

X-ray absorption studies on the early development of *Xenopus laevis* (frog) oocytes

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Introduction

The pervasive role of zinc in the biology and biochemistry of all living systems requires the existence of regulatory mechanisms that insure that apoproteins and enzymes receive the metal in the quantities needed and at the time required for their functions to be carried out in the broad range of zinc-dependent processes [1]. Until recently, such regulatory mechanisms have been hypothesized, but knowledge of the chemical species and processes involved is limited.

Using x-ray absorption fine structure (XAFS), we have examined the quantitative aspects of zinc metabolism in the maturation of frog oocytes and in their developing embryos [2]. The zinc site of a specific protein, lipovitellin, has been identified. Lipovitellin might play a key role in zinc nutrition and be involved in a zinc transport and distribution system. We are now trying to use a microbeam to observe the zinc signal at various stages of oocyte development. Here we report preliminary studies of zinc mapping at various stages of fertilized frog eggs.

Methods and Materials

Frogs (7 cm in length) were used in studies of zinc distribution in stage VI egg and stage I embryos. Ovulation was induced by an injection of chorionic gonadotropin into the dorsal lymph sac of the female frogs. Frog testes were carefully dissected and placed in a solution of 80% Steinberg's solution. Ovulated mature eggs were fertilized by placing them directly into the sperm suspension. After a specified number of times, they were diluted to a 20% solution with metal-free water and then placed into a 0.16 M NaOH, 2% L-cysteine solution for 2–3 min. to remove the gelatinous envelope. The embryos were then fixed with 5% glutaraldehyde, 5% ficoll (400 kDa), 25 mM HEPES, pH 7.5 for 60 min. The fixed eggs or embryos were then washed three times with metal-free water and stored in metal-free water at 4° C until used for the XAFS experiment.

X-ray absorption measurements were performed at the Bio-CAT beamline, 18-ID. The beamline was operated with a double-crystal monochromator with Si(111) crystals. The x-ray beam was focused horizontally and vertically using a sagittal focusing monochromator and a mirror, respectively. A focal spot of 0.3 mm horizontal and 0.2 mm vertical was maintained during the measurement. A PI pinhole with 10, 20, 30, and 50 micron openings was used to further restrict the beam size. The fluorescence signals were collected using a multilayer analyzer detector, which is under development at Bio-CAT. This type of detector and its prototype design have been described elsewhere [3, 4].

Results

Preliminary x-ray fluorescence imaging experiments were performed on frog eggs 40, 60, and 100 minutes into fertilization, and on nonfertilized stage V–VI eggs. The images of the frog eggs were recorded in fluorescence. The profile of the egg can be determined by transporting it in the beam and monitoring the transmission and/or scattering. Figure 1 shows the zinc concentration image for an egg 60 minutes into fertilization using a 50 micron pinhole. It is evident from the figure that zinc distributes towards one side within the oocytes after fertilization. The fluorescence image on stage V–VI egg shows a more even distribution of zinc, and the distribution on eggs 40 minutes into fertilization shows a moderately uneven zinc distribution (data not shown).

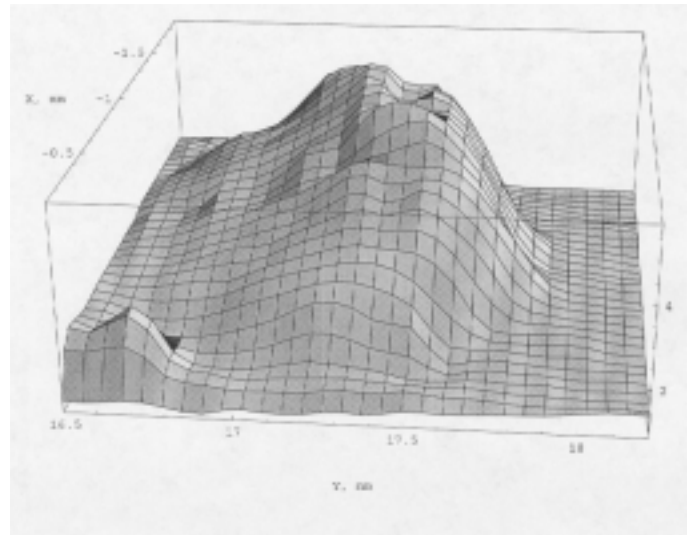


Figure 1: Zinc mapping of a frog egg 60 minutes after fertilization.

Discussion

All the eggs show a clear visual separation of the nutrient part (dark) from the cell itself (white). The redistribution is clearly from the nutrient portion of the egg to the cell that is under development. Thus, our results indicate that a zinc transport system in frog eggs starts redistributing zinc to cells after fertilization. The mechanism of the zinc redistribution is the topic of our ongoing investigations.

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