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Caveolae and Arrhythmogenesis

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Introduction

The plasma membrane is a semipermeable barrier composed of a lipid bilayer, which defines the boundaries between the intracellular and extracellular space. However, unlike the historical model of the "fluid mosaic" for the plasma membrane, in which integral membrane proteins are evenly distributed and free to diffuse, current knowledge suggests a more heterogeneous view of the plasma membrane in which proteins and lipids are clustered within specialized vesicular microdomains termed lipid rafts. This novel scenario for the cell surface has dramatic implications in cardiomyocytes, where the plasma membrane contributes to the correct exchange of electrolytes and ions essential for membrane depolarization and excitation–contraction coupling (ECC), which are at the basis for normal cardiac function. All major ion channels implicated in the regulation of the cardiac action potential mainly localize at the cell surface and the cellular membrane provides these specialized proteins with a formidable interface capable of regulating the cellular response and the modulation of ion channel function upon various stimuli from the extracellular and intracellular environment. The structural support, protein turnover, and functional regulation of ion channels on the plasma membrane occur through self renewal and molecular adaptation, which represent the molecular plasticity of the plasmalemma and its ability to maintain an adequate composition to ensure an adequate cellular performance and response.

Cell trafficking through the endoplasmic reticulum (ER) and the Golgi apparatus, which represent a source of bilipid vesicles and posttranslationally modified proteins, is the mechanism that modifies the cell surface arrangement and intertalks with the main subcellular compartments.

Caveolae (little caves in Latin) are plasmalemmal organelles, deeply involved in vesicular transport from the ER and Golgi compartments to the cell surface. They are characterized by a peculiar "flask-like" shape and are virtually ubiquitous, but are particularly abundant in cells of the cardiovascular system, including endothelial cells, smooth muscle cells, macrophages, cardiomyocytes, and fibroblasts. In addition, many channels, instrumental for ion homeostasis and regulation of the cardiac action potential, have been colocalized within caveolae, suggesting an increasingly important role of these plasmalemmal organelles in trafficking and regulation of ion channel function.

In this chapter, we will discuss the overall function of caveolae and the relationship between caveolae and ion channel function as the base of their involvement in arrhythmogenesis. Caveolae and their major component caveolins may represent a novel molecular machinery implicated in ion channels function.

Caveolae: Discovery

The tale of caveolae started in 1953, when at the dawn of the biological application of electron microscopy (EM) to investigate the cellular

ultrastructure, George Palade observed large numbers of narrow-necked small plasma membrane invaginations in endothelial cells of the heart.[1] Palade named these invaginations "plasmalemmal vesicles." Two years later, in 1955, Yamada confirmed Palade's observation, identifying 50- to 100-nm "flask-shaped" invaginations of the plasma membrane in the gallbladder.[2] Yamada proposed the name caveolae, which in Latin means "little caves," to describe this morphological structure.[2] Since that initial finding, further EM studies have identified caveolae in most cell types, especially endothelial cells and adipocytes, but not in red blood cells, platelets, lymphocytes, some neuronal tissues, and CaCo-2 human fibroblasts.[3–5] All these ultrastructural investigations achieved a detailed morphological definition of caveolae.

In fact, caveolae have been defined as flask-shaped invaginations of the plasma membrane that are regular in shape and size but are distinct from the larger electron-dense clathrin-coated vesicles involved in various phenomena of endocytosis.

Caveolae: Tissue Distribution

Caveolae have been identified in most tissues and cell types, although at a different surface density. In particular, caveolae are intensely abundant in endothelial cells, adipocytes, and type I pneumocytes, which are the major constituents of lung alveoli.[6] However, a distinction can occur within the same cellular type, as has been observed in continuous endothelium, with a higher density of caveolae in contrast to the fenestrated endothelium with a more modest number of caveolae.[7] Although the absolute number of caveolae measured in the aforementioned endothelial cells has been challenged by studies using different tissue preparations, the ratio of caveolae in the two endothelial cell types appears to be conserved.[8–10]

Ultrastructural analysis has demonstrated that adipose tissue is the prevalent source of caveolae with up to 20% of the adipocyte plasma membrane occupied by caveolae.[11] Endothelial and pneumocyte cells of the lung are the second major source of lipid raft vesicles, with a relatively high abundance of these plasma membrane microdomains.[6] Thus caveolae can greatly increase the surface area of numerous cell types, an observation that lends credence to the original speculation that caveolae are involved in macromolecular transport and mechanotransduction events.

In addition, caveolae are also abundant in the skeletal muscle and in the cardiovascular system. In particular, caveolae are particularly abundant in the endothelial and smooth muscle cells of the vasculature as well as in the myocardium.[12]

Caveolae: Structure and Composition

The caveolae are smooth uncoated plasma membrane microdomains of 50–100 nm in diameter that are easily distinguishable from clathrin-coated vesicles. Caveolae can be isolated or can occur in clusters with a peculiar rosette formation deriving from the fusion of individual caveolae. In muscle cells, caveolae usually occur individually, although clusters have been reported.[13,14] In particular, EM analysis of myocardial and skeletal muscle caveolae demonstrated that when they cluster, caveolae occur in ordered linear arrays, suggesting a possible association with the cytoskeletal structure.[15–20] This observation was confirmed by studying caveolae in cell migration. In fact, caveolae are preferentially distributed to the retracting edge of the migrating cell.[21–23] Although extensive investigations revealed the various morphological subsets of caveolae, it is still unclear what the overall function of the archetypal caveolae organelle is, and, in particular, if each different subset of caveolae represents a specialized function.

The first clue to understanding what caveolae are and what their biological significance is comes from biochemical studies of caveolae composition.

Caveolae can be distinguished from the plasma membrane due to their peculiar composition; they are extremely rich in proteins and lipids such as cholesterol, ceramide, and diacylglycerol (DAG), sphingomyelin, and *cis*-unsaturated phospholipids such as phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol biphosphate.[24] Due to their high content in specific lipids, caveolae have also been defined as lipids rafts. Caveolae are formed

Table 14–1. Principal lipids and protein component of caveolae.

Lipids
Cholesterol
Ceramide
Sphingomyelin
Diacylglycerol (DAG)
cis-Unsaturated phospholipids
Phosphatidylserine
Phosphatidylethanolamine
Phosphatidylinositol
Phosphatidylinositol biphosphate (PIP_2)
Protein
Caveolins (-1, -2, and -3)
G proteins
G-α, G-β
Adrenergic receptor (AR)
β_1, β_2, β_3?
Cytokine receptors
Epidermal growth factor receptor (EGFR)
Platelet-derived growth factor receptor (PDGFR)
Insulin receptor
Bradykinin receptor
Endothelin receptor
GRB-2—growth factor receptor bound protein 2
SOS—son of sevenless
Signal transduction
Protein kinase C (PKC) α subunit
Phosphatidylinositol 3-kinase (PI3K)
Mitogen-activated protein kinase (MAPK)
eNOS, nNOS
Calmodulin
Cytoskeletal
Actin
Myosin
Ezrin

by the aggregation of glycosphingolipids and sphingomyelin in the Golgi apparatus, which are transported to the plasma membrane as concentrated units.[6] There are multiple types of rafts categories depending on the presence of specific marker proteins, ultrastructure data, and varying lipid compositions.[25] Caveolae are considered one subpopulation of lipid rafts because of their lipid constituents and biochemical characteristics (Table 14–1). However, their specific morphology and the presence of a scaffolding protein such as caveolin, the principal marker of the caveolae, distinguish them from other raft types.[26]

Caveolins: Markers for Caveolae

Caveolins not only are the main elements of caveolae, but they are the "cornerstone" of these lipid rafts.

