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Molecules and Cells

Quantitative Analysis of Retromer Complex-Related Genes during Embryo Development in the Mouse

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The retromer complex is a heteropentameric protein unit associated with retrograde transport of cargo proteins from endosomes to the *trans*-Golgi network. Functional silencing study of the Vps26a gene indicated the important role of the retromer complex during early developmental stages in the mouse. However, individual expression patterns and quantitative analysis of individual members of the retromer complex during the early developmental stages has not been investigated. In this study, we conducted quantitative expression analysis of six retromer complex genes (Vps26a, Vps26b, Vps29, Vps35, Snx1, and Snx2) and one related receptor gene (Ci-mpr) during the eleven embryonic stages with normal MEF (mouse embryonic fibroblast) and Vps26a$^{-/-}$ MEF cells. Remarkably, except for Vps26a (maternal expression pattern), all tested genes showed maternal-zygotic expression patterns. And five genes (Vps26b, Vps29, Vps35, Snx2, and Ci-mpr) showed a pattern of decreased expression in Vps26a$^{-/-}$ MEF cells by comparative analysis between normal MEF and Vps26a$^{-/-}$ MEF cells. However, the Snx1 gene showed a pattern of increased expression in Vps26a$^{-/-}$ MEF cells. From our results, we could assume that retromer complex-related genes have important roles during oocyte development. However, in the preimplantation stage, they did not have significant roles.

INTRODUCTION

The retromer is a well-conserved heteropentameric complex with a protein sorting function from the endosome to the *trans*-golgi network (TGN), which mediates retrograde transport of transmembrane proteins by vesicle transport (Bonifacino and Hurley, 2008; Seaman, 2005). Well-known transmembrane proteins associated with protein sorting mechanisms include the acid hydrolase receptor, vacuolar protein sorting 10 (Vps10) in yeast *Saccharomyces cerevisiae* (Seaman et al., 1998), and two distinct mannose 6-phosphate receptors (MPR): cation-dependent MPR (Cd-mpr), and cation-independent MPR (Ci-mpr) in mammals (Arighi et al., 2004; Seaman, 2004). The common role of these receptors in the endomembrane system is transport of newly synthesized acid hydrolase from the TGN to the endosome for proper delivery to the vacuole and lysosome (Arighi et al., 2004).

The mammalian retromer complex consists of two functionally distinct sub-complexes, Vps26 - Vps29 - Vps35 heterotrimer for cargo recognition and sorting of nexin (Snx1) - Snx2 homo or hetreodimer for membrane targeting (Seaman, 2005). The Vps35 molecule is a core protein for assembly of the heterotrimer in the cargo recognition sub-complex (Collins, 2008; Seaman, 2005). The Vps35 interacts directly with the cytoplasmic tail of Ci-mpr within a specific domain. Vps35 has direct interaction with Vps26 and Vps29 through its N- and C-terminal, respectively (Hierro et al., 2007; Shi et al., 2006). Mutation analysis of specific residues of Vps35 molecules indicated that Vps35 plays a crucial role in localization of Ci-mpr (Zhao et al., 2007). Vps26, another key member of the retromer complex is a well-investigated molecule for embryo development. In a previous study, a functional silencing experiment for Hβ58 in mice showed Hβ58 (also known as mVps26) derived embryonic lethality at the postimplantation stage (Bachhawat et al., 1994; Lee et al., 1992; Radice et al., 1991). An additional Vps26 gene, termed Vps26b, was recently identified by bioinformatic analysis of the mouse genome (Kerr et al., 2005). The study showed similar protein sequences and function in the retromer complex. Vps26b associates with the retromer complex, interacting with Vps35 of the retromer core protein. And a localization study of Vps26b in the endosome also showed the same results with Vps26. These findings indicate that two different isoforms of Vps26 (Vps26 and Vps26b) might form the independent retromer complex (Collins et al., 2008). Thereafter, the previously identified Vps26 molecule was renamed Vps26a. However, the specific function and structure of each Vps26 isoform in the retromer complex have not been fully characterized. The membrane targeting sub-complex of the retromer complex consists of the Snx1 and Snx2 molecules. Typical features of Snxs are constituted by the phox domain (PX), which binds phosphatidylinositol 3-phosphate (PtdIns3P or

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PI3P), phoinositides, and a Bin-amphiphysin-Rvs domain (BAR) next to the PX domain of the C-terminal, which mediates dimerization and binding to highly curved membranes (Rojas et al., 2007; Worby and Dixon, 2002; Zhong et al., 2005). These domains are used for targeting of the retromer complex for binding of endosomal membranes.

