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Tyrosine phosphorylation-dependence of caveolae-mediated endocytosis

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Abstract

Caveolae are flask-shaped plasma membrane invaginations that mediate endocytosis and transcytosis of plasma macromolecules, such as albumin, insulin and **low-density lipoprotein** (LDL), as well as certain viruses, bacteria and bacterial toxins. Caveolae-mediated transcytosis of macromolecules is critical for maintaining vascular homeostasis by regulating the oncotic pressure gradient and tissue delivery of drugs, vitamins, lipids and ions. Entrapment of cargo within caveolae induces activation of signalling cascades leading to caveolae fission and internalization. Activation of Src tyrosine kinase is an early and essential step that triggers detachment of loaded caveolae from the plasma membrane. In this review, we examine how *Src*-mediated phosphorylation regulates caveolae-mediated transport by orchestrating the localization and activity of essential proteins of the endocytic machinery to regulate caveolae formation and fission.

Keywords: caveolae • endocytosis • phosphorylation • Src • caveolin-1 • actin cytoskeleton

Introduction

Under normal physiological conditions, endothelial cells form monolayers that line blood vessels and serve as an indispensable barrier that controls the transport of macromolecules from blood to the interstitium. Loss of this barrier function results in pathophysiological conditions including oedema [1]. Caveolae-mediated transcytosis is the primary means by which plasma macromolecules cross the endothelium. Caveolae, the omega-shaped plasmalemmal vesicles of 50–80 nm in diameter [2], represent >95% of the endothelial cell vesicles [3]. Transcytosis occurs by the activation of fission and endocytosis of caveolae from the apical membrane [3–5], followed by migration of detached vesicles to the basolateral membrane where they fuse and release their contents into the interstitial space [6–12].

The main structural unit and biological marker of caveolae is the 20–22 kD integral membrane

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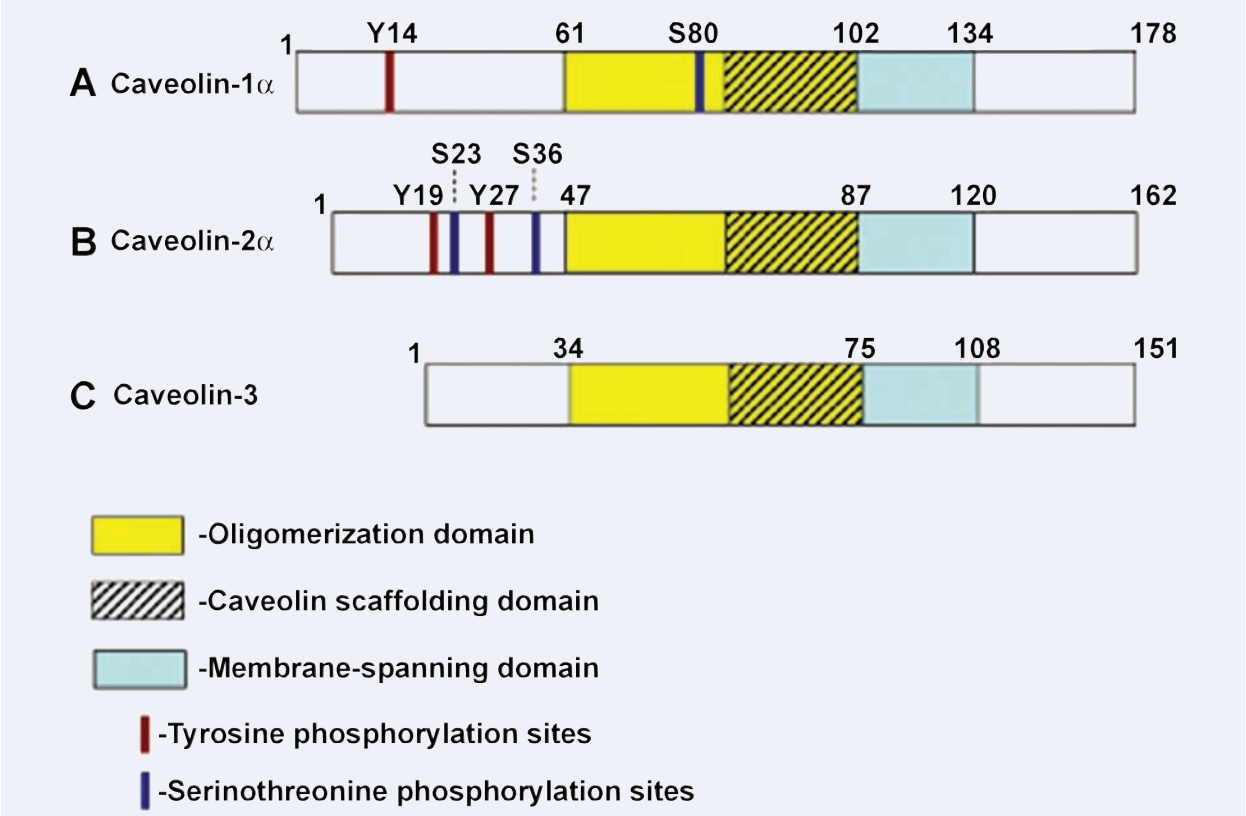

