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Integrin regulation of caveolin function

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Abstract

Caveolae are unique organelles that are found in the plasma membrane of many cell types. They participate in various processes such as lipid recycling, cellular signalling and endocytosis. A variety of signalling molecules localize to caveolae in response to various stimuli, providing a potential mechanism for the spatial regulation of signal transduction pathways. Caveolin-1, a constitutive protein of caveolae, has been implicated in the regulation of cell growth, lipid trafficking, endocytosis and cell migration. Phosphorylation of caveolin-1 on Tyr 14 is involved in integrin-regulated caveolae trafficking and also in signalling at focal adhesions in migrating cells. In this review, we focus on recent studies that describe the role of caveolin-1 in integrin signal transduction, and how this interplay links extracellular matrix anchorage to cell proliferation, polarity and directional migration.

Keywords: integrins • membrane domains • Rho-GTPases • caveolin-1 • signalling • migration • cell proliferation • endocytosis

Introduction

The lipid bilayer of the plasma membrane is organized into domains characterized by different degrees of lipid packing. This domain structure arises as the result of preferential clustering of cholesterol and sphingolipids, together with certain proteins, into highly ordered regions. These regions are known as 'lipid rafts' or cholesterol-enriched membrane microdomains (CEMMs), and are qualitatively different from the bulk membrane (reviewed in [1, 2]). One subset of lipid rafts is found in cell invaginations called caveolae. Caveolae are cholesterol- and sphingomyelin-rich invaginations (50–100 nm) of the plasma membrane and are distinct from other coated pits [3]. They have a characteristic flask shape, no readily visible coat [4], and are highly abundant in many vertebrate cell types, such as smooth-muscle cells, fibroblasts, endothelial cells (EC) and adipocytes [5].

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Caveolae have been proposed to participate in a wide variety of physiological functions, including endocytosis, membrane trafficking [6], lipid recycling [7, 8], cholesterol uptake [9] and various signalling processes [5]. This review will examine and discuss recently published evidence suggesting novel roles for caveolae and caveolin proteins in the control of lipid raft trafficking regulated by integrins and in cell polarization and migration.

Caveolin proteins

The major component of caveolae are caveolin proteins; caveolins stabilize cholesterol rich domains and are essential for the formation and maintenance of caveolae [10]. Caveolins are small proteins (22 KD) but have the ability to form high molecular mass oligomers. These oligomers are filamentous structures that self-associate to stabilize the membrane of caveolae, defining their shape and size [11]. Three caveolin proteins have been described to date. Caveolin-1 (Cav-1) was first identified as a substrate of v-Src-kinase in Rous sarcoma virus-transformed chick fibroblasts [10, 12]. Cav-1 and Cav-2 are commonly co-expressed and are abundant in terminally differentiated cells, such as pneumocytes, epithelial and EC, fibroblasts, adipocytes and smooth muscle. Cav-3 shares higher sequence homology with Cav-1 than does Cav-2, and is restricted to muscle [13, 14]. All three isoforms consist of a hydrophobic central domain that is embedded in the membrane and hydrophilic N- and C-terminal domains, both of which are cytosolic. Three palmitoylation sites in the C-terminal region next to the transmembrane domain contribute to anchoring caveolin proteins to the membrane. The N-terminal region of all caveolins contains a conserved FEDVIAEP motif that has been defined as the 'caveolin signature' sequence, and has been suggested to be important for the binding of caveolin to cholesterol- and glycosphingolipid-rich membrane domains [13, 15]. The central segment of caveolin proteins contains the scaffolding domain, which allows oligomerization of caveolin monomers and direct interaction with other proteins, presumably regulating their activity [16, 17]. In addition, caveolin proteins contain several serine and tyrosine residues within their intracellular domains that are substrates for a variety of kinases, and become phosphorylated in response to different stimuli [18, 19].

Caveolin proteins and disease

Caveolin proteins are implicated in several disease states [20]. The genes encoding Cav-1 and -2 are located on the human 7q31 locus (6-A2 in the mouse genome); this region is frequently altered in tumour cells and is thus likely to contain tumour suppressor genes [21]. Consistent with such a role, Cav-1 appears to have numerous cell-type specific effects on growth-regulatory pathways. Cav-1 null cells show increased proliferation, and loss of Cav-1 expression accelerates tumourigenesis in several models [22, 23]. Mutations in Cav-1 have been found in 16% of human breast cancers [24, 25], and many human tumours have reduced levels of Cav-1 expression (reviewed in [20]). For some tumours, however, Cav-1 expression seems to be related to cell survival and growth [26–28]. Thus, depending on the tumour origin, Cav-1 can function either as a tumour suppressor or as a tumour promoter. The molecular mechanisms underlying these cell-type specific growth-regulatory behaviour are currently unknown. Dys-regulation of Cav-3 results in muscular defects, reflecting its restricted tissue distribution to muscle cells. Patients with Duchenne muscular dystrophy show increased levels of Cav-3 and caveolae at the sarcolemma. The opposite situation occurs in limb girdle muscular dystrophy, where mutations in the Cav-3 gene result in a severe reduction of Cav-3 and caveolae in muscle. Alterations in Cav-3 have also been described for hereditary rippling muscle disease, which is linked to a locus that spans the Cav-3 gene (reviewed in [29]).

The recent generation of knockout models for each of the caveolin proteins has allowed a more precise characterization of their functions. Knockout of Cav-1 also severely represses the expression of Cav-2, and Cav-1 single KO and Cav-1/2 double KO animals completely lack caveolar structures on the plasma membrane of all cells except for Cav-3-expressing myocytes [30, 31]. Caveolae still form in mice lacking Cav-2, although some disruption to Cav-1 expression is observed in some tissues. The phenotypes of caveolin knockout animals include a variety of cardiovascular and pulmonary abnormalities.

