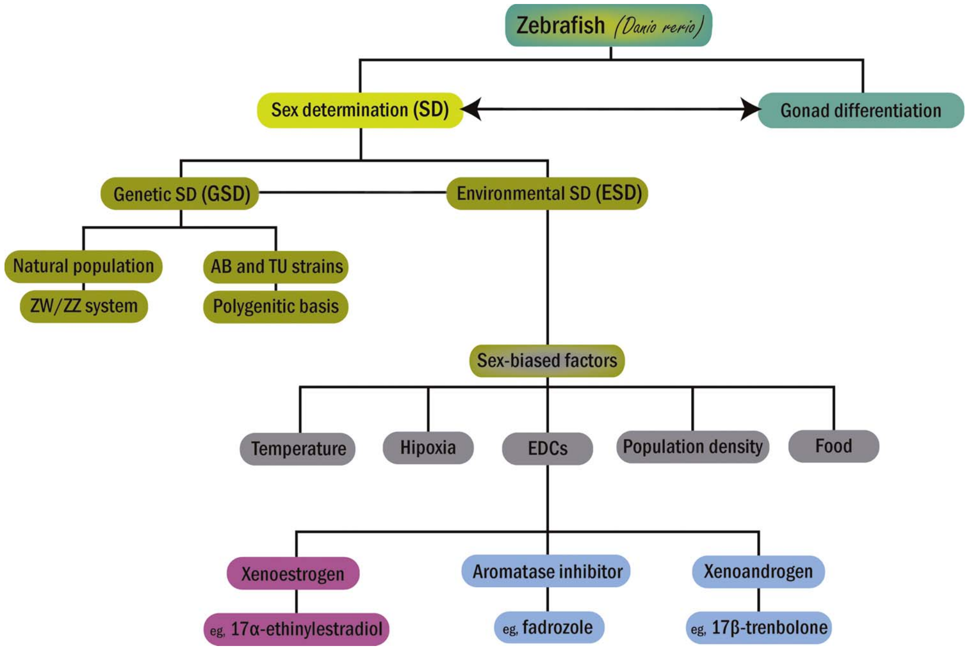

Fig. 1. Schematic representation contextualizing current knowledge of zebrafish sex determination and gonad differentiation.

the current data on this species' sex determination and gonad differentiation. In recent years, it has been proposed that in zebrafish sex is determined by genetic factors (GSD) (Anderson et al., 2012; Bradley et al., 2011; Jørgensen et al., 2008; Liew et al., 2012; Luzio et al., 2015a; Tong et al., 2010), which are secondarily influenced by environmental factors (ESD) (Abozaid et al., 2012; Lawrence et al., 2008; Luzio et al., 2016a; Uchida et al., 2004). The ESD mechanisms have been the less understood and explored. Therefore, this review will focus primarily on ESD and in the main environmental factors that modulate zebrafish sex determination and differentiation. The GSD mechanisms involved in zebrafish sex determination will only be mentioned briefly, since they have been comprehensively reviewed by other authors (Liew and Orban, 2014; Nagabhushana and Mishra, 2016; von Hofsten and Olsson, 2005). A schematic representation of current knowledge on zebrafish sex determination (SD) and gonad differentiation is presented in Fig. 1. Furthermore, several questions associated with zebrafish sex determination will be discussed.

2. Main features of zebrafish gonad differentiation

Zebrafish is classified as an undifferentiated gonochoristic species (Yamamoto, 1969). In this species, the ovarian development is considered to be the default pathway (von Hofsten and Olsson, 2005) and it has been described that all individuals, independently of the genetic background, develop initially an immature ovarian tissue during early life (Uchida et al., 2002), prior to the final differentiation on mature ovaries or testes (Maack and Segner, 2003; Takahashi, 1977; Wang et al., 2007a,b). In general, the process of gonad differentiation begins around 25 days post-fertilization (dpf) (Takahashi, 1977; Uchida et al., 2002) and is completed at 60 dpf (Takahashi, 1977).

However, in recent years, a few studies have questioned the established concept that all males go through the "juvenile ovary-to-testis" transformation process (Hsiao and Tsai, 2003; Luzio et al., 2015b, 2016a,b; Wang et al., 2007a,b), by reporting the existence of two processes of gonad development in male zebrafish. In fact, these works established the existence of three types of males based on the fluorescence emission of the β-actin:EGFP and *vas:egfp* in transgenic zebrafish lines (see Fig. 5 in Hsiao and Tsai, 2003 and Wang et al., 2007a,b) and on a point count strereological approach of the gonads during the differentiation period (see Fig. 3 in Luzio et al., 2015b, 2016a,b). Therefore, males were classified as: "type III" which were the ones showing the highest expression of EGFP, and going through the transformation of the "juvenile ovary" into testis and are only distinguished from females by showing much smaller gonads with larger ovarian cavities; "type II" that presented intermediated fluorescence and that also go through the "juvenile ovary" stage but already evidence male germ line cells; and "type I" that do not express EGFP and only present one or two oocytes at least in one histological section, showing no evidences of a "juvenile ovary" ever being formed (Wang et al., 2007a,b; Luzio et al., 2015b, 2016a,b).

The "juvenile ovary-to-testis" transformation process has been suggested to depend on apoptotic pathways (Luzio et al., 2016b; Rodríguez-Marí et al., 2010, 2011; Shive et al., 2010; Sun et al., 2013; Uchida et al., 2002). In zebrafish, apoptosis has been described as having a crucial role in both testicular and ovarian differentiation. In fact, apoptosis inhibition is crucial to maintain oocyte survival in the female phenotype and essential to the ovary development (Dranow et al., 2013; Luzio et al., 2016b; Pradhan et al., 2012; Uchida et al., 2002). Oppositely, apoptosis occurence is an important contributor to the failure of ovarian development, inducing its transformation into testes (Rodríguez-Marí et al., 2010; Rodríguez-Marí et al., 2011; Shive et al., 2010; Tzung et al., 2015; Uchida et al., 2002). Uchida et al. (2002) showed that oocyte apoptosis could be the mechanism involved in the testicular and ovarian differentiation in zebrafish. These authors identified apoptotic gonadal cells in males, between 23 and 35 days post-hatching (Uchida et al., 2002). Subsequently, Rodríguez-Marí et al. (2010) reported that mutations in the *fanconi anemia complementation group L* (*fancl*) gene induce germ cell apoptosis and consequently the masculinization of zebrafish gonads. These authors observed that the loss of oocytes in *fancl* mutants was accompanied by an abnormal increase of caspase-3-mediated apoptosis. Remarkably, the introduction of a mutation in the *tumor protein p53* (*tp53*) gene in the *fancl* mutants rescued the sex-reversal phenotype by reducing germ cell apoptosis (Rodríguez-Marí et al., 2010). Therefore, these findings support the hypothesis that apoptosis is an important feature of male gonad development (Uchida et al., 2002). More recently, Sun et al. (2013) proposed a novel mechanism, involving the MAPK-sox9/p53 pathway, to explain how the degenerative oocytes undergo apoptosis and how the formation of testis during the juvenile ovary-testis transition occurs. These authors suggested that the *SRY (sex determining region Y)-box 9a gene* (*sox9a*) axis signaling induces the transition of zebrafish "juvenile ovary" into testis, through the increase of the nuclear *sox9a* and *p53*