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Review

Sex determination in amphibians

Masahisa Nakamura*

Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, 2-2 Wakamatsu, Shinjuku-ku, Tokyo 162-8480, Japan

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ABSTRACT

The heterogametic sex is male in all mammals, whereas it is female in almost all birds. By contrast, there are two heterogametic types (XX/XY and ZZ/ZW) for genetic sex determination in amphibians. Though the original heterogametic sex was female in amphibians, the two heterogametic types were probably interchangeable, suggesting that sex chromosomes evolved several times in this lineage. Indeed, the frog *Rana rugosa* has the XX/XY and ZZ/ZW sex-determining systems within a single species, depending on the local population in Japan. The XY and ZW geographic forms with differentiated sex chromosomes probably have a common origin as undifferentiated sex chromosomes resulted from the hybridization between the primary populations of West Japan and Kanto forms. It is clear that the sex chromosomes are still undergoing evolution in this species group. Regardless of the presence of a sex-determining gene in amphibians, the gonadal sex of some species can be changed by sex steroids. Namely, sex steroids can induce the sex reversal, with estrogens inducing the male-to-female sex reversal, whereas androgens have the opposite effect. In *R. rugosa*, gonadal activity of CYP19 (P450 aromatase) is correlated with the feminization of gonads. Of particular interest is that high levels of *CYP19* expression are observed in indifferent gonads at time before sex determination. Increases in the expression of *CYP19* in female gonads and *CYP17* (P450 17α-hydroxylase/C17-20 lyase) in male gonads suggest that the former plays an important role in phenotypic female determination, whereas the latter is needed for male determination. Thus, steroids could be the key factor for sex determination in *R. rugosa*. In addition to the role of sex steroids in gonadal sex determination in this species, *Foxl2* and *Sox3* are capable of promoting *CYP19* expression. Since both the genes are autosomal, another factor up-regulating *CYP19* expression must be recruited. The factor, which may be located on the X or W chromosome, intervenes directly or indirectly, in the transcriptional regulation of the *CYP19* gene for feminization in amphibians. A factor up-regulating *CYP17* expression remains to be identified.

Contents

* Tel.: +81 3 5369 7307; fax: +81 3 5369 7307.
E-mail address: nakamra@waseda.jp.

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1. Introduction

In vertebrate species sex is determined by either chromosomal factors (genetic sex determination, GSD) or environmental factors (environmental sex determination, ESD), or some combination thereof. In humans, for instance, the sex of an individual is determined by combination of sex chromosomes, so-called XY sex-determining system. In some species of reptiles, on the other hand, the sex ratio is determined by an environmental factor, in particular, by incubation temperature of the egg (see Ramsey and Crews and Shoemaker and Crews, this issue). In amphibians, the sex of larvae reared at ambient temperature is generally determined by GSD; an individual with XX or ZW chromosomes becomes female, whereas one with XY or ZZ becomes male. In this case, the sex ratio is 1 male: 1 female. However, at extreme warm and cold temperatures compatible with normal development, sex differentiation is perturbed in one genotypic sex and the sex is biased to male or female. How do genetic and environmental factors determine the sex of amphibians? What is the difference at the molecular level between the two systems? Here I review the available literature on the mechanisms of sex determination in amphibians.

