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Review

Caveolae: Where incoming and outgoing messengers meet

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ABSTRACT **Plasmalemmal caveolae were first identified as an endocytic compartment in endothelial cells, where they appear to move molecules across the cell by transcytosis. More recently, they have been found to be sites where small molecules are concentrated and internalized by a process called potocytosis. A growing body of biochemical and morphological evidence indicates that a variety of molecules known to function directly or indirectly in signal transduction are enriched in caveolae. This raises the possibility that a third function for caveolae is to process hormonal and mechanical signals for the cell. Insights gained from studying potocytosis suggest several different ways that this membrane specialization might function to integrate incoming and outgoing cellular messages.**

The surfaces of most cells are studded with tiny, flask-shaped membrane invaginations called caveolae (1–5). These invaginations are the preferred location for glycosylphosphatidylinositol (GPI)-anchored membrane proteins (6–8). Some of these GPI-anchored proteins function to concentrate small molecules and ions in the sequestered space created when caveolae transiently seal off from the extracellular environment. The concentrated ligands then flow into the cell through carriers or channels embedded in the membrane. This process is called potocytosis (9, 10).

An unexpected complexity is beginning to emerge about caveolae. In addition to being a site of entry for small molecules and ions by potocytosis, evidence is accumulating that they also play a major role in coordinating the interaction of the cell with its environment. The molecular composition of caveolae indicates that they have the capability to store and process messengers such as cAMP, calcium, or adenosine and to use non-receptor tyrosine kinases to initiate crucial phosphorylation cascades. They may actually be able to form chemical synapses between non-neuronal cells. Remarkably, the formation of these signals may be dependent on membrane cholesterol, since the structure of caveolae is disrupted when cells are depleted of this sterol.

In this article, I outline the data that support a role for caveolae in cell signaling. At the same time, this information is used to construct several models that illustrate the different ways that caveolae might function in both intracellular and intercellular communication.

Caveolae

Each caveola is a dynamic piece of membrane that is either open for receiving and releasing material or closed for processing, storage, and delivery to the cell (11). The exact nature of the closed compartment is still unclear. In endothelial cells, caveolae appear to pinch off and form vesicles that sequester low molecular weight tracer molecules introduced into the blood vessel (12–14). Similar vesicles have not been found during receptor-mediated potocytosis, even though a closed caveolar compartment is easily detected by biochemical and morphological methods (11, 15). Caveolae do not merge with other endocytic pathways but appear to remain associated with the cell surface. This is significant because in many instances caveolae are grouped together in specific regions of the cell where they appear to open and close but remain localized.

The invagination of caveolae may be controlled by the distinctive coat material that decorates the inner membrane surface (Fig. 1). In stromal cells such as fibroblasts, this coat has a striated appearance (7, 16, 17), but in epithelial cells it looks more globular (18). The caveolar coat is quite unlike other coats found on budding membranes because it is constructed from membrane proteins that cannot be removed by either high salt or high pH (7). As a consequence, the coat may be permanently attached to caveolae and remain there during all phases of the internalization cycle.

Storage and Processing of Messengers in Caveolae

Caveolae can either be open in direct communication with the extracellular space or be closed to process trapped molecules or store them for later use. Their structure and cellular distribution suggest that caveolae are a semipermanent component of the cell surface that can be moved to different locations to meet the changing demands of the cell. This portable, membrane-bound compartment has been found to contain a number of molecules that are known to participate in cell signaling. There are three classes of molecules: enzymes that generate messengers from substrates in the environment, high-affinity binding sites that concentrate chemical signals, and substrates that are enzymatically converted into messengers.

GPI. Insulin was the first hormone suspected of using inositol phosphoglycan (IPG) or a molecule derived from IPG as a second messenger (19–21). Since then, hormones as diverse as interleukin 2 (22), nerve growth factor (23), TGF-β1 (24), and thyroid-stimulating hormone (25) have been found to stimulate the release of IPG from cells. High concentrations of synthetic IPG (26), IPG from hormone-stimulated cells (21), or IPG isolated from trypanosomes (27) can mimic some of the effects of each of these hormones. Even a GPI toxin from malaria has been identified (28). Finally, an antibody directed against IPG inhibits second messenger-mediated stimulation by insulin (29). These data strongly implicate IPG as a messenger molecule.

