INVITED REVIEW ARTICLE

CELL BIOLOGY OF CAVEOLAE
AND ITS IMPLICATION FOR CLINICAL MEDICINE

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ABSTRACT

Caveolae are small invaginations of the plasma membrane found in many cell types, and caveolins are integral membrane proteins that form the framework of caveolae. In the past several years, research on caveolae has developed explosively, and caveolae and/or caveolins have been shown to play many important roles in cell physiology: in particular, they are thought to be related to signal transduction, cholesterol transport, endocytosis and tumor suppression. On the other hand, some studies have suggested that another membrane domain called rafts is also involved in the same processes, and some confusion remains concerning the relationship between these two domains. Abnormalities in caveolae and/or caveolins have been found in various diseases, including cancer, atherosclerosis, muscular dystrophy and the Alzheimer's disease, which may make this domain a new focus for pharmacological research. This review will focus on the cell biology of caveolae, caveolins and rafts, and then summarize the implications of these findings for clinical studies.

Key Words: caveolae, caveolin, raft, signal transduction, cholesterol

Caveolae are uncoated small invaginations of the cell surface which present as round flask shapes with a narrow neck and a lumen 50–80 nm in diameter (Fig. 1). They are most abundant in adipocytes, endothelial cells and smooth muscle cells, and are also found in many other kinds of cells. They probably do not exist in most blood cells including lymphocytes, neutrophils and erythrocytes. The name, caveolae, was coined by a Japanese electron microscopist, Dr. Eichi Yamada, 45 years ago,[1] but since their recent rediscovery have caveolae been under intensive study in the past several years.

MOLECULES OF CAVEOLAE

A. Caveolins

The recent burst of caveolae research started with the discovery of caveolins, the membrane proteins localized in caveolae.[2,3,4] Three proteins, caveolin-1, 2, 3, encoded by separate genes are known to exist. All the caveolin molecules are about 20 kDa; hydrophilic segments are present at both N and C termini and flanked by a central hydrophobic segment.[5] They are assumed to take a hairpin-like form, whose central segment is inserted in the lipid bilayer with both ends exposed to the cytoplasm. Caveolin-1 is a cholesterol-binding protein[6] covalently

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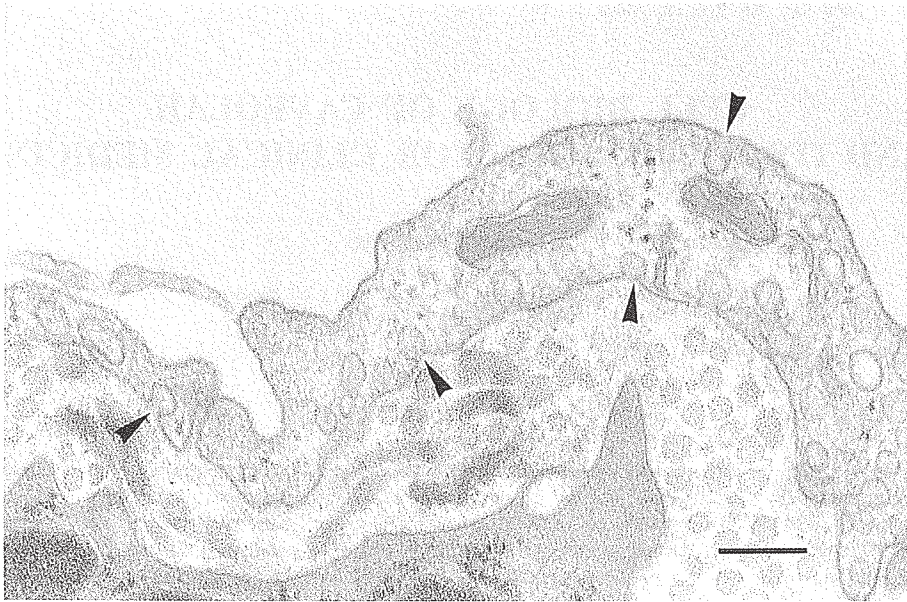
Fig. 1. Electron microscopy of caveolae in the capillary endothelium. Many caveolae are seen on both lumenal and ablumenal surfaces (arrowheads). Scale bar, 200 nm.

modified with three palmitoyl residues.[7] Caveolin-1 and probably caveolin-2 are expressed as two different isoforms; in both proteins a shorter β isoform, lacking the N terminal, is supposed to be generated by alternative translation initiation from the same mRNA as the full-length α isoform.[8] However, we recently found a hitherto unknown mRNA for caveolin-1 which encodes the β isoform alone.[9] Thus it appears that the two caveolin-1 isoforms are translated from two distinct mRNAs so that their ratio is regulated at the transcriptional level.

