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# Chromosomal localization, genomic organization, and developmental expression of the murine caveolin gene family (Cav-1, -2, and -3)

## Cav-1 and Cav-2 genes map to a known tumor suppressor locus (6-A2/7q31)

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**Abstract Caveolins (Cav-1, -2, and -3) are a gene family of cytoplasmic membrane-anchored scaffolding proteins that: (i) help to sculpt caveolae membranes from the plasma membrane proper; and (ii) participate in the sequestration of inactive signaling molecules. In the adult, caveolin-1 and -2 are co-expressed and are most abundant in type I pneumocytes, endothelia, fibroblastic cells and adipocytes, while the expression of caveolin-3 is restricted to striated muscle cells. However, little is known regarding the genomic organization and developmental expression of the caveolin gene family. Here, using the mouse as a model system, we examine the chromosomal localization, the detailed intron-exon organization, and developmental expression pattern of the caveolin gene family. cDNAs encoding caveolin-1, -2, and -3 were used as probes to isolate murine genomic clones containing these genes. Fluorescence in situ hybridization (FISH) analysis using these genomic clones as probes reveals that all three caveolin genes are localized to murine chromosome 6. Specifically, caveolin-1 and -2 co-localize to chromosomal region 6-A2, while caveolin-3 is located within the chromosomal region 6-E1. Searches of the NCBI Human/Mouse Homology map indicate that murine region 6-A2 corresponds to human chromosome 7q31. As this region (6-A2/7q31) is the site of an as yet unidentified tumor suppressor gene(s), our mapping studies clearly define caveolin-1 and caveolin-2 as candidate genes that may be deleted at these loci. All three caveolin genes show similar intron-exon organization, with the last exon of each gene encoding the bulk of the known caveolin functional domains. The boundary position of the last exon is essentially identical in all three caveolin genes, suggesting that they may have arisen through gene duplication events. Developmentally, all three caveolins were expressed late during mouse embryogenesis as assessed by Northern and Western blot analysis. We examined the localization of the caveolin proteins in sections of day 16 mouse embryos using a well-characterized panel of antibody probes. Caveolin-1 and -2 were most abundantly expressed in the developing lung parenchyma, while caveolin-3 was most abundantly expressed in developing tissues that consist primarily of skeletal muscle cells. As the expression of all three caveolins in the adult is highest in terminally differentiated cell types, this is consistent with the idea that caveolins may be viewed as late markers of differentiation during embryogenesis.**



*Key words:* Caveolin; Genomic organization; Gene expression

## 1. Introduction

Caveolae are 50–100-nm vesicular invaginations of the plasma membrane [1]. It has been proposed that caveolae participate in vesicular trafficking events and signal transduction processes [2–4]. Caveolin, a 21–24-kDa integral membrane protein, is a principal component of caveolae membranes in vivo [5–9].

Caveolin is only the first member of a new gene family; as a consequence, caveolin has been re-termed caveolin-1 [10]. Caveolin-2 shows the same tissue distribution as caveolin-1 [11], while caveolin-3 is only expressed in striated muscle cell types (cardiac and skeletal) [12–14]. It has been proposed that caveolin family members function as scaffolding proteins [15] to organize and concentrate specific lipids (cholesterol and glycosphingolipids; [16–18]) and lipid-modified signaling molecules (Src-like kinases, H-Ras, eNOS and G-proteins; [16,19–23]) within caveolae membranes. In support of this idea, caveolin-1 binding can functionally suppress the GTPase activity of heterotrimeric G-proteins and inhibit the kinase activity of Src-family tyrosine kinases, the EGF-receptor kinase, and protein kinase C through a common caveolin domain, termed the caveolin-scaffolding domain [19–21,24,25]. Thus, we have suggested that caveolin may function as a negative regulator of many different classes of signaling molecules through the recognition of specific caveolin-binding motifs [4,26].

The direct interaction of caveolin with signaling molecules leads to their inactivation [2–4]. Since many of these signaling molecules can cause cellular transformation when constitutively activated, it is reasonable to speculate that caveolin itself may possess transformation suppressor activity. Consistent with this hypothesis, both caveolae and caveolin are most abundantly expressed in terminally differentiated cells: type I pneumocytes, endothelial cells, muscle cells, and adipocytes [10,27–31]. In addition, caveolin-1 mRNA and protein expression are lost or reduced during cell transformation by activated oncogenes such as v-abl and H-ras (G12V); caveolae are absent from these cell lines [32].

