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Caveolae: Formation, dynamics, and function

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Abstract

Caveolae are abundant surface pits formed by the assembly of cytoplasmic proteins on a platform generated by caveolin integral membrane proteins and membrane lipids. This membranous assembly can bud off into the cell or can be disassembled releasing the cavin proteins into the cytosol. Disassembly can be triggered by increased membrane tension, or by stress stimuli, such as UV. Here, we discuss recent mechanistic studies showing how caveolae are formed and how their unique properties allow them to function as multifunctional protective and signaling structures.

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Introduction

Caveolae cover the surface of many mammalian cells. These invaginations, of approximately 70-nm diameter, show a characteristic flask-shape as seen by conventional thin section electron microscopy and lack the classical fuzzy coat of clathrin-coated pits. Caveolae are highly abundant in muscle, adipocytes, endothelia, and primary fibroblasts but are scarce in other cell types. Caveolae have been linked to numerous disease conditions including diabetes (reviewed by Stralfors [1]), cardiovascular disease [2,3], pulmonary fibrosis [4], cancer [5–8] and a variety of degenerative muscular dystrophies [9*–11], making their mechanistic analysis of great biomedical importance. In this short review, we will focus on recent advances in three areas; the formation of caveolae, their dynamics, and their role in specific cellular signaling processes. For other aspects of caveola biology including caveola-independent functions of caveolar proteins, regulation of lipids by caveolae, and the dynamic interplay with focal contacts and the actin cytoskeleton, readers are referred to recent reviews [12–18].

Formation of caveolae: an interplay between integral membrane proteins, cytoplasmic proteins, and lipids

Caveolae are formed through the coassembly of two distinct sets of proteins that reach the plasma membrane (PM) by different mechanisms. Caveolins, hairpin-like integral membrane proteins, are synthesized in the endoplasmic reticulum (ER), and then pass through the Golgi complex *en route* to the PM, forming oligomeric assemblies as they progress through the secretory pathway [19]. At the PM, they meet accessory proteins, such as cavins, that are synthesized in the cytosol and then recruited to the caveolin-enriched surface nanodomain by interaction with lipids and, presumably, caveolin. Both caveolins and cavins have distinct properties that are important for generation of the caveolar domain, and these properties have been dissected using a number of model systems. Caveolin-1 (CAV1) can generate caveolar structures when expressed without other accessory proteins in model systems in a process that requires stable insertion of a large portion of the protein (residues 81 to 147) in the membrane and oligomerization [20–22]. In addition, *in vitro* studies suggest that caveolins can cluster specific lipids, such as cholesterol and phosphatidylserine (PtdSer) [23,24]. The cytoplasmic cavin proteins also have lipid binding activity, undergo oligomerization (forming trimers and then higher-order homo- and hetero-oligomers), and have the ability to sculpt membranes *in vitro*. Cavin1, which is essential for caveola formation, binds to phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P_2) and to PtdSer through distinct domains. The cavins are elongated proteins with a very characteristic domain organization; two positively charged helical regions (HR1, crystal structure solved [25] and HR2 based on predictions) are flanked by acidic disordered regions. The HR1 region of all cavins forms a trimeric coiled coil domain and contains a region of positively charged amino acids that mediate binding to PtdIns(4,5)P_2 [25]. The HR2 region of cavin1 differs from the other cavins in containing a unique undecad (11 amino acid) repeat region, termed the UC1 domain,

that binds PtdSer [26**]. The PtdIns(4,5)P_2 binding region of the cavins is not absolutely essential for caveola formation [25] but plays an important role as a sensor of membrane association [27]. When bound to the cytoplasmic surface of caveolae via PtdIns(4,5)P_2, this domain is masked but when free in the cytosol this region is ubiquitinated and the cavin protein is degraded by the proteasome. In contrast, the PtdSer-binding UC1 domain of cavin1 plays an important role in caveola formation and stability [26**].

Taken together, these and other studies (reviewed in [15]) suggest a model for caveola formation as follows (Figure 1). Caveolins reach the PM, arriving from the Golgi complex as oligomeric cholesterol-rich structures, termed scaffolds [28*] to generate nanodomains enriched in a specific subset of membrane lipids. These domains act as platforms to recruit oligomers of cytoplasmic cavin proteins, in a process that depends on the affinity of the elongated cavin oligomers for specific lipids and possibly the special architecture (the target lipids within a curved membrane domain) of the caveolin-rich nanodomains of the PM.