Caveolins-1 and 2 are coexpressed in many kinds of cells, whereas caveolin-3 occurs only in the skeletal muscle, heart muscle, and a few other cell types. Nerve cells had been assumed to lack caveolins, but a recent report showed that dorsal spinal ganglion cells contain caveolins-1 and 2.[10] Caveolins-1 and 3 form homo-oligomers in vitro, whereas caveolin-2 only makes dimers by itself.[3] However, caveolin-2 can form hetero-oligomers with caveolin-1. Caveolin oligomers of 200–400 kDa are thought to be formed in the endoplasmic reticulum (ER), with larger complexes made in the Golgi complex.[11] Caveolin oligomers are necessary for the formation of the caveolar structure; transfection of caveolin-1 cDNA to cells lacking endogenous caveolins induces de novo formation of caveolae.[12] On the other hand, caveolins alone may not be sufficient for caveolae formation. The number of caveolae does not appear to be proportional to the expressed amount of caveolin-1. Furthermore, in the polarized Madin-Darby canine kidney cells, the apical plasma membrane does not contain typical caveolae even though caveolin-1 exists.[13] These results suggest that factors other than caveolins (e.g., cholesterol) are involved in caveolae formation.

Caveolin-1 contains a stretch of 20 amino acids (82–101) called the "caveolin-scaffolding domain"; the domain is localized in the hydrophilic segment proximal to the central hydrophobic stretch.[14] In vitro experiments showed that the caveolin-scaffolding domain can interact with various signaling molecules (e.g., G_α, Src, PKC_α, eNOS, Ha-Ras) and receptors (e.g., endothelin, PDGF) through a common motif (Ψ-X-Ψ-X-X-X-X-Ψ or Ψ-X-X-X-X-Ψ-X-X-Ψ; Ψ

is an aromatic amino acid, and X can be any amino acid).[15] These signaling molecules interact with caveolin-1 more effectively in the inactive state than in the active state. Caveolin-1 appears to function as a regulatory molecule; for example, caveolin-1 binds to the GDP-bound form of G_α and works as a GDP-dissociation inhibitory factor.[16] Based on these experimental results, the "caveola-signaling hypothesis" proposed that caveolae are sites to regulate cross-talk between different signaling pathways through regulatory interaction with caveolins.[17]

Another important function of caveolin-1 is related to cholesterol. Typical caveolae invaginations are known to disappear when total cellular cholesterol is reduced or plasmalemmal cholesterol is extracted. This phenomenon is now explained by the cholesterol-binding property of caveolin-1.[6] Interestingly, when intact cells were treated with cholesterol oxidase, caveolin-1 was redistributed from the plasma membrane to the Golgi apparatus.[18] Furthermore, caveolin-1 is assumed to be involved directly in intracellular cholesterol transport. Transportation of de novo synthesized cholesterol from the ER to the plasma membrane occurs very rapidly (within 10–20 min), and the rate of transport appears to be correlated with the expression level of caveolin-1.[19] This transport pathway needs to be explored in detail, but one report showed that cholesterol forms a soluble complex with caveolin-1, a heat shock protein and immunophilins, and is transported through the cytosol.[20] Once incorporated in the plasma membrane, cholesterol is supposed to diffuse from the caveolae to the surrounding membrane areas; excess cholesterol appears to be removed from the membrane through binding with the plasma high-density lipoproteins.[21] On the other hand, the content of free cholesterol affects the expression level of caveolin-1. The transcription of the caveolin-1 gene is enhanced when the cellular cholesterol content is increased; this activity is thought to be mediated by the binding of sterol-regulatory element binding proteins to the 5' untranslated region of the caveolin-1 gene.[22] To summarize briefly, the expression of caveolin-1 is controlled by the cellular cholesterol content, and the efflux of excess cholesterol is mediated by caveolin-1. Whether caveolins-2 and 3 are also related to cholesterol is not known.

