발생과 생식 제 8권 제 1호, 35~42 (2004)
Dev. Reprod. Vol. 8, No. 1, 35~42 (2004)

Differentially Expressed mRNA Profiles between Immature Germinal Vesicle(GV) and Mature Metaphase II(MII) Mouse Oocytes

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미성숙 난자와 성숙 난자에서 서로 다르게 발현하는 유전자에 관한 연구

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ABSTRACT : Oocyte maturation refers to the process that prophase I arrested germinal vesicle(GV) drives the progression of meiosis to metaphase II(MII) to have the capacity for fertilization and embryo development. To better understand the molecular mechanism(s) involved in oocyte maturation, we identified differentially expressed genes(DEGs) between GV and MII mouse oocytes using a new innovative annealing control primer (ACP) technology. Using 20 ACPs, we successfully cloned 32 DEGs between GV and MII oocytes, and 26 out of these 32 DEGs were functionally known genes. Four genes including Pscd2 were GV-specific, 10 genes including PKD2 and CSN3 were highly expressed in GV oocytes(GV-selective), and 12 genes including Diva were highly expressed in MII oocytes (MII-selective). All of the genes identified in this study were first reported in the oocyte expression using ACP system and especially, we could characterize the existence of PKD-CSN signaling pathwayin the mouse oocytes. Results of the present study would provide insight for studying molecular mechanisms regulating oocyte maturation.

Key words : Oocyte maturation, Differentially expressed genes, Annealing control primer.

요 약 : 미성숙의 Germinal Vesicle(GV) 단계에서 성숙한 Metaphase II(MII) 단계가 되는 난자성숙 과정은 핵과 세포질의 성숙을 통해 이루어지며, 이를 통해 수정과 배 발달을 할 수 있는 능력을 갖게 된다. GV 난자는 prophase I 단계에 arrest 되어 있다가 meiosis 과정을 거쳐 성숙한 MII로 되는데 이를 조절하는 기작에 대해서는 거의 알려져 있지 않다. 따라서 본 연구는 미성숙 난자와 성숙 난자간의 유전자 발현의 차이를 동정함으로써 난자성숙에 관여하는 유전인자를 밝히고자 하였다. GV와 MII 난자에서 mRNA를 정제한 후 ACP System을 이용하여 두 그룹간의 유전자 발현 차이를 분석하여 양적으로 서로 다르게 발현하거나 한쪽에서만 특이적으로 발현하는 유전자를 cloning하여 Sequencing과 BLAST search를 통해 분석하였다. ACP 1번부터 20번까지를 사용하여 32개의 유전자를 찾았으며 이중 26개가 기능적으로 알려진 유전자였다. Pscd2를 포함한 4개의 유전자는 GV에 특이적으로 발현하였고, PKD2와 CSN3를 포함하는 10개의 유전자는 GV에서 더 높게 발현하였으며 Diva를 포함하는 12개의 유전자는 MII에서 더 높게 발현하였다. 본 연구를 통해 분석된 모든 유전자는 난자에서의 발현은 보고되지 않은 것으로 ACP System을 통해 최초로 동정되었으며 특히 PKD-CSN Signaling pathway가 난자에서 발현함을 알 수 있었다. 본 연구는 난자 성숙 과정에서 서로 다르게 발현하는 유전인자를 성공적으로 동정하였으며 향후 이들의 기능을 연구함으로써 난자성숙 조절기전을 연구하는데 기여할 것으로 사료된다.

INTRODUCTION

During oocyte maturation, oocytes at the germinal vesicle (GV) stage acquire competence to undergo nuclear and cytoplasmic maturation resulting in the formation of an egg having the capacity for fertilization and embryo development(Barnes & Sirard, 2000; Sirard, 2001; Ali & Sirard, 2002). Nuclear maturation refers to the processes reversing meiotic arrest at prophase I and driving the progression of meiosis to metaphase II(MII), while cytoplasmic maturation refers to the processes that prepare

This work was supported by grant No. R01-2003-000-10174-0 from the Basic Research Program of the Korea Science & Engineering Foundation (K.A.L.).

