Reviews in Undergraduate Research, Vol. 1, 44-50 , 2002

The Role of Caveolae and the Caveolins in Mammalian Physiology

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Caveolae are 50-100 nm invaginations of the plasma membrane that have captured the interest of scientists for many decades. However, the wide-ranging and physiologically important roles of these curious structures have only recently been addressed. Among the important milestones in the understanding of caveolae is the discovery of a family of proteins that are intimately involved in caveolar function (the caveolins). It has become clear now that caveolae and their caveolin "marker proteins" are involved in a variety of cellular processes including endocytosis, lipid homeostasis, signal transduction, and tumorigenesis. In this review, we will highlight the current view of caveolae in cell biology and discuss the relevance of these structures to mammalian physiology.

Introduction to Caveolae

Examination of a cell at the ultrastructural level reveals numerous intricate components that contribute to its appropriate function. Since the advent of electron microscopy in the 1940s and 50s, structures such as the mitochondrion, endoplasmic reticulum, golgi apparatus, and clathrin-coated endocytic vesicles were discovered for the first time and their distinct functions in cells speculated upon. In this same period, another cellular entity, a 50-100 nm vesicle that was found to be either directly invaginated from or in close proximity to the plasma membrane was also described (Figure 1A). Based on this conspicuous "cave-like" morphology at the membrane, these structures were named "*caveolae*" and were added to the growing list of newly discovered cellular organelles (Palade, 1953; Yamada, 1955).

In the ensuing decades and with the incipience of cellular and molecular biology, research on many of these cellular organelles led to a precise understanding of their function (e.g. implication of mitochondria in ATP production, the ER/golgi in protein synthesis and sorting, and clathrin-coated pits in endocytosis).

Unfortunately, due to difficulty in characterizing their biochemical and molecular nature, caveolae remained enigmatic structures with no definitive function(s). Based on their structural resemblance to clathrin-coated vesicles and their seemingly dynamic movement between the plasma membrane and intracellular compartments, caveolae were initially thought to serve solely an endocytic role akin to clathrin-coated pits (Palade, 1953; Simionescu et al., 1975).

Now, based on work in the last decade, caveolae are being recognized as rather complex organelles with important roles not only in endocytosis but also lipid homeostasis, signal transduction, and tumorigenesis. In addition, they seem to play very specific roles in distinct cell types, making these structures one of the most interesting and multi-functional entities in cells. In this review, we will discuss the salient features of these structures and the current understanding of their function in mammalian organisms.

The Biochemical/Structural Nature of Caveolae: Introduction to the Caveolins

Based on numerous biophysical and biochemical analyses of plasma membranes, it is now known that the traditional view of a lipid bilayer as a "fluid mosaic" is not entirely accurate (Brown and London, 1998). Although a membrane solely made of phospholipids does indeed act as a fluid-mosaic, cell membranes which are also composed of cholesterol, sphingolipids, and various lipid-modified and transmembrane proteins, behave differently (Brown and London, 1998). In cell membranes, depending on the local concentration of cholesterol, sphingolipids, and some phospholipids, more rigid patches of membrane can form. Floating among the bulk phospholipids bilayer, these biochemically distinct patches of membrane have now been termed lipid rafts, the study of which is an active area of research (see (Simons and Toomre, 2000) for review) (Figure 1B).

Interestingly, research in the past decade has shown that caveolae are biochemically indistinguishable from lipid

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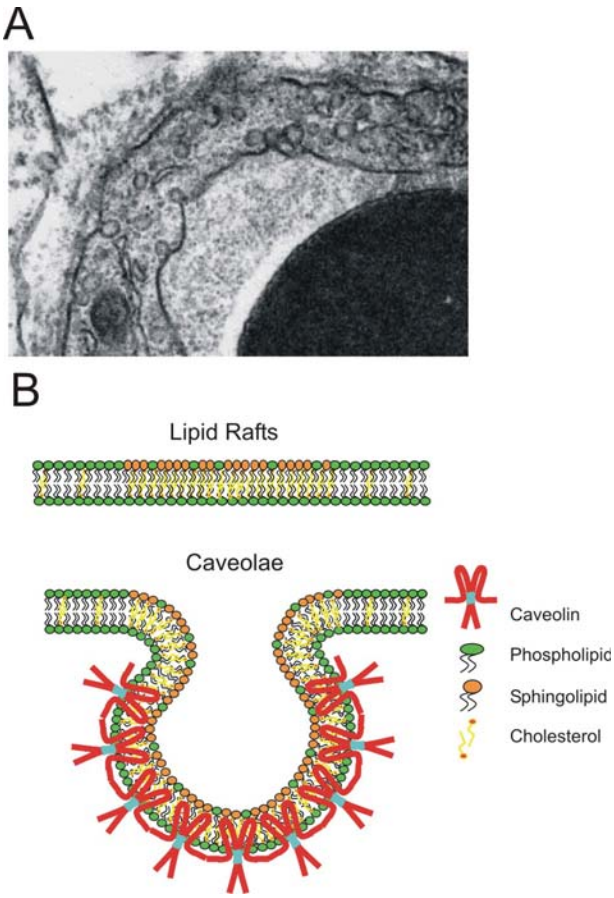