These are accompanied by disorders in fatty acid metabolism and adipose tissue homeostasis, as well as hyperplasia in some tissues, with the defects of Cav-3 KO animals being restricted to muscle (reviewed in [32]). Nevertheless all caveolin KO animals are viable, with only minor abnormalities, suggesting that other mechanisms compensate for the absence of caveolar structures.

Caveolae biogenesis, dynamics and trafficking

Caveolae are motile organelles and their functional roles are linked to their trafficking and dynamics. Cav-1 is synthesized as an integral membrane protein and starts to oligomerize in the endoplasmic reticulum. Cav-1 is then transported through the Golgi complex and, at some point, associates with cholesterol and other lipid raft components (reviewed in [33]). At this stage, Cav-1 is organized into higherorder oligomers characteristic of the surface pool of caveolin. Exit from the Golgi complex is associated with further oligomerization and association with cholesterol and glycosphingolipid-rich lipid raft domains to form a mature 'exocytic caveolar carrier', which migrates to the plasma membrane in a process that requires the SNARE protein syntaxin-6 and maybe other as yet unidentified carriers (reviewed in [5]). Despite the preferred caveolar location of caveolins, these proteins are also expressed away from morphological caveolae, including focal adhesions and lipid droplets [5], and they are also secreted as soluble proteins [34].

Caveolae show a limited motility and dynamics under basal conditions, but interaction with specific ligands, such as simian virus SV40 (a non-enveloped virus that uses caveolae for cell entry) and cholera toxin (CTx) can trigger their rapid internalization, with the involvement of dynamin-II, Src kinases, protein kinase C and actin recruitment [35–38]. After internalization, endocytosed caveolae travel as carrier vesicles that can move into the cytosol and fuse with pre-existing structures called caveosomes in a RAB5-independent manner, or with the early endosome in a RAB5-dependent event [39]. Alternatively, internalized caveolae can fuse back to the plasma membrane to form caveolae, without the involvement of an endosomal intermediate [37, 39, 40]. Caveolae are not disassembled after fusing with these membranes, and still preserve their morphology and unique lipid and protein composition [39].

Caveolae mediate signalling through direct and indirect mechanisms

The abundance of signalling proteins in caveolae led to the hypothesis that caveolae might serve as platforms for processing diverse extracellular signals [41]. Further studies have shown that caveolin acts as a scaffold protein that concentrates lipid-modified proteins at caveolae and regulates their activity through direct interaction. Direct inhibition by caveolin has been reported for several proteins, such as Src, the EGF receptor, endothelial nitric-oxide synthase (eNOS), G protein α subunits, and H-Ras, (reviewed in [42]). Direct activation by caveolin has been described for the insulin receptor [43]. Since many of the signalling molecules that are regulated by caveolin are important for cell cycle progression, it is likely that dys-regulation of caveolin expression will have an impact on cell proliferation. Indeed, many of the above-mentioned caveolin-regulated pathways are constitutively activated in cancer cells.

An alternative, indirect mechanism of signalling regulation by caveolin and caveolae arises from their role in cholesterol/CEMM trafficking. CEMMs arise by the preferential clustering of sphingolipids and cholesterol into moving platforms, or lipid rafts, and it has been proposed that some proteins selectively bind to these rafts while others are excluded [1, 44]. In this way, receptors that become activated upon ligand binding can be concentrated in close proximity to their effector molecules in membrane microdomains from which potential inhibitors, such as membrane phosphatases would be excluded. This is represented in Figure 1A. Supporting this idea, specific phosphotyrosine-kinase activities are known to concentrate at lipid rafts [45], what seems to be an effective mechanism for modulating cross-talk among different pathways. By regulating the trafficking of lipid domains, caveolin could thus indirectly modulate signalling. Through these mechanisms, caveolae may serve as a matrix for early signal transduction events, and reorganization of these domains could critically determine the functional outcomes of signalling cascades.

