Role of Calcium/calmodulin dependent protein kinase II on mouse oocyte maturation in vitro

Sook Young Yoon and Dawon Kang
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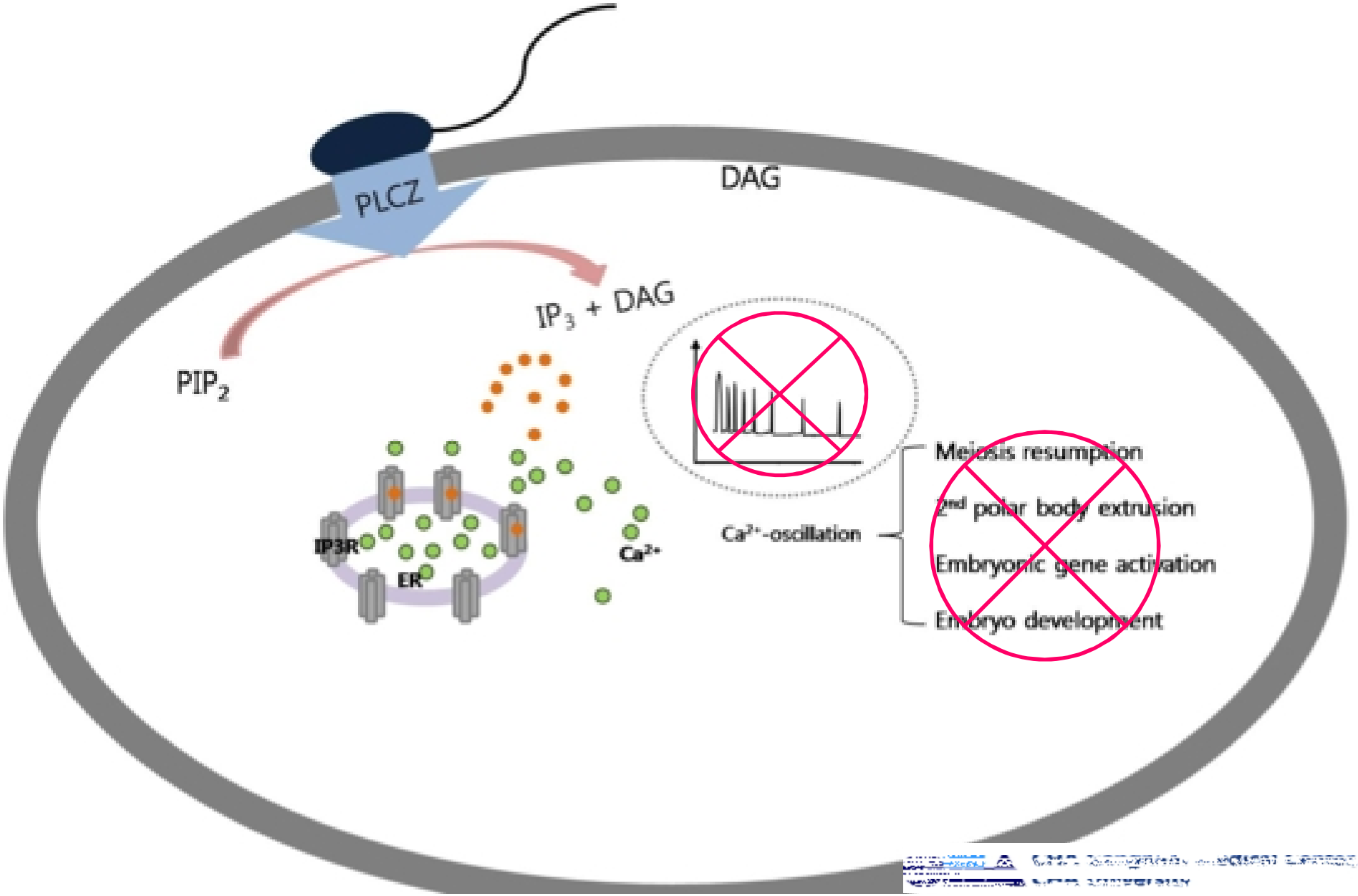
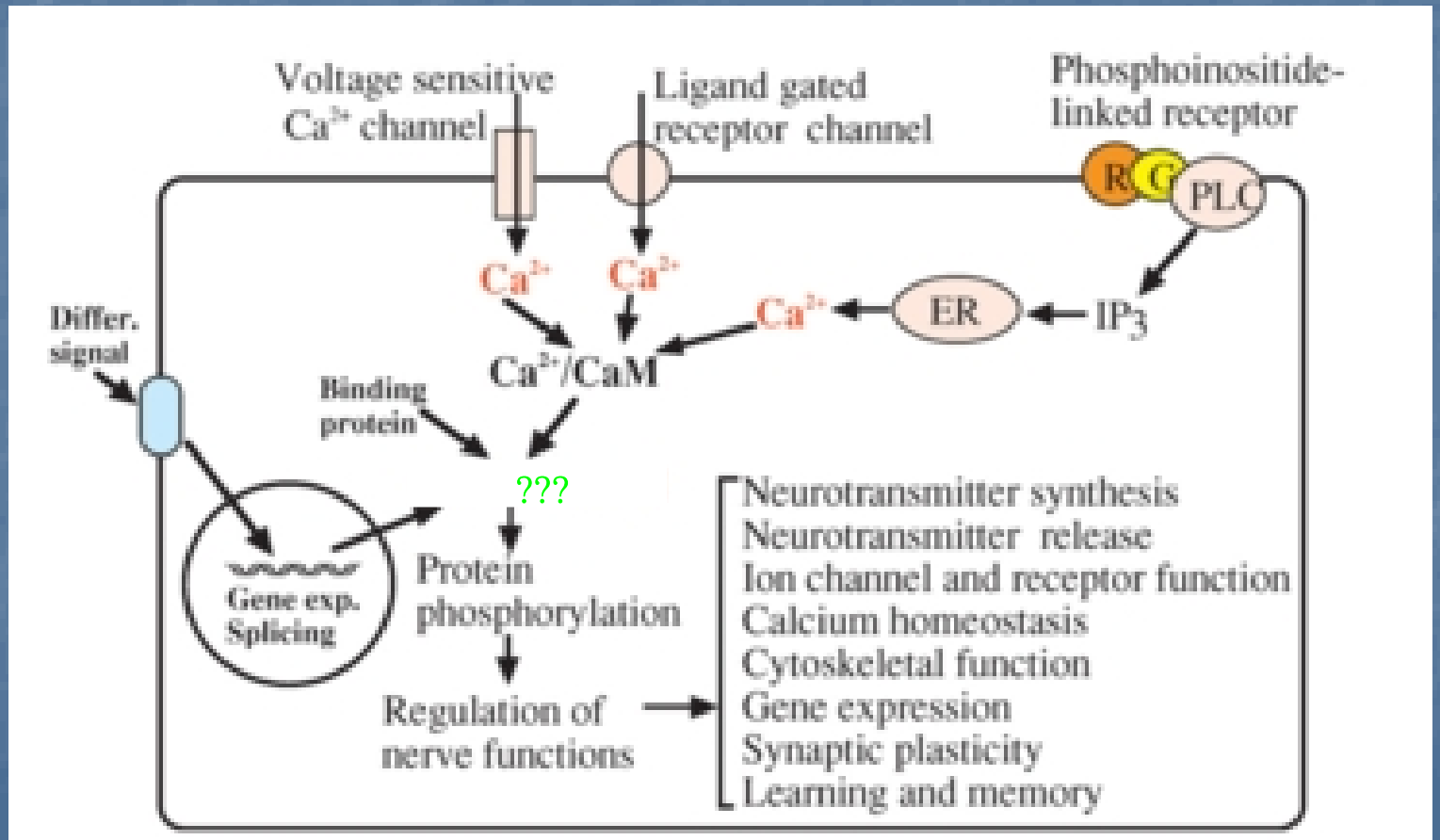
Fertilization and Ca^{2+}-oscillation



Review

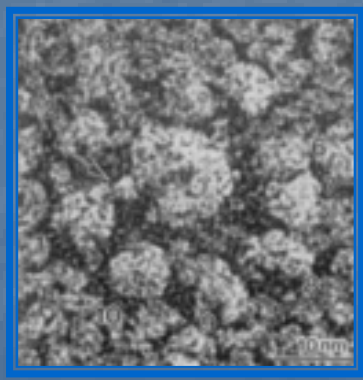
Neuronal Ca^{2+}/Calmodulin-Dependent Protein Kinase II—Discovery, Progress in a Quarter of a Century, and Perspective: Implication for Learning and Memory