Fig. 1. Figure 1. Caveolae, a unique "cellular organelle" with a unique "marker protein"

A) Electron micrograph of an endothelial cell showing caveolae, 50-100 nm structures that are either direct invaginations or in close proximity to the plasma membrane. Caveolae are estimated to make-up an estimated 30-70% of the plasma membrane area in certain cells such as endothelial cells, adipocytes, or Type I pneumocytes .

B) Diagram comparing the biochemical composition of lipid rafts and caveolae (adapted from (Galbiati et al., 2001)). Lipid rafts form via a coalescence of cholesterol and sphingolipids; as a result, these microdomains have vastly different biochemical properties than the bulk phospholipids bilayer. Caveolae are generally considered to be "invaginated" lipid rafts primarily due to an enrichment in a family or proteins known as the caveolins. Here, the caveolin oligomer is depicted as a dimer for simplicity.

rafts and are composed of a similar local enrichment of cholesterol and sphingolipids (Simons and Toomre, 2000) (Figure 1B). The primary difference between these two entities is the invaginated, vesicular morphology of caveolae. This difference arises due to the presence of a set of proteins unique to caveolae but absent from lipid rafts, the caveolins.

The caveolin protein family is composed of three distinct proteins, caveolin-1, -2, and -3 (Cav-1, -2,-3) (Glenney, 1992; Rothberg et al., 1992; Scherer et al., 1996; Tang et al., 1996). Not surprisingly, these proteins are expressed in tissues with a high abundance in caveolae; Cav-1 and -2 are co-expressed in many cell types with especially high levels in endothelial cells, adipocytes, and type I pneumocytes, while Cav-3 is exclusively expressed in skeletal and cardiac muscle cells (Scherer et al., 1994; Scherer et al., 1996; Tang et al., 1996). The main exception is smooth muscle cells, where intriguingly all three proteins are expressed (Tang et al., 1996).

The caveolin proteins have several properties which are important not only for selective localization to caveolae but also for driving the invagination of these structures. Cav-1 has been shown to have high binding affinity for cholesterol and sphingolipids (Fra et al., 1995; Murata et al., 1995; Thiele et al., 2000). This property, along with three carboxy-terminal lipid-modifications (palmitoylations), stabilizes and targets Cav-1 to caveolae (Dietzen et al., 1995; Monier et al., 1996). The caveolins can also oligomerize into a complex of 14-16 subunits and thereafter form even larger mega-complexes by oligomer-oligomer interactions (Monier et al., 1995; Sargiacomo et al., 1995; Song et al., 1997). Although it is still largely speculative, it is thought that the high affinity for cholesterol, the oligomerization, and the oligomer-oligomer interactions can together form an environment in the lipid bilayer conducive for the creation of 50-100 nm caveolar invaginations.

Caveolae, Caveolins, and Endocytic Processes

The observation that caveolae can exist as invaginations of the plasma membrane, as completely enclosed vesicles, or as aggregates of several vesicles, led investigators to infer that these structures were conduits for the endocytosis of macromolecules (Simionescu et al., 1975). Indeed, tracer studies and high resolution electron microscopy has revealed that cells predominantly use caveolae for the selective uptake of molecules as small as folate to full size proteins such as albumin and alkaline phosphatase (Anderson et al., 1992; Parton et al., 1994; Predescu et al., 1997; Schnitzer et al., 1994) (Figure 2A). In endothelial cells, the uptake of such molecules is complicated by the fact that the endocytosed caveolae seem to migrate from the luminal side to the abluminal side, thereby transferring specific serum molecules to the underlying tissue (a process referred to as transcytosis) (Predescu et al., 1997; Simionescu et al., 1975) (Figure 2A).

Interestingly, several studies have also shown that caveolae-mediated uptake of materials is not limited to macromolecules; in certain cell-types, viruses (e.g. simian virus 40) and even entire bacteria (e.g. specific strains of E. Coli) are engulfed and transferred to intracellular compartments in a caveolae-dependant fashion (Anderson et al., 1996; Montesano et al., 1982; Shin et al., 2000).