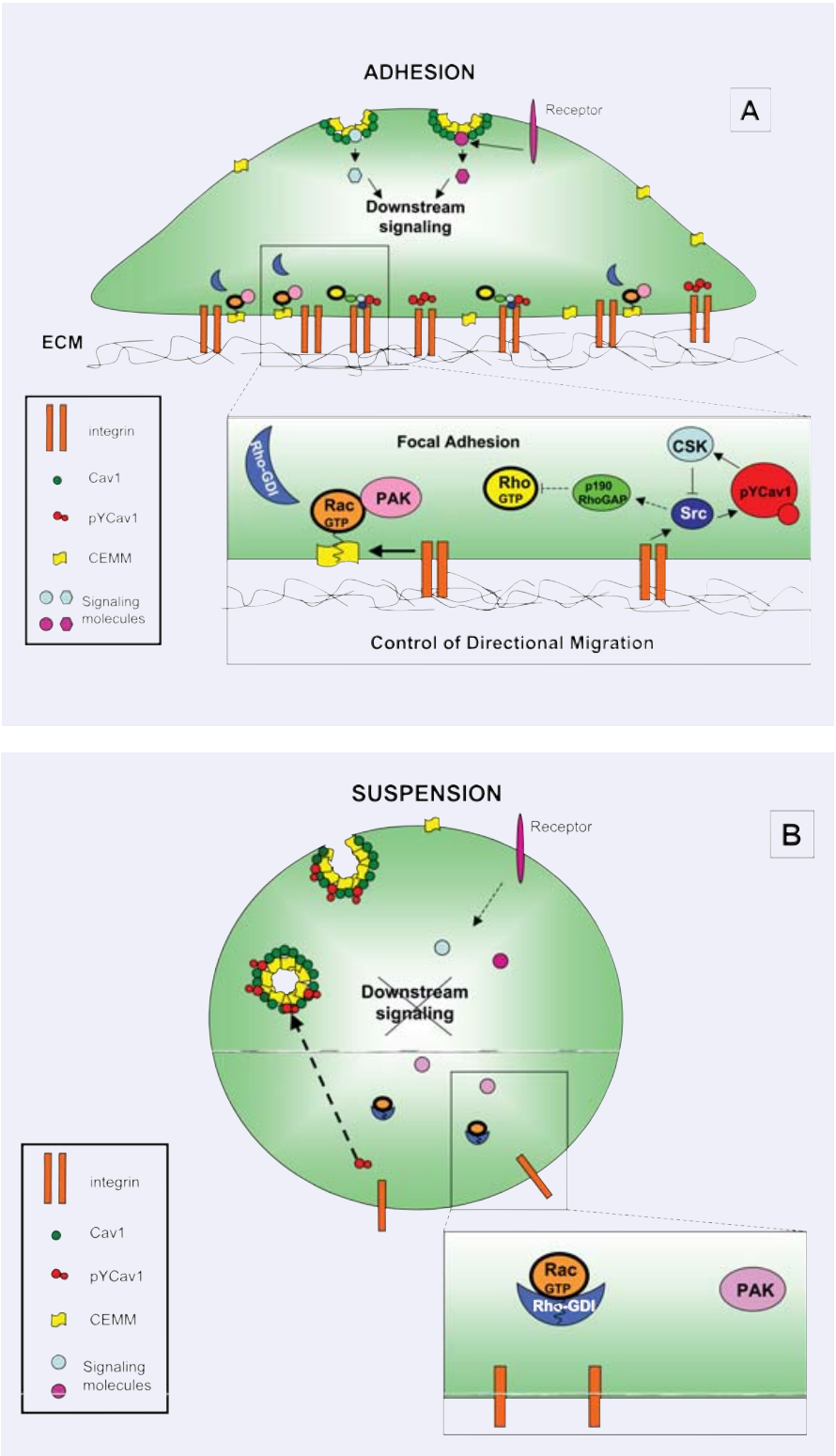

Fig. 1 Dual role of Cav-1 Y14 phosphorylation: phosphorylated caveolin (pYCav-1) is involved in the control of integrin-mediated signal transduction and caveolae-mediated cholesterol-enriched membrane microdomains (CEMM) endocytosis. In adherent cells (**A**), pYCav-1 is retained mainly at focal adhesions and its concentration at caveolae is low. In focal adhesions (FA), pYCav-1 is involved in controlling cell polarity and directional migration. We propose a model in which pYCav-1 regulates Src activity by recruiting C–terminal Src kinase (Csk) to FA; Csk inhibits Src by phosphorylation and this modulates Src-dependent inactivation of Rho through p190RhoGAP. Moreover, integrin activation targets Rac and other proteins to caveolae/CEMMs, allowing interaction with effector molecules. In the case of Rac, integrins increase the affinity for Rac by modulating CEMMs, thus displacing Rac from its cytosolic ligand, Rho-GDI (magnification in A). This process may be controlled directly by integrins or by an unknown pathway that regulates the localization of pYCav-1 at focal adhesions, preventing CEMM internalization. The presence of CEMMs in the plasma membrane allows the activation of multiple signalling pathways. When integrin signalling is interrupted, as in suspended cells or at areas of the cell that transiently detach from the substrate (**B**), CEMMs are endocytosed by a mechanism involving pYCav-1 translocation to caveolae. This process uncouples many signalling intermediates from their effectors, thereby shutting down associated signalling pathways.

Signalling pathways at rafts: targeting by integrins

Examples of signalling pathways that involve lipid rafts include immunoglobulin E signalling, T lymphocyte activation, Glia cell line-derived growth factor (GDNF) signalling and H-Ras-mediated Raf activation (reviewed in [46]). A factor common to all these signalling events is that the interaction between the activated receptors and their immediate downstream effectors takes place in the raft fraction of the plasma membrane, and downstream signalling is inhibited by cholesterol depletion. Recent work in this area has focused on the activation of signalling by the small GTPase Rac in response to integrin-mediated cell adhesion to the extracellular matrix (ECM). Activation of Rac by integrins upon fibronectin binding induces GTP loading, similar to the activation triggered by growth factor receptors; but, distinct from growth factor regulation, integrins also target Rac to specific plasma membrane microdomains, where Rac can interact with its downstream effector molecule PAK to induce signalling [47, 48]. Thus, when β1- and probably other fibronectin-binding integrins are uncoupled from downstream signalling by detaching cells from the ECM, PAK is not activated by Rac, even though Rac-GTP (activated by growth factor receptors) is present in these detached cells [48]. These data suggest that integrin-mediated adhesion facilitates the coupling of Rac to PAK by modulating the plasma membrane so as to target Rac to specific microdomains, where the interaction with its effector can take place. There is strong evidence that the membrane microdomain targets of integrin-modulated Rac affinity are lipid rafts or CEMMs: (*i*) association of Rac with CEMMs within the plasma membrane has been reported in several studies [49–53], including an unbiased proteomic approach [53]; and (*ii*) the loss of integrin signalling promoted by cell detachment induces a rapid internalization of CEMMs, and this prevents the targeting of Rac to the plasma membrane and its coupling to PAK. Replatement of cells on fibronectin or anti-β1-integrin antibody reversed these effects [52]. These data suggest a model in which integrin-mediated cell adhesion promotes plasma membrane localization of Rac and its subsequent coupling to its effector by preventing internalization of the Rac-containing CEMMs [52].

Localization to CEMMs has also been described for the other two members of the Rho family of GTPases, Rho and Cdc42 [49, 54–56]. Rho and its effector mDia regulate microtubule stabilization [56], and this process occurs only at the leading edges of migrating cells, which are enriched in Rho and CEMMs [57]. The local coupling between Rho and its effector is regulated by integrin-mediated cellular adhesion to the ECM, and appears to require active signalling by focal adhesion kinase [55]. Cdc42 targeting to the plasma membrane is also integrin-dependent [47]. Therefore, evidence for association with CEMMs in an integrin-dependent manner has been described for all three major members of Rho family GTPases.