The potential 'transformation suppressor' activity of caveolin-1 has recently been evaluated by using an inducible expression system to up-regulate caveolin-1 expression in oncogenically transformed cells. Induction of caveolin-1 expression in v-Abl- and H-Ras(G12V)-transformed NIH 3T3 cells abrogated the anchorage-independent growth of these cells in soft agar and resulted in the de novo formation of caveolae [33]. Thus, down-regulation of caveolin-1 expression and cav-

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eolae organelles may be critical to maintaining the transformed phenotype.

In collaboration with Minetti and colleagues, we have recently identified two different families in Italy with an autosomal dominant form of limb girdle muscular dystrophy that is due to a deficiency in caveolin-3 expression [34]. In these patients, by quantitative immunofluorescence and Western blot analysis, levels of the caveolin-3 protein are reduced by >95%. Analysis of their genomic DNA reveals two distinct mutations: (i) a 9 base-pair micro-deletion that removes the sequence TFT from the caveolin-scaffolding domain; and (ii) a mis-sense mutation that changes a proline to a leucine (P→L) in the transmembrane domain [34]. As these mutations are heterozygous and show an autosomal dominant form of transmission, we must conclude that these mutations cause the formation of a dominant-negative form of caveolin-3 [34]. As caveolin-1, -2, and -3 are known to form both hetero- and homo-oligomers [10,11,13,15,35], this may lead to the degradation of wild-type caveolin-3.

Interestingly, comparison of the known protein sequences of mammalian caveolin-1, -2, and -3 with *Caenorhabditis elegans* caveolin-1 and -2, reveals that only 12 amino acid residues are invariant between worms and man [35]. These include two charged residues (R, D), five aromatic residues (3F, W, Y), proline (2P), serine (2S), and glycine (G). Of these 12 invariant residues, two are affected by the mutations identified in caveolin-3. One of the invariant prolines is changed to leucine in Family A, while one of the invariant phenylalanines is deleted in Family B [34]. These results suggest that mutation of these evolutionarily conserved residues may have dire consequences for the structure or functioning of the entire caveolin gene family. In an independent report, we have previously shown that alanine scanning mutagenesis of a peptide encoding the caveolin-scaffolding domain reveals that the FTVT/S sequence in caveolin-1 and -3 is important for the correct recognition of caveolin-binding signaling molecules [26]. Note that the FT portion of this FTVT/S sequence is deleted in Family B. This finding provides genetic evidence that this region of the caveolin-scaffolding domain is critical in vivo.

Thus, mouse models of caveolin deficiencies will be important for elucidating the potential role of caveolins in cancerous cell transformation and in muscular dystrophy. However, little is known regarding the genomic organization and developmental expression of the caveolin gene family in humans or mice. Only the overall genomic structure of the chicken caveolin-1 gene has been reported in detail [36].

Here, using the mouse as a model experimental system, we examine the chromosomal localization, the detailed intron-exon organization, and developmental expression pattern of the caveolin gene family. More specifically, we show that: (i) all three caveolin genes are located on murine chromosome 6; (ii) caveolin-1 and -2 co-localize to murine chromosomal region 6-A2; (iii) caveolin-3 is located within the murine chromosomal region 6-E1; (iv) all three caveolin genes show similar intron-exon organization, with the last exon of each gene encoding the bulk of the known caveolin functional domains; and (v) developmentally, all three caveolins were expressed late during mouse embryogenesis as assessed by Northern and Western blot analysis, but in a tissue-specific fashion as seen by immunocytochemistry.

## 2. Materials and methods

### 2.1. Materials

The cDNAs encoding caveolin-1, -2, and -3 were as we described previously [10,13,37]. Antibodies and their sources were as follows: anti-caveolin-1 IgG (mAb 2297; gift of Dr. John R. Glenney, Transduction Labs); anti-caveolin-1 (pAb; rabbit anti-peptide antibody directed against caveolin-1 residues 2–21; Santa Cruz Biotech, Inc.). Mouse monoclonal antibodies directed against caveolin-2 (clone 65) or caveolin-3 (clone 26) were as we described previously [11,12]. A variety of other reagents were purchased commercially: fetal bovine serum (FBS; JRH (Biosciences); pre-stained protein markers (Gibco-BRL); Slow-Fade anti-fade reagent (Molecular Probes, Eugene, OR). The mouse embryo Northern blot was purchased from Clontech, Inc. Paraffin sections containing day 16 mouse embryos were purchased from Novagen, Inc. Whole mouse embryos were harvested on days 11 and 15 and used for Western blot analysis. Cy 3-conjugated donkey anti-mouse and donkey anti-rabbit IgG were from Jackson Laboratories, Inc.

