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REVIEW

**Richard D. Minshall · Chinnaswamy Tiruppathi
Stephen M. Vogel · Asrar B. Malik**

Vesicle formation and trafficking in endothelial cells and regulation of endothelial barrier function

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Abstract Endothelial barrier function is regulated in part by the transcellular transport of albumin and other macromolecules via endothelial caveolae (i.e., this process is defined as transcytosis). Using pulmonary microvascular endothelial cells, we have identified the specific interactions between a cell surface albumin-docking protein gp60 and caveolin-1 as well as components of the signaling machinery, heterotrimeric G protein (G_i)- and *Src*-family tyrosine kinase. Ligation of gp60 on the apical membrane induces the release of caveolae from the apical membrane and activation of endocytosis. The formed vesicles contain the gp60-bound albumin and also albumin and other solutes present in the fluid phase. Vesicles are transported in a polarized manner to the basolateral membrane, releasing their contents by exocytosis into the subendothelial space. The signaling functions of G_i and *Src* are important in the release of caveolae from the plasma membrane. The *Src*-induced phosphorylation of caveolin-1 is crucial in regulating interactions of caveolin-1 with other components of the signaling machinery such as G_i, and key signaling entry of caveolae into the cytoplasm and endocytosis of albumin and other solutes. This review addresses the basis of transcytosis in endothelial cells, its central role as a determinant of endothelial barrier function, and signaling mechanisms involved in regulating fission of caveolae and trafficking of the formed vesicles from the luminal to abluminal side of the endothelial barrier.

Keywords Endothelial cells · Caveolae · Transcytosis · Signaling · Transendothelial albumin transport · Albumin-binding proteins

R.D. Minshall · C. Tiruppathi · S.M. Vogel · A.B. Malik (✉)
Department of Pharmacology (m/c 868),
The University of Illinois, College of Medicine,
835 S. Wolcott Avenue, Chicago, Illinois 60612, USA
e-mail: abmalik@uic.edu
Tel.: +1-312-9967635, Fax: +1-312-9961225

R.D. Minshall
Department of Anesthesiology, The University of Illinois,
College of Medicine, Chicago, Illinois 60612, USA

Summary

A transcellular pathway in endothelial cells has been implicated in the transport of albumin and lipids, hormones, and peptides that bind avidly to albumin. Studies in microvascular endothelial cells have identified specific interactions between a 60-kDa endothelial cell surface albumin-binding glycoprotein, gp60, and caveolin-1 (Schnitzer et al. 1994; Tiruppathi et al. 1997; Minshall et al. 2000). Signaling pathways activated by the interaction of gp60 and caveolin-1 may regulate albumin permeability in endothelial cells via transcytosis. Endothelial cells also transport insulin and transferrin by a transcellular mechanism, but transferrin uptake relies on clathrin-coated pits (Goldberg et al. 1987; Anderson 1991), in contrast to albumin uptake and transport which requires caveolae. This review focuses on the process of transcytosis in endothelial cells, its central role as a determinant of endothelial permeability, and signaling mechanisms involved in regulating endocytosis and trafficking of vesicles from the luminal to abluminal side of the endothelial barrier.

Concept of transcytosis in endothelial cells

Transport of the most abundant plasma protein, albumin, occurs by means of two discrete structural features of the endothelium, that is, the paracellular pathway consisting of the restrictive intercellular junction and the transcellular pathway consisting of a highly mobile set of vesicles that shuttle across the endothelial barrier from its luminal aspect to the abluminal side. Junctional permeability as regulated by components of intercellular junctions, VE-cadherin complex and zonula occludens has been the subject of intense study for several decades, and the efforts of many investigators have led to the quantitative description of endothelial transport known as the “pore theory” (Pappenheimer et al. 1951). In its simplistic form, the pore theory defines the endothelial barrier as a sieve capable of discriminating the transported solutes

according to their molecular weights through "size-selective pores." The interendothelial junction is described as a "fiber matrix" consisting of a fibrillar structure through which macromolecules are transported as in a gel filtration column (Schnitzer 1992a). The basis of this theory has recently been reviewed by Michel and Curry (1999), and thus will not be discussed in this review. While junctional transport occurs in response to mediators such as thrombin and histamine that "dilate" the intercellular space and increase endothelial permeability (Lum and Malik 1994; Garcia et al. 1996; Moy et al. 1996; Rabiet et al. 1996), it appears unlikely that junctional permeability of albumin and other macromolecules is important under non-pathological circumstances. The primary challenge to the pore theory has been the detailed electron microscopic studies employing a variety of electron-dense tracers such as gold-labeled albumin by Palade and his associates (Milici et al. 1987; Predescu and Palade 1993; Predescu et al. 1994, 1997). These and subsequent biochemical studies have shown convincingly that transport of albumin and other macromolecules across the endothelium in situ can be fully explained by transcytosis of vesicles originating in plasma membrane structures known as caveolae (Predescu and Palade 1993; Schnitzer et al. 1994). Endothelial junctions are normally impermeable to macromolecules (diameter 10–15 nm) such as the tracer ferritin or albumin, but these molecules can be transported by shuttling of vesicles from the luminal to abluminal side (Predescu and Palade 1993). This review will address aspects of endothelial transport involving vesicles and the known signaling mechanisms responsible for vesicular transport in endothelial cells.