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the egg for activation and preimplantation development(Eppig, 1996). Until now, except for slight changes in the morphology of the nucleolus that is associated with the prematuration of oocyte capacitation event(Hyttel et al., 1997), it is truly difficult to find the cause or the mechanism of oocyte maturation. For oocyte maturation, the oocyte accumulates RNAs to drive fertilization and early stages of embryonic development and this molecular profile is a crucial part of oocyte maturation.

The identification of oocyte-specific genes or oocyte maturation-regulatory factors is now possible with the use of powerful tools, such as mRNA differential display RT-PCR(DDRT) analysis, cDNA library analysis, suppression subtractive hybridization(SSH), and cDNA microarry. Although efforts have been made to identify these genes, the methods employed to date have major drawbacks(Zeng & Schultz, 2003). For example, DDRT is labor intensive, has a high degree of false positives, and is biased toward detecting more abundant transcripts(Ma et al., 2001). Likewise, analysis of cDNA libraries derived from oocytes is only as robust as the quality of the libraries, in which rare transcripts may be underrepresented(Ko et al., 2000). To screen for differentially expressed gene(DEG) transcripts in low concentration, PCR amplification is required. PCR enables the amplification of minute amounts of material as available in oocytes and embryos. Thus using the PCR based techniques, only a few oocytes are required. We describe here the most accurate and extensive PCR technology controlled by annealing control primer(ACP; Seegene, Seoul, Korea). This primer anneals specifically to the template and allows only genuine product to be amplified, thus eliminating false-positive results. The ACP system is based on two principles: the unique tripartite structure of the primers, which have distinct 3'- and 5'-end regions that are separated by a polydeoxyinosine[poly(dI)] linker, and the interaction of each region during two-stage PCR(Hwang et al., 2003; Kim et al., 2004). Using this ACP system, we identified genes which were differentially expressed in GV and MII mouse oocytes, and this is the first report characterizing DEGs of GV and MII oocytes.

MATERIALS AND METHODS

1. Oocyte Collection

To identify the differential expressed genes between GV and MII oocytes, fully grown, GV-intact oocytes were obtained from 4-week-old female ICR mice 48 h after intraperitoneal injection of 5 IU of pregnant mare's serum gonadotropin(PMSG; Folligon, Intervet, Holland). The collection medium was TCM-199 medium (Gibco, Grand Island, NY) supplemented with 10 % fetal bovine serum(FBS; Gibco). Germinal vesicle breakdown was inhibited by including 0.2 mM 3-isobutyl-1-methyl-xanthine(IBMX; Sigma, St. Louis, MO) in the collection medium. For MII oocytes, female mice were injected with 5 IU of PMSG, followed by injection of 5 IU human chorionic gonadotrophin(hCG, Chorulon, Intervet) 48 h later. Superovulated MII oocytes were obtained 16 h after hCG injection. Cumulus cells surrounding MII oocytes were removed by treatment with hyaluronidase(300 U/ml in PBS).

2. mRNA Isolation and First-Strand cDNA Synthesis

Messenger RNA was isolated from the same number of GV and MII oocytes using Dynabeads mRNA DIRECT ki(Dynal, Oslo, Norway). Briefly, oocytes were resuspended in 300 μL lysis/binding buffer(100 mM Tris-HCl pH 7.5, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM DTT) for 5 min at room temperature. Prior to RNA extraction, 0.03 pg of rabbit α-globin mRNA per oocyte was added to normalize for RNA extraction and reverse transcription reaction efficiency(Ma et al., 2001; Zeng & Schultz, 2003). After vortexing, 20 μL of prewashed Dynabeads oligo$(dT)_{25}$ was mixed with the lysate and annealed by rotating for 5 min at room temperature. The beads were separated with a Dynal MPC-S magnetic particle concentrator and poly$(A)^+$ RNAs were eluted from the beads by incubation in 10 μL of Tris-HCl(10 mM Tris-HCl, pH 7.5) at 65℃ for 2 min, and the aliquot was used for reverse transcription.

For first-strand cDNA synthesis, the reaction was carried out by dT-ACP1, wherein the 3'-end core portion comprises a hybridizing sequence complementary to a poly A region of mRNA transcripts. Purified mRNA was incubated with 1 μL of dT-ACP1 at 80℃ for 3 min, then reverse transcription(RT) reaction was carried out in 50 mM Tris-HCl(pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM of each dNTPs, 20 U of RNase inhibitor, and 200 U of Superscript III(Invitrogen, Carlsbad, CA). The reaction mixture was incubated at 42℃ for 90 min and then, 94℃ for 2 min.

