In situ Elemental Analysis of Frog Eggs and Oocytes by LA-ICP-MS

490-8P

Hannigan, R., Machaca, K., and Sun, L.; Arkansas State University Dept. of Chemistry, State University AR 72467; hannigan@astate.edu; Machaca@astate.edu

ABSTRACT
In order to quantify metal concentrations in the oocytes we used a matrix matched standard, NIST 1946 (Lake Superior Fish Tissue). This tissue is freeze-dried and kept at -80°C until used. The solid frozen tissue was used as a matrix to calibrate the LA-ICP-MS. Our data indicate that this method provides unique insights into the ionic changes occurring during oocyte maturation.

INTRODUCTION
LA-ICP-MS is routinely used for the determination of major, minor, and trace element concentrations in solid media. More and more this technique is applied for studies of spatial variability in the chemistry of biodiverse tissues such as fish ear stones (Histid, Campagna, 2005), corals (e.g., Sweet and Grottoli, 2004), and coralline algae (Cadrin et al., 2000). More recently the capabilities of LA-ICP-MS to spatially resolve chemical variability in soft biological tissues has been explored (e.g., Harden and Harwood, 2005; Campana, 2005; Legrand et al., 2004). The development of LA-ICP-MS for the study of elemental variations in soft biological tissues has been limited by the need for the biological community to be able to quantify the concentrations of the analytes of interest. In many cases the relative abundances of metals provided little information regarding fundamental biological processes.

The purpose of this study was to develop an LA-ICP-MS method that would provide quantitative information regarding the concentration of Ca, Cu, and Zn to allow the elucidation of cell cycle progression during oocyte maturation.

RESULTS
In order to quantify metal concentrations in the oocytes we used a matrix matched standard, NIST 1946 (Lake Superior Fish Tissue). This tissue is freeze-dried and kept at -80°C until used. The solid frozen tissue was used as a matrix to calibrate the LA-ICP-MS. Our data indicate that this method provides unique insights into the ionic changes occurring during oocyte maturation.

SIGNIFICANCE
Cell cycle progression is essential for the development of multicellular organisms and when disrupted leads to serious consequences such as cancer. Transition metals are essential for cellular survival as they are structural elements of many important proteins, yet their concentration needs to be carefully controlled because they are toxic at high doses (Vallee and ,$^{2+}$, 2000). Rotella and Bolis (2000) argue that Cu and Zn are dynamic and that these metals play no other structural roles. Cellular concentrations of these metals are low and are difficult to measure using traditional solution based atomic spectroscopic methods. Much more is known about Ca signaling due to the higher cellular abundance of this metal and the development of fluorescent dyes and detection systems (Tsien, 1981; Poenie et al., 1985; Grynkiewicz et al., 1985).

Cellular free transition metal concentrations are undetectable experimentally arguing that these metals are bound to their target proteins or chelated and transported by carrier proteins. Given that Cu and Zn are known to play a dynamic role in regulation it is critical that the cellular distribution of these metals be quantified.

METHODS
Taking advantage of the large size of Xenopus oocytes (Figure 1), we developed a high sensitive and reproducible method that allows us to map the transition metal content at the subcellular level over time. We analyzed the animal and vegetal poles of unfertilized eggs and stimulated oocytes using a Cl- ICP-MS (PerkinElmer) and a 266 nm Nd:YAG laser ablation system (Cetac Technologies) (Figure 2). Instrument parameters are shown in Table 1.

REFERENCES