This model may represent the simplest explanation for the coordinated role of caveolins and cavin1 in caveola formation, but it is clearly an oversimplification. While caveolins (specifically CAV1 in nonmuscle cells, and CAV3 in striated muscle) and cavin1 are essential for caveola formation in vertebrate cells, other factors are clearly involved. We will consider two recent examples, the transmembrane orphan receptor, ROR1, and the muscle-specific pacsin/syndapin protein, pacsin3. ROR1 is expressed in a number of embryonic tissues but not in all adult tissues (including those with abundant caveolae) yet in some cell types ROR1 is required for caveola formation [29]. This involves binding of ROR1 to both CAV1 and cavin1 through distinct protein domains in the cytoplasmic tail of the transmembrane protein. It seems unlikely that ROR1 is an essential ubiquitous factor in caveola formation and the stoichiometry with respect to other caveolar components is also unclear. An additional question is how this transmembrane protein is incorporated into the caveolar domain when other transmembrane proteins are excluded [30]. A second example has come from studies of mice lacking pacsin3. Pacsins are associated with caveolae and other membrane domains, and have been linked to caveola endocytosis. Intriguingly, skeletal muscle lacking pacsin3 shows a lack of caveolar invaginations but normal levels of surface CAV3 and cavin1 [31**]. This demonstrates that in this system pacsin3 is essential for caveola formation, and that CAV3 and cavin1 alone are not sufficient, and also that cavins can still associate with the PM in the absence of apparent curvature.

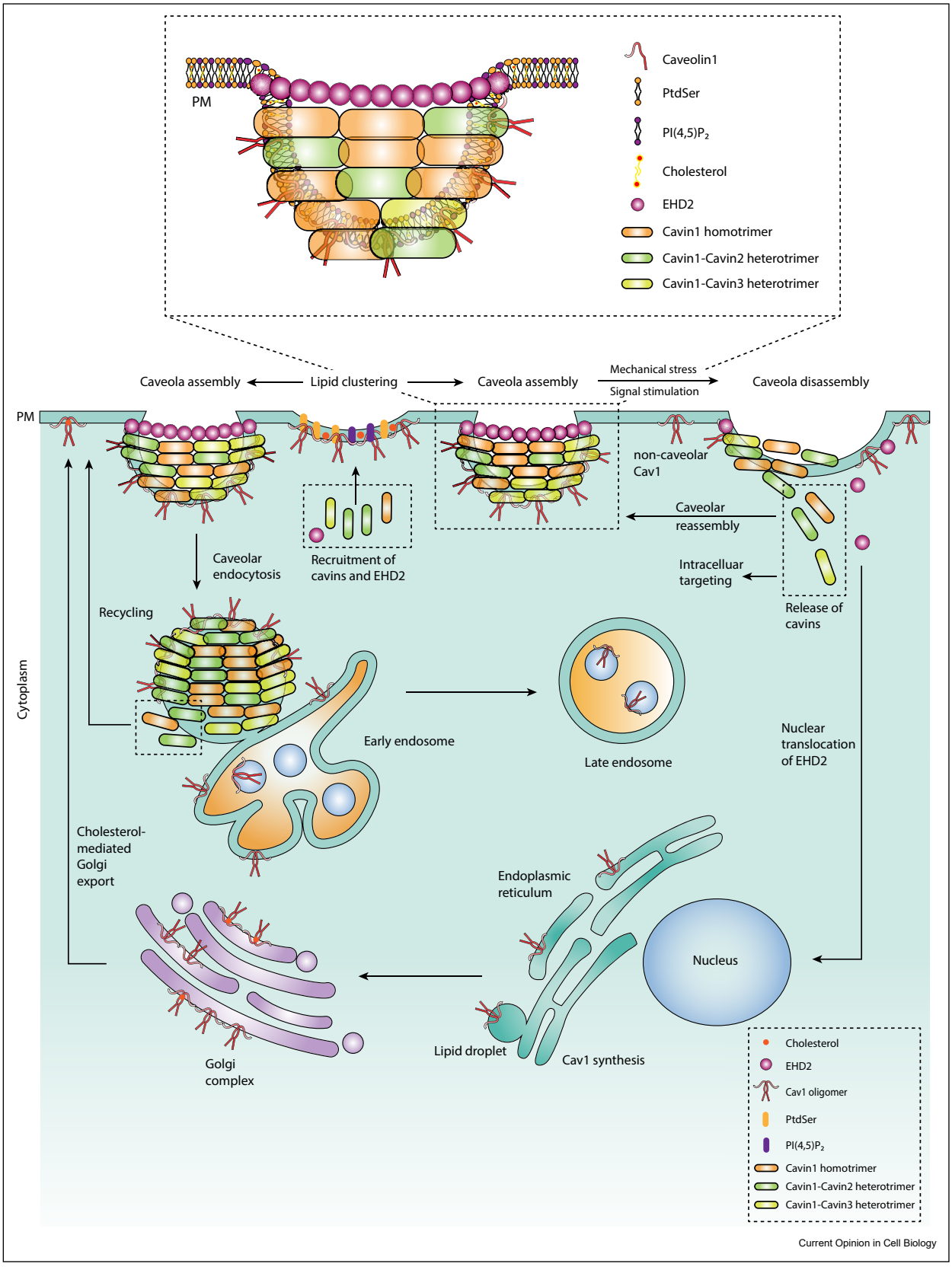
The other components that integrate into the caveolin-cavin assembly are the other cavin family proteins, cavin2 and cavin3 (and in muscle cavin4). These proteins co-oligomerize with cavin1 via the HR1 domain, forming a trimeric coiled coil but cavin2 and cavin3 do not co-oligomerize [32,33]. This results in distinct striations on the cytoplasmic face of caveolae made up of cavin1/2 and cavin1/3 oligomers [32]. EHD2 is a large mechanical ATPase that specifically associates with the neck of caveolae forming rings made up of 14 monomers. This involves binding of the N-terminus of EHD2 that is important for regulating targeting and stability of EHD2 to caveolae [34]. *In vitro*, EHD2 assembles in an ATP-dependent manner to form ring-like oligomers that induce the formation of tubular liposomes with an inner diameter of 20 nm [35].

