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Phospholipid Transfer Protein (PLTP) mRNA Expression Is Stimulated by Developing Embryos in the Oviduct

Kai-Fai Lee,[1,2]* Ka-Leung Kwok,[1] Man-Kin Chung,[1] Yin-Lau Lee,[1] Judy F.C. Chow,[1] and William S.B. Yeung[1,2]

[1]Department of Obstetrics and Gynaecology, The University of Hong Kong, Pokfulam, Hong Kong, Peoples' Republic of China

[2]Center of Reproduction, Development and Growth, Hong Kong Jockey Club Clinical Research Centre, Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, Peoples' Republic of China

Abstract In mammal, fertilization and early preimplantation embryo development occurs in the oviduct. Evidence is accumulating that the oviductal epithelia secrete various biomolecules to the lumen during the secretory phase of the estrus cycle to enhance embryo development. This secretory activity of the oviduct is under the regulation of steroid hormones. Observations also suggested that the gametes and embryos modulate the physiology and gene-expressing pattern of the oviduct. However, the underlying molecular changes remain elusive. We hypothesize that the developing embryos interact with the surrounding environment and affect the gene expression patterns of the oviduct, thereby modulating the oviductal secretory activity conducive to the preimplantation embryo development. To test this hypothesis, suppression subtractive hybridization (SSH) was used to compare the gene expressions in mouse oviduct containing transferred in vitro cultured preimplantation embryos with that of oviduct containing oocytes during the preimplantation period. We reported here the identification and characterization of phospholipids transfer protein (PLTP), which is highly expressed in the embryo-containing oviduct and localized at the oviductal epithelium by in situ hybridization. PLTP contains signal peptide putative for secretory function. More importantly, PLTP mRNA increases in the oviductal epithelia of pregnant, but not pseudo-pregnant mice when assayed by real-time PCR. Taken together, our data suggested that PLTP may play important role(s) during in vivo preimplantation embryo development. This molecule would be a target to delineate the mechanisms and the roles of oviductal secretory proteins on early embryonic development. J. Cell. Biochem. 95: 740–749, 2005.

Key words: oviduct; embryo-containing; PLTP; signal peptide; in situ hybridization; real-time PCR

In mammals, fertilization and early embryonic development starts in the oviduct. It is known that the metabolic needs of preimplantation embryos change during development [Leese, 1995]. In order to cope with the changing needs of developing embryos, the environment of the maternal reproductive tract undergoes biochemical and physiological changes [Buhi et al., 2000; Yeung et al., 2002]. Evidence is accumulating that interaction exists between the embryo and the female reproductive tract before implantation [Downing et al., 2002; Lee et al., 2002; Wolf et al., 2003]. This interaction can be achieved via the paracrine growth factors (reviewed in [Hardy and Spanos, 2002]) and/or cytokines including interleukin-1 alpha [Yeung et al., 1996], insulin-like growth factor-binding proteins [Lai et al., 1996; Watson et al., 1999], vascular endothelial growth factor and receptors [Gabler et al., 1999], transforming growth factor-β [Chow et al., 2001], platelet-activating factor [Downing et al., 2002], embryotrophic factors (ETFs) [Mermillod et al., 1993; Liu et al.,

Abbreviations used: SSH, suppression subtractive hybridization; ISH, in situ hybridization; PCR, polymerase chain reaction; poly(A)+, polyadenylated.

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*Correspondence to: Kai-Fai Lee, PhD, Department of Obstetrics and Gynaecology, The University of Hong Kong, Pokfulam, Hong Kong. E-mail: ckflee@hkucc.hku.hk

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1995; Yeung et al., 2002; Lee et al., 2004], and other factor/peptide of unknown identity [Bauersachs et al., 2003].

The ratio of the ciliated cells to the secretory cells in the mammalian oviduct undergoes cyclic changes [Shirley and Reeder, 1996] and the ciliated cells may be transformed to secretory cells following loss of their cilia [Reeder and Shirley, 1999]. Oviductal biosynthetic activity is modulated by estrogen and progesterone. It is more active at the estrus stage in pig, sheep, and cow and at mid-cycle in human (reviewed in [Buhi et al., 2000]). Porcine oviductal epithelial cells secrete at least 14 major proteins into the culture medium [Buhi et al., 1990], and only five of these proteins have been identified, characterized and localized [Buhi et al., 2000]. These proteins include oviduct-specific glycoprotein, tissue inhibitor of metalloproteinase and plasminogen activator inhibitor-1. Interestingly, these proteins are expressed in a unique temporal and spatial pattern consistent with the existence of physiological interactions of the oviduct with the gametes at fertilization and with the embryos during early embryonic development [Buhi and Alvarez, 2003].

