[Frontiers in Bioscience 5, d929-937, December 1, 2000]

CAVEOLINS IN CHOLESTEROL TRAFFICKING AND SIGNAL TRANSDUCTION: IMPLICATIONS FOR HUMAN DISEASE

Amnon Schlegel [1,2], Richard G. Pestell [1,3,4], and Michael P. Lisanti [1,2]

[1] The Albert Einstein Comprehensive Cancer Center, and [2] Departments of Molecular Pharmacology, [3]Medicine, and [4]Developmental and Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA

TABLE OF CONTENTS

1. ABSTRACT

Caveolins are a family of proteins that coat the cytoplasmic face of caveolae, vesicular invaginations of the plasma membrane. These proteins are central to the organization of the proteins and lipids that reside in caveolae. Caveolins transport cholesterol to and from caveolae, and they regulate the activity of signaling proteins that reside in caveolae. Through studying the genes encoding the caveolae coat proteins, we have learned much about how they perform these multiple functions.

2. INTRODUCTION

Prior to the identification, cloning, and characterization of their coat proteins, the functions of caveolae– flask-shaped invaginations of the plasma membrane (1) also termed plasmalemmal vescicles (2) – remained obscure (3). Several investigations had implicated caveolae in a specialized form of endocytosis termed potocytosis, the large-scale uptake of fluids and small molecules (reviewed in ref. 4). However, application of molecular biological techniques to the study of caveolae dramatically widened our knowledge of their roles in cellular physiology. The simultaneous identification of a 22 kDa phosphoprotein first detected in Rous Sarcoma Virus-transformed fibroblasts (5) as caveolin, the principal caveolae membrane coat protein (now termed caveolin-1) (6), and as VIP21 (vesicular integral membrane protein of 21 kDa), a component of detergent resistant trans-Golgi derived vesicles (7), added signal transduction and intracellular transport to the possible functions of caveolae. Both of these potential roles were realized (see below). Notably, two other mammalian caveolin proteins (caveolin-2 and caveolin-3) have been cloned, and have unique distributions and functions (Table 1).

The essential role of caveolin-1 in caveolae organelle biogenesis has been documented in cell lines that do not express any known caveolins endogenously. Heterologous expression of caveolin-1 is sufficient to form caveolae in lymphocytes (8), insect cells (9, 10), and transformed fibroblasts (11), i.e., cells that otherwise lack caveolin-1 expression and morphological caveolae. Caveolin-1 expression is sufficient to induce this architectural change in the plasma membrane because caveolin-1 actively contributes to the organization of cholesterol and saturated lipids within these vesicular invaginations of the plasma membrane (12-15). The high concentration of cholesterol in caveolae and the tight association of cholesterol with caveolins (13, 16) reflects another function of caveolae and caveolins. They are components of the cell's transport machinery, contributing to the influx and efflux of cellular cholesterol (17-24).

A third function for caveolin proteins was also suggested by the early descriptive reports of caveolins as transformation-dependent substrates of v-Src (5, 25).

Table 1. Properties of Caveolin Proteins

Human Protein	Length (amino acyl residues)	% Similarity (Identity) to Caveolin-1	%Similarity (Identity) to Caveolin-2	Oligomerization[a]	Palmitoylation	Caveolae Formation[b]	Regualtion of Signaling Protein Activity[c]	Expression Pattern
Caveolin-1	178	100(100)	59(40)	+	+	+	+	Epithelia, endothelia, adipocytes, fibroblasts and smooth muscle cells
Caveolin-2	162	58 (38)	100(100)	+/-[d]	?	-	+/-[d]	Similar to caveolin-1
Caveolin-3	151	85 (65)	60 (39)	+	+	+	+	Smooth, skeletal, and cardiac myocytes astrocytes

a: Formation of a high mass complex comprized of ~15 monomers, b: Expression is sufficient to form caveolae as assessed by electron microscopy in cells that otherwise lack these structures, c:As assessed by binding and functional assays (e.g. enzyme activity), d: Caveolin-2 hetero-oligomerizes with caveolin-1, and may interact with signaling proteins.

Namely, caveolin-1 is a component of the cell's signaling machinery. It interacts with an array of lipid-anchored (including Src), integral membrane, and soluble signaling proteins that are recruited to caveolae during their activation cycles (reviewed in ref. 26). These numerous interactions and their significance in cell function were uncovered after the development of several complementary methods for purifying caveolae away from the bulk of cellular membranes (27-30). What these various methods share in common is the dramatic enrichment of caveolin-1 protein, and the exclusion of markers of the plasma membrane proper, and internal membrane compartments (31).

Here, we review the discovery and study of caveolin proteins, emphasizing results that address how these proteins give rise to morphological caveolae, how they contribute to cholesterol traffic, and how they regulate signaling protein function.

3. CAVEOLIN IDENTIFICATION

Using anti-phosphotyrosine IgG (PY20), Glenney identified several proteins selectivley phosphorylated in v-Src-transformed cells. One of these phosphoproteins was a 22 kDa (p22) molecule that was resistant to extraction with Triton X-100, migrated as a high mass complex through glycerol gradients, and resided at the plasma membrane (5, 25).

Immunoelectron microscopy revealed that the anti-p22 antibody binds an antigen that is found in the coat of caveolae membranes. Since p22 was the first marker of caveolae membranes, it was termed caveolin (caveolin-1). Unlike clathrin lattices, the caveolar coats resist destruction (as assessed by scanning electron microscopy with anti-p22 antibodies) when exposed to high concentrations of salt. Surprisingly, caveolae are exquisitely sensitive to nystatin, a cholesterol-binding drug. Caveolin-immunoreactivity and the characteristic striated morphology of caveolae are lost from the plasma membrane upon nystatin treatment (6).

