



Title	Disruption of the Vad1.3 gene causes defective spermatogenesis and loss of preimplantation embryo
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and glycolysis genes fructose 1,6 bisphosphate aldolase (*4ALD*) and lactate dehydrogenase C (*LDH-C*) were more highly expressed in prepubertal derived oocytes ($P < 0.05$). No differences were found in the remaining analyzed transcripts. The differential expression patterns of genes involved in cholesterol synthesis, fatty 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.

391. GnRH Signals Through Calcineurin, NFAT and JUN to Regulate Expression of at Least Three Genes Required for Gonadotrope Function. April K. Binder, Jean C. Grammer, Maria K. Herndon, and John H. Nilson. Washington State University, Pullman, WA, USA

Gonadotropes of the anterior pituitary synthesize and secrete the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are essential for normal reproductive function and act on the ovary and testes to regulate steroidogenesis 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 $G_{\alpha q}$ 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 *Atf3*, *Jun* and *Egr1*. These IEGs confer hormonal responsiveness to the gonadotrope specific genes *Gnhr*, *Cga*, *Fshb* 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 L β T2 cells with either a Ca^{++} blocker, BAPTA-AM, or a calcineurin specific inhibitor, cyclosporin A, reduces the ability of GnRH to regulate the accumulation of *Atf3* and *Jun* mRNA while *Egr1* mRNA is not affected. Furthermore, pretreatment with thapsigargin, an intracellular Ca^{++} protein pump inhibitor, increases Ca^{++} and accumulation of *Atf3* and *Jun* mRNA in the presence of both vehicle and GnRH. Calcineurin also regulates the transcriptional activity of NFAT proteins. Additional data indicate that L β T2 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 API response elements suggesting that API responsive genes including *Jun* may also require NFAT. Together, these data suggest a functional link between GnRH, calcineurin, NFAT and a specific immediate early gene (*Jun*) that regulates expression of at least three genes required for gonadotrope function. These data also suggest that NFAT is a new member of the gonadotrope transcriptional network and may provide another input that confers hormonal responsiveness to several key genes required for gonadotropin synthesis and secretion. This work was supported by NIH R01 HD055776 to J.H.N.

392. Disruption of the *Vad1.3* Gene Causes Defective Spermatogenesis and Loss of Preimplantation Embryo. Jing Gao, Yan Zuo, William S.B. Yeung, and Kai-Fai Lee. The University of Hong Kong, Pokfulam, Hong Kong

Subfertility affects 10-15 percent of couples worldwide, and male factor is one of the dominant causes accounted for one third of these cases. Notably 40-90 percent male subfertility is due to idiopathic defective sperm production. Today, hundreds of genes have been shown to be involved in the regulation of spermatogenesis. Previously, we identified from the rat testis a novel acrosome-specific gene *Vad1.3* in a retinol-treated Vitamin A-deficiency (VAD) model. *VAD1.3* was expressed in the testes from postnatal day 25 and its immunoreactivity was localized to the acrosomal cap of the rat, mouse, human, monkey and porcine spermatids. To explore the functional role of *VAD1.3* in spermatogenesis in vivo, we used the gene-targeting approach to generate *Vad1.3* knockout mice. *Vad1.3* targeted allele was germline transmitted. No gross defect in spermatogenesis was found in the heterozygous mice testes. Crossing of heterozygous mice produced wild-type and heterozygous offsprings in a ratio of 1 : 2.6 ($n = 92$), but no *Vad1.3*^{-/-} offspring was found. There were no significant difference in corpus luteum numbers between the wild-type and heterozygous mice, suggesting these animals ovulated comparable number of oocytes. However, the number of implantation site on day 8 and 15 post coital (dpc) ($n = 7$) as well as the litter size ($n = 9$) was consistently lower in the crossing of the heterozygous (8.0 ± 0.8 ; 7.3 ± 0.8 ; 7.2 ± 0.6 , respectively) than the wild-type (9.1 ± 0.4 ; 8.3 ± 0.3 ; 8.1 ± 0.6 , respectively) mice. Since no resorption site was found on 8 dpc in both groups, the difference in implantation site may result from failure in fertilization, preimplantation embryonic development or implantation of homozygous knockout embryos. To study the time of loss of these homozygous embryos, genotyping of individual preimplantation embryos after whole genome amplification was performed. It was found that *Vad1.3*^{-/-} embryos existed at the zygotic and 2-cell stages but was absent at the blastocyst stage, suggesting that the *Vad1.3*^{-/-} embryos died before blastocyst formation. Interestingly, spermatogenesis in 9-14 months old heterozygous mice exhibited higher incidence of germ cell loss when compared with the control mice of the same ages (4/15 vs. 1/15, respectively). Yet, spermatogenesis were comparable between the wild-type and heterozygous mice on post-natal day 25, 35, 45 and 60. In sum, our results indicate that *VAD1.3* is critical for early

preimplantation embryo development and spermatogenesis in adult mice. The use of tissue-specific knockout approach may help to answer the functional role of *VAD1.3* in fertility. This project is supported in part by an RCG grant HKU7537/05M to KFL.

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

repro34 is an ENU-induced mutation in mice showing male-specific infertility caused by defective spermatogenesis. The homozygous mice (*repro34/repro34*) 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 *repro34*. 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/MII 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 previously revealed, was in concordance with the timing of the onset of multinucleation at pachytene stage. Since multinucleation occurs in pachytene spermatocytes, in which homologous chromosome pairing occurs, we examined to find any abnormalities in chromosome synapsis. 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 synapsis of homologous chromosomes was observed in the *repro34* 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 way as above. 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 homozygous germ cells cannot undergo normal cell division at meiosis I and possibly meiosis II as well. In addition, TUNEL assay revealed some multinucleated metaphase spermatocytes, but neither multinucleated pachytene spermatocyte nor round spermatid, were apoptotic, suggesting that multinucleated spermatocytes at metaphase might be excreted 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.

394. Cloning of Candidate Genes for TMEM48 Binding Proteins, Which Could Be Involved in Gametogenesis. Shimpei Kajita, Kouyou Akiyama, Michiko Hirose, Narumi Ogonuki, Atsuo Ogura, Takehito Tsuji, and Tetsuo Kunieda. Okayama University, Okayama, Japan; RIKEN Bioresource Center, Ibaraki, Japan

The skeletal fusions with sterility (*sk*s) is a mutation of mouse showing defects of gametogenesis and axial skeletal formation. Recently, we have identified a mutation of *Tmem48* (*Ndc1*) gene in the *sk*s mutant mouse. To confirm that the mutation is responsible for the phenotypes of the *sk*s mutant mouse, we attempted to rescue the phenotypes of the *sk*s 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 *sk*s mutant mouse including defective gametogenesis. Thus, *Tmem48* is confirmed to be the causative gene for *sk*s. *TMEM48* is highly conserved in eukaryotes and known for a member of nuclear pore complexes (NPC). *TMEM48* is predicted to be required for NPC assembly and binds to several NPC proteins in somatic cells. Since the *sk*s 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 *TMEM48* in the mouse testis. We employed yeast two hybrid system to screen *TMEM48* binding proteins. For preparation of the yeast two hybrid screening, mouse *Tmem48* cDNA and mouse testis cDNAs were cloned into bait and prey vectors, respectively. As a result of yeast two hybrid screening, we obtain 58 positive clones, and determined their nucleotide sequences. Consequently, 29 independent genes were identified as candidate genes for *TMEM48* binding proteins. Some of these candidate genes encode proteins which could be involved in nucleocytoplasmic transport through NPC or intracellular membrane fusion. Next, we examined expression of these 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.