The majority of research has concentrated on the specific functions of the retromer complex in the sorting mechanism of acid hydrolase receptors. However, recent studies have reported on the various functions of the retromer complex, including regulation of amyloid β peptide production in Alzheimer disease (Muhammad et al., 2008; Small, 2008; Small et al., 2005), secretion of Wnt morphogens and generation of Wnt gradients during embryo development (Belenkaya et al., 2008; Port et al., 2008), transcytosis of polymeric immunoglobulin receptor (PIgR) (Verges et al., 2004), and apoptotic cell clearance by phagocytic receptor recycling (Chen et al., 2010). Most of these processes are dependent on retromer-mediated transport for recycling of its receptors.

Insertional mutagenesis of Vps26a (Radice et al., 1991) and double knock-out of Snx1 and Snx2 (Schwarz et al., 2002) genes induced embryonic lethality in mice. And the importance of Ci-mpr for late embryonic development and growth regulation was reported by knock-out of the Ci-mpr gene (Wang et al., 1994). Moreover, siRNA induced knock-down experiments with Vps26a resulted in reduction of Vps35, Vps29, and Ci-mpr at the protein level (Arighi et al., 2004; Seaman, 2007). From these results, we could assume two different hypotheses. One is that of the specific roles of the retromer complex during the early developmental stages. Others are the specific roles of Vps26a molecules, which could affect the expression activity of other retromer-related molecules. However, individual expression patterns and quantitative analysis of retromer complex-related genes have not been performed during the early developmental stages of the mouse. Therefore, to investigate the function of retromer complex-related genes in mouse oocytes and preimplantation embryos, as well as the specific roles of the Vps26a gene, quantitative expression analysis of retromer-related genes using normal mouse embryos and Vps26a$^{-/-}$ MEF cells using real-time RT-PCR are required.

In this study we investigated quantitative expression analysis of retromer complex-related genes and one target receptor in mouse oocytes and preimplantation embryos using real-time RT-PCR. In addition, to determine whether knock-out of the Vps26a gene is involved in transcriptional regulation of other retromer complex-related genes and receptors, comparative analyses of quantitative expression levels between normal MEF and Vps26a$^{-/-}$ MEF cells were also conducted.

MATERIALS AND METHODS

Mouse embryo collection

All experiments involving animals were approved by and performed in strict accordance with the guidelines of the appropriate institutional animal care and use committees (KRIBB-AEC-09142). C57BL/6J female mice were super-ovulated with 5 IU of pregnant mare serum gonadotropin (PMSG; Sigma-Aldrich), followed by 5 IU of human chorionic gonadotropin (hCG; Sigma-Aldrich) 48 h apart and mated with male C57BL/6J mice. Successful mating was determined on the following morning by detection of a vaginal plug. Fertilized embryos were collected from the oviducal ampullae at 18 h after injection of hCG. Cumulus cells were removed by a brief exposure to 0.1 mg/ml of highly purified hyaluronidase (Sigma-Aldrich) in M2 medium (Sigma-Aldrich). Harvested one cell embryos were cultured in M16 (Sigma-Aldrich) medium at 37°C, 5% CO_2 in air, and were collected at given stages (Supplementary Fig. S1).

Cell culture

In a previous study, the recessive lethal mutation of the Vps26a transgenic mouse line was established by insertional mutagenesis (Radice et al., 1991). From Kazuhiko Imakawa, we donated Vps26a heterozygous mice and its Vps26a$^{-/-}$ MEF cells. MEF (Mouse Embryonic Fibroblast) and Vps26a$^{-/-}$ MEF cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) containing 10% fetal bovine serum (FBS), and were grown at 37°C and 5% CO_2 in a cell culture incubator.

