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PERSPECTIVE

Caveolae and lipid sorting: Shaping the cellular response to stress

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Caveolae are an abundant and characteristic surface feature of many vertebrate cells. The uniform shape of caveolae is characterized by a bulb with consistent curvature connected to the plasma membrane (PM) by a neck region with opposing curvature. Caveolae act in mechanoprotection by flattening in response to increased membrane tension, and their disassembly influences the lipid organization of the PM. Here, we review evidence for caveolae as a specialized lipid domain and speculate on mechanisms that link changes in caveolar shape and/or protein composition to alterations in specific lipid species. We propose that high membrane curvature in specific regions of caveolae can enrich specific lipid species, with consequent changes in their localization upon caveolar flattening. In addition, we suggest how changes in the association of lipid-binding caveolar proteins upon flattening of caveolae could allow release of specific lipids into the bulk PM. We speculate that the caveolae-lipid system has evolved to function as a general stress-sensing and stress-protective membrane domain.

Introduction

Caveolae are small, 60–80-nm, pits of the plasma membrane (PM) generated by membrane proteins, termed caveolins, and cytoplasmic proteins termed cavins (Echarri and Del Pozo, 2012; Hansen and Nichols, 2010; Parton and del Pozo, 2013; Parton and Simons, 2007). Despite being first observed more than six decades ago, an overarching function of these abundant PM microdomains has continued to elude cell biologists. Caveolae have been proposed to function in a wide range of cellular processes, including endocytosis (Boucrot et al., 2011; Pelkmans and Zerial, 2005), transcytosis (Ghitescu et al., 1986), lipid homeostasis (Harder and Simons, 1997), cholesterol homeostasis (Frank et al., 2006; Fu et al., 2004; Ikonen and Parton, 2000), regulation of cellular signaling (Couet et al., 1997; García-Cardeña et al., 1997), regulation of membrane composition and organization (Ariotti et al., 2014; Chaudhary et al., 2014), and mechanoprotection (Gervásio et al., 2011; Sens and Turner, 2006; Sinha et al., 2011). The latter is thought to involve the flattening of caveolae in response to increased membrane tension (Lee and Schmid-Schönbein, 1995; Sinha et al., 2011). In cells with abundant caveolae, this can protect the cell against damage upon cell stretching by providing a reservoir of membrane (Cheng et al., 2015; Lo et al., 2015; Seemann et al., 2017; Sinha et al., 2011; Yeow et al., 2017).

Caveolar architecture: Shape is crucial for function

Caveolae were initially characterized and differentiated from other invaginations of PM by EM. Under conventional EM fixation and embedding protocols, caveolae lack a discernible protein coat, unlike the better-understood clathrin-coated pits (CCPs; Simionescu et al., 1972, 1975). Despite similarities in general morphology, detailed comparisons of caveolae and CCPs offer insights into the unique roles these different PM-connected structures play in the cell. Comprising 1–2% of the PM, CCPs function in receptor-mediated endocytosis (Doherty and McMahon, 2009). CCPs demonstrate a range of curved membrane shapes that start as flat 2D lattice arrangements and mature into 3D pits with sequentially increasing bending that eventually bud from the PM to become intracellular vesicles (Doherty and McMahon, 2009; Heuser, 1980). Caveolae, in contrast, can occupy up to 50% of the surface of some mammalian cells (Lo et al., 2015; Thorn et al., 2003). The very characteristic and extremely uniform shape of caveolae (Fig. 1) resembles an omega in a side-oriented 2D EM image, and importantly, caveolae do not generally show the range of intermediate shapes described for CCPs (Avinoam et al., 2015). This structure is driven by the coordinated action of the caveolin integral membrane proteins together with the cavin peripheral membrane proteins, which both associate with the caveolar bulb (Ariotti et al., 2015a; Gambin et al., 2013; Ludwig et al., 2013;

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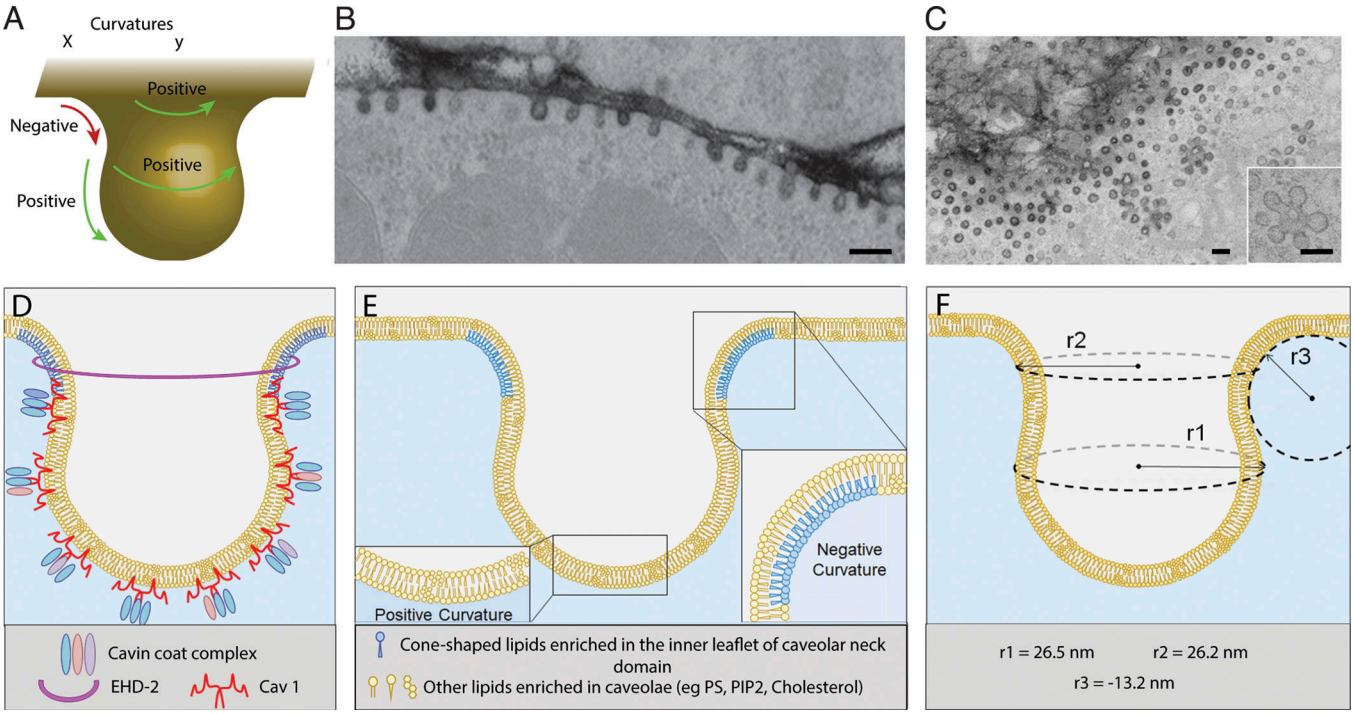

