CALCIUM TRANSIENTS DURING THE FIRST AND SECOND MITOTIC DIVISIONS OF THE FERTILIZED MEDAKA EGG

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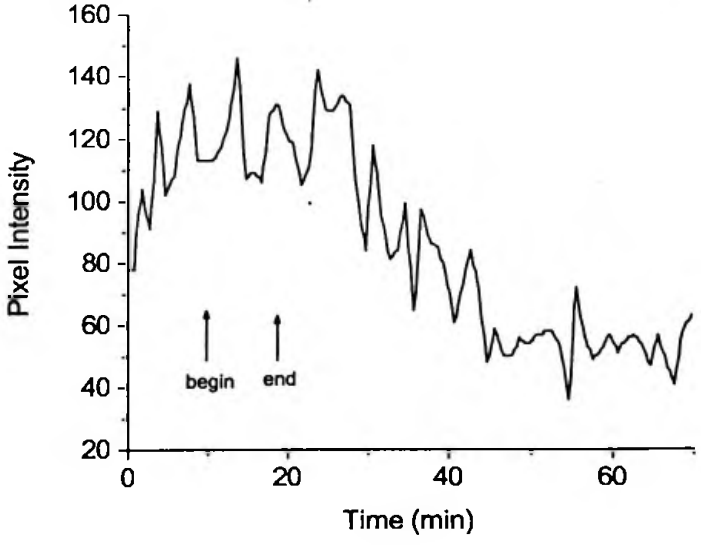
Recently, considerable evidence has emerged that calcium ions play an important role in the regulation of mitosis (e.g.; Whitaker M. Larman MG. Calcium and mitosis. *Seminars in Cell & Developmental Biology* 12, 53-58: 2001). Cell cycle changes in cytosolic free Ca were first observed in 1986 by Roger Tsien's laboratory (*Science* 233, 886-889: 1986), where it was shown that Ca rises at the onset of anaphase. These and other studies have led to the hypothesis that calcium transients regulate the progression of the cell cycle. However, the hypothesis remains incompletely investigated and not universally accepted because calcium transients are not always observed during mitosis, especially in mammalian cells in culture. To obtain more insight into the role of calcium in cell cycle, we examined calcium transients dividing eggs of the Medaka fish (*Oryzias*).

Fertilized eggs prior to the first mitotic division were collected from female Medaka fish early in the morning immediately after fertilization. The eggs were microinjected with ~ 1 nl of the fluorescent Ca-sensitive dye fluo-4 (10 mM of the free acid in Ca-free water). The microinjection pipet was pulled from 1.5 mm diameter glass capillary and injection was controlled by air pressure delivered to the back of the pipet. Injection was monitored on a fluorescent dissecting microscope. The eggs were then oriented on the stage of an Olympus confocal microscope and Fluo-4 fluorescence was imaged in a plane predicted to be perpendicular to the plane of the first cleavage furrow. Images were recorded once very minute.

The figure below plots the pixel intensity measured in a region of cytosol about 10 μm below the plasma membrane parallel to the cleavage furrow. "Begin" and "end" refer to the



onset completion of formation of the cleavage furrow. Approximately 10 min prior to the onset of the first cytokinesis (which probably corresponded to the onset of anaphase), the cytosolic Ca concentration began to rise and Ca oscillations were recorded. Initially, the oscillations were not localized to the cleavage furrow, but were global in nature. As cytokinesis progressed, the oscillations localized to the site of the cleavage furrow. Ca concentration at the cleavage furrow remained elevated for 10-15 minutes after cytokinesis was completed, but during this time Ca oscillations were smaller in amplitude. After 15 min, the Ca concentration returned to basal levels similar to those recorded at prophase.

In several cases, we were able to observe the eggs through 2 or more cycles of cell division. Interestingly, the increase in Ca at the cleavage furrow was most dramatic during the first division and was either greatly diminished or absent in subsequent divisions. This suggests that local Ca transients may play a role in the determination of the axis of embryo. This may explain why Ca transients are not always observed during the cell cycle: perhaps the Ca transients are not essential for cell cycle progression but are important in determining the axis of cytokinesis. Additional studies are planned to investigate these questions.

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