Albumin-binding proteins and their role in transcytosis

A key event initiating the release of the plasmalemmal-derived vesicles is the binding of albumin to a set of albumin-binding proteins; that is, proteins that have been identified by ligand blotting and crosslinking studies with molecular weights of 18, 31, 60, and 75 kDa (Ghitescu et al. 1986; Ghinea et al. 1988, 1989; Schnitzer et al. 1988; Siflinger-Birnboim et al. 1991; Schnitzer 1992b; Antohe et al. 1993; Tiruppathi et al. 1996). Figure 1 shows that some of these, the 60- and 18-kDa proteins, are particularly abundant in bovine lung microvascular endothelial cell membranes, and they can be purified in the membrane fraction. Studies have primarily concentrated on the 60-kDa glycoprotein (gp60 or albondin) because it binds to native albumin and is potentially important in regulating albumin transport (Schnitzer et al. 1988; Schnitzer 1992b; Schnitzer and Oh 1994; Tiruppathi et al. 1996, 1997; Minshall et al. 2000; Vogel et al. 2001a, b). Albumin binding to cell surface gp60 appears to be important in signaling endocytosis and the release of vesicles from the membrane (Minshall et al. 2000). Shasby and Peterson (1987) showed that albumin binding to the endothelial cell (presumably by gp60) can activate albumin transport, consistent with a receptor-mediated mechanism. Vesicles containing the gp60-bound albumin as well as albumin in the fluid phase compartment are thus transferred to the basolateral membrane, releasing the contents by exocytosis into the subendothelial space (Ghitescu et al. 1986; Milici et al. 1987; Simionescu and Simionescu 1991; Minshall et al. 2000).

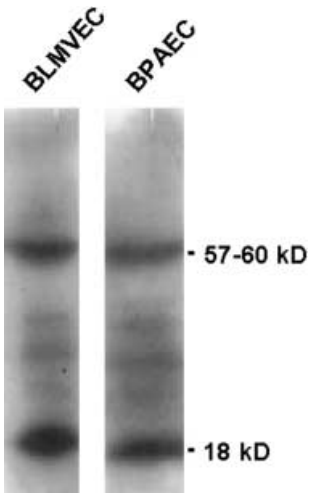

Fig. 1 Identification of albumin-binding proteins in endothelial cell membranes using ^{125}I-labeled albumin. *BLMVEC* Bovine lung microvessel endothelial cells, *BPAEC* bovine pulmonary artery endothelial cells. From Tiruppathi et al. (1996), with permission

In contrast to gp60, the 18- and 31-kDa polypeptides bind to conformationally modified or denatured albumin forms (for example, formaldehyde- or maleic anhydride-treated albumins) with a 1,000-fold greater affinity than monomeric albumin (Schnitzer et al. 1992; Schnitzer and Bravo 1993; Schnitzer and Oh 1994). These albumin binding proteins appear to be similar in their function to scavenger receptors on macrophages and may transfer albumin to the lysosomal compartment for degradation; hence, they are not likely to be important in the transcytosis of albumin.

The 60-kDa albumin-binding protein was initially characterized by its affinity to the galactose-binding lectins *Limax flavus* agglutinin and *Ricinus communis* agglutinin, which in competition assays inhibited albumin binding to rat fat tissue microvessel endothelial gp60 (Schnitzer et al. 1988). Siflinger-Birnboim et al. (1991) showed that *Ricinus communis* agglutinin precipitated gp60 in bovine lung endothelial cell membranes, and importantly, it inhibited transendothelial albumin transport. With the availability of anti-gp60 antibodies, studies showed that the antibody (Ab) blocked albumin binding and albumin permeability in the rat lung microvascular bed (Schnitzer and Oh 1994). These results indicated an important role of gp60 in the transendothelial transport of albumin. What remains unclear from these studies is whether gp60 is responsible for the transcytosis of albumin, defined as transfer of albumin-laden vesicles across the endothelial barrier so as to avoid lysosomes and albumin degradation.