Studies on embryo-maternal interaction have been advanced by the use of both transcriptomic and proteomic approaches to delineate the role of oviduct on preimplantation embryo development [Wolf et al., 2003]. It has been reported that the hamster oviduct can distinguish the developing embryos from the oocytes [Ortiz et al., 1986] and embryos at different ages [Ortiz et al., 1989] as shown by the ability of the oviduct to transport the oocytes/embryos to the uterus at different rates. However, Freeman et al. [1992] demonstrated it was the embryos themselves that controlled the transport in mare. Yet, the molecular mechanisms that govern these changes remain unknown. Recently, Bauersachs et al. compared the gene expression patterns between the ipsilateral and contralateral bovine oviductal epithelial cells by suppression subtraction hybridization (SSH) at day 3.5 after standing heat. They identified a number of differentially expressed genes related to immunity or proteins involved in cell-cell interactions [Bauersachs et al., 2003]. These genes and their protein products would be the potential targets for us to understand the embryonic-maternal interaction in details [Barnea, 2001].

Previously, we demonstrated that oviductal cells affect the gene expression of developing embryos in vitro [Lee et al., 2001] partly via embryotrophic factor-3 [Lee et al., 2003]. The developing embryos, in turn, altered the gene expression in the mouse oviduct [Lee et al., 2000, 2002]. By comparing the gene expression in the oocyte-containing oviduct with that of the embryo-containing oviduct, we successfully isolated more than a dozen genes differentially expressed in the embryo-containing oviduct [Lee et al., 2002]. In the present study, we characterized the PLTP gene in the mouse oviduct and studied their expression during pregnancy. In long-term, detailed expression and functional characterization of the gene and its related secretory proteins should allow us to elucidate their roles in in vitro embryo development.

MATERIALS AND METHODS

Collection of Tissue

The oocyte-containing oviducts and embryo-containing oviducts of MF1 female mice were collected and subjected to SSH with the PCR-Select cDNA Subtraction Kit (Clontech Laboratories, Inc., Palo Alto, CA) as described previously [Lee et al., 2002]. In brief, embryos and oocytes were collected from the MF1 female mice 24 h post-hCG with or without mating, respectively. Ten to 12 1-cell embryos were transferred to one oviduct (embryo-containing oviduct) and a similar number of oocytes were transferred to the contralateral oviduct (oocyte-containing oviduct) of pseudo-pregnant female mice. The mice were killed at 48 h after embryos/oocytes transfer. The oviducts of the mice were removed and their content was flushed out. These oviducts were used for subsequent mRNA study when three to four cell embryos and oocytes were recovered from them if they were previously transferred with 1-cell embryos and oocytes, respectively. The oviducts from three mice of each group were collected and stored at $-70^{\circ}C$ until use. The research protocol was approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong. The oviduct from the estrus cycle (proestrus, estrus, metestrus, and postestrus), pregnant and pseudo-pregnant mice oviduct ($n = 5$) from day 1 to 4 were collected. Half of them were fixed in 4% paraformaldehyde in phosphate buffered

saline (PBS) for in situ hybridization, and the remaining halves were store at −70°C until use.

Messenger RNA Isolation

Poly(A) + RNA and total RNA from mouse tissues were isolated by QuickPrep Micro mRNA Purification kit (Amersham Biosciences, Piscataway, NJ) and TRIzol reagent (GIBCO BRL, Grand Island, NY), respectively, according to the manufacturer's instructions. The RNA was quantified by spectrophotometry and stored at −70°C until use.