B. Molecules related to Ca^{2+}

Because of morphological characteristics, caveolae have been assumed to be related to intracellular Ca^{2+} regulation. We found that a transmembrane protein structurally similar to the type I IP_3R and the plasmalemmal Ca^{2+}-pump (Ca^{2+}-ATPase) are concentrated in caveolae.[23,24] The type I IP_3R in the ER is a Ca^{2+} channel that opens upon IP_3 binding; although not proven yet, the caveolar IP_3R-like protein might be a plasmalemmal Ca^{2+} channel. On the other hand, the Ca^{2+}-pump extrudes Ca^{2+} from the cytosol to the extracellular space. Double labeling indicates that most caveolae have both the IP_3R-like protein and the Ca^{2+}-pump. Based on these results, we proposed that caveolae may be engaged in the regulation of the intracellular Ca^{2+} concentration. In the capillary endothelium, caveolae are closely related to the ER. We observed that caveolae are aligned along the ER rim, implying the presence of a structural linkage between caveolae and the ER.[25] The ER is a major intracellular Ca^{2+} store in non-muscle cells, and may be functionally correlated with caveolae.

The caveolar localization of the Ca^{2+} transport machinery suggests that the local Ca^{2+} concentration beneath caveolae changes in a different way than the rest of the cytoplasm. In fact, the local Ca^{2+} concentration measured by a membrane-anchored Ca^{2+} indicator dye showed a different pattern of fluctuation from that observed by commonly used water-soluble Ca^{2+} indicators, such as fura-2.[26] The local alteration of the Ca^{2+} concentration should influence caveolar proteins preferentially. In fact, the function of several proteins localized in caveolae and/or caveola-like domains is known to be regulated by Ca^{2+}. A notable example is endothelial nitric oxide synthase (eNOS); its enzymatic activity is enhanced by binding to Ca^{2+}/calmodulin, but

inhibited by caveolin-1.[27] These properties indicate that the caveolar localization of eNOS is critical for proper functional regulation. Additionally, limiting an increase of Ca^{2+} to a restricted area would reduce the risk of Ca^{2+} toxicity for a whole cell.

TIFF AND RAFT

Treatment with non-ionic detergents such as Triton X-100 followed by sucrose density-gradient equilibrium ultracentrifugation produces a small amount of Triton-insoluble floating fraction (TIFF);[28] some authors call this or similar material detergent-resistant membrane, detergent-insoluble glycolipid-rich domain, or caveolae-like membrane. Because caveolins are highly enriched in TIFF, the fraction was once thought to be equivalent to purified caveolae.[29] But later it was revealed that TIFF can be obtained from cells lacking caveolae and caveolins,[30] and several molecules enriched in TIFF were found by immunohistochemistry to exist outside of caveolae.[31,32] TIFF consists of membrane vesicles reaching up to 1 μm in diameter,[28] and it is difficult to believe that domains of this size exist in live cells. At present, it is generally thought that TIFF is derived not only from caveolae but also from rafts and other intracellular membranes.

Rafts are a membrane domain enriched with glycolipids, cholesterol and GPI-anchored proteins. They were first proposed as a platform involved in vesicular transport to the apical membrane in the epithelial cell.[33] Compared to glycerophospholipids, sphingomyelin and glycolipids contain longer and less unsaturated acyl chains, and thus show a higher phase transition tem-

Table 1. Molecules enriched in TIFF.

These molecules may be present in caveolae, rafts, and/or other membrane domains.