Figure 1. **The structure of caveolae. (A)** Scheme showing a caveola and the principle membrane curvatures of the caveolar domain. Curvature is described in two perpendicular directions. The bulb of the caveola is positive in both directions (x and y), but the neck shows negative and positive curvature, as indicated. **(B)** Electron micrograph showing the bulblike morphology of caveolae. Bar, 100 nm. **(C)** Higher-order rosette organization of caveolae in a cultured adipocyte. Bar, 100 nm. **(D)** The protein composition of caveolae. **(E)** The neck domain accounts for the overall mean negative curvature of the caveolar domain. Highlighted are the lipids of the inner leaflet of the PM. Blue, cone-shaped lipids. **(F)** Average dimensions of neck and bulb domains from endothelial caveolae preserved by high-pressure freezing and freeze substitution published previously (Richter et al., 2008). Curvature calculations were based on these dimensions. Diagram, including bilayer thickness, is to scale.

Rothberg et al., 1992). These proteins work together with EH domain–containing protein 2 (EHD2), a large ATPase localized at the caveolar neck (Ariotti et al., 2015a; Morén et al., 2012; Stoeber et al., 2012) that shapes membranes in an ATP-dependent manner (Hoernke et al., 2017; Melo et al., 2017). Even in model membrane systems, caveolins and cavins can drive membrane deformation in the absence of other proteins (Kovtun et al., 2014; Uytterhoeven et al., 2015; Walser et al., 2012). Caveolae also adopt a higher-level organization: complex and convoluted arrangements of interconnected structures comprising multiple caveolar units, described as caveolar clusters or rosettes, which have been likened to a bunch of grapes (Fig. 1 C; Lo et al., 2015; Stan, 2005). Prominent in skeletal muscle cells, these striking structures have a large membrane area packaged into a small cytoplasmic volume. Recent work suggests that caveolar rosette formation is driven by an interaction between the caveolar bulbs mediated by membrane bending deformation and is supported by weak membrane tension (Golani et al., 2019). Increased membrane tension preferentially flattens out caveolar rosettes, enabling a large change in cell volume as a cell expands (Golani et al., 2019; Lo et al., 2015). Even in rosettes or these extended networks, the basic morphological unit remains the same; a bulb-shaped structure with a neck connecting those structures to the bulk PM or other caveolae. Altogether, these unique and highly conserved morphological features of caveolae are likely crucial for their function.

Caveolae: A specialized lipid domain

Caveolae have long been considered stabilized cholesterol-dependent lipid microdomains with specialized functions in signal transduction and lipid regulation (Ikonen and Parton, 2000; Parton and Simons, 1995; Rothberg et al., 1992). Caveolin-1 (Cav-1) is a cholesterol-binding protein (Murata et al., 1995) with a cholesterol recognition/interaction amino acid consensus motif (Epand et al., 2005) that significantly enriches cholesterol within the caveolar domain; up to 22,000 cholesterol molecules per caveolae have been estimated (Ortegren et al., 2004). Moreover, cholesterol homeostasis is intimately linked to Cav-1 and caveolae, as cholesterol distribution within the Golgi complex and PM is highly dependent on Cav-1/caveolae expression (Hayer et al., 2010; Pol et al., 2005). The importance of cholesterol in caveola structure is highlighted by the loss of caveolar shape upon depletion of PM cholesterol with extracellular addition of nystatin or methyl-β-cyclodextrin (Breen et al., 2012; Rothberg et al., 1992; Westermann et al., 2005). The substitution of cholesterol for a precursor, desmosterol, also results in an increase in caveolar structural heterogeneity (Jansen et al., 2008). The precise composition of both the headgroups and acyl chains of other lipids in the membrane of caveolae is still not completely clear. Detergent-free lipidomic

Key concepts and hypotheses

Caveolae form a highly curved membrane domain that can transform into a flat membrane under different experimental conditions.

