Cell-type and Tissue-specific Expression of Caveolin-2

CAVEOLINS 1 AND 2 CO-LOCALIZE AND FORM A STABLE HETERO-OLIGOMERIC COMPLEX IN VIVO*

(Received for publication, August 6, 1997, and in revised form, September 10, 1997)

Philipp E. Scherer‡, Renée Y. Lewis‡, Daniela Volonté§, Jeffrey A. Engelman§§, Ferruccio Galbiati§§, Jacques Couet¶, D. Stave Kohtz***, Elly van Donselaar‡‡, Peter Peters‡‡, and Michael P. Lisanti §§

From the Departments of ‡Cell Biology and §Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461, the ¶Laval Hospital Research Center, Sainte-Foy, Quebec, Canada, the **Mount Sinai School of Medicine, Department of Pathology, New York, New York 10029, and the §§Faculty of Medicine, Cell Biology, Heidelberglaan100-H02, 3143584 CX Utrecht, The Netherlands

Caveolae are microdomains of the plasma membrane that have been implicated in organizing and compartmentalizing signal transducing molecules. Caveolin, a 21–24-kDa integral membrane protein, is a principal structural component of caveolae membrane in vivo. Recently, we and other laboratories have identified a family of caveolin-related proteins; caveolin has been termed caveolin-1.

Here, we examine the cell-type and tissue-specific expression of caveolin-2. For this purpose, we generated a novel mono-specific monoclonal antibody probe that recognizes only caveolin-2, but not caveolins-1 and -3. A survey of cell and tissue types demonstrates that the caveolin-2 protein is most abundantly expressed in endothelial cells, smooth muscle cells, skeletal myoblasts (L6, BC3H1, C2C12), fibroblasts, and 3T3-L1 cells differentiated to adipocytes. This pattern of caveolin-2 protein expression most closely resembles the cellular distribution of caveolin-1. In line with these observations, co-immunoprecipitation experiments with mono-specific antibodies directed against either caveolin-1 or caveolin-2 directly show that these molecules form a stable hetero-oligomeric complex. The in vivo relevance of this complex was further revealed by dual-labeling studies employing confocal laser scanning fluorescence microscopy. Our results indicate that caveolins 1 and 2 are strictly co-localized within the plasma membrane and other internal cellular membranes. Ultrastructurally, this pattern of caveolin-2 localization corresponds to caveolae membranes as seen by immunoelectron microscopy. Despite this strict co-localization, it appears that regulation of caveolin-2 expression occurs independently of the expression of either caveolin-1 or caveolin-3 as observed using different model cell systems. Although caveolin-1 expression is down-regulated in response to oncogenic transformation of NIH 3T3 cells, caveolin-2 protein levels remain unchanged. Also, caveolin-2 protein levels remain unchanged during the differentiation of C2C12 cells from myoblasts to myotubes, while caveolin-3 levels are dramatically induced by this process. These results suggest that expression levels of caveolins 1, 2, and 3 can be independently up-regulated or down-regulated in response to a variety of distinct cellular cues.

Caveolae are small omega-shaped indentations of the plasma membrane that have been implicated in signal transduction and vesicular transport processes (1, 2). Caveolae are found in most cell types but are extremely abundant in terminally differentiated cell types: adipocytes (3–5), simple squamous epithelia (type I pneumocytes and endothelial cells) (6), smooth muscle cells (7), and fibroblasts (8). Caveolin, a 21–24-kDa integral membrane protein, is a principal structural component of caveolae membranes in vivo (9–13).

Several independent lines of evidence suggest that caveolin functions as a scaffolding protein within caveolae membranes. Caveolin forms a high molecular mass oligomeric complex (14, 15) that is thought to represent the assembly unit of caveolae membranes (16), and recombinant expression of caveolin in caveolin-negative cells is sufficient to drive the formation of caveolae-sized vesicles (17–19). As caveolin interacts directly with cholesterol (20, 21) and glyco-sphingolipids (22), it has been proposed that the caveolin-mediated selection of endogenous lipid components could provide the driving force for caveolae formation (18).

Loss or dramatic reduction of caveolin expression and caveolae occurs in NIH 3T3 cells transformed by activated oncoproteins other than v-Src (23). Caveolin expression was monitored in normal NIH 3T3 cells and compared with NIH 3T3 cells transformed with known oncopgenes, such as bcr-abl, v-abl, middle T antigen, and activated Ras. In all cases, quantitation of caveolin protein expression revealed that the caveolin levels were dramatically reduced, from 25- to 100-fold depending on the specific oncogene examined. Transformed cells that expressed little or no caveolin did not contain any caveolae, as visualized by transmission electron microscopy (23). In addition, caveolin expression levels correlated inversely with the ability of these cells to grow in soft agar. That is, the cells expressing the least amount of caveolin and containing no detectable caveolae formed the largest colonies in soft agar. These results identify caveolin as a candidate tumor suppressor gene (23).

Recently, we have expressed caveolin in oncogenically transformed cells under the control of an inducible expression system (19). Regulated induction of caveolin expression was monitored by Western blot analysis and immunofluorescence microscopy. Our results indicate that the caveolin protein is

* This work was supported by a National Institutes of Health FIRST Award (to M. P. L.), a grant from the Elsa U. Pardee Foundation (to M. P. L.), a grant from the G. Harold and Leila Y. Mathers Charitable Foundation (to M. P. L. and P. E. S.), and a Scholarship in the Medical Sciences from the Charles E. Culpeper Foundation (to M. P. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡‡ Contributed equally to this work.

§§ To whom correspondence should be addressed: 1300 Morris Park Ave., Bronx, NY 10461. Tel: 718-430-8828; Fax: 718-430-8830; E-mail: lisanti@aecom.yu.edu.

This paper is available on line at http://www.jbc.org

Vol. 272, No. 46, Issue of November 14, pp. 29337–29346, 1997
Printed in U.S.A.
expressed well using this system and correctly localizes to the plasma membrane. Induction of caveolin expression in v-Ab-transformed 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 as seen by transmission electron microscopy (19). Consistent with its antagonism of Ras-mediated cell transformation, caveolin expression dramatically inhibited both Ras/mitogen-activated protein kinase-mediated and basal transcriptional activation of a mitogen-sensitive promoter (19). Using an established system to detect apoptotic cell death, it appears that the effects of caveolin may, in part, be attributed to its ability to initiate apoptosis in rapidly dividing cells (19). In addition, we find that caveolin expression levels are reversibly down-regulated by two distinct oncogenic stimuli. Taken together, our results indicate that down-regulation of caveolin expression and caveolae organelles may be critical to maintaining the transformed phenotype in certain cell populations (19).