Integrin triggered CEMM internalization is mediated by caveolae

The internalization of CEMMs that occurs when integrin signalling is lost by cell detachment is mediated by endocytosis of caveolae [58]. In fact, in detached cells, caveolin shows a time-dependent internalization from the plasma membrane to an intracellular compartment. Electron microscopy and immunogold labelling studies in detached cells show that the flask-shaped caveolae easily observed in the plasma membrane of adherent cells are internalized shortly after detachment into large vacuoles surrounded by invaginated caveolae, similar to the reported caveosomes. Detachment causes internalization of both CEMMs and caveolae, suggesting that CEMM internalization might occur through a caveolin-dependent endocytic pathway. Detachment-induced CEMMs internalization is completely abolished by the expression of a dominant negative dynamin-II mutant, but not by the expression of the C-terminal domain of Eps15, a selective inhibitor of the classic clathrin pathway [58]. In addition, inhibition of Cdc42-dependent internalization with either the Cdc42-binding domain (CBD) of WASP or N17-Cdc42 had no effect in this process [58]. Finally, this endocytosis of CEMMs only takes place in cells expressing the phosphorylable form of Cav-1 and not in those expressing the unphosphorylable Cav-1 mutant Y14F [58]. In conclusion, this process is independent

of clathrin and Cdc42, but requires dynamin-II and phosphorylation of Cav-1 on Tyr 14 (pYCav-1). Supporting these observations, two-photon microscopy studies with the fluorescent probe Laurdan, which has been used extensively to define ordered domains in artificial membranes [59] and in live and fixed cells [60], revealed that loss of integrin signalling (detachment of the cell from the ECM) triggers a biphasal decrease in the plasma membrane order: an initial rapid, caveolin-independent phase followed by a slower, caveolin-dependent decrease [61]. This correlates with the internalization of CEMMs upon loss of cell adhesion [52, 58]. Another link between caveolar endocytosis and integrin-mediated cell adhesion has been recently provided by a high-throughput RNAi screening of the kinome. In this study, specific silencing of several kinases related to integrin signalling and adhesion influenced the caveolae-mediated SV40 virus uptake [62]. Moreover, cell detachment and caveolar endocytosis are also induced by antibody cross-linking of β1-integrins [63].

All these studies support the idea that integrins regulate cellular processes by controlling the localization of CEMMs at the plasma membrane. Caveolae-mediated internalization of CEMMs is an effective means of terminating signalling upon loss of adhesion, as represented in Fig. 1B. In this scenario, depletion of lipid rafts from the plasma membrane could prevent the activation of multiple integrin signalling pathways that are related to CEMMs [46] and that are known to control cell proliferation [64]. In fact, at least three integrin-dependent growth pathways (Ras/Erk, PI3K/Akt and Rac/Pak) are impaired by Cav-1-mediated CEMM internalization [65]. The presence of caveolin thus links integrin signalling to growth-regulatory pathways; alteration of this regulatory mechanism in the absence of caveolin would uncouple integrins from growth-regulatory pathways and therefore break the requirement of integrins for active signalling, resulting in anchorage-independent growth, a characteristic of most tumour cells [66]. These findings may thus have identified a new mechanism for Cav-1 mediated control of anchorage-dependent cell growth. However, absolute lack of caveolae does not hamper normal development and signalling in Cav-1$^{-/-}$ mice, and cells derived from Cav-1$^{-/-}$ mice displayed an unaltered pattern of protein expression and lipid composition of rafts [30, 31]. All this suggests the existence of compensatory mechanisms that replace caveolae function in these organisms and points out the need of further research in the field of caveolae-mediated regulation of cell function.

Massive raft internalization concomitant with global cell detachment is unlikely to occur in physiological situations. Rather, cell detachment occurs in a regional manner, frequently during cell migration, where integrins provide local and spatial means of regulation [67]. A role for caveolin is this process has also been reported and is discussed in the next section.

Dual role of caveolin Y14 phosphorylation: endocytosis *versus* cell migration

Integrins connect the ECM to the actin cytoskeleton at special structures called focal adhesions (FA) through a protein complex that includes vinculin, paxillin, talin and α-actinin [67]. In addition, activation of integrins results in the recruitment of a number of signalling molecules to FA, including focal adhesion kinase (FAK). FAK plays a central role in signalling from FA, participating in integrin-mediated regulation of migration, proliferation and spreading [68]. Cav-1 protein is also found at FA, where most of the phosphorylated Cav-1 pool resides [18, 69]. pYCav-1 appears to be essential for maintaining a highly ordered state in the membranes around these adhesion complexes, and this is likely to be due to the recruitment of membrane components that induce order, such as cholesterol [61]. Besides its structural role, Cav-1 also participates in active signalling at FA. This function seems to rely on its ability to scaffold signalling molecules around integrins. Several studies have reported interaction between Cav-1, integrins and other proteins that localize to FA. Through association with β1 integrins and the Src-related kinase Fyn, Cav-1 promotes Fyn-dependent Shc phosphorylation and MAPK activation in response to integrin ligation [70–72]. Cav-1 signalling at FA also seems to be important for radiation resistance in pancreatic cells [73]. In response to various stimuli, Src and other kinases phosphorylate Cav-1 on Tyr 14, and this phosphorylation is crucial for a number of functions attributed to Cav-1. Activation of adenylyl cyclase (AC), which increases cyclic AMP

(cAMP), results in Src- and PKA-dependent Cav-1 phosphorylation, and pYCav-1 in turn scaffolds AC at FA. This interaction contributes to the disruption of actin organization and FA assembly mediated by dephosphorylation of FAK upon AC activation [74]. pYCav-1 has also been reported to regulate Src activity by recruiting C-terminal Src kinase (Csk) to FA [19, 75]. Csk inhibits Src activity by phosphorylating a conserved Tyr residue, and, consistent with this, overexpression of Cav-1 in 293 cells results in Src inhibition [72]. Conversely, Cav-1 deficiency in 293 cells and mouse embryonic fibroblasts (MEF) increases Src activity [72, 76].