Caveolins were identified quite fortuitously at the end of the 1980s, when investigators studying chicken embryonic fibroblasts transformed with Rous sarcoma virus (RSV) purified several phosphotyrosine-containing proteins, one of these being resistant to nonionic detergent extraction.[27] This 22-kDa protein, later identified in caveolae by EM analysis and thus termed caveolin, showed an immunohistochemical pattern consistent with parallel arrays of individual vesicles along the actin stress fibers, and with altered localization after cellular transformation.[28] The latter observation supported the hypothesis that the phosphorylation of caveolin occurred upon transformation with v-Src, suggesting a possible role of caveolin and caveolae in oncogenesis.[28]

In 1992, studies on cellular trafficking determined that caveolin (also called VIP21) localized to the Golgi apparatus, the plasma membrane, and membrane-bound vesicles.[29]

Caveolin Genes and Their Products

Caveolin-1 and -2

Caveolin is the general term used to define the three members of the three caveolin (*CAV1–3*) gene families identified so far, each encoded by a separate gene (Figure 14–1). The gene coding for caveolin-1 (*CAV1*) is the first gene to be identified, and is composed of three exons that are highly conserved in sequence and structure across species, while the gene coding for caveolin-2 (*CAV2*) was discovered by protein microsequencing of purified adipocyte caveolae membrane domains in which the polypeptide revealed a remarkable similarity to caveolin-1, but differed in some key conserved caveolin-1 residues.[30]

Finally, using the sequence of *CAV1* as a probe, Lisanti's group cloned the gene coding for caveolin-3 (*CAV3*), and characterized its expression to be mainly, if not exclusively, muscle specific.[31]

The *CAV1* and *CAV2* genes are expressed almost ubiquitously, and, in particular, they are coexpressed in most differentiated cells types, especially in adipocytes, endothelial cells, fibroblasts, and type I pneumocytes, but they are absent in striated muscle.[32]

Unfortunately, the caveolin family became more complex with the discovery of multiple isoforms for both caveolin-1 and -2. Caveolin-1

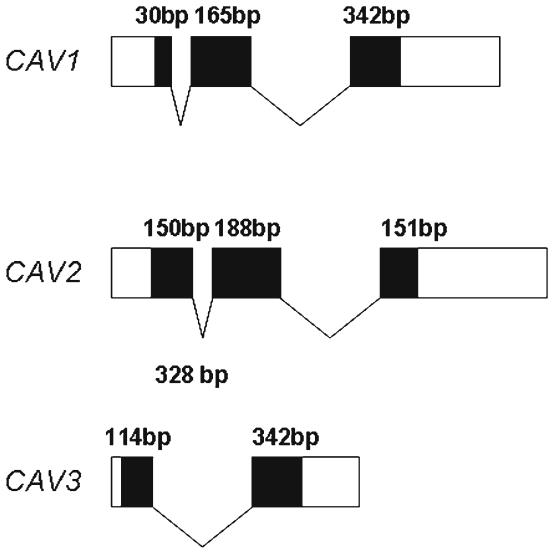

FIGURE 14–1. Schematic depiction of the caveolin gene family. Black boxes indicate the exon coding sequence for each caveolin family member. The numbers indicate the number of coding sequence nucleotides in each exon.

presents with two isoforms, termed α and β; caveolin-1α consists of residues 1–178, while caveolin-1β, originating from an alternate translation initiation site occurring at a methionine in position 32, contains residues 32–178, resulting in a protein ~3 kDa smaller in size.[32] Although shorter than caveolin-1α, caveolin-1β represents a functional isoform able to drive caveolae formation similar to caveolin-1α in *Drosophila melanogaster* Sf21 cells, which lack endogenous caveolae.[33]

The functional significance of these distinct caveolin-1 isoforms remains uncertain, although caveolin-1α appears to be localized primarily to deeply invaginated caveolae and more efficiently drive the formation of caveolae than caveolin-1β.[34,20]

Three isoforms have been identified for caveolin-2, the full-length caveolin-2α and two alternate splice variants, called caveolin-2β and caveolin-2γ (see Figure 14–3 later), which show a subcellular distribution distinct from caveolin-2α, although their functional significance is largely unknown.[6]

Caveolin-3

The tissue distribution of CAV3 has been intensely studied in mouse, in which CAV3 expression appears to be restricted to differentiated skeletal components and cardiomyocytes, while it is absent in nonproliferating C2C12 skeletal murine myoblasts compared to the proliferating precursor myoblasts, which express both caveolin-1 and -2.[35] In addition, caveolin-3 levels are intimately associated with muscle development, and experiments on the differentiation of C2C12 skeletal myoblasts in culture showed CAV3 upregulation,[36] while treatment with a *CAV3* antisense prevented myotube fusion *in vitro*.[37]

Caveolin-3 has been shown to function in a manner similar to caveolin-1, with which it shares high protein homology. In fact, caveolin-3 is connected to the sarcolemma and modulates the function of the dystrophin glycoprotein complex (DGC), the major protein ensemble linking the contractile apparatus to the plasma membrane in striated muscle, and is associated with a variety of muscular dystrophies with cardiac involvement and primary heart diseases all presenting with frequent arrhythmias.[38–40] Dystrophin, as well as several members of the DGC including α-sarcoglycan and β-dystroglycan, cofractionate with caveolin-3 in cultured mouse C2C12 myocytes.[36] In addition, coimmunoprecipitation (Co-IP) experiments demonstrated that dystrophin forms a stable complex with caveolin-3 and that dystrophin and caveolin-3 colocalize.[41] In addition, since the WW-like domain of caveolin-3 binds the C-terminus of β-dystroglycan, a region containing a PPXY motif, it is possible that caveolin-3 competes with dystrophin for the binding to β-dystroglycan; this will inhibit dystrophin binding with subsequent reduced dystrophin presentation to the sarcolemma.[41] In fact, in muscle biopsies from patients suffering from Duchenne muscular dystrophy (DMD) with progressive muscle weakness, respiratory failure and the development of dilated cardiomyopathy (DCM) associated with arrhythmias, caveolin-3 is shown to be upregulated along with an increased number and size of caveolae on the plasma membrane.[42,43] Similarly, caveolin-3 overexpression and increased caveolae number and size also occur in dystrophin-deficient *mdx* mice.[43,44]

Caveolins and Animal Models

One of the first experimental approaches to study the unknown role of a known protein is to generate an animal model in which the target gene has

been ablated. Knockout mice have been generated for all known caveolins, but only the *CAV1*$^{-/-}$ and *CAV3*$^{-/-}$ mice have been particularly interesting in studying caveolin function in the biology of the myocardium.

It is interesting to note that all the caveolin-deficient mouse models generated (*CAV1*$^{-/-}$, *CAV2*$^{-/-}$, *CAV3*$^{-/-}$, *CAV1/3*$^{-/-}$ double knockout mice) are viable and fertile, despite the tissue distribution and the involvement in a variety of biological processes observed for caveolins.