A single mature assembled caveola comprises approximately 140 CAV1 molecules [36], 50–80 cavin1 molecules [19,32,37], other cavins (approx. 20) and the assembled EHD2 oligomeric ring structure (approx. 40; [35]). These numbers provide a starting point for comparative studies with published proteomic studies that have determined the copy numbers of each of these proteins in a single cell (Table 1; Quantitative aspects of caveolae). Such comparisons can provide insights into the number of caveolae per cell if one makes the assumption that all the protein is associated with caveolae. As Table 1 indicates, this is clearly an oversimplification but, nevertheless, an interesting starting point for understanding the caveolar system in quantitative terms.

Dynamics of caveolae: trafficking and disassembly of a metastable domain

We now have a picture of how this assembly of membrane proteins, lipids and cytoplasmic proteins is brought together to make a functional unit. Assembly of caveolae only occurs at the PM showing that the caveolin synthesized in the ER, and while transiting through the secretory pathway, cannot recruit cavins and other proteins. This suggests that caveolae are not formed by simple heterotypic protein–protein interactions between, for example, cavins and caveolins, but that the unique environment of the PM, presumably its specialized lipid composition, is required for caveola formation. The dynamic structure formed in this way is therefore poised to disassemble. The first studies to dissect the process of caveola disassembly involved the use of mechanical stress [38]. Increased membrane tension, through use of hypotonic media or direct physical stretch, caused flattening of caveolae in an energy-independent physical process. The flattening of caveolae can protect cells against physical damage by allowing a cell to change shape — the caveolae acting as springs within the membrane to buffer membrane tension. This has now been shown in muscle [9*,39,40], in endothelia [41], and in the notochord of the zebrafish, a mechanically important embryonic structure [42,43]. It