Fig. 1 Phosphorylation map of the caveolin family of proteins. Caveolins are highly homologous and conserved proteins. All isoforms contain membrane-spanning, oligomerization and caveolin-scaffolding domains. (**A**) Caveolin-1α isoform consists of 178 amino acids with phosphorylation sites at tyrosine 14 and serine 80. Caveolin-1β isoform lacks the first 31 amino acids, and thus it does not contain the tyrosine phosphorylation site. Residues 75–158 (part of scaffolding domain, membrane-spanning domain, and most of the C-terminus) are involved in binding Dynamin 2. Scaffolding and membrane-spanning domains of caveolin-1 also participate in homo-oligomerization and formation of heteroligomers with caveolin-2. (**B**) Caveolin-2 is ~50% homologues to caveolin-1 and caveolin-2α isoform contains 162 amino acids with phosphorylation sites at tyrosines 19 and 27, and serines 23 and 36. Caveolin-2β isoform, produced by alternative splicing of mRNA, is truncated by 13 N-terminal amino acids, while no information is yet available about the phosphorylation of the caveolin-2α isoform. (**C**) Caveolin-3 is a muscle-specific isoform, which contains 151 amino acids and is very homologous to caveolin-1. No phosphorylation sites have been demonstrated for caveolin-3 to date.

protein, caveolin. To date, several different caveolin iso-forms have been identified: caveolin-1α, caveolin-1β, caveolin-2α, caveolin-2β, caveolin-2γ and caveolin-3 [13] (Fig. 1). Caveolin-1 and -2 are ubiquitously expressed, while caveolin-3 is confined to muscle cells [14–16]. The α and β isoforms of caveolin-1 result from alternative splicing from a single gene; the β form is 32 amino acids shorter than the α form [14]. Caveolin-1 is required for the biogenesis of non-muscle caveolae since the overexpression of caveolin-1 in cells that lack endogenous caveolin-1 results in the *de novo* formation of caveolae [17, 18]. In cells that loose caveolin-1 expression during transformation, caveolae are no longer present [19]. Moreover, endothelium and adipose tissue of mice defective in caveolin-1 are devoid of caveolae [20–24]. Suppression of caveolin-1 expression by siRNA leads to a dramatic decrease in the number of caveolae in endothelial cells, which returns to normal following recovery of caveolin-1 expression [25].

By tracing the movement of labelled albumin and other molecules from the luminal side of the

endothelium by electron microscopy [9, 26–28], it was shown that caveolae are involved in the transcellular transport of molecules through endothelial cells. In fibroblasts derived from caveolin-1 knockout mouse embryos, uptake of fluorescent-labelled albumin was abolished [24]. Both electron microscopy and radio-iodinated albumin uptake studies confirmed the failure of caveolin-1 knockout mouse endothelium to internalize albumin *in vivo,* further supporting the importance of caveolae in the transport of macromolecules [29]. However, mice lacking caveolae are viable and the albumin concentration in cerebrospinal fluid of caveolin-1 knockout mice is not different from that of wild-type mice [20]. It was subsequently suggested that in the absence of caveolin-1, elevated levels of nitric oxide (NO) leads to destabilization of the normally restrictive paracellular pathway, thus compensating for the loss of caveolae by generating shorter and more permeable transendothelial junctions [25, 29]. Despite our current understanding of the importance of caveolae-mediated transcytosis in maintenance of the structural integrity of the endothelial barrier, the signalling mechanisms by which caveolin-1 and transcytosis in general regulate endothelial barrier integrity have not been well established.