CAV1$^{-/-}$ Mice

Two independent groups generated *CAV1* null mice. Drab *et al.*, in 2001, showed that upon targeted disruption of *CAV1*, the animals showed an absence of caveolae in all tissues usually expressing caveolin-1; this led to impaired nitric oxide and calcium signaling in the cardiovascular system, causing aberrations in endothelium-dependent relaxation, contractility, and maintenance of muscular tone.[45] In addition, the lungs of *CAV1*$^{-/-}$ mice exhibited alveolar thickening due to unrestrained endothelial cell proliferation and fibrosis.[45]

In the same year, Razani and colleagues generated viable and fertile caveolin-1 null mice demonstrating the same caveolar aberration previously affirmed and showing that absence of caveolin-1 could not prevent the formation of normal caveolae in tissues expressing caveolin-3, such as skeletal and cardiac muscles.[46] In addition, the *CAV1*$^{-/-}$ mouse demonstrated an almost complete loss of caveolin-2, confirming the role of caveolin-1 in heterooligomerization with caveolin-2, and the ability of caveolin-1 to recruit caveolin-2 from the Golgi to the plasma membrane.[45,46]

Although *CAV1* expression cannot be detectable in cardiomyocytes, it is surprising that *CAV1*$^{-/-}$ animals also demonstrate cardiovascular abnormalities including DCM, which was shown by transthoracic echocardiographic (TEE) analysis of 5-month-old *CAV1*$^{-/-}$ mice.[47] Similarly, another group analyzed *CAV1*$^{-/-}$ mice at age 2, 4, and 12 months by cardiac- gated magnetic resonance imagining (MRI) and TEE, revealing progressive concentric left ventricular hypertrophy, severe right ventricular dilation, and sudden cardiac death (SCD).[48] The SCD phenotype in *CAV1*$^{-/-}$ mice has been associated with the occurrence of hypertrophic cardiomyopathy (HCM), which is classically associated with arrhythmias and premature sudden death secondary to the progressive disorganization of cardiac tissue.[49,50]

CAV3$^{-/-}$ Mice

The caveolin-3 null mouse demonstrates the absence of muscular caveolae, while expression of caveolin-1 and -2 was normal in nonmuscle cells.[51,52] The animals developed a muscular dystrophy phenotype similar to limb girdle muscular dystrophy (LGMD) observed in patients with LGMD-1C. In addition, *CAV3*$^{-/-}$ demonstrates a cardiomyopathy phenotype similar to that described above in *CAV1*$^{-/-}$ animals.

Using cardiac-gated MRI and TEE, Woodman *et al.* found that at 4 months of age, *CAV3*$^{-/-}$ showed significant cardiac hypertrophy and reduced fractional shortening.[53]

Cardiac histological analysis revealed perivascular fibrosis, myocyte hypertrophy, and cellular infiltration, derived from the exclusion of the DGC from lipid rafts and hyperactivation of the Ras-p42/44 mitogen-activated protein (MAP) kinase cascade in *CAV3* null cardiac tissue.[53] These changes are consistent with the known role of p42/44 MAP kinase activation in cardiac hypertrophy and the role of caveolin-3 as a negative regulator of the Ras-p42/44 MAP kinase cascade, similar to that of caveolin-1.[53]

Interestingly, the caveolin-3 T63S mutation has been recently identified in patients with HCM and DCM, although the exact pathological mechanism is unknown.[54]

CAV1/3$^{-/-}$ Mice

The generation of truly caveolae-deficient animals was accomplished by interbreeding Cav-1 and Cav-3 null mice, to produce caveolin-1/3 double knockout mice (Cav-1/3 dKO). Surprisingly, Cav-1/3 dKO mice are viable and fertile, despite a complete absence of morphologically identifiable caveolae in muscle and nonmuscle cells.[55] Additionally, these mice are deficient in the expression of all three caveolin family members, as caveolin-2 is unstable and degraded in the absence of caveolin-1.

The dKO mice present with a severe cardiomyopathy phenotype, already evident at 2 months of age, with more pronounced left ventricular wall thickness and ventricular septum thickness, accompanied by dramatic cardiac hypertrophic, interstitial inflammation, perivascular fibrosis, and myocyte necrosis.[55] Thus the combined ablation of both caveolin-1 and -3 profoundly alters cardiac structure and function leading to a remarkable risk of SCD.

Caveolins: Biochemistry

Caveolin Protein Domains

Regardless of isoform variability, all three caveolin proteins contain some protein motifs highly conserved throughout evolution such as the eight amino acid FEDVIAEP, which constitutes the "caveolin signature sequence" and is localized proximal to the N-terminus of the protein.[6]

Another characteristic protein domain present in caveolins is the "scaffolding domain," a motif proximal to the hydrophobic transmembrane domain, which interacts with different signal transduction molecules (Figure 14–2).[56,57] Although researchers recognize the importance of each protein domain present in caveolins, it appears that proper caveolin function, such as lipid raft association, extrusion from the Golgi apparatus, and caveolae targeting, greatly if not solely depends on the overall protein folding and tertiary structure.[58]

Despite the high amino acid sequence similarity among the different caveolins, human caveolin-1 and -3 share the highest protein homology with ~65% identity and ~85% similarity, while caveolin-2 shares only ~38% identity and ~58% similarity to human caveolin-1. This remarkable resemblance between caveolin-1 and -3 and their divergence from caveolin-2 may explain why both caveolin-1 and -3, but not caveolin-2, can form caveolae alone or in association with other caveolins in cells lacking caveolae structures such as Madin–Darby canine kidney (MDCK) and epithelial Caco-2 cells.[59] In addition, caveolin-1 and -3, but not caveolin-2, undergo an irreversible posttranslational modification inserting a palmitoyl acid molecule on a cysteine residue at their C-terminus.[60] It is believed that palmitoylation of caveolin-1 and -3 stabilizes the caveolin oligomers and increases the association with the membrane through hydrophobic domains.[61,62] The expression of both caveolin-1 and -3 is also necessary to stabilize caveolin-1/-2 and caveolin-3/-2 heterodimers and to ensure the correct membrane localization of caveolin-2.[63]

Caveolin Protein Topology

Both the N- and the C-termini of the prototypical caveolin are localized to the cytoplasm, while the central hydrophobic domains are "embedded" in the plasma membrane, and no extracellular segments were detected (Figure 14–3).[64] This spatial conformation allows the N-terminal phosphorylation of caveolin-1 by the α subunit of protein kinase C (PKCα) and, as previously mentioned, the palmitoylation at the C-terminus of both caveolin-1 and -3.[33,60] The ultimate protein structure and topology of caveolins originate in the ER, where caveolins are believed to be introduced in the plasma membrane and form a hairpin loop configuration through a transmembrane domain containing 32 hydrophobic amino acids (residues 102–134), which prevents caveolins from completely extending across the plasma membrane.[65]

In addition, the scaffolding domain (residues 82–101) proximal to the N-terminus and the segment 135–150 proximal to the C-terminus are tightly connected to the membranes.[6] Caveolin-1 constructs containing residues 1–101 are

Figure 14–2. Prototypic caveolin structure and critical protein motifs.

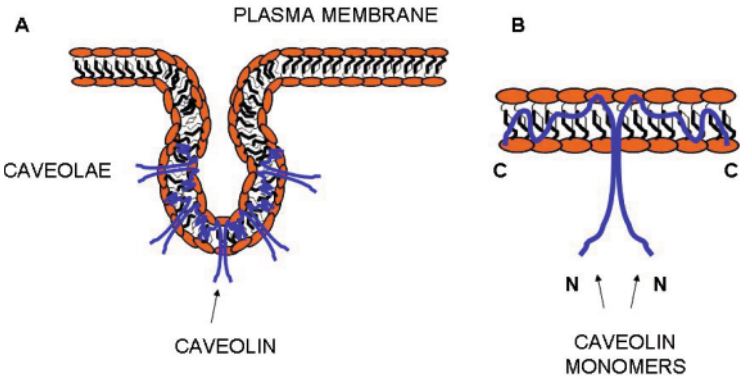

FIGURE 14–3. Caveolae and caveolin topology. (A) Caveolae form plasma membrane invaginations that differ from the clathrin-coated endocytic vesicles. (B) Caveolins exhibit both N- and C-terminus ends into the cytoplasm, although their central portion is tightly embedded into the plasma membrane, where most of the interactions with ion channels are hypothesized to occur. Caveolin topology dramatically determines the role of these proteins in lipid raft internalization and functional modulation of its binding partners.

sufficient for membrane localization, while segment 1–82 remained in the cytoplasm.[66] It has been established that the (KYWFYR) motif was sufficient to confer membrane localization to a green fluorescent protein (GFP) fusion protein.[67] In addition, residues 135–150 include a unique Golgi-targeting sequence, and a construct including only this segment of caveolin shows a prevalent Golgi localization.[68,69]

Caveolin-1 contains an oligomerization domain (residues 61–101), which promotes the homooligomerization of up to 16 individual caveolin-1 molecules,[56] while a second stage of oligomerization occurs during transport from the *trans*-Golgi to the plasma membrane, thus forming a large network of caveolin.[70] In contrast, caveolin-2 is unable to generate large homooligomeric complexes and so develop caveolae by itself,[30] while it can form heterooligomers with caveolin-1 in the ER to stabilize caveolin-2 against proteasomal degradation and consent to its transport from the Golgi apparatus to the plasma membrane.[46,71–73]

Similar to caveolin-1, caveolin-3 forms large homooligomeric complexes in striated muscle, and Co-IP and membrane cofractionation studies in rat neonatal cardiomyocytes demonstrated that caveolin-3 associates with caveolin-2 in heterooligomers as occurs for caveolin-1/-2.[74,75]

Caveolae: Function

Caveolae and Lipid Regulation

Due to their high lipid content, it is not surprising that caveolae are involved in lipid metabolism, in particular in the regulation of cholesterol.

