and glycolysis genes fructose 1,6 bisphosphate aldolase (ALD) and lactate dehydrogenase C (LDH-C) were more highly expressed in prepregnant derived oocytes (P < 0.05). No differences were found in the remaining analyzed transcripts. The differential expression of these genes may reflect the presence of cholesterol-synthesizing enzymes and acid oxidation and glycolysis in good and poor quality porcine oocytes suggest that activities of these metabolic pathways may be important mechanisms involved in oocyte competence.


Gonadotropes of the anterior pituitary synthesize and secrete the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are essential for reproductive function and act on the ovary and testes to regulate steriodogenesis and gametogenesis. The genes that encode these two hormones are regulated by gonadotropin-releasing hormone (GnRH), which is secreted from the hypothalamus and binds to its receptor on gonadotropes. Upon binding its receptor, GnRH activates G alpha, that in turn activates multiple members of the mitogen-activated protein kinase (MAPK) signaling family, including extracellular regulated kinase (ERK), JUN N-terminal kinase (JNK), and p38 MAPK, as well as increases intracellular Ca++ concentrations. Increased activity of the MAPK family members leads to mRNA accumulation of several key immediate early genes (IEGs) including c-Fos, Jun and Egr1. These IEGs confer hormonal responsiveness to the gonadotrope spliced-v heavy chains Gnrb, Cea, Fishb and Lhb. Calcium has been implicated in regulation of several of these genes, although the role for Ca++ within gonadotropes has been controversial depending on the cell line utilized for the study. In this study we test the hypothesis that GnRH regulates activity of the protein phosphatase calcineurin and consequently several immediate early genes through increased intracellular Ca++.

Herein we report that pretreatment of murine LIT2 cells with either a Ca++ blocker, BPAT-AM, or a calcineurin specific inhibitor, cyclosporin A, reduces the ability of GnRH to regulate the accumulation of cFos and Jun mRNA while Egr1 mRNA is not affected. Furthermore, pretreatment with thapsigargin, an intracellular Ca++ protein pump inhibitor, increases Ca++ and accumulation of cFos and Jun mRNA in the presence of both vehicle and GnRH. Calcineurin also regulates the transcriptional activity of NFAT proteins. Additional data indicate that LIT2 cells express NFAT and that GnRH increases the luciferase activity of an NFAT-dependent promoter reporter. We also show that GnRH regulates NFAT-dependent transcription through intracellular Ca++ and calcineurin activation. NFAT proteins have been shown to cooperate with proteins bound to AP1 response elements suggesting that AP1 and NFAT activities of these metabolic pathways may be important mechanisms involved in gametogenesis.

392. The Roles of Syntaxin2/Epiporphin (Stx2/Epim) in Progression of Meiosis During Spermatogenesis. Yasuhiro Fujimura, Kouyou Akiyama, Yuka Asano, Takehito Tsui, Junko Noguchi, and Tetsuo Kunieda. Okayama University, Okayama, Japan; National Institute of Agrobiological Sciences, Tsukuba, Japan

