JCB Mini-Review

Caveolae/raft-dependent endocytosis

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Although caveolae are well-characterized subdomains of glycolipid rafts, their distinctive morphology and association with caveolins has led to their internalization being considered different from that of rafts. In this review, we propose that caveolae and rafts are internalized via a common pathway, caveolae/raft-dependent endocytosis, defined by its clathrin independence, dynamin dependence, and sensitivity to cholesterol depletion. The regulatory role of caveolin-1 and ligand sorting in this complex endocytic pathway are specifically addressed.

Introduction

Clathrin-independent endocytosis includes the constitutive pinocytotic pathway as well as endocytosis mediated by caveolae and glycolipid rafts. Glycolipid rafts are detergent-insoluble, low-density membrane fractions that are rich in cholesterol and sphingolipids; caveolae are cholesterol- and sphingolipid-rich smooth invaginations of the plasma membrane that partition into raft fractions and whose expression is associated with caveolin-1. Caveolae are therefore a subdomain of the biochemically defined glycolipid raft (Anderson, 1998; Kurzchalia and Parton, 1999). The consequent sensitivity of endocytosis, via both caveolae and rafts, to nonacute cholesterol depletion with agents such as filipin, nystatin, or methyl-β-cyclodextrin distinguishes these pathways from both the clathrin-dependent and constitutive pinocytotic pathways. Caveolae and raft pathways mediate the internalization of sphingolipids and sphingolipid binding toxins (cholera toxin [CTX]* and shiga toxin), GPI-anchored proteins, the autocrine motility factor (AMF), endothelin, growth hormone, and IL2 receptors, viruses (including SV40), and bacteria (Nichols and Lippincott-Schwartz, 2001; Duncan et al., 2002; Johannes and Lamaze, 2002; Pelkmans and Helenius, 2002; Conner and Schmid, 2003).

The GTPase activity of dynamin is required for the budding of caveolae from purified endothelial plasma membranes (Oh et al., 1998). Microinjection of antidynamin antibodies, or expression of a dominant–negative dynamin K44A mutant (dynK44A) deficient in GTP hydrolysis, prevents the caveolae- and raft-mediated internalization of various molecules (Henley et al., 1998; Dessy et al., 2000; Lamaze et al., 2001; Puri et al., 2001; Le et al., 2002; Pelkmans et al., 2002; Le and Nabi, 2003). In contrast, a defining feature of the pinocytotic pathway is its insensitivity to dynamin inhibition (Damke et al., 1994; Llorente et al., 1998; Contamin et al., 2000; Sabharanjak et al., 2002). The caveolae- and raft-dependent pathways are therefore characterized by a common sensitivity to cholesterol depletion and inhibition of dynamin function.

Few to no caveolae are present in cells in which caveolin-1 expression levels are significantly reduced or absent, and the reintroduction of caveolin-1 into these cells induces the formation of caveolae at the plasma membrane (Fra et al., 1995; and others). A central dogma of the caveolae/glycolipid raft field, therefore, has been that the invaginated flask-shaped morphology of caveolae is a specific consequence of the association of caveolin-1 with select raft domains. However, even in the absence of caveolin, the internalization of rafts must invoke the invagination and budding of a vesicular structure that is cholesterol and sphingolipid rich and necessarily related to caveolae. The commonly accepted view that caveolae and rafts mediate distinct endocytic pathways ignores the extensive fundamental similarities between these two processes. In this review, we argue that caveolae and rafts mediate a common endocytic pathway, caveolae/raft-dependent endocytosis, defined by its clathrin independence, dynamin dependence, sensitivity to cholesterol depletion, and the morphology and lipid composition of the vesicular intermediate.

Endocytosis of caveolae/raft domains

The internalization of caveolae is facilitated by disruption of the actin cytoskeleton, inhibited by the kinase inhibitors staurosporine and genistein, and enhanced by the phosphatase inhibitors okadaic acid and vanadate (Parton et al., 1994; Pelkmans et al., 2001; Mundy et al., 2002; Nichols, 2002; Pelkmans et al., 2002; Thomsen et al., 2002). SV40 binding to the cell surface activates a tyrosine kinase–based signaling cascade, disrupting the local actin cytoskeleton, and recruiting dynamin II to the site of internalization where it is endocytosed together with caveolin-1-GFP (Pelkmans et al., 2002). Albumin binding to its receptor, gp60, triggers caveolae endocytosis via a Gi-coupled src kinase–mediated pathway (Minshall et al., 2000). Caveolin-1 interaction with gp60 is required for albumin uptake, and albumin uptake is inhibited in caveolin-1 null cells (Minshall

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*Abbreviations used in this paper: AMF, autocrine motility factor; CTX, cholera toxin; dynK44A, dynamin K44A mutant.

