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Review

Relationship between cholesterol trafficking and signaling in rafts and caveolae

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Abstract

Caveolae and lipid rafts are two distinct populations of free cholesterol, sphingolipid (FC/SPH)-rich cell surface microdomains. They differ in stability, shape, and the presence or absence of caveolin (present in caveolae) or GPI-anchored proteins (enriched in lipid rafts). In primary cells, caveolae and rafts support the assembly of different signaling complexes, though signal transduction from both is strongly dependent on the presence of FC. It was initially thought that FC promoted the formation of inactive reservoirs of signaling proteins. Recent data supports the concept of a more dynamic role for FC in caveolae and probably, also lipid rafts. It is more likely that the FC content of these domains is actively modulated as protein complexes are formed and, following signal transduction, disassembled. In transformed cell lines with few caveolae, little caveolin and a preponderance of rafts, complexes normally assembled on caveolae may function in rafts, albeit with altered kinetics. However, caveolae and lipid rafts appear not to be interconvertible. The presence of non-caveolar pools of caveolin in recycling endosomes (RE), the trans-Golgi network (TGN) and in mobile chaperone complexes is now recognized. A role in the uptake of microorganisms by cells ascribed to caveolae now seems more likely to be mediated by cell surface rafts.

Keywords: Lipid raft; Caveolae; Caveolin; Cholesterol; Signal transduction

1. Introduction

Cells receive information from their extracellular space in the form of signals transmitted via cell surface receptors. While most signals are protein-dependent, their transduction is strongly influenced by the local composition of lipids within the plasma membrane. These, mainly phospholipids (PL) and free cholesterol (FC), are present in a heterogeneous array of microdomains of different compositions. In artificial membranes made from lipid mixtures with a composition similar to that of cells, domains rich in FC and sphingolipids (SPH), resembling those associated with signaling intermediates in native membranes, separate as a liquid-ordered phase. In turn, these FC/SPH-rich microdomains are influenced by the presence of proteins.

Originally considered to form a single, detergent-insoluble glycolipid-enriched (DIG) membrane fraction, two major kinds of FC/SPH-containing microdomains are now recognized to coexist within the plasma membrane. In this review, lipid rafts are defined as planar domains enriched in glycosylphosphatidylinositol (GPI)-anchored proteins and deficient in caveolin. Caveolae are cell surface invaginations stabilized by structural proteins (caveolins) and deficient in GPI-anchored proteins. Though occasional planar cell surface domains containing caveolin, as well as caveolin-free flask-shaped invaginations have been reported, these appear to be rare structures concerning whose composition and further existence nothing is known. Several general reviews of lipid rafts and caveolae have recently appeared [1–4]. The present account focuses on the divergent roles of these microdomains in biology, and the interaction between their lipid and protein moieties in regulating signal transduction.

2. Structure and composition of rafts and caveolae

Recent studies, using fluorescence resonance techniques, suggest that rafts have a mean diameter between ~25 and several hundred microns [5,6]. It was suggested earlier that

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rafts moved within the exofacial leaflet of the membrane bilayer [7]. It now seems more likely that rafts also involve the cytofacial leaflet via organized (though not necessarily identical) lipids imposed by the association of rafts with transmembrane signaling proteins such as *rho-A*, *fyn* and the interleukin receptor IL2R-beta [8–10]. Rafts are also enriched in GPI-anchored proteins [11,12] (Table 1). As these do not traverse the membrane bilayer, they probably have little effect on the organization of the inner membrane leaflet. Rafts contain unusual amounts of ethanolamine plasmalogens and phosphatidylserine [13]. Ganglioside GM3 was concentrated in a raft fraction prepared from detergent-extracted cells [11].

Caveolae are cell surface invaginations, the diameter of whose opening at the cell surface is typically 60–80 nm. Caveolae contain more FC (relative to SPH) than rafts [11,13]. Ganglioside GM3 was reported to be undetectable in caveolae [11]. Caveolae were enriched in intact ganglioside GM1 in A431 cells as evidenced by their selective binding of cholera toxin B-subunit [14] but in cells in which caveolae are decreased or absent, GM1 was recovered in lipid rafts [15,16].

Caveolae contain the structural protein caveolin-1, present in caveolae in the form of high molecular weight oligomers. A second caveolin (caveolin-2) is usually also present. GPI-anchored proteins are largely or completely absent from caveolin-containing microdomains [11,12]. Caveolae co-purify with receptor kinases including the platelet-derived growth factor receptor (PDGF-R), the insulin receptor protein (IR), and with related linkers and signaling intermediates such as *shc* and *h-ras*. Protein kinase A, adenyl cyclase, and several isoforms of protein kinase C are recovered in purified caveolae fractions [17]. Each of these proteins can bind directly to caveolin via a 'scaffold' site containing the sequences $\phi xxxx\phi xx\phi$ or $\phi xx\phi xxxx\phi$, where ϕ is F, Y or W, and x is any amino acid. Caveola-associated proteins (such as scavenger receptor BI, SR-BI) involved in lipid homeostasis lack a formal caveolin-binding domain [18]. The association of these proteins with caveolae probably involves their lipid, rather than their protein moiety. It seems likely that this class of proteins would normally distribute freely between lipid rafts and caveolae, though experimental evidence for this is presently limited.