Reverse Dot-Blot Analysis

Two hundred and fifty clones were screened by reverse dot-blot analysis as described [Lee et al., 2002]. The DNA inserts from the selected clones were amplified by PCR. In brief, a bacterial colony of 2 mm in size was picked and re-suspended in 50 μl water. Five microliter of bacterial suspension was mixed with 20 μl 1X PCR reaction buffer (10 mM Tris-HCl; pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% TritonX-100), 200 nM primers (NP1: 5′-TCGAGCGGCCGCCCGGGCAGGT-3′; NP2R: 5′-AGCGTGGTCGCGGCCGAGGT-3′), 200 μM dNTPs, and 5 U Taq DNA polymerase (Roche Molec. Biochem., Indianapolis, IN). PCR was carried out at 94°C for 10 min, then 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The PCR products (50 ng) were purified and dotted onto Hybond N + membranes (Amersham Pharmacia Biotech.) as described previously [Lee et al., 2000, 2002]. Non-subtracted cDNA probes from testers and drivers were ^{32}P-labeled using PCR-select Subtraction Hybridization Screening Kit (Clontech, Inc.) in the presence of [α−^{32}P]-dCTP (Dupont NEN, Boston, MA). The labeled probes were purified by spin column. The membranes were pre-hybridized with Rapid Hyb-buffer (Amersham Pharmacia Biotech.) for 2 h at 65°C. Hybridizations were performed at 65°C for 18 h in the Rapid Hyb-buffer containing radioactive probes (1×10^6 cpm). Membranes were washed once in 2 × standard saline citrate (SSC), 0.1% SDS, and once in 1 × SSC, 0.1% SDS at 45°C for 20 min each, then subjected to autoradiography (BioMax autoradiographic film, Eastman Kodak, Rochester, NY) for 12–48 h. The hybridization signals were analyzed using LabWorks analysis software (Ultra-Violet Products, Cambridge, UK). Three membranes were used for each probe and the signals were normalized with the β-actin house-keeping gene.

DNA Sequencing and Analysis

DNA Sequencing was performed using an automated 3730 DNA Analyzer (Perkin-Elmer, Applied BioSys, Foster City, CA) in the Genome Research Center (Core Facilities, The University of Hong Kong). Sequencing reactions were carried out with the T7 and SP6 primers. The sequences obtained were compared against GenBank/EMBL database using the online computer BLAST program. The in silico prediction of cDNA encoding secretory protein was carried out by online computer program SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) [Nielsen et al., 1997; Bendtsen et al., 2004].

Tissue Distributions

Northern blot analysis using cDNA probes on mouse tissues have been described previously [Lee et al., 2000, 2002]. Briefly, OD107 fragment (BC003782, nt 1358–1736) in pGEM-T Easy was PCR amplified and used for radiolabeling reaction. Normalized mouse cDNA panels (Clontech, Inc.) were used to examine the expression of the SSH clones in other mouse tissues. In brief, 5 μl of cDNA was mixed with 45 μl 1 × PCR reaction buffer (10 mM Tris-HCl; pH9.0, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% TritonX-100), 200 nM primers (Table I), 200 μM dNTPs, and 5 U Taq DNA polymerase (Roche Molec. Biochem., Indianapolis, IN). The PCR conditions were 26–38 cycles of 95°C for 15 s and 68°C for 5 min. The amplified PCR products were separated on a 2% agarose gel, stained with ethidium bromide and visualized by UV illumination.

In Situ Hybridization

Paraffin embedded mouse oviducts were sectioned at 5 μm thick and subjected to in situ hybridization using the mRNAlocator in situ hybridization kit (Ambion, Inc., Austin, TX). In brief, the dewaxed tissues were washed in PBS and treated with Proteinase K for 10 min at room temperature. The tissues were washed sequentially with PBS for 5 min, 1.32% triethanolamine for 3 min, triethanolamine with 0.25% acetic anhydride for 10 min, and PBS for another 5 min at room temperature. The probes for one of the oviductal clones, OD107, (sense and antisense, Table I) were generated using the SP6/T7 riboprobe combination system

TABLE I. Primer Sequences Used For PCR Analysis and Generation of Full-Length cDNA for In Situ Hybridization (ISH) Study

Target	Primer[a]	Sequence	Product size (bp)
PCR			
OD107	QPCR-5′	5′ CTTTGCTGAACGAGCGTACC 3′	212
	OD107-3′	5′ GA**GAATTC**AAGGCAGCTGCAGCAGAGGG 3′	
β-actin	Actin-5	5′ GTGCCCATCTACGAGGGCTATGCT 3′	319
	Actin-6	5′ TACCCAAGAAGGAAGGCTGGAAAA 3′	
GAPDH	GAPDH-5′	5′ ACCACAGTCCATGCCATCAC 3′	450
	GAPDH-3′	5′ TCCACCACCCTGTTGCTGTA 3′	
ISH			
OD107	OD107-5′	5′ GA**GAATTC**CATGGTCCTGCTCTGGGCC 3′	1496
	OD107-3′	5′ GA**GAATTC**AAGGCAGCTGCAGCAGAGGG 3′	

[a]Primers for OD107 (phospholipid transfer protein (PLTP), accession no. BC003782) flanked the region 71–1549 nt. An adaptor sequence (bold) containing an *Eco*RI restriction site (underlined) was added at the 5′-end of the primers.