### 2.2. Isolation and characterization of genomic clones

Probes corresponding to the cDNAs of caveolin-1, -2, and -3 were used to screen a previously described murine genomic library (for caveolin-1; generous gift of H. Wu and R. Jaenisch, Whitehead Institute) [38,39] or a mouse ES-129/SvJ II BAC library (for caveolin-2 and -3; Genome Systems, Inc.). To determine the genomic organization of the caveolin genes, portions of these genomic inserts were subcloned into the vector pBS-SK$^+$. The genomic organization of each caveolin gene was then established by using Southern blotting to develop a detailed restriction map of the region. The intron-exon boundaries were established by dideoxy sequencing using specific primers to the coding sequence of the caveolins.

### 2.3. Fluorescence in situ hybridization (FISH) analysis

Chromosomal localization of the murine caveolin gene family was carried out in collaboration with Genome Systems. Briefly, a given mouse genomic clone was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with sheared mouse DNA and hybridized to normal metaphase chromosomes (derived from mouse embryo fibroblasts). Hybridization was carried out in a solution containing 50% formamide, 10% dextran sulfate and 2×SSC. Hybridization signals were detected by incubating the hybridized slides with FITC-labeled anti-digoxigenin IgGs. Chromosomes were also counterstained with DAPI. Probes specific for the centromeric and telomeric region of chromosome 6 were used to confirm the localization of the caveolin gene family. For each caveolin genomic clone, a total of 80 metaphase cells were analyzed with 54, 74, and 71 showing specific labeling for caveolin-1, -2, and -3, respectively.

### 2.4. Northern blot analysis

Hybridizations were performed in 50% formamide, 5×SSC, 25 mM Na-phosphate, pH 7.0, 10× Denhardts, 5 mM EDTA, 1% SDS, 0.1 mg/ml PolyA at 42°C overnight and subsequently washed in 2×SSC/0.1% SDS and 0.5×SSC/0.1% SDS at 50°C; radiolabeled DNA concentrations were at $2\times10^6$ cpm/ml.

### 2.5. Western blot analysis

Mouse embryo extracts were prepared with IP buffer and homogenized on ice with a Polytron tissue grinder, as described [28]. Equal amounts of protein (50 μg) were loaded on an SDS-PAGE gel (12% acrylamide) and transferred to nitrocellulose. After transfer, nitrocellulose sheets were stained with Ponceau S to visualize protein bands and subjected to immunoblotting. For immunoblotting, incubation conditions were as described by the manufacturer (Amersham), except we supplemented our blocking solution with both 1% BSA and 1% non-fat dry milk (Carnation).

### 2.6. Immunofluorescence microscopy

Paraffin embedded sections were first cleared with xylene and rinsed twice (2 min each) with 100% alcohol, 95% alcohol, and 80% alcohol. (Note that alcohol for all washes is 90% ethanol/5% methanol/5% isopropanol). Sections were then equilibrated with de-ionized water for 5 min, followed by several rinses with fresh de-ionized water. Slides were placed face-up in an incubation container and each section was covered with a solution of 1% SDS in Tris-buffered saline