Shortly after caveolin-1's identification came the cloning of VIP21 (7). This protein is a component of trans-Golgi derived transport vesicles. Immunogold microscopy revealed that it resides in non-coated vesicles on the plasma membrane as well as in the trans-Golgi. VIP21 resists extraction with alkaline sodium carbonate (an agent that strips membranes of peripherally associated proteins) and non-ionic detergents (7). Cloning of the caveolin-1 cDNA revealed that it is identical to VIP21 (32, 33).

4. CAVEOLIN TOPOLOGY AND STRUCTURE

The primary sequence of caveolin-1 contains a central hydrophobic domain (residues 102-134) that is believed to anchor the protein to membranes (33) (Figure 1). Given its tight association with membranes and its unusual primary structure (i.e. a single hydrophobic domain, no signal sequence), a major thrust in the study of caveolin-1 has been the determination of its membrane topology. Several studies have convincingly established that caveolin-1 is split into two large cytoplasmic domains by the central hyrophobic membrane anchor (Figure 1). Evidence for this model includes (i) the failure of antibodies directed against either the N- or C-terminus of caveolin-1 to detect the molecule in unpermeablized cells (34, 35); (ii) the lack of sugar modification when artificial glycosylation sites are placed at either the extreme N- or C-termini (36); (iii) the inaccessibility of caveolin-1 in MDCK cells to surface biotinylation (37); and (iv) the presence of cytoplasmic post-translational modifications (phosphorylation and palmitoylation) at the N- and C-termini (35, 38).

The unusual membrane topology remains an important area of investigation, and work performed over the last two years by our group (39, 40) and by others (41, 42) has revealed that caveolins bind to biological membranes through two unconventional Membrane Attachment Domains (MADs). More specifically, the portions of the molecule flanking the central hydrophobic domain can each bind to membranes with high affinity. Furthermore, these two MADs target to different cellular

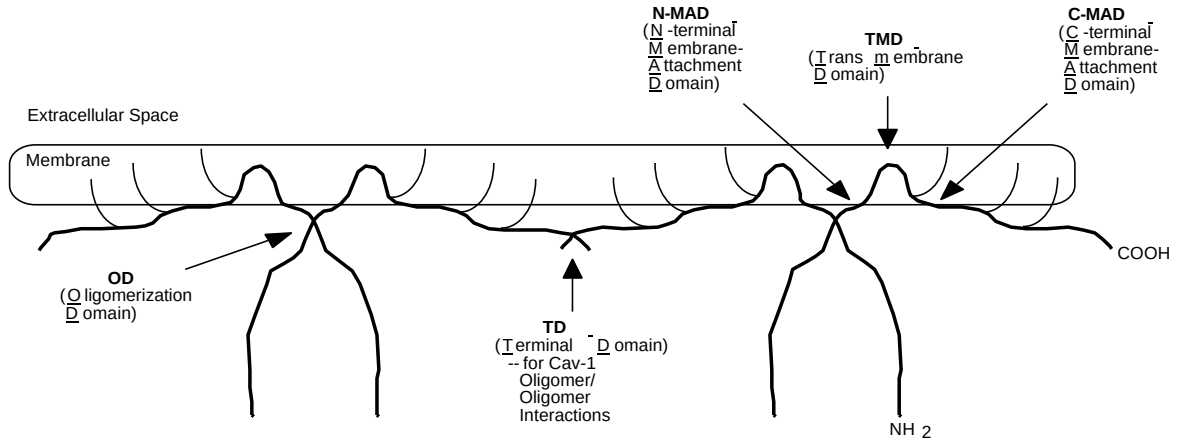

Figure 1. Caveolin-1 Membrane Topology. Two caveolin-1 oligomers (shown as dimers for simplicity) are drawn bound to the cytoplasmic face of the membrane. The three palmitic acid modifications are shown as upward hatches. The free amino (NH_2) and carboxyl (COOH) domains are separated by a central hydrophobic (TM) domain that is believed to penetrate the membrane. The protein is bound to membranes through tight association between the N-terminal membrane attachment domain (N-MAD) and the C-terminal MAD (C-MAD). Homo-oligomerization is mediated by a 40 amino-acyl residue domain (OD) in the N-terminal portion of the molecule. Adjacent oligomers interact via the terminal domain (TD). This model is a composite of work from several independent laboratories (7, 13, 14, 34, 35, 37, 39-41).

compartments with the N-terminal MAD (N-MAD) targeting to caveolae membranes and the C-MAD targeting to the trans-Golgi. Further work is required to clarify the biological consequences of these multiple targeting sequences, but they provide mechanistic insights into how "monotopic" proteins like caveolins associate with biological membranes.

Caveolin-1 and -3 can each form homotypic high molecular mass oligomers containing ~ 14-16 individual molecules (36, 37, 43). In the ER (44), homo-oligomerization is mediated by a forty amino-acyl residue domain (residues 61-101 in caveolin-1) within the N-terminal portion of the molecule (37). In the Golgi, adjacent homo-oligomers undergo a second stage of oligomerization; they interact with one another through contacts between the C-terminal domains of caveolin, in a side-by-side packing scheme (Figure 1) (40, 45). These oligomer/oligomer interactions then produce an interlocking network of caveolin molecules that gives rise to the striated caveolar coat seen by scanning electron microscopy.

5. CAVEOLAE, CAVEOLIN, AND SUBCELLULAR CHOLESTEROL TRAFFIC

After establishing that caveolae are sensitive to cholesterol depletion (and oxidation), Anderson and colleagues assessed the effects of nystatin and cholesterol oxidase treatment on caveolin-1 localization. They found that depletion of cellular cholesterol with either of these agents causes a retreat of caveolin-1 from caveolae to internal membrane compartments (46, 47). Restoring cellular cholesterol causes caveolin-1 to return to plasma membrane caveolae. Independent studies performed with bacterially expressed caveolin-1 confirmed an essential role for cholesterol in caveolin-1 attachment to membranes. Caveolin-1 incorporates into reconstituted lipid vesicles only when the vesicles contain high cholesterol concentrations (13, 14). Furthermore, caveolin-1 is tightly associated with cholesterol *in vitro* (13).