Studies employing cholesterol-binding agents such as filipin and nystatin resulted in the disruption of caveolae, suggesting the importance of cellular cholesterol balance on caveolar structure.[26] In addition, an increase in cholesterol levels upregulates caveolin-1 expression through specific binding to two steroid regulatory elements (SRE) on the caveolin-1 promoter, while decreased cholesterol levels result in *CAV1* downregulation.[76,77] Moreover, oxidation of cholesterol into cholesterone by cholesterol oxidase alters the cellular cholesterol balance and results in caveolin-1 internalization from the plasma membrane to the ER and the Golgi apparatus.[78]

Caveolin-1 transports newly synthesized cholesterol from the ER to membrane caveolae, and than to plasma high-density lipoproteins (HDL), while extracellular cholesterol primarily enters cells via clathrin-mediated endocytosis of low-density lipoproteins (LDL).[79] Therefore, caveolae may represent the principal location for cholesterol exchanged between HDL and the cell membrane.

Caveolae and Endocytosis

Caveolae are believed to play a role in endocytosis, oncogenesis, and internalization of pathogenic bacteria.[80]

Caveolae represent a clathrin-independent mechanism of endocytosis for the turnover of adhesive complexes, and since their discovery, they were believed to play a role in endocytosis because they protrude from the plasma membrane into the cytoplasm. However, there is conflicting evidence involving caveolae in constitutive endocytic trafficking. Caveolin-1 cloned as N- and

C-terminus GFP fusion protein and expressed in HeLa, A431, and MDCK cells demonstrated that caveolae require both cholesterol and an intact actin cytoskeleton to maintain their integrity.[81] This may support the hypothesis that caveolae are intimately connected to the actin network. Conversely, Pelkmans and colleagues demonstrated that caveolae play an instrumental role in the internalization of SV40 viral particles, and that this internalization was triggered by molecular signals from the virus, able to activate the caveolae otherwise "dormant" in the stable state previously described.[82]

Caveolae and Signal Transduction

Caveolae play an essential role in signal transduction since biochemically purified caveolae contain multiple signaling molecules, including heterotrimeric G proteins, which in the myocardium are involved in sympathetic tone response though β-adrenergic receptors (β-AR).[83,84] In addition, other signaling factors such as H-Ras, Src-family kinases, and endothelial nitric oxide synthase (eNOS) have been isolated from caveolae.[6]

Other pathways important in cardiac remodeling have been associated with caveolae. For instance, it has been shown that key components of the Ras-p42/44 MAP kinase cascade (MEK and ERK), involved in cardiac hypertrophy, reside within caveolae and are negatively regulated by a direct interaction with caveolin.[36,85–89]

Transient transfection of caveolin dramatically inhibits Raf-1/MEK/ERK and p42/44 MAP kinase signaling, while *in vitro* expression of the caveolin scaffolding domain inhibits the kinase activity of MEK-1 and ERK-2.[83,85] The finding of such a concentration of signaling molecules in caveolae suggests that caveolins act as scaffolding proteins, through the amino acid 82–101 motif, to concentrate and localize these elements for a rapid and specific cell response.[83]

Caveolae and Ion Channels

Being highly expressed in excitable cells of the nervous system, skeletal muscle, and myocardium, a variety of ion channels are localizing to caveolae (Table 14–2). However, it took more than a decade to obtain evidence that these plasmalemmal vesicles not only cluster and scaffold a plethora of cell membrane proteins including signaling molecules, but also anchor, distribute, transport, and possibly modify ion channels on the cell surface.

Table 14–2. Ion channels localizing to cardiac caveolae.

Cardiac ion channels
Cardiac sodium channel (Nav1.5)[94]
L-type Ca^{2+} (Cav1.2a)[123,124]
Voltage-gated Kv1.5[91]
Pacemaker channel HCN4[125]
Na^{+}–Ca^{2+} exchanger (NCX)[126]

In 2000, Martens and colleagues identified the relationship of Shaker-like K^{+} channels (Kv2.1) to noncaveolar lipid rafts[90]; the role of these vesicles in Kv2.1 function regulation was elucidated a year later by the same laboratory, showing that cyclodextrin treatment not only led to cholesterol depletion, but also caused a significant shift in steady-state inactivation of the Kv2.1 channel.[91] This first evidence that ion channels localize to lipid rafts suggested that other ion channels may be present in caveolae and that caveolins may regulate ion channel function.

In fact, in 2001 Martens and colleagues localized the voltage-gated potassium channel Kv1.5 to caveolae in transfected fibroblasts.[91] It is interesting to note that Kv1.5 binds the major protein of the Z-line, α-actinin-2,[92] which is connected to the actin network, supporting the concept that caveolae interact with the cytoskeleton (Figure 14–4).

The Kv1.5 channels are expressed in the intercalated disk of human cardiomyocytes and are associated with connexin 43 and N-cadherin.[93] Cytoskeletal alteration using cytochalasin D, which disrupts the filament actin (F-actin), leads to a massive increase in I_{K+}, while the phenomenon was totally abolished when the cells were preincubated with phalloidin, an F-actin-stabilizing agent.[92]

Probably the most intriguing discovery concerning the relationship between cardiac ion channels and caveolae was that the *SCN5A*-coded voltage-gated Na^{+} channels (Nav1.5) have been reported to localize to "caveolin-rich membranes" in cardiac myocytes.[94] In addition, the α subunit of the L-type Ca^{2+} channel (Cav1.2) was originally found in caveolin-enriched membranes in smooth muscle.[95]

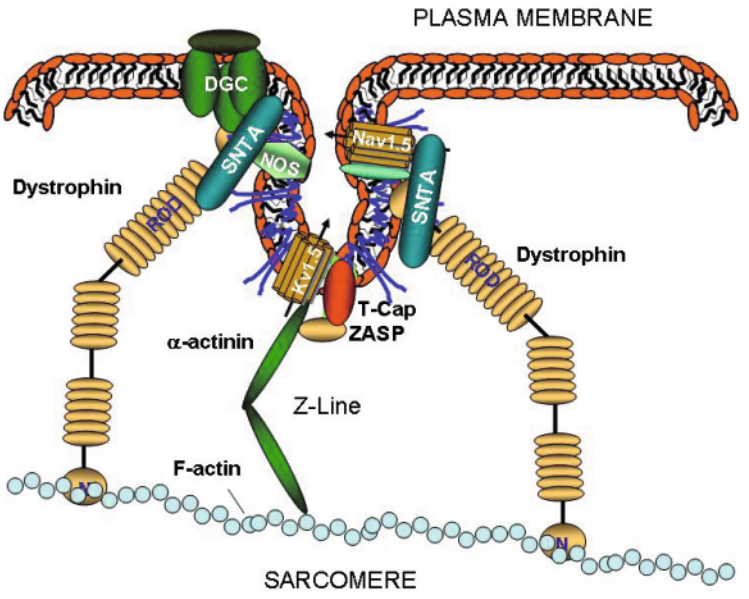

FIGURE 14–4. Cytoskeleton, caveolae, and caveolins. Caveolae are intimately associated with the cytoskeleton of the cardiomyocytes, and are ultimately linked to the contractile apparatus of the myocardial cells. DGC, dystrophin glycoprotein complex; SNTA, α_1-syntrophin.