Total RNA isolation and cDNA preparation

Total RNA was extracted from mouse oocytes and preimplantation embryos using the RNeasy Plus Mini kit (Qiagen), according to the manufacturer's instructions. Also, total RNA was extracted from MEF and Vps26a$^{-/-}$ MEF cell-lines using Trizol reagent (Invitrogen). The turbo DNA-free™ kit (Ambion) was used for eradication of DNA contamination in total RNA preps. Total RNA was quantified using a NanoDrop® ND-1000 UV-Vis Spectrophotometer. M-MLV (Moloney-Murine Leukemia Virus) reverse transcriptase with an annealing temperature of 42°C was used for the reverse transcription reaction with RNase inhibitor (Promega). We performed PCR amplification without the reverse transcription reaction using pure RNA samples and determined that the prepared mRNA samples did not contain genomic DNA.

Primer design and standard curve analysis

For development of specific primers for seven retromer complex-related genes, different primer pairs were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3/) (Table 1) (Rozen and Skaletsky, 2000). BLAST searches were performed to confirm the total gene specificity of the primer sequences, and the results showed the absence of multi-locus matching at the individual primer sites. All primers spanned at least two exons in order to avoid false positive amplification by genomic DNA contamination in RNA samples (Supplementary Fig. S2). Amplification efficiencies and correlation coefficients (R2) of seven retromer complex-related genes were generated using the slopes of the standard curves obtained by serial dilutions. Standard curves with a 10-fold dilution series were used for calculation of amplification efficiency (Table 2). Amplification efficiency was calculated according to the formula: efficiency (%) = $(10^{(-1/\text{slope})} - 1) * 100$. The efficiency range of the real-time RT-PCR amplifications of all tested genes was 84-105%.

Real-time RT-PCR amplification

SYBR green real-time RT-PCR was performed on a Rotor Gene Q (Qiagen). In each run, 1 ul of cDNA was used as template for amplification per reaction. Samples were added to 19 ul of reaction mixture containing 7 ul H2O, 10 ul QuantiTect® SYBR® Green PCR Master Mix (Qiagen), and 1 ul forward and reverse primers. Real-time RT-PCR amplification of the retromer complex-related genes was carried out for 50 cycles of 94°C for 10 s, 60°C for 15 s, and 72°C for 15 s. Amplification specificity for each real-time RT-PCR analysis was confirmed by melting curve analysis. The temperature range for analysis of melting curves was 55°C to 99°C for 5 s. As shown in Supplementary Fig. S3, each primer pair showed a single, sharp peak, indicating that the primers amplify only one specific PCR product. Primer dimers were not observed. In all experiments, normalization factor (NF) of Hprt1, H2afz, and Ppia as reference genes was used in analyses of relative mRNA expression

Table 1. Primers for Real-Time RT-PCR

Gene	Forward Primer Sequence [5′ → 3′]	Position in cDNA	Reverse Primer Sequence [5′ → 3′]	Position in cDNA	Product size
Vps26a	GAAGTGGGCATTGAAGACTG	5th Exon	GTGCTGGGTCCAATTCCTG	6, 7th Exon	170 bp
Vps26b	GGACAGAATGTGAAGCTCCG	2th Exon	CAATCCTCAATGCCAACTTC	3th Exon	152 bp
Vps29	CGTCCACATCGTGAGAGGAG	2th Exon	TGTCCTGAGATAAGAATGTCCAC	3th Exon	180 bp
Vps35	AACACAGAAATCGTCTCTCAGG	11th Exon	CAGATGAATAAATCGGCCAAC	12th Exon	150 bp
Snx1	CCCTTACTTCTCATCCTCCG	3th Exon	CATAGGCATTCATACCATCCC	5th Exon	150 bp
Snx2	GATCTTTTCGCAGAAGCCAC	2, 3th Exon	CTTCAATCTCGTCCCTGGAT	3, 4th Exon	184 bp
Ci-mpr	TCCTTGTGGTGGGAATAAGAC	42th Exon	GTTGGTGAGCGTTCCGTT	43th Exon	184 bp
Hprt1	GCTTGCTGGTGAAAAGGACCTCTCGAAG	7th Exon	CCCTGAAGTACTCATTATAGTCAAGGGCAT	8th Exon	117 bp
H2afz	ACAGCGCAGCCATCCTGGAGTA	3th Exon	TTCCCGATCAGCGATTTGTGGA	5th Exon	202 bp
Ppia	CGCGTCTCCTTCGAGCTGTTTG	2th Exon	TGTAAAGTCACCACCCTGGCACAT	3th Exon	150 bp