GTP-binding protein:	$G_{i\alpha}$, $G_{s\alpha}$, G_β, Rab5, Rap1, Ras, Rho-A, Rac1
Kinase and phosphatase:	Src, Yes, Lyn, Fyn, Lck, Fgr, Nck (p59), JAK-2, PKA, PKCα, PKCβ, Casein kinase II, MAP kinase, PI3-kinase, Raf1, Syp, PTP-1D
Other enzyme:	PLCγ, PLD1, eNOS
Signaling molecule:	Shc, Grb 2, Nck, mSos-1, 14-3-3, vav
Cytoskeletal protein:	Actin, Myosin II, Gelsolin, Dystrophin, α-Sarcoglycan, β-Dystroglycan, Annexin II, Annexin VI
Receptor:	Muscarinic acetylcholine receptor, Endothelin receptor type A, Bradykinin receptor, u-PA receptor, EGF receptor, GH receptor, PDGF receptor, Insulin receptor, Atrial natriuretic peptide receptor, Angiotensin II receptor (AT1), AMF receptor, RAGE, CD36, SR-BI, SR-BII, p75NTR, Ca^{2+}-sensing receptor, TrkA, estrogen receptor α
Channel and pump:	Porin, Aquaporin-1, IP_3 receptor, Ca^{2+} pump
Other protein:	caveolin-1,2,3, GPI-linked proteins (including PrPc, PrPsc, folate receptor, CD73), VAMP, SNARE, NSF, Duffy antigen, Atrial natriuretic peptide, Hyaluronan, Tissue Factor, Axonal amyloid precursor protein, Flotillin, Epidermal surface antigen, T-cadherin, PSD-95, CD39
Lipids:	Sphingomyelin, Ceramide, Ganglioside GM1, Other glycolipids, PtdIns, PtdInsP, $PtdInsP_2$

perature. Due to these characteristics, sphingolipids are assumed to be packed closely and to form raft domains in conjunction with cholesterol.[34] It has been difficult to prove the existence of rafts, but two recent reports showed that rafts really exist as a domain up to 70 nm in diameter.[35,36] The lipid composition of caveolae and rafts are thought to be similar; whereas caveolae with the caveolin framework appear relatively static, rafts may be highly dynamic and change size and shape continuously.

Table 1 is a list of molecules found in TIFF or similar membranes. It is noteworthy that many membrane receptors and intracellular molecules involved in different signaling pathways are included. Several different methods were devised to isolate caveolae, and they also showed enrichment of similar molecules.[37-39] But most of the methods utilized the low buoyant density of the membrane in the final isolation process. As discussed above, however, the property appears to be shared by rafts. Thus it is likely that rafts as well as caveolae are contained in the low buoyant density membrane preparations obtained by biochemical procedures. In this context, it needs to be noted that a membrane preparation purified by anti-caveolin-1 affinity did not show any enrichment of the signaling proteins.[40] The result indicates that there remain problems to be solved concerning caveolae and rafts. In fact the two domains are not independent, but are most likely in a dynamic equilibrium with one another. For example, GPI-anchored proteins and glycolipids, which are assumed to be in rafts in undisturbed cells, accumulate to caveolae when crosslinked by antibodies (Fig. 2).[32] The mechanism that governs the caveolae-raft relationship is not clear, and needs to be studied further.
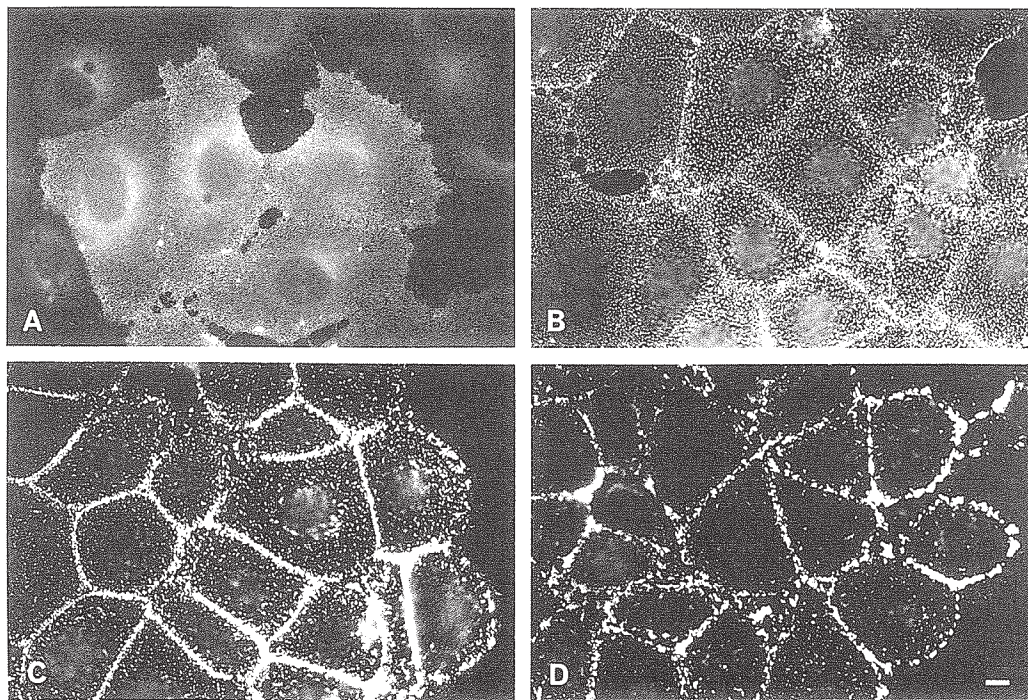
Fig. 2. Distributional change of Thy-1, a GPI-anchored protein, induced by cross-linking with antibodies. A: Fixed before application of antibodies; B: Treated with antibodies on ice; C, D: Incubated at 37°C for 10 min (C) or 30 min (D) after binding with antibodies on ice. Scale Bar, 10 μm. Reproduced from reference 32.