It is interesting to note that mutations in both Nav1.5 and Cav1.2 have been associated with a primary arrhythmogenic disorder such as the long QT syndrome (LQTS) in human subjects.[96–98]

Similarly, skeletal muscle caveolae have also been found to contain the 1,4-dihydropyridine receptor (DHPR or RyR) in the subsarcolemmal region of the myofibers.[99] In addition, caveolin expression induces Cl_2 channel function.[100]

It is hypothesized that ion channels, which localize to caveolae probably through the scaffolding domain of caveolin-3, reach these lipid rafts after posttranslational modifications such as palmitoylation and myristoylation, or by glycophosphatidylinositol (GPI) membrane anchors, mostly occurring in the Golgi apparatus.[101]

It is still unclear whether caveolin, which can independently drive caveolae formation, can organize membrane lipids and stabilize transient membrane rafts, so that channel proteins might perform as a focal point in raft development.

Alternatively, the association between ion channels and caveolae may not occur through protein/lipid interactions but rather through protein/protein interactions with the direct involvement of caveolins such as caveolin-3, or the direct binding of other caveolin-associated proteins such as the PDZ motif containing protein PSD95 (postsynaptic density protein 95), which has been reported to associate with low-density lipid rafts in mammalian cells.[102] However, the PDZ protein domain may be only one player in the localization of ion channels to raft domains. In fact, Kv2.1 channels do not contain standard PDZ binding sequences,[103] and removal of the PDZ binding motif from the Kv1.5 channel does not prevent its lipid raft association.[91]

Caveolae: Cytoskeleton and Ion Channels

It is interesting to note that many key structural elements, such as the Z-band alternatively spliced PDZ motif protein (ZASP), signaling molecules such as eNOS, and an Nav1.5 modulator, such as α_1-syntrophin (SNTA), contain a PDZ domain, and both eNOS and SNTA also localize to caveolae and directly bind caveolin-3.[104–107] In addition, all the aforementioned proteins localize or are associated with the sarcolemma via direct or indirect interaction with the large protein dystrophin and the DGC, thus modulating ion channel regulation.[38–40]

Caveolin-3; Cytoskeleton and Ion Channels

Syntrophins are known to contain two pleckstrin homology (PH) domains, a PDZ domain, and a syntrophin-unique (SU) at its C-terminus. Syn-

trophins directly bind dystrophin through their PH domain distal to the N-terminus and the highly conserved SU domain.[108,109] The PH domain proximal to the N-terminus and the PDZ domain interact with other membrane components such as phosphatidylinositol-4,5-bisphosphate (PIP_2),[110] neuronal NOS (nNOS),[111] aquaporin-4,[112] stress-activated protein kinase-3,[113] and Nav1.5,[114] thereby linking all these molecules to the dystrophin complex (Figure 14–4).[115]

In addition, syntrophins bind the C-terminus of Nav1.5 through its PDZ domain, regulating the gating properties of the sodium channel.[116]

Remarkably, caveolae not only cluster syntrophins, but caveolin-3 directly binds syntrophin[107] as well as other important factors such as the Na^+–Ca^{2+} exchanger,[117] the L-type Ca^{2+} channel,[107] eNOS and nNOS, and the DGC (Figure 14–4).[104–106] Therefore, the association of caveolin-3 with α_1-syntrophin, through its binding to F-actin, nNOS, and Nav1.5, appears to be involved in regulating structural and electrical functions as well as signal transduction in heart failure.[107]

Caveolae and Ion Channel Regulation

As previously discussed, due to their physical localization into caveolae and their direct binding to caveolins, it should not be surprising that ion channels might be functionally regulated by lipid rafts such as caveolae. However, the precise mechanism by which caveolins modulate ion channel function, either via direct protein/lipid or protein/protein interactions, as well as through indirect signaling mechanisms, remains unknown.

It is known that caveolin-3 is associated with G protein-coupled receptors (GPCR), which upon stimulation of sympathetic adrenergic receptors triggers G protein-mediated activation of both cAMP-dependent protein kinase A (PKA) and phospholipase C (PLC)-activated protein kinase C (PKC). Both PKA and PKC are known to directly phosphorylate Nav1.5 and modulate its function.[118]

In addition, Kv channels can be phosphorylated by tyrosine kinase such as c-Src, present particularly in caveolae leading to a reduced I_{K+}.[24,119] The two proteins interact through the N-terminal proline-rich sequence of the Kv1.5 channel and the Src homology region 3 (SH3) of Src.[24]

It is also known that CAV3 binds calmodulin (CaM), which in response to a regional increase of Ca^{2+} concentration binds SCN5A increasing its slow-inactivation kinetics.[118] Moreover, SNTA binds Nav1.5 through its PDZ domain at the C-terminus of the sodium channel, altering its gating properties, markedly shifting its activation kinetics, and reducing Na^+-current availability. Therefore, it is possible that mutated CAV3 could modulate ion channel activity directly or via altered structural support on the plasma membrane resulting in uncoupling of ion channels from the cytoskeleton.

Also altered membrane cholesterol can directly modulate ion channel function.[120–122]

All this evidence points toward an increasingly important role of caveolae and caveolin-3 in particular in the regulation of gating, activation/inactivation kinetics, as well as conductance of cardiac ion channels.

Conclusions and Future Directions

Caveolae are peculiar plasma membrane vesicles with an important biological function and role in various cellular processes. The discovery of their main constituents, the caveolins, suggested that caveolae and caveolins are implicated in a variety of important cellular activity, including vesicular trafficking, cholesterol homeostasis, signal transduction, and ion channel regulation.

Since caveolins act as oligomeric scaffolding elements responsible for the clustering and localization of numerous proteins, it was not surprising to discover that caveolin aberration is associated with human diseases.

The ultrastructural, genetic, and molecular analysis of caveolae and caveolins both *in vitro* and *in vivo* provided increasing evidence that these components were instrumental in a range of pathological processes such as muscular dystrophy, cardiac dysfunction, and probably arrhythmias.

The generation of caveolin-deficient mice, and in particular the *CAV3*$^{-/-}$, provides further evidence that although caveolins are not indispensable for life, caveolin alterations are significant in the pathogenesis of human striated muscle abnormalities such as muscular dystrophies and cardiomyopathies, often associated with

arrhythmogenesis. Only recently have investigators accumulated increasing evidence of the important role of caveolins in arrhythmogenesis.

There is still a need for further investigation of caveolin-3 abnormalities in patients with primary arrhythmogenic syndromes such as LQTS and Brugada syndrome (BrS). This may help in understanding pivotal protein domains for ion channel function regulation and in designing novel therapeutic approaches to prevent lethal arrhythmic events such as sudden cardiac death.