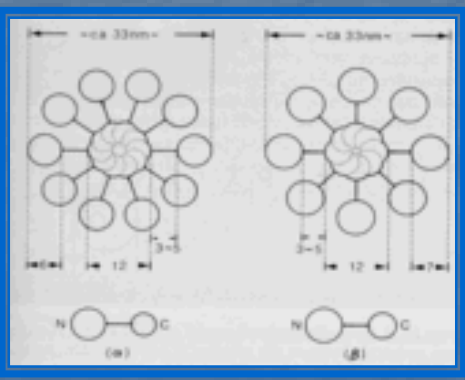


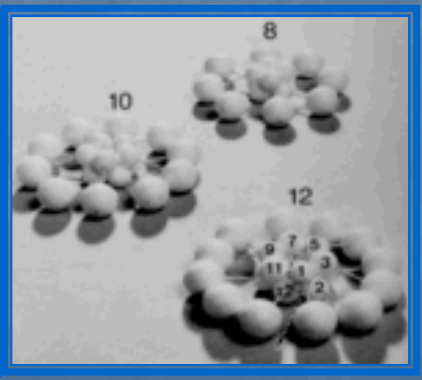
Ca^{2+}/calmodulin-dependent protein kinase II(CaM KII)

- serine/threonine-specific protein kinase
- rat brain CaM KII: α, β, β', γ and δ
- 28 different isoforms
- catalytic domain and calmodulin-binding domain: 550-560kDa

- petal-like shape: octamer and decamer







Intracellular distribution :

interphase - cytoplasm and nucleus and nucleoli

mitosis- mitotic apparatus(microtubule-organizing center)

metaphase/anaphase - centrosome and spindle

Meiosis resumption:

Xenopus oocyte / Mouse egg activation - meiotic spindle regulator

Cell cycle

G1/S phase and G2/M phase

(Tombes et al., 1995; Rasmussen and Rasmussen, 1995 Planas-Silva and Means, 1992)

Pronuclear fusion ; sea urchin eggs, *anti*-CaM KII or specific peptide inhibitor microinjection(Baitinger et al., 1990; Santella, 1998)

Annu. Rev. Biochem. 2002. 71:473–510
DOI: 10.1146/annurev.biochem.71.110601.135410

NEURONAL Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II: The Role of Structure and Autoregulation in Cellular Function

Andy Hudmon and Howard Schulman

Science
AAAS

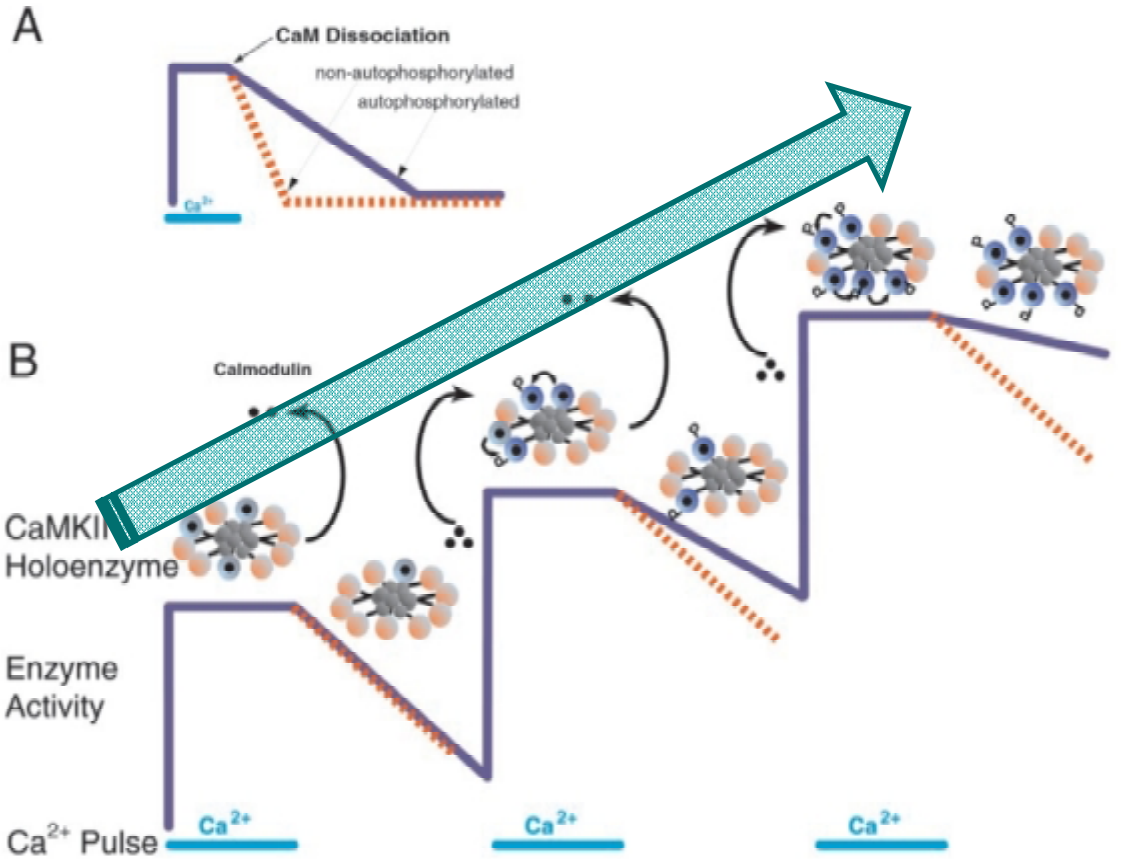
Sensitivity of CaM K
Paul De Koninck and
Science **279**, 227 (19
DOI: 10.1126/science

CaM kinase II as frequency decoder of Ca^{2+} oscillations

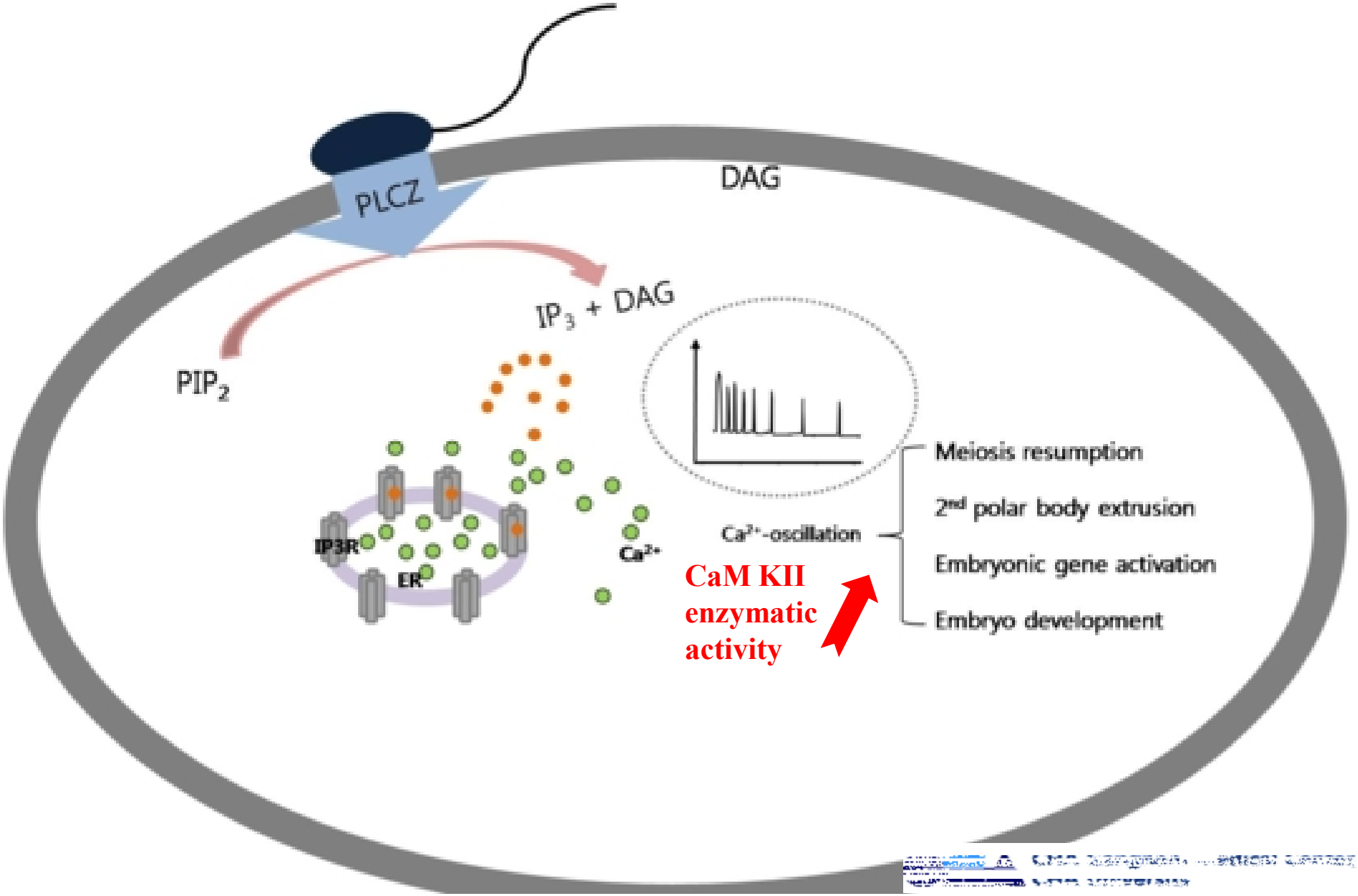
Geneviève Dupont and Albert Goldbeter

Summary

In many cell types, Ca^{2+} signals are organized in the form of repetitive spikes ... frequency of these intracellular Ca^{2+} oscillations increases with the level of stim... suggesting the existence of a frequency-encoding phenomenon. The question ... as to how the frequency of Ca^{2+} oscillations can be decoded inside the cell ... calmodulin kinase II has long been proposed as an attractive candidate, as it ... target of Ca^{2+} signals. By mimicking the Ca^{2+}/calmodulin kinase II and subject... pulses of Ca^{2+} of variable amplitude, duration, and frequency, De Koninck and ... man[1] have shown for the first time that the autonomous activity of Ca^{2+}/calm... kinase II is highly sensitive to the temporal pattern of Ca^{2+} oscillations. ...