3. Amplification of cDNA Target Sequence

PCR was conducted using arbitrary ACPs to synthesize second-strand cDNAs under conditions that the 3'-end core portion of the dT-ACP2 is prevented from annealing to the first-strand cDNAs and only the 3'-end core portion of the arbitrary ACP comprising a hybridizing sequence sufficiently complementary to a region of the first-strand cDNAs is involved in annealing to the first-strand cDNAs. A two-stage amplification reaction was conducted in 50 μL reaction mixture comprising 20 mM Tris-HCl(pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 2 μL of arbitary ACP(5 μM), 1 μL of dT-ACP2(10 μM), and 2.5 U of *Taq* DNA polymerase(Gene-Craft, Munster, Germany). For the PCR reaction, 2 oocytes-equivalents of cDNA were used as template for each amplification using arbitrary ACP. We used the following PCR conditions: one cycle of 94℃ for 3 min, 50℃ for 3 min, and 72℃ for 1 min; followed by 40 cycles of 94℃ for 40 sec, 65℃ for 40 sec, 72℃ for 40 sec, and post-extension was done at 72℃ for 5 min. Following PCR, the amplified products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. Differentially expressed bands were identified and extracted using a gel extraction kit(QIAGEN, Valencia, CA) and cloned into a TOPO TA cloning vector(Invitrogen). The cloned DNA was sequenced and analyzed by BLAST search.

4. Quantitative Real-Time RT-PCR

To confirm the DEGs of selected genes of interest, quantitative real-time PCR was carried out with an equal number of GV and MII mouse oocytes. Three GV-specific or -selective genes such as Pscd2, PKD2, and CSN3 and 2 MII-selective genes such as Diva and TEMO were amplified and quantitated. Messenger RNA was extracted with the Dynabeads mRNA Direct Kit(Dynal) and first-strand cDNA was synthesized using Superscript III First- Strand Synthesis System(Invitrogen). After incubation of isolated mRNA with 0.5 μg oligo$(dT)_{12\text{-}18}$ primer at 65℃ for 5 min, the reaction was carried out in 50 μM Tris-HCl(pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 10 mM DTT containing 0.5 mM of each dNTPs, 20 U of RNase inhibitor, and 200 U of Superscript III mix in a final volume of 20 μL. The reaction mixture was incubated at 42℃ for 60 min and then, 75 ℃ for 15 min. For the PCR reaction, 2 oocytes-equivalents of cDNA were used as template for amplification of each gene with gene-specific primers listed in Table 2. Quantitative real-time PCR analysis was performed using MyiQ apparatus(Bio-Rad, Hercules, CA) and the result was evaluated by iCycler iQTM real-time detection system software. PCR reaction was carried out in template cDNA, 20 pmol of each primer, and 2X SYBR Green Supermix(Bio-Rad) containing 100 mM KCl, 40 mM Tris-HCl(pH 8.4), 0.4 mM each dNTP, 50 U/mL iTaq DNA polymerase, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluoresein, and stabilizers. Template was amplified by 40 cycles of denaturation at 95℃ for 40 sec, annealing at 57℃ for 40 sec, and extension at 72℃ for 40 sec. At the completion of PCR, a melt-curve was produced by monitoring fluorescence continuously with slow heating the samples from 60℃ to 95℃ at 0.5℃ intervals to identify nonspecific product. Quantitation of gene amplification was made by determining the cycle threshold(C_T) based on the fluorescence detected within the geometric region of the semi-log amplification plot. Relative quantitation of target gene expression was evaluated using the comparative C_T method as described previously(Hettinger et al., 2001). Expression of each mRNA species was normalized to the level of rabbit α-globin mRNA, and the ratio was calculated between GV and MII oocytes. The PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining.

5. Statistical analysis

Statistical analysis of the real-time RT-PCR data was evaluated using one-way analysis of variance(ANOVA) and a log linear model. Data were presented as mean±SEM derived from at least three separate and independent experiments and a value of $p<0.05$ was considered to be statistically significant.

