# Roles of Calmodulin-dependent Protein Kinase Ⅱ in Meiotic Maturation and Fertilization of Oocytes*

FAN Heng-Yu, HUO Li-Jun, SUN Qing-Yuan**

*(State Key Laboratory of Reproductive Biology, Institute of Zoology, The Chinese Academy of Sciences, Beijing* 100080, China)

**Abstract** Calmodulin-dependent protein kinase (CaMK), activated by auto-phosphorylation at the presence of calcium and calmodulin, is widely distributed in eukaryotes. CaMKs are important mediators of calcium signal in eukaryotes. Recent researches have suggested that CaMK Ⅱ is involved in the regulation of meiotic cell cycle of oocytes. It plays functional roles in meiotic maturation, polar body extrusion, fertilization and egg activation. As one of the down-stream signaling molecules of calcium, CaMK Ⅱ facilitates the inactivation of maturation promoting factor (MPF) and cytostatic factor (CSF) following fertilization, as well as the spindle microtubule organization and centrosome duplication. Although the functions of CaMK Ⅱ in oocyte meiosis are versatile and essential, the present results are primarily obtained from low vertebrates and mouse. In future studies, the function and regulation of this kinase in other mammals should be stressed.

**Key words** CaMK Ⅱ, oocyte, meiosis, fertilization, cell cycle

The $Ca^{2+}$/calmodulin-dependent protein kinases (CaM kinases) comprise a structurally related subfamily of serine/threonine kinases that include CaMKⅠ, CaMKⅡ, and CaMKⅣ. CaMKⅡ is a ubiquitously expressed serine/threonine protein kinase that is activated by $Ca^{2+}$ and calmodulin and has been implicated in regulation of the cell cycle and transcription. There are four CaMKⅡ isoforms designated α, β, γ and δ ranging in molecular mass from 52 ku (α) to 61 ku (58~61 ku: β, γ and δ). CaMKⅡ is normally maintained in an inactive state by the interaction of the catalytic domain with an autoinhibitory domain located on the same polypeptide. In the nonphosphorylated form, CaMKⅡ requires calcium and calmodulin for activity. In the presence of calcium and calmodulin, the enzyme is autophosphorylated on the threonine 286 (T286) and become calcium- and calmodulin-independent. Thus, the generation of this autonomous kinase may underlie some long-term potentiation of transient calcium signals. Several lines of evidence from amphibian or mammalian models suggest that calcium-mediated and CaMKⅡ-dependent signaling pathways are essential for the normal progression of meiotic cell cycles in oocytes.

## 1 CaMKⅡ and meiotic maturation of oocytes

Fully grown mammalian oocytes are arrested at the diplotene stage of the first meiotic prophase, which is also termed germinal vesicle (GV) stage because of the presence of a vascularized nucleus. The GV stage arrested mammalian oocytes can resume meiosis spontaneously when they are released from the inhibitory environment of follicles[1]. Oocytes also mature *in vitro* under the stimulation of gonadotropin when the spontaneous maturation is prevented by meiotic inhibitors, such as hypoxanthine and cAMP-elevating agents. In these two experimental models, different mechanisms are employed in regulating the progression of meiotic cell cycles[2, 3].

The culture of denuded mammalian (rat, cow, and pig) oocytes in calcium-deficient medium does not appear to have any significant effect upon their ability to undergo meiotic resumption. However, calcium-dependent pathways are essential for gonadotropin-induced oocyte meiotic resumption. Spontaneous calcium oscillations occur in GV-stage mouse oocytes, and chelation of intracellular calcium blocks FSH-induced meiotic resumption in mouse, cow and pig cumulus-enclosed oocytes (CEOs). Calcium probably interacts with calmodulin to regulate oocyte maturation, since calmodulin antagonists inhibit GVBD in mouse and rabbit oocytes, and prevent meiotic progression to MⅡ stage in cow and rabbit oocytes[4].

Recent reports indicated that CaMKⅡ was likely a molecular linkage between the calcium signal and the cell cycle regulatory molecules in the oocytes. The FSH-induced GVBD of mouse CEO, instead of the spontaneous meiotic resumption, is inhibited by CaMKⅡ inhibitors KN-93 and myristoylated AIP[5]. It is not clear whether CaMKⅡ functions in the cumulus cells or in the oocyte. Since CaMKⅡ inhibitors does not affect spontaneous GVBD of denuded oocytes (DOs), it is most likely that the CaMKⅡ-dependent step in the induction of meiotic resumption takes place in the cumulus cells. Activation of CaMKⅡ in the cumulus cells might be involved in stimulating the production

* This work was supported by grants from The Special Funds for Major State Basic Research of China (G1999055902), Knowledge Innovation Project of The Chinese Academy of Sciences (KSCX2-SW-303), and The Outstanding Young Scientists from The National Natural Sciences Foundation (30225010).