Tiruppathi et al. (1996, 1997) in a series of studies showed that anti-gp60 Ab inhibited the specific binding

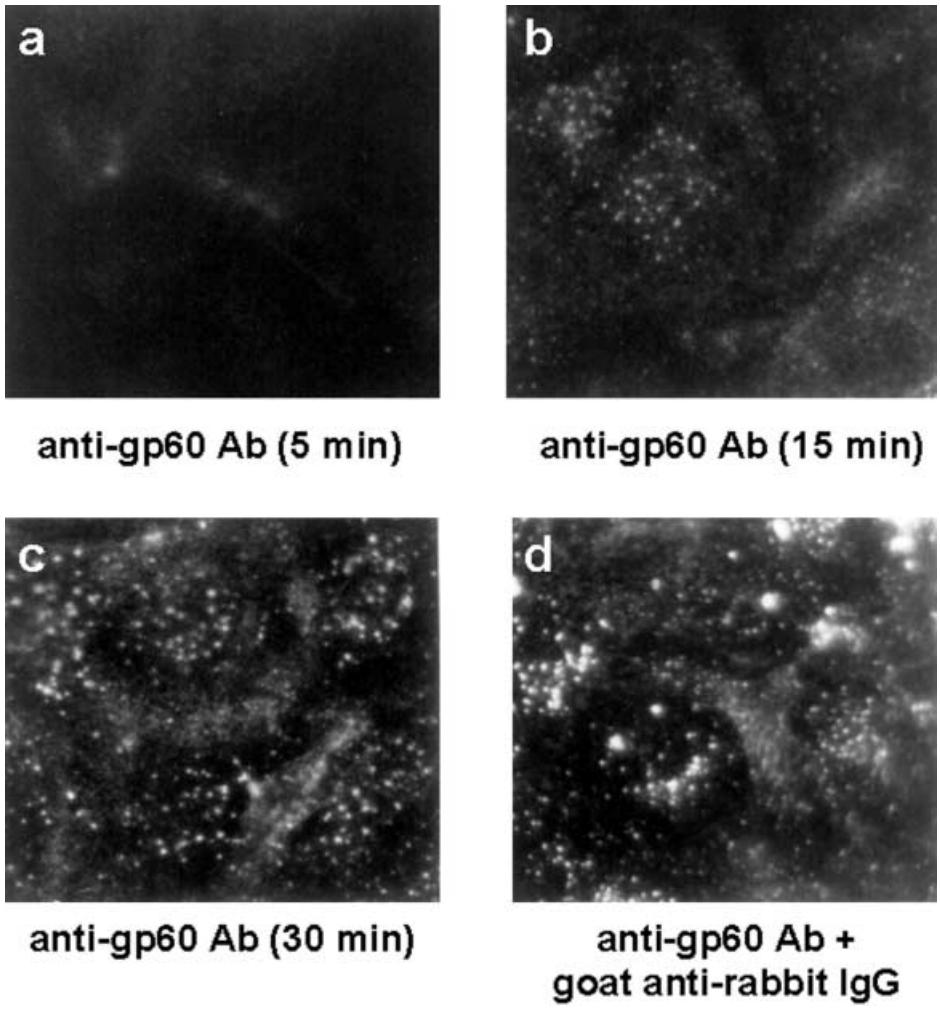

Fig. 2a–d Immunofluorescence localization of gp60 on the endothelial cell surface. Bovine endothelial cells cultured on glass coverslips were incubated with Cy3-anti-gp60 antibody (Ab) for 5 (**a**), 15 (**b**), and 30 min (**c**) at 22°C. In **d**, the cells were treated as in **c** followed by goat anti-rabbit IgG for 20 min to enhance Ab crosslinking and clustering of gp60. From Tiruppathi et al. (1997), with permission

of albumin to the endothelial cell surface at 4°C and that activation of gp60 by Ab-induced crosslinking stimulated albumin uptake in endothelial cells and migration of vesicles to the basolateral membrane. These studies demonstrated an important functional role of gp60 in activating endothelial permeability of albumin by means of increased transendothelial vesicle trafficking.

The gp60 Ab-induced crosslinking of gp60 shows some interesting features characteristic of all receptors. Upon incubation of endothelial cells at 22°C with fluorescently tagged anti-gp60 Ab for up to 30 min followed by addition of a secondary Ab, punctate structures resembling clusters of gp60 appeared on the cell surface (Fig. 2). This membrane receptor clustering phenomenon suggested that gp60 upon binding to albumin activates endocytosis in a receptor-dependent manner (Tiruppathi et al. 1997). Membrane clustering increased the uptake of tracer albumin as well as transendothelial albumin flux (Fig. 3). As predicted by the model in which budding of plasmalemmal vesicles should also carry with it fluid phase solutes, it was shown (Tiruppathi et al. 1996) that gp60 clustering induced the uptake and transport of horseradish peroxidase, a tracer without endothelial cell surface binding proteins (Fig. 3).