(Promega, Madison, WI) and allowed to hybridize overnight with hybridization buffer containing probe at 1×10^6 cpm/slide. The slides were stored in a humidified chamber at 55°C. After hybridization, the slides were washed extensively to remove non-specifically bound riboprobe. Tissues were washed with $4 \times$ SSC buffer ($1 \times$ SSC containing 0.15 M NaCl, and 15 mM sodium citrate; and 1mM DTT) for 5 min at 55°C, followed by 2× SSC/1mM DTT for 30 min at 55°C, and then treated with RNase A (20 μg/ml) at 37°C for 15 min. Slides were then washed in 2× SSC/1mM DTT for 30 min at 55°C and then $0.1 \times$ SSC for another 30 min at 55°C before dehydrating in ethanol, and air-drying. RNase A resistant hybrids were detected after 1–2 weeks of autoradiography using Kodak NTB-2 (Amersham BioSciences) liquid emulsion. The slides were post-stained with hematoxylin. Tissues were examined with a Zeiss Axioskop microscope (Photometrics Sensys, Roper Scientific, Tuesoa, AZ) under bright- and dark-field optics.

Real-Time PCR

Total RNA template was used per 20 μl PCR with $1 \times$ SYBR Green I (Molecular Probes, Eugene, OR) and the Access Quick RT-PCR kit (Promega, Madison, WI) as described [Lee et al., 2001]. The primer sequences used is shown in Table I. Duplicate samples of 0.5 μl total RNA were amplified in the M x 3000P real-time PCR system (Stratagene, La Jolla, CA). Amplification conditions were as follows: 48°C for 45 min for cDNA synthesis; 95°C for 2 min to inactivate the reverse transcriptase; then followed by 45 cycles at 95°C for 30 s for denaturation, 60°C for 30 s for annealing, and 72°C for 45 s for polymerization. The relative amount of gene expression was calculated using the expression of β-actin as an internal standard. The PCR cycle number that generated the first fluorescence signals above a threshold value (threshold cycle [C_T]) was determined. Threshold was calculated as a value 10 times SDs (standard deviation) above the mean fluorescence generated during the baseline cycles. A comparative C_T method ($2^{-\Delta\Delta C_T}$ method) was used to detect relative gene expression [Aarskog and Vedeler, 2000; Livak and Schmittgen, 2001]. The following formula was used to calculate the relative amount of the transcript of interest in the treated sample (X) and the control sample (Y), both normalized to an endogenous reference (β-actin): $2^{-\Delta\Delta C_T}$, where ΔC_T is the difference in C_T between the gene of interest and β-actin, and $\Delta\Delta C_T$ is the difference for sample $X = \Delta C_{T,X} - \Delta C_{T,Y}$. Pregnancy day 1 or proestrus stage was used as calibrators for different PCR experiments.

Statistical Analysis

All results are expressed as means ± SEM. Statistical comparisons were performed by One Way Analysis of Variance (ANOVA) followed by Tukey test. A probability of $P < 0.05$ was used to indicate a significant difference.

RESULTS

Identification of Oviductal Clones Containing Secretory Signal Peptides

Two mouse oviduct cDNA libraries were constructed for the enrichment of transcripts preferentially expressed in the embryo-containing oviduct ($n = 3$) relative to the oocyte-containing oviduct. Two hundred and fifty randomly selected clones were dotted onto Hybond N + membranes, and probed with reverse transcribed ^{32}P-labeled cDNA from the

unsubtracted embryo-containing and oocyte-containing oviducts (Fig. 1). The expression levels were normalized with the housekeeping gene β-actin. Positive signals with higher expression in the embryo-containing oviductal samples were detected in 97 of 250 re-amplified clones (39%, Table II). All these putative positive clones from reverse dot-blot were further analyzed by DNA sequencing and compared with the GenBank/EMBL databases using the BLAST search. Sixty of 97 clones that showed >90% identity to known genes and contained full-length cDNA sequences in the databases were selected for SignalP 3.0 analysis. Using this program, we identified 17 of the 60 full-length clones containing signal peptides at the 5′-end of the coding sequences (Table II) and their corresponding signal peptide cleavage sites were shown (Table III). No signal peptide was identified for the remaining 43 clones.