RESULTS

Using ACP technology, we isolated differentially expressed bands between GV and MII oocytes and the PCR product sizes ranged from 200 bp to 2 kb. Fig. 1 shows representative GV and MII oocyte used for ACP-PCR and bands identified using each of the corresponding ACPs. We conducted PCR analysis using 20 primers(No. 1 to 20) out of 120 commercially available ACPs, and successfully cloned and identified the sequences of 32 DEGs using BLAST search. Among the 32 DEGs, 26 genes had strong homology with known genes while 6 were hypothetical proteins. As listed in Table 1, 4 genes including Pscd2 were GV-specific,

Table 1. List of differentially expressed genes obtained from ACP-PCR results

Description of genes	Gene Bank Accession No.
GV-specific genes(4/26)	
eukaryotic translation initiation factor 3, subunit 8(Eif3s8)	NM_019646
myomesin 2(Myom2)	NM_008664
platelet-activating factor acetylhydrolase isoform Ib γ subunit(PAF-AH γ)	U57746
plecstrin homology, Sec7 and coiled-coil domains 2(Pscd2)	NM_011181
GV-selective genes(10/26)	
COP9 complex, subunit 3(CSN3)	AF071313
DEAD(Asp-Glu-Ala-Asp) polypeptide 48(Ddx48)	NM_138669
Elongation factor 2(EF-2)	NM_007907
ELOVL family member 5, elongation of long chain fatty acids(Elovl5)	NM_0134255
membrane associated progesterone receptor component 2(MAPRC2)	AK014543
mitochondrial ribosomal protein L10(Mrpl10)	NM_026154
phospholysine phosphohistidine inorganic pyrophosphate phosphatase homolog	AK009207
protein kinase D2 homolog(PKD2)	AK037030
ribosomal protein, large, P1(Rplp1)	NM_018853
solute carrier family 25, member 15(Slc25a15)	NM_011017
MII-selective genes(12/26)	
Bcl-2 homolog(Diva)	AF067660
farnesyltransferase alpha subunit	D49744
female sterile homeotic-related gene 3(Fsrg3)	AF358660
general transcription factor IIB(Gtf2b)	BC016637
growth arrest specific 6(Gas 6)	NM_019521
malate dehydrogenase, soluble(Mor2)	NM_008618
mouse minichromosome maintenance 2(mMCM2)	D86725
palmitoyl-protein thioesterase 2(Ppt2)	NM_019441
sodium-dependent taurine transporter(Slc6a6)	L03292
suppressor of cytokine signaling 4(Socs4)	NM_018821
surfeit gene 5(Surf5)	BC018225
TEMO	AF296169

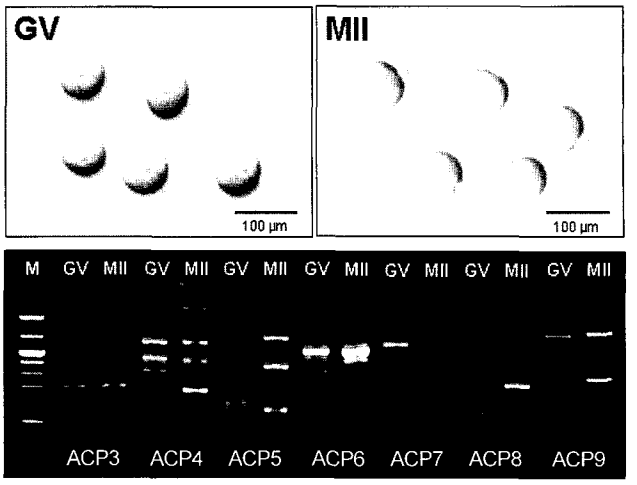

Fig. 1. Representative GV and MII oocytes and band patterns following ACP-PCR. mRNA was isolated from equal numbers of GV and MII oocytes and subjected to RT-PCR using ACP. The PCR products were separated by electrophoresis on 2% agarose gels. GV; germinal vesicle, MII; metaphase II, M; 100 bp DNA ladder.

10 genes including PKD2 and CSN3 were highly expressed in GV oocytes(GV-selective), and 12 genes including Diva were highly expressed in MII oocytes(MII-selective). We found no MII-specific gene with these 20 ACPs at this moment.