Src signalling in caveolae-mediated endocytosis

Selective internalization of certain proteins occurs within caveolae upon ligation of cell surface receptors [10, 30–33]. We and others have shown that endocytosis *via* caveolae is critically dependent on stimulation of tyrosine kinase signalling [10, 31, 34–41]. Dephosphorylation may also be involved in the control of caveolae-mediated endocytosis [34, 40]. However, the mechanism of caveolae formation and release from the plasma membrane in response to caveolin-1 phosphorylation remains unknown.

Early on, studies showed that the binding of some proteins to their receptors induces receptor clustering in caveolae, which in turn activates phosphorylation cascades [31, 34, 36]. Clustering of albumin receptor gp60 or alkaline phosphatase, induced by cross-linking with antibodies, is sufficient for activation of signalling events associated with caveolae internalization [31, 34, 40]. Virions of SV40 virus, another caveolae marker, initially do not bind to caveolae, but once ligated translocate to caveolae [36, 42, 43]. Major histocompatability complex-I (MHC-I), the surface receptor for SV40, also moves to caveolae upon cross-linking with antibodies [44]. Treatment with nystatin, a drug that sequesters cholesterol and flattens caveolae, does not inhibit SV40 binding to the cell surface, but impairs SV40-induced signalling and endocytosis [36], as well as endocytosis of albumin and glycosphingolipids [39]. These studies suggest that receptor clustering and movement into caveolae is crucial for the induction of endocytosis.

Caveolae are also known to scaffold and concentrate signalling molecules, and entrapment of cargo in caveolae is required for initiation of signalling. As mentioned already, inhibition of phosphatases augments caveolar internalization whereas inhibition of kinases decreases it, implying that phosphorylation events play an essential role in the mechanism of caveolae-mediated endocytosis. While searching for the kinases that could initiate this phosphorylation cascade, it became apparent that Src family non-receptor tyrosine kinases, Src and Fyn, are activated immediately after addition of cargo [31, 40]. Tiruppathi *et al*., as well as others [39, 40] showed that the tyrosine kinase inhibitors tyrphostin A, genistein and PP2 block albumin uptake. Additionally, expression of dn-Src (dominant-negative Src, Y527F/K295M) inhibited gp60-mediated internalization of caveolae in endothelial cells [10, 41]. Furthermore, a peptide encoding the caveolin-1 scaffolding domain (CSD) dose-dependently inhibited the auto-activation of purified Src kinases (c-Src and Fyn) [45] and also decreased transcytosis of albumin by 50% when introduced to the cells [46]. Finally, down-regulation of Src with siRNA induced aggregation of caveolae and a decrease in the mobility of caveolae, which resulted in defective endocytosis of SV40 in HeLa cells [47, 48]. A recent screening for kinases important for caveolae-mediated endocytosis revealed a large number of these molecules are involved in co-ordinating caveolae internalization [48]. The present review focuses on Src family kinases as they represent the most well studied signalling mechanism involved in caveolae-mediated endocytosis.

The mechanism by which receptor clustering in caveolae activates Src kinases is not completely understood. Activation of Src kinase and Src-mediated phosphorylation of caveolin-1, caveolin-2 and