Table 2. Slope, r2 Values, and Efficiency by Standard Curve in Real-Time RT-PCR

Gene	Efficiency	R^2	Slop
Yps26a	0.92	0.99072	-3.528
Yps26b	0.84	0.99772	-3.792
Yps29	0.94	0.99967	-3.469
Yps35	0.90	0.99272	-3.583
Snx1	0.99	0.99493	-3.342
Snx2	1.05	0.99035	-3.203
Ci-mpr	0.93	0.99938	-3.493

(Mamo et al., 2007). Three independent experiments were performed.

RESULTS

Quantitative expression analysis of retromer complex-related genes in mouse oocytes and preimplantation embryos

Using the specific primer pairs of seven retromer complex-related genes (Vps26a, Vps26b, Vps29, Vps35, Snx1, Snx2, and Ci-mpr), real-time RT-PCR amplification was performed during eleven different developmental stages [germinal vesicle (GV), MII, 1-cell, 2-cell, 4-cell, 8-cell, morula, blastocyst, inner cell mass (ICM), 7.5 days and 10.5 days] (Fig. 1). In general, similar expression patterns were observed in six retromer complex-related genes (Vps26b, Vps29, Vps35, Snx1, Snx2, and Ci-mpr), except for the Vps26a gene (Fig. 1). In the case of the Vps26a gene, dominant expression patterns were observed in germinal vesicle, MII oocytes and 1-cell, 2-cell preimplantation stages (Fig. 1A). However, its expression level was nearly depleted at the 4-cell stage. Also, its expression level had not changed at the 4-cell to 10.5 day stage. The Vps26b gene showed a pattern of increased expression from germinal vesicle oocyte to 2-cell stage and it showed a pattern of decreased expression at blastocyst stage (Fig. 1B). However, after blastocyst stage to 10.5 day stage, transcription activity of the Vps26b gene increased. The high expression level of the Vps29 gene was observed in the germinal vesicle oocyte and1-cell stage (Fig. 1C). However, expression level of Vps29 was very low in the MII stage and not detected at 2-cell stage. After 2-cell stage, it showed a continual increase to 10.5 day stage. The Vps35 gene showed a pattern of increased expression from the germinal vesicle to the 1-cell stage and its expression level was not detected at the 2-cell stage (Fig. 1D). However, it showed a pattern of increased expression from the 4-cell to the 10.5 day stage. The Snx1 gene showed dominant expression at the 1-cell stage and the transcript level showed a pattern of decreased expression at the morula stage (Fig. 1E). Thereafter, its level increased to 10.5 day stage. The expression level of Snx2 increased continuously at germinal vesicle oocyte to 1-cell stage and was nearly depleted at 2- and 4-cell stage (Fig. 1F). Although expression level of the Snx2 gene at blastocyst was higher than at front and rear stage, its level was increased at 10.5 day stage. A high level of expression of the Ci-mpr gene was observed in germinal vesicle, MII, at the 7.5 day and 10.5 day stages; however, it was not detected in the 1- and 2-cell stages (Fig. 1G). The Ci-mpr gene showed a low level of expression and a pattern of increased and decreased expression at the 4-cell stage to inner cell mass, and its expression level showed a considerable increase at the 7.5 and 10.5 day stages.

Comparative quantification analysis of retromer complex-related genes in Vps26a$^{+/+}$ MEF and Vps26a$^{-/-}$ MEF cells

Examination of knock-out phenotypes of the mouse is one of the best methods for investigation of specific gene functions. To date, knock-out mice of the Vps26a, Snx1, Snx2, and Ci-mpr genes have been reported among retromer complex-related genes (Radice et al., 1991; Schwarz et al., 2002; Wang et al., 1994). In this study, we used Vps26a$^{-/-}$ MEF (mouse embryonic fibroblast) cells and Vps26a$^{+/+}$ wild-type (normal) MEF cells. We compared the transcription activity of retromer complex-related genes between Vps26a$^{+/+}$ wild-type (normal) MEF and Vps26a$^{-/-}$ MEF cells. Validation of the absence of the Vps26a gene in Vps26a$^{-/-}$ MEF cells was conducted by RT-PCR experiments (Fig. 2A).

