Cell Type-specific Occurrence of Caveolin-1α and -1β in the Lung Caused by Expression of Distinct mRNAs*

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Two isoforms of caveolin-1, α and β, had been thought to be generated by alternative translation initiation of an mRNA (FL mRNA), but we showed previously that a variant mRNA (5′V mRNA) encodes the β isoform specifically (Kogo, H., and Fujimoto, T. (2000) FEBS Lett. 465, 119–123). In the present study, we demonstrated strong correlation between the expression of the caveolin-1 protein isoforms and mRNA variants in culture cells and the developing mouse lung. The α isoform protein and FL mRNA were expressed constantly during the lung development, whereas expression of the β isoform protein and 5′V mRNA was negligible in the fetal lung before 17.5 days post-coitum, and markedly increased simultaneously at 18.5 days post coitum, when the alveolar type I cells started to differentiate. Immunohistochemical analysis revealed the cell type-specific expression of the two isoforms; the alveolar type I cell expresses the β isoform predominantly, while the endothelium harbors the α isoform chiefly. The mutually exclusive expression of caveolin-1 isoforms was verified by Western blotting of the selective plasma membrane preparation obtained from the endothelial and alveolar epithelial cells. The present result indicates that the two caveolin-1 isoforms are generated from distinct mRNAs in vivo and that their production is regulated independently at the transcriptional level. The result also suggests that the α and β isoforms of caveolin-1 may have unique physiological functions.

Caveolae are small invaginations of the plasma membrane and have been implicated in many cell functions, including endocytosis, cholesterol transport, signal transduction, and tumor suppression (for reviews, see Refs. 1–4). Although caveolae are present in most tissues, lung has abundant caveolae in both endothelial and alveolar type I cells. Caveolins (caveolin-1, -2, and -3) are major constituents of caveolae (5–7). Among them, caveolin-1 was discovered first and has been characterized most extensively. Caveolin-1 has been shown to interact with many signaling proteins by the scaffolding domain (8, 9). The cholesterol binding property of caveolin-1 (10) appears to be related to the unique lipidic composition of the caveolar membrane and its involvement in cholesterol transport (11). Furthermore, ectopic expression of caveolin-1 is sufficient for de novo formation of caveolae in cells lacking this organelle (12). These observations suggest that caveolin-1 is an indispensable protein for both structure and function of caveolae.

There are two isoforms of caveolin-1, termed α and β. They are identical except for the additional 31 amino acids of the α isoform at its N terminus. The two isoforms were reported to show an overlapping, but slightly different subcellular distribution in culture cells (13). Our detailed observation by immunogold electron microscopy of a freeze fracture replica revealed that the α isoform preferentially distributed to caveolae with deep invagination in cultured fibroblasts (14). Specific phosphorylation of the α isoform in v-Src transformed cells (15, 16) and that of the β isoform by insulin treatment of 3T3-L1 cells have been reported (17). Despite these differences between the two isoforms, the β isoform has been generally considered to be a by-product formed by translation initiation from the second AUG codon (13). In addition, the specific function of the β isoform is hard to speculate under the current knowledge, as most functional domains, i.e. those related to membrane attachment, oligomer formation, intracellular trafficking, and inhibitory interaction with many signaling molecules, are common in the two isoforms (18, 19). As a consequence, most experiments on the involvement of caveolin-1 in various cell functions have dealt only with the α isoform, leaving the functional significance of the β isoform elusive.

Previously, we identified an mRNA variant of caveolin-1, termed 5′V, which specifically encodes the β isoform (20). The result indicates that the expression of the β isoform is regulated independently from that of the α isoform. But to the best of our knowledge, preferential suppression of the α isoform by active c-Src (21) and that of the β isoform in follicular thyroid carcinoma (22) have been the only examples of differential regulation. In the present study, we demonstrated that the production of the two caveolin-1 isoforms is regulated at the transcriptional level by showing the correlation between the expression of the protein isoforms and the corresponding mRNAs in culture cells and the mouse tissue in vivo. Furthermore, we found that the α and β isoforms of caveolin-1 are expressed in the endothelial and alveolar epithelial cells of the mouse lung, respectively, in a mutually exclusive manner. This result supports the contention of Ramirez et al. (23), which suggested the expression of caveolin-1β in the fetal and neonatal alveolar epithelial cell based on the absence of caveolin-1α. Our result suggests that the two isoforms may have distinct physiological functions in different cell types.