Expression and Localization of OD107 in Mouse Tissues

We used both Northern blot and multiple tissue cDNA panels to determine the transcript sizes and the tissue distribution of the putative secretory clones. By using Northern blot analysis, the transcript sizes of 10 of the 17 oviductal clones were successfully determined (data not shown). One clone (OD107) that shares a high homology with phospholipid transfer protein (PLTP) from the GenBank database (accession no. BC003782) was highly expressed in the oviduct and moderately in the lung as demonstrated by the Northern blot analysis (Fig. 2A).

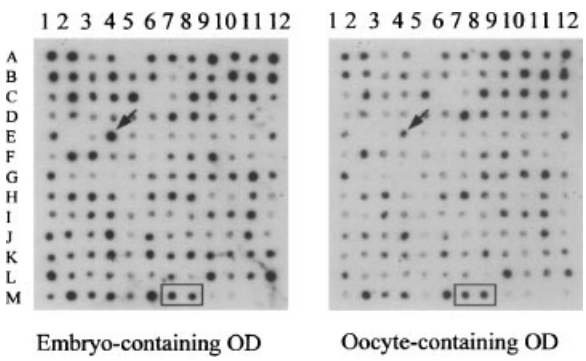

Fig. 1. A Representative reverse dot-blot analysis of the subtracted oviductal clones. Two identical membranes were hybridized with radioactive labeled probes prepared from unsubtracted embryo-containing and oocyte containing oviductal mRNA. A differentially expressed clone (E4) was marked with an arrowhead. β-actin gene was dotted onto the membrane as control for hybridization signals (boxed).

TABLE II. Summary of SSH Clones From Embryo-Containing Oviduct Containing Signal Peptides by SignalP Program

	Number
Number of clones sequenced[a]	97
Signal peptide containing proteins	17
Non-secretory proteins	43
Not determined[b]	30

[a]Seven clones contained no inserts or yielded poor sequence data.
[b]The full-length cDNA sequences of these clones were not found in the GenBank database and SignalP search were not performed.

OD107 had a transcript size of 1.8-kb. Using a more sensitive PCR method, OD107 was also detected in the heart and skeletal muscle. The expression levels of GAPDH were similar for all the cDNA samples studied in this experiment (Fig. 2B). SignalP 3.0 analysis determined the potential cleavage site OD107 of the signal peptide at amino acid position 18 (Table III). Using ISH, OD107 (PLTP) mRNA was localized mainly to the luminal epithelium of the cyclic mouse oviduct (Fig. 3A). Very weak or no hybridization signal was detected when the sense probe was used (data not shown). To quantify the expression of OD107 in different stages of the estrus cycle, real-time PCR was used. It was found that the expression levels of OD107 were similar throughout the estrus cycle (Fig. 3B) when β-actin gene was used as the PCR control.

OD107 Expression Is Up-Regulated in Pregnant Mouse Oviduct

The expression level of OD107 in pregnant and pseudo-pregnant mouse oviduct between days 1 to 4 were studied by real-time PCR (Fig. 4). There was a gradual increase (day 1–4) of the OD107 mRNA transcript in the pregnant oviduct (Fig. 4A) but not in the pseudo-pregnant (Fig. 4B) mouse oviduct. The increase reached statistically significant ($P < 0.05$) at day 4 when compared to the expression at day 1, confirming that the presence of embryos induced the expression of the OD107 gene in the oviduct.

DISCUSSION

In mammals, the steroid hormones affect the physiology of the oviduct which in turns provides suitable environment for fertilization, transport and development of the embryos. Recent studies reported that various

TABLE III. Identities of SSH Clones Containing Signal Peptides Analyzed by SignalP Program

Clone	Accession number	Identities	Homology (E-value)	Signal peptide cleavage site (a.a.)
OD1	NM_010145	Mus musculus epoxide hydrolase 1	1.00E-59	22
OD2	X53825	Mus musculus heat stable antigen	3.00E-21	27
OD14	AY029764	Mus musculus TRAM-1 mRNA	7.00E-98	47
OD17	AK004853	Mus musculus adult male liver cDNA	9.00E-81	22
OD18	NM_025516	Mus musculus serologically defined breast cancer antigen 84	2.00E-62	47
OD20	NM_007570	Mus musculus B-cell translocation gene 2	1.00E-79	29
OD68	J01420	Mus musculus mitochondrion (homology to nt 3459-3619)	2.00E-72	23
OD73	X14376	Mus musculus mRNA for ZP3	0.001	23
OD97	NM_009941	Mus musculus cytochrome c oxidase, subunit 4a	2.00E-75	26
OD107	BC003782	Mus musculus phospholipid transfer protein	1.00E-162	18
OD133	U47328	Mus musculus MHC class I heavy chain precursor	1.00E-150	22
OD135	NM_019910	Mus musculus demilune cell and parotid protein	2.00E-31	24
OD136	J01420	Mus musculus mitochondrion (homology to nt 15112-15282)	8.00E-74	49
OD145	BC012413	Mus musculus RIKEN cDNA 2700049I22	4.00E-16	17
OD202	AK002875	Mus musculus adult male kidney cDNA	1.00E-110	19
OD214	J01420	Mus musculus mitochondrion (homology to nt 3691-3429)	1.00E-108	23
OD233	U16818	Mus musculus UDP glucuronosyltransferase	2.00E-69	27