Caveolin also interacts directly with signaling molecules, preferring their inactive conformation. Using a variety of domain-mapping approaches (deletion mutagenesis, glutathione S-transferase fusion proteins, and synthetic peptides), a region within caveolin has been defined that mediates the interaction of caveolin with itself and other proteins. This cytoplasmic 41-amino acid membrane proximal region of caveolin is sufficient to mediate the formation of caveolin homo-oligomers (14), and the C-terminal half of this region (20 amino acids; residues 82–101) mediates the interaction of caveolin with G-protein α subunits, H-Ras, and Src-family tyrosine kinases (24–26). This caveolin region preferentially recognizes the inactive conformation of these molecules, as mutationally activated Gα subunits (Gαq; Q227L), v-Src, and H-Ras (G12V) fail to interact with caveolin (24–26). As this caveolin domain (residues 82–101) is critical for caveolin homo-oligomerization and the interaction of caveolin with certain caveola-associated proteins (G-proteins, H-Ras, and Src-family kinases), we have previously termed this protein domain the caveolin-scaffolding domain or CSD (25, 27).

We have suggested that the caveolin scaffolding domain may function like other modular protein domains (SH-2, SH-3, PH, WW, and others) to generate membrane-bound oligomeric complexes that contain signaling molecules and cytoskeletal elements (2, 27). In essence, caveolin may act as molecular “velcro” to nucleate the formation of signal transduction complexes, holding these molecules in the off state. These findings would also explain the ability of caveolin expression to abrogate the anchorage-independent growth of cancerous/transformed cells (19).

Recent studies have shown that caveolin is only the first member of a growing gene family of caveolin proteins; caveolin has been re-terminalized caveolin-1. Three different caveolin genes (Cav-1, Cav-2, and Cav-3) encoding four different subtypes of caveolin have been described thus far (2). There are two subtypes of caveolin-1 (Cav-1α and Cav-1β) that differ in their respective translation initiation sites (28). The tissue distribution of caveolin-2 mRNA is extremely similar to caveolin-1 mRNA (5). In striking contrast, caveolin-3 mRNA and protein are expressed mainly in muscle tissue types (skeletal, cardiac, and smooth) (29–31).

Although caveolins-1 and -3 are now well-characterized, the study of caveolin-2 has been hampered by a lack of caveolin-2-specific antibody probes. Here, we have generated and characterized a novel mAb probe that recognizes the caveolin-2 protein but not other known members of the caveolin gene family. Using this novel mAb probe, we (i) characterize the cell-type and tissue-specific expression of the caveolin-2 protein, (ii) report co-expression, co-localization, and co-immunoprecipitation with caveolin-1, a well established caveolar marker protein, and (iii) demonstrate that caveolin-2 is localized to caveolae membranes as seen by immunoelectron microscopy.

EXPERIMENTAL PROCEDURES

Materials—The cDNAs for caveolins-1, -2, and -3 were as described previously (5, 30, 32). Antibodies and their sources were as follows: anti-caveolin-1 IgG (mAb 2297; gift of Dr. John R. Glenney, Jr., Transduction Labs); anti-caveolin-2 IgG (mAb 2254; gift of John R. Glenney, Jr.); anti-myc epitope IgG (mAb 9E10; Santa Cruz Biotech); anti-caveolin-1 (pAb; rabbit anti-peptide antibody directed against caveolin-1 residues 2–21; Santa Cruz Biotech). A mAb directed against caveolin-3 (clone 26) was as described previously (29). The anti-GDP dissociation inhibitor (GDI) antibody was a gift from Dr. Ferry Bickel, Whitehead Institute, Cambridge, MA (33). A variety of other reagents were purchased commercially: fetal bovine serum (SRH Biosciences); pre-stained protein markers (Life Technologies, Inc.); Slow-Fade anti-fade reagent (Molecular Probes, Eugene, OR).

Hybridoma Production—A monoclonal antibody to caveolin-2 was generated by multiple immunizations of Balb/c female mice with a fusion protein encoding the full-length human caveolin-2 protein. Mice showing the highest titer of anti-caveolin-2 immuno-reactivity were used to create fusions with myeloma cells using standard protocols (34). Positive hybridomas were cloned twice by limiting dilution and injected into mice to produce ascites fluid. IgGs were purified by affinity chromatography on protein A-Sepharose. These antibodies were produced in collaboration with Drs. Roberto Campos-Gonzalez and John R. Glenney, Jr. (Transduction Laboratories, Lexington, KY).

Transient Expression of Caveolin mRNAs in 293T Cells—Constructs encoding C-terminally myc-epitope-tagged forms of caveolin-1, -2, or -3 were as described previously (5, 28, 30). These constructs (~5–10 μg) were transiently transfected into 293T cells using standard calcium-phosphate precipitation. Forty-eight hours post-transfection, cells were scraped into lysis buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100, 60 mM octyl-glucoside). Reombinant expression was analyzed by SDS-PAGE (15% acrylamide) followed by Western blotting. Epitope-tagged forms of caveolin-1, -2, and -3 were detected using the monoclonal antibody, 9E10, which recognizes the myc-epitope (EQKQLISEEDLN).

Tissue Western—Approximately 200 mg of various mouse tissues were lysed in immunoprecipitation buffer and homogenized on ice with a Polytron tissue grinder, as described (4). Equal amounts (100 μg) were loaded on an SDS-PAGE gel (12% acrylamide). After transfer to nitrocellulose, the blot was probed with antibodies directed against caveolin-1, -2, -3 and GDI.