EXPERIMENTAL PROCEDURES

Culture Cells and Animals—A preadipocyte cell line, ST-13 (24), was kindly provided by Dr. Fumio Fukai (Tokyo University of Science). 3T3-L1 (JCRB 9014) was obtained from Health Science Research Resources Bank (Osaka, Japan). Pam212 (25) was a gift from Dr. Koji...
Hoshimoto (Ehime University). ST-13 cells were cultured in Dulbecco’s modified Eagle’s medium/Ham’s F-12 (1:1) supplemented with 10% fetal serum. 3T3-L1 and Pam212 were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Mouse embryos were obtained by breeding BALB/c mice. The presence of a vaginal plug at noon was considered as gestational day 0.5. Lungs were isolated from embryos at gestational days 16.5 (E16), 17.5 (E17), 18.5 (E18), and 19.5 (E19) and from mice at the postnatal age of 1 day, 3 weeks, and 12 months. Wistar rats weighing 100–150 g were used for isolating the plasma membrane from pulmonary endothelial and epithelial cells. All experiments using laboratory animals were approved by the University Committee in accordance with the Guidelines for Animal Experimentation in Nagoya University Graduate School of Medicine.

RNA Extraction and Ribonuclease Protection Assay—Total RNA of culture cells and mouse lung was extracted by TRIzol reagent (Invitrogen). The template for the caveolin-1 antisense probe was subcloned into a pSPT18 plasmid (Roche Applied Science). This probe corresponds to a 294-bp sequence of mouse caveolin-1 5’ mRNA (bp 31–324 in AB023993). Cyclophilin was used as an internal control probe. The template for the cyclophilin antisense probe (bp 50–180 in X52803) was obtained by reverse transcriptase-PCR and subcloned into a pBluescript II plasmid (Stratagene). Antisense riboprobes were transcribed using T7 RNA polymerase and DIG1 RNA labeling mix (Roche Applied Science). A cDNA fragment of FL (bp 28–614 in AB023992) and that of 5’ (bp 51–662 in AB023993) were subcloned into a pSPT18 plasmid for the synthesis of sense caveolin-1 RNAs, which were transcribed using T7 RNA polymerase (Roche Applied Science). Ribonuclease protection assay (RPA) analysis of caveolin-1 mRNA was performed using RPA III kit (Ambion) with DIG-labeled riboprobes according to the manufacturer’s instruction using 10 μg of total RNAs and 1 ng of antisense riboprobes and anti-rabbit and anti-mouse antibodies. The protected RNA fragments were separated by electrophoresis in 5% polyacrylamide gel with 7M urea and electropherogram was visualized and analyzed as described above.

Western Blotting—Culture cells and the plasma membrane preparations from the rat lung were directly dissolved with a SDS-containing buffer (125 mM Tris-HCl, 4% SDS, pH 6.8). Mouse lungs were homogenized in a buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 1 μM phenylmethylsulfonyl fluoride) before being treated with SDS sample buffer. Ten μg of proteins per lane were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The blotted membranes of culture cells and developing mouse lungs were probed with anti-caveolin-1 antibody (clone 2297, BD Transduction Laboratories, at 1:1,000 dilution), anti-GAPDH monoclonal antibody (clone 1A4 (Sigma) at 1:1,000 dilution), and anti-PGECAM-1 antibody (sc-1506 (Santa Cruz Biotechnology Inc.) at 1:2,000 dilution), mouse monoclonal anti-α-smooth muscle actin antibody (clone 1A4 at 1:400 dilution), and hamster monoclonal anti-mouse gp38/TIα antibody (clone 8.11 (Developmental Studies Hybridoma Bank, University of Iowa) at 1:100 dilution) (26, 27), and mouse monoclonal anti-thyroid transcription factor-1 (TTF-1) antibody (clone 8G7G3/1 (Neomarkers) at 1:20 dilution) were used as markers for epithelial cells, smooth muscle cells, alveolar epithelial type I, and type II cells, respectively. Alexa488-conjugated goat anti-rabbit and anti-hamster antibodies (Molecular Probes), fluorescein isothiocyanate-conjugated donkey anti-goat antibody (Jackson Immunoresearch Laboratories), and Cy3-conjugated donkey anti-mouse and anti-rabbit antibodies (Jackson Immunoresearch Laboratories) were used for the fluorescent detection of the antigens. Negative control staining was performed with normal mouse and rabbit sera at 1:100 dilution replacing the primary antibodies.