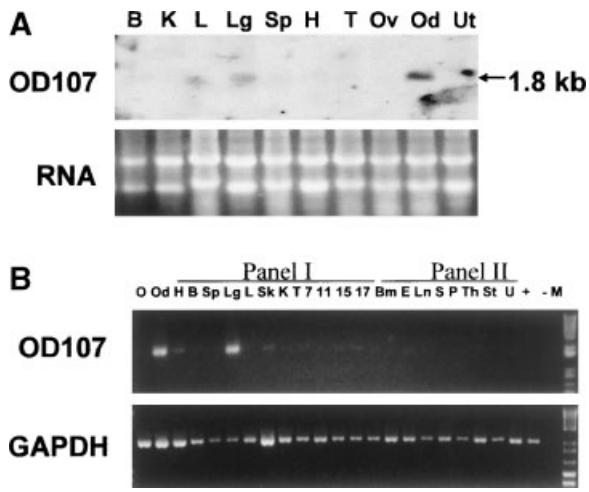

Fig. 2. Tissue distribution and localization of OD107 mRNA in mouse tissues. **A**: Northern blot analysis of the OD107 (PLTP) in mouse tissues. The membranes were hybridized with radioactive labeled cDNA probe prepared from the subtracted oviductal clone. The sizes of the transcripts were indicated on the right. The RNA gel stained with ethidium bromide was used as loading control for the representative membranes. B, brain; K, kidney; L, liver; Lg, lung; Sp, spleen; H, heart; T, testis; Ov, ovary; Od, oviduct and Ut, uterus. **B**: cDNA panels analysis of the OD107 in mouse tissues. The amplified PCR products from the mouse ovary, oviduct and multiple tissue cDNA panels (MTC I and II) were resolved in 2% agarose gel. The gel was stained with ethidium bromide and visualized by UV illumination. B, brain; Bm, bone marrow; E, eye; H, heart; K, kidney; L, liver; Lg, lung; Ln, lymph node; Ov, ovary; Od, oviduct; P, prostate; Sk, skeletal muscle; S, smooth muscle; Sp, spleen; St, stomach; T, testis; Th, thymus; U, uterus; 7, 7-day embryo; 11, 11-day embryo; 15, 15-day embryo; 17, 17-day embryo; +, mouse MTC II positive control; −, negative control and M, 1-kb plus DNA marker (Invitrogen). A housekeeping gene, GAPDH was used as PCR loading control.

biomolecules secreted by the oviduct enhance embryo development in vitro. On the other hand, sperm, oocyte and embryos may modulate the physiology and gene expressing pattern in the oviduct. We have previously demonstrated by SSH the differences in the gene expression patterns between the embryo-containing oviduct and the oocyte-containing oviduct of the same animals [Lee et al., 2002].

SSH and mRNA differential display (DDRT-PCR) are two commonly used techniques to compare gene expression profiles from two or more samples [Liang and Pardee, 1992; Diatchenko et al., 1996]. These techniques have been used successfully to identify differentially expressed genes from the oviduct [Lee et al., 2000, 2002] and preimplantation embryos [Natale et al., 2000; Lee et al., 2001, 2003; Ponsuksili et al., 2002; Yao et al., 2003]. In combination with cDNA microarray, SSH has been used to compare the gene expression patterns between the ipsilateral and contralateral bovine oviduct in the post-ovulation period [Bauersachs et al., 2003]. In our previous study, we used SSH and successfully isolated 97 clones that are differentially expressed in the embryo-containing oviduct [Lee et al., 2002]. In order to investigate whether the proteins encoded by these genes are secretory in nature, we used SignalP 3.0 program [Nielsen et al., 1997; Bendtsen et al., 2004] to search for signal peptide sequence for these genes. Compared with other prediction programs, SignalP provides the best performance (85% prediction in 1568 positive sets) and had been used for the signal sequence annotation in the SWISS-

