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Caveolae: Structure, Function, and Relationship to Disease

Robert G. Parton

Institute for Molecular Bioscience and Centre for Microscopy and Microanalysis, University of Queensland, Brisbane, Queensland 4060, Australia; email: R.Parton@imb.uq.edu.au

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Abstract

The plasma membrane of eukaryotic cells is not a simple sheet of lipids and proteins but is differentiated into subdomains with crucial functions. Caveolae, small pits in the plasma membrane, are the most abundant surface subdomains of many mammalian cells. The cellular functions of caveolae have long remained obscure, but a new molecular understanding of caveola formation has led to insights into their workings. Caveolae are formed by the coordinated action of a number of lipid-interacting proteins to produce a microdomain with a specific structure and lipid composition. Caveolae can bud from the plasma membrane to form an endocytic vesicle or can flatten into the membrane to help cells withstand mechanical stress. The role of caveolae as mechanoprotective and signal transduction elements is reviewed in the context of disease conditions associated with caveola dysfunction.

Contents

CAVEOLAE: GENERAL CONSIDERATIONS AND DEFINITIONS

Caveolae are one of the most striking morphological features of mammalian cells. Some cell types, such as adipocytes, endothelial cells, and muscle cells, show an incredibly high density of these plasma membrane pits, which can increase the surface area of the plasma membrane twofold (**Figure 1**). Caveolae show a characteristic morphology by transmission electron microscopy, described as a bulb-shaped or cuplike uncoated invagination. This morphology is used for their identification and remains a key defining feature of caveolae. In addition, we can now further define caveolae by the presence of key structural proteins, such as caveolins and cavins, that associate with the caveola bulb. As described below, both of these structural components can have functions outside of caveolae.

Caveolae have often been compared to clathrin-coated pits, dynamic structures that form at the plasma membrane to concentrate receptor-ligand complexes and mediate efficient endocytosis. However, there are key differences. First, in contrast to the constant density of clathrin-coated pits in different cell types, the density of caveolae in different tissues is extremely variable; caveolae can occupy a major fraction of the cell surface, more than 50%, in some tissues (e.g., skeletal muscle), but caveolae in other locations can be very rare (in, e.g., the liver) or even completely absent (in, e.g., the kidney proximal tubule). Second, whereas clathrin-coated pits form single pits, with a rapid progression from a flat clathrin lattice to an increasingly invaginated structure that eventually buds into the cell, caveolae show a very constant shape with consistent curvature and a defined neck region (**Figure 1**). In addition, caveolae form complex higher-order structures not seen in clathrin-coated pits. Termed caveola clusters or rosettes, they represent multiple caveolae connected to the plasma membrane through a single neck (Lo et al. 2015, Yeow et al. 2017) (**Figure 1**). This striking organization is unique to caveolae. A full understanding of caveolae therefore requires an explanation of why caveola density varies so greatly between different cell types (and even the same cell type in different tissues, such as endothelia of the peripheral vasculature versus the blood-brain barrier) and the role of the complex multicaveolar structures in cellular function.

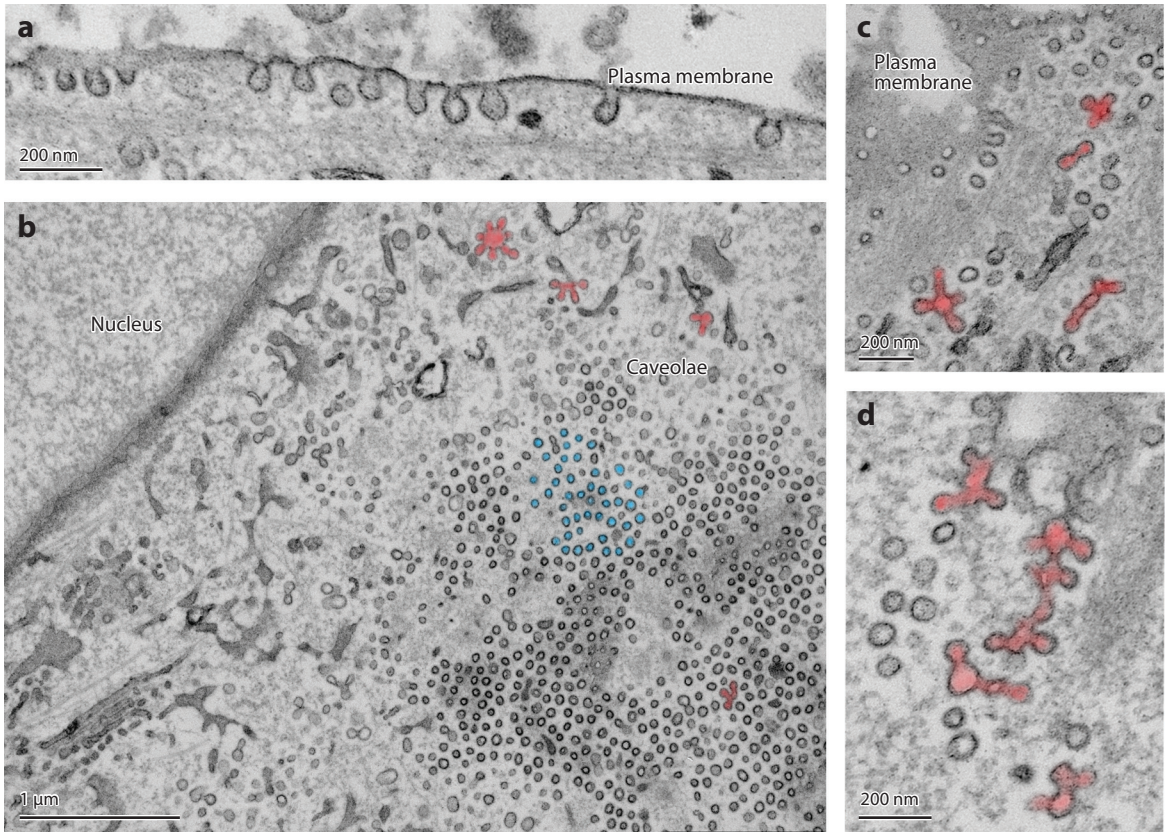

Figure 1

The characteristic morphology of caveolae. Gallery of transmission electron micrographs of 3T3-L1 adipocytes. Caveolae exist as single pits in the plasma membrane as shown in cross section in panel *a* and in sections almost parallel to the cell surface in panels *b–d* (see *blue highlighted structures* in panel *b*). Caveolae are also organized into multilobed structures termed rosettes or clusters (highlighted in *red*). Note the extremely high density of caveolae in regions of the cell surface.

Caveolae have been implicated in numerous disease conditions, including muscle diseases such as limb girdle muscular dystrophy, cardiomyopathy, lipodystrophy, and pulmonary arterial hypertension (PAH), all caused by mutations in caveola proteins. In addition, caveolae have been linked more indirectly to many other conditions, including breast and prostate cancer. Uncovering the diverse physiological roles of caveolae will provide new insights into these conditions.

MOLECULAR COMPOSITION OF CAVEOLAE

Proteins

The protein components of caveolae can be provisionally classified into two groups: core structural components and key accessory proteins (**Figure 2**; **Supplemental Table 1**). Core structural components required for the formation of caveolae include caveolin-1 (CAV1), caveolin-3 (CAV3), cavin1, and pacsin/syndapin proteins. ROR1 (receptor tyrosine kinase–like orphan receptor 1), a transmembrane receptor tyrosine kinase, may also be required for caveola formation in some