Isolation of Endothelial and Alveolar Epithelial Cell Plasma Membranes—The luminal plasma membrane of the rat lung endothelium was silica-coated by perfusing the cationized colloidal silica solution from the pulmonary artery as reported previously (29, 30). The silica coating of the alveolar epithelial cell plasma membrane was performed by filling and removing the solution manually through trachea using a 13.5-gauge and a 16-gauge intravenous catheter. The lung with catheterized trachea was excised and lavaged four times with 5 ml of HEPES-buffered saline (136 mM NaCl, 5.3 mM KCl, 2.5 mM sodium phosphate buffer, 10 mM HEPES at pH 7.4) to remove macrophages and then twice with 5 ml of MES-buffered saline (MBS: 125 mM NaCl, 20 mM MES, pH 6.0). The lung was then filled with 5 ml of 1% cationic colloidal silica in MBS and settled for 1 min, followed by lavaging twice with 5 ml of MBS to remove excess colloidal silica. The lung was then filled with 5 ml of 1% sodium polycarlylate in MBS and settled for 1 min, followed by lavaging twice with 5 ml of HEPES-buffered sucrose solution (HBSS: 0.25 mM sucrose, 25 mM HEPES, pH 7.4, supplemented with 1 mM phenylmethylsulfonyl fluoride). The silica-coated lung (about 1 g) was minced and homogenized in 5 ml of cold HBSS and processed as reported previously (31). In brief, after filtration through 200-μm nylon mesh, the homogenate was mixed with 75% (w/v) Nycodenz (Nycodan AS) to make a 50% final solution and was layered over a cushion of 70% Nycodenz (in HBSS) and then overlaid with HEPES-buffered saline. After a centrifugation at 20,000 × g for 30 min, the pellet formed at the bottom of the tube was washed with HBBS. To obtain a purer preparation, the pellets were resuspended with 50% Nycodenz solution and recentrifuged over a 70% Nycodenz cushion. The silica pellets were dissolved with a standard SDS sample buffer and analyzed by Western blotting as described above.

RESULTS

Detection of Two Caveolin-1 mRNAs by RPA—In a previous study, we identified two mRNAs for caveolin-1. They were named as FL and 5’-5’ and were thought to encode the α and β isoforms, respectively (20). However, it has not been determined whether the isoform production is principally regulated at the transcriptional level or caused by alternative translation initiation (13). If the former is the case, the expression of the α and β isoform proteins should be correlated with that of the FL and 5’-5’ mRNAs. To examine this, we quantified isoform proteins and mRNAs. For quantitative detection of two mRNAs in a single assay, we employed the RPA using the DIG-labeled antisense riboprobe that hybridizes to 5’ mRNA with 293 bases and to FL mRNA with 168 bases. We confirmed that the two caveolin-1 mRNAs can be quantitated by this method using known amounts of synthesized sense RNAs as samples (Fig. 1).