The modulation of Src activity at FA has many implications for the integrin-dependent control of cell adhesion, spreading, and cytoskeletal organization. Src regulates members of the Rho family of GTPases, activating Rac [77, 78] and Cdc42 [79–81], and inhibiting Rho *via* the activation of p190RhoGAP [82–84]. In agreement with this, Rac1 and Cdc42 activities are increased in Cav-1$^{-/-}$ MEFs, whereas RhoA activity is decreased. These cells display abnormalities in cell polarization and directional migration, processes known to be commanded by members of the Rho family of GTPases. Moreover, the wild-type phenotype can be rescued in Cav-1$^{-/-}$ MEFs by re-expression of wild-type Cav-1 but not by expression of a non-phosphorylatable mutant isoform (Y14FCav-1) [76]. This indicates a role for Tyr 14 phosphorylation of Cav-1 in the control of fibroblast polarization and directional migration. Focal complexes are newly assembled and mature into larger FA at the leading edge of migrating cells, while they disassemble at the trailing edge to allow contractility [67]. Accordingly, EC show a polarized distribution of Cav-1 during planar migration, with caveolae containing non-phosphorylated Cav-1 accumulating at the cell rear, while non-caveolar pYCav-1 localizes to the FA at the frontal lamellipodia [85–87]. EC expressing a non-phosphorylatable Cav-1 mutant (where Tyr 14 is replaced by Ala) fail to polarize Cav-1 [86]. Together these results reinforce the importance of Cav-1 phosphorylation in the regulation of integrin signalling at FA, contributing to the precise control of cell polarity and migration.

In rat EC, high levels of pYCav-1 correlate with fewer caveolae at the cell surface and increased numbers of cytoplasmic caveolin-containing vesicles, suggesting that Cav-1 phosphorylation could be important for caveolae internalization [88]. This is supported by the observation that caveolae-dependent endocytosis is dependent on kinase activity [38, 89, 90]. Caveolar dynamics on the cell surface of CV-1 cells is stimulated by treatment with vanadate (a tyrosine phosphatases inhibitor) to a similar extent that SV40 virus, and conversely, SV40-induced activation is blocked by the tyrosine kinase inhibitor genistein [36]. Cells expressing the non-phosphorylatable mutant Y14FCav-1 have morphologically normal caveolae, but are unable to internalize CEMMs after loss of adhesion. Moreover, constitutively low levels of pYCav-1 correlate with impaired CEMM internalization in detached COS-7 cells, which is overcome upon increased caveolin Tyr 14 phosphorylation after vanadate treatment [58]. Vanadate-treated COS-7 cells showed similar levels of pYCav-1 to NIH-3T3 fibroblasts which did not affect CEMM membrane localization on adherent cells, but allowed internalization after detachment. Whereas in adherent cells, pYCav-1 is mainly retained at FA, cell detachment triggers a shift in phospho-caveolin localization from FA to caveolae [58]. Thus, relocalization of pYCav-1 from FA to caveolae appears to be necessary for the clearance of lipid rafts from the cell surface, although it can not be excluded that pools of Cav-1 present at caveolae are phosphorylated *de novo* upon cell detachment. Independently of this question, given the small proportion of cellular Cav-1 that is phosphorylated (less than 1%) [58], it is likely that pYCav-1 acts by activating other signalling cascades to trigger internalization. Further investigation is required to unveil the precise molecular mechanism involved in this process. A consequence of CEMM internalization is the termination of certain signalling cascades associated with rafts (see above and Fig. 1). Loss of adhesion causes PAK inactivation in wt MEFs but not in their Cav-1$^{-/-}$ counterparts, consistent with the retention of CEMMs and associated Rac at the plasma membrane of the mutant cells. Similarly, other integrin-regulated signalling cascades, such as MAPK-Erk and PI3K-Akt remain active in suspended Cav-1$^{-/-}$ MEFs but are strongly down-regulated in suspended wt cells [58]. Thus, we propose that by modulating the subcellular distribution of lipid rafts, pYCav-1 connects integrin-dependent adhesion to the targeting of signalling molecules to the plasma membrane and their subsequent coupling to downstream effector molecules.

Conclusions

Caveolae are specialized structures at the membranes of most cell types. They participate in a number of processes including lipid trafficking, signalling and cholesterol uptake. However, some cells are naturally devoid of caveolae without their metabolism being affected. Also, caveolin knockout mice, which completely lack caveolae in their cells, display a relatively mild phenotype. This indicates that caveolae, although important, are not essential and that other mechanisms might be compensating for the absence of these structures. Caveolin proteins are the major component of caveolae and are required for their formation, but new roles for these proteins at locations away from caveolae continue to emerge. For instance, Cav-1 is found at FA of adherent cells and at the leading edge of migrating EC, where no caveolae are present. Phosphorylation of Tyr 14 seems to be important for localizing Cav-1 pools away from caveolae to allow other functions in signalling and protein scaffolding. Thus, phosphorylated Cav-1 appears to accomplish different roles in different locations: a number of studies link pYCav-1 to signalling and clustering of proteins at FA, and re-location of pYCav-1 to caveolae triggers endocytosis. In this paper, we have reviewed possible implications of caveolin phosphorylation in the integrin-mediated control of membrane lipid dynamics, cell proliferation, migration and cell polarity. Integrin binding to the ECM targets a number of proteins to CEMMs, allowing coupling to downstream effectors. When integrins are uncoupled from the downstream signalling machinery, for example by loss of adhesion, caveolae mediate endocytosis of CEMMs and certain associated signalling pathways are inhibited. It is thus possible that pYCav-1-mediated control of caveolar endocytosis is relevant to the integrin-mediated control of proliferation, and therefore dys-regulation in this mechanism could result in anchorage-independent growth. This is a common feature in tumour cells, and could partly explain the observed alterations in caveolin expression in human cancers. Two other aspects that define normal 'untransformed' cells-cell polarity and migration-seem also to rely on pYCav-1. The integrin-associated pool of pYCav-1 at FA seems to be involved in the control of these features. In summary, recent studies in this field link Cav-1 expression and its phosphorylation on Tyr 14 to the regulation of many molecular processes that define normal cell behaviour, but intense study will be needed to gain a full understanding of the mechanisms involved.