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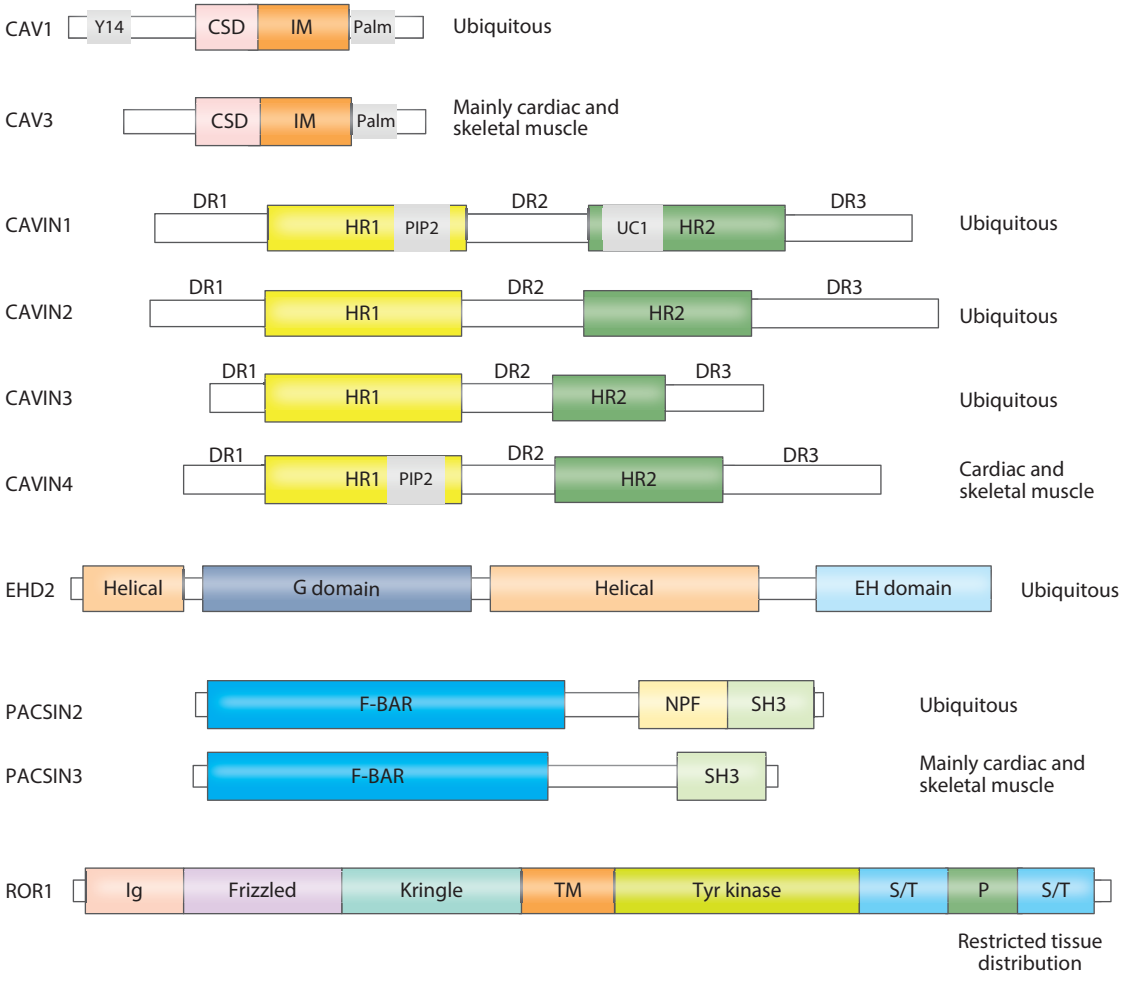

Figure 2

The major proteins of caveolae: domains and features. Abbreviations: CSD, caveolin scaffolding domain; DR, disordered region; EH domain, Eps15 homology domain; HR, helical region; IM, intramembrane; NPF, Asn-Pro-Phe motif; P, proline-rich domain; palm, palmitoylation sites; PIP2, PtdIns(4,5)P_2 binding site; ROR, receptor tyrosine kinase–like orphan receptor; S/T, serine/threonine-rich domain; TM, transmembrane domain; UC1, undecad repeat domain of cavin1.

tissues, while EHD (Eps15 homology domain) proteins 1, 2, and 4 also play a key role in the stability of caveolae.

Mammals possess three caveolin genes: *CAV1*, *CAV2*, and *CAV3*. Genetic loss of *CAV1* (expressed predominantly in nonmuscle cells) and *CAV3* (expressed in muscle cells) leads to a loss of caveolae in their respective tissues (Drab et al. 2001, Minetti et al. 2002). Caveolins are small (17–24-kDa) oligomeric cholesterol-binding proteins. Both the N and C termini of caveolins face the cytoplasm, and the proteins are believed to adopt a hairpin conformation in the membrane. No complete atomic structure of CAV1 is yet available, but various studies have examined isolated domains of caveolins or caveolin complexes purified from cells. Four distinct domains have been operationally defined: the N terminus (residues 1–81 in human CAV1), the conserved scaffolding domain region (residues 82–102 in CAV1), the intramembrane domain (residues 103–133 in

CAV1), and the C terminus (residues 134–178 in CAV1). The precise membrane-inserted regions of the caveolin protein are unclear, but protease protection studies suggest that a large portion of the protein, including the scaffolding domain region, may be tightly membrane associated (Ariotti et al. 2015b). The C terminus of CAV1 is palmitoylated, and the N terminus of CAV1 shows highly regulated phosphorylation on a conserved tyrosine residue (Tyr14).

Expression of CAV1 in a model bacterial system produces vesicles with the size and caveolin composition of budded caveolae (Walser et al. 2012). This observation shows that CAV1 can generate membrane curvature, at least in a model system. In vivo, however, cavin proteins, in particular cavin1, are also required for caveola formation (Hill et al. 2008, Liu et al. 2008). Cavins are peripheral membrane proteins with lipid-binding activity (Kovtun et al. 2015). Cavins show a particularly striking charge distribution, with two positively charged helical regions (HR1 and HR2) flanked by three negatively charged disordered regions (DR1, DR2, and DR3; **Figure 2**). Cavins form oligomeric complexes that are then recruited to caveolae to stabilize caveola formation. Cavin1 binds to both PtdIns(4,5)P_2 (phosphatidylinositol 4,5-bisphosphate) and phosphatidylserine (Hill et al. 2008, Kovtun et al. 2014), and such binding contributes to the stabilization of the CAV1-enriched domain.

Unlike the caveolins and cavins, which associate primarily with the caveola bulb, EHD proteins associate with the neck of caveolae (Ariotti et al. 2015a, Moren et al. 2012, Yeow et al. 2017). EHD2 was the first of the EHD proteins shown to associate with caveolae, but recent studies showed that EHD1 and EHD4 can also associate with caveolae with compensatory recruitment when EHD2 levels are reduced (Yeow et al. 2017). Loss of these three EHD proteins does not affect the caveola bulb but alters the neck morphology. In addition, cells lacking EHD proteins show decreased clustering of caveolae into multicaveolar assemblies, suggesting a vital role for these neck proteins in stabilizing these higher-order assemblies of caveolae.

Until recently, only caveolins and cavins were believed to be essential for caveola formation. However, other proteins have now been implicated in this process. Knockdown of pacsin2/syndapin2 in cultured cells causes a loss of morphological caveolae (Hansen et al. 2011, Senju et al. 2011), and genetic ablation of pacsin3/syndapin3, the muscle-enriched member of the pacsin/syndapin family, in mice causes a loss of muscle caveolae (Seemann et al. 2017). These findings have led to changes in the models of caveola formation and suggest that the pacsins, which contain membrane-sculpting F-BAR domains, are crucial for the membrane deformation required for caveola generation. In model systems, each individual caveolin, cavin, and pacsin has membrane-sculpting activity, but in vertebrate cells, the coordinated action of all these proteins is required for efficient caveola formation. This cooperativity suggests that in a vertebrate cell each of these components can help to regulate caveola formation and disassembly and to ensure that these processes occur at the right place and time. Pacsins have also been proposed to play another important role in caveola function by recruiting dynamin II to caveolae and by binding to EHD proteins (Senju et al. 2011). Interestingly, pacsins may be enriched close to the neck of the caveolae rather than around the caveola bulb (Seemann et al. 2017), consistent with the proximity of these potential interacting proteins.

Another protein recently implicated in caveola formation is ROR1 (Yamaguchi et al. 2016). This transmembrane protein does not appear to be present in stoichiometric amounts with caveolins or cavins in caveolae but facilitates the association of caveolin and cavin by binding both proteins through independent binding sites in the cytoplasmic domain to allow for caveola formation, preventing CAV1 degradation. Whether ROR1 is a universal regulator of caveola formation and a rare transmembrane protein associated with caveolae is not yet clear. ROR1 expression is present during embryonic and fetal development but is generally absent in most mature tissues. Expression is high, however, in a number of malignancies, including colon, lung, and pancreatic cancers and B

cell chronic lymphocytic leukemia (Borcherding et al. 2014). Despite the general lack of correlation between ROR1 expression and the tissue distribution of caveolae in mature tissues, there are clearly strong links with caveolae. Knockdown of CAV1 showed identical effects as knockdown of ROR1 on receptor tyrosine kinase–mediated phosphorylation patterns in non–small cell lung cancer cells (Yamaguchi et al. 2016).