Expression of Caveolin-1 Isoforms and mRNAs in Murine Culture Cells—We screened several mouse cell lines showing various expression ratios of caveolin-1 isoforms and present here the results of three culture cell lines (Fig. 2). The ratio of signal intensity for the two isoforms (β/α) by Western blotting (the mean ± S.D., n = 4) was 3.59 ± 0.62 for ST13, 0.55 ± 0.10 for 3T3-L1, and 0.03 ± 0.04 for Pam212 (Fig. 2, WB). The signal ratio of two mRNAs (5’/FL) by RPA (the mean ± S.D., n = 3) was 6.00 ± 2.63 for ST13, 0.80 ± 0.24 for 3T3-L1, and 0.36 ± 0.11 for Pam212 (Fig. 2, RPA). The expression ratio of protein isoforms and mRNA variants was obviously correlated each

1 The abbreviations used are: DIG, digoxigenin; RPA, ribonuclease protection assay; MES, 4-morpholineethanesulfonic acid; MBS, MES-buffered saline; HBSS, HEPES-buffered sucrose solution.

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Fig. 1. Quantitative detection of two caveolin-1 mRNAs by RPA using a single DIG-labeled antisense riboprobe. A, RPA was performed to synthesized sense caveolin-1 RNAs, FL and 5′, which were mixed in indicated amounts in a sample. The size of the DIG-labeled antisense riboprobe is 384 base, having the extra sequence derived from the plasmid vector. Length of protected fragments was 294 base for 5′ and 168 base for FL. B, signal intensity of chemiluminescent bands was plotted on the graph. Data are mean ± S.D. of three independent assays. Signal intensity for the two mRNAs was linearly proportional to the amount of the input sense RNAs.

Expression of caveolin-1 isoforms and mRNAs in murine culture cells. Three cell lines (ST-13, ST3L-L1, and Pam212) expressing the two isoforms in different ratios were examined by Western blotting (WB) using an anti-caveolin-1 antibody (2297). The cells were also analyzed for caveolin-1 mRNA expression by RPA. Comparing the results of Western blotting and RPA, the expression of the α and β isoform proteins was correlated well with that of FL and 5′ mRNAs, respectively. β-Actin in Western blotting and cyclophilin in RPA were used as internal controls for the amount of loaded samples.

Expression of Caveolin-1 Isoforms and mRNAs during the Murine Lung Development—Next, we examined the caveolin-1 expression in the developing mouse lung. We found that the β isoform was hardly detectable at E16 and E17, but became prominent at E18 and thereafter (Fig. 3A). On the other hand, the α isoform was already detectable at E16 and constantly expressed during the lung development (Fig. 3A). The expression of the two mRNAs also showed a similar change; namely, the expression of 5′ mRNA increased drastically between E17 and E18, while that of FL mRNA was rather constant (Fig. 3B), consistent with the assumption that the 5′ mRNA is responsible for the production of the β isoform. Quantitative analysis of the signal intensity ratio for caveolin-1 isoforms (β/α, Fig. 3C) and that of mRNA variants (5′/FL, Fig. 3D) clearly shows the marked up-regulation of the β isoform protein and 5′ mRNA between E17 and E18. The protein β/α ratio and the mRNA 5′/FL ratio both reached a plateau around birth and remained the same in the following ages at least until 12 months old (Fig. 3, C and D, part of the data is not shown). The apparent continuous increase of the β/α ratio after birth in Fig. 3A with the same protein loading is due to saturation of the α isoform signal in the older aged mice. By avoiding signal saturation by a decreased sample loading (Fig. 3A), the β/α ratio remained the same after birth as shown in Fig. 3C.

Immunohistochemical Identification of Caveolin-1-expressing Cell Types in the Developing Mouse Lung—To examine whether the expression of the β isoform increases specifically in some cell type at E18, we histologically observed caveolin-1-positive cells in the developing mouse lung by immunofluorescence labeling. In the lung at E16 and E17, in which the α isoform protein and FL mRNA were dominant, caveolin-1 was detected in cells in the lung mesenchyme (Fig. 4A), as well as in the endothelial cell of large blood vessels (Fig. 4, A and B, asterisks). The cells positive for caveolin-1 in the mesenchyme were identified as the endothelial cell of developing small vessels because they expressed endothelial markers, PECAM-1 (Fig. 4D) and eNOS (data not shown). This result is consistent with the previous report on the expression of caveolin-1α in the fetal lung (23). Caveolin-1 was labeled only weakly in other cell types such as the smooth muscle cell of the blood vessel (Fig. 4E, asterisk) and of the bronchial tubule (Fig. 4E, br) and the interstitial fibroblast. The bronchial epithelium (Fig. 4, A and B, br) was negative for caveolin-1.

