Nava Dekel

Sagit Abramovich
Liat B. Josefsberg
Dalia Galiani
Eran Gershon
Irit Granot
Miri Kandli
Yael Kalma
Shlomi Lazar

Mechanisms involved in control of the meiotic cell cycle

Department of Biological Regulation

Tel. 972 8 934 3716 **Fax.** 972 8 934 4116
E-mail: nava.dekel@weizmann.ac.il

The oocyte, which is present in the ovary of the mammalian female from birth, is arrested at a G2-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:

The gating mechanism of gap junctions in the ovarian follicle

Our recent studies directed at the hormonal regulation of the ovarian gap junction protein connexin 43(Cx43) revealed that: (i) expression of the gap junction protein in the ovary is developmentally regulated; (ii) after sexual maturation both the amount of Cx43 and its phosphorylation are subjected to regulation by gonadotrophins; (iii) the LH-induced gating mechanism of the gap junctions in rat ovarian follicles is comprised of two steps: an immediate response is represented by a change in the phosphorylation state of Cx43, and a later response manifested by a reduction of Cx43 concentration, due to attenuation of its gene expression. Site directed mutagenesis of tentative phosphorylated sites on the Cx43 molecule is presently employed for identification of the specific kinase/s that mediate LH action in Cx43 phosphorylation and clarification of its/their role in regulating the gating of the gap junction channels.

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 is subjected to cdc25 phsophatase regulation and results in MPF activation. MPF inactivation occurring between the two rounds of meiosis is subsequent to cyclin degradation. Activation of MPF in rat oocytes resuming meiosis is followed by an increase in the activity of 42 and 44 kDa MAP kinases (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. Using rat oocytes as our experimental model we generated evidence suggesting that cAMP acts as a negative regulator of meiosis by preventing p34cdc2 dephosphorylation and inhibiting cyclin B synthesis. A possible effect of cAMP on cdc25 phsophatase is presently examined. A drop in intracellular concentrations of cAMP allows MPF activation resulting in meiosis reinitiation and its progression to the second metaphase. It also leads to cmos mRNA polyadenylation, Mos translation and further elevation of MAPK activity. An active MAPK is required for maintaining oocytes in the second metaphase arrest. MPF inactivation is subjected to regulation by proteasomal degradation of cyclin and is required for the exit from the first round of meiosis. MPF reactivation upon the transition from the first to the second meiotic division is absolutely necessary for the suppresion of interphase between the two meiotic divisions. Some of our findings are presented in Fig. 1.

A-kinase anchoring protein (AKAP) in regulation of meiosis

The negative regulation of cAMP on meiosis is mediated by the cAMP-dependent protein kinase (PKA). To understand the mechanism of PKA regulation, we examined rat oocytes for the presence and developmental modulations of A-kinase anchoring proteins (AKAPs) that target PKA to specific subcellular locations through interaction with its regulatory subunit (R). Using the modified RII overlay procedure, we demonstrated the presence of a novel 140 kDa AKAP, AKAP140 that is phosphorylated in association with progression of meiosis. Inhibition of p34cdc2 kinase also prevented AKAP140 phosphorylation. Our

experiments are presently directed at AKAP 140 cloning and its biochemical characterization. We will also examine the physiological consequence of PKA/AKAP interaction.

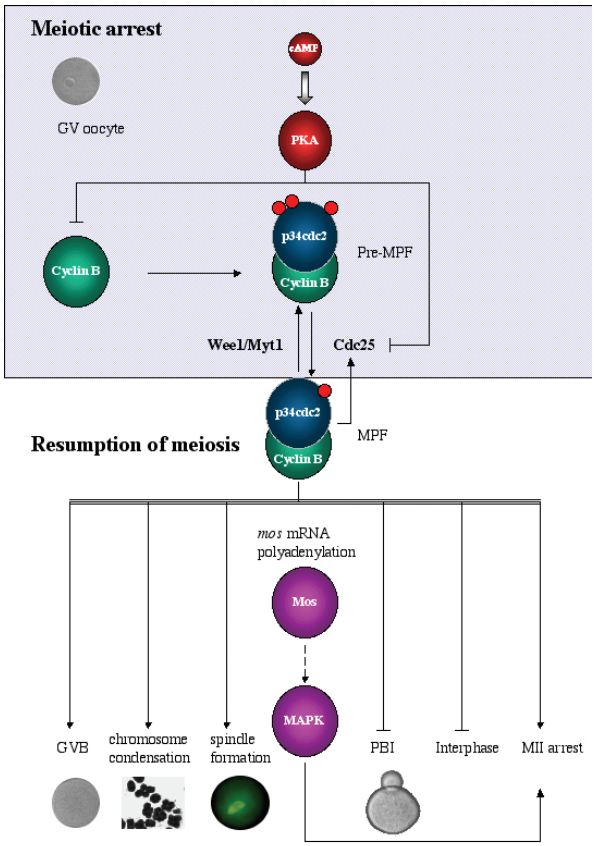

Fig. 1 *Regulation of meiosis in mammalian oocytes. The GV arrested oocyte is maintained at prophase due to the inhibitory effect of PKA mediated cAMP action. The inhibition is conferred on two levels: the prevention of pre-MPF activation due to sustained phosphorylation on p34cdc2 and repression of de-novo synthesis of cyclin B1. Following the release of the oocyte from the ovarian follicle, intraoocyte cAMP concentration drops and pre-MPF undergoes activation. Active MPF elicits resumption of meiosis, namely GVB, chromosome condensation and spindle formation. The polyadenylation of mos mRNA follows MPF activation, leading to Mos expression and activation of MAPK. Inactivation of MPF at MI is necessary for the extrusion of PBI, whereas its reactivation at the onset of the second meiotic division suppresses entry into interphase. The MII arrest of the oocyte is endured until fertilization by the action of both MAPK and MPF.*

Selected Publications

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