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CHARACTERIZATION OF Ca2+ INFLUX PATHWAY(S) DURING MOUSE OOCYTE MATURATION

Banyoon Cheon, *bcheon@mcb.umass.edu*

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First Advisor
RAFAEL A. FISSORE

Second Advisor
PABLO E. VISCONTI

Third Advisor
JESSE MAGER

Fourth Advisor
ALEJANDRO P. HEUCK

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Abstract
Ca^{2+} signaling induced at fertilization, also known as oscillations, is essential in mammalian eggs to initiate early embryonic development. The

generation of the oscillations relies on optimization of Ca^{2+} toolkit components during oocyte maturation. In this dissertation, we intend to deepen our understanding of how this differentiation of the Ca^{2+} toolkit, especially those components associated with Ca^{2+} influx, is achieved during maturation, and how it contributes to the filling of the ER Ca^{2+} store during the maturation and fertilization.

We first identified the expression and characterized the function of the components of the Store Operated Ca^{2+} Entry (SOCE) during oocyte maturation. We observed that SOCE underwent suppression during maturation concomitant with an increase in $[Ca^{2+}]_{ER}$content. We demonstrated that the suppression of SOCE coincided with the inability of Stim1, the Ca^{2+} sensor in ER, to form puncta near the PM, which prevented interaction with Orai1, the channel on the PM. Consistent with a possible role on Ca^{2+} homeostasis in oocytes, overexpression of Stim1 and Orai1 increased basal Ca^{2+} levels during maturation, especially during the GV stage, but this influx was suppressed in MII eggs. These results suggest that Ca^{2+} uptake during maturation is closely related to the Ca^{2+} content of the ER. Bypassing this inactivation via expression of mutant versions of Stim1 prevented oocytes from resuming meiosis.

The inactivation of SOCE was due in part to changes in Stim1 organization during maturation. We found that Stim1 reorganization was occurred largely due to CDK1. We confirmed the effect of phosphorylation by expressing several non-phosphorylated mutants of Stim1. These mutants displayed cortical location and puncta, and interact with Orai1, and enhanced Ca^{2+} influx at the MII stage.

Thus, our study also demonstrates that Ca^{2+} influx and SOCE are actively regulated during mouse oocyte maturation by the MII stage, the stage of fertilization. The suppression of SOCE relies on phosphorylations on the C-terminal end of Stim1 by CDK1.Therefore our studies show that down-regulation of Ca^{2+} influx is required for oocyte maturation and meiotic progression, although it is still unclear how the sperm manages to re-activate Ca^{2+} influx after fertilization, and what channel(s) underlie Ca^{2+} influx during fertilization. Future studies should address how this is accomplished and the channel(s) that mediate it.