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 cavin (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., 2005), 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 at 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 caveolae bulb (Ariotti et al., 2015a; Gambin et al., 2013; Ludwig et al., 2013;
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. 1C; 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 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...
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, phosphatidylyserine (PtdSer) and phosphoinositide 4,5-bisphosphate (PtdIns(4,5)P2) 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 curvature 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, $\varphi_c$, and that in the plane of the flat PM, $\varphi_f$, can be estimated using the relationship

$$
\frac{\varphi_c}{\varphi_f} = \exp \left( \frac{k_a}{k_B T} J S \right),
$$

where $k_B$ is the product of the Boltzmann constant and the absolute temperature, $\kappa = 10k_B T$ 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 $a \sim 0.7$.
nm² 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 cytoplastic 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 \( J \sim -0.075 \) 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 surface. LPA is characterized by an effective molecular curvature of \( J_S \sim 0.5 \) 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. I). 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 cytoplastic 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 \( J_S \sim -0.91 \) nm⁻¹, predicts an \( \sim 30\% \) 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 cytoplastic membrane leaflet exceeds that of the extracellular leaflet by \( \sim 15-20\% \). Therefore, as caveolae are flattened by imposed lateral tension, this area difference must be eliminated by lipid flux directed from the cytoplastic to the extracellular membrane leaflets and/or by release of protein domains inserted into the cytoplastic side of the membrane. Thus, the membrane would be able to compensate for these area differences only if every fifth molecule of the cytoplastic 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 cytoplastic 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 cytoplastic surface of caveolae in response to stresses (McMahon et al., 2019; Sinha et al., 2011), and so lipid interactions can be regulated. Cavins bind
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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 caveola 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 caveola 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 caveola 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 UCI 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 UCI 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 nm², a 1:1 ratio of cavin to PtdIns(4,5)P_2 would equate to a concentration in caveola of cavin-associated PtdIns(4,5)P_2 of >8,000 molecules per µm². 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 µm² (but see Hilgemann [2007] for higher estimates of PtdIns(4,5)P_2 levels; 20,000 to 60,000 per µm²), 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
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 caveola-like invaginations in the required resolution/sensitivity to make these distinctions in methods of lipid analysis and lipid localization are approaching the required resolution/sensitivity to make these distinctions in methods of lipid analysis and lipid localization are approaching the required resolution/sensitivity to make these distinctions in methods of lipid analysis and lipid localization are approaching the required resolution/sensitivity to make these distinctions in methods of lipid analysis and lipid localization are approaching the required resolution/sensitivity to make these distinctions in methods of lipid analysis and lipid localization are approaching.

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

| 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; Ishihki 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; Albinnsson et al., 2008; Milovanova et al., 2008; Müller-Marschhausen et al., 2008; Tian et al., 2010; Yamamoto et al., 2011; Choi et al., 2013; Figureoa 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; Chretien et al., 2008; Jin et al., 2008; Milovanova et al., 2008; Percy et al., 2008; Wang et al., 2008; Volonte and Galbiati, 2009, 2011; Luannipong et al., 2010; Tian et al., 2010; Bosch et al., 2011; Martinez-Outschoorn et al., 2011; Yuan et al., 2011; Yun et al., 2011; Gorton 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; Riwalidt et al., 2015; Wang et al., 2015a; Zhou et al., 2015a; Shi et al., 2016 |
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 nano-clustering 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 caveolar-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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