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Retinoic acid homeostasis regulates meiotic entry in developing anuran gonads and in Bidder's organ through Raldh2 and Cyp26b1 proteins

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ABSTRACT

The vitamin A (retinol) and its metabolites such as retinoic acid (RA) affect vertebrate gametogenesis. The level of RA in cells relies on the balance between its synthesis and degradation. The sex-dependent equilibrium is reached in different ways in various species. It is known that RA induces meiosis in developing gonads in mouse, chicken and urodel amphibians, but its role in anuran amphibians has not been studied. Here we show in six anuran species (*Xenopus laevis*, *Bombina bombina*, *Hyla arborea*, *Bufo viridis*, *Rana arvalis* and *Rana temporaria*) that cultured undifferentiated gonads were insensitive to RA treatment, but the RA induced ectopic meiosis in cultured larval testes. In larval testes of all studied species, the exogenous RA induced leptotene phase of I meiotic prophase in gonia, but only in *H. arborea* and *B. viridis* gonia progressed to zygotene phase. In the cultured developing ovaries, exogenous RA led to increase in the number of oocytes as compared to the control. Inhibition of either RA synthesis or RA-receptors prevented meiotic entry in larval gonads of all species. Exogenous RA rescued this inhibitory effect demonstrating that the balance in RA homeostasis plays a key role in meiotic entry in anuran gonads. The localization of two enzymes, Raldh2 and Cyp26b1, which antagonistically control RA levels and whose abundance suggests the sites of RA synthesis and degradation respectively, showed two distinct expression patterns specific for (i) *X. laevis*, *H. arborea*, *R. arvalis*, *R. temporaria* and (ii) *B. bombina*, *B. viridis*. Thus, RA, in correlation with specific expression patterns of Raldh2 and Cyp26b, induces meiosis during gonad development in anurans. In addition, in *B. viridis*, RA signalling seems important for development of the Bidder's organ containing oocytes both in males and females.

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1. Introduction

Germ cells are unique due to their ability to giving rise to gametes of both sexes. This divergence depends on the commitment to either spermatogenic or oogenic pathway of differentiation during the larval or foetal period. Thus, the somatic environment of gonad determines the fate of germ cells (Evans et al., 1977; McLaren, 1975, 1981; Palmer and Burgoyne 1991; Adams and McLaren, 2002). The key step of the commitment to oogenesis involves induction of the pre-meiotic DNA repair and the entry into prophase of the first meiotic division (Bowles and Koopman, 2010). In mammalian female foetus all existing oogonia enter meiosis and develop into diplotene oocytes (Bukovsky et al., 2005). It was thought that in amphibians subpopulation of oogonia enters meiosis every consecutive year (Witschi, 1929; Bukovsky et al., 2005), however, a recent study suggests that at least in *Rana temporaria* the stockpile of oocytes is established once for a life span as in mammals (Ogielska et al., 2013). In contrast to females, the commitment to spermatogenesis involves avoidance of meiotic entry during foetal or larval life, until puberty (Bowles and Koopman, 2010). In consequence meiotic entry in male gonads is dramatically delayed in comparison to female gonads. For instance in the mouse, meiosis is initiated at 13.5 dpc in ovaries and around a week after birth in testes (McLaren, 1984, 2003). Thus, it has been postulated that foetal ovaries produce a meiosis-initiating substance (MIS) and foetal testes produce meiosis-preventing substance (MPS) (reviewed by Kocer et al., 2009). Organ co-culture studies showed that murine foetal ovaries initiated meiosis when cultured in the presence of mesonephros indicating that in mice the mesonephros is the source of MIS (Byskov, 1974; Baker et al., 1976). However, surprisingly both the male and female mesonephroi have the ability to induce meiosis specifically in the ovary. This suggests that murine foetal testes produce a substance preventing meiotic entry (Byskov and Saxen, 1976; Evans et al., 1982; Dolci and DeFelici, 1990). Even less is known about these processes in amphibian gonads, beyond the fact that in all studied amphibians, the first oocytes appear in developing ovaries before metamorphosis, whereas in the testes spermatocytes appear usually after metamorphosis (Kobayashi and Iwasawa, 1988; Ogielska, 2009; Piprek et al., 2010).