Key accessory proteins include caveolin-2 (CAV2), cavin2, cavin3, and cavin4. CAV2 forms an oligomeric complex with CAV1 (Scherer et al. 1997) but is generally not required for caveola formation [as shown in mice lacking CAV2 (Razani et al. 2002b)], and expression in model systems does not generate morphologically normal caveolae (Walser et al. 2012). However, CAV2 has been suggested to facilitate caveola formation (Lahtinen et al. 2003). Mice lacking CAV2 show lung defects characterized by endothelial cell hyperproliferation as well as by striking skeletal muscle abnormalities, including tubular aggregates, mitochondrial defects, and increased numbers of satellite cells (Schubert et al. 2007).

While cavins2–4 are homologous to cavin1, they are unable to form caveolae on their own, requiring cavin1 for their recruitment (Bastiani et al. 2009). All the cavin family members can hetero- and homo-oligomerize, but cavin1/cavin2 and cavin1/cavin3 form separate subcomplexes that associate with distinct striations on caveolae (Gambin et al. 2014). These distinct complexes are generated due to competition between the cavin2 and cavin3 proteins for the same binding interface in the HR1 domain of cavin1 (Mohan et al. 2015). Cavin3 also interacts with the scaffolding domain of CAV1, although this interaction is not required for caveola formation. The interaction is increased by cholesterol depletion, raising the possibility that this domain of CAV1 can either be associated with cholesterol within the bilayer or be associated with cavin3 outside the membrane. Functionally, cavin3 regulates caveola dynamics (McMahon et al. 2009, Mohan et al. 2015). Genetic ablation in mice has shown a role for cavin3 in regulating the balance between ERK and AKT signaling, in regulating cell proliferation, and in suppressing apoptosis (Hernandez et al. 2013), although some questions have been raised about genotype-specific phenotypes of some cavin3-null mice strains (Liu et al. 2014).

Cavin2 plays a role in shaping caveolae (Hansen et al. 2009) but is not required for caveola formation/stability in all tissues. Intriguingly, genetic ablation of cavin2 in mice caused a loss of caveolae in some tissues such as cardiac and adipocyte endothelia, but not in endothelia of the heart or other tissues (Hansen et al. 2013). It remains to be seen whether this tissue-specific loss of caveolae represents a requirement for biogenesis in certain tissues or a change in caveola stability that renders caveolae lacking cavin2 more susceptible to flattening. Cavin2 has also been implicated in the plasma membrane recruitment, stabilization, and activation of endothelial nitric oxide synthase (eNOS) that is required for endothelial cell maintenance and function (Boopathy et al. 2017).

Taken together, these findings suggest that the essential structural components of caveolae, each of which has its own membrane-sculpting ability, come together to generate a relatively stable core caveola domain. The stability of this domain can be further tuned by other components, including CAV2, cavin2, and ROR1. Other components, such as cavin3 and cavin4 (in striated muscle), associate with the caveola domain to mediate specific caveola functions.

Lipids

Caveolae are likely to represent a specialized lipid domain. As the majority of caveolae at steady state are connected to the plasma membrane, biochemical methods must rely on their release and separation from the bulk plasma membrane and are currently unable to differentiate lipid compositions of subdomains such as the caveola bulb and the caveola neck. Nevertheless, detailed quantitative lipid analyses of purified caveolae have shown an enrichment of cholesterol and specific

glycosphingolipids, such as the ganglioside GM3, relative to the bulk plasma membrane (Ortegren et al. 2004).

The major structural components of caveolae show specific lipid-binding properties. CAV1 binds cholesterol (Murata et al. 1995) and is palmitoylated (Dietzen et al. 1995). Peptides derived from caveolin can reorganize lipids when incorporated into liposomes with particular specificity for cholesterol, PtdIns(4,5)P_2, and phosphatidylserine (Wanaski et al. 2003). Together, these experiments suggest that oligomers of caveolin could generate a specific lipid environment. Cavin proteins also show lipid-binding activity, possessing both a PtdIns(4,5)P_2 binding site in the HR1 domain and phosphatidylserine-binding activity. The concentration of these abundant lipid-binding proteins within caveolae (~140–150 caveolins and ~50 cavins per caveola) would therefore be expected to generate a lipid domain highly enriched in these specific lipids. In a cell with abundant caveolae, such as a skeletal muscle cell, where 50% of the cell surface is occupied by caveolae, a major fraction of certain plasma membrane lipids may be present within caveolae.

Other studies have focused on the functional requirements for specific lipids in caveola formation and function. It has long been known that cholesterol depletion causes both a flattening of caveolae (Rothberg et al. 1992) and the dissociation of cavins from the membrane (Breen et al. 2012, Hill et al. 2008). Phosphatidylserine has recently been shown to be essential for caveola formation and stability (Hirama et al. 2017). Phospholipid scrambling, a feature of apoptotic cells, contributes to caveola instability with potential functional consequences. The particular morphology of caveolae, with a very tight radius of curvature at the neck (see **Figure 1**), may also contribute to enrichment of certain lipids in particular nanodomains of the caveolae. Only lipids with a specific shape will be enriched in these regions of the caveolae and may be required for caveola formation. In fact, specific lipids may not only be required for caveola formation but may also be able to perturb caveola formation. The blood-brain barrier generally maintains a high impermeability and shows a very low density of caveolae. Genetic ablation of the lipid flippase Mfsd2a, which is responsible for transporting long-chain unsaturated fatty acid–containing phospholipid species from the outer to the inner cytoplasmic leaflet of the endothelial cell plasma membrane, causes the appearance of caveolae and increases vascular permeability (Andreone et al. 2017). This finding suggests that, in the wild-type blood-brain barrier, specific polyunsaturated fatty acid–containing phospholipids in the cytoplasmic leaflet of the plasma membrane create an inhibitory environment for caveola formation.

CAVEOLA FORMATION

Caveola formation in vertebrate cells requires the coordinated action of the key structural proteins described above that come together to generate the sculpted domain at the plasma membrane. Caveolins are synthesized in the endoplasmic reticulum (ER) (Monier et al. 1995) and are then exported as oligomers to the Golgi complex via a COPII-dependent pathway (Hayer et al. 2010a) (**Figure 3**). A steady-state pool of caveolin exists in the Golgi complex in many cell types and can be depleted if protein synthesis is inhibited. Exit from the Golgi complex is inhibited by cholesterol depletion and is accelerated by cholesterol addition, suggesting that cholesterol is required for the maturation of caveolin oligomers to a form that can be exported (Pol et al. 2005). Interestingly, antibodies against the scaffolding domain of CAV1 recognize the protein in the Golgi complex, but not at the plasma membrane. Cholesterol depletion from the plasma membrane, however, reveals the surface pool of the protein (Pol et al. 2005), suggesting that part of the maturation process may involve cholesterol-dependent insertion of this region of CAV1 into the bilayer. The caveolin-containing exocytic carriers leaving the Golgi complex and destined for the plasma membrane contain a defined number of caveolin molecules, similar to that of mature caveolae,