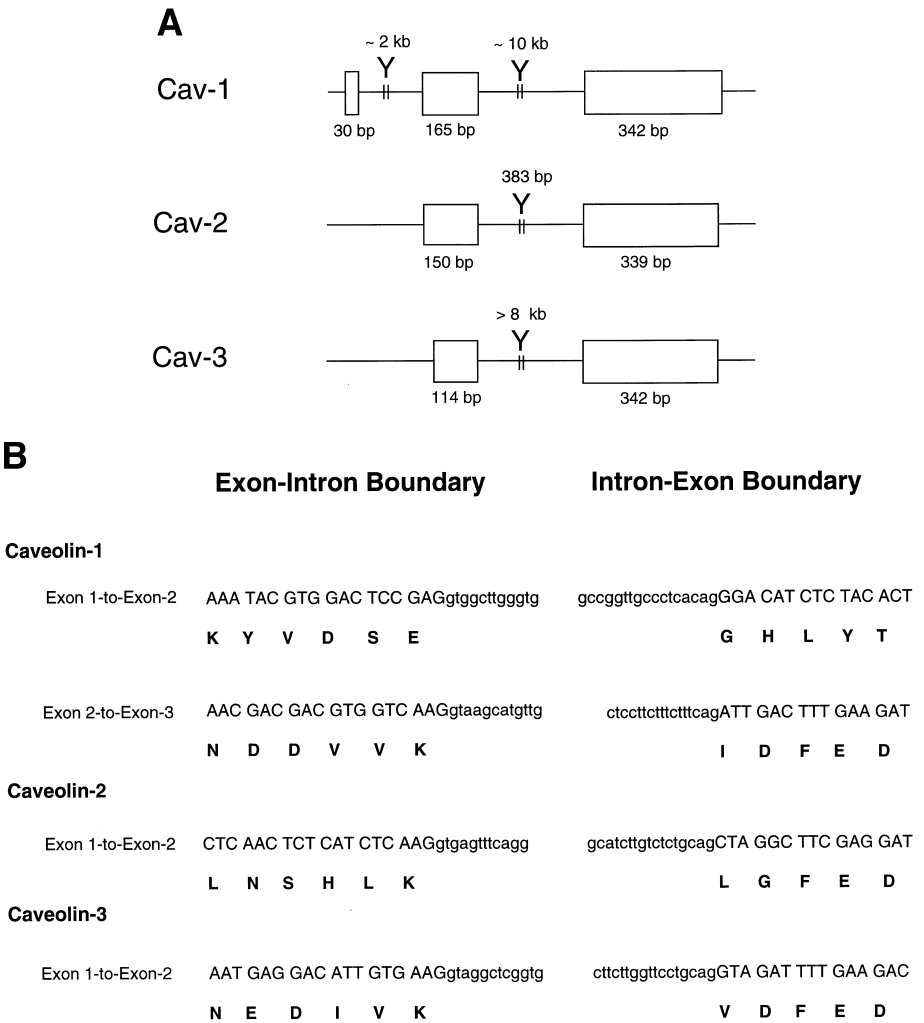


Fig. 1. Genomic organization of the murine caveolin gene family. The relative sizes of exons and introns are as indicated and were determined by direct sequencing and restriction mapping. A: Overall genomic organization of murine caveolin-1, -2, and -3. B: Specific DNA sequences surrounding various intron-exon boundaries.

(100 mM Tris, pH 7.4, 138 mM NaCl, and 27 mM KCl). After 5 min, slides were washed 3× with TBS (5 min each). To block non-specific binding, sections were incubated with a 1:10 dilution of horse serum in TBS for 1 h at 37°C. After blocking, sections were incubated with antibodies directed against either caveolin-1, -2, or -3 for 2 h at 37°C. After rinsing with TBS (3×, 5 min each), sections were incubated with Cy 3-conjugated donkey anti-mouse or donkey anti-rabbit IgG (1:200 dilution in blocking buffer; Jackson Laboratories) for 1 h at 37°C. Note that when the primary antibody used was a mouse monoclonal, the blocking solution also contained anti-mouse Fab fragments (20 μg/ml) to prevent the Cy 3-labeled secondary antibody from recognizing endogenous antibodies in the embryo sections. After rinsing with TBS (3×, 5 min each), coverslips were mounted with Slow-Fade anti-fade reagent and slides were examined by fluorescence microscopy using a Bio-Rad confocal microscope. Note that pre-treatment of sections with 1% SDS has recently been shown to be an effective method for antigen retrieval, especially with anti-caveolin-1 IgG [40].

## 3. Results

### 3.1. Chromosomal localization and genomic organization of murine caveolin gene family (Cav-1, Cav-2, and Cav-3)

The cDNAs encoding caveolin-1, -2, and -3 were used as probes to isolate their corresponding genomic clones from a murine genomic library. The clone numbers and the insert sizes of these caveolin-containing genomic clones are summarized in Table 1.

These genomic clones were next used as probes to determine the chromosomal localization of the caveolin gene family by FISH analysis. Perhaps surprisingly, our results indicate that all three caveolin genes are localized to murine chromosome 6. Caveolin-1 and -2 are co-localized to region 6-A2, while the caveolin-3 gene is localized to region 6-E1 (Table 1).