Given the exquisite sensitivity of caveolae morphology and caveolin-1 localization to cholesterol, it comes as no surprise that caveolae and caveolin-1 are important components of the cell's cholesterol transport machinery. In a series of pulse-chase experiments, Fielding and Fielding established that caveolae mediate the efflux of cellular cholesterol. Both LDL-derived cholesterol (which is internalized by clathrin-coated pits) and newly synthesized cholesterol reach the plasma membrane through caveolae (17, 18). Figure 2A summarizes the known modes of cholesterol uptake and delivery to lipoprotein particles.

As expected, caveolin-1 is an active component of the cholesterol transport machinery (19), and its expression in cell lines lacking endogenous caveolin-1 markedly enhances the rate of delivery of newly synthesized cholesterol to caveolae. Kinetic, cellular fractionation, and immuno-precipitation studies by Smart and his colleagues have uncovered a novel mode of caveolin-1 trafficking from the endoplasmic reticulum (the site of cholesterol synthesis) to caveolae membranes (Figure 2). More specifically, caveolin-1 travels between these two membrane compartments as a cytosolic complex containing chaperone proteins and cholesterol (20). Although caveolin-1 palmitoylation is not required for targeting to caveolae (35), the ability of cytosolic caveolin-1 to bind and transport cholesterol requires palmitoylation (24).

The physiological significance of caveolae in cholesterol homeostasis is supported by the observation

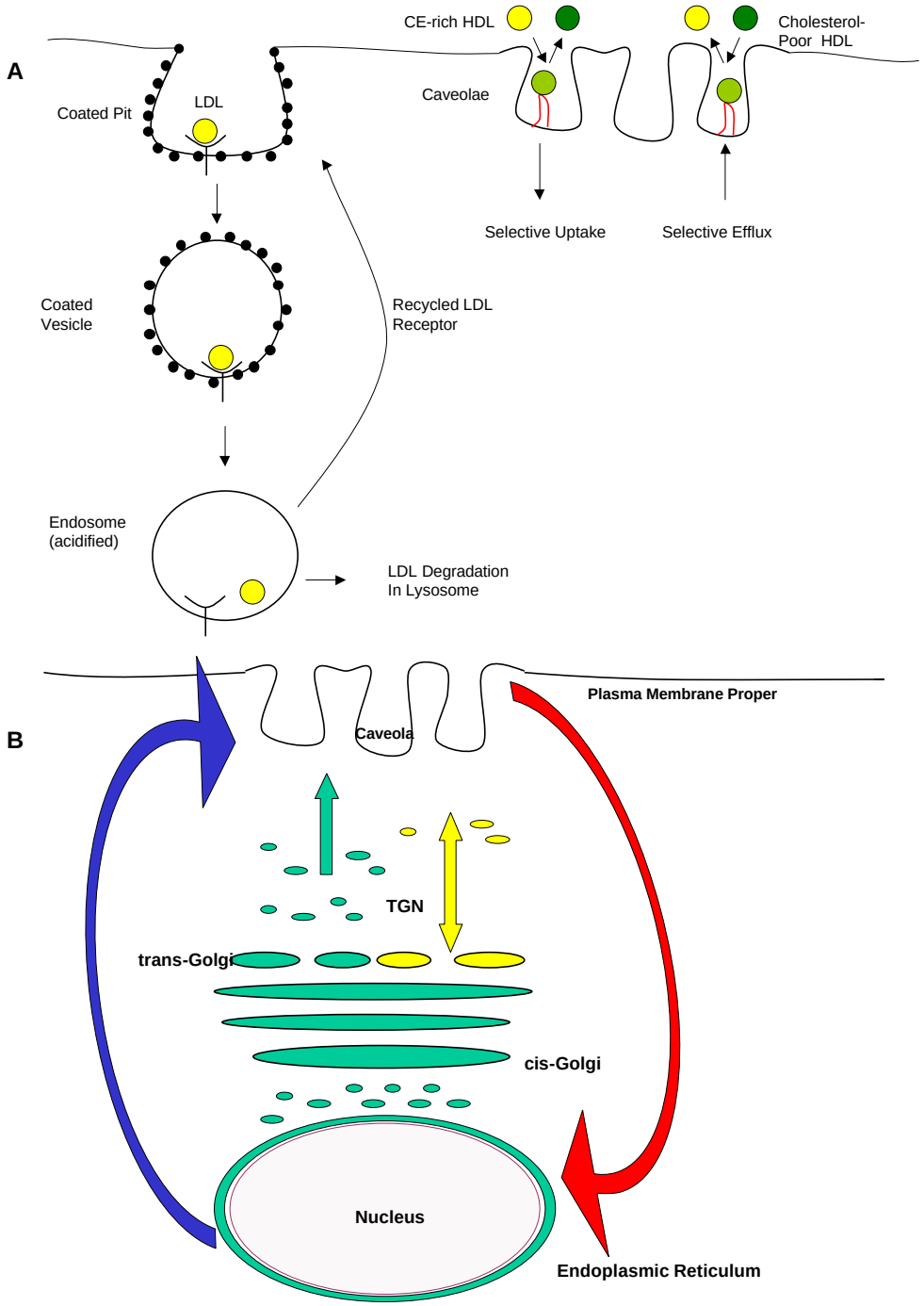

Figure 2. Cholesterol transport and caveolin-1 traffic. A) LDL particles are bound to the LDL receptor and internalized via clathrin coated pits. The complex dissociates as the coated vesicle's pH drops. The proteins in the LDL particle are then degraded by lysosomal enzymes, while the receptor recycles to the plasma membrane. HDL particles are not internalized. Rather, bidirectional flow of cholesterol to and from these lipoprotein particles occurs in caveolae, and is mediated by the HDL receptor, SR-BI (red). B)The various routes caveolin-1 takes in its intracellular traffic are shown using different colors. The biosynthetic pathway (green) involves co-translational insertion of the protein into the endoplasmic reticulum (ER) membrane, traffic through the ER, the Golgi complex, trans-Golgi network (TGN), and ultimate residence in caveolae vesicles (7, 13, 44). The yellow arrow and vesicles indicate the cycling of caveolin-1 between the trans-Golgi and caveolae membranes (34). The blue arrow indicates the path of cytosolic caveolin-1 in complex with chaperone proteins in delivering newly synthesized cholesterol from the ER to caveolae (19, 20, 24). The red arrow shows the retreat of caveolin-1 from caveolae membranes to the lumen of the ER in cells depleted of plasma membrane cholesterol (46, 47). For simplicity, the ER is shown as a perinuclear ring.