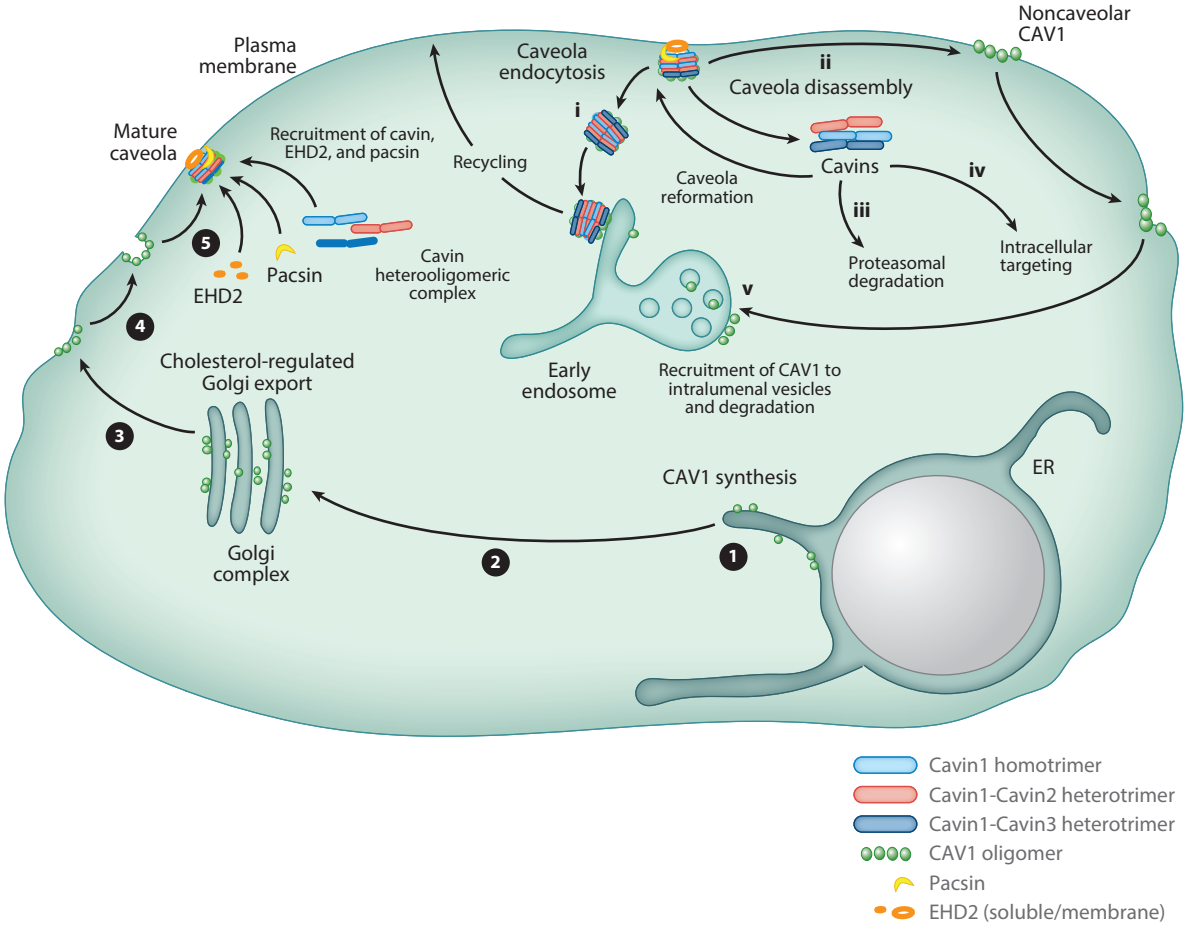

Figure 3

Formation and dynamics of caveolae. Caveola formation (as indicated by numbers ❶ through ❺): Caveolin-1 (CAV1) is synthesized and oligomerizes in the endoplasmic reticulum (ER) (❶). CAV1 oligomers are transported to the Golgi complex (❷). Golgi exit (❸) is stimulated by cholesterol and leads to a defined quantum of CAV1 being delivered to the plasma membrane. CAV1-rich domains (❹) associate with EHD proteins, the cavin complex, and pacsins (❺) to generate caveolae. Caveola dynamics (as indicated by roman numerals *i* through *v*): Caveolae at the plasma membrane can bud off to form an endocytic carrier (*i*), which fuses with the early endosome and which can be recycled back to the plasma membrane. In response to high membrane tension, caveolae can flatten (*ii*), releasing cavins into the cytoplasm and CAV1 oligomers into the bulk plasma membrane, where CAV1 can be endocytosed. Cytosolic cavins are degraded (*iii*) unless they interact with intracellular targets (*iv*). Noncaveola CAV1 within the early endosome is recruited into intraluminal vesicles and is degraded (*v*). Adapted from Parton et al. (2018).

and do not disperse on fusion with the plasma membrane (Tagawa et al. 2005). Maturation of caveolae, as indicated by recruitment of cavins, EHD2, and pacsin proteins, then occurs. The specific lipid composition of the plasma membrane presumably plays a role in the recruitment of these components. The binding of cavin proteins to both PtdIns(4,5)P_2 and phosphatidylserine as well as the cholesterol sensitivity of cavin recruitment is consistent with this model.

Detailed models of caveola formation still await atomic models of the key caveola components and their relationship to the fine architecture of caveolae. The classical striations on the surface of caveolae observed by scanning and deep-etch transmission electron microscopy were originally

proposed to be made up of caveolin (Rothberg et al. 1992). However, vesicles generated by caveolin in bacteria show no such striations and show only a polygonal symmetry (Walser et al. 2012), and striations are lacking from cells expressing caveolin but not cavins. It is now thought that cavin oligomers, which form extended structures with dimensions compatible with forming the striated coat (Kovtun et al. 2014), may be a major component of the striations, and this idea is consistent with the high-resolution immunogold labeling of plasma membrane sheets (Gambin et al. 2014) and other advanced electron microscopic techniques (Ludwig et al. 2016). An alternative model for cavin association with caveolae was recently proposed on the basis of the formation of a cavin lattice on the surfaces of liposomes (Stoeber et al. 2016). As methods of reconstitution are improved and additional components are introduced into the reconstitution system, we can envisage the precise molecular details of caveola formation to be further refined and the mechanisms clarified. As further discussed below, understanding caveola formation is also crucial for our understanding of how caveolae are rapidly disassembled in response to specific stimuli.

DYNAMICS OF CAVEOLAE: ENDOCYTOSIS AND DISASSEMBLY

Endocytosis

Caveolae can bud from the plasma membrane to form an endocytic carrier (reviewed in Mayor et al. 2014) (**Figure 3**). This process has now been extensively studied in cultured cells by using a range of techniques, including live microscopy with tagged proteins, quantitative electron microscopy with specific markers, and genome editing. Caveola budding is a constitutive process that leads to the formation of an endocytic caveola carrier; this carrier has the morphology of a spherical budded caveola (Kirkham et al. 2005) and contains both CAV1 and cavin1 (Boucrot et al. 2011, Shvets et al. 2015). Budding is dependent on the membrane scissioning GTPase dynamin (as revealed by inhibitor studies, knockdown studies, and dominant negative inhibitor studies) (Boucrot et al. 2011, Henley et al. 1998, Oh et al. 1998, Stoeber et al. 2012) but is negatively regulated by EHD2 (Moren et al. 2012, Stoeber et al. 2012), a process that can also involve EHD1 and EHD4 (Yeow et al. 2017). Pacsin2 is crucial in assembling the endocytic machinery of caveolae by recruiting dynamin to caveolae and binds EHD2 (Senju et al. 2011). Cavin3 regulates caveola dynamics; depletion of cavin3 causes a shift to more stable caveolae and causes a loss of highly dynamic caveolae (McMahon et al. 2009, Mohan et al. 2015).

In a fibroblast, the budded caveola (or endocytic caveola carrier) either fuses back to the plasma membrane or fuses with the early endosome in a Rab5-dependent process (Pelkmans et al. 2004); colocalization with internalized cargo in transferrin-negative structures, distinct from early endosomes, has also been described in genome-edited cells (Shvets et al. 2015), possibly representing caveola clusters that have detached from the plasma membrane. On the early endosome, the caveola domains form a defined structure, still positive for both CAV1 and cavin1, and then bud off the endosome to fuse back to the plasma membrane (Boucrot et al. 2011, Pelkmans et al. 2004). This internalization and recycling cycle also involves the cytoskeleton. Actin, microtubules (MTs), and intermediate filaments associate with caveolae. In mice lacking the integrin-linked kinase (ILK), caveola density is dramatically decreased; caveolae remain associated with dynamic MTs, fail to stably fuse with the plasma membrane, and accumulate in intracellular structures (Wickstrom et al. 2010). ILK controls this process by regulating MT stability through the recruitment of the scaffold protein IQGAP1 and its downstream effector, mDia1 (Wickstrom et al. 2010). These results suggest a very dynamic cycle of caveola internalization and recycling in this cell type. Caveolae associate with actin filaments and cavin3 interacts with myosin1c in a CAV1- and cavin1-dependent manner (Hernandez et al. 2013). Actin also regulates endocytosis of multilobed caveola clusters.

This regulation depends on the actin-binding protein filamin A, mDia1, and Abl (reviewed in Echarri & Del Pozo 2015, Parton & del Pozo 2013). The complex bidirectional interplay between caveolae and the actin cytoskeleton is also shown by the flattening of caveolae in response to excessive actin polymerization.