** Corresponding author.

Tel: 86-10-62563923, E-mail: sunqy1@yahoo.com

Received: September 23, 2002 Accepted: November 29, 2002

of a factor that promotes the resumption of meiosis in the oocyte, as proposed for other kinases.

Unlike GVBD, results of studies investigating the role of extracellular calcium on the progression of meiosis beyond metaphase I are more consistant. In both the FSH-induced and the spontaneous meiotic maturation systems, CaMK II inhibitors prevent the emission of first polar bodies and arrest the oocytes at M I stage. Calmodulin antagonist W7 could also inhibit the extrusion of PB1 in spontaneously matured oocytes. Culture of pig and bovine oocytes in calcium-deficient medium or calcium-depleted medium in the presence of EDTA or EGTA suppresses polar body formation. These results suggest that CaMK II is involved in not only the initiation of meiotic resumption, but also the M I to M II transition of the oocytes. Furthermore, there is evidence that the influx of extracellular calcium, instead of the release of intracellular calcium, is essential for activation of CaMK II and polar body extrusion, since the nifedipine, a kind of membrane calcium ion channel blocker, inhibits the polar body release and block the meiosis I at metaphase[5].

In the mouse, meiotic maturation is accompanied by development of cell polarity, resulting in the establishment of a subdomain of the egg plasma membrane (known as the microvillar region) to which sperm bind. Establishment of cell polarity in the mouse egg correlates with the localization of chromatin, and this appears to depend, at least partially, on intracellular calcium transients that occur during meiotic maturation. Treatment of oocytes during maturation *in vitro* with the cell-permeable calcium chelator BAPTA-AM perturbed development of normal cell polarity in the egg[6]. But it is not known what roles does the CaMK II play in this process. $Ca^{2+}$ and its downstream mediators are likely involved in the regulation of polarized cytoskeleton organization in the egg, especially the cortical area.

## 2 CaMK II and egg activation

In most vertebrates, unfertilized eggs are arrested at second meiotic metaphase by a cytostatic factor (CSF), an essential component of which is the product of the *c-mos* proto-oncogene (MOS). CSF prevents ubiquitin-dependent degradation of metaphase cyclins and thus inactivation of the M phase-promoting factor (MPF). The evolutionarily conserved stimulus which releases the egg from cell cycle arrest in all animal species studied so far is a rise in intracellular free $Ca^{2+}$, which is triggered by fertilization or parthenogenetic activation[7]. An undefined mechanism activated by $Ca^{2+}$ inactivates both CSF and MPF, and releases eggs from meiotic metaphase arrest.

An early and transient activation of CaMK II was detected in *Xenopus* eggs after parthenogenetic activation[8]. When $Ca^{2+}$ was added to egg extracts, the activity of CaMK II increased dramatically, and earlier than the drop of MPF activity associated with cyclin degradation. Microinjection into M II-arrested *Xenopus* eggs of the constitutively active CaMK II inactivated MPF and triggered MOS degradation. Conversely, unfertilized *Xenopus* eggs microinjected with the CaMK II inhibitory peptide failed to undergo cyclin degradation and inactivation of either Cdc2 kinase or CSF upon electrical stimulation, a treatment that causes parthenogenetic activation in non-injected eggs. These results indicate that CaMK II mediates the $Ca^{2+}$-dependent inactivation of MPF and CSF accompanying fertilization or egg activation. However, the mechanisms by which CaMK II induces MPF and CSF inactivation are still under investigation.

CaMK II is activated in mouse oocytes following a rise in intracellular calcium concentration, as observed in *Xenopus* eggs and *in vitro* extracts. Treatments that increase the intracellular calcium lead to the activation of the mouse eggs, which is a calmodulin- and CaMK II-dependent process[9]. Calmodulin is present in a dispersed pattern throughout the unfertilized egg with an enrichment near the periphery of the egg and around the meiotic spindle. Calmodulin forms a tight association with CaMK II on the meiotic spindle immediately after egg activation. This tight association with calmodulin disappears, while CaMK II remains on the spindle as the chromosomes transit into anaphase II. Further, CaMK II becomes localized in the region of the midzone microtubules between anaphase II and telophase II[10].

Mitogen-activated protein kinase (MAPK) is another kind of protein kinase that plays key roles in the regulation of oocyte meiosis (for review, see Fan *et al*[11]). MAPK was active at M II stage and its activity was kept after egg activation until the pronucleus formation. Results from confocal microscopy revealed that MAPK and CaMK II were co-localized on the meiotic spindle, suggesting their potential mutual regulation at vicinity. There is evidence supporting this hypothesis. Suppression of CaMK II activity during egg activation results in reduction in the amount of MAPK as well as a decreased level of MAPK activity[12]. Since CaMK II becomes active as a result of fertilization, the former kinase could serve to potentiate MAPK activity and the co-localization of these two kinases may facilitate such an interaction.