References

1. Palade GE. An electron microscope study of the mitochondrial structure. *J Histochem Cytochem* 1953;1(4):188–211.
2. Yamada E. The fine structure of the gall bladder epithelium of the mouse. *J Biophys Biochem Cytol* 1955;1(5):445–458.
3. Fra AM, Williamson E, Simons K, Parton RG. De novo formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Proc Natl Acad Sci USA* 1995;92(19):8655–8659.
4. Gorodinsky A, Harris DA. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. *J Cell Biol* 1995;129(3):619–627.
5. Mirre C, Monlauzeur L, Garcia M, Delgrossi MH, Le Bivic A. Detergent-resistant membrane microdomains from Caco-2 cells do not contain caveolin. *Am J Physiol* 1996;271(3Pt. 1):C887–894.
6. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev* 2004;84(4):1341–1379.
7. Simionescu M, Simionescu N, Palade GE. Morphometric data on the endothelium of blood capillaries. *J Cell Biol* 1974;60(1):128–152.
8. McGuire PG, Twietmeyer TA. Morphology of rapidly frozen aortic endothelial cells. Glutaraldehyde fixation increases the number of caveolae. *Circ Res* 1983;53(3):424–429.
9. Noguchi Y, Shibata Y, Yamamoto T. Endothelial vesicular system in rapid-frozen muscle capillaries revealed by serial sectioning and deep etching. *Anat Rec* 1987;217(4):355–360.
10. Wood MR, Wagner RC, Andrews SB, Greener DA, Williams SK. Rapidly-frozen, cultured, human endothelial cells: An ultrastructural and morphometric comparison between freshly-frozen and glutaraldehyde prefixed cells. *Microcirc Endothelium Lymphatics* 1986;3(5–6):323–358.
11. Fan JY, Carpentier JL, van Obberghen E, Grunfeld C, Gorden P, Orci L. Morphological changes of the 3T3-L1 fibroblast plasma membrane upon differentiation to the adipocyte form. *J Cell Sci* 1983;61:219–230.
12. Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circ Res* 2004;94(11):1408–1417.
13. Ishikawa H. Formation of elaborate networks of T-system tubules in cultured skeletal muscle with special reference to the T-system formation. *J Cell Biol* 1968;38(1):51–66.
14. Parton RG, Way M, Zorzi N, Stang E. Caveolin-3 associates with developing T-tubules during muscle differentiation. *J Cell Biol* 1997;136(1):137–154.
15. Gabella G, Blundell D. Effect of stretch and contraction on caveolae of smooth muscle cells. *Cell Tissue Res* 1978;190(2):255–271.
16. Sawada H, Ishikawa H, Yamada E. High resolution scanning electron microscopy of frog sartorius muscle. *Tissue Cell* 1978;10(1):179–190.
17. Frank JS, Beydler S, Kreman M, Rau EE. Structure of the freeze-fractured sarcolemma in the normal and anoxic rabbit myocardium. *Circ Res* 1980;47(1):131–143.
18. Severs NJ. Plasma membrane cholesterol in myocardial muscle and capillary endothelial cells. Distribution of filipin-induced deformations in freeze-fracture. *Eur J Cell Biol* 1981;25(2):289–299.
19. Izumi T, Shibata Y, Yamamoto T. Striped structures on the cytoplasmic surface membranes of the endothelial vesicles of the rat aorta revealed by quick-freeze, deep-etching replicas. *Anat Rec* 1988;220(3):225–232.
20. Fujimoto T. Cell biology of caveolae and its implication for clinical medicine. Nagoya *J Med Sci* 2000;63(1–2):9–18.
21. Isshiki M, Ando J, Korenaga R, *et al*. Endothelial Ca2+ waves preferentially originate at specific loci in caveolin-rich cell edges. *Proc Natl Acad Sci USA* 1998;95(9):5009–5014.
22. Isshiki M, Ando J, Yamamoto K, Fujita T, Ying Y, Anderson RG. Sites of Ca(2+) wave initiation move with caveolae to the trailing edge of migrating cells. *J Cell Sci* 2002;115(Pt. 3):475–484.
23. Parat MO, Anand-Apte B, Fox PL. Differential caveolin-1 polarization in endothelial cells during migration in two and three dimensions. *Mol Biol Cell* 2003;14(8):3156–3168.
24. Anderson RG. The caveolae membrane system. *Annu Rev Biochem* 1998;67:199–225.
25. Edidin M. The state of lipid rafts: From model membranes to cells. *Annu Rev Biophys Biomol Struct* 2003;32:257–283.

26. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. *Cell* 1992;68(4):673–682.
27. Glenney JR Jr, Kindy MS, Zokas L. Isolation of a new member of the S100 protein family: Amino acid sequence, tissue, and subcellular distribution. *J Cell Biol* 1989;108(2):569–578.
28. Glenney JR Jr. Tyrosine phosphorylation of a 22-kDa protein is correlated with transformation by Rous sarcoma virus. *J Biol Chem* 1989;264(34): 20163–20166.
29. Kurzchalia TV, Dupree P, Parton RG, *et al*. VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. *J Cell Biol* 1992;118(5):1003–1014.
30. Scherer PE, Okamoto T, Chun M, Nishimoto I, Lodish HF, Lisanti MP. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc Natl Acad Sci USA* 1996;93(1): 131–135.
31. Tang Z, Scherer PE, Okamoto T, *et al*. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem* 1996;271(4):2255–2261.
32. Stan RV. Structure of caveolae. *Biochim Biophys Acta* 2005;1746(3):334–348.
33. Li S, Song KS, Koh SS, Kikuchi A, Lisanti MP. Baculovirus-based expression of mammalian caveolin in Sf21 insect cells. A model system for the biochemical and morphological study of caveolae biogenesis. *J Biol Chem* 1996;271(45):28647–28654.
34. Scherer PE, Tang Z, Chun M, Sargiacomo M, Lodish HF, Lisanti MP. Caveolin isoforms differ in their N-terminal protein sequence and subcellular distribution. Identification and epitope mapping of an isoform-specific monoclonal antibody probe. *J Biol Chem* 1995;270(27):16395–16401.
35. Way M, Parton RG. M-caveolin, a muscle-specific caveolin-related protein. *FEBS Lett* 1995;376(1–2): 108–112.
36. Song KS, Scherer PE, Tang Z, *et al*. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J Biol Chem* 1996;271(25):15160–15165.
37. Galbiati F, Volonte D, Engelman JA, Scherer PE, Lisanti MP. Targeted down-regulation of caveolin-3 is sufficient to inhibit myotube formation in differentiating C2C12 myoblasts. Transient activation of p38 mitogen-activated protein kinase is required for induction of caveolin-3 expression and subsequent myotube formation. *J Biol Chem* 1999;274(42):30315–30321.
38. Ahn AH, Yoshida M, Anderson MS, *et al*. Cloning of human basic A1, a distinct 59-kDa dystrophin-associated protein encoded on chromosome 8q23–24. *Proc Natl Acad Sci USA* 1994;91(10): 4446–4450.
39. Adams ME, Dwyer TM, Dowler LL, White RA, Froehner SC. Mouse alpha 1- and beta 2-syntrophin gene structure, chromosome localization, and homology with a discs large domain. *J Biol Chem* 1995;270(43):25859–25865.
40. Piluso G, Mirabella M, Ricci E, *et al*. Gamma1- and gamma2-syntrophins, two novel dystrophin-binding proteins localized in neuronal cells. *J Biol Chem* 2000;275(21):15851–15860.
41. Sotgia F, Lee JK, Das K, *et al*. Caveolin-3 directly interacts with the C-terminal tail of beta-dystroglycan. Identification of a central WW-like domain within caveolin family members. *J Biol Chem* 2000;275(48):38048–38058.
42. Bonilla E, Fischbeck K, Schotland DL. Freeze-fracture studies of muscle caveolae in human muscular dystrophy. *Am J Pathol* 1981;104(2):167–173.
43. Repetto S, Bado M, Broda P, *et al*. Increased number of caveolae and caveolin-3 overexpression in Duchenne muscular dystrophy. *Biochem Biophys Res Commun* 1999;261(3):547–550.
44. Vaghy PL, Fang J, Wu W, Vaghy LP. Increased caveolin-3 levels in mdx mouse muscles. *FEBS Lett* 1998;431(1):125–127.
45. Drab M, Verkade P, Elger M, *et al*. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 2001; 293(5539):2449–2452.
46. Razani B, Engelman JA, Wang XB, *et al*. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem* 2001;276(41):38121–38138.
47. Zhao YY, Liu Y, Stan RV, *et al*. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci USA* 2002;99(17):11375–11380.
48. Park DS, Cohen AW, Frank PG, *et al*. Caveolin-1 null (–/–) mice show dramatic reductions in life span. *Biochemistry* 2003;42(51):15124–15131.
49. Maron BJ. Cardiology patient pages. Hypertrophic cardiomyopathy. *Circulation* 2002;106(19):2419–2421.
50. Tin LL, Beevers DG, Lip GY. Hypertension, left ventricular hypertrophy, and sudden death. *Curr Cardiol Rep* 2002;4(6):449–457.
51. Galbiati F, Engelman JA, Volonte D, *et al*. Caveolin-3 null mice show a loss of caveolae, changes in