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et al., 2000; Razani et al., 2001). The internalization of some caveolar ligands is, therefore, a signal-mediated process that requires caveolin-1 expression.

However, internalization of other caveolae/raft ligands occurs independently of caveolin expression. For instance, AMF follows an essentially equivalent cholesterol- and dynamin-dependent pathway to the ER in caveolin-expressing NIH-3T3 cells as well as in transformed NIH-3T3 cells that express little caveolin and few cell surface caveolae (Benlimame et al., 1998; Le et al., 2002). Internalization of CTX via caveolae to the Golgi apparatus is cholesterol sensitive and occurs via a caveolin-1–positive endosomal intermediate (Parton et al., 1994; Henley et al., 1998; Nichols et al., 2001; Puri et al., 2001; Nichols, 2002; Wolf et al., 2002; Le and Nabi, 2003). At the same time, CTX is internalized via a filipin sensitive "raft" pathway in CaCo-2 cells and jurkat lymphoma cells that do not express caveolin-1 (Orlandi and Fishman, 1998). Furthermore, reduction of caveolin-1 expression in Cos-7 cells with RNAi does not prevent CTX delivery to the Golgi (Nichols, 2002). Due to the absence of caveolae in caveolin-deficient cells and of a defined vesicular intermediate for the raft pathway, the common nature of these two pathways has gained only limited acceptance.

Morphologically equivalent vesicles mediate endocytosis of caveolae and rafts

In lymphocytes that do not express caveolin, cross-linked GPI-anchored proteins cluster in smooth vesicles morphologically equivalent to caveolae before endocytosis (Deckert et al., 1996). Expression of dynK44A in abl-transformed NIH-3T3 cells that express little caveolin and few caveolae results in the expression of smooth invaginations that are morphologically indistinguishable from caveolae induced in the same cells by expression of caveolin-1 (Fig. 1). The dynK44A-induced smooth invaginations are derived from cholesterol-rich glycolipid raft domains as they are not present in cells treated with methyl-β-cyclodextrin (Le et al., 2002). The fact that these "caveolae" can only be visualized when budding is inhibited indicates that, upon invagination, they exhibit a limited residence time at the plasma membrane. Instability of invaginated rafts at the plasma membrane would necessarily limit morphological detection of these structures, especially in ultrathin electron microscopy sections that represent but a fraction of the total plasma membrane surface. The extent to which, in the absence of caveolin-1, invagination and budding are intrinsic properties of the raft domain remains to be determined. Ligand binding and antibody cross-linking may serve not only to recruit receptors to rafts but also contribute to the formation and internalization of these domains (Parton et al., 1994; Deckert et al., 1996; Verkade et al., 2000; Lamaze et al., 2001; Fivaz et al., 2002).

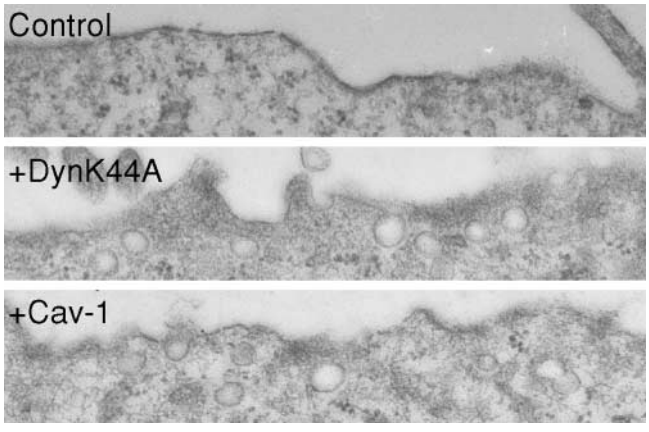

Figure 1. **Expression of dynaminK44A or caveolin-1 results in the formation of morphologically equivalent caveolar invaginations.** v-abl-transformed NIH-3T3 cells that exhibit minimal caveolin expression and few cell surface caveolae were infected with adenoviruses coding for either the dynamin K44A mutant (dynK44A) or caveolin-1 (cav-1), and the cells were then processed for electron microscopy. For details, see Le et al., 2002.