Caveolae are detectable to some extent at the surface of most cells though their density varies many-fold in different lines. Primary lymphocytes and blood monocytes were thought earlier to be devoid of caveolins; more sensitive techniques have identified low levels of these proteins in these cells [19,20]. Caveolin was not detected under baseline conditions in several cancer or transformed cell lines [21] but even here, caveolae in large numbers can be expressed under appropriate conditions, for example following the induction of drug resistance in MCF-7 human cancer-derived cells [22], or the transformation of monocyte-derived cell lines with phorbol esters [23]. In summary, probably all nucleated mammalian cells retain an inherent ability to form both caveolae and lipid rafts.

3. Stability of rafts and caveolae

Rafts are mobile within the cell surface, to an extent proportional to their FC content [5]. The association between FC and SPH is spontaneous in films made from sphingolipid and phospholipid mixtures [24], but may be modified by interactions with GPI-anchored and signaling proteins. Conversely, complex formation between signaling proteins at the cell surface may facilitate separation of the liquid-ordered phase [25]. Single-particle tracking of gold-linked thy-1 (a GPI-anchored protein) indicated its retention for 7–9 s within raft domains 260–330 nm in diameter [6]. A somewhat longer lifespan (1 min) was reported earlier using different techniques [5]. All recent studies are consistent in suggesting that lipid rafts have a relatively short lifespan.

In contrast to the ephemeral nature of caveolin-free lipid rafts [1,5,6], a recent investigation indicated that caveolae were stable and possibly semi-permanent membrane structures in epithelial cells. Their turnover under normal conditions was negligible compared to that of other differentiated surface domains, such as clathrin-coated pits [26]. The expression of caveolae is reduced in the presence of cyclodextrin, which depletes cell FC nonspecifically [27,28]. Hyperphosphorylation of caveolin (induced by the protein phosphatase inhibitor okadaic acid) [29] and dissociation of microtubules with nocodazole [30] also reduced the numbers of cell surface caveolae. Though the level of caveolae at the cell surface is responsive to metabolic inhibitors, this finding need not imply that caveolae recycle spontaneously between cell surface and intracellular pools under physiological conditions, and there is little evidence to date that they do.

In some cell lines—for example, Fisher rat thyroid (FRT) cells—overexpression of caveolin is associated with an increase in the numbers of cell surface invaginations [31]. Morphologically, these appear to be similar or identical to normal caveolae, though a quantitative comparison of their effectiveness in signal transduction and lipid homeostasis has not been undertaken. In other cells—for example, human prostate carcinoma (NcaP) cells—overexpression of caveolin leads to the formation, not of surface pits, but of caveolin bound to intracellular lipid vesicles [31]. A similar result was obtained in cells transfected with a dominant-negative mutant caveolin [32]. The presence of several additional kinds of intracellular complexes containing caveolin has been described. Caveolin co-purifies with markers of the trans-Golgi network (TGN) in fibroblasts as part of a recycling pathway for internalized preformed lipoprotein FC [33]. Caveolin was also associated with transferrin receptors in recycling endosomes (RE), mildly acidified vesicles which bypass the lysosomal pathway to

return undegraded proteins to the cell surface [34]. Chaperone complexes of caveolin, heat shock proteins, and cyclophyllins transferring FC to caveolae from intracellular lipid pools have also been described [35]. These data emphasize that caveolin is present in multiple pools in the cell (Fig. 1) and that the presence of caveolin within a vesicle need not imply its origin from caveolae, or the expression of functional caveolae at the cell surface.

Despite lipid compositional similarities between caveolae and lipid rafts, there is little evidence that the two major classes of FC/SPH-rich microdomains are interconvertible. Fusion of chaperone complexes or transport vesicles with preexisting caveolae is probably responsible for replacing lipid (mainly FC) lost via efflux from the cell, or by leakage to other membrane domains. Present evidence strongly suggests that caveolae and lipid rafts are distinct structures, which normally play different roles in cell biology. Nevertheless, there is recent evidence that rafts and caveolae can partially substitute for each others' signaling functions, although with modified kinetics, if one class is experimentally deleted. When caveolin is completely absent from cells, signaling complexes which are usually associated with caveolae may bind, though less effectively, to cell surface

