Program/Abstract # 297
Localization of the Vasa homolog and formation of germ granules during oogenesis of the sea urchin Strongylocentrotus intermedius
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In sea urchins, germ line segregation occurs during embryogenesis by selective accumulation of germine-specific markers in the small micromere lineage, which descendants contribute to gamete production in adults. However, the accumulation and distribution of germine determinants during oogenesis and the role of maternal contribution to germine formation in sea urchin embryogenesis are still poorly known. Vasa is widely used as a germine marker and is a component of unique ribonucleoprotein complexes known as nuage, chromatoid bodies, and germ granules that are essential for gametogenesis and germine segregation in embryos. Despite the presence of Vasa in the sea urchin eggs, germ granules were not detected until micromere segregation occurred. Therefore, in this study, we analyzed the distribution of the sea urchin homolog of a Vasa protein during oogenesis of the sea urchin Strongylocentrotus intermedius and also studied its intracellular localization. We found that Vasa localized in the cytoplasm of all germ cells and eggs and had two distinct intracellular granular patterns. In mitotic germ cells, oogonia, and primary oocytes, Vasa often localized in perinuclear nuage-like structures. In eggs, Vasa was detected only in the cortical layer; this pattern was initiated in the full-grown primary oocytes that had lost cortical restriction during the first embryonic cleavage. These preliminary results show that the Vasa is not only accumulated during sea urchin oogenesis, but also is incorporated in maternal germ granules associated with the egg cortex. Supported by FEB RAS grant 09-I-P22-04.
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Program/Abstract # 298
Cyp26b1 regulates sex-specific timing of meiotic initiation independent of retinoic acid
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Entry into meiosis was initially proposed to be an intrinsic property of fetal germ cells unless prevented by a meiosis-inhibiting factor produced in the testis. Other studies suggested that sex-specific initiation of meiosis requires retinoic acid (RA) synthesized by Raldh2 in the mesonephros for induction of Stra8 in fetal ovary, with expression of the RA-degrading enzyme Cyp26b1 in testis delaying meiosis until postnatally. Here, investigation of Raldh2−/− mice lacking RA synthesis in mesonephros indicated that Stra8 expression in the ovary does not require RA signaling; meiotic markers Scp3 and g-H2AX were also expressed normally. We also found that Stra8 is expressed in the absence of physiologically detectable levels of RA in either mesonephros or gonad using the RARE-lacZ transgene which was validated to be a sensitive reporter for physiological levels of RA (25 nM). Chromatin immunoprecipitation studies demonstrated that RARs do not bind efficiently to a putative RARE upstream of Stra8. In addition, ketoconazole inhibition of Cyp26b1 in Raldh2−/− testis allows RA-independent induction of Stra8, but only when the mesonephros remains attached, pointing to a non-RA signal from the mesonephros that induces Stra8 in the adjacent gonad. These findings demonstrate that Cyp26b1 prevents the onset of meiosis by metabolizing a substrate other than RA which regulates Stra8 expression.
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Program/Abstract # 299
The role of Geminin in germinal stem cells
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Stem cells preserve tissue stability by balancing self-renewal with differentiation into somatic cells. The unstable regulatory protein Geminin is one of the factors that determine if stem cells will continue to divide or terminally differentiate. Geminin is required for maintenance of embryonic stem cells and is thought to maintain cells in an undifferentiated state while they proliferate. Geminin is a bifunctional protein. It prevents a second round of DNA replication during S and G2 phases by inhibiting the essential replication factor Cdt1. It also inhibits cell differentiation by binding to and inhibiting several transcription factors, including members of the Homeobox family and the retinal development protein Six3. To test this model, we have developed a mouse model in which Geminin is specifically deleted from germ cells. This mouse carries a loxP-ranked Geminin allele and expresses Cre-recombinase under the control of the germ cell-specific Vasa promoter. We find that Vasa-Cre+/GemininloxP/loxP males are viable but not fertile. The number of germ cells in Vasa-Cre+/GemininloxP/loxP mice is normal at birth but they are lost within the first week of life. Geminin is not required for meiosis and spermiogenesis. Our results indicate that the defect arises in the proliferative phase of spermatogenesis. We are now testing whether the spermatogonial defect is caused by replication abnormalities or by a change in the spermatogonial transcription pattern.
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