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The Role of MAPK Cascade and Its Regulation during Pig Oocyte Maturation and Fertilization

Qing-Yuan Sun

State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China

sunqy@ioz.ac.cn or sunqy1@yahoo.com

Mitogen-activated protein kinase (MAPK) is a family of Ser/Thr protein kinases that are widely distributed in eukaryotic cells. Studies were designed to investigate the expression, phosphorylation, localization, possible roles and regulation of MAPK/p90rsk in porcine oocytes during meiotic maturation and fertilization. MAPK began to be phosphorylated around the time of GVBD, reached a peak at MI stage and kept highly activated till MII stage. P90rsk kinase was partially phosphorylated in oocytes at germinal vesicle (GV) stage through a MAPK-independent mechanism, but its full phosphorylation is dependent on MAPK activity. After fertilization or electrical activation, MAPK/p90rsk was dephospho-rylated shortly before pronucleus formation. A protein phosphatase inhibitor, okadaic acid, accelerated the phosphorylation of MAPK/p90rsk during meiotic maturation and induced its rephosphorylation in activated eggs. MAPK kinase (MAPKK or MEK) inhibitor U0126 inhibited the acti-vation of MAPK and p90rsk in both cumulus-enclosed and denuded pig oocytes, but prevented GV breakdown (GVBD) only in cumulus-enclosed oocytes. Active MAPK and p90rsk were detected in pig cumulus cells, and U0126 induced their dephosphorylation. In meiosis II arrested eggs, U0126 led to the inactivation of MAPK and p90rsk, as well as the interphase transition of the eggs. P90rsk was distributed evenly in GV oocytes, but it accumulated in the nucleus before GVBD. It was localized to the meiotic spindle after GVBD and concentrated in the spindle mid zone during emission of the polar bodies. All these results suggest that MAPK/p90rsk

play functional roles in the regulation of nuclear status and microtubule organization. Although MAPK/p90rsk activity is not essential for the spontaneous meiotic resumption in denuded oocytes, activation of this cascade in cumulus cells is indispensable for the gonadotropin-induced meiotic resumption of pig oocytes.

Calcium signal is important for the regulation of meiotic cell cycle in oocytes, but its downstream mechanism is not well known. Next we investigated the functional roles of calcium/calmodulin-dependent protein kinase II (CaMKII) pathway in meiotic maturation and activation of pig oocytes and regulation on MAPK cascade. The results indicated that meiotic resumption of both cumulus-enclosed and denuded oocytes was prevented by CaMKII inhibitor KN-93, Ant-AIP-II, or CaM antagonist W7 in a dose-dependent manner, but only germinal vesicle breakdown (GVBD) of denuded oocytes was inhibited by membrane permeable Ca^{2+} chelator BAPTA-AM. When the oocytes were treated with KN-93, W7, or BAPTA-AM after GVBD, the first polar body emission was inhibited. A quick elevation of CaMKII activity was detected after electrical activation of mature pig oocytes, which could be prevented by the pretreatment of CaMKII inhibitors. Treatment of oocytes with KN-93 or W7 resulted in the inhibition of pronuclear formation. The possible regulation of CaMKII on maturation promoting factor (MPF), mitogen-activated protein kinase (MAPK), and ribosome S6 protein kinase (p90rsk) during meiotic cell cycles of pig oocytes was also studied. KN-93 and W7 prevented the accumulation of cyclin B and the full phosphorylation of MAPK and p90rsk during meiotic maturation. When CaMKII activity was inhibited during parthenogenetic activation, cyclin B, the regulatory subunit of MPF, failed to be degraded, but MAPK and p90rsk were quickly dephosphorylated and degraded. Confocal microscopy revealed that CaM and CaMKII were localized to the nucleus and the periphery of the GV stage oocytes. Both proteins were concentrated to the condensed chromosomes after GVBD. In oocytes at the meiotic metaphase MI or MII stage, CaM distributed on the whole spindle, but CaMKII was localized only on the spindle poles. After transition into anaphase, both proteins were translocated to the area between separating chromosomes. All these results suggest that CaMKII is

a multifunctional regulator of meiotic cell cycle and spindle assembly and that it may exert its effect via regulation of MPF and MAPK/p90rsk activity during the meiotic maturation and activation of pig oocytes.

Degradation of proteins mediated by the ubiquitin-proteasome pathway (UPP) plays essential roles in the eukaryotic cell cycle. We further analyzed the functional roles of UPP in pig oocyte meiotic maturation and activation and its regulation on MAPK cascade and cyclin B. By using the hypoxanthine-maintained meiotic arrest model, we showed that the meiotic resumption of both cumulus-enclosed oocytes and denuded oocytes was stimulated in a dose- and time-dependent manner by two potent and cell-permeable proteasome inhibitors. Both the mitogen-activated protein kinase (MAPK) kinase inhibitor U0126 and the maturation-promoting factor inhibitor roscovitine overcame the stimulation of germinal vesicle break-down induced by proteasome inhibitors. The phosphorylation of MAPK and p90rsk and the expression of cyclin B1 increased in a dose- and time-dependent manner when treated with proteasome inhibitors during oocyte *in vitro*-maturation culture. Both U0126 and roscovitine inhibited the phosphorylation of MAPK and p90rsk, and the synthesis of cyclin B1 stimulated by proteasome inhibitors. When matured oocytes were pretreated with proteasome inhibitors and then fertilized or artificially activated, the second polar body emission and the pronuclear formation were inhibited, and the dephosphorylation of MAPK and p90rsk as well as the degradation of cyclin B1 that should occur after oocyte activation were also inhibited. We also investigated, to our knowledge for the first time, the subcellular localization of 20S proteasome alpha subunits at different stages of oocyte maturation and fertilization. The 20S proteasome alpha subunits were accumulated in the germinal vesicle, around the condensed chromosomes at prometaphase, with spindle at metaphase I and II, the region between the separating chromosomes, and especially the midbody at anaphase I and telophase and the pronucleus. Our results suggest that the UPP regulates cyclin B1 degradation and MAPK/p90rsk phosphorylation as well as multiple steps of pig oocyte meiosis and fertilization.