Immunofluorescence Microscopy—All reactions were performed at room temperature. 3T3-L1 fibroblasts were briefly washed three times with PBS and fixed for 45 min in PBS containing 3% paraformaldehyde. Fixed cells were rinsed with PBS and treated with 25 mM NH4Cl in PBS for 10 min to quench free aldehyde groups. Cells were then permeabilized with 0.1% Triton X-100 for 10 min at room temperature and washed with PBS, four times at 10 min each. The cells were then successively incubated with PBS, 2% BSA containing: (i) a 1:200 dilution of anti-caveolin-2 IgG (mAb 65) and anti-caveolin-1 IgG (pAb; directed against caveolin-1 residues 2–21), and (ii) lissamine rhodamine B sulfonyl chloride-conjugated goat anti-mouse antibody (5 μg/ml) and fluorescein isothiocyanate-conjugated donkey anti-rabbit antibody (5 μg/ml). The first incubation was 30 min while primary and secondary antibody reactions were 60 min each. Cells were washed three times with PBS between incubations. Slides were mounted with Slow-Fade anti-fade reagent and observed under a Bio-Rad MR600 confocal fluorescence microscope.

Cell Culture Models of Adipocyte and Skeletal Muscle Differentiation—3T3-L1 mouse fibroblasts were propagated in 10-cm dishes and differentiated according to the conventional protocol (35). C2C12–3 cells (36) were derived from a single colony of C2C12 cells (37) cultured at clonal density and display a more stable phenotype than the parental cell line. C2C12–3 myoblasts were cultured as described elsewhere (36). Briefly, proliferating C2C12–3 cells were cultured in high mitogen amide gel electrophoresis; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary; IPTG, isopropyl-1-thio-β-D-galactopyranoside; Mes, 4-morpholinethanesulfonic acid.
medium (Dulbecco's modified Eagle's containing 15% fetal bovine serum and 1% chicken embryo extract) and induced to differentiate at confluence in low mitogen medium (Dulbecco's modified Eagle's containing 3% horse serum). Overt differentiation was indicated by the assembly of multi-nucleated syncyta, which commenced 36–48 h after the cells were switched to low mitogen media.

**Velocity Gradient Centrifugation**—Estimation of the molecular mass of caveolin-2 was performed as described previously for caveolin-1 (14). Briefly, samples were dissociated by incubation with 500 μl of Mes-buffered saline (25 mm, pH 6.5, 0.15 mM NaCl) plus 60 mM octyl-glucoside. Solubilized material was then loaded atop a 5–40% linear sucrose gradient (4.3 ml) and centrifuged at 50,000 rpm (~340,000 × g) for 10 h in a SW-60 rotor (Beckman Instruments, Palo Alto, CA). Note that the entire gradient was prepared in MBS plus 60 mM octyl-glucoside. After centrifugation, gradient fractions were collected from the top. Molecular mass standards for velocity gradient centrifugation were as follows: carbonate anhydrase (29 kDa); bovine serum albumin (66 kDa); alcohol dehydrogenase (150 kDa); β-amylose (200 kDa); apoferritin (445 kDa) (Sigma).

**Immunoblotting**—Samples were separated by SDS-PAGE (15% 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 Life Science, Inc.) except that we supplemented our blocking solution with both 1% bovine serum albumin and 1% non-fat dry milk (Carnation).

**Immunoprecipitation**—Immunoprecipitations were carried out using protein-A Sepharose CL-4B (Pharmacia Biotech Inc.) as described previously (38), with minor modifications. Briefly, cells were lysed in a buffer containing 10 mM Tris, pH 8.0, 0.15 mM NaCl, 5 mM EDTA, 1% Triton X-100, 60 mM octyl-glucoside and subjected to immunoprecipitation with anti-caveolin-1 (mAb 2224) or anti-caveolin-2 (mAb 65). After extensive washing, samples were separated by SDS-PAGE (15% acrylamide) and transferred to nitrocellulose. Blots were then probed with IgGs directed against caveolin-1 (mAb 2297) or caveolin-2 (mAb 65).

**Immunogold Electron Microscopy**—The procedures used were as described previously (39). Briefly, samples were fixed in 2% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 for 24 h at room temperature. Cells were transferred in 0.2% paraformaldehyde, scraped, and collected. Samples were then processed for cryo-electron microscopy using a Leica ultra-cryomicrotome and Diatome diamond knife. Sections of 45 nm were cut at 125 °C and collected with a mixture of sucrose and cellulose (40). Cryosections were incubated at room temperature with antibodies for 30 min, washed, and incubated for 20 min with protein A gold. Electron micrographs were made with a JEOL 1010 electron microscope at 80 kV.

**Immunostaining of Human Skeletal Muscle Tissue**—All solutions were prepared in PBS. Frozen sections (4–6 μm) from normal human muscle biopsies were fixed with 4% paraformaldehyde and blocked for 1 h with 5% horse serum and 5% non-fat dry milk. Primary antibodies were diluted in blocking solution (1:400) and incubated at 4 °C overnight. After washing 4 times, sections were incubated with horseradish peroxidase-conjugated anti-mouse antibody (diluted 1:1,500) for 2 h at 4 °C. To visualize bound secondary antibodies, sections were further incubated with 3,3'-diaminobenzidine (1 mg/ml) and 0.03% hydrogen peroxide. Note that endogenous peroxidase activities were inactivated after fixation and prior to antibody incubations.

**RESULTS**

**Generation and Characterization of a mAb Probe Specific for Caveolin-2**—Caveolins-1, -2, and -3 are distinct gene products with different molecular masses, all in the range of ~18–24 kDa. C-terminal myc-tagged forms of caveolins-1, -2, and -3 were transiently expressed in 293T cells. Lysates were generated and used to determine the specificity of caveolin antibody probes by immunoblotting. As a control for equal loading, immunoblotting was first performed with mAb 9E10 that recognizes the myc-epitope; this antibody reveals all three myc-tagged caveolin gene products (top panel). Note that mAb 65 only recognizes caveolin-2 (bottom panel). Molecular weight markers are as indicated.

**Caveolin-2 Protein Is Induced during Adipocyte Differentiation**—Cultured 3T3-L1 fibroblasts offer a convenient system to study adipocyte differentiation (3). These cells can be induced to differentiate over a period of 8 days from precursor fibroblasts into adipocytes. Caveolin-2 protein is strongly induced during this differentiation process (Fig. 3B, bottom panel).