To determine the genomic organization of the caveolin genes, portions of these genomic inserts were subcloned. The intron-exon boundaries were directly established by dideoxy sequencing using specific primers to the coding sequence of the caveolins. The genomic organization of the genes encoding caveolin-1, -2, and -3 is shown schematically in Fig. 1A. The

Table 1
Chromosomal localization of murine caveolin-1, -2, and -3

| Gene | Clone # | Insert size (kb) | FISH analysis |
|---|---|---|---|
| Cav-1 | 3-2 | ~12-15 | 6 A2 |
| Cav-2 | 17031 | ~80-120 | 6 A2 |
| Cav-3 | 14316 | ~80-120 | 6 E1 |

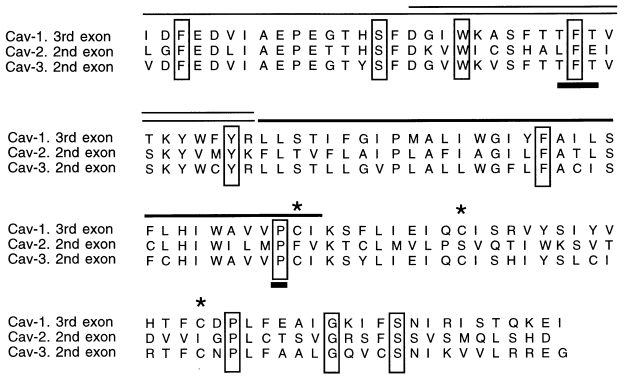


Fig. 2. Alignment of the protein sequences and functional domains encoded by the last exon of the murine Cav-1, -2, and -3 genes. The positions of the oligomerization domain (single overline), the caveolin scaffolding domain (double overline), the membrane spanning domain (boldface overline), and cysteines conserved in caveolin-1 and -3 that are sites of palmitoylation (asterisks) are shown. In addition, 10 of the 12 residues that are invariant between all known mammalian caveolins and caveolins from *C. elegans* are localized to this last exon and are boxed. Conserved residues that are deleted or mutated in human caveolin-3 and lead to an autosomal dominant form of limb-girdle muscular dystrophy [34] are indicated by a boldface underline.

caveolin-1 gene contains 3 exons, while both caveolin-2 and caveolin-3 genes contain only 2 exons. The corresponding protein sequences encoded by each of these exons are summarized in Table 2. Donor and acceptor splice junctions for each exon are shown in Fig. 1B, and conform well to the known consensus for these sites.

Interestingly, the boundary position of the last exon is essentially identical in all three caveolin genes. An alignment of the protein sequences encoded by this segment is shown in Fig. 2. Note that most of the functional domains of the caveolins are contained within this last exon, including the homo-oligomerization domain, the scaffolding domain, the membrane spanning domain, and the C-terminal domain. In addition, this last exon encodes 10 of the 12 invariant residues that are conserved from worms to man in all members of the caveolin gene family. Mutations that involve these invariant residues in human caveolin-3 have been identified in two distinct families with an autosomal dominant form of limb-girdle muscular dystrophy [34].

Given that the murine caveolin gene family is (i) localized to the same murine chromosome, and (ii) they share a similar genomic organization with a virtually identical boundary for the last exon, it is likely that the caveolin gene family arose through gene duplication of this last exon.

Table 2
Genomic organization of the murine caveolin gene family

| Gene | Exon | Size (bp) | Protein segment encoded (aa) |
|---|---|---|---|
| Cav-1 | 1 | 30 | 1–10 |
| | 2 | 165 | 11–65 |
| | 3 | 342 | 66–178 |
| Cav-2 | 1 | 150 | 1–50 |
| | 2 | 339 | 51–162 |
| Cav-3 | 1 | 114 | 1–38 |
| | 2 | 342 | 39–151 |

### 3.2. Comparison with the organization of the caveolin gene family in C. elegans

We compared the intron-exon organization of all three murine caveolin genes with the known organization of a caveolin gene from *C. elegans*, termed Cav$^{ce}$-1 ([35]; Fig. 3, upper panel). Interestingly, the position of the last exon in the Cav$^{ce}$-1 gene corresponds well with the position of the last exons of the murine caveolin gene family (compare Figs. 1 and 3).

The genomic organization of a second caveolin gene expressed in *C. elegans* (Cav$^{ce}$-2) was deduced here by comparing the published cDNA sequence of Cav$^{ce}$-2 with the cosmid C56A3 (accession number Z77655) and is shown in Fig. 3 (lower panel). Note that the Cav$^{ce}$-2 gene contains 10 exons and does not generally resemble any of the murine caveolin genes or the Cav$^{ce}$-1 gene in terms of its intron-exon organization. These findings suggest that the Cav$^{ce}$-2 may be an ancestral predecessor of Cav$^{ce}$-1 and the mammalian caveolin genes. Alternatively, the Cav$^{ce}$-2 gene may be the first member of a new gene family of caveolins that have yet to be discovered in mammals. In support of the latter notion, the genes encoding Cav$^{ce}$-1 and Cav$^{ce}$-2 are located on different chromosomes in *C. elegans* (chromosomes IV and V, respectively).