In the GV-specific or selective genes, Pscd2(pleckstrin homology, Sec7 and coiled-coil domains 2), PKD2 homolog (protein kinase D2 homolog), and CSN3(COP9 complex, subunit 3) have been reported to function in the axis of PKD(containing pleckstrin homology domain) - CSN signaling pathway(Sturany et al., 2001; Uhle et al., 2003). In the MII-selective genes, Diva and TEMO were functionally interested. Diva(Boo/Bcl-B) is highly expressed in the ovary and it is likely that cellular context will be important for Diva-regulated apoptosis(Inohara et al., 1998; Song et al., 1999; Russell et al., 2002). However, there is no report in the mRNA expression of Diva in oocytes. Interestingly, TEMO has been submitted to BLAST as a novel testicular marker characterized by cDNA library screening (Strausberg et al., 2002) without supportive data regarding the characterization of TEMO in any of cells or tissues including oocytes.

Differential expression of 5 genes between GV and MII was confirmed by quantitative real-time PCR analysis(Fig. 2). With real-time PCR, amplification can be monitored at each cycle to

Table 2. Sequences of oligonucleotide primers and their annealing temperature(AT) and expected RT-PCR product sizes

Genes	Gene Bank Accession No.	Oligonucleotide Sequences	AT (℃)	Size (bp)
Pscd2	NM_011181	F 5'-GCGAGAAGAGCTAAGTGAAG-3' R 5'-AGAGGTTCCTGAGAAGATCC-3'	58	596
PKD2	AK037030	F 5'-GGTTACAGGAGTACCAGACG-3' R 5'-ACTCAAGTTGTTAGGCTTGG-3'	58	436
CSN3	AF071313	F 5'-CAGCTGCCTAAATACACCTC-3' R 5'-GAGTCATCTTCTTGTGAGCC-3'	58	527
Diva	AF067660	F 5'-CTCTGTGACTAGGCAGATCC-3' R 5'-GTCTCTAGGCTGGAGGACTT-3'	60	551
TEMO	AF296169	F 5'-AGGGAGGATATGATAGAGGC-3' R 5'-GTGCAATAGTTCAGGGACTC-3'	58	558
Globin	V00875	F 5'-GCAGCCACGGTGGCGAGTAT-3' R 5'-GTGGGACAGGAGCTTGAAAT-3'	60	257

F, forward primer; R, reverse primer.

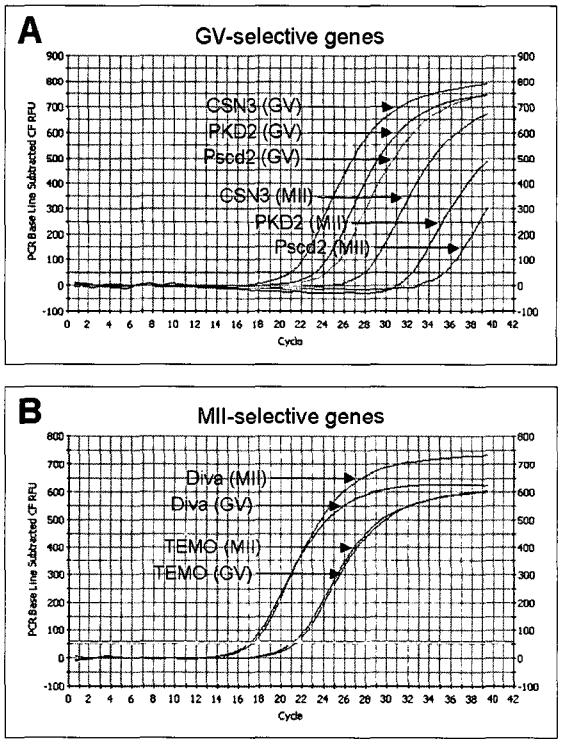

Fig. 2. Quantitative real-time PCR analysis with gene specific primer to confirm differential expression. A. Amplification profile of Pscd2, PKD2, and CSN3 between GV and MII mouse oocytes. B. Amplification profile of Diva and TEMO between GV and MII mouse oocytes. The C_T values wereas follow: Pscd2(GV = 24.27; MII = 35.44), PKD2(GV = 23.16; MII = 32.40), CSN3(GV = 20.91; MII = 27.92), Diva(GV = 17.7; MII = 16.5), and TEMO(GV = 23.5; MII = 22.6).