**Cell-type and Tissue-specific Expression of the Caveolin-2 Protein**—To identify model cell systems to study caveolin-2, we examined the expression of caveolin-2 in a variety of commonly used cell lines and primary cultured cells (Fig. 2). The expression patterns of caveolin-1 and -3 are shown for comparison; antibodies to the ubiquitously expressed GDI were used to confirm equal loading. Note that caveolin-2 is most widely expressed, whereas caveolin-1 shows a more restricted distribution, and caveolin-3 is found only within a cell line derived from skeletal muscle. More specifically, caveolin-2 is most abundant in endothelial cells, smooth muscle cells, skeletal myoblasts (L6 and BC3H1), fibroblasts, and 3T3-L1 adipocytes. Thus, the expression of caveolin-2 protein most closely parallels the distribution of caveolin-1. Also, it is important to note that L6 myoblasts are the only cell line that expresses all three caveolins simultaneously.

To establish the tissue distribution of caveolin-2 protein, we prepared extracts from a number of different murine tissues (Fig. 3A). The tissue distribution of caveolins-1 and -3 are shown for comparison; again, to ensure equal protein loading in all lanes, we also probed these blots with anti-GDI antibodies. Caveolin-2 is most abundantly main in adipose and lung tissues, although longer exposures demonstrate a lower level of caveolin-2 expression in most tissue types. This is consistent with (i) our previous work demonstrating that caveolin-2 mRNA is most abundant in white adipose tissue, differentiated 3T3-L1 adipocytes, and lung tissue (5) and (ii) with previous morphological evidence suggesting that differentiated 3T3-L1 adipocytes are a rich source of caveolae (3, 4).

**Caveolin-2 Protein Is Induced during Adipocyte Differentiation**—Caveolin-2 protein was induced in 3T3-L1 fibroblasts during the terminal differentiation process. The expression of caveolin-2 is induced ~10–20-fold.

**Caveolin-2 Protein Is Induced during Adipocyte Differentiation**—Caveolin-2 protein was induced in 3T3-L1 fibroblasts during the terminal differentiation process. The expression of caveolin-2 is induced ~10–20-fold.

**Fig. 1. Characterization of a mAb probe specific for caveolin-2.** Caveolins-1, -2, and -3 are distinct gene products with different molecular masses, all in the range of ~18–24 kDa. C-terminal myc-tagged forms of caveolins-1, -2, and -3 were transiently expressed in 293T cells. Lysates were generated and used to determine the specificity of caveolin antibody probes by immunoblotting. As a control for equal loading, immunoblotting was first performed with mAb 9E10 that recognizes the myc-epitope; this antibody reveals all three myc-tagged caveolin gene products (top panel). Note that mAb 65 only recognizes caveolin-2 (bottom panel). Molecular weight markers are as indicated.
Caveolins-1 and -2 Form a Stable Hetero-oligomeric Complex—Given the similarity between the tissue and cellular distributions of caveolins 1 and 2, we wondered whether these two distinct caveolin gene products interact in vivo. To address this issue, we performed a series of co-immunoprecipitation experiments. 3T3-L1 adipocytes were lysed and subjected to immunoprecipitation with a mAb directed against caveolin-1 (2234) that recognizes a unique N-terminal epitope that is not found in other caveolin family members (28). These immunoprecipitations were then probed by Western analysis using anti-caveolin-1 IgG (mAb 65). Conversely, lysates were also immunoprecipitated with IgGs directed against caveolin-2 and then probed by Western analysis using anti-caveolin-1 IgG (mAb 2234).

Fig. 4 demonstrates that mAb 2234 directed against caveolin-1 (2234) can be used to co-immunoprecipitate both caveolins-1 and -2. In addition, mAb 65 directed against caveolin-2 can be used to co-immunoprecipitate both caveolins-1 and -2. This is despite the fact that these antibodies are monospecific as demonstrated by Western blot analysis (Ref. 28, and this report). Thus, it appears that caveolins 1 and 2 form a stable complex in vivo.

To estimate the amount of caveolin-2 that forms a complex with caveolin-1, a 3T3-L1 adipocyte lysate was divided into two parts. Part A was loaded directly onto an SDS-PAGE gel to quantitate the total amount of caveolin-2 in the extract. Part B was immunoprecipitated with anti-caveolin-1 IgG (mAb 2234). Fig. 5 shows that immunoprecipitation of the lysate with anti-caveolin-1 IgG (mAb 2234) resulted in a dramatic reduction of the caveolin-2 signal by >90%. These results clearly demonstrate that under steady-state conditions the bulk of caveolin-2 is associated with caveolin-1.

Immunolocalization of Caveolin-2 to the Plasma Membrane and Intracellular Membranes: Co-localization with Caveolin-1—To further examine whether caveolins 1 and 2 are physically associated as a discrete complex in intact cells, we performed double-labeling with mAb 65 (caveolin-2-specific) and an anti-caveolin-1-specific polyclonal IgG directed against a unique N-terminal region of caveolin-1 (residues 2–21). These two primary antibodies were chosen for double-labeling experiments as they were elicited in different animal species (mouse versus rabbit), minimizing possible cross-reaction of the individual primary antibodies with distinctly tagged secondary antibodies. Immunostaining was visualized by traditional fluorescence microscopy.

The immunostaining pattern obtained in 3T3-L1 fibroblasts with caveolin-2 is very similar to immunostaining patterns observed previously for caveolins-1 and -3 (data not shown) (5, 12, 13, 28, 30–32). Many small micro-patches are present throughout the cell and along the cell surface. In addition, double-labeling experiments employing 3T3-L1 fibroblasts that co-express caveolin-1 and caveolin-2 demonstrate significant co-localization of these two distinct gene products (not shown). The intense immunostaining may represent the leading edge of the cell as caveolae are known to be morphologically concentrated in this area of the cell (12).

As our initial experiments using traditional fluorescence microscopy showed co-localization of caveolins 1 and 2, we used confocal laser scanning microscopy to more stringently assess their co-localization. Fig. 6A shows a series of optical sections taken from the top (panel 1) to the bottom (panel 10) of a single 3T3-L1 fibroblast. A stacked composite of these images is presented in Fig. 6B. Note that in all the optical planes examined, caveolin-1 and -2 demonstrate the same pattern of localization, indicating that these two caveolins co-exist within the same regions of a given cell.