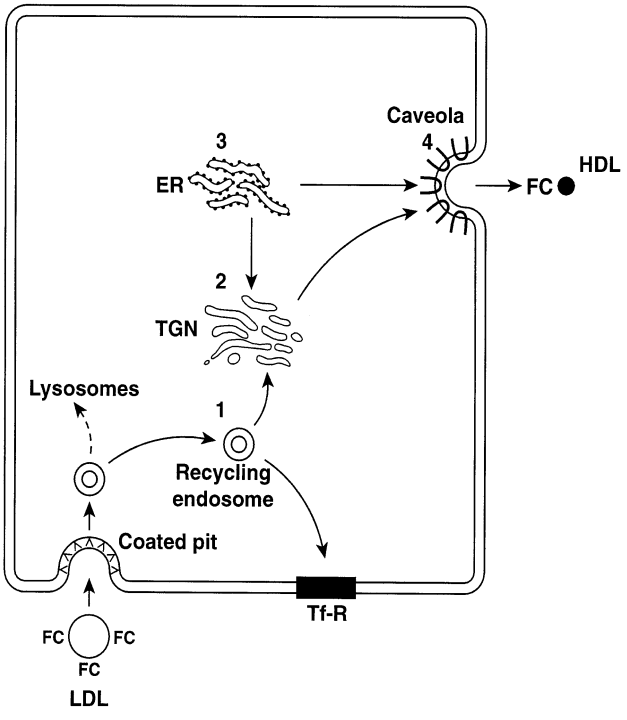

Fig. 1. The identity and possible relationships of intracellular and plasma membrane caveolin pools. The continuous arrows show intracellular FC transfers. The lysosomal pathway (dashed arrow) is highly downregulated in the presence of normal (lipoprotein-containing) media. The numbered sites (1–3) represent membrane compartments where caveolin has been detected (in addition to caveolae, 4) and from which FC–caveolin complexes may be transported. TGN (Ref. [33]); RE (Ref. [34]); ER (Ref. [35]). Also shown in the figure are coated pits (CP). Transferrin receptors (TfR), which are recycled non-caveolar cell surface domains from recycling endosomes.

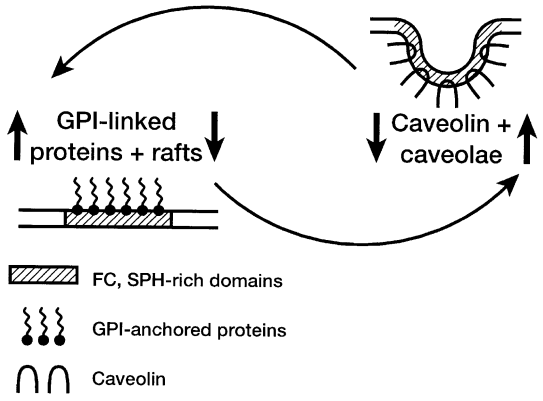

Fig. 2. Crosstalk between caveolae and lipid rafts. Reciprocal changes in the numbers of caveolae and GPI-anchored proteins following the selective deletion of one or other structure are illustrated. Cells deficient in GPI-anchored protein production were obtained following selection for aerolysin-resistance; cells overexpressing caveolin were selected following transfection with caveolin-1 cDNA [12]. Loss of caveolae was accompanied by upregulation of the expression of GPI-anchored proteins within rafts, while deletion of GPI-anchored proteins was associated with an upregulation of caveolae.

rafts [31]. The IR protein whose primary sequence includes a caveolin-binding scaffold sequence, and which is located in caveolae in normal adipocytes, partitioned into lipid rafts in caveola-free liver-derived cells [36]. Endothelial nitric oxide synthase (eNOS), much of which normally functions within caveolae, associated with lipid rafts in the absence of caveolae [31]. In CHO cells, where both species of FC/SPH-rich domain coexist, crosstalk between lipid rafts and caveolae was identified by depleting them of either GPI-anchored proteins or caveolin [12]. In the former case, caveolin and the expression of caveolae increased and overexpression of caveolin decreased the synthesis of GPI-anchored proteins. When caveolin was absent, the synthesis of GPI-anchored proteins, and their incorporation into rafts, was stimulated (Fig. 2).

4. Functions of rafts and caveolae

Caveolae function in the transduction of cell surface signals, and in the regulation of cholesterol homeostasis. These activities now appear to be closely interrelated [17,18]. The former depends on the capacity of caveolin to organize the complexes of receptor kinases, adapters and small GTP-binding proteins which initiate signal transduction from the cell surface. The latter depends on access to extracellular FC acceptors, particularly high-density lipoprotein (HDL). Interrelationship between the lipid- and protein-dependent activities of caveolae is suggested by the following observations. The effectiveness of signal transduction from caveolae is sensitive to their FC content [18]. The rate of cholesterol transfer into and out of caveolae responds to the phosphorylation of signaling proteins during signal transduction [37].

The composition of signaling complexes associated with rafts differs from those in caveolae. In part this relates to the enrichment in caveolae of proteins with a caveolin-binding domain, and the absence of these proteins from caveolin-free lipid rafts [17]. It also reflects the use of leukocytes, which lack caveolae, for isolation of rafts. In contrast, signaling proteins in caveolae have usually been characterized from caveola-rich cells such as endothelial and smooth muscle cells, adipocytes and fibroblasts.

