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Xenopus laevis oocyte maturation is affected by metal chlorides

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ABSTRACT

Few studies have been conducted using *Xenopus laevis* germ cells as oocytes, though these cells offer many advantages allowing both electrophysiological studies and morphological examination.

Our aim was to investigate the effects of metal (cadmium, lead, cobalt and zinc) exposures using cell biology approaches. First, cell survival was evaluated with both phenotypical and electrophysiological approaches. Secondly, the effect of metals on oocyte maturation was assessed with morphological observations and electrophysiological recordings.

From survival experiments, our results showed that metal chlorides did not affect cell morphology but strongly depolarized *X. laevis* oocyte resting potential. In addition, cadmium chloride was able to inhibit progesterone-induced oocyte maturation. By contrast, zinc, but also to a lesser extent cadmium, cobalt and lead, were able to enhance spontaneous oocyte maturation in the absence of progesterone stimulation. Finally, electrophysiological recordings revealed that some metal chlorides (lead, cadmium) exposures could disturb calcium signaling in *X. laevis* oocyte by modifying calcium-activated chloride currents. Our results demonstrated the high sensitivity of *X. laevis* oocytes toward exogenous metals such as lead and cadmium. In addition, the cellular events recorded might have a predictive value of effects occurring later on the ability of oocytes to be fertilized. Together, these results suggest a potential use of this cellular lab model as a tool for ecotoxicological assessment of contaminated fresh waters.

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1. Introduction

Among animal models used in biological research areas, amphibians play a prominent part. They have been regarded for decades as key species allowing to gather new insights and to unravel original concepts, which were extended to other animal models. One cannot doubt that *Xenopus*, or other amphibian species, have been since the mid-fifties models for developmental biology. Amphibians were first privileged by pioneer embryologists such as Hans Spemann, Nobel prized in 1935 for its work on the organizing effect of a cellular group during embryogenesis. Moreover, Nobel Prized Sir John Gurdon performed the first nuclear transfer with success in 1958 (Gurdon et al., 1958), opening a road driving to Dolly's birth after nuclear transplantation. At the same time, in the end of the sixties, beginning of the seventies, the existence of a cytoplasmic activity responsible for the onset of M-phase was discovered in amphibian (Brachet et al., 1975; Masui and Markert, 1971), laterly explored in other model, such as starfish (Kishimoto et al., 1984). Namely M-Phase Promoting Factor (MPF, characterized in 1988 in *Xenopus* oocytes (Lohka et al., 1988), was rapidly found be a universal key factor for division, not restricted to amphibian.

As prototypic lab animals, amphibians exhibit advantages that include a well-characterized physiology, tolerance to temperature and oxygen variations, and a greater resemblance to humans than many other animal models (Brown, 2004; Ferrell, 1999; Schultz and Dawson, 2003). Such assertion is especially relevant in physiological research and in evolutionary biology, but also in environmental studies (Burggren and Warburton, 2007). Thus, observations concerning growth, development, reproduction and physiology might be gathered easily and could be applied in aquatic ecotoxicology. In this respect, and by being aquatic throughout all

Abbreviations: FETAX, Frog Embryo Teratogenesis Assay Xenopus; GVBD, germinal vesicle breakdown; ICl, calcium-activated chloride currents; ICl1-S, calcium-activated chloride current enhanced by calcium release from internal stores; ICl1-T, calcium-activated chloride current enhanced by calcium release and calcium entry; ICl2, calcium-activated chloride current enhanced by calcium entry from external medium; MPF, M-phase Promoting Factor; MS222, tricaine methane sulfonate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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its embryonic and post-embryonic life, *Xenopus laevis* is one of the most studied amphibians. This species is strictly dependent upon aquatic media (Hillman, 1980), and its embryos and larvae are directly exposed to freshwater environmental micropollutants. *X. laevis* is considered as a valuable sentinel species to evaluate environmental health (Collins and Storfer, 2003). Surprisingly, few studies were undertaken on this amphibian, though *Xenopus* tadpoles and adults are strictly aquatic living animals. Thus, adults' tissues including ovaries and testis, eggs and embryos could be indirectly or directly exposed to environmental pollutants. Most studies with *X. laevis* have been developed for many years with embryos to assess early developmental toxicity of environmental pollutants (Frog Embryo Teratogenesis Assay—*Xenopus*, FETAX) (ATSM, 1998).

X. laevis oocyte has been used for decades as a model for studying calcium signaling (Delisle, 1991; DeLisle and Welsh, 1992; Marin, 2012; Marin et al., 2010), cell cycle transitions (Bodart et al., 2002; Heikkila et al., 2007; Machaca, 2007). However, this model has been seldom used in toxicological studies in spite of the advantages provided by different species of *Xenopus* genus (Khokha, 2012; Schultz and Dawson, 2003). Forty years ago, Dumont described *X. laevis* oogenesis (Dumont, 1972). According to his classification, six stages (I to VI) may be distinguished, which are related to vitellogenesis. Fully-grown Stage VI oocytes have a diameter ranging from 1 to 1.3 mm. Referred as immatures, these oocytes are blocked at prophase of meiosis I, in a G2-like state. The release from the block at prophase I can be experimentally triggered by progesterone, mimicking the steroid stimulation provided by follicular cells *in vivo*. Then, the immature oocytes resume meiosis and a typical white spot appears at the top of the animal pole. This white spot is relative to the germinal vesicle breakdown (GVBD) and the migration of nuclear material to the apex of the cell. Oocytes exhibiting a white spot are often referred as mature oocytes. These oocytes progress from meiosis I until metaphase of meiosis II. Thus, mature oocytes are arrested in a second block, at metaphase of meiosis II, waiting for fertilization (Nebreda and Ferby, 2000). It is to note that meiotic resumption is analogous to the transition from G2-like state to a M-phase, and is therefore considered as an M-phase entry. At the molecular level, the meiotic resumption is enabled by the activation of a universal factor, the MPF (M-Phase Promoting Factor), which is activated by Cdc25, a pivotal enzyme in the regulation of M-phase progression (Nebreda and Ferby, 2000).