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References

1. **Jacobson K, Mouritsen OG, Anderson RG.** Lipid rafts: at a crossroad between cell biology and physics. *Nat Cell Biol.* 2007; 9: 7–14.
2. **Hancock JF.** Lipid rafts: contentious only from simplistic standpoints. *Nat Rev Mol Cell Biol.* 2006; 7: 456–62.
3. **Palade GE.** Fine structure of blood capillaries. *J. Appl Phys.* 1953; 24: 1424.
4. **Stan RV.** Structure of caveolae. *Biochim Biophys Acta.* 2005; 1746: 334–48.
5. **Parton RG, Simons K.** The multiple faces of caveolae. *Nat Rev Mol Cell Biol.* 2007; 8: 185–94.
6. **Anderson RG.** The caveolae membrane system. *Annu Rev Biochem.* 1998; 67: 199–225.
7. **Fielding CJ, Fielding PE.** Relationship between cholesterol trafficking and signaling in rafts and caveolae. *Biochim Biophys Acta.* 2003; 1610: 219–28.
8. **Liu P, Ying Y, Zhao Y, Mundy DI, Zhu M, Anderson RG.** Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. *J Biol Chem.* 2004; 279: 3787–92.
9. **Murata M, Peranen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K.** VIP21/caveolin is a cholesterol-binding protein. *Proc Natl Acad Sci USA.* 1995; 92: 10339–43.
10. **Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG.** Caveolin, a protein

component of caveolae membrane coats. *Cell.* 1992; 68: 673–82.

11. **Fernandez I, Ying Y, Albanesi J, Anderson RG.** Mechanism of caveolin filament assembly. *Proc Natl Acad Sci USA.* 2002; 99: 11193–8.
12. **Glenney JR Jr., Soppet D.** 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 USA.* 1992; 89: 10517–21.
13. **Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, Nishimoto I, Lodish HF, Lisanti MP.** Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem.* 1996; 271: 2255–61.
14. **Biederer C, Ries S, Drobnik W, Schmitz G.** Molecular cloning of human caveolin 3. *Biochim Biophys Acta.* 1998; 1406: 5–9.
15. **Machleidt T, Li WP, Liu P, Anderson RG.** Multiple domains in caveolin-1 control its intracellular traffic. *J. Cell Biol.* 2000; 148: 17–28.
16. **Couet J, Sargiacomo M, Lisanti MP.** Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. *J Biol Chem.* 1997; 272: 30429–38.
17. **Li S, Couet J, Lisanti MP.** 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.* 1996; 271: 29182–90.
18. **Lee H, Volonte D, Galbiati F, Iyengar P, Lublin DM, Bregman DB, Wilson MT, Campos-Gonzalez R, Bouzahzah B, Pestell RG, Scherer PE, Lisanti MP.** 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.* 2000; 14: 1750–75.
19. **Cao H, Courchesne WE, Mastick CC.** A phosphotyrosine-dependent protein interaction screen reveals a Role for phosphorylation of caveolin-1 on tyrosine 14: Recruitment of C- terminal Src kinase. *J Biol Chem.* 2002; 22: 22.
20. **Williams TM, Lisanti MP.** Caveolin-1 in oncogenic transformation, cancer, and metastasis. *Am J Physiol Cell Physiol.* 2005; 288: C494–506.
21. **Engelman JA, Zhang XL, Lisanti MP.** Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers. *FEBS Lett.* 1998; 436: 403–10.
22. **Lee SW, Reimer CL, Oh P, Campbell DB, Schnitzer JE.** Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. *Oncogene.* 1998; 16: 1391–7.
23. **Koleske AJ, Baltimore D, Lisanti MP.** Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc Natl Acad Sci USA.* 1995; 92: 1381–5.
24. **Williams TM, Cheung MW, Park DS, Razani B, Cohen AW, Muller WJ, Di Vizio D, Chopra NG, Pestell RG, Lisanti MP.** Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. *Mol Biol Cell.* 2003; 14: 1027–42.
25. **Hayashi K, Matsuda S, Machida K, Yamamoto T, Fukuda Y, Nimura Y, Hayakawa T, Hamaguchi M.** Invasion activating caveolin-1 mutation in human scirrhous breast cancers. *Cancer Res.* 2001; 61: 2361–4.
26. **Yang G, Timme TL, Frolov A, Wheeler TM, Thompson TC.** Combined c-Myc and caveolin-1 expression in human prostate carcinoma predicts prostate carcinoma progression. *Cancer.* 2005; 103: 1186–94.
27. **Sunaga N, Miyajima K, Suzuki M, Sato M, White MA, Ramirez RD, Shay JW, Gazdar AF, Minna JD.** Different roles for caveolin-1 in the development of non-small cell lung cancer *versus* small cell lung cancer. *Cancer Res.* 2004; 64: 4277–85.
28. **Thompson TC, Timme TL, Li L, Goltsov A.** Caveolin-1, a metastasis-related gene that promotes cell survival in prostate cancer. *Apoptosis.* 1999; 4: 233–7.
29. **Hnasko R, Lisanti MP.** The biology of caveolae: lessons from caveolin knockout mice and implications for human disease. *Mol Interv.* 2003; 3: 445–64.
30. **Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, Kurzchalia TV.** Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science.* 2001; 293: 2449–52.
31. **Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di Vizio D, Hou H Jr., Kneitz B, Lagaud G, Christ GJ, Edelmann W, Lisanti MP.** Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem.* 2001; 276: 38121–38.
32. **Le Lay S, Kurzchalia TV.** Getting rid of caveolins: phenotypes of caveolin-deficient animals. *Biochim Biophys Acta.* 2005; 1746: 322–33.
33. **Parton RG, Hanzal-Bayer M, Hancock JF.** Biogenesis of caveolae: a structural model for caveolin-induced domain formation. *J Cell Sci.* 2006; 119: 787–96.