In a series of studies in the intact microvessels, Vogel et al. (2001a, b) showed that gp60 activation is also capable of inducing active transport of albumin across the continuous endothelial cell barrier of skeletal muscle and pulmonary microvessels. These studies demonstrated that gp60 activation increased transendothelial albumin transport, but did so without increasing liquid permeability (as measured by hydraulic conductivity) (Vogel et al. 2001b). Thus, gp60 activation uncouples hydraulic conductivity (likely the diffusive paracellular pathway) from the transcellular pathway involving the shuttling of vesicles.

Confocal imaging studies further defined the nature of the transcellular pathway. Studies carried out using fluorescent-tagged albumin and Cy3 fluorophore-labeled anti-gp60 Ab showed that both probes were colocalized in vesicles near the luminal membrane (Fig. 4). Gp60 activation increased transcellular migration of styryl pyridinium dye-filled vesicles (used as a marker of vesicle traffic) in the endothelial monolayer (Minshall et al. 2000). Thus, that gp60 activates membrane traffic and thereby triggers increased transendothelial albumin permeability.

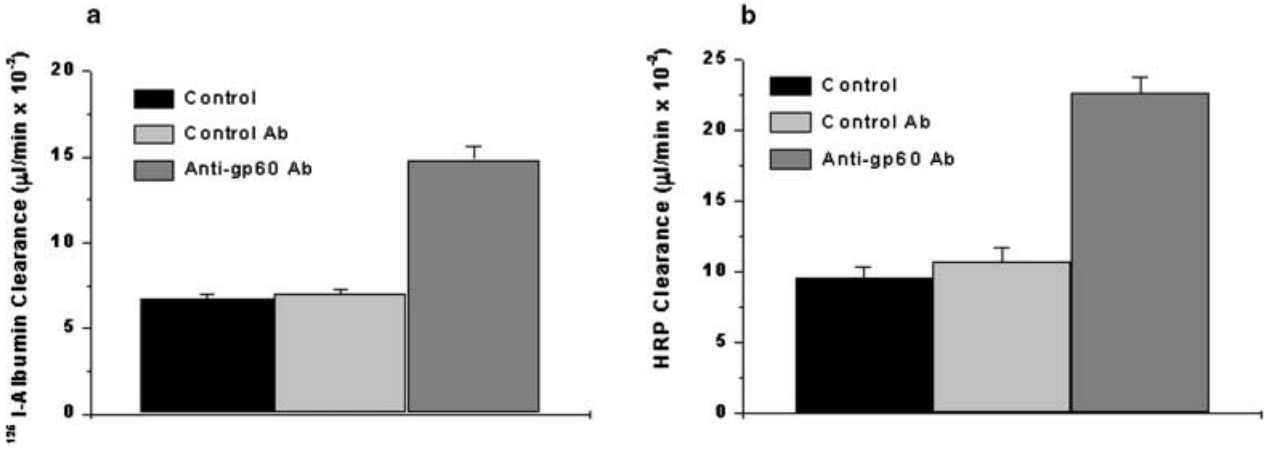

Fig. 3 Effects of crosslinking of gp60 on the transendothelial permeability of ^{125}I-albumin (**a**) and horseradish peroxidase (*HRP*; **b**). Bovine pulmonary microvessel endothelial cells cultured on microporous filters were washed twice and incubated with serum free HEPES/DMEM containing either anti-gp60 Ab or control Ab (100 µg/ml) for 30 min at 22°C. The cells were then washed twice with HEPES/DMEM, incubated with secondary Ab for 20 min, and transendothelial clearance of ^{125}I-albumin or HRP was measured at 37°C. Since both the luminal and abluminal compartments contained 1 mg/ml unlabeled albumin, transendothelial hydrostatic or oncotic pressure gradients were not imposed and thus can not account for albumin and HRP clearance. From Tiruppathi et al. (1997), with permission