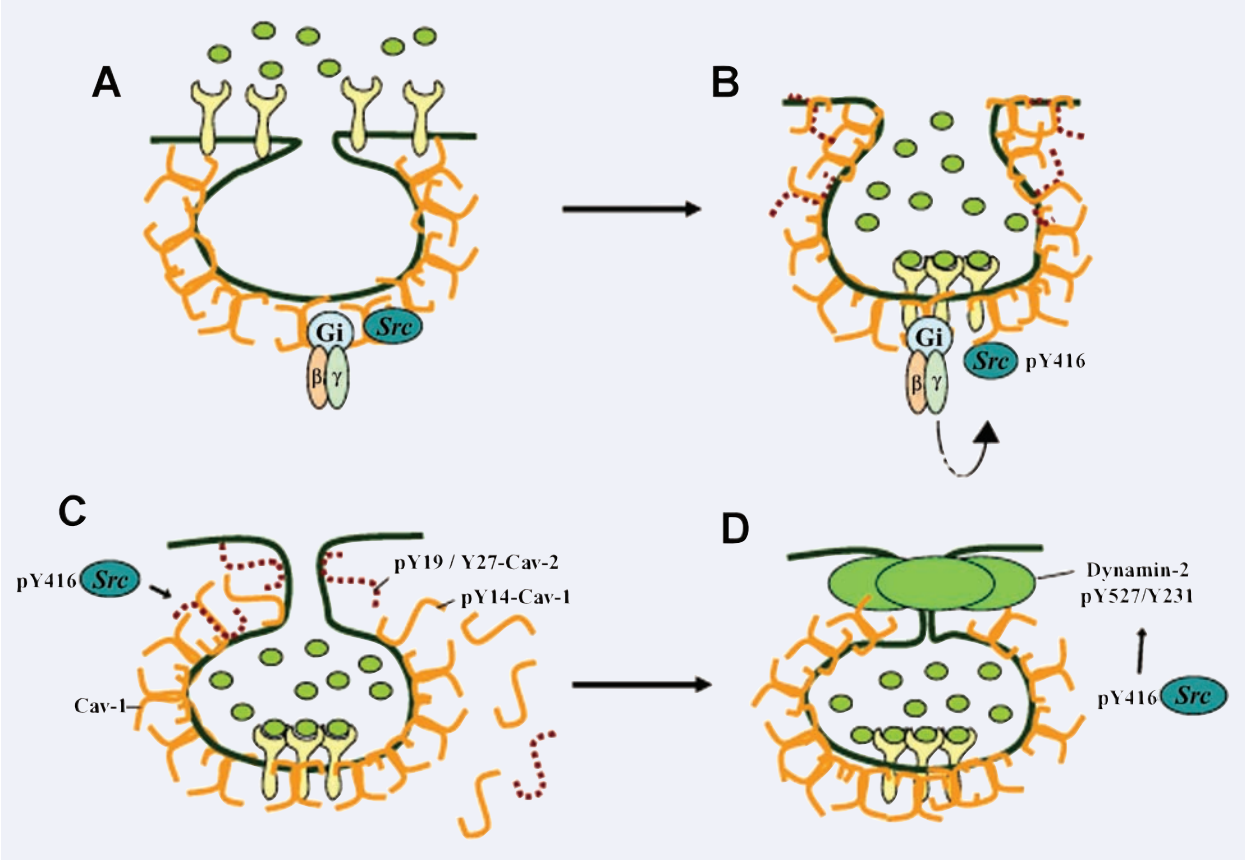

Fig. 2 Src-dependent signalling of caveolae-mediated endocytosis. Caveolae are the primary vesicular transporters or 'carriers' in endothelial cells. Clustering of various receptors, for example albumin binding protein gp60, initiates endocytosis *via* caveolae by associating with caveolin-1 and activating Src-family tyrosine kinase signalling (**A**, **B**). Caveolin-1 plays a central role as it serves a scaffolding function for components of the signalling machinery responsible for endocytosis and also stabilizes caveolae at the membrane. Gi/βγ -linked Src-family kinase activation (*via* autophosphorylation of Src Y416) (**B**) in turn phosphorylates tyrosine residues on caveolin-1 (Y14) and caveolin-2 (Y19, Y27) (**C**), which may destabilize membrane-associated hetero-oligomers (**C**), and the GTPase dynamin-2 (Y231, Y597) which is thought to 'pinch' caveolae from the plasma membrane (**D**). Src-dependent phosphorylation is hypothesized to be the trigger that activates caveolar fission by decreasing the rigid structure of the caveolar coat and activating dynamin pinchase function.