### 3.3. Developmental expression of murine caveolin-1, -2, and -3 in whole mouse embryos

In order to study the developmental expression of caveolins, we performed Northern blot analysis on mRNA isolated from mouse embryos (days 7, 11, 15 and 17). The expression of β-actin mRNA was used as a control for equal loading. Our results indicate that caveolin-1 and -2 show a biomodal pattern of expression (Fig. 4A). mRNA species for both caveolin-1 and -2 were expressed on day 7, down-regulated by day 11, and upregulated by day 15, and remained elevated on day 17. In contrast, caveolin-3 mRNA was first detectable on day 15 and remained elevated on day 17 (Fig. 4A).

To confirm the strong upregulation of caveolins between days 11 and 15 at the protein level, we performed Western blot analysis with isoform specific antibody probes on extracts prepared from embryos harvested on days 11 and 15. Fig. 4B shows that on day 11, little or no protein expression of caveolin-1, -2, and -3 was observed. However, in accordance with our results from Northern blot analysis, all three caveolin

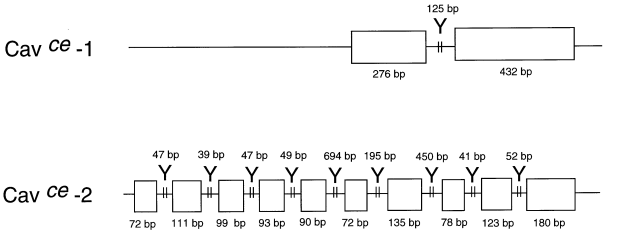


Fig. 3. Genomic organization of the caveolin gene family in *C. elegans*. The genomic organization of Cav$^{ce}$-1 was described previously [35] and is reproduced here. The genomic organization of a second caveolin gene expressed in *C. elegans* (Cav$^{ce}$-2) was deduced here by comparing the published cDNA sequence of Cav$^{ce}$-2 [35] with the cosmid C56A3 (accession number Z77655) and is shown. Note that the Cav$^{ce}$-2 gene contains 10 exons and does not generally resemble any of the murine caveolin genes or the Cav$^{ce}$-1 gene in terms of its intron-exon organization. Interestingly, the position of the last exon in the Cav$^{ce}$-1 gene corresponds well with the position of the last exons of the murine caveolin gene family.

proteins were upregulated on day 15. Similarly, the protein levels of all three caveolins also remained elevated on day 17 (not shown). These results further demonstrate that levels of caveolin mRNA strictly correlate with protein expression, as we have consistently observed previously in adult tissues and cultured cells [10–13,28,32,41].

*3.4. Localization of caveolin-1, -2 and -3 during development*

As the mRNA and protein expression of all three caveolins peaked on days 15–17, we next examined the localization of caveolins in paraffin embedded sections of day 16 mouse embryos using a well-characterized panel of isoform specific antibody probes.

Fig. 5A shows that both caveolin-1 and -2 proteins were most abundantly expressed in lung parenchyma and also within the epithelial cells that line the developing bronchioles. This is in accordance with previous studies showing that both caveolin-1 and -2 are abundantly expressed at the mRNA and protein level in adult lung tissue. Interestingly, it has been recently reported that caveolin-1 levels are down-regulated in type I pneumocytes during lung fibrosis, suggesting that