Membrane shape can enrich specific lipids within distinct highly curved subdomains of caveolae; flattening of caveolae would eliminate this shape-induced concentration of lipids and release them into the bulk membrane.

Lipid-binding peripheral membrane proteins densely coat the cytoplasmic face of caveolae; flattening of caveolae can change their interaction with caveolar lipids.

Caveolae have evolved to respond to a range of cellular stresses; lipid sorting might be a crucial aspect of this response.

analyses have shown that sphingomyelin, glycosphingolipids, and gangliosides, components of the extracellular leaflet of the bilayer, are enriched within caveolae (Ortegren et al., 2004). Extracellular addition of glycosphingolipids and cholesterol stimulates caveolar endocytosis (Le Lay et al., 2006; Sharma et al., 2003, 2004, 2005), and cells lacking Cav-1 show aberrant trafficking of excess glycosphingolipids and cholesterol (Shvets et al., 2015). In the cytoplasmic leaflet of caveolae, phosphatidylserine (PtdSer) and phosphoinositide 4,5-bisphosphate (PtdIns(4,5)P_2) may be enriched (Fairn et al., 2011; Fujita et al., 2009). Functionally, PtdSer has a critical role in regulating caveola stability/formation, as depletion of PtdSer reduces caveolae detectable by EM (Hirama et al., 2017a).

While caveola formation and dynamics appear to be dependent on specific lipid species, caveolae have conversely been implicated in the regulation of PM lipid organization (Parton, 2018). Loss of caveolae, or their flattening in response to increased membrane tension, affects the lipid-based organization of the bulk PM, as revealed by changes in organization of distinct lipid-anchored Ras species (Ariotti et al., 2014). This has dramatic effects on specific lipid-based signal transduction processes. For example, K-ras activity is enhanced but H-ras signaling is reduced (Ariotti et al., 2014). Nanoscale clustering of PtdSer in the bulk PM was also significantly affected when caveolae were rapidly disassembled, showing effects on lipid organization that extended over the entire PM.

How is the lipid composition of caveolae established, and how does this translate into effects on bulk PM lipid organization as caveolae flatten? We will consider two models. The first involves the tight curvature of the caveolar membrane driving concentration of lipids of specific effective molecular shape. The second involves the lipid-binding ability of the caveolar constituents and the impact of modulation of the membrane-binding activity of these components on lipid organization (see text box). These models are not mutually exclusive but will be considered in turn.

Caveolar curvature and lipid sorting

The extent of membrane curving is quantitatively characterized by a geometrical notion of curvatures (e.g., Spivak, 1970). The membrane surface is described at every point by curvatures of two circular arcs representing the surface cross sections in two particular mutually perpendicular directions (Fig. 1 A). The curvatures of these arcs are referred to as the principle curvatures. The physics behind the membrane shape is determined by the sum and the product of the two principle curvatures called the mean and Gaussian curvatures (Helfrich, 1973). In the following, for brevity, we refer to the mean curvature as simply the curvature.

Eukaryotic membranes comprise hundreds of different lipid species that differ in their headgroups and acyl chains. Most importantly for this discussion, lipid species also differ in their effective molecular shape, being classified as (a) cylindrical (where the area of the polar head group projection to the membrane plane is roughly equal to that of the hydrophobic chain, such as phosphatidylcholine); (b) cone-shaped (where the acyl chains occupy a substantially greater in-plane area than the head group, such as DAG or dioleoylphosphatidylethanolamine); or (c) inverted cone–shaped (whereby the polar headgroup occupies a considerably larger in-plane area than the acyl chain, e.g., lysophosphatidic acid [LPA] or lysophosphatidylcholine). Cone- or inverted cone–shaped lipids can facilitate the formation of membrane curvature (Zimmerberg and Kozlov, 2006; McMahon and Boucrot, 2015); enrichment of the cytoplasmic membrane leaflet in cone-shaped lipids favors negative curvature (here defined as bulging out away from the cytoplasm as used previously; McMahon and Gallop, 2005; Fig. 1 A); a preferential distribution of the inverted cone–shaped lipids into the cytoplasmic leaflet favors positive curvature (bulging into the cytoplasm).

Caveolae possess regions of positive and negative mean curvature. Positive mean curvature characterizes the bulb subdomain (Fig. 1 A). The neck subdomain has a saddle-like shape, which is convex in one principle direction and concave in the second. The resulting positive and negative principle curvatures compete, but typically, their sum (representing the mean curvature of the neck) is negative (see below; Fig. 1). The neck negative curvature is favored by the lateral tension usually existing in the PM (Golani et al., 2019). This curvature distribution along the caveolar surface would favor the enrichment of specific cone- and inverted cone–shaped lipids in the cytoplasmic leaflets of the neck and bulb subdomains, respectively (Fig. 1). This would also need to be balanced by specific lipids in the extracellular leaflet, although we will restrict our considerations to the cytoplasmic leaflet here.

The extent of specific lipid enrichment in the caveolar domains can be estimated by assuming that lipid molecules redistribute along the cytoplasmic monolayer according to minimization of the membrane bending energy. Thermodynamic consideration of lipid molecule partitioning between membrane regions of different curvatures, taking into account a competition between the curvature energy and the translational entropy of lipid molecules, predicted that the ratio between the concentration of a specific lipid in the curved membrane region, φ_c, and that in the plane of the flat PM, φ_f, can be estimated using the relationship

$$\frac{\phi_c}{\phi_f} = \exp\left(\frac{\kappa a}{k_B T} J J_S\right),$$

where k_BT is the product of the Boltzmann constant and the absolute temperature, $\kappa \approx 10 k_B T$ is the bending modulus of a lipid monolayer, J is the membrane mean curvature, J_S is the effective molecular curvature of the lipid under consideration, and $a \sim 0.7$

nm^2 is the area per lipid molecule in the membrane plane. Conventionally, J_S is defined as positive for the inverted cone–shaped lipids and negative for the cone-shaped lipids (e.g., Zimmerberg and Kozlov, 2006).