FC-depletion, induced by the presence of cyclodextrin, led to the dissociation of signaling proteins from lipid rafts [38] and from caveolae [39]. Probably as a result of their brief lifetime, no data has yet been obtained to show if lipid rafts can serve as selective donors for FC efflux to HDL in the manner described for caveolae [18].

Both caveolae and lipid rafts have also been implicated in the uptake of viruses and bacteria into cells. Such a pathway would permit the microorganism to bypass lysosomal degradation. Echovirus, HIV, SV40, polyoma and Ebola and Marburg viruses are among those reported to be internalized via FC/SPH-rich microdomains [40–44] (Fig. 3). *Chlamydia*, *Brucella* and *E. coli* are among the bacteria reported to be taken up via these pathways [45–47], though these microorganisms are much larger in size than caveolae. It has been suggested that such targeting might involve the co-opting of a signal transduction pathway as a means of inducing uptake by the host cell, but the identity of the mechanism involved has not been identified in most cases.

The experimental criteria used in different studies to establish caveola- or raft-dependent uptake of microorganisms include an association of bacterial endocytic vesicles with caveolin during infection; colocalization of microorganisms with GPI-anchored proteins; and inhibition of infection by reactions that sequester cholesterol, such as filipin and cyclodextrins. Several of these criteria apply to both caveolae and rafts. Also, caveolin is a component of several intracellular vesicle populations in addition to caveolae [33,34]. As a result, it has been difficult to definitively determine if a given microorganism makes its primary entrance into the host cell via a caveola or a lipid raft. The situation is made more complicated by the observation that the same organism may infect simultaneously via several different endocytic pathways [47]. In cases where a dependence of infection on GPI-anchored proteins was established [48], the data shown in Table 1 suggest that rafts are more likely to have been involved. This conclusion was recently strengthened by the demonstration that activated integrins, which are localized to rafts [49] facilitate the attachment and uptake of Echo and Hanta viruses by mammalian cells [50]. However, further research is still needed to fully characterize the molecular association of bacteria and viruses with FC/SPH-rich domains.

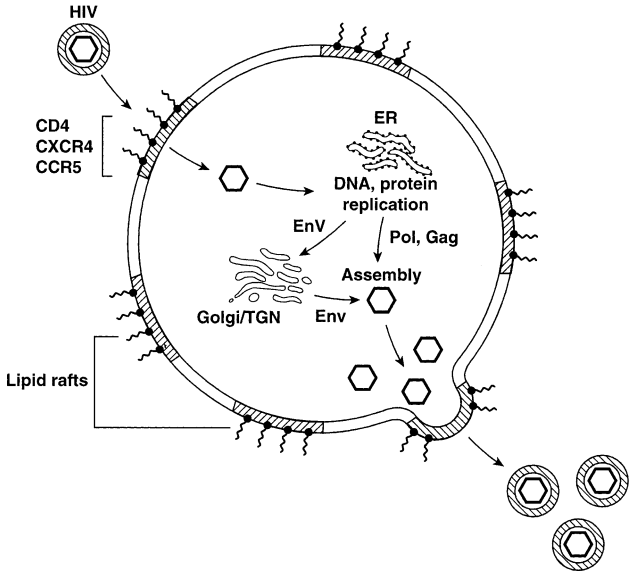

Fig. 3. Internalization, maturation and shedding of HIV virus—relationship to lipid rafts. Infection involves recognition of lipid rafts by viral *Nef* protein [95]. Following replication and assembly within the TGN and cytoplasm, newly synthesized virus appear to be targeted by the same protein to preexisting or newly assembled rafts for shedding. Specific interaction between *Nef* and host GPI-anchored protein *Lck* has been identified [96].

Table 1
Properties of lipid rafts and caveolae

	Lipid rafts	Caveolae
Major lipids	FC, SPH	FC, SPH
Structure	planar	invaginated
Major proteins	GPI-anchored proteins	caveolins
Major signal binding motif	acylation	caveolin-binding 'scaffold' sequence[a]
Half-life	short (min)	long (hours)
FC-dependent	yes	yes
FC dynamically regulated	?	yes

[a] While acylation appears to be fundamentally more important in binding signaling proteins to rafts than to caveolae [94], eNOS is both caveolin-targeted and functionally acylated [87] while EGF-R, with a caveolin-binding sequence, can distribute between lipid rafts and caveolae [16,91].

Lipid rafts (though not caveolae) have also been implicated in viral shedding from the cell surface [51,52]. Many of the same microorganisms which enter cells via caveolae or lipid rafts are enveloped by a host-derived FC/SPH-rich membrane when shed from mammalian cells. Conceivably, the mature bacterium or virus may be targeted for budding to preexisting rafts in the plasma membrane of the host cell. Alternatively, FC and SPH might be recruited from intracellular pools such as the TGN. Finally, targeting of the microorganism to the cell surface could induce raft formation by recruitment of FC and SPH from other membrane microdomains. These alternatives have yet to be distinguished.