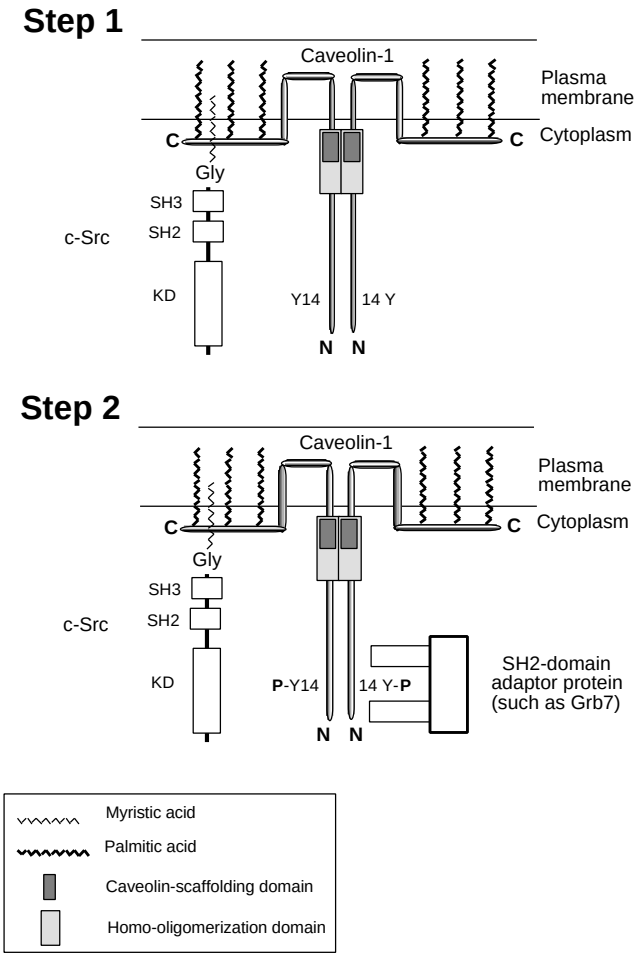

Figure 3. Ternary complex containing caveolin-1, Src, and GRB7. Upon growth factor stimulation, the non-receptor tyrosine kinase Src is activated and catalyzes the phosphorylation of caveolin-1 on Tyr^{14}. Phosphorylated caveolin-1 then binds the adapter protein GRB7, transmitting the signal through a novel signaling cascade. Src has three domains: a kinase domain (KD, also called Scr homology 1, SH1), and two tandem protein-protein interaction domains, SH-2 and SH-3. Also, Src bears a myristoyl group on the N-terminal glycine residue.

that the HDL receptor SR-BI (type I, class B scavenger receptor) is a resident caveolar protein (48). Furthermore, the bidirectional flow of cholesterol between HDL particles and the cell occurs in caveolae (21, 22). However, genetic ablation studies will be required to establish the role of caveolin-1 in cholesterol traffic in whole animals. More specifically, the effects of caveolin-1 deficiency on reverse cholesterol transport (i.e. periphery to liver), and on the development of dyslipidemia (and its sequelae) must be examined in mice lacking the caveolin-1 gene. Other unresolved areas include the relationship between caveolin-1 and sterol carrier proteins (see ref. 49 and citations therein).

The oxidized low-density lipoprotein receptor CD36, which mediates the conversion of macrophages to foam cells in the intima of blood vessels undergoing atherosclerosis, also resides in caveolae (50). The role of this subcellular localization and of caveolae and caveolins in this disease can also be examined in *CAV1* null mice. Crosses with animals with a propensity to develop atherosclerosis (e.g. *APOE* null) or hypertension (e.g. *ENOS* null) will also be informative. Other areas of interest include the role of caveolins in the uptake of dietary cholesterol by enterocytes (51).

6. CAVEOLIN-1 AND SIGNAL TRANSDUCTION

6.1. The Caveolae Signaling Hypothesis and the Caveolin Scaffolding Domain

The unique lipid and protein composition of caveolae membranes renders caveolae resistant to non-ionic detergent membranes (52). Development of purification methods that exploit this detergent-insolubility of caveolae membranes led to the identification of caveolae-associated proteins (27). Many caveolae resident proteins are signaling molecules (53). This discovery prompted us to propose that caveolae are a sub-compartment of the plasma-membrane where signal transduction events occur (50); clustering of signaling proteins in this environment may result in more rapid cross-talk and promote efficiency of signal transmission (50).

The 'Caveolae Signaling Hypothesis' was extended to include a more active role for caveolin-1 after the discovery that caveolin-1 interacts with and regulates the activity of several caveolae-associated signaling proteins (54-56). This interaction is direct, and is mediated largely by a 20 amino-acyl residue domain termed the caveolin scaffolding domain (residues 82-101).