The capacity of the caveola endocytic pathway appears to be low relative to clathrin-mediated endocytosis (Shvets et al. 2015). Approximately 1% of caveolae were estimated to detach from the cell surface in 1 min in a fibroblastic cell line, and similar estimates were made for caveolae in cultured adipocytes (Kirkham et al. 2005, Le Lay et al. 2006). Of CAV1/cavin1-positive structures in fibroblastic cells, 5–10% were dynamic and contained endocytosed cargo, and approximately 4% localized with endosomal markers (Shvets et al. 2015). Thus, it is now evident that caveolae are dynamic structures, at least in cultured fibroblastic cells, but the role of caveola endocytosis is not as clear. While a number of proteins and infectious agents have been proposed to be endocytosed through caveolae, subsequent work has shown that these agents are rarely dependent on caveolae for entry. For example, the nonenveloped virus simian virus 40 is internalized partially through caveolae, but genetic loss of caveolae does not impede infectious entry; in fact, entry is more efficient (Damm et al. 2005). Similarly, cholera toxin, which binds to the ganglioside GM1 and is incorporated into budded caveolae, is also internalized efficiently in cells lacking caveolae (Chaudhary et al. 2014, Kirkham et al. 2005, Shvets et al. 2015). The general exclusion of transmembrane proteins from caveolae would also argue against a role in classical receptor-mediated ligand endocytosis (Shvets et al. 2015) but is consistent with the observed inclusion of lipids and lipid-anchored proteins (e.g., GPI-anchored proteins) in caveolae (Parton et al. 1994). If caveola endocytosis is not a major pathway for internalization of cargo molecules, what is its role? Caveola budding and recycling may allow caveola density at the plasma membrane to be regulated without disassembly of caveolae. For example, in mitotic cells, the balance between endocytosis and recycling is altered to cause a net loss of caveolae from the plasma membrane during metaphase (Boucrot et al. 2011). This pattern is reversed during cytokinesis, restoring levels of surface caveolae. Caveola endocytosis is also important for internalization of ordered plasma membrane domains, including GM1, as cells detach from their substratum to regulate specific signaling pathways (del Pozo et al. 2005). Recycling endosomes and Rab7-positive late endosomes, as well as the actin and MT networks, have been implicated in this process (del Pozo et al. 2005, Hertzog et al. 2012). Recycling of CAV1 back to the surface involves MTs for long-range translocation, the actin cytoskeleton for short-range movement in the periphery, and the exocyst subunit Exo70. A third possibility relates to the role of CAV1 phosphorylation in endocytosis. Multiple stimuli can cause CAV1 tyrosine phosphorylation, which triggers CAV1 internalization. As these processes are closely linked, the internalized phosphorylated CAV1 could form a signaling platform on the early endosome to recruit downstream signaling components.

Most studies on caveola endocytosis have focused on cultured cells. A vital unanswered question relates to the role of caveola endocytosis in different tissues in vivo. Are caveolae dynamic in all tissues in which they are found, and if so, are there distinct tissue-specific functions of caveolae endocytosis? In this respect, endothelia have received the most attention because caveolae have long been proposed as vital mediators of transcellular transport of solutes and ligands across the endothelium (Palade & Bruns 1968). In addition to extensive electron microscopic tracer studies on transport in vivo, this process has been visualized in real time by using antibodies to an endogenous GPI-anchored protein (Oh et al. 2007). Transport was extremely rapid and was dependent on caveolae, as shown using mice genetically lacking *Cav1*. Recent work focusing on the blood-brain barrier has further strengthened the possibility of a proposed role of caveolae (Andreone et al. 2017). The blood-brain barrier is characterized by an extremely low rate of transcellular transport and by a lack of caveolae. Remarkably, genetic ablation of a lipid flippase

caused an increase in caveolae in the blood-brain-barrier endothelium, increasing transcellular transport in a CAV1-dependent manner. Although there is considerable evidence for a role of caveolae in transcytosis in endothelia, there is also controversy (Rippe et al. 2002). Mice lacking CAV1 show a complete absence of endothelial caveolae yet show increased albumin clearance from the bloodstream (Rosengren et al. 2006, Schubert et al. 2002); in both wild-type and knockout mice, transendothelial transport was shown to be through a passive porous pathway rather than through transcytosis or upregulation of an alternative transcellular pathway (Rosengren et al. 2006). Endothelial caveolae are important in other crucial processes such as mechanoprotection, mechanotransduction, and calcium signaling (Cheng et al. 2015, Isshiki et al. 1998, Lee & Schmid-Schonbein 1995, Park et al. 2000, Yu et al. 2006). How these functions would integrate with the role of caveolae as a high-capacity constitutive endocytic pathway is not yet clear.

Disassembly of Caveolae

One aspect of the caveola life cycle that is now receiving great attention is the idea that caveolae can flatten, particularly as a result of increases in membrane tension (**Figure 3**). This phenomenon was originally characterized by electron microscopy in muscle fibers subjected to extreme stretching (Dulhunty & Franzini-Armstrong 1975) and then in endothelial cells of muscle capillaries subjected to increased transmural pressure (Lee & Schmid-Schonbein 1995). More recently, this phenomenon was further characterized in fibroblasts, muscle cell lines, muscle fibers, endothelia, and the notochord of fish (Cheng et al. 2015, Y.W. Lim et al. 2017, Lo et al. 2015, Sinha et al. 2011, Yeow et al. 2017). Increases in membrane tension cause a loss of caveolae, as revealed by electron microscopy, and are associated with decreased association of caveolins and cavins and, in some cases, with increased mobility of caveolin (Sinha et al. 2011; see also Yang & Scarlata 2017). Caveola disassembly is an energy-independent physical process and can be observed in isolated plasma membrane sheets (Sinha et al. 2011). In cells with abundant caveolae, the flattening of caveolae can allow a cell to change shape and withstand physical stress. Cells and tissues lacking caveolae show increased membrane damage, relative to wild-type cells, when they are subjected to various physical challenges. The same has been shown in cell culture systems, for example, using hypotonic treatment or direct stretching of cultured cells, and in various in vivo systems, including manipulation of blood pressure to study endothelial caveolae and increased muscle activity to study the effect on muscle caveolae and on caveolae of the zebrafish notochord. Primary adipocytes from mice lacking caveolae also show greatly increased fragility relative to wild-type cells. Direct measurement of membrane tension in cells with and without caveolae shows that, as cells swell in response to hypotonic medium, the flattening of caveolae buffers tension changes (Sinha et al. 2011). Muscle caveolae show a particularly striking organization into multilobed caveola clusters. Detailed quantitative 3D studies of the effect of hypotonic medium on isolated muscle fibers showed that these complex structures, rather than individual caveolae, preferentially disassemble (Lo et al. 2015), with the membrane presumably rapidly incorporated into the bulk membrane. These studies provided the first evidence for a functional role of these complex structures. Support for this model came from study of EHD proteins. EHD2, in cooperation with EHD1 and EHD4, is important for the formation of these multicaveolar clusters, and, consistent with the above results, fibroblasts lacking EHD proteins show greater sensitivity to stretch (Yeow et al. 2017). These studies confirm EHD2 as a vital regulator of cluster formation and further support the crucial role of the multicaveolar clusters in mechanoprotection.

Many of the molecular details of the disassembly process are still unresolved. Caveolins and cavins show a change in association, as revealed by fluorescence resonance energy transfer measurements, and caveolae, as shown through electron microscopy, are flattened into the

membrane in response to hypotonicity-induced membrane stretching (Sinha et al. 2011). Complexes of cavin1/cavin2 and cavin1/cavin3 are also detectable in the cytosol as caveolae disassemble in response to hypotonic treatment (Gambin et al. 2014). However, at the level of individual caveolae, it is not yet clear whether there is total caveola disassembly or an all-or-nothing response or whether some cavins can be released without total caveola flattening. Caveolae reassemble, and caveolins/cavins reassociate rapidly, after removal of a swelling stimulus (Sinha et al. 2011), and the details of this energy-dependent process are also unclear. The idea that a change in the curvature of a caveola would dictate cavin disassembly/reassembly is attractive, but clear evidence for this model is lacking. In fact, skeletal muscle lacking pacsin3 shows a lack of caveolae, as shown through electron microscopy, yet both CAV3 and cavin1 proteins are detectable at the muscle plasma membrane (Seemann et al. 2017). This intriguing result suggests that flattening of the caveolae in this case does not induce cavin dissociation. Whether the genetic deficiency of pacsin3 is compensated by lipid changes that retain the cavin proteins at the cell surface (consistent with observation of cavin1 association with CAV3-negative sarcolemmal regions) or whether other interactions maintain cavin1 at the cell surface is not yet clear.