rep34 is an ENU-induced mutation in mice showing male-specific infertility caused by defective spermatogenesis. The homozygous mice (rep34/rep34) show abnormal spermatogenesis with multinucleated germ cells, and no mature spermatozoon nor elongated spermatid was observed in the seminiferous epithelium. We have previously identified Stx2/Epim as the gene responsible for rep34. In the present study, we performed detailed phenotypic analysis of the homozygotes in order to reveal the function of Stx2/Epim in germ cell differentiation of mice. Since several types of cells showed multinucleation in the seminiferous epithelium of the homozygotes, we first performed immunohistochemical staining of the testis using gammaH2AX, IZUMO, and HSC70T antibodies to identify the cells which multinucleated. It was clear that multinucleation occurred in various types of cells including pachytene spermatocytes, spermatocytes at MI/III and round spermatids. Histological analysis of the homozygotes during the first-wave of spermatogenesis confirmed these three types of multinucleation. The increase of Stx2/Epim expression in the testes of mice at about day 18 of the first-wave of spermatogenesis, which we have already revealed, was in accordance with the timing of the onset of multinucleation at pachytene stage. Since multinucleation occurs in pachytene spermatocytes, in which homologous chromosome paring occurs, we examined to find any abnormalities in chromosome synopsis. Surface spread chromosome preparation of pachytene spermatocytes (not multinucleated ones) was stained with gammaH2AX and SCP3 antibodies and was observed under a fluorescence microscope. However, normal synopsis of homologous chromosomes was observed in the rep34 homozygotes. Next, we examined Giemsa stained chromosome preparation of metaphase spermatocytes and found abnormal karyotype, containing aneuploidy. Similar abnormality was detected in the surface spread chromosome preparation in the same sample. Furthermore, immunohistochemical staining of the metaphase spermatocytes with alpha-TUBULIN antibody revealed more than one pair of spindle bodies and abnormal shape of metaphase plates, suggesting that the homoginous germ cells cannot undergo normal cell division at meiosis I and possibly meiosis II as well. In addition, TUNEL assay revealed some multinucleated round spermatids. Anti-apoptotic activity of Stx2/Epim to round spermatid, were apoptotic, suggesting that multinucleated spermatocytes at metaphase might be excrated from the seminiferous epithelium. On the other hand, some spermatocytes might go through normal meiotic progression, resulting in multinucleated round spermatids. Together, it was revealed that the loss of Stx2/Epim causes a series of abnormalities in meiotic progression, most significantly during metaphase, resulting in a formation of multinucleated germ cells in various stages of spermatogenesis. Thus, Stx2/Epim plays an important role in meiosis during spermatogenesis.

393. Cloning of Candidate Genes for TME484 Binding Proteins, Which Could Be Involved in Gametogenesis. Shunpei Kajita, Kouyou Akiyama, Michiko Hirose, Norumi Oguni, Atsuo Ogura, Takehito Tsui, and Tetsuo Kunieda. Okayama University, Okayama, Japan; RIKEN Bioresource Center, Ibaraki, Japan

The skeletal fusions with sterility (sks) is a mutation of mouse showing defects of gametogenesis and axial skeletal formation. Recently, we have identified a mutation of Tmem48 (Ndk1l) gene in the sks mutant mouse. To confirm that the mutation is responsible for the phenotypes of the sks mutant mouse, we attempted to rescue the phenotypes of the sks mutant mouse by using bacterial artificial chromosomes (BAC) transgenic mouse, containing a normal Tmem48 gene. As a result, the BAC transgene completely rescued phenotypes of the sks mutant mouse including defective gametogenesis. Thus, Tmem48 is confirmed to be the causative gene for sks. TME484 is highly conserved in eukaryotes and known for a member of nuclear pore complex (NPC), TME484 is predicted to be required for NPC assembly and binds to several NPC proteins in somatic cells. Since the sks mutant mouse show defects of gametogenesis, Tmem48 should have an essential role in gametogenesis in mouse. However, functions of Tmem48 and NPC in gametogenesis are still elusive. The purpose of this study is, therefore, to reveal the functions of Tmem48 in gametogenesis, and we attempted to identify proteins that bind to TME484 in the mouse testis. We employed yeast two hybrid system to screen TME484 binding proteins. For preparational of the yeast two hybrid screening, mouse testis cDNAs were cloned into bait and prey vectors, respectively. As a result of yeast two hybrid screening, we obtained 58 positive clones, and determined their nucleotide sequences. Consequently, 29 independent genes were identified as candidate genes for TME484 binding proteins. Some of these candidate genes encode proteins which could be involved in nucleocytoplasmic transport through nuclear pore complex (NPC). For example, the C. elegans cDNA and mouse testis cDNAs were cloned into bait and prey vectors, respectively. As a result of yeast two hybrid screening, we obtained 58 positive clones, and determined their nucleotide sequences. Consequently, 29 independent genes were identified as candidate genes for TME484 binding proteins. Some of these candidate genes encode proteins which could be involved in nucleocytoplasmic transport through nuclear pore complex (NPC). Some candidate genes and Tmem48 in various mouse tissues by RT-PCR. A significant part of these candidate genes were expressed strongly in testis, and some of them showed expression patterns similar to that of Tmem48. These findings suggested that Tmem48 is involved in germ cell specific nucleocytoplasmic transport.