Phage display revealed that the caveolin scaffolding domain binds to a motif (ΦXΦXXXXΦ, ΦXXXXΦXXΦ, and ΦXΦXXXXΦXXΦ, where Φ is a phenylalanine, tyrosine, or tryptophan residue; and X is any aminoacyl residue, ref. 57) shared by receptor tyrosine kinases and their down stream targets (e.g. EGFR, c-Neu, H-Ras, MEK, ERK, refs. 55, 58-60); non-receptor tyrosine kinases (e.g. Src and Fyn, refs. 56, 61); G-protein coupled receptors and their downstream signaling molecules (e.g. endothelin receptor, various Gα subunits, adenylyl cyclase, cyclic AMP-dependent protein kinase, refs. 54, 62-66); and regulated enzymes (e.g. endothelial nitric oxide synthase, eNOS, ref. 67).

With some notable exceptions (reviewed in ref. 68) caveolin-1 inhibits the activity of its interaction partners. In some cases, caveolin-1 binding maintains a signaling protein in an inactive state until a stimulus is presented (e.g. eNOS). For other signaling proteins, caveolin-1 binding serves to terminate signal transmission after activation (e.g. Gα). In both cases, caveolin-1 interaction is dynamic and tightly regulated (64, 69).

Although many proteins interact with caveolins during different parts of their activation cycle, several recent studies reveal that caveolins form ternary complexes with Src family kinases and signaling proteins that lack inherent catalytic activity. For example, caveolin-1 and Fyn associate with the short cytoplasmic tails of beta-1 integrins. Anchorage dependent growth requires the ligation of extracellular ligands to the latter and the concomitant association and activation of caveolin-bound Fyn (reviewed in ref. 70). Other proteins like the glycosylphosphatidyl inositol-linked (GPI) urokinase receptor u-PAR also regulate integrin function and are part of the integrin/caveolin complex (71).

The association of GPI anchored proteins with caveolae was first deemed an experimental artifact (72, 73). However, studies of u-PAR, caveolin-1, Src family kinases and integrins indicate that the compartmentalization of signaling proteins in caveolae is highly dynamic and subject to multiple regulatory inputs. Indeed, other GPI-anchored proteins like the cellular prion protein (PrP) signal through caveolin-1 and Src family kinases upon immunoglobulin-dependent clustering of PrP in caveolae (74).

6.2. Caveolin-1 tyrosine phosphorylation and SH2-domain bearing signaling proteins

In addition to the caveolin scaffolding domain, caveolins contain other protein-protein interaction domains (39, 40, 45, 66, 75, 76). We recently uncovered a novel interaction between caveolin-1 and the SH2 domain containing adapter molecule GRB7 (Figure 3). Caveolin-1 binds GRB7 following growth factor-stimulated (and Src-catalyzed) phosphorylation of caveolin-1 tyrosine residue 14. Mutation of this residue to prevent phosphorylation undermines GRB7 association. When co-expressed, Src, Cav-1, and GRB7 dramatically increase anchorage-independent growth and cell migration (77). Down stream effector molecules remain to be identified, but this series of protein-protein interactions defines the first biological function for caveolin-1 tyrosine phosphorylation.

6.3. Caveolin-1 and Cellular Transformation

The majority of studies examining caveolin-mediated regulation of signal transduction have been performed in cell culture. However, recent reports indicate that the role of caveolin-1 in regulating signaling is not limited to this setting. For instance, down-regulation of caveolin-1 through RNA-interference in the gonad of *Caenorhabditis elegans* hermaphrodites results in a phenotype (accelerated meiotic progression and egg-laying) that is identical to that observed when activated Ras and MAP kinase mutants are expressed in this organ (78).

Notably, a phenocopy of caveolin-downregulation is observed when worms are depleted of cholesterol with methyl-β-cyclodextrin (78). These results confirm earlier findings obtained in mammalian cell culture: cholesterol depletion or transfection of caveolin-1 anti-sense constructs result in hyperactivation of the Ras/MAP kinase signaling cascade (79, 80).

The importance of caveolin-1 as an antagonist of mitogenic signaling is underscored by the observation that caveolin-1 is downregulated in cancer cell lines (81). Indeed, re-expression of caveolin-1 in Ras transformed fibroblasts abrogates anchorage-independent growth (11). Other tumor cell lines cease to proliferate when caveolin-1 is over expressed (82). Conversely, stable transfection of anti-sense caveolin-1 vectors results in anchorage-dependent growth *in vitro* (i.e. in soft agar), and *in vivo* (i.e. tumors in nude mice, ref. 80).

Other lines of evidence suggest that *CAV1* is the tumor suppressor gene residing at chromosome 7q3.1. Namely, *CAV1* maps close to the CA-repeat microsatellite marker D7S522, a marker frequently deleted in a variety of human epithelial tumors including breast, prostate, ovarian, and renal cell carcinomas (83). Also, D7S522 spans the common fragile site, FRAG7, that resides at 7q31.1 (68).

7. PERSPECTIVE

The cloning of caveolin proteins over the last decade has stimulated research into the functions of caveolae in cell biology. During this period many exciting results have been gathered from test tubes and cell culture dishes. More studies in these familiar settings will be performed in the future. However, we anticipate many exciting new results in whole animals as well.

8. ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (NIH), the Muscular Dystrophy Association, and the Komen Breast Cancer Foundation (all to M.P.L.). R.G.P. was supported by National Institutes of Health grants (R01-CA70897, R01-CA75503, and P50-HL56399), an Irma T. Hirschl Award, and an award from the Susan G. Kommen Breast Cancer Foundation. A.S. was supported by an NIH Medical Scientist Training Program Grant (T32-GM07288). Portions of this review were submitted as part of a thesis in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Sue Golding Graduate Division of Medical Sciences of the Albert Einstein College of Medicine.