the microdomain distribution of the dystrophin-glycoprotein complex, and T-tubule abnormalities. *J Biol Chem* 2001;276(24):21425–21433.
52. Hagiwara Y, Sasaoka T, Araishi K, *et al*. Caveolin-3 deficiency causes muscle degeneration in mice. *Hum Mol Genet* 2000;9(20):3047–3054.
53. Woodman SE, Park DS, Cohen AW, *et al*. Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. *J Biol Chem* 2002;277(41): 38988–38997.
54. Hayashi T, Arimura T, Ueda K, *et al*. Identification and functional analysis of a caveolin-3 mutation associated with familial hypertrophic cardiomyopathy. *Biochem Biophys Res Commun* 2004; 313(1):178–184.
55. Park DS, Woodman SE, Schubert W, *et al*. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. *Am J Pathol* 2002;160(6):2207–2217.
56. Sargiacomo M, Scherer PE, Tang Z, *et al*. Oligomeric structure of caveolin: Implications for caveolae membrane organization. *Proc Natl Acad Sci USA* 1995;92(20):9407–9411.
57. Schlegel A, Arvan P, Lisanti MP. Caveolin-1 binding to endoplasmic reticulum membranes and entry into the regulated secretory pathway are regulated by serine phosphorylation. Protein sorting at the level of the endoplasmic reticulum. *J Biol Chem* 2001;276(6):4398–4408.
58. Ren X, Ostermeyer AG, Ramcharan LT, Zeng Y, Lublin DM, Brown DA. Conformational defects slow Golgi exit, block oligomerization, and reduce raft affinity of caveolin-1 mutant proteins. *Mol Biol Cell* 2004;15(10):4556–4567.
59. Vogel U, Sandvig K, van Deurs B. Expression of caveolin-1 and polarized formation of invaginated caveolae in Caco-2 and MDCK II cells. *J Cell Sci* 1998;111(Pt. 6):825–832.
60. Dietzen DJ, Hastings WR, Lublin DM. Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. *J Biol Chem* 1995;270(12): 6838–6842.
61. Monier S, Dietzen DJ, Hastings WR, Lublin DM, Kurzchalia TV. Oligomerization of VIP21-caveolin in vitro is stabilized by long chain fatty acylation or cholesterol. *FEBS Lett* 1996;388(2–3):143–149.
62. Parat MO, Fox PL. Palmitoylation of caveolin-1 in endothelial cells is post-translational but irreversible. *J Biol Chem* 2001;276(19):15776–15782.
63. Razani B, Woodman SE, Lisanti MP. Caveolae: From cell biology to animal physiology. *Pharmacol Rev* 2002;54(3):431–467.
64. Dupree P, Parton RG, Raposo G, Kurzchalia TV, Simons K. Caveolae and sorting in the trans-Golgi network of epithelial cells. *EMBO J* 1993;12(4): 1597–1605.
65. Monier S, Parton RG, Vogel F, Behlke J, Henske A, Kurzchalia TV. VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol Biol Cell* 1995;6(7): 911–927.
66. Schlegel A, Schwab RB, Scherer PE, Lisanti MP. A role for the caveolin scaffolding domain in mediating the membrane attachment of caveolin-1. The caveolin scaffolding domain is both necessary and sufficient for membrane binding in vitro. *J Biol Chem* 1999;274(32):22660–22667.
67. Woodman SE, Schlegel A, Cohen AW, Lisanti MP. Mutational analysis identifies a short atypical membrane attachment sequence (KYWFYR) within caveolin-1. *Biochemistry* 2002;41(11):3790–3795.
68. Luetterforst R, Stang E, Zorzi N, Carozzi A, Way M, Parton RG. Molecular characterization of caveolin association with the Golgi complex: Identification of a cis-Golgi targeting domain in the caveolin molecule. *J Cell Biol* 1999;145(7):1443–1459.
69. Schlegel A, Pestell RG, Lisanti MP. Caveolins in cholesterol trafficking and signal transduction: Implications for human disease. *Front Biosci* 2000;5:D929–937.
70. Song KS, Tang Z, Li S, Lisanti MP. Mutational analysis of the properties of caveolin-1. A novel role for the C-terminal domain in mediating homo-typic caveolin-caveolin interactions. *J Biol Chem* 1997;272(7):4398–4403.
71. Mora R, Bonilha VL, Marmorstein A, *et al*. Caveolin-2 localizes to the Golgi complex but redistributes to plasma membrane, caveolae, and rafts when co-expressed with caveolin-1. *J Biol Chem* 1999;274(36):25708–25717.
72. Parolini I, Sargiacomo M, Galbiati F, *et al*. Expression of caveolin-1 is required for the transport of caveolin-2 to the plasma membrane. Retention of caveolin-2 at the level of the golgi complex. *J Biol Chem* 1999;274(36):25718–25725.
73. Scherer PE, Lewis RY, Volonte D, *et al*. Cell-type and tissue-specific expression of caveolin-2. Caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex in vivo. *J Biol Chem* 1997; 272(46):29337–29346.
74. Rybin VO, Grabham PW, Elouardighi H, Steinberg SF. Caveolae-associated proteins in cardiomyocytes: Caveolin-2 expression and interactions with caveolin-3. *Am J Physiol Heart Circ Physiol* 2003;285(1):H325–332.