Although the molecular mechanism for these endocytic events are not completely understood, there are indications that the same machinery operating traditional vesicle budding and fusion processes is functional in this setting (Henley et al., 1998; Oh et al., 1998; Schnitzer et al., 1995). Thus, the cell utilizes similar endocytic techniques to differentially traffic cellular materials.

Caveolae, Caveolins, and Cholesterol Homeostasis

Caveolae are highly enriched in cholesterol as compared to the bulk plasma membrane and Cav-1 binds this cholesterol with high affinity (estimated at 1 cholesterol molecule per caveolin molecule) (Murata et al., 1995; Thiele et al., 2000). Furthermore, pharmacological depletion of plasma membrane cholesterol leads to a loss of morphologically identifiable caveolae (i.e. "flattening" against the membrane) and dissipation of the caveolin-matrix (Rothberg et al., 1992). Due to these observations, it was suggested that caveolae and caveolins are involved in maintaining intracellular cholesterol balance; indeed, there is evidence for such a role.

Cellular cholesterol is derived from two main sources, de novo production or extracellular uptake (via low density lipoprotein (LDL) receptors localized in clathrin-coated vesicles) (Fielding and Fielding, 1997; Simons and Ikonen, 2000) (Figure 2B). Once inside, the caveolins seem to function as intracellular escorts for the transport of this cholesterol from the endoplasmic reticulum to plasma membrane caveolae (Smart et al., 1996; Uittenbogaard et al., 1998) (Figure 2B). Upon delivery, this cholesterol has three fates: (1) to remain as a component of caveolar cholesterol, aiding in the invagination and proper function of these structures, (2) to be siphoned into the bulk plasma membrane, repleting the lipid bilayer with appropriate amounts of cholesterol, or (3) to be effluxed to serum cholesterol-transporting units like high density lipoproteins (HDLs) (Fielding et al., 1999; Fielding and Fielding, 1995; Smart et al., 1996) (Figure 2B). In essence, the caveolins deliver intracellular cholesterol to a "relay station" wherein the overall fate of cholesterol is determined; the cholesterol needs of the cell are met and excesses are effluxed.

Caveolae, Caveolins, and Signal Transduction

The intimate relationship between caveolae and their protein components, the caveolins, is obvious. An important question that remained was whether other plasma membrane proteins can also preferentially localize to these structures. This issue has been addressed using biochemical purification, wherein caveolae can be selectively isolated from other cellular constituents and their protein components analyzed (Lisanti et al., 1994; Sargiacomo et al., 1993).

Caveolae are highly enriched in numerous membrane-bound proteins, especially signaling proteins with lipid-modified groups (e.g. H-ras, src-family tyrosine kinases, heterotrimeric G-proteins, eNOS, etc) (Lisanti et al., 1994; Smart et al., 1999) (Figure 2C). Furthermore, it appears that the caveolins are not innocent by-standers in this environment and can bind and functionally regulate (mostly inhibit) several of these caveolae-localized molecules (Feron et al., 1996; Garcia-Cardena et al., 1996; Li et al., 1996; Li et al., 1995; Song et al., 1996; Song et al., 1997) (Figure 2C). The caveolins possess a 20 amino acid juxtamembrane domain (now appropriately called the scaffolding domain) that mediates this functional binding (Okamoto et al., 1998).

The predilection for signaling proteins to localize to caveolae and the capacity for the caveolins to regulate their function has led some to refer to caveolae as "signalosomes" (or bodies where signal transduction events and cross-talk between different signaling pathways can take place efficiently and in regulated fashion) (Lisanti et al., 1994; Smart et al., 1999). This aspect of caveolae is currently an active area of research since it brings together the interests of investigators conducting research in seemingly disparate areas.

Caveolae, Caveolins, and Tumorigenesis

An interesting corollary to the above-mentioned signalosome concept arises during tumorigenesis. Several of the proteins localized to caveolae and inhibited by Cav-1 (namely EGFR, Her2/Neu, and PDGF receptor tyrosine kinases, components of the Ras/p42/44 MAP kinase cascade, and members of the PI-3-kinase cascade)) (Couet et al., 1997; Engelman et al., 1998; Liu et al., 1996; Yamamoto et al., 1999; Zundel et al., 2000) are extremely important in pro-proliferative/anti-apoptotic signaling. If functionally deranged, such proteins can result in cells with hyperactive cell cycles and eventually tumor formation. In this regard, caveolae and Cav-1 might be expected to be essential members of the cellular tumor suppressor repertoire, acting to dampen the action of tumorigenic signals.