9. REFERENCES

1. Yamada E; The fine structure of the gall bladder epithelium of the mouse. *J Biophys Biochem Cytol* 1,445-458 (1955)
2. Palade GE; Fine structure of blood capillaries. *J Appl Physics* 24,1424 (1953)
3. Severs NJ; Caveolae: static inpocketings of the plasma membrane, dynamic vesicles or plain artifact? *J Cell Sci* 90(Pt 3):341-8 (1988)
4. Anderson RG, BA Kamen, KG Rothberg, SW Lacey; Potocytosis: sequestration and transport of small molecules by caveolae. *Science* 255,410-1 (1992)
5. Glenney JR, Jr.; Tyrosine phosphorylation of a 22-kDa protein is correlated with transformation by Rous sarcoma virus. *J Biol Chem* 264,20163-6 (1989)
6. Rothberg KG, JE Heuser, WC Donzell, YS Ying, JR Glenney, RG Anderson; Caveolin, a protein component of caveolae membrane coats. *Cell* 68,673-82 (1992)
7. Kurzchalia TV, P Dupree, RG Parton, R Kellner, H Virta, M Lehnert, K Simons; VIP21, a 21-kD membrane protein is an integral component of trans-Golgi- network-derived transport vesicles. *J Cell Biol* 118, 1003-14 (1992)
8. Fra AM, E Williamson, K Simons, RG Parton. De novo formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Proc Natl Acad Sci U S A* 92:8655-9 (1995)
9. Li S, KS Song, SS Koh, A Kikuchi, MP Lisanti. Baculovirus-based expression of mammalian caveolin in Sf21 insect cells. A model system for the biochemical and morphological study of caveolae biogenesis. *J Biol Chem* 271, 28647-54 (1996)
10. Li S, F Galbiati, D Volonté, M Sargiacomo, JA Engelman, K Das, PE Scherer, MP Lisanti; Mutational analysis of caveolin-induced vesicle formation. Expression of caveolin-1 recruits caveolin-2 to caveolae membranes. *FEBS Lett* 434, 127-34 (1998)
11. Engelman JA, CC Wykoff, S Yasuhara, KS Song, T Okamoto, MP Lisanti; Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. *J Biol Chem* 272, 16374-81 (1997)
12. Fra AM, M Masserini, P Palestini, S Sonnino, K Simons; A photo-reactive derivative of ganglioside GM1 specifically cross-links VIP21-caveolin on the cell surface. *FEBS Lett* 375, 11-4 (1995)
13. Murata M, J Peranen, R Schreiner, F Wieland, TV Kurzchalia, K Simons; VIP21/caveolin is a cholesterol-binding protein. *Proc Natl Acad Sci U S A* 92, 10339-43 (1995)
14. Li S, KS Song, MP Lisanti; Expression and characterization of recombinant caveolin. Purification by polyhistidine tagging and cholesterol-dependent incorporation into defined lipid membranes. *J Biol Chem* 271568-73 (1996)
15. Trigatti BL, RG Anderson, GE Gerber; Identification of caveolin-1 as a fatty acid binding protein. *Biochem Biophys Res Commun* 25534-9 (1999)
16. Thiele C, MJ Hannah, F Fahrenholz, WB Huttner; Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat Cell Biol*. 2, 42-9 (2000)
17. Fielding PE, CJ Fielding; Plasma membrane caveolae mediate the efflux of cellular free cholesterol. *Biochemistry*. 34, 14288-92 (1995)
18. Fielding PE, CJ Fielding; Intracellular transport of low density lipoprotein derived free cholesterol begins at clathrin-coated pits and terminates at cell surface caveolae. *Biochemistry*. 35:14932-8 (1996)
19. Smart EJ, Y Ying, WC Donzell, RG Anderson; A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. *J Biol Chem* 271, 29427-35 (1996)
20. Uittenbogaard A, Y Ying, EJ Smart; Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. Involvement in cholesterol trafficking. *J Biol Chem* 273, 6525-32 (1998)
21. Babitt J, B Trigatti, A Rigotti, EJ Smart, RG Anderson, S Su, M Krieger; Murine SR-BI, a high density lipoprotein receptor that mediates selective lipid uptake, is N-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae. *J Biol Chem* 272, 13242-9 (1997)
22. Graf GA, PM Connell, DR van der Westhuyzen, EJ Smart; The class B, type I scavenger receptor promotes the selective uptake of high density lipoprotein cholesterol ethers into caveolae. *J Biol Chem* 274, 12043-8 (1999)
23. Fielding CJ, A Bist, PE Fielding; Intracellular cholesterol transport in synchronized human skin fibroblasts. *Biochemistry* 38, 2506-13 (1999)
24. Uittenbogaard A, EJ Smart; Palmitoylation of caveolin-1 is required for cholesterol binding, chaperone complex formation, and rapid transport of cholesterol to caveolae. *J Biol Chem* 275, 25595-9 (2000)
25. Glenney JR, Jr., L Zokas; Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are present in the membrane skeleton. *J Cell Biol* 108, 2401-8 (1989)
26. Okamoto T, A Schlegel, PE Scherer, MP Lisanti; Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem* 273, 5419-22 (1998)
27. Sargiacomo M, M Sudol, Z Tang, MP Lisanti; Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *J Cell Biol* 122, 789-807 (1993)