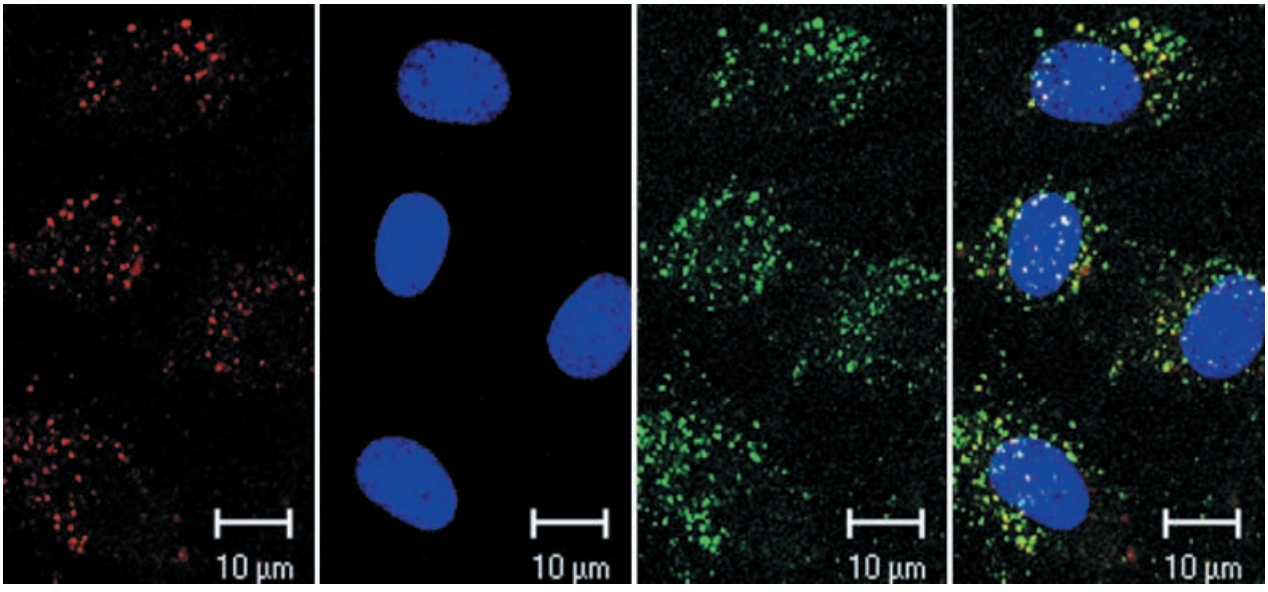

Fig. 4 Colocalization of gp60 with albumin in endocytic vesicles. Confocal image (<1.0 µm thick) of bovine PMVEC shows Cy3-gp60 Ab (*red*) and Alexa 488-albumin (*green*) uptake near the apical membrane surface and DAPI staining of the nucleus (*blue*). The overlay of gp60 and albumin fluorescent images shows coincident staining of gp60 and albumin as *yellow*. From Minshall et al. (2000), with permission

Endothelial caveolae and their role in vesicle trafficking

Caveolae, the non-clathrin coated pits, are ubiquitous features of endothelial cells: 95% of cell surface vesicles in endothelial cells consist of caveolae and they comprise ca 20% of endothelial cell volume (Predescu and Palade 1993). Caveolar release from the plasma membrane is also implicated in endocytosis (Lisanti et al. 1994; Parton et al. 1994; Anderson 1998). Portion of the vesicles thus formed migrate to the opposite membrane of the endothelial cell (Predescu and Palade 1993; Schnitzer et al. 1994; Niles and Malik 1999; Minshall et al. 2000). The question of what determines whether a vesicle is destined to the basolateral membrane, lysosomal compartment, or another intracellular organelle remains unanswered. Immunoprecipitation studies showed that gp60 and caveolin-1 become physically associated following gp60 activation (Minshall et al. 2000; Fig. 5). Endocytosis of the fluorescent-tagged albumin is blocked by filipin (Minshall et al. 2000), a sterol-binding agent that disassembles the cholesterol-rich caveolae

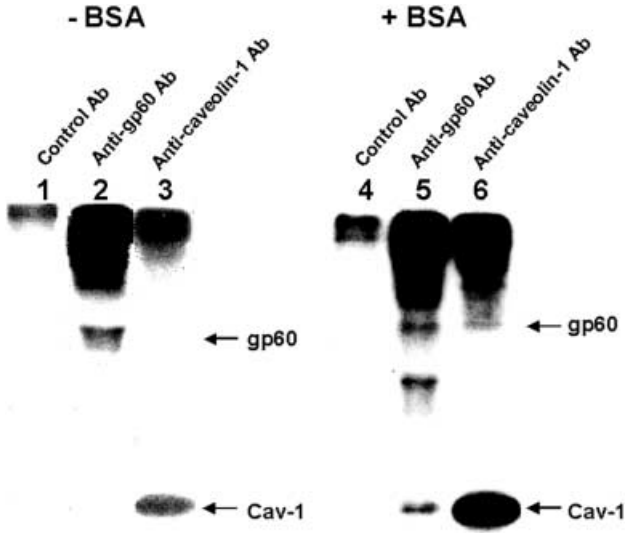

Fig. 5 Coimmunoprecipitation and migration of caveolin-1 and gp60. Confluent bovine PMVEC were metabolically labeled for 4 h with 200 µCi/ml ^{32}P-orthophosphate. Cells were then incubated at 37°C for 20 min with or without 6 mg/ml BSA and lysed. Total cell lysate was immunoprecipitated with preimmune rabbit IgG (*lanes 1, 4*), rabbit anti-gp60 IgG (*lanes 2, 5*), or rabbit anti-caveolin-1 IgG (*lanes 3, 6*), separated by SDS-PAGE, transferred to a nitrocellulose membrane, and visualized by autoradiography. As shown in *lane 5*, cell lysate immunoprecipitated with the anti-gp60 Ab also contained a 22-kDa protein which migrated similarly to that immunoprecipitated with anti-caveolin-1 Ab (*lane 6*). *Lane 6* shows that the cell lysate immunoprecipitated with anti-caveolin-1 Ab also pulled down a protein similar to that immunoprecipitated with the anti-gp60 Ab (*lane 5*). Control Ab did not immunoprecipitate either ^{32}P-labeled protein. In the absence of albumin stimulation, gp60 was not co-immunoprecipitated with the anti-caveolin-1 Ab and visa versa (*lanes 2, 3*). From Minshall et al. (2000), with permission