dynamin-2 represent early and essential steps in this cascade (Fig. 2). The phosphorylation state of these proteins may regulate their three-dimensional structure, activity, localization and set of binding partners. Therefore, the primary function of Src in caveolae-mediated endocytosis has been proposed to be the regulation and assembly of multi-protein complexes responsible for caveolae fission and internalization [10]. Treatment of bovine lung endothelial cells with pertussis toxin or a dominant negative Gαi construct encoding the carboxyl-terminal 11 amino acids of Gαi inhibited endocytosis of iodinated albumin and vesicle formation induced by gp60 cross-linking in endothelial cells, illustrating that caveolar internalization may be regulated by a Gi-linked pathway [10]. Also, both activation and inhibition of Gβγ had a profound effect on Src activation and caveolae-mediated endocytosis of cholera toxin subunit B and albumin [40], suggesting that heterotrimeric G-protein Gi is upstream of Src in the signalling cascade initiated by albumin which is then internalized and transported in caveolae.

When Src is activated, a number of proteins concentrated on the cytoplasmic surface of caveolae are

phosphorylated. Caveolin-1, perhaps the primary substrate of Src, is phosphorylated on tyrosine 14 [45] (Fig. 1). It is implied by several studies that phosphorylation of caveolin-1 is essential for caveolae-mediated endocytosis; the possible role phospho-caveolin-1 plays in this process will be discussed below. Caveolin-2, which forms hetero-oligomers with caveolin-1, represents another Src substrate on caveolae. Caveolin-2 does not appear to be essential for caveolae formation, since caveolin-2 knockout mice were reported to have normal non-muscle caveolae [49]. Additionally, in the absence of caveolin-1, caveolin-2 not only fails to form oligomers, but is also unable to incorporate into lipid rafts, emphasizing the role of caveolin-1 in proper function and localization of caveolin-2 *in vivo* [50]. However, caveolin-2 appears to be involved in the regulation of caveolae size since co-expression with caveolin-1 in insect cells lacking endogenous caveolins leads to the formation of smaller and more uniform vesicles than those formed by caveolin-1 alone [51]. Formation of free cytoplasmic caveolae (*i.e.*, vesicles that have detached from the plasma membrane) in HepG2 cells that do not express endogenous caveolins was dependent on co-expression of caveolin-1 and caveolin-2 [52]. Another group showed that caveolae formed by caveolin-1 alone lack the characteristic neck structure and were not connected to the plasma membrane [53], suggesting that expression of both caveolin-1 and caveolin-2 is necessary for the formation of functional caveolae. In the same study, the authors proposed that caveolin-2 phosphorylation on serines 23 and 36 regulates the attachment of caveolae to the plasma membrane [53]. Caveolin-2 also contains two tyrosines that can be phosphorylated by Src: tyrosine 19 and 27 [54, 55]. In NIH3T3 cells, caveolin-2 phosphorylated on tyrosine 19 localizes mainly to the cell borders, whereas caveolin-2 phosphorylated on tyrosine 27 was observed in small puncta throughout the membane and cytosol [55]. Despite different cellular localization, both phosphorylated forms of caveolin-2 dissociate from high molecular weight caveolin-1 oligomers but remain in lipid rafts [54, 55]. However, there are no data on the importance of caveolin-2 or its phosphorylated forms in caveolae internalization. It may be possible, by dissociating from caveolin-1 oligomers upon phosphorylation, that caveolin-2 participates in the regulated fission of caveolae. Also, since caveolin-2 is only 36% homologous to caveolin-1 [50], its tyrosine-phosphorylated forms can recruit signalling molecules distinct from those recruited by phosphorylated caveolin-1 [54].