It appeared that *Stra8* (stimulated by retinoic acid gene 8) is up-regulated in germ cells starting from 12.5 dpc during female mouse development, i.e. right before meiotic entry (Baltus et al., 2006; Menke et al., 2003; Oulad-Abdelghani et al., 1996). Moreover, Cyp26b1, the enzyme degrading RA, is expressed in the mouse male gonads at 12.5 dpc, i.e. at the time when male germ cells lose their ability to transdifferentiate into female germ cells (Bowles et al., 2006; Koubova et al., 2006). These data indicate the key role of RA in meiosis entry in mice.

In the mouse, RA is synthesized from retinol by three retinaldehyde dehydrogenases (Raldh1, Raldh2, Raldh3; Rhinn and Dollé, 2012). Raldh1 and Raldh3 play roles mainly in the development of ectodermal origin organs such as brain and eyes, while Raldh2 is broadly distributed in developing mesodermal tissues, thus also in the gonads. The degradation of RA depends on three P450 cytochromes (Cyp26A1, Cyp26B1, Cyp26C1). However, Cyp26B1 being expressed in mouse fetal urogenital system seems to be the key enzyme in RA degradation in the gonads (Abu-Abed et al., 2001; Yashiro et al., 2004; Bowles et al., 2006; Koubova et al., 2006; Uehara et al., 2007; Kashimada et al., 2011).

The origin of RA in the urogenital system differs in various species. In mouse RA is produced in mesonephroi and it diffuses to the gonads. However, the meiotic entry is triggered exclusively in ovaries. In testes, RA is degraded, most probably in Sertoli cells and Leydig cells, and this action prevents meiotic entry (Bowles et al., 2006; Koubova et al., 2006; Kashimada et al., 2011). In humans, RA is produced directly in foetal gonads (Childs et al., 2011). Also in birds RA is synthesized in the gonads (in the ovarian cortex as well as in the testis cords) and it is degraded by Sertoli cells (Smith et al., 2008). This indicates that the regulation of the meiotic onset varies among the vertebrates and may differ even within related clades (e.g. in mouse and human). Anura is an interesting old group, whose diversification time has begun about 240 MYE (Roelants et al., 2007), containing highly divergent branches. Additionally, in anurans the establishment of germ cell lineage occurs via preformed germ plasm in contrast to urodels and mammals in which intercellular signals are crucial in this process (Extavour and Akam, 2003). Considering the long time evolution of anurans, we chose six divergent species to investigate the variety of mechanisms of meiotic entry: *Bombina bombina* (Bombinatoridae), *Xenopus laevis* (Pipidae), *Bufo viridis* (Bufonidae), *Hyla arborea* (Hylidae), *Rana arvalis* and *Rana temporaria* (Ranidae) (Schmid and Steinlein, 2001; Roelants et al., 2007), which differ in their heterogametic status (both XX/XY and ZZ/ZW) (Fig. 1). *X. laevis* is a sub-Saharan African species and the rest of studied species are mostly European. *B. bombina* and *X. laevis* are representative of more primitive anurans (Archaeobatrachia), whereas Bufonidae, Hylidae and Ranidae belong to more derived anurans – Neobatrachia (Fig. 1). Eventual diversity in molecular control of gonadogenesis may reflect a high time of divergence among anuran amphibians. We also analyzed the localization pattern and level of Raldh2 and Cyp26B1 expression (which indicate sites of RA synthesis and degradation) in developing Bidder's organs in *B. viridis*. The Bidder's organs are unique

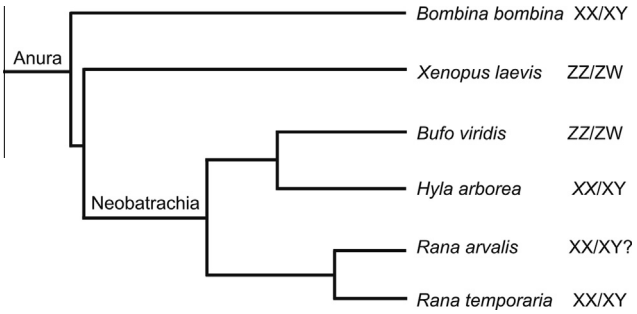

Fig. 1 – Phylogenic tree of studied anuran species and their sex chromosome status (on the basis of Schmid and Steinlein, 2001; Roelants et al., 2007).