Real-time RT-PCR amplification was applied for the purpose of quantitative comparison of retromer complex-related genes between Vps26a$^{+/+}$ wild-type (normal) MEF and Vps26a$^{-/-}$ MEF cells (Fig. 2B). Six retromer complex-related genes, including Vps26b, Vps29, Vps35, Snx1, Snx2, and Ci-mpr were used for comparative analysis. Of particular interest, we observed conflicting expression patterns between major groups of Vps26b, Vps29, Vps35, Snx2, and Ci-mpr genes and minor groups of Snx1 genes. In these results, we designated that the major group showed a pattern of decreased expression and that the minor group showed a pattern of increased expression in Vps26a$^{-/-}$ MEF cells (Fig. 2B). Specifically, dramatically decreased expression patterns of Vps26b (2.5 fold) and Ci-mpr genes (5.2 fold) were observed in Vps26a$^{-/-}$ MEF cells. However, minor groups of the Snx1 gene (approximately 13.5 fold) showed increased expression patterns in Vps26a$^{-/-}$ MEF cells.

DISCUSSION

Due to its specificity, accuracy, and broad range of applications, real-time RT-PCR amplification is an abundantly used and technically validated method for quantification of gene expres-

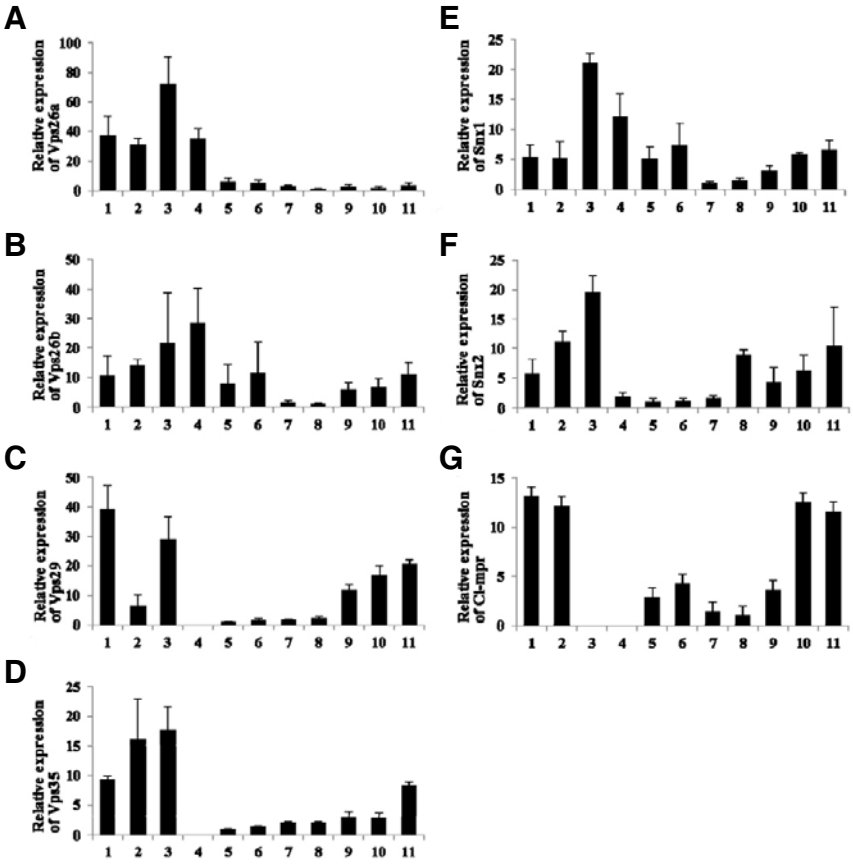

Fig. 1. Quantitative expression analysis of retromer complex-related genes in oocytes and preimplantation embryos. In the figure, (A, B, C, D, E, F, and G) correspond to the expression analysis of each retromer complex-related gene Vps26a, Vps26b, Vps29, Vps35, Snx1, Snx2, and Ci-mpr, respectively. Each value represents the mean ± SD. 1, Germinal vesicle (GV); 2, MII; 3, 1 cell; 4, 2 cell; 5, 4 cell; 6, 8 cell; 7, Morula; 8, Blastocyst; 9, Inner cell mass; 10, 7.5 day; 11, 10.5 day.

