D105 Inhibition of S6 Kinase by Rapamycin Blocks Maturation of *Rana dybowskii* Oocytes

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Experiments were carried out to assess whether S6 kinase is involved in hormone induced maturation of *Rana dybowskii* oocyte. Rapamycin, a specific inhibitor of S6 kinase, blocked progesterone-induced oocyte germinal vesicle breakdown (GVBD) in a dose dependent manner indicating that S6 kinase is required for meiotic maturation of *Rana* oocyte. Time dependent addition of rapamycin to progesterone stimulated oocytes showed that rapamycin inhibited GVBD when added within 3 h, but not 6 h of progesterone treatment. In contrast, cycloheximide, a general protein synthesis inhibitor, blocked GVBD even when added 9 h after progesterone addition. Within 1 h of progesterone stimulation, increase in S6 kinase activity occurred in oocytes and rapamycin inhibited this activity. A marked increase in protein synthesis occurred between 3 h and 9 h of culture and changed little subsequently. Rapamycin suppressed, in a dose dependent manner, progesterone-induced protein synthesis during the first 12 h and less effectively later whereas cycloheximide completely inhibited protein synthesis throughout the 24 h culture period. Histone H1 kinase activity (MPF) in oocyte extracts was markedly increased at 6 and 9 h and at 24 h after progesterone stimulation while rapamycin and cycloheximide blocked its activity. Results suggest that proteins synthesized in response to progesterone during the early stage of oocyte maturation are closely associated with S6 kinase activation that are essential for maturation of *Rana* oocytes.

D106 Requirement of Protein Kinase C Pathway during Progesterone-induced Oocyte Maturation in Amphibians, *Rana dybowskii*

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Previously we have shown that protein kinase C (PKC) activation induced meiotic maturation (germinal vesicle breakdown, GVBD) of *Rana dybowskii* follicular oocytes cultured *in vitro* without hormonal stimulation. However, the oocyte GVBD induced by PKC activation with 12 - *O* - tetradecanoyl phorbol - 13 - acetate (TPA) exhibited abnormal cytoplasmic mixing and thus it is still unclear whether PKC play an essential role during oocyte maturation in amphibians. Experiments were carried out to ascertain whether activation of PKC and phospholipase C (PLC), which is closely associated with PKC, are involved in the oocyte maturation of *Rana dybowskii.* Stimulation of oocytes with progesterone caused a transient increase (30 min) in PKC activity. Pre-treatment of oocytes with U107 (a PLC inhibitor), or staurosporine (a PKC inhibitor), suppressed progesterone-induced PKC activation throughout the course of maturation. When oocytes were cultured in presence of progesterone or progesterone with various doses of staurosporine or U107 , oocyte GVBD was inhibited in a dose dependent manner. Inhibition of either PKC or PLC in oocytes also suppressed the $p34^{cdc2}$ kinase activity, which is a component of maturation promoting factor (MPF), stimulated by progesterone. Thus, the data clearly showed that activation of PKC and PLC is necessary for the activation of MPF, and protein kinase C pathway plays a key role in the progesterone - induced oocyte maturation in amphibians.