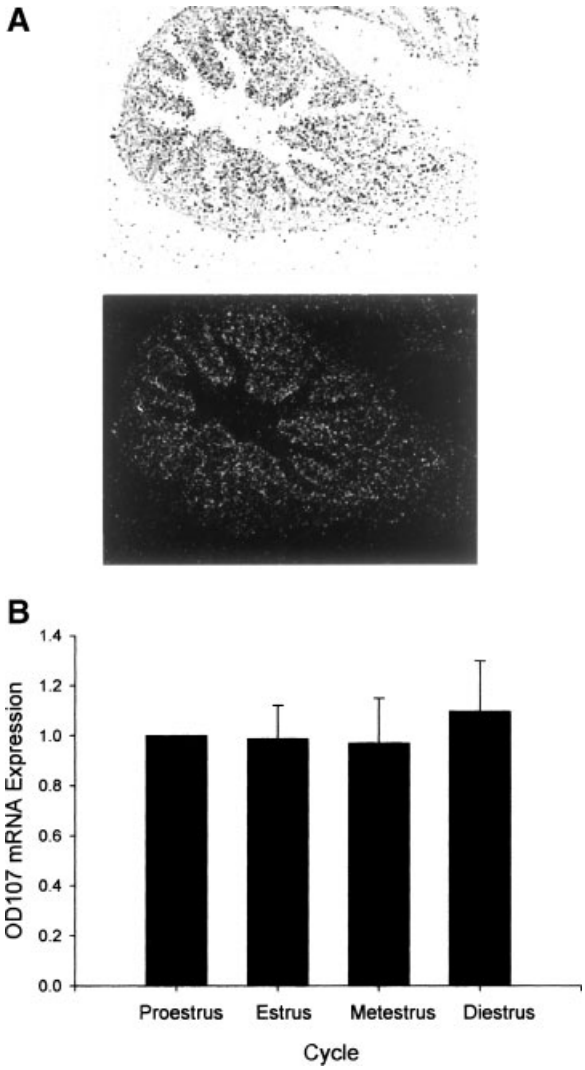

Fig. 3. Localization and expression of OD107 mRNA in oviduct. **A**: In situ hybridization of OD107 mRNA on mouse oviduct. OD107 mRNA was localized mainly to the luminal epithelium of the oviduct. Weaker signal was found in the stromal cell of the oviduct. The bright field (**top panel**) and the dark field (**bottom panel**) images of oviduct at estrus stage were shown. Magnification 200×. **B**: The relative expression OD107 in mouse uterus at different stages (proestrus, estrus, metestrus and diestrus) of the estrus cycle was quantified by real-time RT-PCR. The proestrus stage was arbitrary set to a value of 1. There was no significant change of OD107 throughout the cycle. The data were represented by mean ± SEM from three independent experiments.