Turnover of Caveola Components

Earlier studies of caveolins showed that mutants of caveolins, particularly muscular dystrophy–associated mutants of *CAV3*, trafficked abnormally and that patients showed a reduction in plasma membrane caveolae (Galbiati et al. 1999). Mutant CAV3 showed Golgi complex association and rapid turnover through the ubiquitin/proteasomal pathway (Galbiati et al. 2000). Inhibition of proteasomal degradation caused increased CAV3 in the ER, suggesting an ER-associated degradation pathway. This pathway may be important for ensuring that caveolin levels in the ER are always low and may be relevant to other disease conditions. Several heterozygous mutations in the C terminus of *CAV1* have been described in patients with PAH (Copeland et al. 2017, Han et al. 2016), a disease that is associated with increased pulmonary vascular resistance and that leads to progressive right ventricular failure. One of the mutant forms of *CAV1* has a novel C terminus with a functional ER-retention motif and is defective in ER export (Copeland et al. 2017). The ER accumulation of the mutant protein has a number of functional consequences, including hyperphosphorylation of Smad, increased proliferation (Marsboom et al. 2017), and increased sensitivity to hypotonic treatments (Copeland et al. 2017). More recently, the ubiquitin ligase ZNRF1 was shown to interact with the C terminus of CAV1 and to mediate its ubiquitination and turnover in macrophages (Lee et al. 2017). This functional interaction was stimulated by endotoxin and led to downregulation of CAV1, resulting in proinflammatory responses.

Intriguingly, CAV1 protein levels are also regulated by a lysosomal pathway (Ritz et al. 2011), possibly reflecting the need for mechanisms to turn over mature caveolin oligomers in the post-Golgi compartments as well as newly synthesized caveolin in the ER through proteasomal degradation. Loss of cavin1 causes concomitant downregulation of CAV1 protein levels (Hayer et al. 2010b, Hill et al. 2008) by increased endocytosis and degradation of CAV1 through noncaveola pathways (**Figure 2**). CAV1 degradation requires valosin-containing protein (VCP)/p97, which specifically binds to monoubiquitinated CAV1 oligomers and recruits them into the intraluminal vesicles of multivesicular endosomes (Ritz et al. 2011). Loss of VCP/p97 or pharmacological inhibition increases CAV1 levels. In this way, CAV1 and cavin levels can be balanced. This mechanism appears to be unbalanced in prostate cancer cells, in which CAV1 levels are high but cavin1 levels negligible (Moon et al. 2014).

Consistent with the general cooperation between the CAV1 and cavin1 proteins, loss of CAV1 similarly causes loss of cavin1 protein (Hill et al. 2008). Turnover of cavin1 is also increased in

cells subjected to cyclic stretch (Tillu et al. 2015). In both of these conditions, the cytosolic pool of cavin1 is increased. Cytosolic cavin1 is degraded by the proteasome due to ubiquitination on a basic patch in the HR1 region, the same region involved in binding to PtdIns(4,5)P_2 (**Figure 2**). It has been proposed that this site is exposed only when cavins dissociate from the membrane in response to stimuli that cause caveola disassembly (Tillu et al. 2015). In this way, the PtdIns(4,5)P_2 binding site acts as a sensor for membrane association, balancing cavin1 levels with those of caveolin and maintaining low cytosolic levels.

FUNCTIONS OF CAVEOLAE AND RELATIONSHIP TO DISEASE

After the identification of the caveolin protein family came the discovery that a number of disease conditions are caused by loss, or mutation, of caveolins (Garg & Agarwal 2008, Kim et al. 2008, McNally et al. 1998, Minetti et al. 1998; see **Supplemental Table 2**). These diseases include lipodystrophy and PAH, which are caused by mutations in *CAV1*, and skeletal muscle disorders and cardiomyopathies, which are linked to mutations in *CAV3*. With the discovery of the cavins came further disease associations, with cavin1 linked to lipodystrophy, muscular dystrophies, and cardiomyopathies and cavin4 linked to cardiac disease (Hayashi et al. 2009, Rajab et al. 2010, Rodriguez et al. 2011). Cavins and caveolins are also strongly linked to cancer. Early studies showed a loss of caveolae in tumor cells and inhibition of tumor growth by CAV1 expression (Cerezo et al. 2009, Koleske et al. 1995, Lee et al. 1998). Additionally, cavins are generally linked to tumor suppression, with cavin3 expression lost in breast and lung tumors and expression of cavin1 and cavin2 reduced in breast cancer (Bai et al. 2011, Ozturk et al. 2016, Xu et al. 2001). However, CAV1 has a tumor-promoting role in other cancers, including prostate cancer, in which CAV1 is expressed without cavin1 (Moon et al. 2014, Thompson 1998, Thompson et al. 2010). This imbalance is associated with a poor prognosis in prostate cancer patients. In mouse models, expression of cavin1 in the prostate cancer cells dampens the tumorigenic properties of CAV1 (Moon et al. 2014). This finding demonstrates that CAV1 in cells without caveolae behaves differently than when localized to caveolae.

Supplemental Material >

These observations emphasize the complexity of the functions of the caveola components and the need to understand the entire caveola system. Further research should include the role of caveola invagination and all the caveola proteins, not just CAV1, both when they are associated with caveolae and when they are released from caveolae to reach other cellular destinations. Recent studies have started to directly address the role of caveolae, as distinct from caveola components, in cellular function and disease.

Signal Transduction

The idea that caveolae can play a vital role in regulation of signal transduction, with implications for caveolae as negative regulators of cancer progression, first arose from biochemical studies showing enrichment of numerous signaling proteins in detergent-insoluble complexes prepared from cells and tissues (Sargiacomo et al. 1993). While the equivalence of these biochemically defined complexes to caveolae was shown to be incorrect [similar complexes were isolated from cells lacking caveolae (Fra et al. 1994)], the proposed role of caveolae as signaling platforms was further strengthened by evidence for direct interactions between caveolin and numerous signaling proteins, i.e., the caveolin signaling hypothesis (Couet et al. 1997, Okamoto et al. 1998). Screening of a phage display peptide library identified a number of peptides with specificity for the scaffolding domains of CAV1 and CAV3, but not CAV2. The subsequent recognition that various signaling proteins contain motifs similar to those identified in the screen led to a profusion of studies identifying caveolin-binding motifs (CBMs) in signaling proteins. These studies identified

numerous proteins that contained putative CBMs, that showed immunoprecipitation with caveolin, and that in some cases were dysregulated by loss of caveolin. Perhaps the most extensively studied interaction is with eNOS. eNOS interacts with CAV1 (as shown by immunoprecipitation) and is hyperactivated in cells and animals lacking CAV1, and its activity can be selectively modulated in vitro and in vivo by peptides corresponding to the caveolin scaffolding domain (Garcia-Cardena et al. 1997, Sowa et al. 2001). These peptides show striking functional effects when added to cells or when administered to animals, convincingly supporting the direct interaction model for the caveolin signaling hypothesis (Bucci et al. 2000, Gratton et al. 2003).

So why is there controversy regarding the caveolin signaling hypothesis? The first and most crucial consideration relates to the interaction between the two proteins. Structural data are available for many of the proteins proposed to interact with the caveolin scaffolding domain via their identified CBMs. Examination of the protein structure shows that the putative CBMs adopt no common structure and are actually inaccessible, as they are buried within the hydrophobic core of the protein (Byrne et al. 2012, Collins et al. 2012). Modification of these amino acids is likely to yield unfolded and thus nonfunctional proteins (for example, mutation of certain CBM proteins causes their intracellular accumulation rather than plasma membrane association). Putative CBMs are also extremely common in organisms lacking caveolins. There are also doubts about the accessibility of the caveolin scaffolding domain in caveolin. This highly amphipathic region of caveolin juxtaposes the putative hairpin intramembrane domain, and some studies suggest that this amphipathic region is at least partially buried within the bilayer. For example, protease treatment of isolated caveolae yields a protected central portion that contains the scaffolding domain (Ariotti et al. 2015b). This region is degraded if detergents are included during the protease treatment or if charged residues are introduced in this region.