Although raft-derived smooth vesicles are technically "raft invaginations," use of this nomenclature implies that raft-derived smooth invaginations mediate different endocytic processes than do caveolae. We propose that the term "caveolar" be used as a morphological descriptor for endocytic raft-derived invaginations. Caveolar invaginations and caveolar vesicles therefore encompass both endocytosis-competent caveolin-positive caveolae and more transient caveolin-negative raft-derived morphological equivalents. This represents a nomenclature that reflects the similar morphology, lipid composition, and role in endocytosis of these domains. It nevertheless recognizes that stable cell surface–associated caveolae are a functionally distinct organelle whose expression and function are associated with caveolin-1 expression (Fig. 2 A). Caveolae are not observed in endothelial cells of caveolin-1 knock-out mice, although some smooth caveolae-like invaginations were reported (Drab et al., 2001; Razani et al., 2001; Zhao et al., 2002). The extent to which the caveolae/raft-dependent pathway functions in caveolin-1 null cells and whether expression of the dynK44A mutant induces the expression of caveolar invaginations in these cells represent critical experiments that remain to be performed.

Caveolin-1 stabilizes the cell surface expression of raft domains

FRAP studies have shown that caveolin-1 GFP is highly immobile at the plasma membrane and that only a minority of caveolin-1 GFP-positive vesicles actually internalize (Pelkmans et al., 2001; Mundy et al., 2002; Thomsen et al., 2002). Decreased expression of caveolin-1 in ras and abl-transformed NIH-3T3 cells is associated with the increased endocytosis of AMF to the ER; reintroduction of caveolin-1 specifically reduces the ER delivery of AMF identifying caveolin-1 as a negative regulator of caveolae/raft-dependent endocytosis (Le et al., 2002). Caveolin-1 therefore appears not to induce raft invagination but rather to stabilize the plasma membrane association of invaginated rafts retarding their dynamin-dependent budding and detachment (Fig. 2 A).

Association of caveolin-1 with raft domains may act as a lock that regulates their dynamic constitutive endocytosis and that can be opened by specific signaling events. If so, ligand internalization via caveolae/raft-dependent endocytosis may be signal mediated in cells expressing caveolin-1 but not in cells exhibiting minimal caveolin-1 expression levels. Caveolin may act by regulating the cholesterol content of raft domains (Roy et al., 1999), by retarding the dynamin-depen-

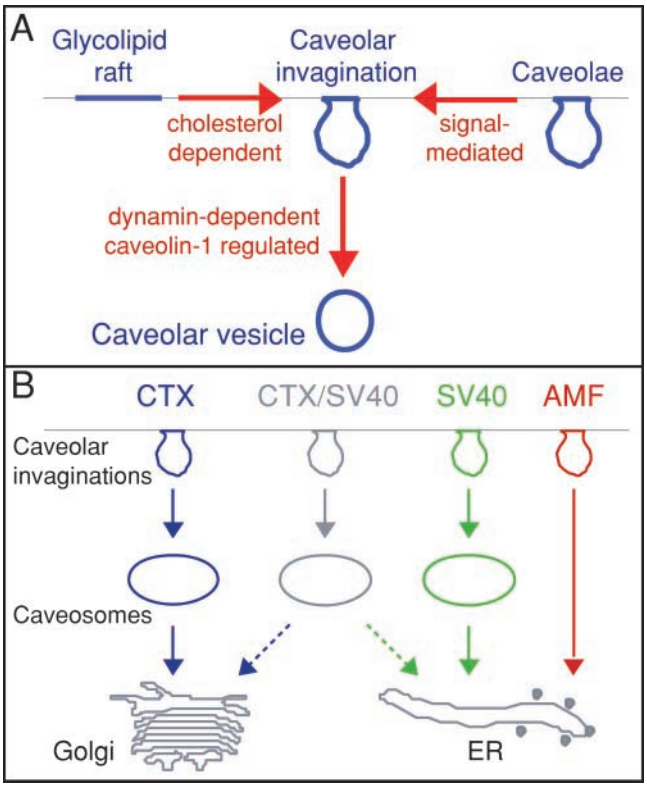

Figure 2. **Caveolae/raft-mediated endocytosis.** (A) The cholesterol-dependent invagination of glycolipid rafts occurs independently of caveolin-1 expression and results in the formation of caveolar invaginations that remain only transiently associated with the plasma membrane. Caveolin-1 is a negative regulator of the budding of caveolar invaginations, and caveolin-1–expressing stable cell surface caveolae can become endocytosis competent aftert specific signaling events. Caveolar invaginations bud in a dynamin-dependent manner from the plasma membrane to form caveolar vesicles. (B) COP-dependent pathways target CTX (blue) and SV40 (green) via the caveosome for delivery to the Golgi and ER, respectively, whereas AMF (red) is targeted via a distinct pathway that is apparently direct to the ER. CTX and SV40 could alternatively be targeted to a common caveosome (gray) and subsequently segregated for delivery to the Golgi and ER, respectively (dashed lines).