Another target of Src shown to be critical to the regulation of caveolae-mediated endocytosis is the large molecular weight GTPase dynamin-2, which mediates fission of caveolae from the plasma membrane [40, 41, 56–58]. Src phosphorylation of dynamin at Tyr^{231} and Tyr^{597} increases its GTPase activity, assembly into oligomers [59, 60], and association with caveolin-1 at the plasma membrane [41, 61]. Dynamin-2 with mutated sites of Src phosphorylation, at tyrosine 231 and 597, fails to migrate to the membrane and also has a reduced ability to bind caveolin-1 [41]. Such binding, which was recently shown to be direct [63], is required for caveolae-mediated endocytosis of albumin and cholera toxin [41]. Interestingly, SV40-induced internalization of caveolae was also shown to be dependent on tyrosine kinase activity [36] and recruitment of dynamin to the membrane [37]. Dynamin-2 localizes to the neck of caveolae and mediates their release from the plasma membrane [56, 62], which is supported by studies that showed expression of the GTPase-defective dynamin mutant (K44A) or microinjection of antibodies against dynamin-2 prevented caveolae-dependent internal-ization of cholera toxin subunit B, albumin and glycosphingolipid [39, 41, 56].

Potential role of Src-mediated phosphorylation of caveolin-1 in caveolae-mediated endocytosis

Caveolin-1 is a well-known substrate of Src [31, 40, 64–68]. Src phosphorylation of caveolin-1 occurs within the extreme N-terminal region, between residues 6 and 26 which contains three tyrosine residues at positions 6, 14 and 25. Studies involving *in vitro* phosphorylation of caveolin-1-derived synthetic peptides and site-directed mutagenesis revealed that Tyr^{14} is the primary residue phosphorylated by Src [45]. Therefore, only caveolin-1α, which contains residues 1-178, can undergo tyrosine phosphorylation since caveolin-1β only contains residues 32–178 (Fig. 1).

The initial approach to understand the role of the caveolin-1 N-terminus included comparisons between α and β caveolin-1 isoforms. Although different by just 32 amino acids, caveolin-1 isoforms play non-redundant roles in zebrafish development

[69]. A recent study also detected an alternative promoter in the caveolin-1 gene, suggesting that caveolin-1 isoforms can be produced by mRNA splicing or perhaps transcribed from two distinct mRNA transcripts [70]. By tracking the level of both mRNA and protein in mouse lungs, it was shown that caveolin-1α is expressed earlier in development and predominates in endothelial cells, while caveolin-1β is expressed primarily in epithelial cells and at a later stage of development [70]. Within each cell, the distribution of caveolin-1α and -1β isoforms only partially overlaps. For example, it was shown that an α-isoform specific antibody stains micropatches throughout the cell, whereas a non-selective caveolin-1 antibody that recognizes both α and β caveolin-1 isoforms additionally stains cell borders [14]. Another study suggested that there are different populations of caveolae and that the ratio of caveolin-1α to caveolin-1β was greater in deeply invaginated caveolae [52]. These studies lead to the proposal that the α-isoform possesses a greater internalization potential than the β-isoform.

Different approaches have been used to study the functional role of caveolin-1α phosphorylation in caveolae-mediated endocytosis. Staining of normal rat tissues with an antibody specific to phospho-caveolin-1 (Y14) revealed that *in vivo*, caveolin-1 is phosphorylated in endothelial cells but not in pericytes, fibroblasts and other cells abundant in caveolin-1 [35]. These data correlate with the constant engagement of endothelial cells in the endocytosis and transcytosis of macromolecules. In cultured cells, vanadate, an inhibitor of protein tyrosine phosphatases, increases the level of caveolin-1 phosphorylation and also stimulates its translocation from peripheral membrane patches to intracellular vesicles [35, 65, 71], enhancing the internalization of albumin [40]. Interestingly, co-transfection of v-Src, which is constitutively active, induces both caveolae internalization and aggregation. Phosphorylation of additional tyrosines in caveolin-1 by v-Src was suggested as an explanation for the differences in response to vanadate *versus* v-Src [65]. Thus, the N-terminus of caveolin-1, where tyrosine 14 is located, seems to be critical for caveolae internalization. This point is strengthened by observations that in CV-1 cells expressing caveolin-1 with its N-terminus blocked by a green fluorescent protein (GFP)-tag, SV40 internalization was attenuated, while cells transfected with caveolin-1 tagged with GFP at the C-terminus showed normal SV40 endocytosis [43]. A recent study conducted in epithelial cells illustrated that the Src-dependent increase in the level of caveolin-1 phosphorylation upon epidermal growth factor (EGF) treatment corresponded to caveolae formation and internalization [72]. In the same study, the authors also provided evidence that caveolae formation in response to EGF was dependent on caveolin-1 phosphorylation since a phosphorylation-defective caveolin-1 mutant containing tyrosine 14 substituted with phenylalanine (Y14F) was not able to form visible caveolae. Moreover, we observed that expression of Y14F caveolin-1 mutant in endothelial cells significantly reduced endocytosis and transcytosis of albumin [23, 46] suggesting the caveolin-1 N-terminus plays a regulatory role and its phosphorylation by Src kinase may be crucial for caveolae formation and detachment from the membrane.