Fertilization and Ca^{2+}-oscillation

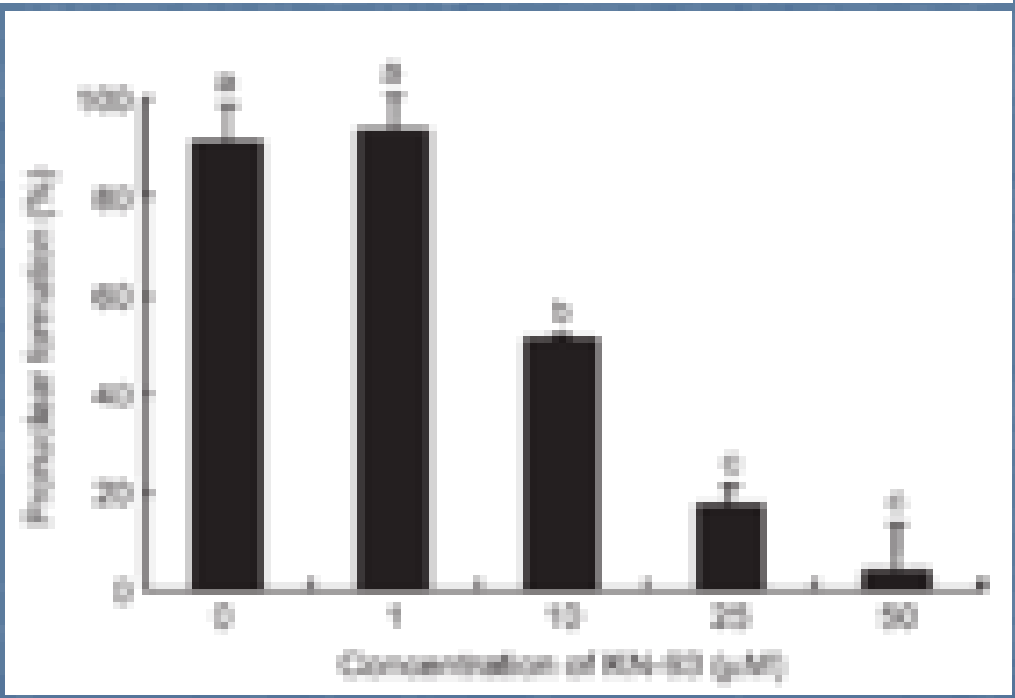


REPRODUCTION
RESEARCH

The role of calcium/calmodulin-dependent protein kinase II on the inactivation of MAP kinase and p34^{cdc2} kinase during fertilization and activation in pig oocytes

Junya Ito[1,2], Natsuko Kawano[1], Masumi Hirabayashi[2,3] and Masayuki Shimada[1]

CaM KII inhibitor, KN-93



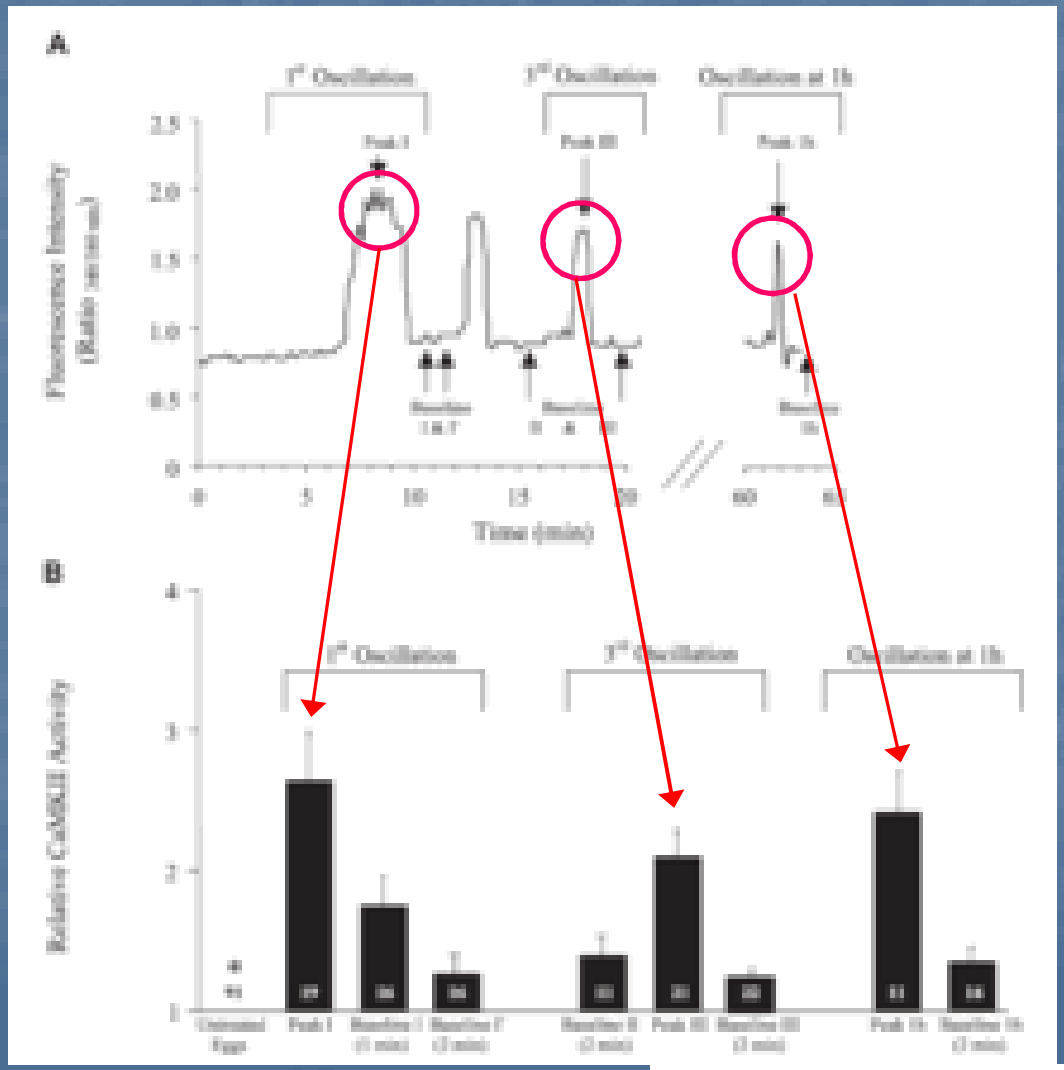
IER

Developmental Biology 272 (2004) 15–25

www.elsevier.com/loc

Fertilization stimulates long-lasting oscillations of CaMKII activity in mouse eggs