Based on this relationship, the concentration of the inverted cone–shaped molecules of LPA within the cytoplasmic leaflet of the bulb region can be predicted. When processed by high-pressure freezing and low-temperature freeze substitution, the radius of the bulb domain of caveolae is ∼26–27 nm (Richter et al., 2008), so the bulb mean curvature is J ∼0.075 nm^{-1}. It has also been shown that caveolins (Ariotti et al., 2015b; Walser et al., 2012) and cavins (Stoeber et al., 2016) can impart a polygonal structure on the caveolar membrane, suggesting higher membrane curvature along the polygon edges and around the vertices, but for the purpose of our semiquantitative discussions, we will assume a uniformly curved structure. LPA is characterized by an effective molecular curvature of J_S ∼0.5 nm^{-1} (Kooijman et al., 2005). Using the above relationship, the LPA concentration in the bulb can be calculated to exceed by 25–30% that in flat membrane regions (note that LPA and DAG, below, are used simply as model lipids for these analyses; enrichment in caveolae is not implied).

This shape-driven mechanism might also be important in the neck region of caveolae, which is highly curved (cross-sectional radius 13 nm; Richter et al., 2008; Fig. 1). The negative principle curvature in the narrowest region of the neck is approximately −1/13.2 nm^{-1} and exceeds the positive curvature contribution in this region of ∼1/26.2 nm^{-1} (Fig. 1 F). This suggests that, as mentioned above, the overall mean curvature of the neck region is negative, J, approximately −0.038 nm^{-1}. Therefore, we hypothesize that the cytoplasmic leaflet of the neck region would be enriched in cone-shaped lipids. An estimation for a representative common cone-shaped lipid, DAG, which is characterized by an effective molecular curvature of J_S ∼−0.91 nm^{-1}, predicts an ∼30% enrichment of DAG in the neck region compared with the flat membrane. This is consistent with work in model systems showing only a slight enrichment of specific lipid species in lipid tubules of relevant diameters pulled out of flat membranes (Sorre et al., 2009) and with the limited curvature preference of specific lipid species observed by using a fluorescence-based method with unilamellar lipid vesicles (Kamal et al., 2009).

The effectiveness of lipid redistribution into the curved regions of caveolae is expected to greatly exceed the above estimates if the lipid molecules undergo even a slight segregation in the membrane plane, i.e., by forming lipid raft–like nanodomains (Callan-Jones et al., 2011; Tian and Baumgart, 2009). This might be a reason that PtdSer distribution is tightly linked to cholesterol (Cho et al., 2012; Maekawa and Fairn, 2015), which can promote domain formation. In fact, recent work has shown curvature-dependent sorting of specific PtdSer species in a model cellular system (Liang et al., 2019). While proteins could potentially contribute to this sorting, that study demonstrated that the acyl composition of the lipids that shared the same headgroup was essential for curvature-dependent segregation in the membrane plane of a lipid-anchored GTPase K-ras (Liang et al., 2019). The headgroup charge can also potentially contribute to this effect, as shown for PtdSer (Hirama et al., 2017b). While the biophysical understanding of this effect is rudimentary, it strengthens the idea that highly curved membrane domains can sort lipids. Most importantly, the suggested and estimated recruitment of specific lipids to the bulb and neck regions would be solely based on the geometrical aspects of the unique caveolar domain: shape would drive lipid composition.

This lipid-shape model proposes that simple biophysical principles would provide a mechanism for a regulated concentration of specific lipids in caveolae. Could this have relevance for their function? Flattening of caveolae would release specific lipids that are usually concentrated at the neck and the bulb of the caveolae into the bulk membrane. Thus, the shape of caveolae (flattened or deeply invaginated) would dictate lipid composition. A shape change generated, for example, by lateral tension imposed on the membrane by external, cytoskeletal, or osmotic forces that induce caveolar flattening (Ariotti et al., 2014; Sinha et al., 2011) would result in alterations to the PM lipid environment.

The shape of caveolae relative to the lamellar organization of the PM allows for further interesting predictions regarding the effect of caveolar flattening. Given that the interleaflet area difference is proportional to the membrane mean curvature averaged over the membrane surface, and given that, assuming that caveolae represent 50% of the membrane area, a caveola radius is 26.5 nm (Fig. 1 F) and the area of the caveola bulb considerably exceeds that of the neck (Fig. 1), we estimate that the area of the cytoplasmic membrane leaflet exceeds that of the extracellular leaflet by ∼15–20%. Therefore, as caveolae are flattened by imposed lateral tension, this area difference must be eliminated by lipid flux directed from the cytoplasmic to the extracellular membrane leaflets and/or by release of protein domains inserted into the cytoplasmic side of the membrane. Thus, the membrane would be able to compensate for these area differences only if every fifth molecule of the cytoplasmic monolayer was to flip. The most probable candidates for fast flipping would be lipid molecules with small polar heads such as cholesterol, as they cross the hydrophobic moiety of the membrane in a relatively short time (Bennett and Tieleman, 2012; Ingólfsson et al., 2014). This would mean that loss of caveolae could potentially generate a temporary and considerable depletion of the cytoplasmic leaflet of these components, and a concomitant enrichment of these lipids in the extracellular monolayer.