dent budding of caveolae (Henley et al., 1998; Oh et al., 1998; Le et al., 2002), or by sequestering signaling molecules, such as G proteins, required for caveolae/raft internalization (Minshall et al., 2000; Oh and Schnitzer, 2001). Increasing caveolin-1 association with individual raft domain levels may progressively reduce their endocytic potential. Alternatively, the requirement of a threshold level of cholesterol for caveolae invagination (Hailstones et al., 1998) suggests that a threshold level of caveolin-1 association with individual rafts may be required to stabilize the plasma membrane association of caveolae. The latter possibility envisions the existence within the same cell of dynamic, highly endocytic raft domains as well as more stable, plasma membrane-associated, less endocytic caveolae (van Deurs et al., 2003).

Sorting in caveolae/raft-dependent endocytosis

Caveolae and rafts represent highly heterogeneous populations of functionally distinct membrane domains (Maxfield, 2002). Multiple raft-associated proteins may therefore serve to segregate and define raft domains that exhibit differential endocytic capacities. The existence of multiple caveolin-1 binding partners (Liu et al., 2002) implicates caveolin-1 as a scaffolding molecule that determines the cargo for caveolae/raft-dependent endocytosis. Caveolae/raft ligands are also internalized by other endocytic pathways. For instance, cholera, shiga, and anthrax toxins bind to cell surface raft domains and yet are internalized via clathrin-dependent pathways (Sandvig et al., 1989; Shogomori and Futerman, 2001; Abrami et al., 2003). Raft association cannot therefore be considered a criterion in and of itself for internalization via the caveolae/raft-dependent pathway.

Two caveolar ligands, SV40 and CTX, are delivered to a caveolin-1–positive endocytic compartment or caveosome (Parton et al., 1994; Pelkmans et al., 2001; Nichols, 2002). The caveosome is distinguished from the early endosome by its neutral pH and by the expression of caveolin-1 (Pelkmans et al., 2001). Whether the caveosome is a fusion station for budding caveolar vesicles or rather a sorting site is not yet clear. Distinct caveolin-1–positive endocytic structures are labeled for SV40 and CTX (Nichols, 2002), suggesting that sorting of these two caveolar ligands for delivery to the Golgi and ER, respectively, may occur before delivery to distinct endosomal populations. Although SV40 has been reported to be targeted to the ER without traversing the Golgi (Kartenbeck et al., 1989), the intracellular targeting of both SV40 and CTX is COP-mediated (Norkin et al., 2002; Richards et al., 2002). CTX and SV40 could conceivably take the same retrograde route to the ER with significantly different resident times in the Golgi. Alternatively, sorting of these two ligands may occur either at the plasma membrane, targeting different caveosome populations, or via segregation within the caveosome, resulting in different intracellular targeting routes (Fig. 2 B).

Interestingly, although treatment with the microtubule-depolymerizing agent nocodazole, brefeldin A, or a 20°C temperature block disrupts the delivery of CTX and/or SV40 to the Golgi and ER, respectively (Pelkmans et al., 2001; Norkin et al., 2002; Richards et al., 2002), none of these treatments affects AMF delivery to the smooth ER. Furthermore, AMF and CTX internalized via caveolae/raft-dependent pathways do not localize to common intracellular compartments (Le and Nabi, 2003). Endocytic ligands can therefore be sorted at the plasma membrane to different caveolae/raft domains for internalization to distinct intracellular compartments, including an apparently direct route to the ER (Fig. 2 B). Segregation of caveolar endocytic cargo has also been demonstrated in endothelial cells in which albumin and insulin are localized to distinct caveolae populations (Bendayan and Rasio, 1996). The multiple mechanisms that exist to regulate recruitment to caveolae/raft domains may also serve to segregate and sort lipids and proteins to functionally distinct raft domains that follow varied intracellular targeting pathways.

Conclusion

Caveolae- and raft-mediated endocytosis therefore represent essentially equivalent clathrin-independent, dynamin-dependent, cholesterol-sensitive endocytic routes with similar ligand specificity and morphology of the vesicular interme-

diate. Caveolin-1 acts not as a determinant of caveolae invagination and internalization but rather as a regulator that stabilizes caveolae at the plasma membrane and reduces the endocytic potential of caveolae/raft domains. The existence of distinct endocytic routes for caveolae/raft-internalized ligands demonstrates that raft heterogeneity at the plasma membrane functionally segregates raft-associated proteins and lipids to generate distinct caveolar vesicles. Although caveolins are certainly implicated in this sorting event, other factors yet to be identified are necessarily determinants of the formation, endocytic potential, and intracellular targeting of raft domains.

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