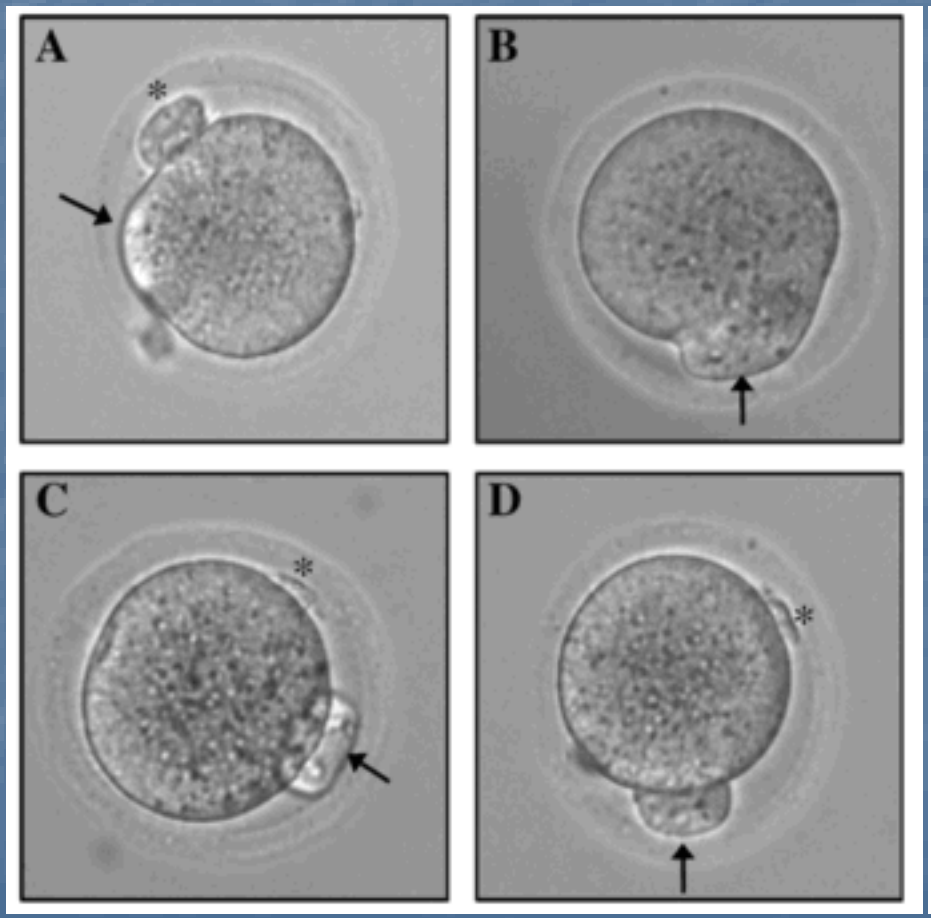
Styliani Markoulaki,[a] Sara Matson,[b] and Tom Ducibella[a,b,*]

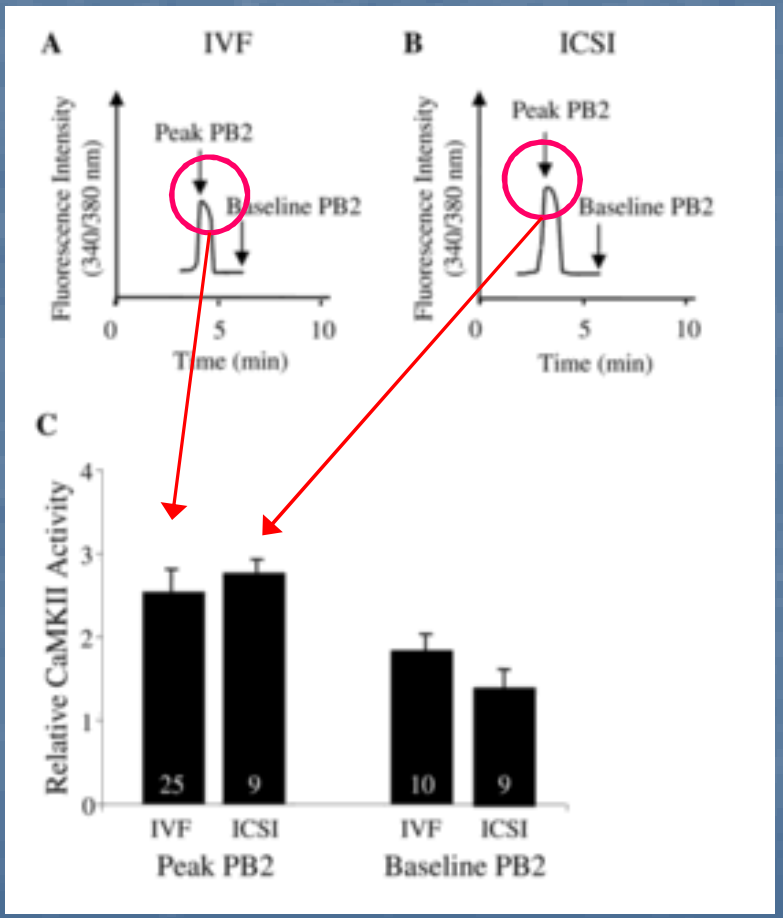


Molecular Human Reproduction Vol.13, No.4 pp. 265–272, 2007
Advance Access publication February 27, 2007
doi:10.1093/molehr/gal121

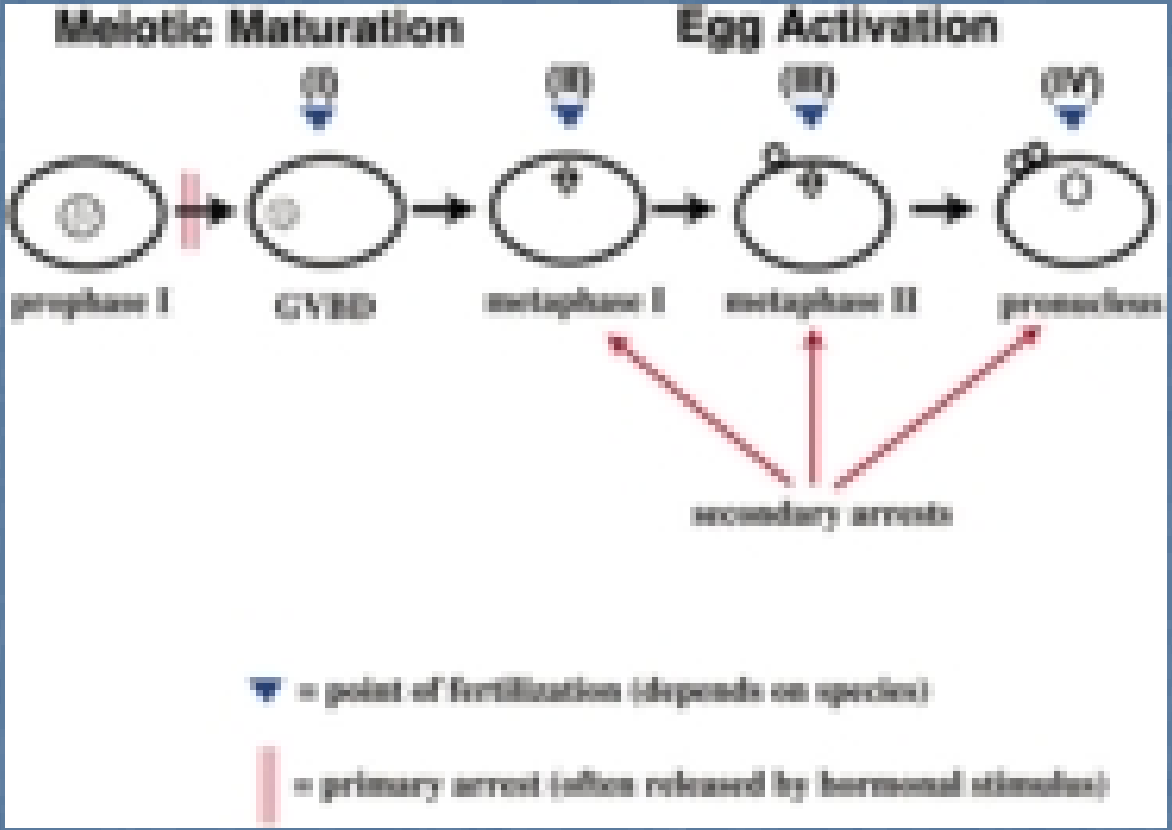
Comparison of Ca^{2+} and CaMKII responses in IVF and ICSI in the mouse

Styliani Markoulaki[1,†,‡], Manabu Kurokawa[2,†,¶], Sook-Young Yoon[2,†], Sara Matson[3], Tom Ducibella[1,3,4] and Rafael Fissore[2]





Oocyte Meiotic Maturation and Egg Activation.



The oocytes of most animal species arrest in meiotic prophase I (reviewed by Masui and Clarke, 1979; Masui, 2001)

Present studies were performed to investigate the role of CaM KII during resumption of meiotic arrest *in vitro* of mouse oocytes.

