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Mechanisms involved in control of cell cycle: meiosis in oocytes and mitosis in early embryos

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The oocyte, which is present in the ovary of the mammalian female from birth, is arrested at a G2-like phase of meiosis. It is not before its entry into the M-phase and progression to the second meiotic metaphase that the oocyte acquires maturity and can be fertilized. Fertilization triggers the completion of meiosis and is followed by successive mitotic divisions of the newly formed embryo. Reinitiation of meiosis is stimulated by luteinizing hormone (LH). We have previously shown that intraoocyte concentrations of cAMP negatively regulate the meiotic status of the oocyte and that this cyclic nucleotide is not generated by the oocyte, but rather supplied by the surrounding follicle cells through gap junctions. We have further demonstrated that LH interrupts cell-to-cell communication in the ovarian follicle, leading to a decrease in intraoocyte concentrations of cAMP and resumption of meiosis. Our studies are presently extended to the following related topics:

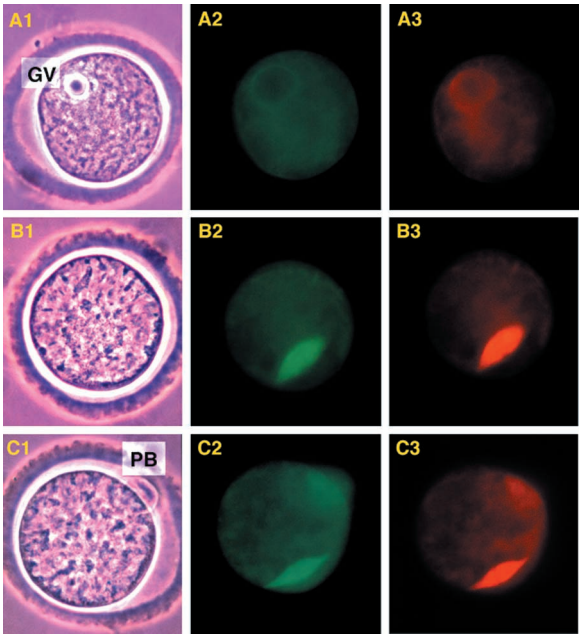

Fig. 1. *Transloation of the proteasome to the spindle apparatus of rat oocytes in the first (B) and second (C) meiotic metaphase. Rat oocytes were immunostained with anti 20S proteasome antibodies (red) and anti β-tubulation (green).*

1. The gating mechanism of gap junctions in the ovarian follicle

Connexin 43 (Cx43), the ovarian gap junction protein, was undetectable in ovaries of rats on the first postnatal day and increased gradually with age. A dramatic elevation accompanied by Cx43 phosphorylation was observed in the large antral follicles of sexually mature rats on proestrus and was followed by elimination of the protein on estrous. Disappearance of Cx43 could be prevented by cancellation of the proestrous LH surge. The estrous-cycle associated pattern of Cx43 modifications was mimicked by exogenous administration of gonadotropins to sexually immature rats. Analysis of the Cx43 mRNA revealed a similar pattern for Cx43 gene expression. We conclude that after antrum formation, transcription, translation, posttranslational modifications and degradation of the ovarian Cx43 are regulated by gonadotropins. These conclusions agree with our previous and recent findings obtained in intact ovarian follicles incubated in vitro.

2. Protein translation, posttranslational, modifications and protein degradation in regulation of the meiotic cell cycle

We and other laboratories have previously demonstrated that meiosis in the oocyte is associated with a characteristic pattern of oscillation of the activity of maturation promoting factor (MPF), a heterodimer composed of p34cdc2 kinase and cyclin B. Dephosphorylation of p34cdc2 on Thr 14 and Tyr 15 at entry into M-phase of the first meiotic division results in MPF activation. MPF inactivation occurring between the two rounds of meiosis is associated with cyclin degradation. Activation of MPF in oocytes resuming meiosis is followed by an increase in the activity of a 42 kDa MAP kinase (MAPK) that remains elevated until the completion of the second meiotic division. The upstream regulator of MAPK in oocytes is Mos kinase, the product of the c-mos protooncogene. Mos is responsible for the second metaphase arrest of unfertilized oocytes. We have recently shown that rat oocytes maintained in G2-arrest by relatively high intracellular concentrations of cAMP do not elevate the activity of MPF. Mos translation in

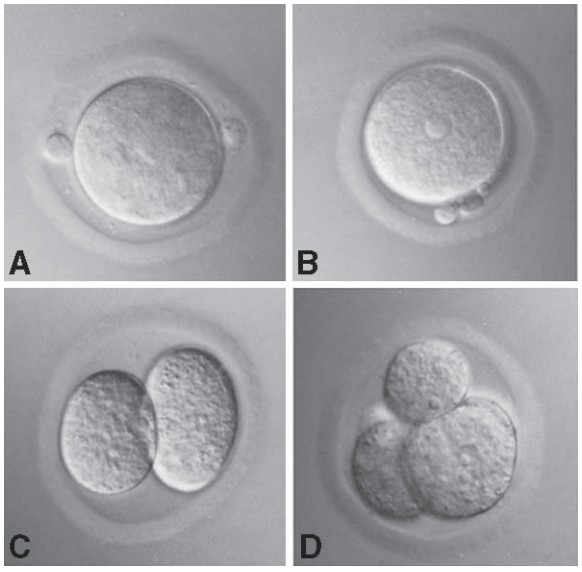

Fig 2. *Parthenogenically activated rat oocytes treated with PD098059. Formation of the second polar body and the female pronucleus (A,B) followed by the first (C) and the second embryonic cell divisions (D).*

these oocytes is inhibited and MAPK activity stays low. Inhibition of MAPK activation by PD 098059 that acts downstream to Mos in the MAPK signaling cascade does not interfere with G2 to M-phase transition. However, these oocytes failed to arrest at the second meiotic metaphase undergoing parthenogenic activation. We also demonstrated that lactacystin and MG-132, that are selective and potent inhibitors of the proteasome, prevented completion of the first meiotic division arresting the oocytes at the first metaphase/anaphase transition. The MG-132 arrested oocytes contained a relatively high amount of cyclin associated with a sustained elevated activity of MPF. A role for the proteasome in the regulation of meiosis is also suggested by its translocation to the spindle apparatus. We conclude that cAMP acts as a negative regulator of meiosis by inhibiting p34cdc2 dephosphorylation preventing MPF activation. A drop in intracellular concentrations of cAMP allows the translation of Mos and further elevation of MAPK activity. An active MAPK is required for maintaining oocytes in the second metaphase arrest. MPF inactivation required for exit from the first round of meiosis is subjected to regulation by proteasomal degradation. Cyclin, as well as other, as yet unidentified proteins, should be degraded in order to allow the completion of the first meiotic division.

3. Role(s) of the proteasome at early embryogenesis

Downregulation of MPF activity is also associated with termination of mitosis. MPF inactivation in most cases is brought about by the ubiquitin-proteasome pathway. The possible role of the proteasome in regulation of early embryogenesis was studied in fertilized rat oocytes. We found that at the first embryonic mitosis the proteasome translocates to the spindle apparatus. We further demonstrated that the proteasome inhibitors lactacystin and MG-132 effectively prevented the formation of two-cell embryos. Inhibition of proteasomal action was associated with relatively high amounts of cyclin and elevated MPF activity. Our study implies that proteasomal degradation of cyclin, and possibly other, as yet unidentified proteins allows the formation of the two-cell embryo.

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