The only known cellular sources of these glycans are the externally oriented, GPI-anchored proteins and lipids of surface membranes (30, 31). How could an IPG derived from these molecules ever get into the cell unless they were first captured by an endocytic pathway? This is where caveolae might have an important function. Nearly every caveolae has a cluster of GPI anchored proteins, and within a cluster the protein density can reach as high as 30,000 molecules per μm^2 (15). Therefore, the IPG is naturally at a high concentration in caveolae. If the hormone-stimulated release of glycans were coordinated with the closure of the caveolae (Fig. 2*A*), then just a few IPG molecules generated in this space would be sufficient to establish a concentration gradient capable of driving transport through a membrane carrier into the cell. Another possibility is that a subset of caveolae may be kept closed and used as IPG storage sites. Hormones such as insulin would then allow IPG entry into

Abbreviations: GPI, glycosylphosphatidylinositol; IPG, inositol phosphoglycan.

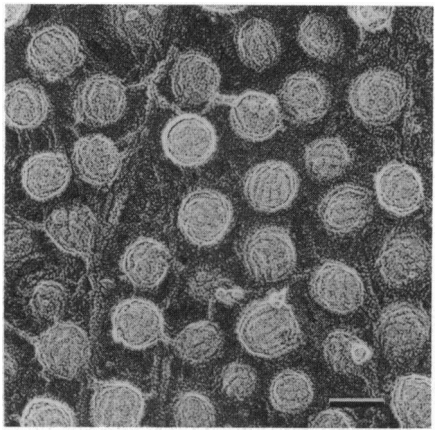

FIG. 1. Rapid-freeze, deep-etch view of the striated coat that decorates the inside membrane surface of each human fibroblast caveola. (Bar = 50 nm.)

the cell by opening a carrier in the caveolar membrane.

cAMP-Binding Protein. Some GPI-anchored proteins might be binding sites for molecules or ions that function as messengers. Analysis of the folate receptor in MA104 monkey kidney epithelial cells suggests that GPI-anchored, high-affinity binding sites can very effectively concentrate molecules in caveolae. For example, this receptor can sequester as many as 700 molecules of folate in each caveola (15). Recently a set of related cAMP-binding proteins that contain a GPI anchor have been found in mammalian (32) and yeast (33) cells. Therefore, in certain cells caveolae may function as storage and release sites for cAMP (Fig. 2*A*).

Membrane Carriers and Pumps for Calcium. Ca^{2+} is a ubiquitous second messenger (34). For some time there has been speculation that caveolae might be a Ca^{2+} entry site, similar to the T-tubule of muscle cells (35). Histochemical methods have even indicated that high concentrations of Ca^{2+} are present at these sites, which suggests a storage role (36, 37). Two recent reports now put these observations on a firmer footing: a form of the inositol 1,4,5-trisphosphate-regulated Ca^{2+} channel (38) and a Ca^{2+}-pump ATPase have been localized to caveolae (39). This places two membrane molecules known to be crucial for regulating Ca^{2+} fluxes (40) in a location where they can be used to store and release this messenger. These findings also suggest that caveolae have the capability to function as an intracellular Ca^{2+} store.

Packaging high concentrations of Ca^{2+} in a vesicle-like compartment at the cell surface could offer several unique ways to use this ion as a signal (Fig. 2*A*). If Ca^{2+} were to enter the cell from a highly concentrated source such as the caveolae, then steep gradients of Ca^{2+} would be created across the membrane. At the same time, only the amount of Ca^{2+} that is stored in the vesicle would enter the cell, which would help control Ca^{2+} toxicity and allow for a more precise delivery of the signal (41). The quantal release of Ca^{2+} from caveolae could also be used to generate Ca^{2+} oscillations that code for vital intracellular messages (42, 43). A final idea is based on the finding that caveolae are often asymmetrically distributed on the surface of the cell. Most of the caveolae in migrating fibroblasts, for example, are positioned at the cell margin and the leading edge (7). In these locations caveolae could control the spatial distribution of cytoplasmic Ca^{2+} by regulating the entry and retrieval of the ion at these sites.