34. **Liu P, Rudick M, Anderson RG.** Multiple functions of caveolin-1. *J Biol Chem.* 2002; 277: 41295–8.
35. **Sharma DK, Brown JC, Choudhury A, Peterson TE, Holicky E, Marks DL, Simari R, Parton RG, Pagano RE.** Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol. *Mol Biol Cell.* 2004; 15: 3114–22.
36. **Tagawa A, Mezzacasa A, Hayer A, Longatti A, Pelkmans L, Helenius A.** Assembly and trafficking of caveolar domains in the cell: caveolae as stable, cargo-triggered, vesicular transporters. *J Cell Biol.* 2005; 170: 769–79.
37. **Pelkmans L, Kartenbeck J, Helenius A.** Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. *Nat Cell Biol.* 2001; 3: 473–83.
38. **Pelkmans L, Puntener D, Helenius A.** Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. *Science.* 2002; 296: 535–9.
39. **Pelkmans L, Burli T, Zerial M, Helenius A.** Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. *Cell.* 2004; 118: 767–80.
40. **Sharma DK, Choudhury A, Singh RD, Wheatley CL, Marks DL, Pagano RE.** Glycosphingolipids internalized *via* caveolar-related endocytosis rapidly merge with the clathrin pathway in early endosomes and form microdomains for recycling. *J Biol Chem.* 2003; 278: 7564–72.
41. **Anderson RG.** Caveolae: where incoming and outgoing messengers meet. *Proc Natl Acad Sci USA.* 1993; 90: 10909–13.
42. **Okamoto T, Schlegel A, Scherer PE, Lisanti MP.** Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem.* 1998; 273: 5419–22.
43. **Yamamoto M, Toya Y, Schwencke C, Lisanti MP, Myers MG Jr., Ishikawa Y.** Caveolin is an activator of insulin receptor signaling. *J Biol Chem.* 1998; 273: 26962–8.
44. **Simons K, Ikonen E.** Functional rafts in cell membranes. *Nature.* 1997; 387: 569–72.
45. **Harder T, Simons K.** Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. *Eur J Immunol.* 1999; 29: 556–62.
46. **Simons K, Toomre D.** Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol.* 2000; 1: 31–9.
47. **del Pozo MA, Kiosses WB, Alderson N, Meller N, Hahn KM, Schwartz MA.** Integrins regulate GTP-Rac localized effector interactions through dissociation of RhoGDI. *Nature Cell Biol.* 2002; 4: 232–9.
48. **del Pozo MA, Price LS, Alderson NB, Ren XD, Schwartz MA.** Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *EMBO J.* 2000; 19: 2008–14.
49. **Prag S, Parsons M, Keppler MD, Ameer-Beg SM, Barber P, Hunt J, Beavil AJ, Calvert R, Arpin M, Vojnovic B, Ng T.** Activated ezrin promotes cell migration through recruitment of the GEF Dbl to lipid rafts and preferential downstream activation of Cdc42. *Mol Biol Cell.* 2007; 18: 2935–48.
50. **Michaely PA, Mineo C, Ying YS, Anderson RG.** Polarized distribution of endogenous Rac1 and RhoA at the cell surface. *J. Biol. Chem.* 1999; 274: 21430–6.
51. **Grimmer S, Van Deurs B, Sandvig K.** Membrane ruffling and macropinocytosis in A431 cells require cholesterol. *J Cell Sci.* 2002; 115: 2953–62.
52. **del Pozo MA, Alderson NB, Kiosses WB, Chiang HH, Anderson RG, Schwartz MA.** Integrins regulate Rac targeting by internalization of membrane domains. *Science.* 2004; 303: 839–42.
53. **Foster LJ, De Hoog CL, Mann M.** Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc Natl Acad Sci USA.* 2003; 100: 5813–8.
54. **Golub T, Caroni P.** PI(4,5)P2-dependent microdomain assemblies capture microtubules to promote and control leading edge motility. *J Cell Biol.* 2005; 169: 151–65.
55. **Palazzo AF, Eng CH, Schlaepfer DD, Marcantonio EE, Gundersen GG.** Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. *Science.* 2004; 303: 836–9.
56. **Palazzo AF, Cook TA, Alberts AS, Gundersen GG.** mDia mediates Rho-regulated formation and orientation of stable microtubules. *Nat Cell Biol.* 2001; 3: 723–9.
57. **Pertz O, Hodgson L, Klemke RL, Hahn KM.** Spatiotemporal dynamics of RhoA activity in migrating cells. *Nature.* 2006; 440: 1069–72.
58. **del Pozo MA, Balasubramanian N, Alderson NB, Kiosses WB, Grande-Garcia A, Anderson RG, Schwartz MA.** Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. *Nat Cell Biol.* 2005; 7: 901–8.
59. **Bagatolli LA, Sanchez SA, Hazlett T, Gratton E.** Giant vesicles, Laurdan, and two-photon fluorescence microscopy: evidence of lipid lateral separation in bilayers. *Methods Enzymol.* 2003; 360: 481–500.
60. **Gaus K, Gratton E, Kable EP, Jones AS, Gelissen I, Kritharides L, Jessup W.** Visualizing lipid structure

and raft domains in living cells with two-photon microscopy. *Proc Natl Acad Sci USA.* 2003; 100: 15554–9.