The role of rafts in signal transduction has been demonstrated in lymphocytes and eosinophils, both of which lack caveolae and caveolins.[41,42] For example, in T lymphocytes, crosslinking of GPI-anchored proteins and glycolipids leads to activation of Lck and Fyn, and then causes an increase of the intracellular Ca^{2+} concentration. It is not known whether rafts mediate the same phenomenon in cells expressing caveolae, but it appears reasonable to think that rafts are the primary locus of signal transduction, and that caveolae are an additional component which modifies the process in one way or another.

The molecular mechanism of signal transduction in rafts is not well understood: GPI-anchored proteins and glycolipids are confined to the outer leaflet of the plasma membrane, whereas most signaling molecules including Lck and Fyn, are only anchored to the inner leaflet with acyl chains. Interaction of long acyl chains between the two leaflets, dimer formation of cholesterol molecules in the two leaflets, and/or involvement of transmembrane protein(s) are postulated to provide linkage between the two leaflets,[43] but there has not been much experimental evidence in support of any particular hypothesis in many cases.

CAVEOLAE, CAVEOLIN, AND DISEASES

In cells that were transformed by various oncogenes and lost anchorage dependency, the expression of caveolin-1 and the number of caveolae decreased drastically.[44] When the expression of caveolin-1 was induced by transfection, the anchorage dependency was recovered. Furthermore, simply by reducing the expression level of caveolin-1 using the antisense protocol, cells exhibited anchorage-independent growth, formed tumors in immunodeficient mice, and show hyperactivation of the MEK/ERK cascade.[45] These results strongly indicate that caveolin is intimately related to the regulatory mechanism of cell proliferation, and that its deficiency may lead to cell transformation. Consistent with this, the human caveolin-1 gene was mapped to a suspected tumor suppressor locus (7q31.1) which is often deleted in human cancers.[46]

On the other hand, in prostate cancer that has lost sensitivity to androgen deprivation therapy, the expression of caveolin-1 was found to increase.[47] By suppressing the caveolin-1 expression using the antisense method, the cancer cells recovered their androgen-sensitivity. In cells transformed by v-Src, caveolin-1 is not markedly decreased, but highly phosphorylated. In fact, caveolin-1 was first identified as a substrate of tyrosine-phosphorylation in v-Src transformed cells.[48] In those cells, the number of typical invaginated caveolae decreased, and caveolin-1 was found in the flat region of the plasma membrane and in small cytoplasmic vesicles (Fig. 3);[49] the morphological change is likely to insulate the caveolae domain from the extracellular milieu, and might be related to transformation. These results suggest that a decrease of caveolin-1 may not be directly related to transformation and that different mechanisms may function in different cases.