Materials and Methods

- **Animals: ICR mouse**
- **Culture condition: M16 or M2 medium**
- **$[Ca^{2+}]i$ measurement: $[Ca^{2+}]i$ indicator, fluo- 3AM,**
  confocal laser scanning microscope(CLSM)
- **CaM KII inhibitor ; KN-93, KN-92**
- **GVBD block; dbcAMP**
- **Immunohistochemisty / Western blot**
  monoclonal anti-CaM KII(α-subunit, Oncogene, USA),
  anti-tubulin,
  anti-MAPs

Results

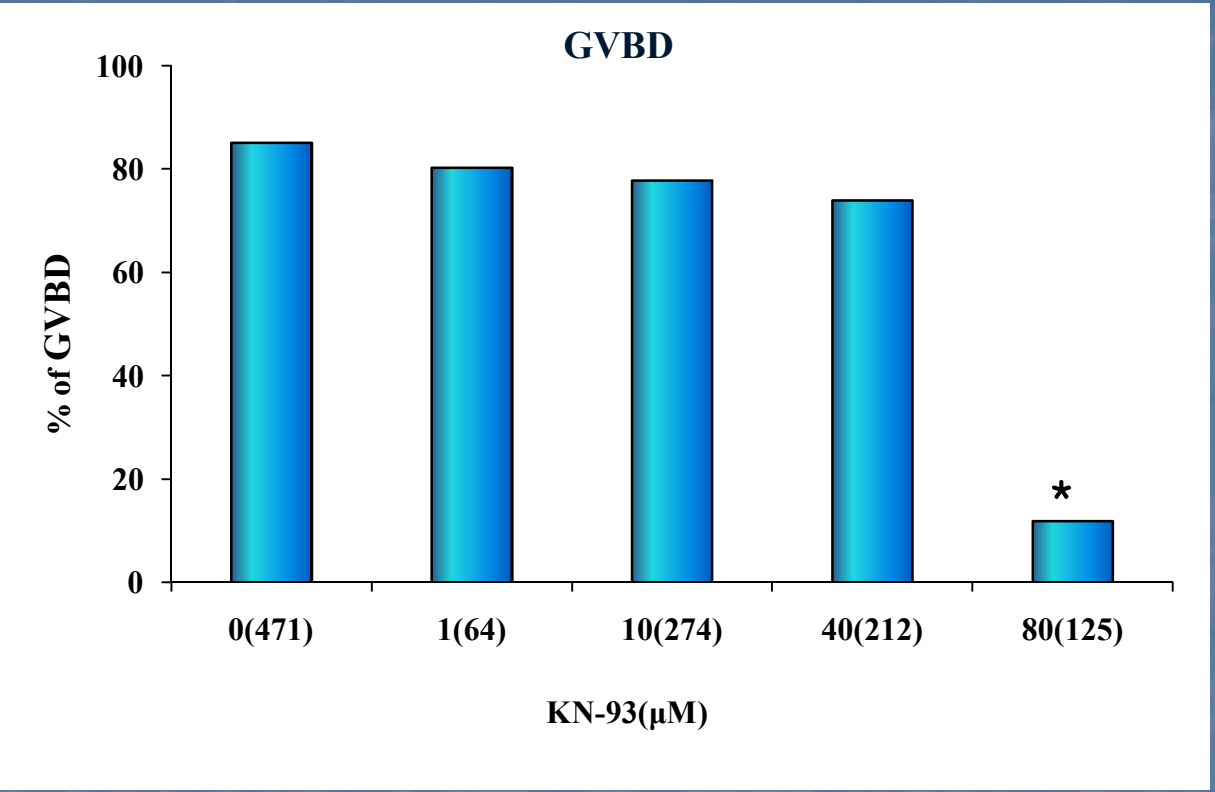


Figure 1. Effect of KN-93 on germinal vesicle breakdown of mouse GV oocytes. GVBD was assessed at 3hr after culture, respectively. The number in the parenthesis represents the total number of oocytes examined. Results were obtained by pooling the seven replicates (mean ± SEM). * significantly differ from the control, $p<0.05$.

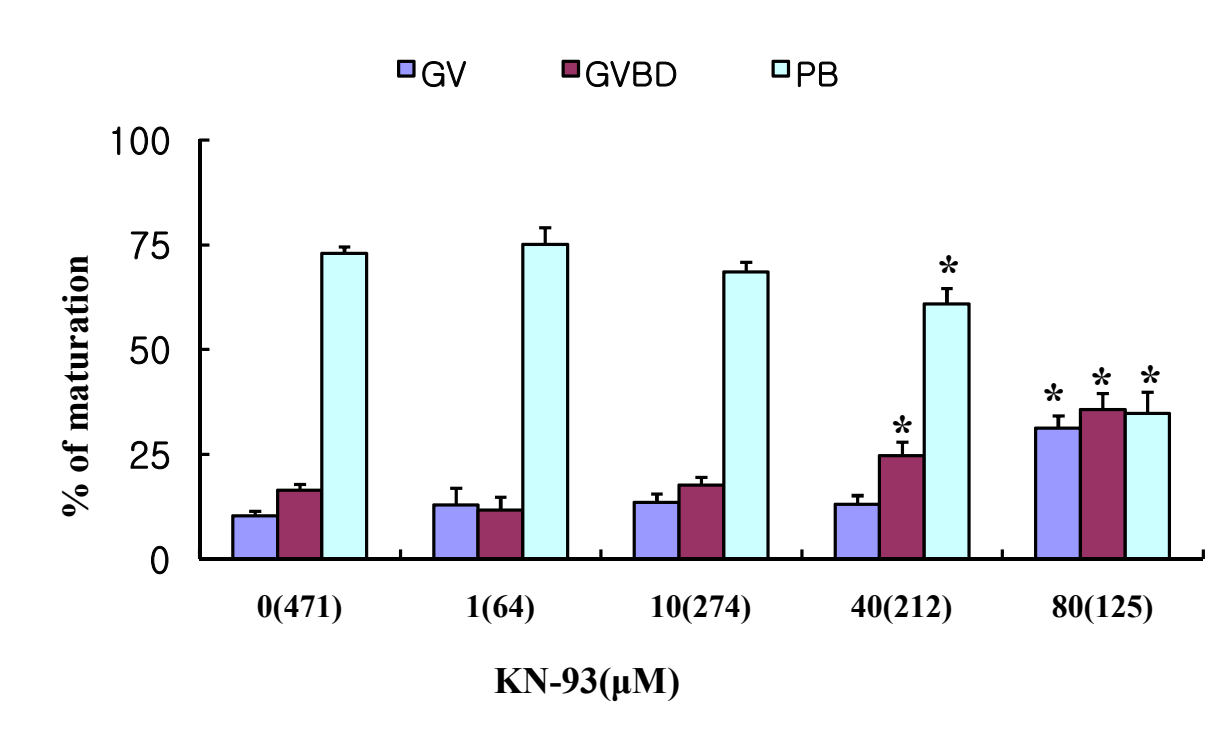


Figure 2. Effect of KN-93 on GVBD and (PB) of mouse GV oocytes. GVBD and PB was assessed at 3hr and 17hr after culture, respectively. The number in the parenthesis represents the total number of oocytes examined. Results were obtained by pooling the seven replicates (mean ± SEM). * significantly differ from the control, $p<0.05$.

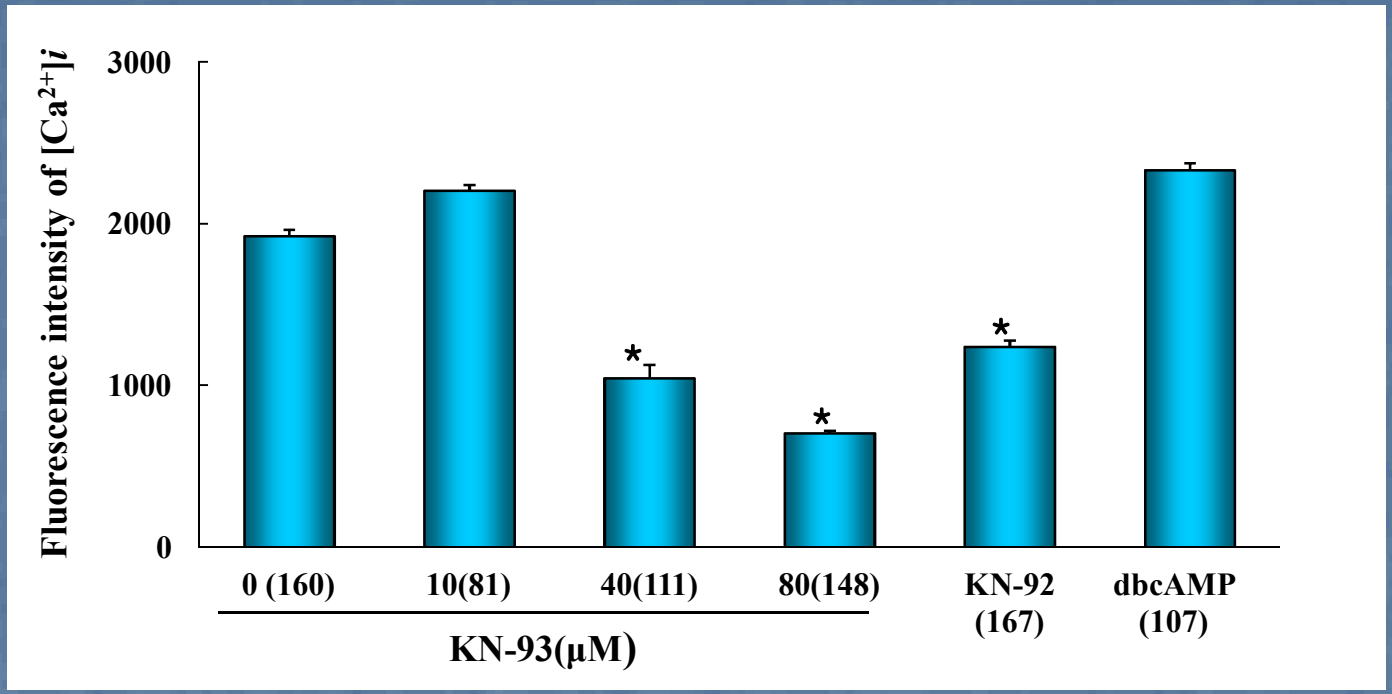


Figure 3. $[Ca^{2+}]i$ of mouse GV oocyte after treatment with various concentration of KN-93(40μM) , KN-92(40μM) or dbcAMP(200mM). The number in the bars represents the total number of oocytes examined. Results were obtained by pooling the six replicates (mean±SEM). * significantly differ from the control, $p<0.001$.