If the scaffolding domain–CBM interaction is not occurring in vivo, how are the effects of synthetic scaffolding domain peptides explained? This is clearly an important avenue of further research, as the in vivo effects of the peptides are striking. Peptides corresponding to the scaffolding domain show lipid-binding activity. Expression of the caveolin scaffolding domain alone can also specifically inhibit clathrin-independent endocytosis in cells lacking caveolae, without affecting clathrin-dependent endocytosis (Chaudhary et al. 2014). In addition, the scaffolding domain alone can inhibit bacterial invasion (J.Y. Lim et al. 2017). This inhibition is associated with major changes in the mobility of plasma membrane lipids, an effect modulated by changing the physical properties of the plasma membrane (Chaudhary et al. 2014, Hoffmann et al. 2010). These results point to general changes in the properties of the plasma membrane, presumably due to the effect of the scaffolding domain on the lipid domains and the lipid organization required for specific endocytic events and for signal transduction. Lipid-based effects of caveolin and caveolin-derived peptides could therefore provide an alternative theory to explain caveolin-eNOS functional links.

A further aspect of the caveolin scaffolding hypothesis that has not received great attention is the manner in which an apparently constitutive interaction between caveolin and signaling proteins, as first documented in the peptide interaction screen, could be regulated to allow for movement into and out of caveolae as originally proposed. In this respect, the idea that caveolin phosphorylation plays a crucial role in regulating interactions is an attractive one. CAV1 is phosphorylated on a conserved tyrosine, Tyr14, in response to numerous stimuli, including mechanical stress, growth factors, oxidative stress, and bacterial invasion (Boettcher et al. 2010; Cao et al. 2004; Joshi et al. 2008, 2012; Orlichenko et al. 2006; Radel & Rizzo 2005; Zhang et al. 2007). CAV1 phosphorylation has been suggested to be a trigger for endocytosis of caveolae (Boettcher et al. 2010, del Pozo et al. 2005). In addition, phosphorylated CAV1 interacts specifically with a number of downstream targets. In the case of mechanical stimuli, the phosphorylation of CAV1 has been linked to transcriptional upregulation of key caveola components through a pathway involving

EGR1 (Joshi et al. 2012). Thus, signaling from caveolae can provide long-term protection against physical stress by stimulating new caveola biosynthesis. Other downstream pathways activated by CAV1 phosphorylation are not yet fully defined, but screens for phospho-CAV1 interacting partners have identified a number of potential binding partners, including TRAF2 [tumor necrosis factor (TNF) receptor–associated factor 2], CSK (C-terminal Src kinase) (Cao et al. 2002, Jung et al. 2018), and the RhoA-GEF VAV2 (Boettcher et al. 2010). The last mediates cytoskeletal rearrangement in response to bacterial infection to prevent invasion.

Cavins as Signaling Molecules

The finding that cavins can be released from caveolae in response to mechanical stimuli raised the possibility that the released cavins could act as signaling molecules, interacting with intracellular targets to regulate diverse physiological processes. Cavin1 (originally termed polymerase transcript release factor) was first described as a nuclear protein regulating ribosomal RNA transcription (Jansa et al. 1998, 2001). Direct evidence for a caveola-to-nuclear trafficking pathway came with the demonstration that in adipocytes insulin phosphorylates cavin1 associated with caveolae and this phosphorylation stimulates the translocation of cavin1 to the nucleus (Liu & Pilch 2016). This pathway is involved in the upregulation of ribosomal RNA transcription, which is essential for increased ribosomal biogenesis in response to nutrients and growth factors. Loss of cavin1 causes an imbalance in ribosomal production with an excess of ribosomal proteins, causing nuclear stress and activation of p53.

Cavin4, the muscle-specific cavin isoform, dissociates from surface caveolae when isolated mature muscle fibers are subjected to a hypotonic treatment that causes increased membrane tension and caveola flattening (Lo et al. 2015). Cavin4 also accumulates in the nucleus in mouse muscle lacking cavin1 (Lo et al. 2015), which normally anchors cavin4 in caveolae (Bastiani et al. 2009). Taken together, these observations suggest that cavin4 may redistribute to intracellular compartments, including the nucleus, under conditions in which the muscle sarcolemma is subjected to mechanical forces, for example, in conditions of excessive muscle activity. Overexpression of cavin4 in cultured skeletal muscle cells induces hypertrophy through a pathway involving activation of RhoA/ROCK and transcriptional upregulation of myofibrillar components (Rodriguez et al. 2011, Tagawa et al. 2008). This observation suggests a potential mechanism by which excessive muscle activity could cause cavin4 release to stimulate hypertrophy. Additional evidence exists for noncaveola roles of released cavins, including regulation of lipolysis through phosphorylation of cavin1 (Aboulaich et al. 2011). In view of the different subcomplexes of cavins that can be released from caveolae [e.g., cavin1/cavin2 and cavin1/cavin3 (Gambin et al. 2014)], there may be a range of downstream targets reached by distinct combinations of cavins. As mechanisms exist to maintain low levels of cytosolic cavin1 (and presumably other cavins) (Tillu et al. 2015), the system would be sensitized to respond to stimuli that release cavins and then rapidly reset.

Mechanoprotection

The wide range of studies in different experimental systems showing a mechanoprotective role for caveolae provides an explanation for the very high density of caveolae in certain cell types and why the defined shape of caveolae is so important. The ability of caveolae to act as a spring or membrane reservoir that can be deformed and so prevent membrane damage may underlie many of the disease conditions associated with loss of caveolae, such as muscular dystrophies. Myotubes with defective caveolae are more susceptible to damage (Sinha et al. 2011), and excessive muscle activity in zebrafish lacking the muscle-specific isoform of cavin1 or expressing dominant-acting mutants of CAV3 causes increased membrane damage (Lo et al. 2015). As muscle caveolae are

flattened in response to membrane stretch, a likely explanation for these observations is direct damage to the sarcolemma in the absence of caveolae, consistent with observations in other systems such as endothelial cells subjected to high blood pressure (Cheng et al. 2015). However, there is also evidence that muscle caveolae contribute to the repair of damage after injury (Cai et al. 2009, Corrotte et al. 2013) and CAV3 is important in the trafficking of the muscle repair protein dysferlin (Hernandez-Deviez et al. 2006, Matsuda et al. 2001). CAV3 levels and caveola density are increased in Duchenne muscular dystrophy (Repetto et al. 1999), suggesting that caveolae may be upregulated to compensate for loss of other structural components of the sarcolemma. Lipodystrophy associated with loss of CAV1 may also have a mechanical contribution. Mice lacking CAV1 show normal adipose tissue at birth, but as they age, they show a loss of adipose tissue and increased inflammation. Adipocytes isolated from CAV1-null mice show very fragile adipocytes, which may be partially compensated by increased collagen deposition in vivo (Martin et al. 2012). As mice age and lipid deposits increase, the effect of increased adipocyte fragility may cause a progressive loss of the most highly differentiated adipose tissue. Interestingly, a loss of the major adipocyte isoform of collagen is associated with increased caveola density (Khan et al. 2009), suggesting that the two systems may be interdependent to provide stability to the tissue. Mechanoprotective roles of caveolae may be even more widespread, with the findings that polymorphisms in *CAV1* and *CAV2* are associated with glaucoma and that loss of CAV1 is associated with increased susceptibility to plasma membrane damage upon experimental elevations of intraocular pressure (Elliott et al. 2016).