Caveolar proteins and lipid sorting

The lipid-shape model would likely generate only a moderate concentration of specific lipids at the necks and bulbs of caveolae. Here we consider the role of proteins in recruiting lipids to caveolae and how this can lead to a regulated redistribution of lipids as caveolae disassemble. The major proteins we will discuss are the cavins, but it is important to note that in model systems, caveolins also show a lipid-concentrating ability (Epand et al., 2005; Wanaski et al., 2003). Importantly for this discussion, as peripheral membrane proteins, cavins, unlike caveolins, can dissociate from the cytoplasmic surface of caveolae in response to stresses (McMahon et al., 2019; Sinha et al., 2011), and so lipid interactions can be regulated. Cavins bind

PtdIns(4,5)P_2 via a basic domain in their first helical region, HR1 (Kovtun et al., 2014). Based on the number of basic amino acids, we envisage that cavins have relatively low affinity for PtdIns(4,5)P_2, compared with, for example, the myristoylated alanine-rich C-kinase substrate (MARCKS) protein (McLaughlin and Murray, 2005; McLaughlin et al., 2002). This is sufficient for in vitro binding to PtdIns(4,5)P_2-containing liposomes but, in vivo, is insufficient for membrane association and driving caveola formation (Kovtun et al., 2014). However, the cavin proteins form large oligomeric complexes, potentially bringing multiple PtdIns(4,5)P_2-binding domains into close proximity in the cytoplasmic leaflet of the caveolar domain. An interesting comparison can be made with BAR domain proteins that, like cavins, bind PtdIns(4,5)P_2, oligomerize, and induce tubule formation in vitro (Picas et al., 2014). In both cases, the multiple basic patches within the hetero- and homo-oligomeric complexes, each of low affinity for PtdIns(4,5)P_2, cooperate with each other to bind to the membrane surface as long as the geometry of the membrane conforms to that of the protein complex. We speculate that the curved surface of the caveolae is essential for this lipid-driven interaction. If so, then a change in this geometry could change PtdIns(4,5)P_2 association. In fact, there is evidence for this model. Flattening of caveolae due to increases in membrane tension causes lysine residues within the PtdIns(4,5)P_2-binding basic patch of the HR1 of cavin to become ubiquitinated (Tillu et al., 2015). The modified protein presumably has less affinity for the membrane, preventing rebinding, and ubiquitination triggers degradation. This mechanism maintains low levels of cytosolic cavin proteins (Tillu et al., 2015). But most importantly, these results suggest that one effect of increased membrane tension is to dissociate cavin1 from its interacting PtdIns(4,5)P_2-binding sites. For such a model, PtdIns(4,5)P_2 binding to cavin1 must be of relatively low avidity. Consistent with this, the putative PtdIns(4,5)P_2-binding sites of cavin1, while mediating in vitro association with PtdIns(4,5)P_2-containing liposomes, are not essential for association with caveolae in cells (Kovtun et al., 2014).

In addition to PtdIns(4,5)P_2 binding, cavin1 has another proposed lipid-binding site at the start of the second helical region, HR2. This region, termed the UC1 domain, comprises 11 amino acid (undecad) repeats and binds PtdSer (Tillu et al., 2018). In contrast to the PtdIns(4,5)P_2-binding region in the HR1 region (Kovtun et al., 2014), the UC1 domain plays a structural role; loss of PtdSer binding decreases membrane affinity, and progressive loss of multiple domains causes increased susceptibility to disassembly (Tillu et al., 2018), providing an interesting mechanism by which lipid binding can modulate caveolar stability.

What is the function of lipid binding of caveolar proteins? First, as proposed earlier, the PtdIns(4,5)P_2-binding domain acts as a sensor of the protein–membrane association; PtdIns(4,5)P_2 binding prevents protein ubiquitination, but loss of PtdIns(4,5)P_2 binding allows ubiquitination and degradation (Tillu et al., 2015). Second, and relevant to the current discussion, we speculate that the lipid-binding activity of the caveolar proteins can contribute to the unique lipid composition of caveolae. The cytoplasmic face of the caveolar bulb is densely covered in cavin molecules, with an estimated 50 cavin1 molecules associated with a single caveola (Gambin et al., 2013). Estimating the surface area of the caveola bulb to be ∼6,000 nm^2, a 1:1 ratio of cavin to PtdIns(4,5)P_2 would equate to a concentration in caveolae of cavin1-associated PtdIns(4,5)P_2 of >8,000 molecules per μm^2. Note that for BAR domain proteins such as Bin1 and for other phosphatidylinositol-binding proteins, PtdIns(4,5)P_2 clustering is induced owing to nonspecific electrostatic protein–lipid interactions increasing the stoichiometry of PtdIns(4,5)P_2 association dramatically (Picas et al., 2014). PM PtdIns(4,5)P_2 levels have been estimated to be in the range of 4,000 per μm^2 (but see Hilgemann [2007] for higher estimates of PtdIns(4,5)P_2 levels; 20,000 to 60,000 per μm^2), and so this mechanism could recruit a considerable pool of PtdIns(4,5)P_2 into caveolae. The caveolar proteins EHD2 and dynamin are also PtdIns(4,5)P_2-binding proteins, as are the other cavin family members (Daumke et al., 2007; Kovtun et al., 2015). An additional point worthy of mention is that the most abundant form of PtdIns(4,5)P_2 in mammalian cells is polyunsaturated, with an arachidonic acid in the n2 position (McLaughlin et al., 2002). This would not be expected to favor concentration in the cholesterol-enriched membrane raft domain of caveolae, in which saturated lipids are thought to predominate (McLaughlin et al., 2002). The cavin interaction might be crucial for overcoming this barrier and recruiting the PtdIns(4,5)P_2 to an unfavorable environment.