61. **Gaus K, Le Lay S, Balasubramanian N, Schwartz MA.** Integrin-mediated adhesion regulates membrane order. *J Cell Biol.* 2006; 174: 725–34.
62. **Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, Zerial M.** Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature.* 2005; 436: 78–86.
63. **Sharma DK, Brown JC, Cheng Z, Holicky EL, Marks DL, Pagano RE.** The glycosphingolipid, lactosylceramide, regulates beta1-integrin clustering and endocytosis. *Cancer Res.* 2005; 65: 8233–41.
64. **Assoian RK, Schwartz MA.** Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression. *Curr Opin Genet Dev.* 2001; 11: 48–53.
65. **Del Pozo MA, Schwartz MA.** Rac, membrane heterogeneity, caveolin and regulation of growth by integrins. *Trends Cell Biol.* 2007; 17: 246–50.
66. **Freedman VH, Shin S.** Cellular Tumorigenicity in nude mice: correlation with cell growth in semisolid medium. *Cell.* 1974; 3: 355–9.
67. **Vicente-Manzanares M, Webb DJ, Horwitz AR.** Cell migration at a glance. *J Cell Sci.* 2005; 118: 4917–9.
68. **Cary LA, Guan JL.** Focal adhesion kinase in integrin-mediated signaling. *Front Biosci.* 1999; 4: D102–13.
69. **Mettouchi A, Klein S, Guo W, Lopez-Lago M, Lemichez E, Westwick JK, Giancotti FG.** Integrin-specific activation of Rac controls progression through the G(1) phase of the cell cycle. *Mol Cell.* 2001; 8: 115–27.
70. **Wary KK, Maneiro F, Isakoff SJ, Marcantonio EE, Giancotti FG.** The adapter protein Shc couples a class of integrins to the control of cell cycle progression. *Cell.* 1996; 87: 733–43.
71. **Wary KK, Mariotti A, Zurzolo C, Giancotti FG.** A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell.* 1998; 94: 625–34.
72. **Wei Y, Yang X, Liu Q, Wilkins JA, Chapman HA.** A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. *J Cell Biol.* 1999; 144: 1285–94.
73. **Cordes N, Frick S, Brunner TB, Pilarsky C, Grutzmann R, Sipos B, Kloppel G, McKenna WG, Bernhard EJ.** Human pancreatic tumor cells are sensitized to ionizing radiation by knockdown of caveolin-1. *Oncogene.* 2007;
74. **Swaney JS, Patel HH, Yokoyama U, Head BP, Roth DM, Insel PA.** Focal adhesions in (myo)fibroblasts scaffold adenylyl cyclase with phosphorylated caveolin. J Biol Chem. 2006; 281: 17173–9.
75. **Radel C, Rizzo V.** Integrin mechanotransduction stimulates caveolin-1 phosphorylation and recruitment of Csk to mediate actin reorganization. *Am J Physiol Heart Circ Physiol.* 2005; 288: H936–45.
76. **Grande-García A, Echarri A, de Rooij J, Alderson NB, Waterman-Storer, C.M., Valdivielso JM, del Pozo MA.** Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. *J. Cell Biol.* 2007; 177: 683–94.
77. **Kawakatsu T, Ogita H, Fukuhara T, Fukuyama T, Minami Y, Shimizu K, Takai Y.** Vav2 as a Rac-GDP/GTP exchange factor responsible for the nectin-induced, c-Src- and Cdc42-mediated activation of Rac. *J Biol Chem.* 2005; 280: 4940–7.
78. **Servitja JM, Marinissen MJ, Sodhi A, Bustelo XR, Gutkind JS.** Rac1 function is required for Src-induced transformation. Evidence of a role for Tiam1 and Vav2 in Rac activation by Src. *J Biol Chem.* 2003; 278: 34339–46.
79. **Tu S, Wu WJ, Wang J, Cerione RA.** Epidermal growth factor-dependent regulation of Cdc42 is mediated by the Src tyrosine kinase. *J Biol Chem.* 2003; 278: 49293–300.
80. **Fukuyama T, Ogita H, Kawakatsu T, Fukuhara T, Yamada T, Sato T, Shimizu K, Nakamura T, Matsuda M, Takai Y.** Involvement of the c-Src-Crk-C3G-Rap1 signaling in the nectin-induced activation of Cdc42 and formation of adherens junctions. *J Biol Chem.* 2005; 280: 815–25.
81. **Miyamoto Y, Yamauchi J, Itoh H.** Src kinase regulates the activation of a novel FGD-1-related Cdc42 guanine nucleotide exchange factor in the signaling pathway from the endothelin A receptor to JNK. *J Biol Chem.* 2003; 278: 29890–900.
82. **Meng W, Numazaki M, Takeuchi K, Uchibori Y, Ando-Akatsuka Y, Tominaga M, Tominaga T.** DIP (mDia interacting protein) is a key molecule regulating Rho and Rac in a Src-dependent manner. *EMBO J.* 2004; 23: 760–71.
83. **Arthur WT, Petch LA, Burridge K.** Integrin engagement suppresses RhoA activity *via* a c-Src-dependent mechanism. *Curr Biol.* 2000; 10: 719–22.
84. **Brouns MR, Matheson SF, Settleman J.** p190 RhoGAP is the principal Src substrate in brain and regulates axon outgrowth, guidance and fasciculation. *Nat Cell Biol.* 2001; 3: 361–7.
85. **Sun XH, Flynn DC, Castranova V, Millecchia LL, Beardsley AR, Liu J.** Identification of a novel domain at the N terminus of caveolin-1 that controls rear polarization of the protein and caveolae formation. *J Biol Chem.* 2007; 282: 7232–41.

86. **Parat MO, Anand-Apte B, Fox PL.** Differential caveolin-1 polarization in endothelial cells during migration in two and three dimensions. *Mol Biol Cell.* 2003; 14: 3156–68.
87. **Beardsley A, Fang K, Mertz H, Castranova V, Friend S, Liu J.** Loss of caveolin-1 polarity impedes endothelial cell polarization and directional movement. *J Biol Chem.* 2005; 280: 3541–7.
88. **Aoki T, Nomura R, Fujimoto T.** Tyrosine phosphorylation of caveolin-1 in the endothelium. *Exp Cell Res.* 1999; 253: 629–36.
89. **Pelkmans L, Zerial M.** Kinase-regulated quantal assemblies and kiss-and-run recycling of caveolae. *Nature.* 2005; 436: 128–33.
90. **Parton RG, Joggerst B, Simons K.** Regulated internalization of caveolae. *J Cell Biol.* 1994; 127: 1199–215.