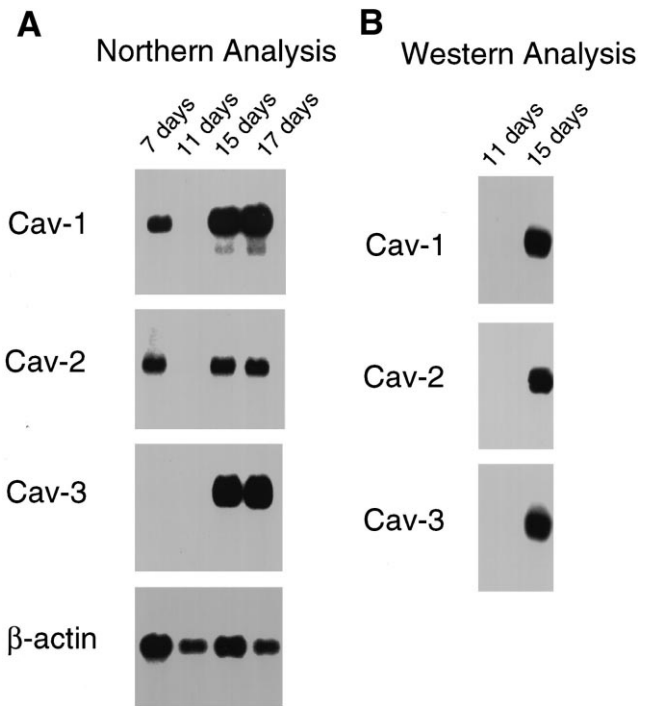


Fig. 4. Northern and Western blot analysis of the developmental expression of the murine caveolin gene family. A: Poly(A)$^+$-RNA from day 7, 11, 15 and 17 mouse embryos was probed with the cDNAs to caveolin-1, -2, and -3. β-Actin was used as a control for equal loading. Note that caveolin-1 and -2 show a biomodal pattern of expression. mRNA species for both caveolin-1 and -2 were expressed on day 7, down-regulated by day 11, and upregulated by day 15, and remained upregulated on day 17. In contrast, caveolin-3 mRNA was first detectable on day 15 and remained elevated on day 17. B: Protein extracts were prepared from day 11 and 15 mouse embryos and probed by Western analysis using a panel of well-characterized monoclonal antibodies specific for either caveolin-1, caveolin-2, or caveolin-3. Note that on day 11, little or no expression of caveolin-1, -2, and -3 was observed. However, in accordance with our results from Northern blot analysis, all three caveolins were upregulated on day 15. Each lane contains an equivalent amount of protein extract (50 μg) and this was confirmed by Ponceau S staining prior to immunoblotting.