Ultrastructural Localization of Caveolin-2 to Caveolae Membranes by Immunoelectron Microscopy—Using ultrathin cryosections, we next explored the ultrastructural localization of caveolin-2 by immunoelectron microscopy. Fig. 7 shows the distribution of caveolin-2 in a fibroblastic cell line (CHO cells, panel A) and endothelial cells (panel B) derived from a human skin biopsy. In CHO cells, immunogold labeling is specifically associated with caveolae (see arrows). In endothelial cells, caveolae on both luminal and basolateral sides of the cell were stained by immunogold labeling with anti-caveolin-2 IgG (mAb 65). In contrast, mitochondria, endoplasmic reticulum, the nu...
Caveolins 1 and 2 Form Stable Hetero-oligomeric Complexes

**Fig. 3.** Western blot analysis of the tissue distribution of caveolin-2: induction of caveolin-2 protein during adipocyte differentiation. **A**, tissue Western. Extracts of mouse tissues were prepared as described under “Experimental Procedures”. In addition, a lane containing differentiated 3T3-L1 adipocytes was included as a comparison. After SDS-PAGE and transfer to nitrocellulose, blots were probed with anti-caveolin IgG. First panel, caveolin-1 (mAb 2297); second panel, caveolin-2 (mAb 65); third panel, caveolin-3 (mAb 26); and fourth panel, GDI polyclonal IgG (as a control for equal loading). Caveolins-1 and -2 are most abundantly expressed in adipose tissue and 3T3-L1 adipocytes. Note that the lane containing skeletal muscle is underloaded as judged by the low signal obtained for GDI immunoblotting.

**B**, adipocyte differentiation. 50 μg of total cellular protein extracted from 3T3-L1 fibroblasts and 3T3-L1 adipocytes (days 2, 4, 6, 8, and 10) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-caveolin-2 (mAb 65; top panel) or anti-Acrp30 (rabbit polyclonal IgG; bottom panel). Note that caveolin-2 and Acrp30 (an adipocyte-specific secretory protein) are both dramatically induced during adipocyte differentiation. Acrp30 served as a positive control for differentiation.

A significant fraction of total caveolin-2 is associated with caveolin-1 at steady-state. One 10-cm plate of 3T3-L1 adipocytes was lysed as described under “Experimental Procedures.” Prior to the immunoprecipitation, 5% of the total lysate was removed (T, Total). The remaining 95% was subsequently immunoprecipitated with anti-caveolin-1 IgG (mAb 2234); 5% of the resulting immunoprecipitate (P, Pellet) and 5% of the remaining supernatant after the extract was immunodepleted for caveolin 1 (S, Supernatant) were then analyzed by SDS-PAGE/Western blotting and probed with anti-caveolin-2 IgG. Note that >90% of caveolin-2 was recovered with anti-caveolin-1 IgG (mAb 2234).

**Fig. 4.** Caveolins 1 and 2 form a stable hetero-oligomeric complex. 3T3-L1 fibroblasts were lysed and subjected to immunoprecipitation with anti-caveolin-1 IgG (mAb 2234) that recognizes a unique N-terminal epitope that is not found in other caveolin family members (28). These immunoprecipitates were then probed by Western analysis using anti-caveolin-2 IgG (mAb 65). Conversely, lysates were also immunoprecipitated with IgGs directed against caveolin-2 (mAb 65) and then probed by Western analysis using anti-caveolin-1 IgG (mAb 2297). Immunoprecipitation with anti-caveolin-3 IgG (mAb 26) was included as a negative control as the expression of caveolin-3 is muscle specific. Note that anti-caveolin-1 IgG (mAb 2234) can be used to co-immunoprecipitate both caveolins-1 and -2. In addition, mAb 65 directed against caveolin-2 can be used to co-immunoprecipitate both caveolins-1 and -2. Thus, it appears that caveolins 1 and 2 form a stable complex in vivo.

**Fig. 5.** Caveolins 1 and 2 form a stable hetero-oligomeric complex. 3T3-L1 fibroblasts were lysed and subjected to immunoprecipitation with anti-caveolin-1 IgG (mAb 2234) that recognizes a unique N-terminal epitope that is not found in other caveolin family members (28). These immunoprecipitates were then probed by Western analysis using anti-caveolin-2 IgG (mAb 65). Conversely, lysates were also immunoprecipitated with IgGs directed against caveolin-2 (mAb 65) and then probed by Western analysis using anti-caveolin-1 IgG (mAb 2297). Immunoprecipitation with anti-caveolin-3 IgG (mAb 26) was included as a negative control as the expression of caveolin-3 is muscle specific. Note that anti-caveolin-1 IgG (mAb 2234) can be used to co-immunoprecipitate both caveolins-1 and -2. In addition, mAb 65 directed against caveolin-2 can be used to co-immunoprecipitate both caveolins-1 and -2. Thus, it appears that caveolins 1 and 2 form a stable complex in vivo.

**Fig. 8A** shows that while caveolin-1 expression is dramatically down-regulated in v-Abl and H-Ras (G12V)-transformed cells, endosomes, intermediate filaments, the basal lamina, and erythrocytes remained completely unlabeled by this technique. Immunogold labeling of caveolae within endothelial cells is consistent with our observation that caveolin-2 is abundantly expressed in endothelial cells by Western blot analysis (Fig. 2). Differential Expression of Caveolins-1 and -2 in Oncogenically Transformed NIH 3T3 Cells—Modification and/or inactivation of caveolin-1 expression appears to be a common feature of the transformed phenotype. Historically, caveolin-1 was first identified as a major v-Src substrate in Rous sarcoma virus-transformed cells (9). Based on this observation, it has been proposed that caveolin-1 may represent a critical target during cell transformation (9, 10). In direct support of this notion, caveolin-1 mRNA and protein expression are reduced or absent in NIH 3T3 cells transformed by a variety of activated oncogenes (such as v-Abl and H-Ras (G12V)); caveolae organelles are also missing from these transformed cells (23). However, it remains unknown whether caveolin-2 is down-regulated in response to oncogenic transformation.
29342 Caveolins 1 and 2 Form Stable Hetero-oligomeric Complexes

NIH 3T3 cells, caveolin-2 expression remains virtually unaffected in v-Abl transformed cells and is slightly induced (~2-fold) in H-Ras (G12V) transformed cells. As we have previously demonstrated that these transformed cells do not contain detectable caveolae (23), it appears that expression of caveolin-2 is not sufficient to drive caveola formation. Thus, caveolin-2 can be expressed within cells that lack morphologically distinguishable caveolae.