28. Chang WJ, YS Ying, KG Rothberg, NM Hooper, AJ Turner, HA Gambliel, J De Gunzburg, SM Mumby, AG Gilman, RG Anderson; Purification and characterization of smooth muscle cell caveolae. *J Cell Biol* 126, 127-38 (1994)
29. Smart EJ, YS Ying, C Mineo, RG Anderson; A detergent-free method for purifying caveolae membrane from tissue culture cells. *Proc Natl Acad Sci U S A* 92, 10104-8 (1995)
30. Schnitzer JE, DP McIntosh, AM Dvorak, J Liu, P Oh; Separation of caveolae from associated microdomains of GPI-anchored proteins. *Science* 269, 1435-9 (1995)
31. Smart EJ, GA Graf, MA McNiven, WC Sessa, JA Engelman, PE Scherer, T Okamoto, MP Lisanti; Caveolins, liquid-ordered domains, and signal transduction. *Mol Cell Biol*. 19, 7289-304 (1999)
32. Glenney JR, Jr., D. Soppet; Sequence and expression of caveolin, a protein component of caveolae plasma membrane domains phosphorylated on tyrosine in Rous sarcoma virus-transformed fibroblasts. *Proc Natl Acad Sci U S A* 89, 10517-21 (1992)
33. Glenney JR, Jr.; The sequence of human caveolin reveals identity with VIP21, a component of transport vesicles. *FEBS Lett* 314, 45-8 (1992)
34. Dupree P, RG Parton, G Raposo, TV Kurzchalia, K Simons; Caveolae and sorting in the trans-Golgi network of epithelial cells. *EMBO J* 12, 1597-605 (1993)
35. Dietzen DJ, WR Hastings, DM Lublin; Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. *J Biol Chem* 270, 6838-42 (1995)
36. Monier S, RG Parton, F Vogel, J Behlke, A Henske, TV Kurzchalia; VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol Biol Cell* 6, 911-27 (1995)
37. Sargiacomo M, PE Scherer, Z Tang, E Kubler, KS Song, MC Sanders, MP Lisanti; Oligomeric structure of caveolin: implications for caveolae membrane organization. *Proc Natl Acad Sci U S A* 92, 9407-11 (1995)
38. Li S, R Seitz, MP Lisanti; Phosphorylation of caveolin by src tyrosine kinases. The alpha-isoform of caveolin is selectively phosphorylated by v-Src in vivo. *J Biol Chem* 271, 3863-8 (1996)
39. Schlegel A, RB Schwab, PE Scherer, MP Lisanti; A role for the caveolin scaffolding domain in mediating the membrane attachment of caveolin-1. The caveolin scaffolding domain is both necessary and sufficient for membrane binding *in vitro*. *J Biol Chem* 274 22660-7 (1999)
40. Schlegel A, MP Lisanti; A Molecular Dissection of Caveolin-1 Membrane Attachment and Oligomerization Two separate regions of the caveolin-1 C-terminal domain mediate membrane binding and oligomer/oligomer interactions *in vivo*. *J Biol Chem* 275, 21605-17 (2000)
41. Luetterforst R, E Stang, N Zorzi, A Carozzi, M Way, RG Parton; Molecular characterization of caveolin association with the Golgi complex: identification of a cis-Golgi targeting domain in the caveolin molecule. *J Cell Biol* 145, 1443-59 (1999)
42. Arbuzova A, L Wang, J Wang, G Hangyás-Mihályné, D Murray, B Honig, S McLaughlin; Membrane Binding of Peptides Containing Both Basic and Aromatic Residues. Experimental Studies with Peptides Corresponding to the Scaffolding Region of Caveolin and the Effector Region of MARCKS. *Biochemistry*. 39, 10330-39 (2000)
43. Tang Z, PE Scherer, T Okamoto, K Song, C Chu, DS Kohtz, I Nishimoto, HF Lodish, MP Lisanti; Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem* 271, 2255-61 (1996)
44. Lisanti MP, ZL Tang, M Sargiacomo; Caveolin forms a hetero-oligomeric protein complex that interacts with an apical GPI-linked protein: implications for the biogenesis of caveolae. *J Cell Biol* 123, 595-604 (1993)
45. Song KS, Z Tang, S Li, MP Lisanti; Mutational analysis of the properties of caveolin-1. A novel role for the C-terminal domain in mediating homo-typic caveolin-caveolin interactions. *J Biol Chem* 272, 4398-403 (1997)
46. Smart EJ, YS Ying, PA Conrad, RG Anderson; Caveolin moves from caveolae to the Golgi apparatus in response to cholesterol oxidation. *J Cell Biol* 127, 1185-97 (1994)
47. Conrad PA, EJ Smart, YS Ying, RG Anderson, GS Bloom; Caveolin cycles between plasma membrane caveolae and the Golgi complex by microtubule-dependent and microtubule-independent steps. *J Cell Biol* 131, 1421-33 (1995)
48. Krieger M, K Kozarsky; Influence of the HDL receptor SR-BI on atherosclerosis. *Curr Opin Lipidol* 10, 491-7 (1999)
49. Atshaves BP, O Starodub, A McIntosh, A Petrescu, JB Roths, AB Kier, F Schroeder; Sterol Carrier Protein-2 Alters HDL-Mediated Cholesterol Efflux. *J Biol Chem* In Press (2000).
50. Lisanti MP, PE Scherer, ZL Tang, M Sargiacomo; Caveolae, caveolin and caveolin-rich membrane domains: a signalling hypothesis. *Trends Cell Biol* 4, 231-5 (1994)
51. Field FJ, E Born, S Murthy, SN Mathur; Caveolin is present in intestinal cells: role in cholesterol trafficking? *J Lipid Res* 39, 1938-50 (1998)
52. Brown DA, E London; Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol* 14, 111-36 (1998)
53. Lisanti MP, PE Scherer, J Vidugiriene, Z Tang, A Hermanowski-Vosatka, YH Tu, RF Cook, M Sargiacomo; Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. *J Cell Biol* 126, 111-26 (1994)
54. Li S, T Okamoto, M Chun; Evidence for a regulated interaction between heterotrimeric G proteins and caveolin. *J Biol Chem*. 270, 15693-701 (1995)
55. Song SK, S Li, T Okamoto, LA Quilliam, M Sargiacomo, MP Lisanti; Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. *J Biol Chem* 271, 9690-7 (1996)
56. Li S, J Couet, MP Lisanti; Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. *J Biol Chem* 271, 29182-90 (1996)
57. Couet J, S Li, T Okamoto, T Ikezu, MP Lisanti; Identification of peptide and protein ligands for the