75. Woodman SE, Sotgia F, Galbiati F, Minetti C, Lisanti MP. Caveolinopathies: Mutations in caveolin-3 cause four distinct autosomal dominant muscle diseases. *Neurology* 2004;62(4):538–543.
76. Bist A, Fielding PE, Fielding CJ. Two sterol regulatory element-like sequences mediate up-regulation of caveolin gene transcription in response to low density lipoprotein free cholesterol. *Proc Natl Acad Sci USA* 1997;94(20):10693–10698.
77. Fielding CJ, Bist A, Fielding PE. Caveolin mRNA levels are up-regulated by free cholesterol and down-regulated by oxysterols in fibroblast monolayers. *Proc Natl Acad Sci USA* 1997;94(8): 3753–3758.
78. Smart EJ, Ying YS, Conrad PA, Anderson RG. Caveolin moves from caveolae to the Golgi apparatus in response to cholesterol oxidation. *J Cell Biol* 1994;127(5):1185–1197.
79. Graf GA, Matveev SV, Smart EJ. Class B scavenger receptors, caveolae and cholesterol homeostasis. *Trends Cardiovasc Med* 1999;9(8):221–225.
80. Frank PG, Lisanti MP. Caveolin-1 and caveolae in atherosclerosis: Differential roles in fatty streak formation and neointimal hyperplasia. *Curr Opin Lipidol* 2004;15(5):523–529.
81. Thomsen P, Roepstorff K, Stahlhut M, van Deurs B. Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking. *Mol Biol Cell* 2002;13(1):238–250.
82. Pelkmans L, Helenius A. Endocytosis via caveolae. *Traffic* 2002;3(5):311–320.
83. Lisanti MP, Scherer PE, Tang Z, Sargiacomo M. Caveolae, caveolin and caveolin-rich membrane domains: A signalling hypothesis. *Trends Cell Biol* 1994;4(7):231–235.
84. Sargiacomo M, Sudol M, Tang Z, Lisanti MP. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *J Cell Biol* 1993;122(4):789–807.
85. Engelman JA, Chu C, Lin A, *et al*. Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. *FEBS Lett* 1998;428(3): 205–211.
86. Galbiati F, Volonte D, Engelman JA, *et al*. Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. *EMBO J* 1998;17(22): 6633–6648.
87. Liu P, Ying Y, Ko YG, Anderson RG. Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. *J Biol Chem* 1996; 271(17):10299–10303.
88. Liu P, Ying Y, Anderson RG. Platelet-derived growth factor activates mitogen-activated protein kinase in isolated caveolae. *Proc Natl Acad Sci USA* 1997;94(25):13666–13670.
89. Smart EJ, Ying YS, Anderson RG. Hormonal regulation of caveolae internalization. *J Cell Biol* 1995;131(4):929–938.
90. Martens JR, Navarro-Polanco R, Coppock EA, *et al*. Differential targeting of Shaker-like potassium channels to lipid rafts. *J Biol Chem* 2000; 275(11):7443–7446.
91. Martens JR, Sakamoto N, Sullivan SA, Grobaski TD, Tamkun MM. Isoform-specific localization of voltage-gated K+ channels to distinct lipid raft populations. Targeting of Kv1.5 to caveolae. *J Biol Chem* 2001;276(11):8409–8414.
92. Maruoka ND, Steele DF, Au BP, *et al*. Alpha-actinin-2 couples to cardiac Kv1.5 channels, regulating current density and channel localization in HEK cells. *FEBS Lett* 2000;473(2): 188–194.
93. Mays DJ, Foose JM, Philipson LH, Tamkun MM. Localization of the Kv1.5 K+ channel protein in explanted cardiac tissue. *J Clin Invest* 1995;96(1): 282–292.
94. Yarbrough TL, Lu T, Lee HC, Shibata EF. Localization of cardiac sodium channels in caveolin-rich membrane domains: Regulation of sodium current amplitude. *Circ Res* 2002;90(4):443–449.
95. Darby PJ, Kwan CY, Daniel EE. Caveolae from canine airway smooth muscle contain the necessary components for a role in Ca(2+) handling. *Am J Physiol Lung Cell Mol Physiol* 2000;279(6): L1226–1235.
96. Jiang C, Atkinson D, Towbin JA, *et al*. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. *Nat Genet* 1994;8(2):141–147.
97. Splawski I, Timothy KW, Sharpe LM, *et al*. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004;119(1):19–31.
98. Splawski I, Timothy KW, Decher N, *et al*. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci USA* 2005;102(23):8089–8096; discussion 8086–8088.
99. Jorgensen AO, Shen AC, Arnold W, Leung AT, Campbell KP. Subcellular distribution of the 1,4-dihydropyridine receptor in rabbit skeletal muscle in situ: An immunofluorescence and immunocolloidal gold-labeling study. *J Cell Biol* 1989;109(1):135–147.
100. Okada Y. A scaffolding for regulation of volume-sensitive Cl^- channels. *J Physiol* 1999;520(Pt. 1):2.

101. Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA. Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many raft proteins are acylated, while few are prenylated. *J Biol Chem* 1999;274(6):3910–3917.
102. Perez AS, Bredt DS. The N-terminal PDZ-containing region of postsynaptic density-95 mediates association with caveolar-like lipid domains. *Neurosci Lett* 1998;258(2):121–123.
103. Scannevin RH, Murakoshi H, Rhodes KJ, Trimmer JS. Identification of a cytoplasmic domain important in the polarized expression and clustering of the Kv2.1 K+ channel. *J Cell Biol* 1996;135(6 Pt. 1):1619–1632.
104. Venema VJ, Ju H, Zou R, Venema RC. Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. *J Biol Chem* 1997;272(45):28187–28190.
105. Campbell KP, Kahl SD. Association of dystrophin and an integral membrane glycoprotein. *Nature* 1989;338(6212):259–262.
106. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: An overview of current mouse models. *Curr Opin Genet Dev* 2002;12(3):349–361.
107. Feron O, Kelly RA. Gaining respectability: Membrane-delimited, caveolar-restricted activation of ion channels. *Circ Res* 2002;90(4):369–370.
108. Ahn AH, Kunkel LM. Syntrophin binds to an alternatively spliced exon of dystrophin. *J Cell Biol* 1995;128(3):363–371.
109. Kachinsky AM, Froehner SC, Milgram SL. A PDZ-containing scaffold related to the dystrophin complex at the basolateral membrane of epithelial cells. *J Cell Biol* 1999;145(2):391–402.
110. Chockalingam PS, Gee SH, Jarrett HW. Pleckstrin homology domain 1 of mouse alpha 1-syntrophin binds phosphatidylinositol 4,5-bisphosphate. *Biochemistry* 1999;38(17):5596–5602.
111. Brenman JE, Chao DS, Gee SH, *et al.* Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. *Cell* 1996;84(5):757–767.
112. Frigeri A, Nicchia GP, Verbavatz JM, Valenti G, Svelto M. Expression of aquaporin-4 in fast-twitch fibers of mammalian skeletal muscle. *J Clin Invest* 1998;102(4.):695–703.
113. Hasegawa M, Cuenda A, Spillantini MG, *et al.* Stress-activated protein kinase-3 interacts with the PDZ domain of alpha1-syntrophin. A mechanism for specific substrate recognition. *J Biol Chem* 1999;274(18):12626–12631.
114. Gee SH, Madhavan R, Levinson SR, Caldwell JH, Sealock R, Froehner SC. Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. *J Neurosci* 1998;18(1):128–137.
115. Adams ME, Mueller HA, Froehner SC. In vivo requirement of the alpha-syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. *J Cell Biol* 2001;155(1):113–122.
116. Ou Y, Strege P, Miller SM, *et al.* Syntrophin gamma 2 regulates SCN5A gating by a PDZ domain-mediated interaction. *J Biol Chem* 2003;278(3): 1915–1923.
117. Bossuyt J, Taylor BE, James-Kracke M, Hale CC. Evidence for cardiac sodium-calcium exchanger association with caveolin-3. *FEBS Lett* 2002; 511(1–3):113–117.
118. Abriel H, Kass RS. Regulation of the voltage-gated cardiac sodium channel Nav1.5 by interacting proteins. *Trends Cardiovasc Med* 2005;15(1): 35–40.
119. Holmes TC, Fadool DA, Ren R, Levitan IB. Association of Src tyrosine kinase with a human potassium channel mediated by SH3 domain. *Science* 1996;274(5295):2089–2091.
120. Romanenko VG, Rothblat GH, Levitan I. Modulation of endothelial inward-rectifier K+ current by optical isomers of cholesterol. *Biophys J* 2002;83(6): 3211–3222.
121. Hajdu P, Varga Z, Pieri C, Panyi G, Gaspar R Jr. Cholesterol modifies the gating of Kv1.3 in human T lymphocytes. *Pflugers Arch* 2003;445(6):674–682.
122. Barrantes FJ. Lipid matters: nicotinic acetylcholine receptor-lipid interactions (Review). *Mol Membr Biol* 2002;19(4):277–284.
123. Galbiat F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, et al., Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophinglycoprotein complex, and t-tubule abnormalities. *J Biol Chem* 2001;276:21425–21433.
124. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation. *Proc Natl Acad Sci USA* 2006;103(19):7500–7505.
125. Barbuti A, Terragni B, Brioschi C, DiFrancesco D. Localization of f-channels to caveolae mediates specific beta2-adrenergic receptor modulation of rate in sinoatrial myocytes. *J Mol Cell Cardiol* 2007;42(1):71–78.
126. Teubl M, Groschner K, Kohlwein SD, Mayer B, Schmidt K. Na(+)/Ca(2+) exchange facilitates Ca(2+)-dependent activation of endothelial nitric-oxide synthase. *J Biol Chem* 1999;274(41);29529–29535.