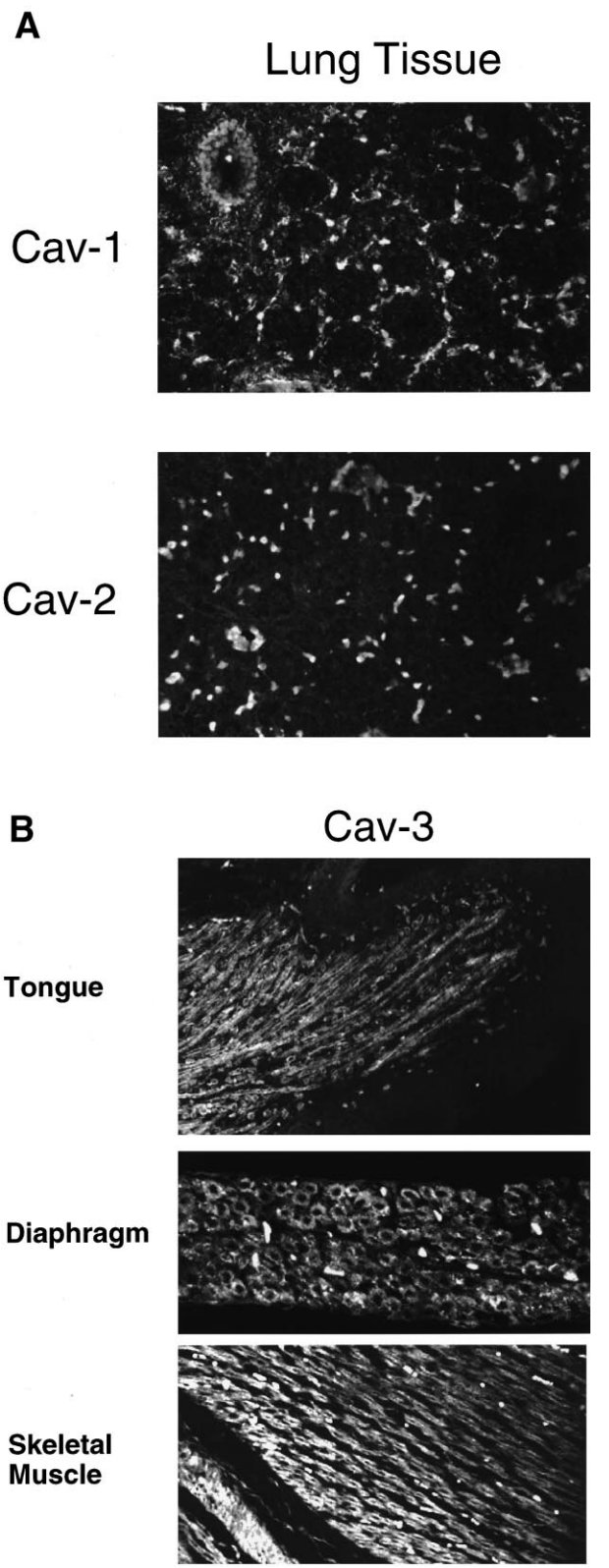


Fig. 5. Immunolocalization of the caveolin gene family in day 16 mouse embryos. Paraffin embedded sections of whole mouse embryos were immunostained with a panel of isoform-specific monoclonal and polyclonal antibodies that recognize either caveolin-1, caveolin-2, or caveolin-3 selectively. A: Localization of caveolin-1 and -2 in lung parenchyma. B: Localization of caveolin-3 in tongue, diaphragm, and skeletal muscle (from the hindlimb).

caveolin-1 may be a negative marker for degenerative lung disorders [42]. Conversely, our results here suggest that caveolin-1 and -2 may be positive markers for lung maturation during embryogenesis.

Fig. 5B shows that the caveolin-3 protein was most abundantly expressed in the developing tongue, diaphragm, and hindlimb muscle that consist primarily of skeletal muscle cells. Similarly, in the adult, caveolin-3 expression is confined primarily to striated muscle cells (cardiac myocytes and skeletal muscle fibers) [12–14]. Interestingly, little or no expression of caveolin-3 was detected in developing cardiac muscle cells (not shown); this is in striking contrast to the adult situation where cardiac and skeletal muscle express equivalent amounts of caveolin-3 [12–14]. However, as the expression of all three caveolins in the adult is highest in terminally differentiated cell types [4], this is consistent with the idea that caveolins may be viewed as late markers of differentiation during embryogenesis.

## 4. Discussion

In this study, we have determined that the genes encoding murine caveolin-1 and caveolin-2 are co-localized within the A2 region of mouse chromosome 6 (6-A2). Recently, the genes encoding human caveolin-1 and -2 have been found to map to the q31 region of human chromosome 7 (7q31; our unpublished observations and [43]). In accordance with these results, a search of the NCBI Human/Mouse Homology Map reveals that the murine chromosomal region 6-A2 corresponds precisely to the human chromosomal region 7q31.

There is now an accumulating body of evidence provided by our lab and others that caveolin-1 mRNA and protein expression is down-regulated during cell transformation of cultured NIH 3T3 cells, in transgenic mouse models of breast cancer, and in cell lines derived from human breast cancers [32,33,44–46]. Furthermore, recombinant expression of caveolin-1 in transformed NIH 3T3 cells or cell lines derived from human breast cancers can suppress their transformed phenotype, such as anchorage-independent growth in soft agar [33,46]. Interestingly, human chromosome 7q31 and murine chromosome 6-A2 are often deleted or are translocational break point sites in a wide variety of tumors. Such tumors include hepatomas [47,48], ovarian cancers [49,50], prostate cancers [51,52], uterine leiomyomas [53], myeloid neoplasms [54], oral cancers [55], breast cancers [52], stomach adenocarcinomas [56], and renal cell carcinomas [57]. Based on these studies it has been concluded that an as yet unidentified tumor suppressor gene resides at 7q31/6-A2. Given that caveolin-1 possesses 'transformation suppressor activity' in vitro and caveolin-1 and -2 are localized to this chromosomal region, this unidentified tumor suppressor gene(s) may be caveolin-1 and/or -2. As many of these normal adult tissues express caveolin-1 and -2, it will be important to determine whether any of these chromosomal rearrangements leads to a loss of caveolin mRNA or protein expression during tumorigenesis.

We have recently shown that mutations in the human caveolin-3 gene, which maps to human chromosome 3p25, cause an autosomal dominant form of limb-girdle muscular dystrophy [34]. Here, we show that murine caveolin-3 localizes to chromosome 6-E1 in the mouse. A search of the NCBI Human/Mouse Homology Map indicates that these two regions (human 3p25 and murine 6-E1) are also homologous. Thus, the information provided here may help identify existing mouse models of muscular dystrophy that are due to mutations within the caveolin-3 gene. In addition, our studies should aid efforts toward constructing 'knock-out' mice that harbor homozygous targeted disruption of the caveolin genes. Such future studies will allow us and others to develop whole animal models to study the functional role of caveolins in normal development and in pathogenic states, such as cancer and muscular dystrophy.

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