assess initial quantity, with much more precision and more sensitivity(Robert et al., 2002). The CT values were as follows: Pscd2(GV=24.27; MII=5.44), PKD2(GV=23.16; MII=32.40), CSN3(GV=20.91; MII=27.92), Diva(GV=17.7; MII=16.5), and TEMO(GV=23.5; MII=22.6). In each reaction, there was a single melting peak indicating the absence of nonspecific amplicon (data not shown). The result of each reaction was further confirmed by subjecting the PCR products to 1.5% agarose gel electrophoresis(Fig. 3A) and the relative mRNA expression was

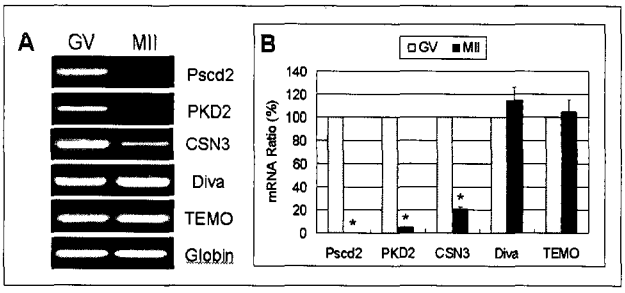

Fig. 3. Differential expression pattern of Pscd2, PKD2, CSN3, Diva, and TEMO. A. Real-time PCR products were subjected to 1.5% agarose gel separation for further confirmation. Rabbit globin mRNA was added before RNA isolation to serve as an external standard. B. Expression amount was calculated from C_T values and the mRNA ratio(%) was calculated against that of GV oocytes. Experiments were repeated at least three times and data were expressed as mean±SEM. Asterisks represent statistical significance at $p<0.05$.

calculated from C_T values(Fig. 3B). All of 3 GV-selective genes followed the ACP-PCR results, but 2 MII-selective genes, Diva and TEMO, showed only trends without statistical significance ($p<0.05$). This lack of significance may due to using external control(rabbit α-globin) rather than internal control for normalization(see discussion).

DISCUSSION

The present study was undertaken to better understand the molecular mechanisms involved in the developmental competence of mouse oocyte. According to our knowledge, this is the first report to identify differentially expressed genes between GV and MII oocytes. The physiological events that occur during oocyte maturation is still poorly understood and the study of oocyte maturation in mammals is progressively changing towards a more molecular approach. Two different approaches, the DDRT and SSH, were used to evaluate differentially expressed mRNA pools from two follicle size categories of bovine oocytes(Robert et al., 2000) and granulose cells(Robert et al., 2001). With the DDRT technique, the average clone size was 150~200 bp and all the sequences were located in 3'-UTR next to the poly(A) tail. Out of 24 series of DDRT comparisons, only 28 bands were considered and three clones showed strong homology with functionallyknown genes. With the SSH technique, the size of the insert ranged from 200 bp to 1.2 kb, with an average size of 550 bp. In that SSH study, 69 of 450 clones were true positive and only 2 of 96 clones for the reverse subtraction, and numerous clones remain unidentified.

Here we describe a novel PCR technique using ACPs that specifically target sequence hybridization to the template via poly(dI) linker(Hwang et al., 2003; Kim et al., 2004). The ACPs comprise 10-mer target sequences at their 3' ends and nontarget universial sequence tails at their 5' ends. The ACP linker in the hinge region prevents the 5' region from annealing under conditions in which the target 3' sequence anneals to the template at the original annealing temperature. This linker facilitates the targeting of the 3' sequences to be hybridized to the template under stringent conditions. This ACP system prevents the nonspecific sequences from annealing to the template due to the effects of the poly(dI) linker, resulting in a significant increase in annealing specificity.