## 3 CaMK Ⅱ and centrosome duplication after fertilization

Centrosome duplicates once and only once during the cell cycle, ensuring the formation of bipolar spindles that distribute replicated chromosomes equally to daughter cells. There are links between the cell cycle and the centrosome duplication cycle. Centrosome generally duplicates at the G1 ~ S transition, and if S phase is prolonged in mammalian cells or cycling *Xenopus* egg extracts, centrosomes can reproduce multiple times. Calcium and calmodulin are required for cells to traverse the G1~ S, G2~ M, and metaphase-anaphase boundaries of the cell cycle. In particular, calcium oscillations occur at the G1~ S boundary and near the G2~ M transition in cycling *Xenopus* egg extracts. These times correlate with centrosome duplication at the G1 ~ S boundary and centrosome separation at the G2~ M transition. Moreover, calcium-modulated proteins, including CaMKII and centrin (a protein essential for centrosome duplication), are localized in the centrosome. In *Xenopus* egg extracts arrested in S phase, centrosme duplication starts after addition of sperm nuclei, and a transient increase in the concentration of intracellular free calcium is required for initiation of centrosome duplication. Correspondingly, peaks of CaMKII activity were detected at the times when centrosomes duplicate. When a specific pseudo-substrate inhibitor peptide of CaMKII was added to the *in vitro* centrosome duplication system at the beginning of the assay, all duplication was blocked. Addition of this pseudo-substrate at the midst of the assay stopped the second round of centrosome duplication[13]. All these results suggest that CaMKII activity is required for the initiation of each round of centrosome duplication.

Cyclin E-Cdk2 is the only Cdk2 complex exists at S phase of *Xenopus* eggs. When cyclin E-Cdk2 is inactivated by its specific inhibitor Δ34Xic1, in contrast to the case of CaMK Ⅱ, the first round of centrosome duplication was not inhibited, but the additional rounds of duplication were blocked[13]. The arrest of the centrosome duplication process at two different points by CaMK Ⅱ pseudo-substrate and Δ34Xic1 suggests that CaMK Ⅱ and cyclin E-Cdk2 regulate different steps of duplication process. Cdk2 may be a licensing factor that restores reproductive components to daughter centrosomes but is not the initiator of the duplication itself, whereas CaMK Ⅱ is required for initiating an essential step in the centrosome duplication process.

## 4 Future directions

Although it has been confirmed that calmodulin and CaMK Ⅱ are among the most important mediators of calcium signal during meiotic maturation and fertilization of mouse oocytes, accumulating information suggests that rodents may be atypical with regard to regulating mechanisms of oocyte maturation and fertilization. The mechanisms for fertilization in domestic animals such as pig and cattle may be more similar to low vertebrates and human than the mouse[14]. However, the knowledge about the importance of calcium in the meiotic cell cycle regulation of large mammals is limited. And no description about the expression and activation of CaMK Ⅱ in oocytes of mammalian species other than mouse is available. Therefore, the studies of the characteristics of calmodulin and CaMK Ⅱ in farm species should be carried out in future. Besides, the development of oocytes and zygotes is under the control of a network of multiple signaling pathways, which most likely to affect each other through the interaction of several important protein kinases, such as MPF, protein kinase C[15, 16], and MAPK. How CaMK Ⅱ regulates the activity of these kinases is still an unsolved problem in revealing the mechanism of meiotic maturation and fertilization.

## References


1 Fan H Y, Tong C, Li S W, *et al*. Microtubule organization during *in vitro* development of rat oocytes. Prog Biochem Biophys, 2002, **29** (5): 708~ 713
范衡宇, 佟 超, 李世文, 等. 生物化学与生物物理进展, 2002, **29** (5): 708~ 713

2 Fan H Y, Tong C, Lian L, *et al*. Characterization of ribosomal S6 protein kinase p90rsk during meiotic maturation and fertilization in pig oocytes: MAPK-associated activation and localization. Biol Reprod, 2003, **68** (3): 968~ 977

3 Bu S M, Xia G L, Xie H R, *et al*. NO produced by the cumulus cells promotes the meiotic maturation of mouse oocytes. Chin Sci Bulletin, 2002, **47** (22): 1730~ 1733
卜淑敏, 夏国良, 谢辉蓉, 等. 科学通报, 2002, **47** (22): 1730~ 1733