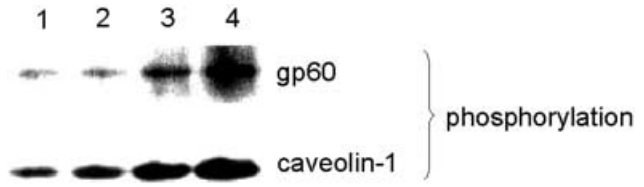

Fig. 6 Binding of albumin to gp60 induces phosphorylation of gp60 and caveolin-1 in microvascular endothelial cells. Cells were labeled with [^{32}P]orthophosphate for 4 h, treated with 5 mg/ml albumin for 0 (*lane 1*), 5 (*lane 2*), 10 (*lane 3*), or 20 min (*lane 4*), and then washed, lysed, immunoprecipitated with anti-gp60 Ab or anti-caveolin-1 Ab, separated by SDS-PAGE, and autoradiographed. Note that albumin induced increases in both gp60 and caveolin-1 phosphorylation. From Tiruppathi et al. (1997), with permission

(Rothberg et al. 1990, 1992; Schnitzer et al. 1994). Gp60-activated albumin endocytosis occurred by fission of membrane-associated caveolae and their release into the cytosol (Minshall et al. 2000). Little is known about the specific signals required for the released caveolae and trafficking of vesicles to the basolateral membrane, in particular the guidance mechanisms or "tracks" such as microtubules and molecular motors that may direct vesicle traffic across the endothelium.

Role of caveolin-1 in endocytosis

There has been much interest in the function of caveolae primarily as the result of the findings that caveolae are able to concentrate signaling molecules [a list of these molecules is provided in a summary of a symposium on caveolae (Galbiati et al. 2001)]. These cholesterol-rich microdomains functionally organize signaling molecules that stand ready to mediate a variety of cellular functions. Caveolin-1 oligomerization and insertion in the cytoplasmic face of the plasma membrane not only serves as a scaffold for signaling molecules (Li et al. 1996a), but also enables the invagination of the membrane into the flask-shaped structure (Anderson 1998; Okamoto et al. 1998). The recent report of caveolin-1 null mice showing the marked absence of caveolae has been an important advance in the field (Drab et al. 2001; Razani et al. 2001). Studies thus far carried out in caveolin-1 knockout animals showed uncontrolled endothelial cell proliferation and lung fibrosis, impaired nitric oxide and Ca^{2+} signaling, and defective endocytosis of albumin that could be reversed by expression of caveolin-1 cDNA (Drab et al. 2001; Razani et al. 2001). These observations are consistent with data showing an important role of caveolin-1 in the mechanism of albumin uptake, but clearly further studies are in order to address whether and how caveolin-1 regulates endothelial transcellular transport in the intact microcirculation.

Signaling pathways mediating release of caveolae from the plasma membrane are poorly understood. Phosphorylation events are likely important since caveolar fission is increased by phosphatase inhibition and decreased by kinase inhibition (Parton et al. 1994). Caveolin-1 is known to be phosphorylated (Glenney 1989) on tyrosine residue 14 by *Src* family kinases (Parton et al. 1994; Li et al. 1996b), suggesting a link between tyrosine kinase activity and release of caveolae. Palmitoylation of caveolin-1 at Cys156 may also contribute to the coupling of *Src* to caveolin-1 (Galbiati et al. 1999; Lee et al. 2000). Interestingly, binding of albumin to gp60 itself induced tyrosine phosphorylation of both gp60 and caveolin-1 (Fig. 6; Tiruppathi et al. 1997). The functional importance of this event is evident from the finding that tyrosine kinase inhibitors, herbimycin A and genistein, prevented the gp60-activated vesicle formation and albumin endocytosis (Tiruppathi et al. 1997).