Interestingly, it has been observed that caveolae are absent or reduced in number and Cav-1 is transcriptionally down-regulated in numerous cancers (both cell-lines and in situ carcinomas) (Engelman et al., 1998; Koleske et al., 1995; Lee et al., 1998; Razani et al., 2000). In addition, both human CAV-1 and -2 genes map to 7q31.1 (a region of the chromosome found to be frequently deleted in several epithelial cancers - e.g. breast, lung, renal, and ovary) (Kerr et al., 1996; Shridhar et al., 1997; Zenklusen et al., 1994). Such observations provide strong evidence for a caveolin-mediated tumor surveillance process and give impetus for researchers to include the caveolins as important factors in the diagnosis and treatment of cancer.

In vivo Relevance of Caveolar Function in Mammalian Physiology

The current understanding of caveolae and caveolin function is based on research conducted either in vitro (biochemical or cell culture systems) or in vivo (namely, morphological assessment by electron microscopy). Although such techniques are useful in providing insights into the functions of these structures, a complete understanding of their physiological relevance can only be attained by experiments conducted in the whole organism (e.g. creation of transgenic or knockout mice, wherein the expression of one or more caveolin proteins is perturbed).

Indeed, in the past year, several groups have reported on the phenotypes of mice with targeted disruptions of the CAV-1, -2, and -3 loci, thereby providing the first rigorous assessment of caveolae function in vivo (Drab et al., 2001; Galbiati et al., 2001; Hagiwara et al., 2000; Razani et al., 2002; Razani et al., 2001; Razani et al., 2002). Mice deficient in Cav-1 or Cav-3 (but not Cav-2) lack morphologically identifiable caveolae in tissues expressing those genes. This observation is important in that it directly proves the importance of caveolin expression in caveolae formation and provides a tool for the study of not only caveolins but caveolae in a mammalian organism.

The phenotypes of Cav-1 null mice (Drab et al., 2001; Razani et al., 2002; Razani et al., 2001):

1) Loss of caveolae in cells expressing Cav-1 (e.g. endothelial, epithelial, adipose cells)
2) Dramatic reduction of Cav-2 protein levels due to destabilization and degradation via the proteosomal pathway - thus, Cav-1 null mice are in essence Cav-1 and -2 deficient
3) Histologically abnormal lungs - thickened alveolar septa due to endothelial cell hyper-proliferation and increased

