

STORE OPERATED CALCIUM ENTRY ACTIVATES AT THE GVBD STAGE OF *XENOPUS* MEIOSIS

Machaca, K. and Haun, S. (2000) *J. Biol. Chem.* **275**, 38710–38715.

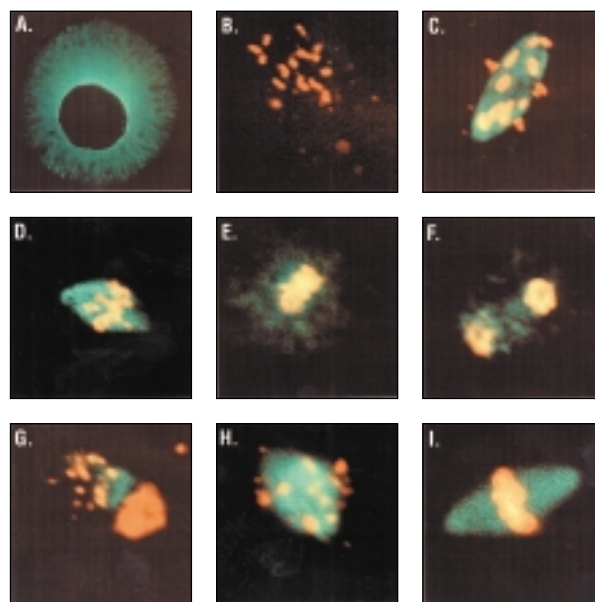
Calcium (Ca^{2+}) is a ubiquitous second messenger in biological systems and controls diverse cellular functions, including gene expression, fertilization and cellular proliferation among others. Ca^{2+} signaling is mediated through a rise in cytoplasmic Ca^{2+} either through Ca^{2+} release from intracellular Ca^{2+} stores (primarily the endoplasmic reticulum) or Ca^{2+} influx from the extracellular space. In nonexcitable cells such as oocytes, Ca^{2+} influx is predominantly through the Store Operated Ca^{2+} Entry (SOCE) pathway. We studied the regulation of SOCE during *Xenopus* oocyte maturation. Before fertilization, immature oocytes undergo meiotic maturation. During this period, immature oocytes become developmentally competent to support embryonic development following fertilization. Immature *Xenopus* oocytes are arrested at the G2-M transition of the cell cycle. During oocyte maturation, oocytes enter meiosis, complete the first meiotic division and arrest at metaphase of the second meiotic division. Meiosis resumes following fertilization. In this study we show that SOCE is inactivated during *Xenopus* oocyte maturation specifically at the germinal vesicle breakdown (prophase I) stage (GVBD). Interestingly, SOCE inactivation coincides with a dramatic increase in Maturation Promoting Factor (MPF) activity. MPF is the primary kinase that regulates entry into meiosis and is composed of the kinase catalytic subunit (p34cdc2) and a cyclin B regulatory subunit. We have measured MPF activity using Promega's SignaTECT[®] cdc2 Protein Kinase Assay System^(a), which employs a small peptide as a specific substrate for MPF activity. This assay allows rapid and convenient determination of the levels of active MPF without having to perform the more tedious "in-gel" kinase assays. Immunofluorescence studies coupled with the MPF assay show that MPF activity cycles in tandem with meiosis progression with minimal levels of activity between meiosis I and II.

The authors used the SignaTECT[®] cdc2 Protein Kinase Assay System (Cat.# V6430) to measure MPF activity in *Xenopus* oocytes.

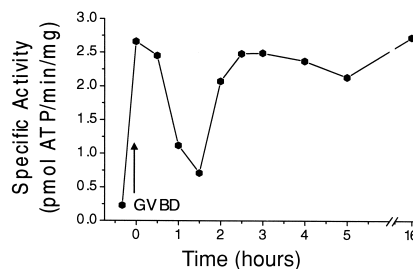
Summary by Khaled Machaca, Ph.D.
University of Arkansas for Medical Sciences

Ordering Information

Product	Size	Cat.#
SignaTECT [®] cdc2 Protein Kinase Assay System	96 reactions	V6430



Panel A



Panel B

▲ Figure 1. Panel A. Stages of meiosis. Oocytes were fixed at different time points after progesterone addition and stained with propidium iodide to visualize the chromosomes and an anti-tubulin antibody to visualize the structure of the spindle. A. Germinal vesicle intact oocytes before GVBD. B. GVBD as indicated by a white spot on the animal hemisphere. C. Prometaphase I, 30 minutes after GVBD. D. Metaphase I, 1–1.5 hours after GVBD. E. Anaphase I, 2–2.5 hours after GVBD. F. Telophase I, 2–2.5 hours after GVBD. G. End of prophase II, 2.5–3 hours after GVBD. Note the diffuse staining of the DNA in the polar body. H. Pro-metaphase II, 2.5–3 hours after GVBD. I. Metaphase II, 3–16 hours after GVBD. The scale bars are 10 μm , except for a, where it is 100 μm . Panel B. MPF Kinase activity during meiotic maturation. Time 0 refers to GVBD as indicated by the arrow. Details on protocols for fixation, staining and kinase assays can be found in Machaca, K. and Haun, S. (2000) *J. Biol. Chem.* **275**, 38710–38715. (Images reprinted with kind permission of Dr. Khaled Machaca, University of Arkansas and the American Society for Biochemistry and Molecular Biology, Inc.)

^(a)U.S. Pat. 6,066,462 and other patents pending.

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