Release of caveolae in endothelial plasma membrane

Caveolin-1 sequesters caveolae-associated proteins such as eNOS, *Src*, and G_i in their inactive forms (Anderson 1998; Song et al. 1997; Okamoto et al. 1998). Tiruppathi et al. (1997) and Minshall et al. (2000) showed that albumin binding to gp60 activated *Src* kinase and the heterotri-

meric GTP binding protein, G_i, which bind to and are normally held in an inactive state by the caveolin-1 scaffolding domain (Chun et al. 1994; Li et al. 1996a; Okamoto et al. 1998). Studies by Minshall et al. (2000) provide the important clues concerning the signaling machinery responsible for activation of endocytosis in endothelial cells (Minshall et al. 2000). The GTPase dynamin, localized at the neck of caveolae, also mediates caveolar release from the plasmalemma by constriction of the neck region of caveolae (Schnitzer et al. 1996; Oh et al. 1998). Docking of vesicles on the basolateral membrane in endothelial cells required the *N*-ethylmaleimide-sensitive fusion factor (NSF) and soluble NSF attachment-protein (SNAP) receptor (SNARE) (Schnitzer et al. 1995; Predescu et al. 1996, 1997, 2001a; Niles and Malik 1999). Thus, components of the canonical vesicle docking machinery are responsible for exocytosis and unloading of vesicular contents in the basal side of the endothelium.

Predescu et al. (2001a) have recently isolated a macromolecular complex consisting of dynamin, Rab5, intersectin, caveolin-1, SNAPs, and SNAREs that may mediate transcytosis in endothelial cells by the release of cell surface caveolae. Components of this complex such as dynamin may be involved in the initial fission event that releases caveolae from the membrane, whereas other components such as intersectin may be involved in fusion of vesicles as they traverse the endothelium (Predescu et al. 2001b). Components such as SNAREs may be involved in the docking of vesicles at the basal membrane.

Overexpression of caveolin-1 resulted in increased binding of $G_{\alpha i}$ and $G_{\alpha q}$ to caveolin-1, consistent with a role of caveolin-1 in sequestering G proteins (Okamoto et al. 1998; Murthy and Makhlouf 2000). Expression of caveolin-1 in endothelial cells also prevented gp60-induced vesicle formation (Minshall et al. 2000). Thus, caveolin-1 by sequestering $G_{\alpha i}$ (Fig. 7) may prevent the release of membrane vesicles and transcytosis.

How does G_i signal the release of caveolae? Although the complete answer to this question is not yet available, there are some clues. Since *Src* activation occurs downstream of G_i (Lutrell et al. 1996; Igishi and Gutkind 1998; Ellis et al. 1999), it is likely that the interaction of G_i with *Src* is important in initiating internalization of caveolae. Expression of the catalytically inactive *Src* mutant prevented vesicle formation in endothelial cells (Minshall et al. 2000). Interestingly, the catalytically inactive *Src* mutant also interfered with the binding of $G_{\alpha i}$ to caveolin-1 (Fig. 7); thus, caveolae release requires that *Src* be in the active state. As the binding of the *Src* mutant to caveolin-1 displaced $G_{\alpha i}$ from caveolin-1, it is possible that *Src* and $G_{\alpha i}$ compete for a common binding site on caveolin-1. Although previous studies have not addressed the binding interactions of signaling molecules on the caveolin-1 scaffold (Li et al. 1996a; Anderson 1998; Okamoto et al. 1998), it appears that *Src*-mediated tyrosine phosphorylation of caveolin-1 is important in the release of $G_{\alpha i}$ from caveolin-1. This potentially important role of *Src* is evident from the observation that there is less binding of $G_{\alpha i}$ to caveolin-1 in cells transfected with the *Src* mutant (Fig. 7).

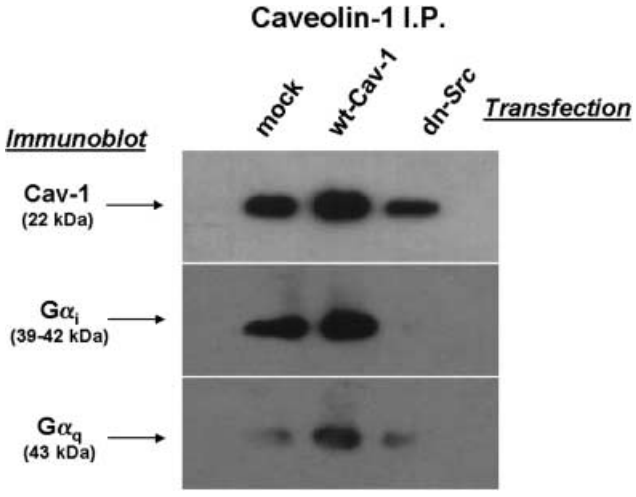

Fig. 7 Effects of caveolin-1 overexpression and dn-*Src* expression on association of $G_{\alpha i}$ and $G_{\alpha q}$ with caveolin-1. PMVEC transfected with pcDNA3.1 alone (*mock*), wt-caveolin-1 cDNA (*wt-cav-1*), or dn-*Src* cDNA were grown to confluence (48 h post-transfection). Cells were washed and lysed, and the lysate was immunoprecipitated using anti-caveolin-1 Ab. Precipitated proteins were separated by SDS-PAGE and transferred to Duralose membrane. Membrane was blotted with anti-caveolin-1 Ab (*top*), anti-$G_{\alpha i}$ Ab (*middle*), and anti-$G_{\alpha q}$ Ab (*bottom*). From Minshall et al. (2000), with permission