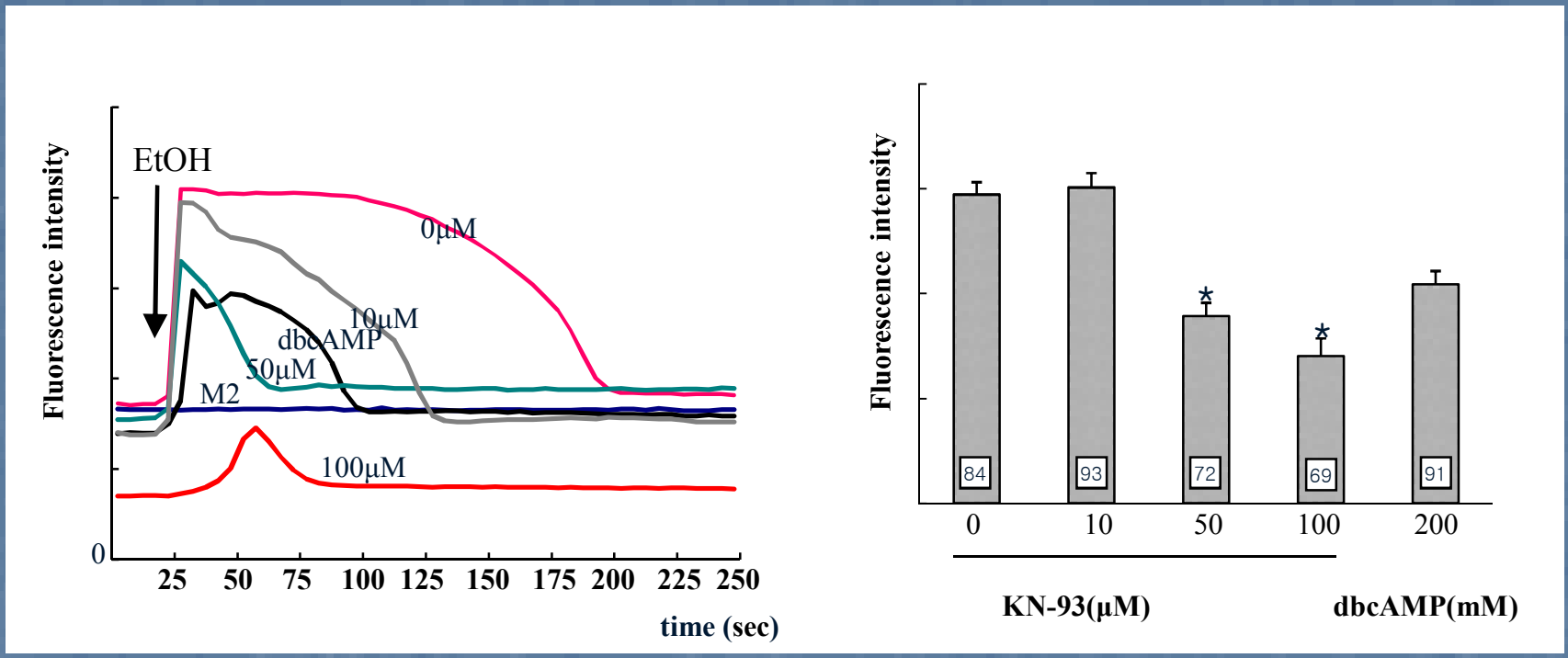
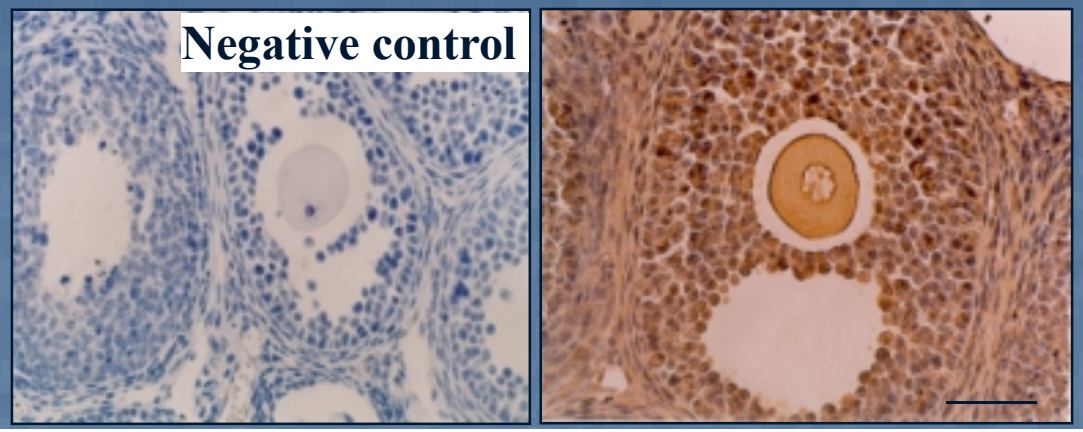
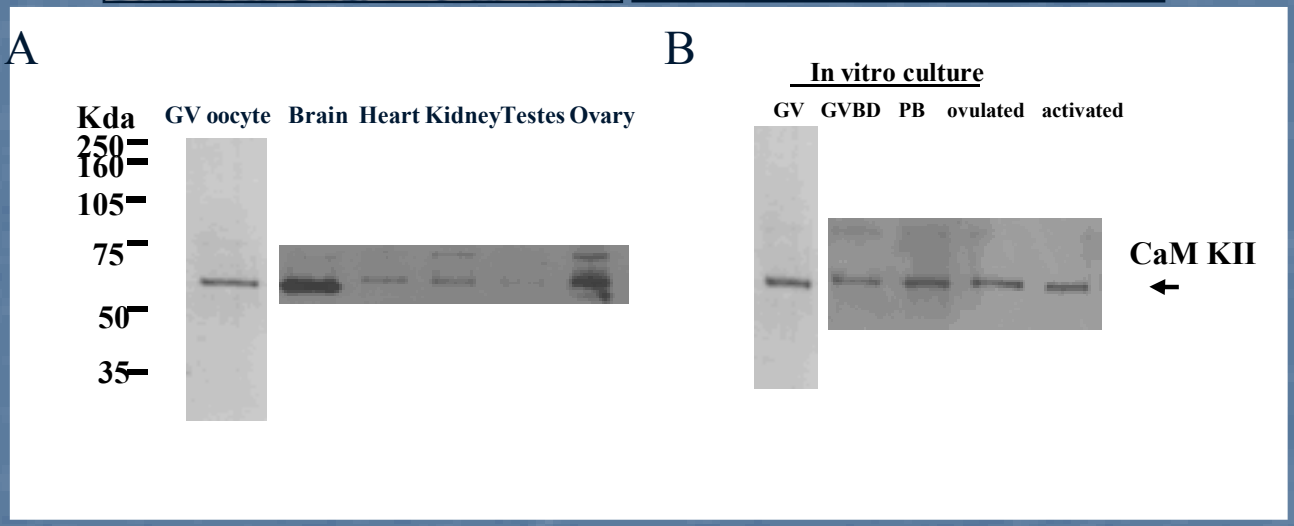


Figure 4. Effect of various drugs on the $[Ca^{2+}]_i$-transient of mouse oocytes in response to EtOH in Ca^{2+}-free medium. Oocytes were preincubated in the control medium or 0~100μM KN-93 or 200mM dbcAMP. Arrow, addition of 6% EtOH M2, M2 medium addition.