As caveolin-1 forms a high molecular mass oligomeric complex (14, 15), we wondered if low levels of caveolin-1 expression would affect the oligomeric state of caveolin-2. Thus, we compared the size of caveolin-2 complexes in normal and v-Abl transformed NIH 3T3 cells (Fig. 8B). Our results indicate that in v-Abl transformed cells that lack normal levels of caveolin-1 expression, a significant fraction of caveolin-2 was present in the monomeric or dimeric state. This is consistent with our previous results demonstrating that recombiant over-expression of caveolin-2 in Cos-7 cells yields primarily dimeric and monomeric caveolin-2 (5). However, in normal NIH 3T3 cells that express caveolin-1, all of the caveolin-2 was present within large oligomeric complexes (>150 kDa). These results suggest that caveolin-1 co-expression facilitates the formation of high molecular mass complexes that contain both caveolins 1 and 2.

The distribution of recombiantly over-expressed caveolin-2 after transient expression in Cos-7 cells is shown for comparison; recombiant caveolin-2 was detected using mAb 9E10 that recognizes the myc-epitope (Fig. 8B). In contrast to endogenous caveolin-2, recombiantly over-expressed caveolin-2 behaves mainly as a dimer in these velocity gradients (5).

Caveolin-1 Embeds Caveolin-2 Tightly within a Hetero-oligomeric Complex—During the course of the current studies, we noticed that there are higher levels of caveolin-2 in immuno-precipitates generated with anti-caveolin-1 IgG than in immunoprecipitates generated with caveolin-2 IgG directly. Given that the anti-caveolin-1 mAb does not cross-react with caveolin-2, we found this observation quite surprising.

A possible explanation for this phenomenon is epitope masking, in which caveolin-1 binding to caveolin-2 would block access to the epitope recognized by the anti-caveolin-2 IgG. To test this hypothesis, we took advantage of a v-Abl-transformed cell line that harbors a copy of the caveolin-1 CDNA under the control of the lacZ promoter (19). While these cells express very low levels of endogenous caveolin-1 due to transformation, caveolin-1 expression levels can be dramatically induced in the presence of IPTG.

Fig. 9 shows that in the absence of IPTG, i.e. very low levels of caveolin-1, small amounts of caveolin-2 can be recovered with anti-caveolin-1 IgG (first lane). Upon induction of caveolin-1, increased levels of caveolin-2 can be recovered with anti-caveolin-1 IgG in agreement with increased incorporation of caveolin-2 into caveolin-1 containing complexes (second lane). However, induction of caveolin-1 decreases the amount of caveolin-2 signal that can be recovered with anti-caveolin-2 IgG (third and fourth lanes).

This observation is in line with our hypothesis that caveolin-1 binding to caveolin-2 masks the epitope recognized by the caveolin-2 IgG. In addition, control experiments confirmed that total caveolin-2 levels remained constant before and after induction of caveolin-1 (not shown). Hence, we conclude that caveolin-2 molecules are tightly embedded within the caveolin-1 oligomer.

Caveolin-2 Is Constitutively Expressed in C2C12 Myoblasts and Myotubes—Cultured C2C12 cells offer a convenient system to study skeletal myoblast differentiation. These cells can be induced to differentiate from myoblasts into myotubes bearing an embryonic phenotype in low mitogen medium over a period of 2 days (36, 37). We and others have previously shown that caveolin-3 mRNA and protein are undetectable in precursor myoblasts and are strongly induced during myoblast differentiation (29–31). In contrast, no caveolin-1 expression was detected in either precursor myoblasts or differentiated myotubes (29, 30). These results are consistent with the selective expression of caveolin-3 in skeletal muscle and other muscle tissues (29–31) and suggest that caveolin-3 may function in muscle from the earliest stages of its development.

As caveolin-2 was expressed in L6 and BC3H1 skeletal myo-
blasts (Fig. 2), we assessed whether caveolin-2 is induced during differentiation of C2C12 cells from myoblasts to myotubes. Fig. 10 shows that caveolin-2 levels remained constant during this process of differentiation. As a positive control for the differentiation process, we also assessed the induction of caveolin-3 within the same samples. In contrast, caveolin-3 was dramatically induced during the transition from myoblasts to myotubes. These results suggest that the expression of caveolins 2 and 3 are independently regulated within skeletal muscle fibers.

Localization of Caveolin-2 within Bona Fide Skeletal Muscle Tissue—Given that caveolin-2 was constitutively expressed in...
three distinct myoblast cell lines (L6, BC3H1, and C2C12) and within differentiated C2C12 myotubes, we next examined the localization of caveolin-2 within human skeletal muscle tissue. Fig. 11 shows that caveolin-2 is primarily expressed within the endothelial cells that line the blood vessels that run between the muscle fibers but not within the myofibers themselves. The distribution of caveolin-3 is shown for comparison. Note that caveolin-3 immunostaining is confined to the sarcolemma (plasma membrane) of the myofibers and is not detected within any other cell types. Thus, co-expression of caveolin-2 and -3 in myoblasts and myotubes does not reflect the state of their expression within human adult skeletal muscle tissue.

**DISCUSSION**

Caveolins 1, -2, and -3 are a family of cytoplasmic membrane-anchored scaffolding proteins that (i) help to sculpt caveolae membranes from the plasma membrane proper and...
Caveolins 1 and 2 Form Stable Hetero-oligomeric Complexes

Fig. 11. Immunolocalization of caveolins 2 and 3 within bona fide skeletal muscle tissue. Two consecutive parallel sections derived from a human skeletal muscle biopsy were immunostained with either anti-caveolin-2 IgG (mAb 65; top panel) or anti-caveolin-3 IgG (mAb 28; bottom panel). Bound primary antibodies were visualized with the appropriate horseradish peroxidase-conjugated secondary antibody. Note that caveolin-2 is primarily expressed within the endothelial cells that line the blood vessels, while caveolin-3 is localized to the sarcolemma (plasma membrane) of the myofibers.