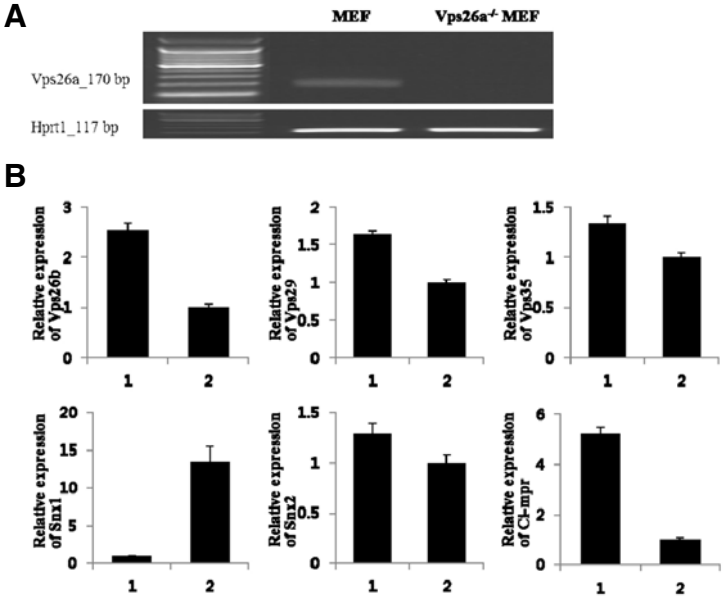

Fig. 2. Expression of retromer complex-related genes in mouse embryonic fibroblast (MEF) and Vps26a$^{-/-}$ MEF cells. (A) Vps26a transcript was not detected in Vps26a$^{-/-}$ MEF cells. (B) Relative expression of retromer complex-related genes. Each value represents the mean ± SD. 1, MEF; 2, Vps26a$^{-/-}$; 3, MEF.

sion (Vandesompele et al., 2002). Hence, it is a very powerful technique for quantification of gene expression during mammalian embryonic developmental stages. However, to obtain accurate gene expression data from various embryonic developmental stages using real-time RT-PCR, suitable reference genes should be determined prior to normalization of gene expression data. In mouse embryonic developmental stages, Ppia, H2afz, and Hprt1 genes were selected as the most stable genes for normalization of gene expression data (Mamo et al., 2007). Therefore, we applied the three well-defined reference genes of Ppia, H2afz, and Hprt1 for investigation of expression levels of retromer complex-related genes and their target receptors in mouse oocytes and preimplantation stages (Table 1).