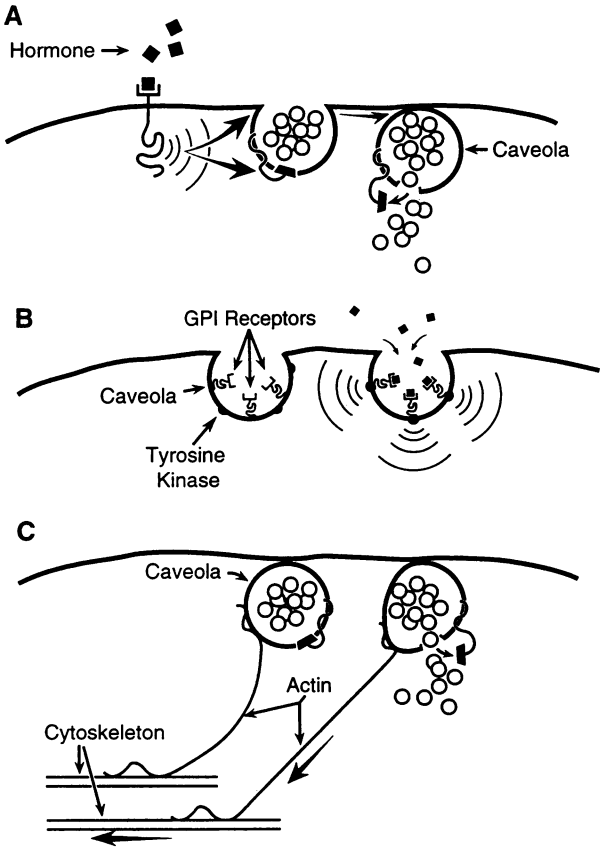

FIG. 2. A model for intracellular signaling by caveolae. (*A*) Hormonal transduction. Hormones (■) such as insulin bind to their receptor on the cell surface and elicit a signal. Caveolae respond to this signal by closing off from the cell surface and opening a membrane channel that allows molecules or ions (○), which are either sequestered or stored in the compartment, to enter the cell. (*B*) Receptor transduction. Some GPI-anchored proteins in caveolae are hormone receptors (GPI receptors). Caveolae have Src kinases (●) on their cytoplasmic surface. Ligand (■) binding causes the GPI receptor to associate with the kinase and stimulate a phosphorylation cascade. Alternatively, stimulation occurs indirectly through an intermediate generated in caveolae after ligand binding. (*C*) Mechanical transduction. Caveolae interact with elements of the cytoskeleton through actin filaments. Stretching or compressing the cell changes the organization of the cytoskeleton, which is transmitted to the caveolae by a change in filament tension. The force applied by the actin filaments deforms the caveolae, which opens membrane channels/carriers that allow messenger molecules or ions (○) to flow into the cytoplasm.

Src Kinases and Caveolin. Antibodies directed against several different GPI-anchored membrane proteins have been found to elicit a signal response in certain cells (28, 44–49). Presumably the stimulation involves the activation of a phosphorylation cascade at the cell surface (50). Several new observations suggest that this cascade might involve membrane-bound tyrosine kinases that are attached to the cytoplasmic surface of each caveola (Fig. 2*B*).

Three different laboratories have reported that the immunoprecipitation of a GPI-anchored membrane protein coprecipitates tyrosine kinase activity (44, 51, 52). In each case, the kinase has been found to be a member of the Src family. Clustered and unclustered (8, 15) GPI-anchored proteins are present on the cell surface, and either type might be the form that interacts with these kinases. However, the GPI-anchored clusters have recently been purified and found to contain multiple GPI-anchored proteins as well as Src-related tyrosine kinase activity (53–55). These studies did not determine whether the tyrosine kinase-containing GPI clusters were derived from caveolae.

Sargiacomo *et al.* (56) now have evidence that they are associated. Using antibodies against caveolin, a membrane marker for caveolae (7), they have developed a purification procedure that yields caveolin-rich fractions of membrane from MDCK canine kidney epithelial cells. These fractions have a 50- to 400-fold enrichment in the non-receptor tyrosine kinase, c-Yes, along with a 150-fold increase in the concentration of GPI-anchored proteins. Taken together, these studies suggest that both Src-related tyrosine kinases and GPI-anchored proteins are resident proteins of caveolae.