What does the protein-driven recruitment of specific lipids mean for function? The fact that disassembly of caveolae, induced by cycles of PM stretch and relaxation, can cause increased accessibility of the PtdIns(4,5)P_2-binding domain of cavin1 to the cellular ubiquitination machinery (Tillu et al., 2015) suggests that PtdIns(4,5)P_2 must be freed from cavin1 interactions. This can release or modify the PM pool of PtdIns(4,5)P_2. In a cell with 50% of its surface occupied by caveolae, this can release (or uncluster) a considerable pool of PtdIns(4,5)P_2 and, presumably, can also release PtdSer. This model is analogous to that proposed for the MARCKS protein, in which calcium releases MARCKS and mobilizes PtdIns(4,5)P_2 pools to regulate processes such as cortical actin dynamics (Laux et al., 2000; McLaughlin et al., 2002). This mechanism would link disassembly of caveolae, caused by changes in membrane tension or other stimuli, to a change in the accessibility of PtdIns(4,5)P_2 and PtdSer. This protein-driven mechanism for lipid concentration could work together with the curvature-induced concentration of specific lipids at the neck of caveolae, with both mechanisms contributing to release of lipids into the bulk membrane when caveolae disassemble. How this then equates to the reported effects of caveolae disassembly, such as reorganization of the nanoscale clustering of specific lipids and lipid-anchored proteins in the bulk membrane, must await further experimentation aimed at understanding the local and global impact of the proposed changes in lipid organization.

Caveolae and stress signaling

These considerations suggest that caveolae represent a specialized lipid domain poised to disassemble, releasing specific lipids into the bulk membrane, in response to mechanical stimuli (see text box). We further speculate on the implications of this model for other caveolar functions. Of particular interest here are the

Table 1. **Caveolae and stress**

Type of stress	References
Osmotic/ stretch	Trouet et al., 1999, 2001; Kang et al., 2000; Sanguinetti et al., 2003; Ullrich et al., 2006; Sinha et al., 2011; Joshi et al., 2012; Ariotti et al., 2014; Guo et al., 2015; Lo et al., 2015; Mougeolle et al., 2015; Gilbert et al., 2016; Dewulf et al., 2019; Hetmanski et al., 2019
Shear	Rizzo et al., 1998a, 1998b; Isshiki et al., 2002; Sun et al., 2002; Boyd et al., 2003; Lungu et al., 2004; Frank and Lisanti, 2006; Shin et al., 2006; Yu et al., 2006; Radel et al., 2007; Albinsson et al., 2008; Milovanova et al., 2008; Müller-Marschhausen et al., 2008; Tian et al., 2010; Yamamoto et al., 2011; Chai et al., 2013; Figueroa et al., 2013; Zeng and Tarbell, 2014; Gilbert et al., 2016; Tran et al., 2016; Yang et al., 2016
Oxidative	García-Cardeña et al., 1996; Aoki et al., 1999; Peterson et al., 1999; Volonté et al., 2001; Volonte et al., 2002, 2009, 2013, 2015; Sanguinetti et al., 2003; Cao et al., 2004; Karaa et al., 2005; Dai et al., 2006; Dasari et al., 2006; Khan et al., 2006; Reddy et al., 2006; Hayashi et al., 2007; Chrétien et al., 2008; Jin et al., 2008; Milovanova et al., 2008; Percy et al., 2008; Wang et al., 2008; Volonte and Galbiati, 2009, 2011; Luanpitpong et al., 2010; Tian et al., 2010; Bosch et al., 2011; Martinez-Outschoorn et al., 2011; Yuan et al., 2011; Yun et al., 2011; Gortan Cappellari et al., 2013; Takeuchi et al., 2013; Chen et al., 2014; Mao et al., 2014; Mougeolle et al., 2015; Paneni et al., 2015; Sun et al., 2016; Jung et al., 2018
Ultraviolet	Volonté et al., 2001; Volonte et al., 2002; Wang et al., 2005; McMahon et al., 2019
Chemical	Bélanger et al., 2004; Cai and Chen, 2004; Pang et al., 2004; Shatz and Liscovitch, 2004, 2008; Martinez-Outschoorn et al., 2011; Wang et al., 2014, 2015b; Shi et al., 2015
Heat	Kang et al., 2000; Chaudhary et al., 2014; Volonté et al., 2001
Gravitational	Spisni et al., 2003, 2006; Riwaldt et al., 2015; Wang et al., 2015a; Zhou et al., 2015a; Shi et al., 2016