To confirm the ACP results, we used quantitative real-time RT-PCR. To justify the variation observed, it requires a better endogenous standard to normalize the RNA expression and to assess mRNA recovery. Robert et al.(2002) measured several housekeeping genes, such as GAPDH, actin, ubiquitin, histone H2a, 18S rRNA, from GV stage to the blastocyst stage in bovine embryos using real-time PCR experiment. Only histone H2a mRNA expression was constant through the preimplantation period and most of them were differentially expressed according to the developmental stages of oocyte or embryo. In our experiment, we used exogenous rabbit globin mRNA to assess RNA recovery because many housekeeping genes such as GAPDH, actin, 18S rRNA including histone H2a expressed higher in GV than MII mouse oocytes(data not shown). This result is in keeping with some studies which have been reported that the amount of mRNA decreases sharply in the hours after beginning of maturation in mice(Bachvarova et al., 1985; Sirard et al., 2003). Consequently, the amount of mRNA available for study is very small and decreases progressively from the beginning of maturation to the maternal to zygotic transition. This highlights an important issue that the definition of a good endogenous standard remains to be clarified.

Using fairly reliable real-time PCR, we confirmed the differential expression patterns of functionally interesting 3 selected genes - Pscd2, PKD2, and CSN3. These 3 genes have been known as being in the same axis of signaling pathway. PKD2 is a novel phorbol ester- and growth factor-stimulated protein kinase(Sturany et al., 2001). The PKD2 mRNA is widely expressed in human and murine tissues and encodes an 878-amino acid protein that is most highly related to human PKCμ(69% identity). It contains a pleckstrin homology domain located between the duplex zinc-finger like motif, and a catalytic domain exhibiting all the characteristic sequence motifs of serine protein kinases. The catalytic domain exhibits the highest similarity to PKD/PKCμ(92% identity). It has reported that PKD is associated with the COP9 signalosome(CSN) through its subunit 3 (CSN3) (Uhle et al., 2003). The CSN is a multimeric complex that is conserved from yeast to man(Deng et al., 2000) andinvolved in regulation of the stability of proteins such as p27 (Tomoda et al., 1999), c-Jun(Naumann et al., 1999), p53(Bech-Otschir et al., 2001) and HY5(Hardtke et al., 2000), which are substrates of the Ub/26S proteasome system. Even though it is

revealed that the CSN is involved in development of Drosophila (Freilich et al., 1999), the exact function of the CSN has not been elucidated yet. Recently, it has been reported that the disruption of CSN subunit 2(CSN2) in mice caused deficient cell proliferation and early embryonic death(Lykke-Andersen et al., 2003). In $Csn3^{-/-}$ mice, CSN subunit 8 was not detected, suggesting that CSN3 is important for maintaining the integrity of the CSN and is crucial to maintain the survival of epiblast cells and thus the development of the postimplantation embryo in mice(Yan et al., 2003).

We also confirmed the differential expression of MII-selective genes such as Diva and TEMO. Diva(also called Boo/Bcl-B) is well known as a member of the Bcl-2 gene family and most likely functions in apoptosis(Inohara et al., 1998; Song et al., 1999). Diva is highly expressed in the ovary, and its pro- and anti-apoptotic functions have been ascribed. In the study of Diva-null mice, they were fertile, responded normally to apoptotic stimuli, and did not have any obvious developmental defects(Russell et al., 2002). However, no data is available about the capacity of oocyte maturation or fertilization in the Diva-null mice model. Moreover, any information on the Diva mRNA expression in oocyte is not available, even though it has been reported that Diva is highly expressed in the granulose cells of ovary by *in situ* hybridization(Inohara et al., 1998). However, we confirmed the expression of Diva mRNA in the oocyte by real-time PCR.

This is the first report of the TEMO mRNA expression in mouse oocytes. TEMO is only known as a novel testicular marker to study sertoli-germ cell interactions(Strausberg et al., 2002). The submitted sequence of TEMO is mostly matched to PA200 which was identified as a novel 200 kDa nuclear protein that activates the proteasome(Ustrell et al., 2002). However, the expression or specific function of TEMO was not elucidated in ovary or oocyte. In the result of real-time PCR, there was no significance in the MII-selective genes Diva and TEMO. However, when we consider the dramatic decrease of mRNA transcripts in MII oocytes after start the maturation process, slightly higher expression of these genes in MII compare to GV implies very large amount of these transcripts.

In conclusion, this is the first report identifying genes that are differentially expressed in GV and MII oocytes using the new ACP technology. The result of the present study with mouse model will be valuable in understanding the mechanism of oocyte maturation and will provide a basis for studies of human oocyte maturation.

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