Until now, various researchers have investigated gene ex-

pression profiling during mouse embryonic developmental stages using microarray analysis and validated their results using real-time RT-PCR experiments. They found that the expression patterns of numerous genes during the mouse embryonic developmental stages were clustered with maternal, maternal-zygotic, 1- cell transient, 2- cell transient, 8- cell transient, and blastocysts of six major expression patterns and sub-clustered nine expression patterns in detail (Amati et al., 2007; Hamatani et al., 2004; Zeng et al., 2004). Although previous insertional mutagenesis studies of various retromer complex-related genes, including Vps26a, Snx1, Snx2, and Ci-mpr have indicated their important roles during the early developmental stages in mice, we could not investigate the exact expression pattern of retromer complex-related genes and their target receptor gene has not been investigated during the early developmental stages. Therefore, we investigated quantitative expression analysis of their genes in mouse oocytes and preimplantation stages using real-time RT-PCR (Fig. 1). Expression profiling in previous studies has commonly been performed in oocyte to blastocyst stages. Thus, to investigate accurate quantitative expression analysis in mouse early developmental stages, we used eleven oocytes and preimplantation stages (from germinal vesicle (GV) to after 10.5 day) in real-time RT-PCR amplification. Overall expression patterns of retromer complex-related genes were clustered with two different groups of Vps26a genes and other genes (Vps26b, Vps29, Vps35, Snx1, Snx2, and Ci-mpr). These groups were designated according to maternal patterns (destruction of many maternal transcripts was initiated in oocyte maturation and continued through the 2-cell stage) and maternal-zygotic patterns (maternal transcripts were replaced by zygotic transcripts), respectively (Zeng et al., 2004). However, only from germinal vesicle to blastocyst stage, except for the Snx2 gene, expression patterns of all retromer complex-related genes are similar to maternal patterns. Maternal expression patterns observed in our investigation of retromer compex-related genes from germinal vesicle to blastocyst stage were also observed in the previous expression profiling study with different stages of embryos using microarray techniques (Hamatani et al., 2004). In previous results from microarray data, Golgi apparatus/intracellular protein transport genes categorized according to gene ontology (GO) terms showed maternal expression patterns (Hamatani et al., 2004). Also, retromer complex-related genes are one of the important units associating with retrograde transport of cargo proteins from endosomes to the trans-Golgi network (Bonifacino and Hurley, 2008; Seaman, 2005). Thus, our result showing maternal expression patterns in retromer complex-related genes is coincident with microarray data. Among retromer complex-related genes, Ci-mpr is an important receptor for insulin-like growth factor 2 (Igf2). Also, Igf2 is involved in development and growth, specifically oocyte maturation (Wang et al., 2006). Therefore, we could assume that retromer complex-related genes carry out important functions for retrograde of the Ci-mpr receptor during mouse oocyte development (before fertilization). In addition, from the result showing very low expression activity of the Ci-mpr gene, we could presume inactivation of Ci-mpr related retromer complex-related genes from 2-cell to blastocyst stages. This assumption coincides with our real-time RT-PCR results showing decreased expression patterns in retromer complex-related genes during the preimplantation stages. After blastocyst stages, expression levels of all retromer complex-related genes showed a continuous increase, except for Vps26a. Thus, reactivation of retromer complex-related genes could have a novel function during postimplantation stages by embryonic genome activation. Although we could not determine the exact reason for inactivation of the Vps26a gene during postimplantation stages, transcription of the Vps26a gene may begin after implantation. However, to demonstrate these results, further studies are needed for investigation of quantitative analysis in the post-implantation stages, *in vivo*.

The retromer complex consists of the Vps26-Vps29-Vps35 heterotrimer for cargo recognition and Snx1-Snx2 heterodimer for membrane targeting (Seaman, 2005). Knock-down analysis derived by the siRNA transfection technique of the Vps26a gene induced reduction of Vps29, Vps35, and their target Ci-mpr protein (Arighi et al., 2004; Seaman, 2007). And expression level of Snx1 and Snx2 proteins did not changed. However, our quantitative real-time RT-PCR analysis between $Vps26a^{+/+}$ wild-type (normal) MEF and $Vps26a^{-/-}$ MEF cells indicated slightly different results in association with Snx1 and Snx2 gene expression. In the Snx2 gene, its expression activity was slightly decreased in $Vps26a^{-/-}$ MEF cells. Furthermore, in the case of the Snx1 gene, completely conflicting results were observed (Fig. 2B). Although previous results from Vps26a knock down analysis showed a constant expression level of Snx1, our result indicated a possible role for Vps26a as a negative regulator of Snx1. Also, these phenomena could be explained by the diverse functions of the Snx1 gene in association with lysomal degradation of the protease-activated receptor (Par1) (Wang et al., 2002), degradation of epidermal growth factor receptor (Egfr) (Kurten et al., 1996), and regulation of $P2Y_1$ receptor recycling (Nisar et al., 2010). Thus, expression level of Snx1 could be regulated by an independent mechanism in association with retromer complex-related genes. To demonstrate this hypothesis, further studies, such as quantitative analysis of mRNA and protein would be required in mouse tissues, *in vivo*, during various stages of embryo development.

In conclusion, we investigated and compared expression analysis of six retromer complex-related genes and one receptor in eleven mouse early developmental stages and MEF and $Vps26a^{-/-}$ MEF cells. Our results indicate that retromer complex-related genes are present in mouse oocytes as maternal mRNAs, and that they could be involved in protein sorting and maturation of oocytes in mouse oocyte stages. However, they could not have involvement in mouse preimplantation stages. Furthermore, they could have a novel function during postimplantation stages, except for Vps26a. Also, we confirmed that absence of the Vps26a gene could influence transcription of other retromer complex-related genes.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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