Caveolin-1 exists as high-order oligomers consisting of 14–16 monomers. Velocity gradient centrifugation results showed the existence of 350–400 kDa complexes of caveolin-1 *in vivo* [73, 74]. Residues 60–101 are suggested to be involved in caveolin–caveolin interactions and formation of homo-oligomers, and thus this region has been designated as the caveolin oligomerization domain [74, 75]. Caveolin-1 oligomerization is thought to facilitate the invagination and release of caveolae into the cytoplasm [76]. Therefore, *Src*-mediated phosphorylation may regulate the state of caveolin-1 oligomerization and subsequently control vesicle formation and fission from the plasma membrane. Another group has shown that caveolin-1 shifts to lighter fractions on a sucrose gradient upon EGF-stimulation in epithelial cells [72]. However, several studies showed that the ability of caveolin-1 to form oligomers incorporate into the lipid rafts, and bind caveolin-2 was not changed by either treatment with vanadate or v-Src co-transfection [54, 65, 77]. Since caveolin-1α and β isoforms co-oligomerize and it is possible that not all caveolin-1α in each oligomeric structure is phosphorylated, it is not yet clear whether caveolin-1 phosphorylation regulates oligomerization state or oligomer stability and thus requires further investigation.

Fernandez *et al.* [78] assessed the structure of caveolin-1 and its oligomers by studying the biophysical properties of a purified caveolin-1 fragment. This study showed that the short N-terminal sequence adjacent to the caveolin-1 membrane spanning domain forms an α-helix which is required for

oligomerization of caveolin [78]. It was also proposed that the rest of the N-terminus wraps around this α-helix, facilitating oligomer formation and stability. In this regard, it is possible that phosphorylation of caveolin-1 changes its tertiary structure and causes destabilization of high molecular weight oligomers [46]. Nevertheless, analysis of caveolin-1-GFP dynamics suggests that the caveolin-1 coating on vesicles do not disassemble and do not exchange with other vesicles during the shuttling of caveolae between the plasma membrane and recycling compartments [79, 80]. Perhaps phosphorylation-mediated destabilization of caveolin-1 oligomers does not result in the complete disassembly of high molecular weight complexes, but rather serves to accommodate an increase in membrane curvature upon formation, fission or closure of caveolae.

Changes in the three-dimensional structure of caveolin oligomers may also affect caveolin-1 binding partners. To date, only two molecules are known to interact with caveolin-1 in a phosphorylation-dependent manner: Csk [81] and adaptor protein Grb7 [77]. Csk is a known regulator of Src family kinases and its recruitment to phosphorylated caveolin-1 provides a negative feedback loop for turning off Src [82]. Grb7 participates in focal adhesion formation and is important for cell migration [77]. Several studies localized caveolin-1 phosphorylated on tyrosine 14 to focal adhesions [77, 83, 84]. del Pozo *et al.* suggested that phospho-caveolin-1 is sequestered in focal adhesions and it is redistributed to caveolae upon cell detachment, inducing internalization of lipid raft marker GM1 [84]. As discussed above, in adherent cells, caveolae do not exchange caveolin molecules [79] and activation of endocytosis leads to phosphoryla-tion of caveolin-1 in the caveolae coat structure. Thus, phospho-caveolin-1 located in focal adhesions and caveolin-1 phosphorylated in the course of endocytosis are likely to represent two different pools of phospho-caveolin-1 in adherent cells.