caveolin- scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. *J Biol Chem* 272, 6525-33 (1997)
58. Couet J, M Sargiacomo, MP Lisanti; Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. *J Biol Chem* 272, 30429-38 (1997)
59. Engelman JA, RJ Lee, A Karnezis, DJ Bearss, M Webster, P Siegel, WJ Muller, JJ Windle, RG Pestell, MP Lisanti; Reciprocal regulation of Neu tyrosine kinase activity and caveolin-1 protein expression in vitro and in vivo. Implications for human breast cancer. *J Biol Chem* 273, 20448-55 (1998)
60. Engelman JA, C Chu, A Lin, H Jo, T Ikezu, T Okamoto, DS Kohtz, MP Lisanti; Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. *FEBS Lett* 428, 205-11 (1998)
61. Wary KK, A Mariotti, C Zurzolo, FG Giancotti A; requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* 94, 625-34 (1998)
62. Chun M, UK Liyanage, MP Lisanti, HF Lodish; Signal transduction of a G protein-coupled receptor in caveolae: colocalization of endothelin and its receptor with caveolin. *Proc Natl Acad Sci U S A* 91, 11728-32 (1994)
63. Song KS, M Sargiacomo, F Galbiati, M Parenti, MP Lisanti; Targeting of a G alpha subunit (Gi1 alpha) and c-Src tyrosine kinase to caveolae membranes: clarifying the role of N-myristoylation. *Cell Mol Biol (Noisy-le-grand)*. 43, 293-303 (1997)
64. Murthy KS, GM Makhlouf; Heterologous desensiti-zation mediated by G protein-specific binding to caveolin. *J Biol Chem* 275, 30211-9 (2000)
65. Toya Y, C Schwencke, J Couet, MP Lisanti; Ishikawa Y. Inhibition of adenylyl cyclase by caveolin peptides. *Endocrinology* 139, 2025-31 (1989)
66. Razani B, CS Rubin, MP Lisanti; Regulation of cAMP-mediated signal transduction via interaction of caveolins with the catalytic subunit of protein kinase A. *J Biol Chem*. 274, 26353-60 (1999)
67. Garcia-Cardeña G, P Martasek, BS Masters, PM Skidd, J Couet, S Li, MP Lisanti, WC Sessa; Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. *J Biol Chem* 272, 25437-40 (1997)
68. Razani B, A Schlegel, MP Lisanti; Caveolin proteins in signaling, oncogenic transformantion and muscular dystrophy. *J Cell Sci* 113, 2103-9 (2000)
69. Gratton JP, J Fontana, DS O'Connor, G Garcia-Cardeña, TJ McCabe, WC Sessa; Reconstitution of an endothelial nitric oxide synthase, hsp90 and caveolin-1 complex in vitro: Evidence that hsp90 facilitates calmodulin stimulated displacement of eNOS from caveolin-1. *J Biol Chem* 275, 22268-72 (2000)
70. Giancotti FG, E Ruoslahti; Integrin signaling. *Science* 285,1028-32 (1999)
71. Chapman HA, Y Wei, DI Simon, DA Waltz; Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb Haemost* 82, 291-7 (1999)
72. Mayor S, KG Rothberg, FR Maxfield; Sequestration of GPI-anchored proteins in caveolae triggered by cross-linking. *Science* 264, 1948-51 (1994)
73. Mayor S, FR Maxfield; Insolubility and redistribution of GPI-anchored proteins at the cell surface after detergent treatment. *Mol Biol Cell* 6, 929-44 (1995)
74. Mouillet-Richard S, M Ermonval, C Chebassier, JL Laplanche, S Lehmann, JM Launay, O Kellermann; Signal transduction through prion protein. *Science* 289,1925-8 (2000)
75. Venema VJ, H Ju, R Zou, RC Venema; Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/ inhibitory domain. *J Biol Chem*. 272, 28187-90 (1997)
76. Das K, RY Lewis, PE Scherer, MP Lisanti. The membrane-spanning domains of caveolins-1 and -2 mediate the formation of caveolin hetero-oligomers. Implications for the assembly of caveolae membranes in vivo. *J Biol Chem* 274, 18721-8 (1999)
77. Lee H, D Volonté, F Galbiati F, P Iyengar, DM Lublin, DB Bregman, MT Wilson, R Campos-González, RG Pestell, PE Scherer, MP Lisanti; Constitutive and growth factor regulated phosphorylation of caveolin-1 occurs at the same site (Tyr-14) *in vivo* : identification of a c-Src/Cav-1/Grb7 signaling cassette. *Mol Endocrinol* 14, 1750-75 (2000)
78. Scheel J, J Srinivasan, U Honnert, A Henske, TV Kurzchalia; Involvement of caveolin-1 in meiotic cell-cycle progression in *Caenorhabditis elegans*. *Nat Cell Biol* 1, 127-9 (1999)
79. Furuchi T, RG Anderson; Cholesterol depletion of caveolae causes hyperactivation of extracellular signal-related kinase (ERK). *J Biol Chem* 273, 21099-104 (1998)
80. Galbiati F, D Volonté, JA Engelman, G Watanabe, R Burk, RG Pestell, MP Lisanti; Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. *EMBO J* 17, 6633-48 (1998)
81. Koleske AJ, D Baltimore, MP Lisanti; Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc Natl Acad Sci U S A* 92, 1381-5 (1995)
82. Lee SW, CL Reimer, P Oh, DB Campbell, JE Schnitzer; Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. *Oncogene* 16, 1391-7 (1998)
83. Engelman JA, X Zhang, F Galbiati, D Volonté, F Sotgia, RG Pestell, C Minetti, PE Scherer, T Okamoto, MP Lisanti; Molecular genetics of the caveolin gene family: implications for human cancers, diabetes, Alzheimer disease, and muscular dystrophy. *Am J Hum Genet* 63, 1578-87 (1998)

Key Words: Caveolin, Caveolae, Cholesterol, Signal Transduction, Cancer, Review

Send correspondence to: Dr. Michael P. Lisanti, Department of Molecular Pharmacology, 1300 Morris Park Avenue, Bronx, New York 10461, USA. Tel: 718-430-8828, Fax: 718-430-8830, E-mail: lisanti@aecom.yu.edu

This manuscript is available on line at:

http://www.bioscience.org/2000/v5/d/schlegel/fulltext.htm