Before caveolae and lipid rafts could be distinguished experimentally, the former had also been broadly implicated in protein transport to and from the cell surface [53–55]. Since ganglioside GM-1, used as a marker for caveolae, can

distribute between both classes of microdomains, the role for GPI-anchored proteins in apical targeting suggests the major role is played by lipid rafts.

5. Caveolae in endothelial cells

While caveolae in endothelial cells possess many of the same properties as those in other cells, in some respects, they appear unusual. The number of caveolae at the endothelial surface is high, relative to that in most other cells. Comparison of the properties of normal and caveola-deficient endothelial cells suggests that endothelial caveolae, in contrast to other cell lines, can generate endocytic vesicles at a rate which allows them to contribute significantly to the transcytosis of albumin and potentially, other plasma proteins [56]. This may reflect the same properties that promote GTP-dependent 'budding' of caveolin-rich vesicles via the activity of dynamin in isolated endothelial cell membranes [57]. The GTPase inhibitor GTPγS was without effect on membranes from fibroblasts (unpublished data). Electron microscopic and biochemical data of endothelial caveolae indicated that the caveolar 'bowl' in endothelial cells was contiguous with a 'rim' of GPI-anchored proteins [58,59]. In contrast, data concerning caveolae and rafts in other cell types has suggested a lack of functional association between microdomains containing caveolin and those containing GPI-anchored proteins (Table 1). If endothelial caveolae are more labile that those in most other cells (and a direct kinetic study has not yet been carried out) the molecular basis for this difference might lie in the degree of oligomerization of caveolin; the presence or absence of specific dynamins; or possibly the presence or organization of ancillary proteins.

6. Cholesterol trafficking in lipid rafts and caveolae

At least in caveola-rich primary cells such as fibroblasts and smooth muscle cells, these microdomains are the preferential cell surface endpoint for the trafficking of both newly synthesized FC, and the preformed FC which recycles from plasma lipoproteins, mainly low-density lipoprotein (LDL) through the cell [60,61]. At equilibrium FC efflux (typically $1.0-1.5 \times 10^2$ nmol min^{-1} μg^{-1} cell FC for fibroblastic cells) is the sum of synthesis de novo (1–2 nmol min^{-1} μg^{-1} cell FC) and the influx of preformed FC [62]. Thus, the greatest part of FC which exits from caveolae is derived from the selective uptake and recycling of preformed FC from other plasma lipoproteins, particularly LDL. This pathway may involve FC, caveolin-rich recycling endosomes [33,34].

Peripheral cells in vivo are surrounded by interstitial fluid rich in lipoprotein acceptors of FC, particularly HDL [63]. In the presence of plasma lipoproteins, caveolar FC was rapidly transferred out of the cell, with a $t_{1/2}$ of <5 min

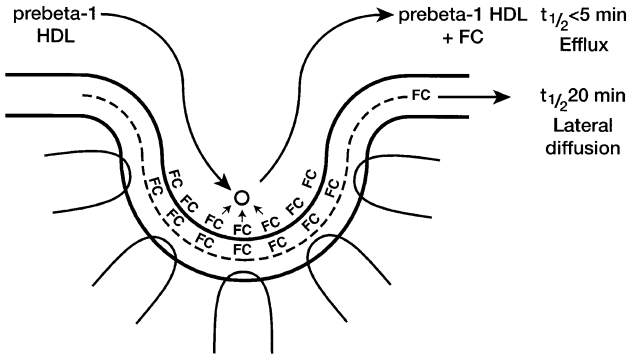

Fig. 4. Spatial and temporal vectors in the transfer of FC from caveolae. The figure compared the kinetics of the movement of FC from caveolae by lateral diffusion or efflux. Transfer of FC through the plane of the plasma membrane to adjacent microdomains, the only pathway available in the absence of an extracellular FC receptor, probably reflects simple diffusion [60]. Loss of FC from caveolae to the extracellular space in the presence of lipid-poor (prebeta-migrating) HDL [61] probably reflects a facilitated transport pathway involving the binding of HDL to the cell surface [97].

[59]. In the absence of an external, FC-binding acceptor, FC was lost from caveolae to non-caveolar membrane domains, by a slower pathway ($t_{1/2}$ 20 min) [61] (Fig. 4).

In cells such as fibroblasts and vascular smooth muscle cells, the expression of caveolin levels rapidly responds to changes in cellular FC content. When FC is increased, caveolin transcription is stimulated by a SREBP-1-dependent mechanism, the number of cell surface caveolae increased, and FC efflux is stimulated [64]. The opposite reaction took place when cell sterol was reduced by loss of FC [65]. In endothelial cells, where the expression of caveolin and caveolae is high under baseline conditions, FC uptake is accompanied not by additional expression of caveolae, but by incorporation of additional FC into preexisting caveolae [66].