Role of actin cytoskeleton in caveolae-mediated endocytosis

The actin cytoskeleton is an essential requirement for endocytosis in several systems [85, 86]. Analysis of endothelial cells by electron microscopy revealed caveolae association with fine actin filaments [87]. This discovery encouraged several research groups to study the role of the actin cytoskeleton in caveolae internalization. Early studies showed that treatment of cells with Cytochalasin D, a drug that disassembles filamentous actin, blocked caveolae-mediated internalization of cholera toxin following treatment of cells with phosphatase inhibitor okadaic acid [34]. Mundy and co-workers illustrated that in Chinese hamster ovary (CHO) cells, cortical actin restricts and organizes caveolae at the plasma membrane since actin depolymerizing drug Latrunculin A induced an increase in lateral mobility of caveolin-1-GFP within the plasma membrane [88]. Other studies showed that the F-actin stabilizing drug Jasplakinolide also blocked caveolae internalization in CV-1 cells [37], implying that dynamic actin cytoskeletal re-modelling is crucial for caveolae-mediated endocytosis. Pelkmans and co-workers proposed a model in which SV40 entrapment in caveolae induces a tyrosine kinase phosphorylation cascade, local disassembly of cortical actin, formation of actin tails on the loaded caveolae and finally vesicle fission and movement into the cytoplasm [37, 89]. SV40 translocation to caveolae was found to be independent of actin or phosphorylation events, whereas virus internalization within caveolae required phosphorylation-mediated actin re-modelling. Furthermore, other kinases that are involved in actin cytoskeletal regulation, such as phosphotidyl inositol (4, 5) kinase, and those that activate the Rho-family small GTPase Cdc42, were shown to inhibit endocytosis by activation of actin polymerization [48]. These and other studies lead to the speculation that caveolae are in fact not involved in constitutive endocytosis, but instead represent a highly stable plasma membrane compartment anchored by the actin cytoskeleton [90] which are mobilized in a phosphorylation dependent manner.

At this point, it is unclear how the actin cytoskeleton connects or communicates with caveolae. Dynamin-2 is known to interact with caveolin-1 [41, 63] as well as actin-binding proteins intersectin and cortactin [91, 92]. Intersectin, a protein with multiple Eps15 homology (EH) and Src homology 3 (SH3) domains, interacts with dynamin at the neck of caveolae in endothelial cells where it is thought to regulate fission and internalization [91]. Another possibility is that actin filaments interact with caveolae through filamin A, an actin cross-linking protein that was shown to directly bind caveolin-1 *in vitro* [93]. Additionally, Csk that is recruited to phosphorylated caveolin-1 has the ability to regulate actin cytoskeletal

dynamics [77, 94, 95]. Src also participates in actin cytoskeletal re-modelling by regulating cortactin [96–98].Therefore, it is possible that Src controls interactions of actin with caveolin-1, thereby regulating caveolar detachment from the membrane and vesicle internalization [88, 98, 99]. Although it is now generally accepted that the actin cytoskeleton plays an important role in caveolae endocytosis, the mechanism of actin re-organization upon activation of caveolae endocytosis and it role in vesicle internalization remains largely unknown.

Conclusion

A large number of kinases are thought to be involved in the regulation of caveolae-mediated endocytosis, including those implicated in integrin and Ca^{2+} signalling as well as actin cytoskeletal regulation [48]. Among them are Src kinases, which are activated early during initiation of caveolae-mediated endocytosis. Activation of Src represents a general requirement for caveolae internalization as it was shown to regulate caveolae internalization in cells of different origin, such as fibroblasts, endothelial and epithelial cells. Src is known to phosphorylate caveolae-associated proteins caveolin-1, caveolin-2 and dynamin-2, regulating the assembly of multi-protein complexes involved in fission and internalization of caveolae. Src-mediated phoshorylation may also regulate the phosphorylation-dependent changes in the actin cytoskeleton that are necessary for caveolae release and migration through the cortical actin network. Ongoing studies by our group and others will hopefully provide greater insight into the mechanisms which regulate caveolae-mediated endocytosis, further defining the physiological importance of caveolae trafficking in health and disease.

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