Mutations in the caveolin-3 gene were found to cause autosomal dominant limb-girdle muscular dystrophy.[50] It was proposed that the mutations may interfere with caveolin-3 oligomerization and disrupt caveolae formation at the muscle cell surface. With respect to vascular diseases, a drastic increase of caveolae was reported in spontaneous hypertensive rats[51] in the arterial endothelium, along with abnormal intracellular Ca^{2+} homeostasis. In the aortic media during the formation of neointimal thickening, caveolae in the smooth muscle decrease concomitant with the transition from the contractile to synthetic phenotype.[52] Furthermore, involvement of caveolins in the pathophysiology of Alzheimer's disease was reported. A dramatic upregulation of caveolin-3 was found in astrocytes around senile plaques.[53] Caveolin-3 is supposed to provide a platform of association for amyloid precursor protein and the presenilins, leading to the overproduction of its toxic amyloid metabolites.
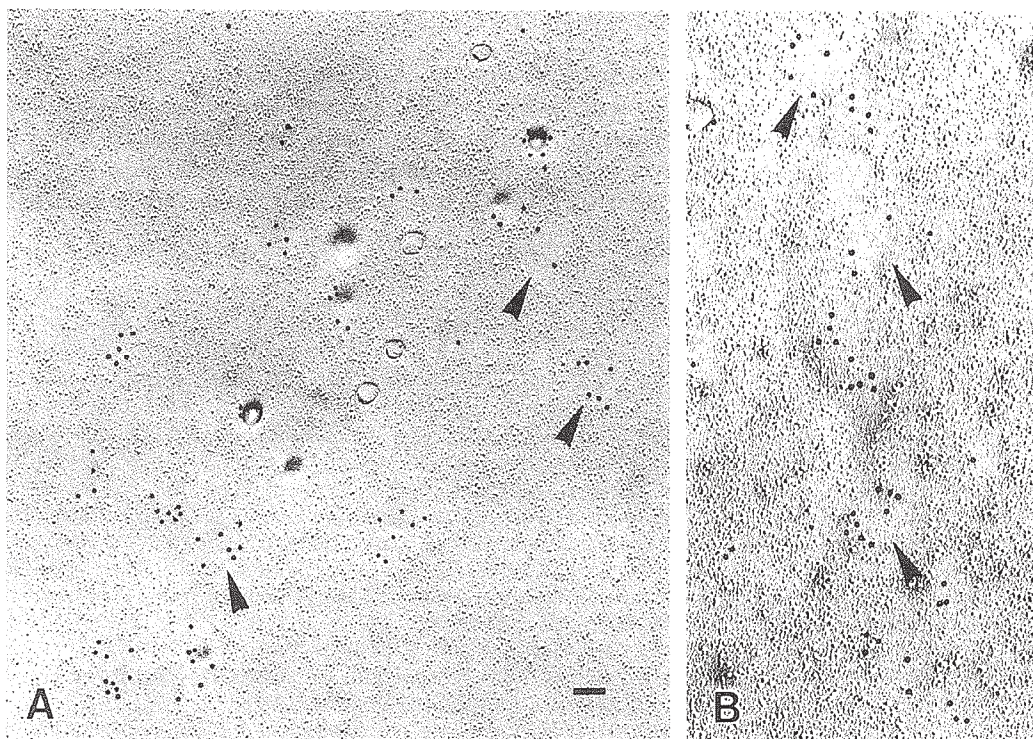
Fig. 3. Immunogold electron microscopy of freeze-fracture replica of v-Src expressing cells. Labeling for caveolin-1 is seen not only in the classical indented form of caveolae, but also in flat membrane areas (arrowheads). Scale Bars, 100 nm (A); 85 nm (B). Reproduced from reference 49.

The above instances indicate that caveolae and/or caveolins play important physiological functions and that their abnormalities lead to various pathological states. It is intriguing that seemingly distant phenomena are linked at caveolae and may underlie some diseases. A notable example is that a mere decrease in the cellular cholesterol content leads to cell proliferation.[54] One explanation for this process is that the reduced cholesterol level disrupts the caveolar environment, which then leads to activation of the MEK/ERK pathway and cell proliferation. Interestingly, cell proliferation was suppressed by replenishing the cellular cholesterol content of transformed cells.[55] These results suggest that caveolae and caveolins are good targets of therapeutic research.

CONCLUSION

This brief review summarized the recent results regarding caveolae and rafts. They are functional interface between the cell interior and the extracellular environment, and a variety of functions they show seems to depend on the cholesterol content. Due to their intriguing characteristics, caveolae and rafts will continue to be the subjects of intensive study, the results of which should have important implications for clinical medicine.

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