There is an almost complete dearth of experimental data on whether FC levels in lipid rafts can be regulated independently of the composition of the bulk phase of membrane lipids. Additionally, it is not known if FC can be transferred directly to preformed rafts via intracellular transport complexes; and if so, whether these are the same complexes that supply FC to caveolae. The transient existence of rafts makes it more likely that their FC content reflects mainly the local lipid composition of the membrane.

7. Signal transduction from lipid rafts and caveolae

Before caveolae and rafts could be experimentally distinguished, the detergent-insoluble fraction of cell membranes was known to be enriched in proteins implicated in the early steps of transmembrane signaling [67]. Depletion of FC from the plasma membrane was carried out with cyclodextrin, a FC-binding oligosaccharide which (though not specific for FC/SPH-rich domains) [68], removed membrane FC, releasing signaling proteins from caveolae and

activating several signal transduction pathways [38,69–73]. In contrast, when plasma levels of FC were increased by preincubation of endothelial cells with FC, association of eNOS with caveolae was increased, and signaling activity or enzyme activity decreased [74]. IR and *h-ras* binding to caveolae were also increased by FC, and decreased by FC depletion [75]. However, the effects of FC depletion in this case were dependent on IR kinase substrate [76]. Together, these data suggest that the effects of FC on caveola-mediated signaling are more complex than originally thought, and involve both positive and negative effects at different points during the signaling reaction.

In lymphocytes and in some transformed cells, lipid rafts are present at high densities while appreciable numbers of caveolae are absent. The rafts are enriched in signaling proteins. A well studied example is the association in T cells of immunoglobulin receptors with the dually acylated *src* family kinase *lyn* [77]. Mutation of its palmitoylation site reduces the association of *lyn* with rafts, and increases the phosphorylation of upstream and downstream intermediates, and intracellular Ca^{++} levels [78]. Mutation of both palmitoyl and myristoyl sites rendered *lyn* incapable of membrane binding and thus inactive in membrane transduction. Signaling via the T cell receptor complex and in particular, the role of *Lck* in initiating signal transduction, was strongly dependent upon the integrity of lipid rafts [79]. Lipid depletion dissociates the complex and inhibits signal transduction. Similarly, IL-2 signaling in human lymphoma cells mediating the phosphorylation of STAT proteins was dependent upon association of the IL-2 and IL-6 receptors with lipid rafts at the cell surface [15,80]. Finally, *fas*-mediated apoptosis of lymphocytes, and the co-proteins FADD and caspase-8, depend on *fas* incorporation into FC/SPH-rich lipid rafts [81]. Disruption of rafts following FC depletion was associated with the inhibition of apoptosis.

One possible explanation of the marked heterogeneity of response which characterizes the relationship between FC and signal transduction from the cell surface would be that protein complexes differ in the affinity of their 'functional' and 'storage' pools for FC/SPH-rich domains. According to this hypothesis, signal transduction would be stimulated by FC in pathways whose reactive intermediates were selectively bound to caveolae and/or rafts; but the process would be inhibited by FC in pathways activated by FC depletion, for example by cyclodextrin. There are several difficulties with this. First, it could hardly explain how investigators using different assays could obtain opposite results for the effect of FC on the same pathway. For example, *h-ras* binding to caveolin is induced by FC-activated *ras*-dependent signaling in endothelial cells. In contrast, a stimulation of *ras*-dependent signaling was reported in FC-depleted Rat-1 fibroblasts [65,72]. A convincing case for membrane microdomains as promoters of the assembly of protein complexes by mass action effects based on the high local concentrations induced was also recently made [82]. It is hard to envisage conditions in which the dilution of aggregates from microdomains promoted their association. In fact, calculations based on the size of rafts and caveolae suggest concentrations of signaling intermediates could be locally increased 10^3-fold over those in cytoplasm. These considerations suggest that both the 'stimulatory' and 'inhibitory' roles of rafts and caveolae could represent dynamic phases of the same reaction cycle, in which signaling proteins, first assembled in FC-rich caveolae or rafts, were phosphorylated then expelled from these microdomains as the result of a coordinated loss of FC (Fig. 5). If this were the case, whether the effect of FC was positive or negative would depend on the reaction step assayed. Gustavsson et al. [75] demonstrated that FC stimulated the association of *h-ras* with caveolae. Furuchi et al. [39] measured the stimulation by FC depletion of PDGF-mediated *ras* activity by assaying the subsequent phosphorylation of ERK. Kranenburg et al. [71] reported that FC negatively regulated the response to epidermal growth factor (EGF).