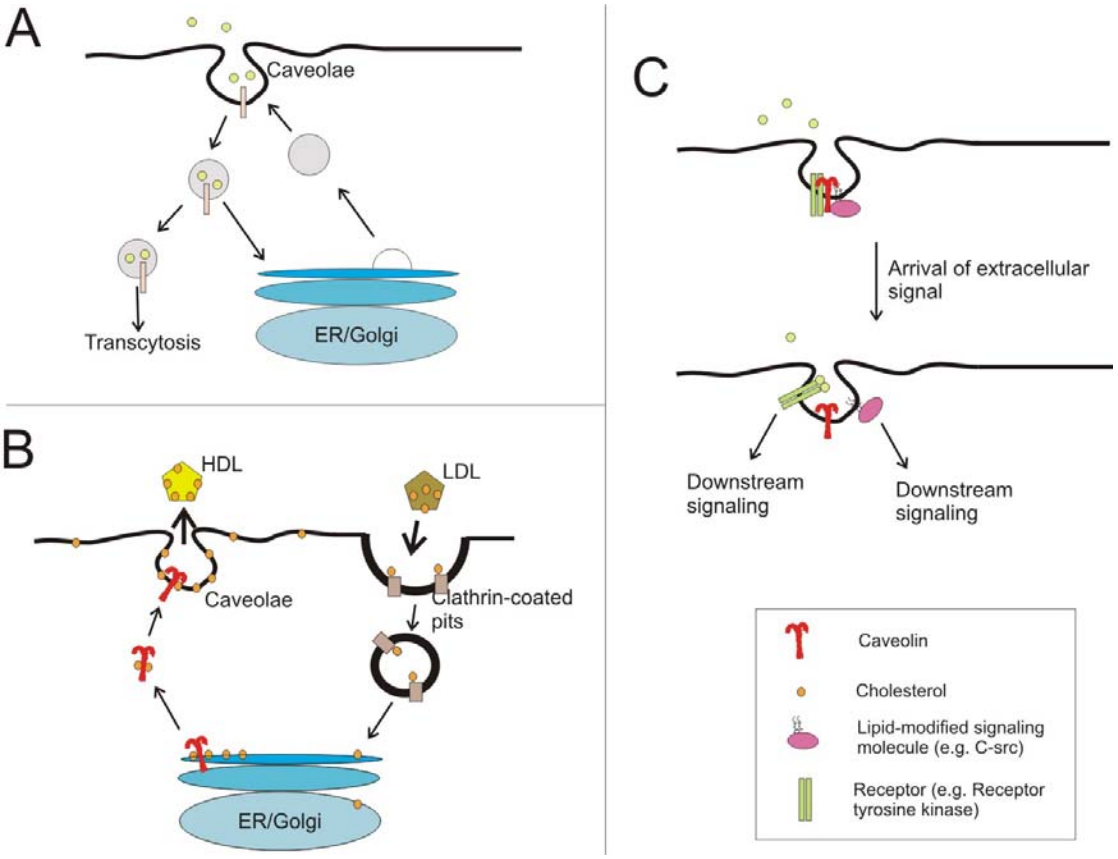

Figure 2. Proposed functions of caveolae and the caveolins (adapted from (Razani and Lisanti, 2001))
A) Certain molecules have been shown to be predominantly endocytosed via caveolae and not clathrin-coated vesicles. The fate the cargo in a fully invaginated caveola is not entirely understood; however, there is evidence to suggest that depending on the cell type, caveolae can deliver their contents to the ER/golgi compartments or to the abluminal side of a cell.
B) Intracellular cholesterol is thought to be transported to plasma membrane caveolae via a golgi-independent caveolin-mediated route. Caveolae then can serve as "relay stations" to deliver the membrane cholesterol to the bulk plasma membrane or to cholesterol-transporters such as HDL particles.
C) Caveolae are now thought to act as signalosomes, or entities in which signal transduction events can take place efficiently. A higher level of regulatory complexity is provided by the caveolins where signaling molecules can be bound until extracellular ligands relieve them of inhibition. Here, the dynamic regulation of a receptor tyrosine kinase (e.g. EGF receptor) and a lipid-modified kinase (e.g. the src-tyrosine kinase) in caveolae are shown.

deposition of extracellular matrix
4) Cell cycle defects - fibroblasts derived from these mice have increased S-phase fractions and proliferate faster than their wild-type counterparts

5) Defects in vasoregulation - the aortas from these mice are hyper-responsive to vasodilatory stimuli due to hyper-activation of the eNOS signaling cascade
6) Defects in endocytosis - the uptake of albumin by endothelial cells is drastically reduced in these mice
7) Defects in lipid homeostasis - these mice are resistant to diet-induced obesity and have histological abnormal adiposities with age. These mice are also hypertriglyceridemic with a reduced capacity to clear serum lipids, a condition likely related to the aberrant adipose function.

The phenotypes of Cav-2 null mice (Razani et al., 2002):

1) Normal or slightly reduced Cav-1 expression with no loss of caveolae - thus, these mice are extremely useful for comparison with Cav-1 deficient mice in that they selectively lack Cav-2
2) Histologically abnormal lungs - in fact, the lung defects in these mice are indistinguishable from Cav-1 null mice, thereby for the first time demonstrating an important role for Cav-2 independent of Cav-1
3) Unperturbed vasoregulation and lipid homeostasis - these observations were important in establishing that Cav-1 and Cav-2 have distinct and non-overlapping roles in physiology.

The phenotypes of Cav-3 null mice (Galbiati et al., 2001; Hagiwara et al., 2000):

1) Loss of caveolae in cells selectively expressing Cav-3 (i.e. skeletal and cardiac muscle)
2) Histologically abnormal skeletal muscle with necrotic muscle fibers and centralized nuclei - indeed, this mild muscular dystrophy phenotype recapitulates the pathology seen in a previously described group of patients with Limb-girdle muscular dystrophy (type 1C) in which mutations in the CAV-3 gene were found (Minetti et al., 1998).
3) Defects in the myocyte T-tubule network with irregularly-oriented tubules

Conclusions and Future Directions

As can be seen from the above description, the initial characterization of these mice has provided a wealth of information ranging from the predicted (e.g. involvement in endocytic processes and signaling cascades such as eNOS) to the completely unexpected (e.g. lung hypercellularity and defects in triglyceride rather than cholesterol homeostasis).
The study of caveolae and their marker proteins, the caveolins, has been an exciting yet challenging endeavor. The ever-changing view of their function in mammalian physiology is in part due to the difficulty of working with such membrane domains and a lack of different but complementary tools available for rigorous analyses. Caveolae and the caveolins have thus far been implicated in endocytosis, lipid homeostasis, signal transduction, and tumorigenesis. Now, with the availability of caveolin-deficient mice, biochemical, cell culture, and genetic approaches can finally be intermeshed to provide a more complete picture of caveolar function in vivo.

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