We subsequently examined the expression of caveolin-1 in the alveolar epithelium. In the fetal lung, the epithelial cell of pulmonary acinus, but not that of bronchioli, was labeled positively by the antibody to mouse gp38/T1α (Fig. 5, A–C, gp38), which is a specific marker for the apical surface of the alveolar type I cells in the adult lung (27). The acinar epithelium at E17 was mostly the cuboidal type II precursor cell (32) and was either negative or only weakly stained by a rabbit polyclonal anti-caveolin-1 antibody (C13630) recognizing both the α and β isoforms (Fig. 5A, ac). A dramatic morphological change of the acinar tubule occurred at E18; the acinar lumen enlarged tremendously and the alveolar epithelial cell became flattened (Fig. 5, B and C, ac) (32). The flat alveolar epithelial cell was labeled positively by C13630 (Fig. 5B, arrows) but not significantly by another rabbit polyclonal anti-caveolin-1 antibody, sc-894, which is specific to the α-isoform (Fig. 5C, arrows). This result indicates that the marked induction of the β isoform protein and 5′ mRNA occurs in the alveolar type I cell at E18 as the cell starts to differentiate.

Immunohistochemical Demonstration of the Differential Expression of Caveolin-1 Isoforms—To clearly visualize the differential expression of the two caveolin-1 isoforms in different cell types, we performed double labeling by using a combination of the two anti-caveolin-1 antibodies, 2297 and sc-894. While sc-894 recognizes only the α isoform, 2297 recognizes both the α and β isoforms evenly. In the lung at E16 and E17, the labeling by sc-894 and 2297 was almost indistinguishable, which result is consistent with the predominance of the α isoform at this stage (Fig. 6A). A little difference was observed in the vascular smooth muscle cell, which appeared reddish compared with the endothelial cell in the merged picture (data not shown). On the other hand, at E18, a marked difference between sc-894 (Fig. 6B, green) and 2297 (Fig. 6B, red) was observed; the alveolar epithelial cell was positive with 2297 but not with sc-894 (Fig. 6B, arrows). We confirmed this result by using another anti-caveolin-1 antibody, C13630, which labels the α isoform better than the β isoform (14). At E19, whereas 2297 labeled the alveolar epithelium and the vascular endothelium in a similar intensity (Fig. 6C, red, arrows), C13630 labeled the endothelium preferentially (Fig. 6C, green, arrows). The difference of
the isoform ratio in the two cell types is clearly visible in the merged picture (Fig. 6C); the alveolar epithelium was stained in orange or red, whereas the endothelium was stained in yellow or green.

We also assessed the expression of caveolin-1 isoforms in the adult lung (Fig. 7). The alveolar type I cell of the adult lung recognized by anti-gp38 (red) was labeled by 2297 (green), thus resulting in yellow coloring (Fig. 7A, arrows), but it was not
labeled by sc-894, resulting in red coloring (Fig. 7B, arrows). By the double staining with two different combinations of anti-caveolin-1 antibodies, the adult alveolar type I cell was intensely labeled by 2297 (Fig. 7, C and D, red, arrows), and only weakly by C13630 (Fig. 7D, green, arrows), but not by sc-894 (Fig. 7C, green, arrows), resulting in orange (Fig. 7D) and red (Fig. 7C) coloring, respectively, in the merged picture. The endothelial cell was intensely labeled by all caveolin-1 antibodies and thus it appeared yellow or green (Fig. 7, C and D). The lack of labeling by sc-894 in the alveolar type I epithelium was observed consistently in 3-, 6-, and 12-month old mice. This result contradicts the previous work (23), which showed the α isoform in the adult alveolar type I epithelium. Most likely this discrepancy was caused by the method applied; we used fluorochrome-labeled antibody and confocal laser microscopy, whereas the cited work (23) used tyramide amplification combined with diaminobenzidine coloring. Due to the thinness of the adult epithelium, it is difficult to observe it differentially from the endothelium by the latter method. In conjunction with the immunoblotting result described below, we concluded that the adult alveolar epithelium contains little, if any, caveolin-1α. All together, our immunofluorescence labeling demonstrated that the alveolar type I cell expresses the β isoform of caveolin-1 predominantly in both the fetal and adult mouse lung. The alveolar type II cell identified by the presence of TTF-1 (28) was negative for both the α and β isoform of caveolin-1 (data not shown).