One of the target substrates for v-Src tyrosine kinase is caveolin (57, 58). The phosphorylation of caveolin by $pp60^{src}$ has been linked to the malignant transformation of chicken embryo fibroblasts (58). This suggests that in normal cells caveolae are important for cell behavior and that in transformed cells tyrosine phosphorylation of caveolin may interfere with an essential signaling activity. Caveolin may function to transmit signals directly from individual GPI-anchored proteins to a resident tyrosine kinase (Fig. 2*B*) (56). Another possibility is that caveolin directly or indirectly controls the opening and closing of caveolae and that phosphorylation of caveolin inhibits the normal internalization cycle. This would prevent the processing and/or storage of any essential messengers that might originate in this compartment.

5′-Nucleotidase. Thus far in the discussion, caveolae have been portrayed as storage and processing sites that ultimately deliver messages to the inside of the cell. They also could be used to store signaling molecules that are released on demand into the extracellular space. Caveolae sometimes aggregate together in specific regions of a cell and form patches containing 10–100 densely packed units (Fig. 1). If a patch were abutting an adjacent cell, then any substance released from those caveolae into the extracellular space would have only a short distance to travel to interact with that cell (Fig. 3). Adenosine is a candidate molecule for intercellular signaling by caveolar patches.

Adenosine monophosphate (AMP) has recently been found to be a paracrine factor secreted by neutrophils that, upon conversion to adenosine, stimulates Cl^- secretion from nearby intestinal epithelial cells (59). GPI-anchored 5′-nucleotidase, the ecto-enzyme responsible for converting AMP to adenosine, is clustered in caveolae (60). Furthermore, many different cells have P_1 purinoceptors that bind adenosine with high affinity (61). These receptors are usually linked to adenylate cyclase and can either stimulate (A_1 receptors) or inhibit (A_2 receptors) the production of cAMP (61). Imagine two closely apposed cells where patches of closed caveolae on one cell are adjacent to clusters of purinoceptors on the other (Fig. 3, *Unstimulated*). 5′-Nucleotidase converts AMP to adenosine in caveolae, where it is stored until an appropriate stimulus causes caveolae to open and release the signal (Fig. 3, *Stimulated*). The released adenosine interacts with the receptor and then is quickly degraded by another ecto-enzyme, adenosine deaminase (62, 63). The result is a chemical "synapse" between two non-neuronal cells. While entirely speculative at this point, such a synapse might be capable of propagating messages by stimulating membrane depolarization or inducing the release of a second chemical signal. Another potential function for caveolar synapses is to separate multiple signaling pathways that are operating in the same cell. This would provide the cell with a way to control the spatial distribution of signal input and output.