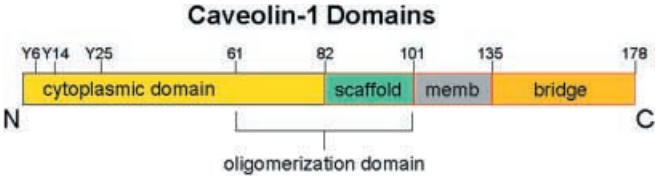

Fig. 8 Domains of caveolin-1. Caveolin-1 has been divided into three main sections: two cytoplasmic domains (N-terminal a.a. 1–101 and C-terminal a.a. 135–178) and a central hydrophobic domain (a.a. 102–134) that forms a hairpin-like structure in the membrane. Homo-oligomerization of caveolin-1 is due to N- and C-domain interactions and bridging, which contributes to the caveolin-rich scaffold on the cytoplasmic side of the plasma membrane. Within the N-terminal cytoplasmic region is the caveolin scaffolding domain (a.a. 82–101) that may be important in in binding signaling molecules. *Src* phosphorylation of N-terminal tyrosines (Y6, Y14, Y25) may be involved in the regulation of vesicle formation and transcellular permeability

Studies are needed to determine the precise role of caveolin-1 phosphorylation induced by *Src* in activating internalization of caveolae, and thereby in initiating transcytosis. Since caveolin-1 phosphorylation is required for endocytosis (Parton et al. 1994), understanding the function of N-terminal tyrosine residues in caveolin-1 (Y6, Y14, and Y25; Fig. 8) in binding the components of the caveolar release complex, *Src* and $G_{\alpha i}$, becomes particularly important. The phosphorylation state of caveolin-1 tyrosine residues may dictate whether *Src* and $G_{\alpha i}$ bind to the caveolin-1 scaffold domain (a.a. 82–101; Okamoto et al. 1998) and trigger the release of caveolae.

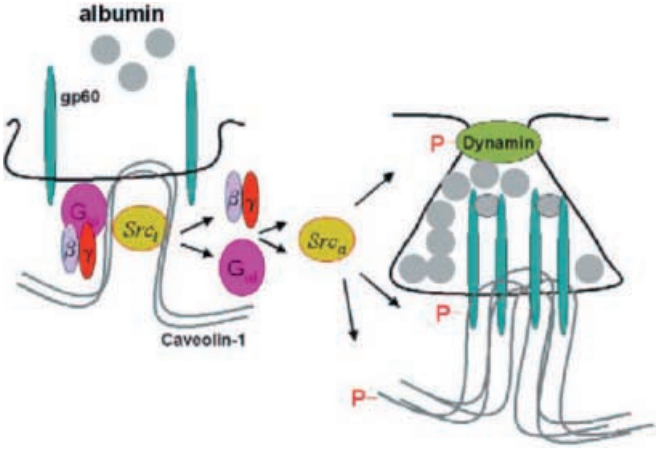

Fig. 9 Hypothesized mechanisms of gp60-activated vesicle formation. Albumin binding to endothelial cell surface 60-kDa glycoprotein (gp60) induces clustering of gp60 and its physical interaction with caveolin-1. The heterotrimeric GTP binding protein, G_i, and *Src* tyrosine kinase, bound to the caveolin-1 scaffolding domain in their inactive conformation, are activated upon albumin binding to gp60. Activated *Src* in turn may phosphorylate caveolin-1, gp60, and dynamin to initiate plasmalemmal vesicle fission and transendothelial vesicular transport. The high-affinity albumin-binding protein gp60 may induce high-capacity receptor-bound and fluid phase albumin transport via caveolae

Figure 9 describes a model of endocytosis in endothelial cells, and the resultant uptake of albumin. Caveolin-1 plays a central role as it serves as a scaffold for components of the caveolar release complex, G_i and *Src*, the signaling machinery responsible for gp60-induced endocytosis. *Src*-family tyrosine kinases, which can be activated by $G_{\beta\gamma}$ subunits upon stimulation of G protein-coupled receptors (Lutrell et al. 1996; Igishi and Gutkind 1998) or directly by $G_{\alpha i}$ (Ma et al. 2000), may phosphorylate tyrosine residues on caveolin-1 (Li et al. 1996b; Tiruppathi et al. 1997) as well as gp60 (Tiruppathi et al. 1997). The caveolae release complex thus engaged may activate vesicle formation and their trafficking across the endothelial barrier. This model will need to be interrogated to define the precise relationships of caveolin-1 with gp60 as well as with G_i and *Src*.

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