We tried to examine the cell type-specific expression of FL and 5 V mRNAs by in situ hybridization using two DIG-labeled antisense riboprobes, which correspond to the unique 5′ ends of FL mRNA (1–117 in AB029929) and of 5′ V mRNA (31–170 in AB029930); however, they were not sensitive enough to detect the mRNAs in tissue sections (data not shown).

Selective Isolation of the Plasma Membrane from the Endothelium and the Alveolar Epithelium by Silica Coating Technique—To verify the cell type-specific expression of the caveolin-1 isoforms in the lung, we isolated the plasma membrane selectively from the endothelial and the alveolar epithelial cells by silica coating technique (see “Experimental Procedures”) and analyzed the expression of caveolin-1 isoforms by Western blotting. In the total homogenate, both caveolin-1α and -1β were detected intensely (Fig. 8, lane H, Cav1). On the other hand, only the α isoform was markedly enriched in the plasma membrane preparation of the endothelium (Fig. 8, lanes En1, Ep1, and Cav1), while in the preparation from the alveolar epithelial cells only the β isoform was enriched (Fig. 8, lanes Ep1, Ep2, and Cav1). The cell type-specific enrichment of isoforms became more evident when the preparation was purified further by an extra centrifugation (Fig. 8, lanes Ep1, Ep2, and Cav1). Although a little reaction for the β and α isoform was observed in En2 and Ep2, respectively (Fig. 8, Cav1 and Cav1α), they are most likely due to a little contamination by other cell types, because a small amount of cytosolic protein (β-actin) and other cell markers (α-SMA and PECAM) were detected in these preparations. The result is fully consistent with the immunofluorescence result, showing the cell type-specific and mutually exclusive expression of the caveolin-1 isoforms in the endothelium and alveolar epithelium. In contrast to the caveolin-1 isoforms, caveolin-2 was detected to a similar extent in the two plasma membrane preparations (Fig. 8, Cav2), indicating that both the α and β caveolin-1 isoforms exist as the hetero-oligomer with caveolin-2.

DISCUSSION

The two isoforms of caveolin-1, α and β, had been believed to be produced by alternative translation initiation from a single mRNA (13). On the other hand, we identified a mRNA variant of caveolin-1 that is transcribed from a downstream promoter and encodes the β isoform and proposed an alternative mechanism for the isoform production (20). The mechanism is used by some genes to produce two isoforms, the shorter isoform of which lacks the N-terminal domain of the longer one (33). In
In the present study, we showed data to support that the two caveolin-1 protein isoforms are generated from the two distinct mRNAs in cells *in vivo*. The ratio of the two mRNAs may be regulated by alternative promoters. In a model cell system, we found that the first intron region of the caveolin-1 gene, which contains the transcription start site for the 5′/H11032 V mRNA, exhibits different promoter activity in different cell types, consistent with the endogenous expression level of the 5′/H11032 V mRNA. The same mechanism is likely to work in the developing lung, but involvement of other mechanisms, such as cell type-specific regulation of mRNA stability, is also possible. Whereas the alternative translation initiation may not be a regulated process, the mRNA level is likely to be controlled independently to meet physiological demands for respective protein products. It will be interesting to examine whether signals known to modulate the caveolin-1 gene expression, such as mitogen-activated protein kinase and protein kinase A signals (34) and free cholesterol (35), affect the expression of the two isoforms differentially.