(ii) participate in the sequestration of inactive signaling molecules (reviewed within Ref. 2). Although caveolins-1 and -3 are now well-characterized, the study of caveolin-2 has been hampered by a lack of caveolin-2-specific antibody probes. Only the distribution of caveolin-2 mRNA was previously studied, and a recombinant epitope-tagged form of the protein has been expressed in cultured cells (5).

Here, we have generated and characterized a novel mAb probe that recognizes the native endogenous caveolin-2 protein but not other known members of the caveolin gene family. This novel probe will greatly facilitate the study of caveolae in adipocytes, endothelial cells, and smooth and striated muscle cells as caveolin-2 is the most widely expressed caveolin family member observed to date. Caveolin-2 is highly expressed in many cell lines that fail to express caveolin-1, suggesting that caveolin-2 does not absolutely require caveolin-1 for its expression.

Immunolocalization of caveolin-2 in 3T3-L1 fibroblasts reveals that caveolin-2 is localized to the plasma membrane and internal membranes; this immunostaining pattern strictly coincides with the subcellular distribution of caveolin-1 within the same cell. In line with these observations, co-immunoprecipitation experiments clearly demonstrate that caveolins 1 and 2 form a stable hetero-oligomeric complex in vivo. This is consistent with our previous report demonstrating that the mRNA’s for caveolins 1 and 2 are co-expressed within the same cell types and both mRNA species are co-induced during adipocyte differentiation (5).

We have previously described the existence of caveolin-1 homo-oligomeric complexes (14). This was at a time when we had no concrete evidence for the existence of additional caveolin genes. A host of experiments prompted us to conclude that caveolin-1 exists as a homo-oligomeric complex. These studies were performed mainly in MDCK cells. Interestingly, MDCK cells are peculiar in that they express caveolin-1 but very little if any caveolin-2 (See Fig. 2). As such, they are an exception since we find here that caveolins-1 and -2 are co-expressed in most other cell systems. However, this does explain our initial findings that caveolin-1 forms a homo-oligomeric complex in MDCK cells (14). In addition, we have shown that purified recombinant caveolin-1 expressed in E. coli and SF 21 insect cells is sufficient to form caveolin-1 homo-oligomers of the same size as native endogenous caveolin-1 (14, 18, 21).

Perhaps surprisingly, we find that the co-expression of caveolins 1 and 2 is uncoupled by cellular transformation by activated oncogenes, such as v-Abl and activated H-Ras (G12V). While caveolin-1 mRNA and protein levels are down-regulated in response to cellular transformation (23), caveolin-2 protein levels remain relatively unchanged. While these cells continue to express caveolin-2 protein (this report), they fail to contain detectable caveolae, as we have reported previously (23). These observations suggest that caveolin-2 expression alone is not sufficient to drive the formation of morphologically detectable caveolae. However, detergent-insoluble caveolin-2-rich domains still exist (in the absence of caveolin-1) that are not distinguishable as “invaginated caveolae” by conventional transmission electron microscopy. These observations suggest that caveolin-rich microdomains may be more versatile structures with greater plasticity than previously imagined. In support of this idea, we have recently expressed caveolin-2 in SF21 insect cells using baculo-virus-based vectors. Caveolin-2 expression in these insect cells fails to drive the formation of caveole-like vesicles 2 while recombinant expression of caveolin-1 within the same cell system is sufficient to drive the formation of hundreds of uniform caveolae-like vesicles (50–100 nm in diameter) (18). These results are consistent with the idea that caveolin-2 may function as an “accessory protein” in conjunction with caveolin-1.

In many experiments we observed a slightly less abundant and faster migrating form of caveolin-2 (See Figs. 2, 3, and 4). This may represent a proteolytic degradation product of caveolin-2 or may be a translationally produced isoform. As a single caveolin-1 mRNA gives rise to caveolin-1α and caveolin-1β via alternate translation initiation (28), we favor the possibility that the faster migrating form of caveolin-2 is also generated as a consequence of alternate translation initiation. In support of this hypothesis, further sequencing and analysis of the 5’ end of the human caveolin-2 cDNA reveals an additional initiator methionine 13 amino acids upstream (MGLETKEADVQLFMDD...) of the previously reported initiator methionine. The updated cDNA sequence can be found under GenBank™ accession number U32114. We are currently investigating the significance of this upstream initiation site. However, one possibility is that this upstream initiator may allow for myristoylation of caveolin-2 as an N-terminal cytoplasmic MG sequence is a co-translational consensus site for N-myristoylation. In addition, these two methionines may serve as alternate translation initiation sites to generate two distinct isoforms of caveolin-2 (Cav-2α and Cav-2β). If this is the case, then only the longer isoform of caveolin-2 (Cav-2α) would be expected to undergo myristoylation.

Caveolin-3 is a muscle-specific caveolin-related protein that is expressed in striated muscle cell types (cardiac and skeletal) (29–31). Unlike caveolin-3, caveolin-1 is not expressed in striated muscle cells. It was, therefore, surprising to observe caveolin-2 expression in three skeletal myoblast cell lines (L6, BC3H1, and C2C12) and in differentiated C2C12 myotubes. As caveolins-1 and -3 are most closely related based on primary sequence homology and caveolin-2 is most distant (30), caveolin-2 may also function in embryonic muscle as a complex with caveolin-3. However, we have been unable to demonstrate a

2 M. P. Lisanti, unpublished observations.
stable association between caveolins 2 and 3 by co-immunoprecipitation (data not shown), as caveolin-2 is not expressed within adult skeletal muscle fibers (See Fig. 11).

Caveolins 1 and 3 may have originated from a common ancestor as we have recently identified only two caveolins within C. elegans, termed Cavα-1 and Cavα-2 (43). Cavα-1 is most closely related to mammalian caveolins-1 and -3; Cavα-2 is most closely related to mammalian caveolin-2 (43). Thus, caveolin-2 appears to be structurally and functionally conserved from worms to man, suggesting an important evolutionary role for caveolin-2 in the regulation of caveolae membranes.