The broad conclusion that caveolin-binding proteins signal from caveolae, while others signal from rafts, is compli-

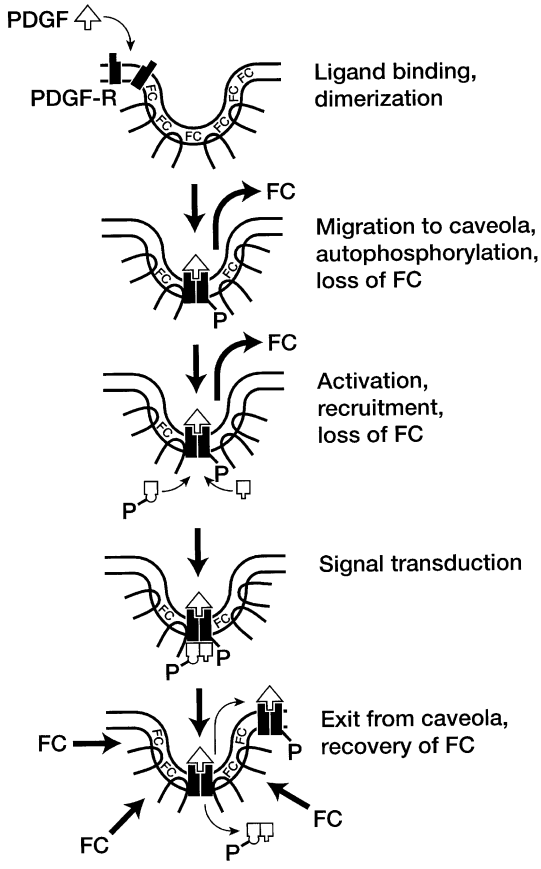

Fig. 5. Steps in PDGF-mediated signal transduction and the role of caveolae. The figure illustrates dimerization and ligand binding of PDGF-R, followed by its autophosphorylation, and signal transduction to signaling intermediate proteins. Following its subsequent exclusion from the caveola, the PDGF/PDGF-R complex is internalized via coated pits, and degraded lysosomally [86]. The exit of FC during signal transduction, and its recovery from internal pools, is also illustrated.

cated by the recent observation that the EGF receptor protein (EGF-R), which includes a caveolin-binding 'scaffold' sequence, can also signal from rafts. In caveola-poor HeLa cells, EGF-R under basal conditions is localized mainly to non-caveolar lipid rafts [16]. Cholesterol-depletion in these cells stimulates EGF binding, by increasing the availability of EGF-R. Cholesterol loading decreases EGF-mediated signal transduction. In contrast, in caveola-rich vascular smooth muscle cells, EGF-R was recovered following immunoprecipitation with caveolin antibody [71]. Angiotensin II induced the transactivation of EGF-R by promoting the insertion of angiotensin receptors into caveolae and activation of the protein kinase B signaling pathway. After phosphorylation of EGF-R, the receptor protein dissociated from caveolae. Activation of signaling by EGF under these conditions was inhibited following cyclodextrin-mediated depletion of FC. These data indicate that even the presence of a caveolin-binding domain is not always sufficient to target a signaling protein exclusively to caveolae.

8. Dynamic aspects of FC trafficking and signal transduction

The examples described above indicate that changes in membrane FC levels had significant but variable effects on signaling from caveolae and lipid rafts. Until recently, it was unclear if these were mainly pharmacological effects, or if systematic changes in FC levels in these domains were an integral part of effective signal transduction. PDGF-mediated signaling was among those first demonstrated to originate from caveolae [73,83,84]. Following ligand binding, PDGF-R is phosphorylated and signal transduction initiated, while PDGF-R moves out of FC-rich caveolae [85] for internalization and degradation via the lysosomal pathway [86]. However, the direct role, if any, of caveolar FC content in these changes was unclear.

Some insight into this question was recently obtained by simultaneously measuring FC homeostasis and PDGF-dependent protein kinase activities in the caveolae of vascular smooth muscle cells [37]. Within 5 min of the addition of PDGF, there was a substantial reduction of caveolar FC content associated with a transient compensatory 3–4-fold increase in FC efflux to the extracellular lipid acceptor apo A-1, the major protein of HDL. Under these conditions, caveolar FC decreased by 60–80% (Fig. 6). This decrease was confirmed using a labeled photoactivable analog of FC, followed by analysis of caveolin-bound radioactivity. In the absence of apo A-1, PDGF was without effect on FC efflux. Caveolar FC was reduced, but significantly less. In the presence of apo A-1 (and accelerated reduction of caveolar FC) protein kinase activity was increased 2–3-fold.