We also demonstrated that the two isoforms of caveolin-1 are expressed in a mutually exclusive manner in the vascular endothelium and the alveolar epithelium of the lung, respectively, by several lines of evidence. First, by the quantitative analysis of caveolin-1 isoforms and mRNAs during the fetal lung development, we showed that the α isoform and FL mRNA are dominant in the lung before E17 in which the vast majority of caveolin-1-positive cell is the endothelial cell, while the β isoform and 5′ V mRNA are markedly induced at E18 when the differentiation of the alveolar type I cell begins. Second, by immunolabeling using three anti-caveolin-1 antibodies, we demonstrated histologically that the two isoforms are differentially expressed depending on the cell types in both fetal and adult mouse lung. A recent paper has also suggested the expression of the α isoform in the fetal and neonatal alveolar type I cells based on the lack of labeling by an antibody specific to the α isoform (23), but the result is inconclusive because the expression of the β isoform was not confirmed. Third, by Western blotting of the selective plasma membrane preparation, we obtained the unequivocal result showing that the endothelium and the alveolar epithelium exclusively contained the α and β isoform of caveolin-1, respectively. The ratio of the two isoforms has been known to vary depending on the cell type, especially in culture cells (unpublished data), but such an exclusive expression of either isoform as seen in the lung *in vivo* has not been known to date. In conjunction with the existence of unique mRNAs for the two isoforms, the result may

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*2 H. Kogo, M. Ishikawa, and T. Fujimoto, unpublished data.*
imply that the α and β isoforms of caveolin-1 have distinct functions that need to be exerted specifically for some cell types.

The cell type-specific expression of the caveolin-1 isoforms in the lung, that is, the α isoform in the endothelium and the β isoform in the alveolar epithelium, might be related to the physiological function of the two cells. Caveolae in the endothelium have been shown to be engaged in transcellular transport by several lines of evidence (36), but it is controversial whether those in the alveolar epithelium have a similar function (37). The molecular machinery for vesicular transport, including dynamin and VAMP-2, was shown to exist in caveolae in the endothelium but not in caveolae in the alveolar epithelium. In this context, the phenotype of the caveolin-1-null mouse (38, 39) is noteworthy; they lack both the α and β isoforms, but only the pulmonary endothelium showed hyperproliferation, whereas the alveolar epithelium did not. This difference might be related to function of the two isoforms, but it may not be so simple because the same phenotype was observed in the caveolin-2-deficient mouse (40). Since the selective plasma membrane preparation is available from the endothelium and the alveolar epithelium, comparative analysis of caveolae of the two cell types can be done to study functions of the two isoforms. The study must also be important for elucidation of the transcellular transport mechanism in the alveolar epithelium, which is of particular interest in terms of pulmonary drug delivery (37).

Comparing the molecular structure of the two caveolin-1 isoforms, the only difference is the N-terminal 31 amino acids of the α isoform that does not exist in the β isoform. Naturally most functional domains are common to the two isoforms (18, 19). Thus it may appear unlikely that the β isoform has some unique function that the α isoform does not have. However, the...
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43. But the presence of caveolae in caveolin-2-null mice is against this assumption (40). Identification of other factors, especially those interacting with the β isoform in the alveolar type I cell, may be a clue to understand the molecular mechanism of caveole formation. Caveolins can give rise to the characteristic filamentous coat of caveolae (44), but whether the caveolin coat alone is capable of forming the invagination is not known. Second, the alveolar type I cell may contain a small amount of caveolin-1α, whereas HepG2 appears to lack it totally (14). The small amount of caveolin-1α combined with caveolin-1β may suffice to form deep caveolae.

Now that we showed that caveolin-1 isoforms are generated from distinct mRNAs at least in some cell types, we can utilize gene targeting and/or RNA interference techniques to do the isoform-specific knockout experiments both in vivo and in vitro. Moreover, the plasma membrane preparation obtained selectively either from the endothelium or from the epithelium enables biochemical analysis of caveolae formed exclusively by the α or the β isoform. By using these techniques, functional difference of caveolin-1 isoforms and diversity of caveolar domains would be defined unequivocally.

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