This hormonal-dependent maturation corresponds to morphologic events (appearance of a typical white spot at the top of the animal pole, relative to the GVBD and migration of nuclear material to the apex of the cell), and biochemical events (initiation of signaling pathways leading to the activation of the MPF). During this maturation (corresponding to G2/M transition), the oocyte also undergoes ionic changes, including calcium fluxes: calcium release from internal stores of endoplasmic reticulum and/or calcium entry from the outside of the cell. With electrophysiological approach, calcium sources/fluxes could be followed, in particular by recording calcium-activated chloride currents (also called ICl currents) which develop in order to block polyspermy during fertilization.

Many characteristics specific to the *X. laevis* oocyte make it an excellent experimental system, especially of interest for aquatic ecotoxicology. The large size of oocytes provides ease of amenability for manipulations varying from electrophysiology to micro-injection, large amounts of proteins facilitating biochemical studies, a system for heterologous expression and a model where cell cycle regulation mechanisms are evolutionary conserved. Easy to dissect manually, oocyte can be used to carry out various assays within a single cell (Cailliau and Browaeys-Poly, 2009; Weber, 1999). Noteworthy, fertilization can be easily reproduced *in vitro*, and external development offers opportunity to test the direct effects of various compounds.

In this first work, we chose metals as representative of freshwater contaminants. Indeed, it is well documented that in the Northern Hemisphere all the water bodies are contaminated with metals such as mercury, cadmium and lead due to long-range atmospheric transport and deposition from anthropic sources (Naimo, 1995). Contaminated environments (water bodies, ditches, ponds) are used by amphibians. Besides, studies on non-point sources revealed that urban storm water and highway runoffs are a major source of pollutants. Metals were found in this order: zinc > lead ≈ copper > cadmium (Davis et al., 2001).

Here, we intended to use *X. laevis* oocytes to evaluate the effect of metal chlorides on cells survival and on their ability to undergo into M-phase. In one hand, healthy oocytes were evaluated using both a phenotypic approach and electrophysiological recordings; in the other hand, M-phase entry was assessed by phenotypical examination of G2/M transition and by measuring associated calcium-dependent chloride channels.

2. Materials and methods

2.1. Reagents and test substances

All compounds were of molecular biology grade of purity. They were obtained from Sigma–Aldrich Chimie (Saint-Quentin Fallavier, France). All tested solutions and media (ND96: 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES-NaOH, pH 7.5) were freshly daily prepared or obtained extempore by appropriate dilutions of metal chloride stock solutions in ND96 medium. Metal salts were of 99.9% grade of purity (Sigma–Aldrich Chimie, Saint-Quentin Fallavier, France).

2.2. Handling of oocytes

The use of living organisms was conducted in accordance to the protocols submitted and approved by the French national guidelines for animal welfare. *X. laevis* females were anesthetized by immersion in 1 g L^{-1} tricaine methane sulfonate (MS222) solution for 45 min. An ovariectomy was performed: clusters of oocytes were surgically removed and placed in ND96 medium. Oocytes were isolated first, by a collagenase treatment (1 mg mL^{-1} of collagenase in ND96) for 45 min then, by a manual dissociation with tweezers under a binocular microscope. Only the stages V and VI oocytes (suitable for maturation, see Fig. 1) were defolliculated and used for all the experiments.

2.3. Metal chloride exposures for meiosis resumption

Oocytes were exposed overnight at 19 °C to metal chloride solutions: cadmium, zinc, lead, or cobalt (Table 1). Maturation was tested in presence of progesterone (4 μg mL^{-1}). The M-phase entry was associated with the appearance of the white spot. In standard conditions, *Xenopus* oocyte maturation occurs in about 12 h. Such experiment allowed obtaining M-phase entry percentage as a function of metal concentrations. Oocytes were placed in metal chloride solutions in 24-wells plates (10 oocytes per test well and 15 for the control one, in triplicates). For each metal, the following concentrations were tested (metal chloride concentrations): 25 mg L^{-1}, 12.5 mg L^{-1}, 2.5 mg L^{-1}, 1 mg L^{-1}, 250 μg L^{-1} and 25 μg L^{-1}. In order to choose the concentrations to be tested, we looked for the EC50 (embryo-toxicity) of metal ions in the FETAX test. The most toxic metal ion (with the lowest reported EC50) was cadmium (1.6 mg L^{-1}) followed by zinc (55.6 mg L^{-1}), lead (96.1 mg L^{-1}) and cobalt (613 mg L^{-1}) (Gungordu et al., 2012;

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