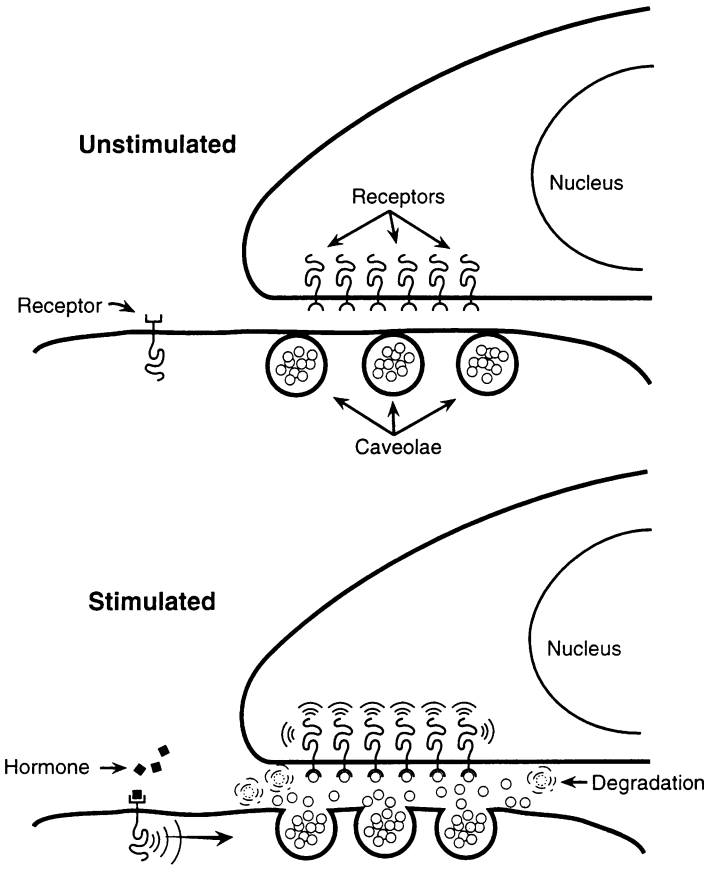

FIG. 3. A model for intercellular signaling by caveolae. Caveolae are sometimes grouped together in patches that are located at sites of cell–cell contact. Closed caveolae could function in this location as storage sites for messenger molecules such as adenosine (○, in caveolae of unstimulated cells). Stimulation of the cell, for example by the binding of a hormone to its receptor (■, stimulated), causes the caveolae to open and release the messenger into the intercellular space. The messenger then binds to a specific receptor on the adjacent cell, which initiates a signal cascade in that cell. The messenger is quickly degraded and the cascade is turned off. Thus, caveolae may be able to form chemical synapses between neuronal and non-neuronal cells.

Release of Messengers from Caveolae

Caveolae may be a versatile location for messenger processing because the cell can potentially control the opening and closing cycle as well as their spatial distribution. In addition, there may also be multiple mechanisms for controlling the entry into the cytoplasm of material stored in caveolae.

Cells are constantly bombarded by mechanical stimuli from surrounding cells and the extracellular matrix. These impulses must be correctly interpreted if a cell is to maintain a proper relationship with its neighboring cells in the tissue. The shape and malleability of the cell is controlled by a cytoskeleton that is partly composed of actin filaments. Recently, an actin-binding protein has been found associated with caveolae (64). In addition, myosin subfragment 1 is able to bind to a molecule in the striated caveolar coat *in vitro* (17, 18). The presence of these binding sites in caveolae suggests that this portion of membrane might be linked to the cytoskeleton. If so, then mechanical perturbations of the cell could directly cause the release of a message that is stored in this compartment (Fig. 2*C*). Actin may also control caveolar patch

formation at regions of the cell that are actively making contact with the substratum or with other cells (65). These patches could be positioned to optimize the conversion of focal, mechanical input into local changes in cell behavior.

Hormones may also control the opening of the caveolar channels that allow potential signals to be released into the cell (Fig. 2*A*). Inositol trisphosphate receptors, for example, are hormonally regulated Ca^{2+} channels. Many different hormones are known to induce inositol trisphosphate formation (43) and one of the targets for inositol trisphosphate is this group of related channel proteins that control Ca^{2+} entry into the cytoplasm. A similar signal cascade may control the entry of other messengers via channels or carriers that reside in caveolar membranes.

Caveolae cannot carry out any of the proposed functions unless they work properly. Recent evidence indicates that membrane cholesterol is essential for the normal function of this organelle (66). Lowering the cholesterol content of a cell causes disruption of the caveolar coat (7), unclustering of GPI-anchored proteins (7), and an inhibition of potocytosis (66). Most likely, cholesterol helps to organize caveolar components in late portions of the Golgi apparatus. This is where GPI anchors begin to cluster (67) and it is a region of the Golgi that contains caveolin (7, 68). This sterol may act like a lipid "glue" that holds together the protein and lipid components of the caveolar membrane. Thus, too much or too little cholesterol may influence the transmission of messages that originate in caveolae.

Outlook

The next step is to learn how caveolae help the cell to receive and interpret environmental stimuli. The vesicular architecture of the caveolae suggests the possibility that they are sites where continuous signals received from a hormonal or mechanical stimulus are converted into quantal signals that control cell behavior. The frequency, the concentration, and the type of messenger released from each caveola may encode the actual message that the cell receives. If caveolae are arranged into patches at specific locations on the cell surface, then these messages provide critical spatial information. And when these messages are passed between cells they become an important form of intercellular communication. Caveolae may be "message centers" for the cell.

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