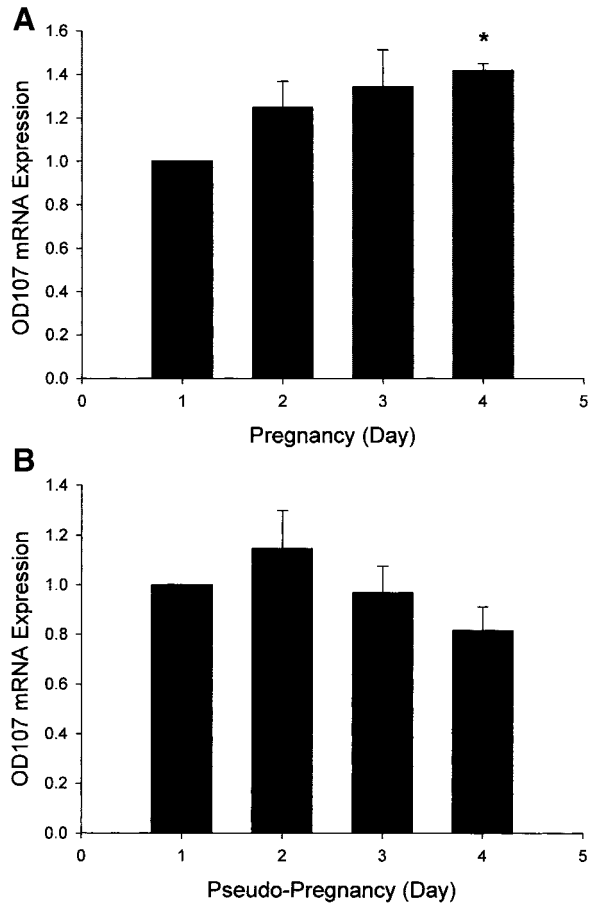

Fig. 4. Reverse transcriptase Real-time PCR of OD107 in pregnant and pseudo-pregnant mouse oviduct. **A**: Mouse oviducts (n = 5) from day 1 to 4 of pregnancy were collected and the expression of OD107 mRNA was quantified. There was a significant increase of OD107 at day 4 of pregnancy. **B**: The expression of OD107 mRNA in pseudo-pregnant mouse oviduct remained constant from day 1 to 4. The expression of OD107 at day 1 was arbitrary set to a value of 1. The data were represented by mean ± SEM from three independent experiments.

PROT and TrEMBL entries [Menne et al., 2000]. Seventeen of 90 oviduct expressing clones contain signal peptide at the 5′-end and their corresponding signal peptide cleavage sites were summarized (Table III).

Northern blot and cDNA panel analysis identified a number of clones that are highly expressed in the mouse oviduct (data not shown). One clone (OD107) which is highly homologous to PLTP (PLTP; accession no. BC003782) was further studied in this report. PLTP was first described as a plasma factor facilitating the transfer of phospholipid between lipoproteins and plays multiple roles in lipoprotein metabolism. Using Northern blot and cDNA panel analysis, the expression of PLTP was high in the oviduct and lung tissues (Fig. 2). It has been reported that the expression of PLTP mRNA in alveolar epithelial cells could be induced by hypoxia condition [Jiang et al., 1998]. Phospholipid transfer activity can also be found in the human ovary, placenta, testis [Albers et al., 1995], and seminal plasma [Masson et al., 2003]. In situ hybridization

study localized PLTP mRNA mostly at the oviductal epithelia. The levels of PLTP mRNA were similar at different stages of estrus cycles suggesting that steroid hormones had no effect on the expression levels of the gene. However, the presence of embryo stimulated the expression of PLTP mRNA in the oviduct of the pregnant mouse. The pseudo-pregnant animals had the hormonal profile as the pregnant animals but without the embryo did not show an increase in the gene expression. These observations further confirm that PLTP is not hormonally regulated.

PLTP in seminal plasma has been suggested to be involved in the capacitation process of spermatozoa [Masson et al., 2003]. However, the action of PLTP on embryo development remains to be elucidated. It is worth to note that phospholipids and their related substances have been implicated in embryo development. Platelet activating factor is another phospholipids with well known action in supporting early embryo development [Lu et al., 2003]. Lysophosphatidic acid stimulates cavitation of embryo [Kobayashi et al., 1994] and accelerates blastocyst differentiation [Liu and Randall, 2004]. Mouse blastocysts take up low-density lipoprotein and may metabolize it to produce cholesterol for steroid biosynthesis [Sato et al., 2003]. These reports warrant further investigation on the role of PLTP in embryo development.

Recently, Buhi and Alvarez [2003] reported identification of three secretory proteins from the porcine oviduct by affinity chromatography and N-terminal amino acid analysis. These proteins were identified as complement component C3b, the carboxy-terminal propeptide of alpha 1 (III) procollagen (PIICP), and the heavy chain variable region of IgA. More importantly, the expression of these proteins has temporal and spatial differences in the oviduct at estrus and during early pregnancy, suggesting that they may play a critical role in protecting the luminal environment, participating in extracellular matrix remodeling and gamete interactions [Buhi and Alvarez, 2003]. Conjointly, our laboratory first reported the purification of embryotrophic factor-3 (ETF-3) from human oviductal cells [Liu et al., 1995]. ETF-3 could enhance embryo development in vitro [Xu et al., 2001; Lee et al., 2003] and it was subsequently identified as C3-like molecules. Mouse embryos incubated with C3b and/or iC3b have large size and higher hatching rate in vitro [Lee et al., 2004]. In line with this, it would be interesting to see if PLTP have the similar effect on embryo development in vitro.

In conclusion, the oviduct provides an optimal microenvironment for the development of embryo partly by the production of growth factors and/or proteins. The identification of oviductal mRNA coding for proteins containing signal peptides in silico, their up-regulation (e.g., PLTP) in the presence of developing embryos, and their localization in oviductal epithelium strongly suggested that these proteins may play important roles on preimplantation embryo development. Detailed expression and functional characterization of these proteins would be important to elucidate their roles on embryo development in future.

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