Acknowledgments—We thank members of the Scherer and Lisanti laboratories for helpful and insightful discussions, Dr. Perry Bickel for donating anti-GDI antibodies, Michael Cammer for help with laser scanning confocal microscopy, Gina Georgescue for expert processing of human muscle biopsies, the darkroom services of Utrecht for printing of electron micrographs, and Drs. John R. Glenney, Jr., and Roberto Campos-Gonzalez for mAb production at Transduction Laboratories (Lexington, KY).

REFERENCES
1. Lisanti, M. P., Scherer, P., Tang, Z.-L., and Sargiacomo, M. (1994) Trends Cell Biol. 4, 231–235
2. Couet, J., Li, S., Okamoto, T., Scherer, P. S., and Lisanti, M. P. (1997) Trends Cardiovasc. Med. 7, 103–110
3. Fan, J. Y., Carpenter, J.-L., van Obberghen, E., Grunfeld, C., Gorden, P., and Orci, L. (1988) J. Cell Sci. 81, 219–235
4. Scherer, P. E., Lisanti, M. P., Baldini, G., Sargiacomo, M., Corley-Mastick, C., and Lodish, H. F. (1994) J. Cell Biol. 127, 1233–1243
5. Scherer, P. E., Okamoto, T., Chun, M., Nishimoto, I., Lodish, H. F., and Lisanti, M. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 131–135
6. Simionescu, N., and Simionescu, M. (1983) in Histology: Cell and Tissue Biology (Weiss, L., ed), Fifth Ed., pp. 371–433, Elsevier Biomedical, New York
7. Forbes, M. S., Rennels, M., and Nelson, E. (1979) J. Ultrastruc. Res. 67, 325–339
8. Bretscher, M., and Whytock, S. (1977) J. Ultrastruc. Res. 61, 215–217
9. Glenney, J. R., Jr. (1989) J. Biol. Chem. 264, 20163–20166
10. Glenney, J. R., and Soppet, D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10517–10521
11. Glenney, J. R. (1992) FEBS Lett. 314, 45–48
12. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y., Glenney, J. R., and Anderson, R. G. W. (1992) Cell 68, 673–682
13. Kurzchalia, T., Dupree, P., Parton, R. G., Kellner, R., Virta, H., Lehner, M., and Simons, K. (1992) J. Cell Biol. 118, 1003–1014
14. Sargiacomo, M., Scherer, P. E., Tang, Z.-L., Kohler, E., Song, K. S., Sanders, M. C., and Lisanti, M. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9407–9411
15. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A., and Kurzchalia, T. (1995) Mol. Biol. Cell 6, 811–827
16. Song, K. S., Tang, Z.-L., Li, S., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 4398–4403
17. Fra, A. M., Williamson, E., Simons, K., and Parton, R. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8655–8659
18. Li, S., Song, K. S., Koh, S., and Lisanti, M. P. (1996) J. Biol. Chem., 271, 28647–28654
19. Engelman, J. A., Wykoff, C. C., Yasuhara, S., Song, K. S., Okamoto, T., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 16374–16381
20. Maritez, M., Peranen, J., Scheinberg, R., Weiland, F., Kurzchalia, T., and Simons, K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10339–10343
21. Li, S., Song, K. S., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 568–573
22. Fra, A. M., Maserin, M., Palestini, F., Sonnino, S., and Simons, K. (1995) FEBS Lett. 376, 11–14
23. Koleske, A. J., Baltimore, D., and Lisanti, M. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1381–1385
24. Li, S., Okamoto, T., Chun, M., Sargiacomo, M., Casanova, J. E., Hansen, S. H., Nishimoto, I., and Lisanti, M. P. (1995) J. Biol. Chem. 270, 15693–15701
25. Li, S., Couet, J., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 29182–29190
26. Song, K. S., Li, S., Okamoto, T., Quilliam, L., Sargiacomo, M., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 9690–9697
27. Couet, J., Li, S., Okamoto, T., Ikeru, T., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 6525–6533
28. Scherer, P. E., Tang, Z.-L., Chun, M. C., Sargiacomo, M., Lodish, H. F., and Lisanti, M. P. (1995) J. Biol. Chem. 270, 16395–16401
29. Song, K. S., Scherer, P. E., Tang, Z.-L., Okamoto, T., Li, S., Chafe, M. C., Kohzt, D. S., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 15160–15165
30. Tang, Z.-L., Scherer, P. E., Okamoto, T., Song, K., Chaf, M. C., Kohzt, D. S., Nishimoto, I., Lodish, H. F., and Lisanti, M. P. (1996) J. Biol. Chem. 271, 2255–2261
31. Way, M., and Parton, R. (1995) FEBS Lett. 376, 108–112
32. Sargiacomo, M., Sudol, M., Tang, Z.-L., and Lisanti, M. P. (1993) J. Cell Biol. 122, 785–807
33. Bickel, P. E., Scherer, P. E., Schnitzer, J., Oh, P., Lisanti, M. P., and Lodish, H. F. (1997) J. Biol. Chem. 272, 13793–13802
34. Harlow, E., and Lane, D. (eds) (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. Baldini, G., Kohzt, D. S., and Lisanti, M. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5049–5052
36. Cole, P., Fasy, T. M., Rao, S., Peralta, M., and Kohzt, D. S. (1993) J. Biol. Chem. 268, 1580–1585
37. Blau, H., Chiu, C.-P., and Webster, C. (1983) Cell 32, 1171–1180
38. Lisanti, M. P., Tang, Z.-L., and Sargiacomo, M. (1993) J. Cell Biol. 123, 595–604
39. Peters, P. J., Neejes, J. J., Oorschot, V., Ploegh, H. L., and Geuze, H. J. (1991) Nature 349, 669–678
40. Liao, W., Geuze, H. J., and Slet, J. W. (1996) Histochem. Cell Biol. 106, 41–58
41. Glenney, J. R., and Zokas, I. (1989) J. Cell Biol. 108, 2401–2408
42. Scherer, P., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995) J. Biol. Chem. 270, 26746–26749
43. Tang, Z., Okamoto, T., Boutsakalopoulos, K., Katada, T., Otsuka, A., and Lisanti, M. P. (1997) J. Biol. Chem. 272, 2437–2445