number of stress conditions linked to disassembly of caveolae. While caveolae have been shown to protect cells against increased membrane tension, a remarkably extensive literature has linked caveolae to other stress conditions, including shear, UV, chemical, oxidative, heat, and gravitational stresses (Shi et al., 2015; Wang et al., 2015b; McMahon et al., 2019; Table 1 summarizes the widespread literature linking caveolae/caveolin to stress response/protection). Indirect evidence for an evolutionary conservation of this role is provided by analysis of the genome of the oyster *Crassostrea gigas*. A remarkable 24 distinct caveolin genes have been identified in the oyster (compared with three mammalian caveolin genes and two in the primitive nematode, *Caenorhabditis elegans*; Zhang et al., 2012). This expansion of caveolin genes in the oyster genome is similar to that of known stress-response gene families such as heat shock proteins and chaperones, potentially reflecting adaptation to the harsh environment of the intertidal zone in which the oyster must survive (Zhang et al., 2012). Caveolae have not been identified, as yet, within the oyster, which like other invertebrates lacks cavin proteins. However, caveolins from other invertebrates including the honey bee, *Apis mellifera*, and the sea squirt, *Ciona*, can induce membrane curvature in model systems (Jung et al., 2018; Kirkham et al., 2008), and evidence now exists for caveola-like invaginations in *C. elegans* (Roitenberg et al., 2018) and *Ciona* (Bhattachan et al., 2020) embryos.

In view of the wide range of stimuli that are linked to caveolae and the apparent evolutionary conservation of stress signaling, we speculate that these two proposed models might be relevant to the effects of these stimuli on caveolae and resultant effects on lipids. In vertebrate cells, increased membrane tension can induce Cav-1 tyrosine phosphorylation, cavin dissociation (with concomitant ubiquitination of cavin1; Tillu et al., 2015), caveolar flattening (Sinha et al., 2011), and membrane lipid alterations (Ariotti et al., 2014). Interestingly, many other stress conditions may have similar effects. For example, UV treatment also causes loss of caveolae, Cav-1 tyrosine phosphorylation, and cavin dissociation (McMahon et al., 2019). This raises the possibility that caveolar disassembly might be a general sensing mechanism for cells to respond to various stressful stimuli. In the case of increased membrane tension, the change in curvature could lead to a change in the interaction of the cavins with the membrane, resulting in broad changes to lipid organization that could have wide-ranging implications for the clustering of other signaling proteins not directly enriched within the caveola domain (Fig. 2). We speculate that the change in curvature at the neck of the caveolae could also potentially decrease sorting of lipids based on shape, as discussed.

We further hypothesize that in invertebrates, caveolins alone could sculpt the membrane and drive lipid enrichment. This would provide a mechanism for their release, as caveolae flatten in response to membrane tension. Conversely, in the lipid-shape model, specific lipid enrichment in caveolar nanodomains would also be important for maintaining caveola shape. Perturbation of specific lipids, for example by lipid peroxidation under conditions of oxidative stress, would then perturb caveola structure, analogous to the effects of UV treatment on caveolae shown in mammalian systems (McMahon et al., 2019).

Perspectives

This model makes a number of predictions and raises many questions, which require experimental verification. First, are distinct lipid species enriched in the different subdomains of caveolae? This is a challenge, as purification of caveolae with the neck subdomain intact would be difficult. However, modern methods of lipid analysis and lipid localization are approaching the required resolution/sensitivity to make these distinctions in cells (Contreras et al., 2012; Owen et al., 2012; Huang et al., 2015; Zhou et al., 2015b). Enrichment of specific acidic lipids such as phosphatidylglycerol and lysophosphatidylglycerol, and an increase in long-chain unsaturated fatty acids, was observed in a model bacterial system for caveola formation compared with the lipidome of the prokaryotic host (Walser et al., 2012). While this