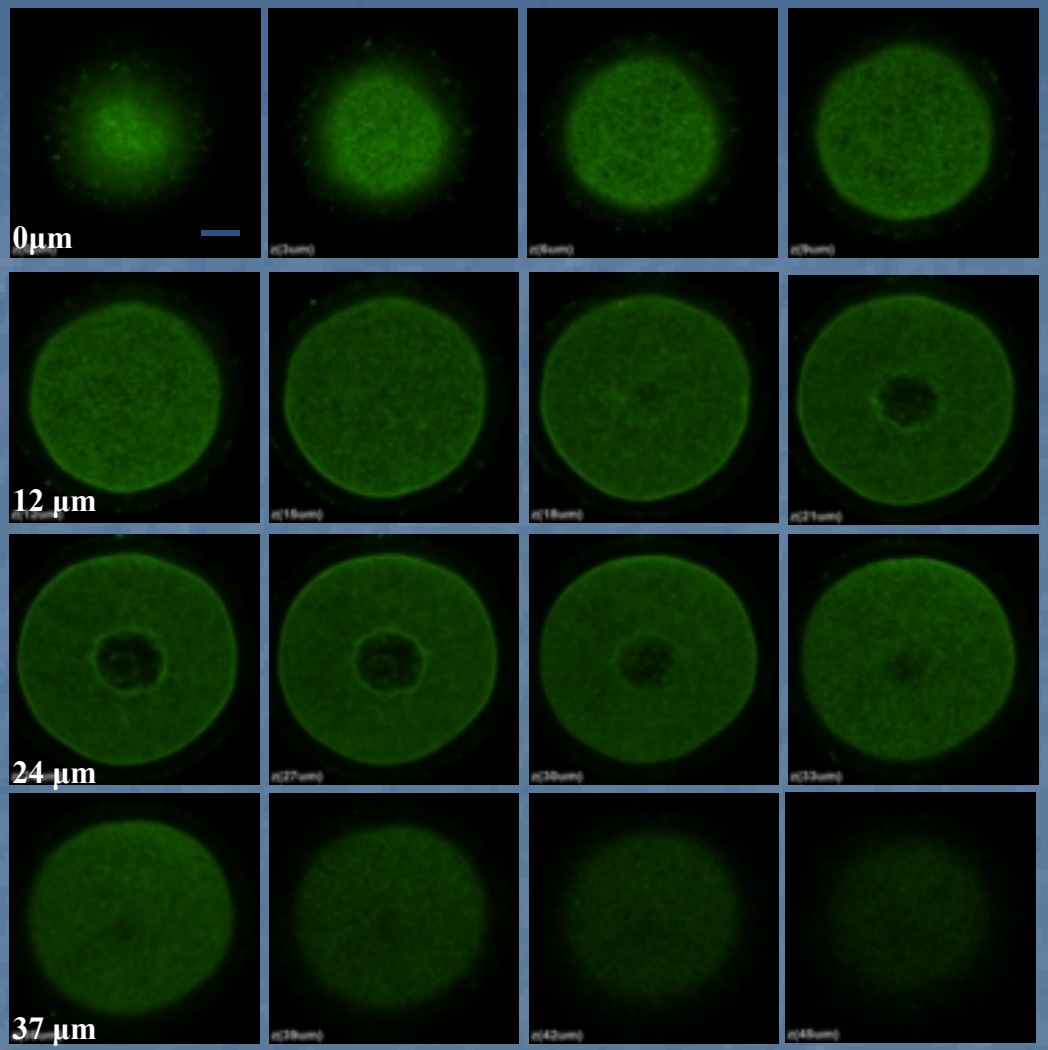
CaM KII might be involved in meiotic resumption via intracellular Ca^{2+} concentration regulation.

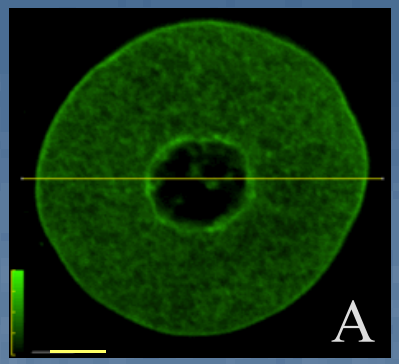
Identification of CaM KII in mouse ovary and tissue

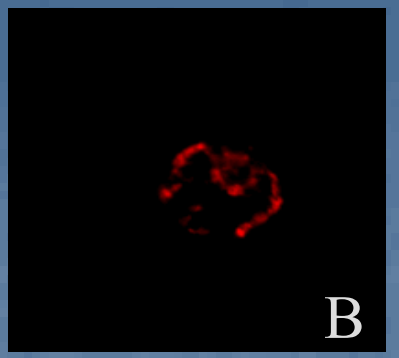


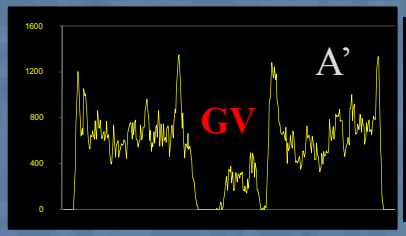


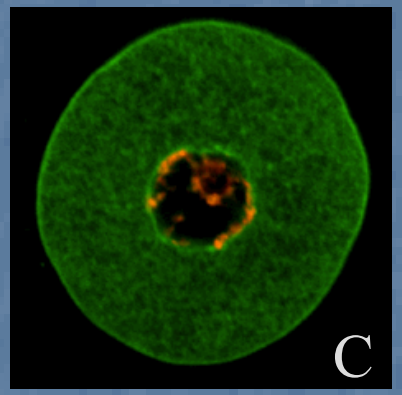
10% SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred on to NC membrane and subsequently hybridized by using CaM KII(monoclonal antibody as primary antibody. One hundred GV oocytes, 10μg of ovarian homogenate or 4mg each tissue homogenate was loaded onto a wall. Activated oocytes after 30min activation by 6% ethanol.