Many of the above studies compared wild-type cells and tissues with cells or tissues lacking CAV1, CAV3, or cavin1. As described above, these proteins generally show reciprocal regulation at the level of the protein (e.g., loss of CAV1 decreases levels of cavin1). In addition, both caveolins and cavins have functions outside caveolae. To address the specific role of the caveola invagination, rather than the function of these caveola proteins, a recent study generated mice lacking pacsin3/syndapin3 (Seemann et al. 2017). Skeletal muscle from these animals showed wild-type levels of CAV3 and cavin1 associated with the sarcolemma but a striking loss of caveolae. Unlike wild-type mice, knockout mice subjected to physical exercise showed muscle damage, including necrotic fibers and inflammation. The similarity of the phenotypes to those associated with CAV3-associated muscle disease provides further evidence for the model of caveola flattening acting to protect muscle fibers against abrupt tear forces and is also consistent with the phenotype of dominant-acting CAV3 mutants in zebrafish subjected to intense physical activity (Lo et al. 2015). Intriguingly, although the cardiac muscle of the pacsin3 knockout mice also lacked caveolae, the cardiac phenotypes observed in CAV3 knockout animals were not detected in mice lacking pacsin3 (Seemann et al. 2017). These results suggest that cardiac defects may be more related to noncaveola functions of caveola proteins than to a loss of the mechanoprotective activity of the caveola invagination.

Lipid Regulation

The first patients who were discovered to totally lack CAV1 expression showed severe lipodystrophy (Cao et al. 2008, Kim et al. 2008). More recent studies described patients lacking cavin1 who also showed lipodystrophy, in addition to presenting clinical symptoms associated with a loss of muscle caveolae (Hayashi et al. 2009, Shastry et al. 2010). Many studies point to a role of caveolae in regulating lipid trafficking, storage, and/or metabolism.

At the level of the plasma membrane, caveolae influence general membrane properties, including the nanoclustering of phosphatidylserine and the nanoscale organization of lipid-based signaling molecules (Ariotti et al. 2014). This effect appears to be dependent on caveola

invagination, not just caveola components, as similar effects were seen upon flattening of caveolae. These results suggest that disassembly of caveolae can lead to significant changes in the bulk plasma membrane, with consequences for the organization of signaling domains. Flattening of caveolae could potentially cause redistribution of lipids through two mechanisms. Disruption of the caveola domain and the associated proteins, including the dissociation of cavins, could lead to a loss of their lipid-concentrating activity. Second, lipids of specific shape may be concentrated in highly curved domains (such as the neck) of intact caveolae and would be released into the bulk membrane as caveolae flatten. Loss or overexpression of caveola components also has general effects on membrane lipid properties, including the degree of lipid order (Gaus et al. 2006) and the diffusion of lipids and lipid-anchored proteins in the membrane (Chaudhary et al. 2014, Hoffmann et al. 2010). These changes have profound physiological effects. Expression of either caveolin or cavin inhibits clathrin-independent endocytosis (Chaudhary et al. 2014), and caveolin inhibits invasion by specific bacteria (Hoffmann et al. 2010, J.Y. Lim et al. 2017). In each case, inhibition by caveolin depends on the scaffolding domain region and can be modulated by general changes in membrane properties (Chaudhary et al. 2014, Hoffmann et al. 2010, J.Y. Lim et al. 2017). These studies point to a general role of caveolae and/or caveola proteins in modulating the lipid-based organization of the plasma membrane.

Caveolae have also been implicated in glycosphingolipid transport (Shvets et al. 2015), and glycosphingolipids regulate caveola formation and endocytosis (Sharma et al. 2004; Singh et al. 2003, 2010). GM3 is enriched in caveolae, and increasing levels of GM3 upregulate levels of CAV1 (Prinetti et al. 2010). These studies emphasize a close relationship between caveolae and specific lipid species. Cholesterol and caveolae are also closely linked. While cholesterol is required for caveola formation and integrity, depletion causes loss of caveola morphology and turnover of cavin2 (Breen et al. 2012, Hailstones et al. 1998, Rothberg et al. 1992), and caveolae are in turn important for maintenance of the cholesterol balance in the cell (Ikonen & Parton 2000). CAV1 deficiency causes cholesterol to accumulate in mitochondria, disrupting mitochondrial function (Bosch et al. 2011). Mitochondrial dysfunction is a feature of many CAV1-null cells (Asterholm et al. 2012, Fernandez-Rojo et al. 2013, Fridolfsson et al. 2012). Caveolin mutants that accumulate in lipid droplets disrupt cholesterol trafficking (Pol et al. 2004), and loss of CAV1 causes decreased cholesterol synthesis and increased esterification (Frank et al. 2006). CAV1 also binds fatty acids and increases fatty acid uptake, and loss of CAV1 causes increased lipotoxicity (Meshulam et al. 2006, 2011; Simard et al. 2010).

At the level of whole animals, CAV1-null mice have small adipocytes, are resistant to diet-induced obesity, and are insulin resistant (Razani et al. 2002a). Similar, although not identical, phenotypes are observed in mice lacking cavin1 (Liu et al. 2008). Cavin1 plays a direct role in regulation of lipolysis (Aboulaich et al. 2011) as well as contributing to caveola formation/stability and to regulation of the ribosome synthesis required for adipocyte maturation (Liu & Pilch 2016). CAV1-null mice also lack efficient lipid droplet formation in hepatocytes during liver regeneration, leading to decreased survival after partial hepatectomy (Fernandez et al. 2006). Despite low levels of CAV1 in liver, loss of CAV1 has profound cell-autonomous effects on multiple facets of lipid metabolism, including compromised signaling by the nuclear hormone receptor PPARα, a key regulator of fatty acid oxidation and ketogenesis, in response to fasting (Fernandez-Rojo et al. 2013). As endogenous fatty acids are ligands for PPARα, these effects may partially relate to the role of CAV1 in regulating fatty acid transport and availability (Astudillo et al. 2011).

These complex phenotypes, observed in cell studies and in vivo, present a confusing picture of the precise way that caveolae control lipid metabolism. The contributions to the lipodystrophy seen in patients may well be through many different molecular mechanisms. Another question is which of these phenotypes requires caveolae and which is dependent only on caveola proteins, such

as CAV1. This question is only starting to be addressed. In some mammalian cells, such as many types of neurons, caveolae are absent, but CAV1 still plays an important role. For example, striatal neurons lack caveolae but have endogenous CAV1, which in these cells regulates cholesterol trafficking and functionally interacts with mutant Huntington's disease (HD) protein (Trushina et al. 2006). In addition, loss of CAV1 accelerates neurodegeneration and aging in hippocampal neurons, whereas increased CAV1 expression in neurons enhances prosurvival and progrowth signaling (Head et al. 2010).

The lipid-transporting role of CAV1 may be its primary role in cells, such as neurons, that lack caveolae. Further clues come from study of nonmammalian caveolins. Caveolins are widely conserved in evolution, including 24 different genes in the oyster genome (Zhang et al. 2012), whereas cavins are restricted to vertebrates. In *Caenorhabditis elegans*, there are two genes encoding caveolins (CAV-1 and CAV-2) (Tang et al. 1997). CAV-1 is expressed fairly ubiquitously in the embryo but, in the adult, is restricted to specific cell types (Parker et al. 2007, Scheel et al. 1999). *C. elegans* CAV-1 does not generate caveolae (Kirkham et al. 2008) but is instead an endocytic cargo protein in oocytes (Sato et al. 2006). *C. elegans* CAV-2 is expressed in the intestine, and loss of CAV-2 leads to reduced apical lipid uptake (Parker et al. 2009). It is interesting to speculate that caveolins may have evolved as lipid-transporting/regulating proteins and that their role in caveola formation may have been a later adaptation.

CONCLUSIONS

Caveola research has now shifted from an exclusive focus on caveolins to a more holistic analysis of the entire caveola system, including the cavins, pacsins, and EHD proteins; the distinct lipid composition of caveolae; and the role of the special morphology of caveolae and that of the characteristic multilobed caveola clusters. With the major proteins now identified, we can anticipate gaining an atomic-scale understanding of caveola formation, which will also be crucial to understanding how caveolae can be rapidly disassembled and how this process is regulated. Studies on caveolae in cultured cells, as used almost exclusively to study caveola dynamics, are now being complemented by studies in vivo, including both mouse and zebrafish models, which will be facilitated by the incredible recent advances in in vivo light microscopic imaging. In addition, researchers are starting to appreciate the need to understand the caveola and noncaveola roles of the major structural and accessory proteins associated with caveolae and to develop new tools to address these issues. Release of distinct subsets of caveola proteins from surface caveolae as they flatten, membrane lipid changes, and the phosphorylation of caveola proteins to generate signaling platforms may contribute to a responsive sensing system. The role of caveolae as a dynamic sensing and mechanoprotective structure, with a specialized lipid composition, clearly impacts diverse areas of biomedical science, and the implications for understanding a range of disease conditions will be immense.

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