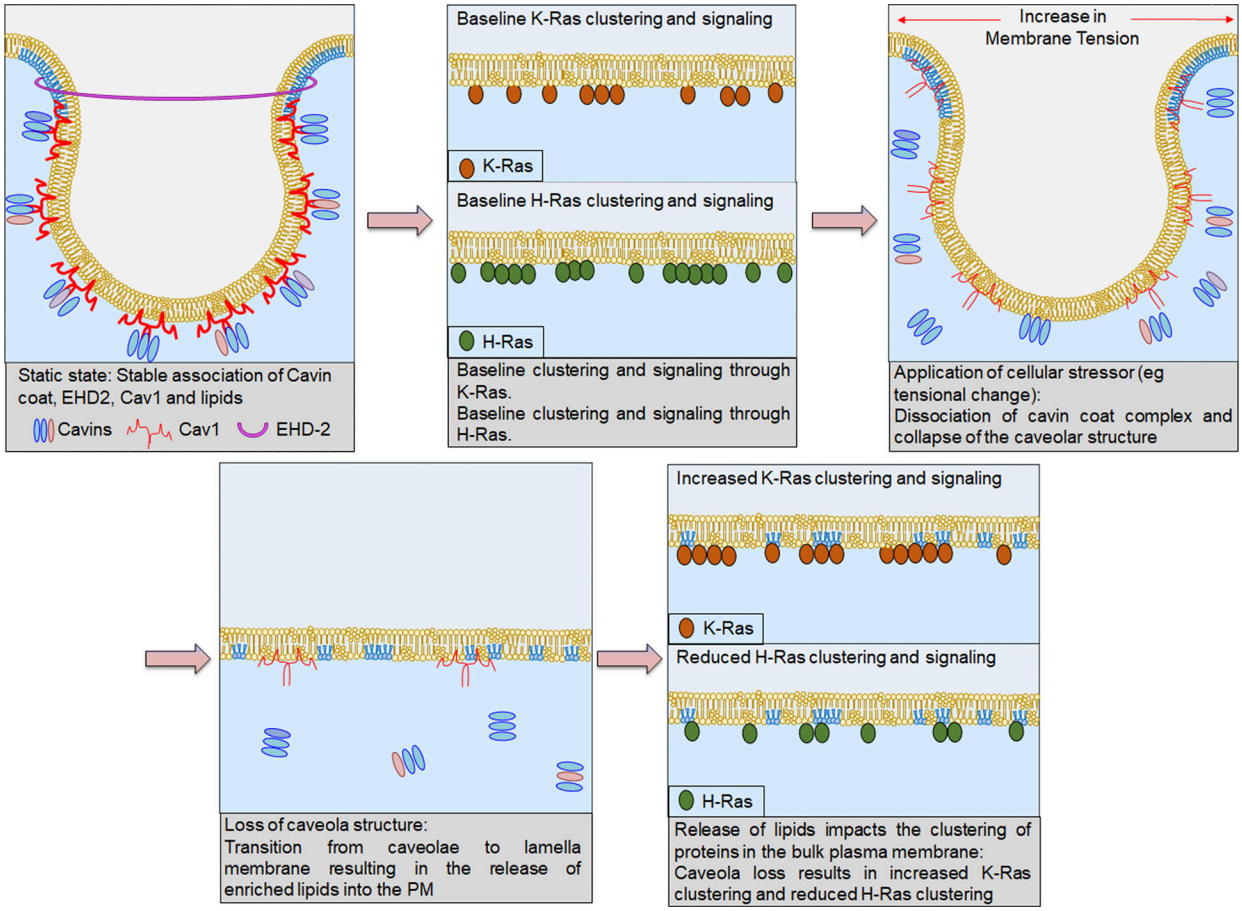

Figure 2. **Model of lipid release from caveolae in response to cell stress.** Schematic of the release of the cavin coat and enriched lipids from caveolae in response to cell stress. Our model proposes that caveolae, signaling proteins clustered on the PM outside of caveolae, and the lipids that make up the PM are in a "baseline/normal" state. However, upon exposure of the cell to an external stressor, such as an increase in membrane tension, caveolae are disassembled (Sinha et al., 2011); we hypothesize that this is a consequence of the release of the cavin coat complex and loss of the stability of the caveolar microdomain (McMahon et al., 2019; Sinha et al., 2011), which in turn releases the lipids enriched within the curved caveolar domain (Ariotti et al., 2014). Destabilization and release of caveolar lipids into the bulk membrane can indirectly affect protein clustering by modulation of the lipid nanoenvironment (as shown for PtdSer and Ras proteins) to modulate cellular signaling cascades (Ariotti et al., 2014). Finally, we hypothesize that this process may help cells respond to challenges from a wide array of cellular stressors (Table 1).

method does not allow for analysis of the lipid composition of the neck subdomain, as expression of caveolin in this system results in constitutive cincture and internalization of complete vesicles, it provides evidence for specific lipid sequestration in a bulblike subdomain, as also shown by earlier studies of purified caveolae from mammalian cells (Ortegren et al., 2004).

Second, it is important to understand the role of specific lipid species in caveola structure and function (Ortegren et al., 2004). Polyunsaturated phospholipids have been shown to modulate caveola formation and structure (Andreone et al., 2017; Ma et al., 2004). Reactive oxygen species are produced in UV or oxidative stress conditions. Could the oxidation of these unsaturated phospholipids play a role in the caveolar response to these stressors? It is notable that another complex membrane system, cubic membranes, protect against oxidative stress (Deng and Almsherqi, 2015). Like caveolar rosettes, cubic membranes contain a high density of curved membranes within a small volume, favoring enrichment in specific small lipid species; but unlike caveolae, cubic membranes are only rarely observed in mammalian cells, and these lipids would be enriched symmetrically within the two membrane leaflets. Cubic membranes are enriched in plasmalogens containing vinyl ether bonds. The increased susceptibility of these bonds to oxidation has been proposed to protect the membrane from lipid peroxidation (Sindelar et al., 1999), and plasmalogen oxidation might also favor a transition away from cubic membranes (Deng and Almsherqi, 2015; Deng et al., 2002, 2009).

Third, the flattening of caveolae in mammalian cells is associated with caveolin phosphorylation (Joshi et al., 2008) and cavin release (Sinha et al., 2011). Do other cellular stresses, which also trigger caveolin phosphorylation (Aoki et al., 1999; Spisni et al., 2006; Takeuchi et al., 2013; Volonté et al., 2001), stimulate similar loss of cavins and flattening of caveolae? A recent study found that UV light does have effects similar to increased membrane tension in causing caveolar disassembly and cavin release into the cytosol (McMahon et al., 2019).

Finally, we need to test the proposed lipid redistribution upon flattening of caveolae. Although it has been shown that

caveolar flattening can be associated with changes in lipid nanoclustering in the bulk PM (Ariotti et al., 2014), we do not yet know if/how this can be linked to the postulated release of lipids from caveolae based on a transition in caveolar shape and whether lipid redistribution can impact on the bulk PM organization. Moreover, we lack insight into how a caveolae-to-lamellar transition at the PM could impact lipid flip-flop dynamics, and if this is the case, which lipids moderate this transition.

In summary, we speculate that caveolae have evolved to produce metastable structures with unique architecture. It is this architecture that works together with the multitude of lipids within the membrane of a eukaryotic cell to generate a device that can respond to a multitude of different stimuli.

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