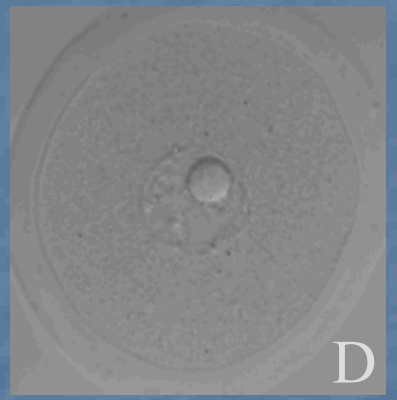




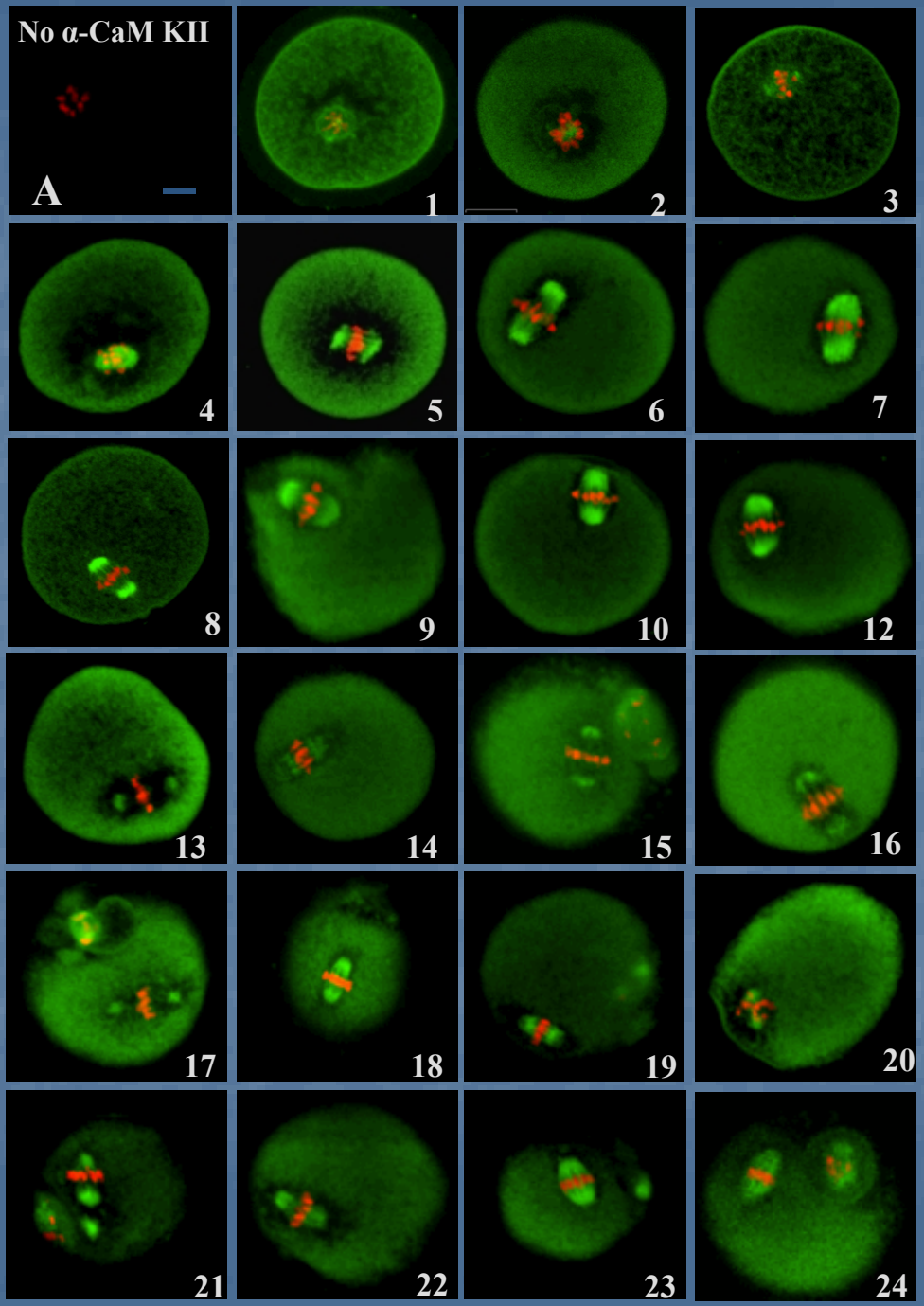






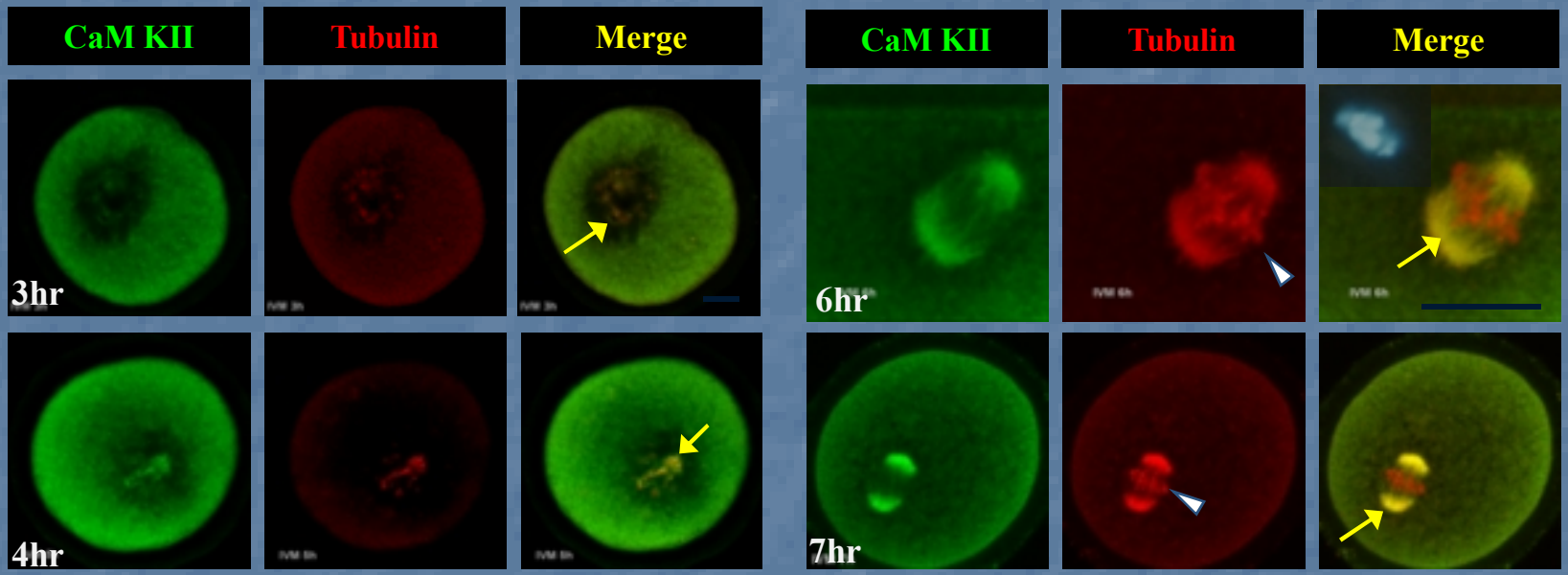


Distribution of CaM KII in the mouse GV oocyte.



Distribution of CaM KII /chromosome in the mouse maturing oocyte

Colocalization of CaM KII and tubulin on the spindle pole in the germinal vesicle breakdown oocyte

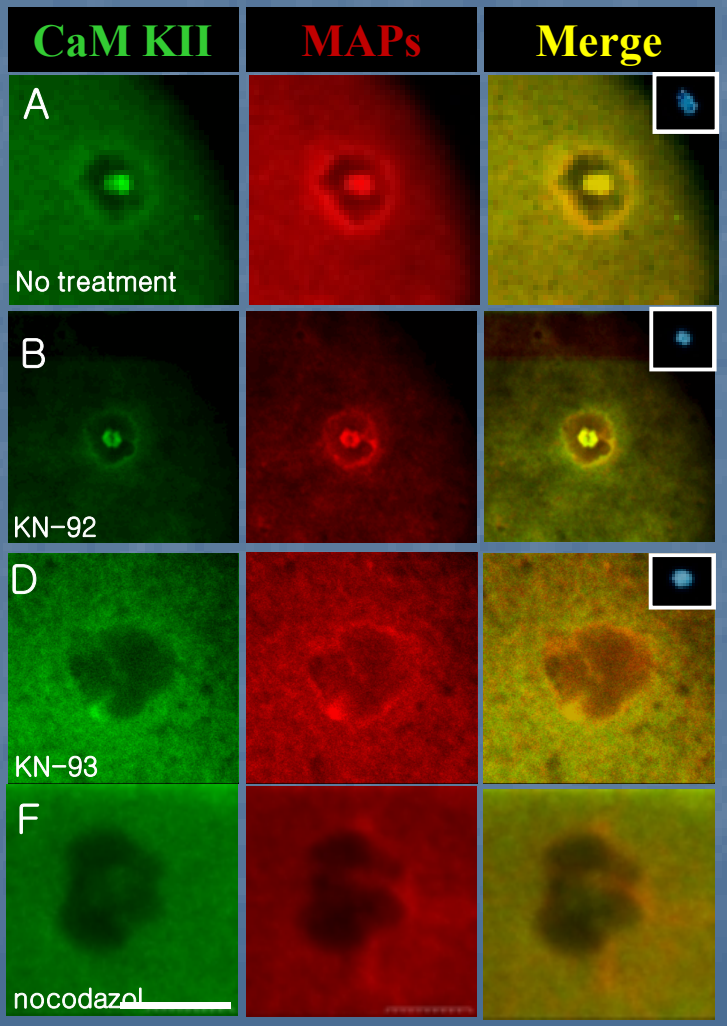


Colocalization of CaM KII and microtubule-associated proteins (MAPs) in the germinal vesicle breakdown oocyte

MAPs bind to the tubulin subunits regulate their stability.

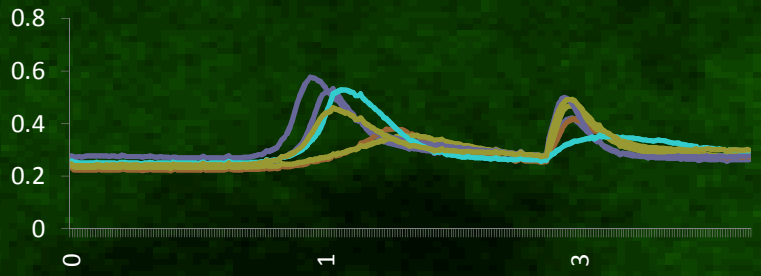
Xenopus MAP230 (XMAP230)stabilizes oocyte MTs and is required for assembly of spindles and cortical MTs……

Andersen, S.S., et al., Effect on microtubule dynamics of XMAP230, a microtubule-associated protein present in Xenopus laevis eggs and dividing cells. J Cell Biol, 1994. 127(5): p. 1289-99.

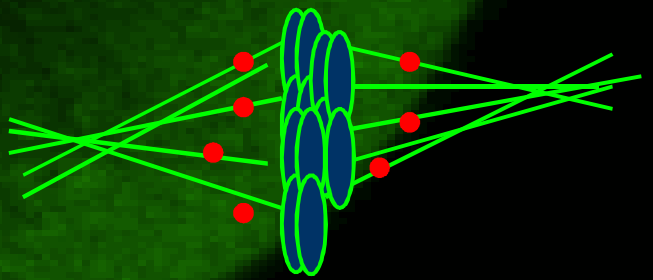


Conclusions

1. **CaM KII might be involved in the regulatory mechanism of meiotic resumption via intracellular Ca^{2+} concentration.**



2. **CaM KII might play a regulatory role in the stabilization of microtubule via MAPs.**




Thanks !