2. Genetic sex determination

2.1. Sex-determining mechanisms

Most amphibians do not exhibit morphologically distinguishable sex chromosomes, but sex is generally determined at fertilization of zygotic sex chromosome composition. A sex-determining mechanism in amphibians had been successfully demonstrated by sex reversal and breeding experiments. Humphrey [1–3] first demonstrated a sex-determining mechanism of the salamanders, *Ambystoma mexicanum* and *A. tigrinum* by using non-cytogenetic methods or transplantation of the primordium of a testis or an ovary. Consequently, it was found that these two species display the ZZ/ZW type sex-determining system. A similar experiment was carried out in the toad *Bufo bufo* [4]. The Bidder's organ, a tissue unique to toads, is the incompletely involuted cortex of the embryonic gonad and is located in the anterior part of the testis of the sexually mature male. This tissue is compared to a rudimentary ovary. After the removal of a testis of an adult toad, the Bidder's organ develops into a functional ovary. When such a sex-reversed male is mated with a normal male, only male progeny are obtained, indicating that the male *Bufo* is homogametic (ZZ) and the female heterogametic (ZW). In the frog *Xenopus laevis*, all tadpoles develop as functional females when they are exposed to estradiol-17β [5]. Half of the female individuals produces only male progeny when they are crossed with normal males, which means that *X. laevis* has the ZZ/ZW sex-determining system. The type (XX/XY or ZZ/ZW) of sex-determining mechanism in many amphibian species is reported [6]. It should also be noted that the sex-determining system of six Japanese and one Korean species in anurans has been determined [7]. In these studies gynogenetic diploids were abundantly produced and allowed to develop up to 1 month after completion of metamorphosis. As a result, all species showed that males are heterogametic whereas females are homogametic; frog species examined were *Rana nigromaculata*, *R. brevipoda*, *R. tsushimensis*, *R. rugosa*, *Hyla arborea japonica* and *Bonbina orientalis* [7]. In the anurans, the sex-determining system was further determined in 24 species. Five species such as *X. laevis*, *Pyxicephalus adspersus*, *Tomopterna delalandii*, *B. bufo*, and *Leiopelma hamiltoni* display the ZZ/ZW sex-determining system. In the urodela, on the other hand, only seven species (*A. mexicanum*, *A. tigrinum*, *A. laterale*, *Pleurodeles watlii*, *P. poireti*, *Aneides ferreus*, and *Chiropterotriton dimidiatus*) have female heterogamety. Most of species in the urodele (salamander) amphibians have the XX/XY sex-determining system [6].

2.2. Evolution of sex chromosomes

All mammals are male heterogametic, whereas all birds are female heterogametic. However, unlike mammals and birds, amphibians possess two types of the sex-determining systems and their sex chromosomes have evolved several times in amphibians. In the ancestral anuran Leiopelmatidae, females are the heterogametic sex [8,9]. Male heterogamety had evolved several times, but a reversion to female heterogamety from a male heterogametic lineage had occurred only once (*Chiropterotriton dimidatus* in Plethodontidae in urodele) [9]. It is important to note that the ancestral amphibian Z chromosome does not share a common ancestor with the Z chromosome of snakes and birds [10]. Mammalian XY and reptilian ZW systems have evolved independently from a common ancestor [11]. Further, the X/Y sex chromosomes of humans do not share any genes with the Z/W chromosomes of birds [12].

Interestingly, the two heterogametic types exist in the three taxonomic groups or fish, amphibians, and reptiles and are shared by closely related species [11]. Both the heterogametic sexes are not always evolutionarily stable, since there is only a tiny genetic difference between them, which means that they are changeable. In birds and mammals, the two types of heterogamety (the Z/W in birds and the X/Y in mammals) are completely different and evolutionarily stable. Heterogametic sex has not been changed as far as highly degenerated W or Y chromosome is concerned, because WW or YY embryos are lethal. In the platyfish (*Xiphophorus maculates*), which has X, Y, and W chromosomes, the male is basically heterogametic (XX/XY), but the W chromosome derived from the X is added. An individual having X, Y, and W chromosomes is female, as the W dominates the Y [13]. In the lemming (*Myopus schisticolor* and *Dicrostonyx torquatus*), XY females are fertile, as a female determiner of X chromosomes, designated X*, dominate a male one of Y [14]. Therefore, heterogametic sex in non-amniote vertebrates does not appear to be fixed permanently.

Heterogametic sex can vary even between populations of a single species, although a species in amphibians has either the XX/XY or the ZZ/ZW type sex-determining system. For example, the pond frog (*R. rugosa*) living in Japan is quite unique. This frog has both male and female heterogametic systems, depending on the local population [15,16]. Populations of *R. rugosa* are divided into four genetic forms, depending on four geographical regions; West Japan, Central Japan, Kanto area and Northwest Japan. Populations living in the former three areas have male heterogamety in the sex determination, whereas frogs in Northwest Japan display female heterogamety [15]. The populations of West Japan and Kanto area have undifferentiated and homomorphic chromosomes in both the sexes, but that in the Central Japan has differentiated sex chromosomes, which are the seventh-largest in the 13 pairs of the chromosomal complement. The population in the Northwest Japan has female heterogamety (ZZ/ZW) with differentiated