4 Homa S T. Calcium and meiotic maturation of mammalian oocyte. Mol Reprod Dev, 1995, **40** (2): 122~ 134

5 Su Y Q, Eppig J J. Evidence that multifunctional calcium/calmodulin-dependent protein kinase Ⅱ (CaM K Ⅱ) participates in the meiotic maturation of mouse oocytes. Mol Reprod Dev, 2002, **61** (3): 560~ 569

6 Evans J P. Getting sperm and egg together: things conserved and things diverged. Biol Reprod, 2000, **63** (1): 355~ 360

7 Stricker S A. Comparative biology of calcium signaling during fertilization and egg activation in animals. Dev Biol, 1999, **211** (1): 157~ 176

8 Lorca T, Cruzalegul F H, Fesquet D, *et al*. Calmodulin-dependent protein kinase Ⅱ mediates inactivation of MPF and CSF upon fertilization of *Xenopus* eggs. Nature, 1993, **366** (2): 270 ~ 273

9 Winston N J, Maro B. Calcium-dependent protein kinase Ⅱ is activated transiently in ethanol-stimulated mouse oocytes. Dev Biol, 1995, **170** (2): 350~ 352

10 Johnson J, Bierle B M, Gallicano G I, *et al*. Calcium/calmodulin-dependent protein kinase Ⅱ and calmodulin: regulators of the meiotic spindle in mouse eggs. Dev Biol, 1998, **204** (2): 464~ 477

11 Fan H Y, Tong C, Chen D Y, *et al*. Roles of MAP kinase signaling pathway in meiosis of oocytes. Chin Sci Bulletin, 2002, **47** (11): 1157~ 1162
12 Hatch K R, Capco D G. Colocalization of CaMK Ⅱ and MAP kinase on architectural elements of the mouse egg: potentiation of MAP kinase activity by CaMK Ⅱ. Mol Reprod Dev, 2001, **58** (1): 69~ 77
13 Matsumoto Y, Maller J L. Calcium, calmodulin, and CaMK Ⅱ requirement for initiation of centrosome duplication in *Xenopus* egg extracts. Science, 2002, **295** (2): 499~ 502
14 Fan H Y, Tong C, Li M Y, *et al*. Translocation of classical protein kinase C (cPKC) isoforms in porcine oocytes: implications of PKC involvement in the regulation of nuclear activity and cortical granule exocytosis. Exp Cell Res, 2002, **277** (2): 183~ 191
15 Fan H Y, Tong C, Li M Y, *et al*. Detection of protein kinase C by confocal microscopy in mouse oocytes. Prog Biochem Biophys, 2001, **28** (6): 900~ 903
范衡宇, 佟 超, 李满玉, 等. 生物化学与生物物理进展, 2001, **28** (6): 900~ 903
16 Fan H Y, Tong C, Li S W, *et al*. Roles of protein kinase C in the meiotic maturation and fertilization of mouse oocytes. Acta Biol Exp Sin, 2003, **36** (1): 37~ 42
范衡宇, 佟 超, 李世文, 等. 实验生物学报, 2003, **36** (1): 37~ 42

# 钙调蛋白依赖的蛋白激酶Ⅱ在卵母细胞减数分裂和受精中的作用*

范衡宇 霍立军 孙青原**
(中国科学院动物研究所, 计划生育生殖生物学国家重点实验室, 北京 100080)

**摘要** 钙调蛋白依赖的蛋白激酶 (CaMK) 是一类分布广泛的丝/苏氨酸蛋白激酶家族, 在钙离子和钙调蛋白存在的条件下发生自磷酸化而被激活, 在细胞内对于钙信号的传递具有重要的介导作用. 近年来的研究表明 CaMK Ⅱ是参与调节卵母细胞减数分裂的重要分子, 在卵母细胞成熟、极体排放、受精和活化等过程中发挥作用. CaMK Ⅱ作为 $Ca^{2+}$ 的下游信号分子, 在受精后促进成熟促进因子 (MPF) 和细胞静止因子 (CSF) 的失活, 并调节纺锤体微管的组装和中心体的复制过程. 虽然 CaMK Ⅱ在减数分裂中的作用广泛而关键, 但目前的研究主要集中于低等动物和小鼠, 今后有待进一步阐明该蛋白激酶在其他哺乳动物中的作用和调节机制.

**关键词** CaMK Ⅱ, 卵母细胞, 减数分裂, 受精, 细胞周期
**学科分类号** Q26

* 国家重点基础研究发展规划项目 (973) (G1999055902), 中国科学院知识创新工程重要方向性项目 (KSCX2-SW-303) 和国家杰出青年基金 (30225010) 资助.
** 通讯联系人.
Tel: 010-62563923, E-mail: sunqy1@yahoo.com
收稿日期: 2002-09-23, 接受日期: 2002-11-29