These data suggest an intimate relationship between FC homeostasis and signal transduction. PDGF-mediated phosphorylation stimulated FC efflux, which in turn amplified

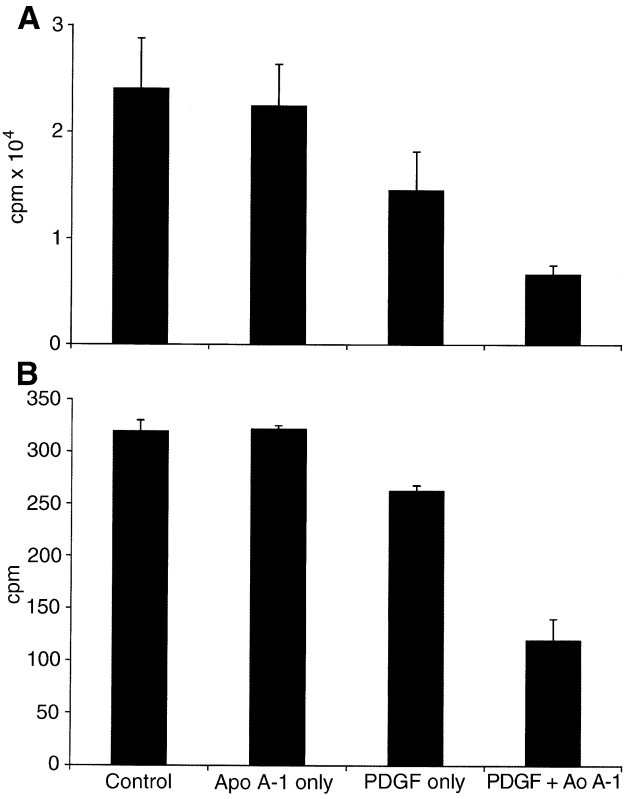

Fig. 6. PDGF/caveola interactions. Regulation of caveolar FC content during signal transduction: caveolar FC unloading stimulated by PDGF-dependent protein kinase activity. Panel A, caveolin-associated FC in ^{3}H-FC labeled vascular smooth muscle cells in the presence or absence of the HDL protein apo A-1 (10 μg ml^{-1}) and PDGF (100 ng ml^{-1}) . Panel B, the same protocol from cells equilibrated with the photoactivable FC analog, FCBP. The data illustrates the major decrease in caveolar FC content which is mediated by PDGF and PDGF-R (from Ref. [37]).

signal transduction, and accelerated the dissociation of the signal complex from the caveola. This reaction was not accompanied by any decrease in cell surface caveolin, suggesting that equilibrium was rapidly restored. Since PDGF-R is not recycled to a significant extent [86] and caveolae are stable [26], it seems likely that the lipid-depleted caveolae are recharged with FC and PGF-R from other cell surface microdomains or intracellular pools.

It is not yet clear if ligand binding to and phosphorylation of other receptor kinases is accompanied by similar induced changes in caveolar FC. eNOS localization to caveolae is dynamically modified by acylation [87], to regulate the activation of the muscarinic cholinergic receptor [88]. The association of eNOS with caveolae is highly FC-sensitive [89,90]. If the activation of eNOS is linked to transient changes in caveolar FC, this could represent an additional example of a self-regulatory cycle within caveolae involving FC. In Swiss 3T3 cells EGF-R, in non-caveolar rafts prior to addition of EGF, was translocated to caveolae in the presence of ligand in a reaction dependent upon caveolin-1 and inhibited by cyclodextrin, before being displaced as signal transduction progressed [73]. If cyclodextrin is acting

here mainly to reduce caveolar FC, this data suggests that the reaction sequence for EGF may be similar to that described for PDGF [37]. In A431 and Hep-2 carcinoma cell lines, EGF-R was absent from both rafts and caveolae in baseline cells, but transferred to rafts (not caveolae) in the presence of EGF [91]. These data contrast with the caveolar location of EGF receptors in vascular smooth muscle cells [16]. One factor determining the distribution of a given receptor kinase between caveolae and lipid rafts may be the relative levels of each in different cells, with primary cells being generally higher, and transformed cells generally lower, in the expression of caveolae.

9. Comparison of the functions of rafts and caveolae

We suggest that caveolae represent a differentiated, evolved species of FC/SPH-rich domains. These structures are stabilized by caveolin, optimized for signaling via receptor kinases by caveolin 'scaffold' binding, and specialized for FC efflux to the small, prebeta fraction of HDL. The development of an invaginated structure may impede the access of larger lipoprotein particles. In combination, these effects magnify signal transduction from the surface of caveola-rich cells. Caveolae, downregulated during mitosis [92] are more developed in a number of highly differentiated cells. Rafts are the major FC/SPH-rich domain of transformed cells and circulating leukocytes.

10. Further research

More information is needed in several areas covered by this review. If loss of caveolar FC is linked to caveolin phosphorylation, the kinase involved remains unidentified. The stability of caveolae [26] suggests that caveolin phosphorylation is reversible. At least one phosphatase colocalizes with caveolae [93], but its specificity has not been determined. Finally, the relationship between FC binding and phosphorylation in caveolin remains undefined. Continued research will thus be required to further establish the molecular basis of the dynamic relationship between signaling and FC outlined in this review.

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