Figure 1


PM
Caveolin1
PtdSer
PI(4,5)P2
Cholesterol
EHD2
Cavin1 homotrimer
Cavin1-Cavin2 heterotrimer
Cavin1-Cavin3 heterotrimer
Caveola assembly
Lipid clustering
Caveola assembly
Mechanical stress
Signal stimulation
Caveola disassembly
PM
non-caveolar Cav1
Caveolar reassembly
Caveolar endocytosis
Recruitment of cavins and EHD2
Intracelluar targeting
Release of cavins
Recycling
Cytoplasm
Early endosome
Late endosome
Nuclear translocation of EHD2
Cholesterol-mediated Golgi export
Endoplasmic reticulum
Nucleus
Golgi complex
Lipid droplet
Cav1 synthesis
Cholesterol
EHD2
Cav1 oligomer
PtdSer
PI(4,5)P2
Cavin1 homotrimer
Cavin1-Cavin2 heterotrimer
Cavin1-Cavin3 heterotrimer
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is also likely that the flattening of caveolae can be controlled by cavins to alter their sensitivity to physical forces. Progressive loss of the repeats within the PtdSer-binding UC1 region of cavin1 makes caveolae more sensitive to mechanical stimuli, and the variability in the number of repeats observed in evolutionary analyses may indicate the need to cope with forces of differing strength in different tissues [26,43].

As well as being mechanoprotective, caveolae can also transmit mechanical signals and so act as mechanosensors (Figure 1). This can happen through a number of different mechanisms. First, the disassembly process can trigger the release of cavins into the cytosol. Cavins are released as subcomplexes of cavin1/cavin2, cavin1/cavin3 [32] or cavin3 monomers [44**]. The released proteins can then potentially interact with, and regulate, intracellular proteins. A second example is shown by studies of EHD2. EHD2 is released from caveolae as membrane tension increases, translocates to the nucleus, and regulates transcription as part of the cellular response to external forces [45**]. Targets include distinct gene sets related to cell cycle, cell division, and cell-cycle checkpoints were all negatively regulated [45**]. Intriguingly, a recent study demonstrates that a third mechanism can also operate, in this case intact caveolae serve as indicators of lower membrane tension. Cells rapidly migrating in a rigid matrix must retract the rear of the cell as they move forward. The lower tension in the rear of the cell allows caveola formation, and these intact caveolae specifically recruit the Rho GTP exchange factor, Ect2 [46**]. Recruitment of Ect2 hyperactivates RhoA and the actomyosin machinery to cause rear retraction and facilitate forward migration [46**]. In contrast to the caveolar disassembly triggered by high membrane tension and the subsequent release of signaling proteins, in this case it is the intact caveolae which are the indicators of the level of (low) membrane tension.

While these studies emphasize the effect of mechanical stress on the disassembly of caveolae, under physiological conditions cavins are tightly associated with caveolae as shown by photobleaching recovery studies [19]. But caveolae can bud from the PM with their associated caveolins and cavins (Figure 1). EHD2 negatively regulates this budding process; loss of EHD2 causes increased endocytosis [47,48], whereas loss of cavin3 decreases caveola dynamics [49,50]. Caveolae then fuse with the early endosome, in a Rab5-dependent process [51]. Caveolae can then cycle back to the PM, and this can regulate surface caveola density, as seen, for example, during mitosis [52]. However, caveola endocytosis might not be a major pathway for uptake of cargo proteins [53] but allow a cell to fine tune the number of caveolae on the cell surface able to protect, or signal, in response to membrane force changes.

Caveolae and stress signaling

The proposed role of caveolae in mechanoprotection and mechanosignaling is consistent with the observation that some of the cell types with the highest density of caveolae, particularly skeletal muscle [9*,40] and the zebrafish notochord [42,43], are all subjected to considerable mechanical stress *in vivo*. However, many other cell types have significant levels of caveolae, and it is clear that the functions of caveolae are not only restricted to mechanical stimuli. Numerous signaling pathways have been linked to caveolae, and these pathways have generally been linked to a direct

Table 1

Quantitative aspects of caveolae.

Protein	Copy number per HeLa cell[a]	Approximate number per caveolae[b]	Estimated number of caveolae per HeLa cell (based on column 1 and 2)[3]
Caveolin-1 (CAV1)	28,069	105–189	267–148
Caveolin-2 (CAV2)	5592	29*	–
Cavin1	39,779	40–50	796–994
Cavin2	1427	20–25	–
Cavin3	4333	20–25	–
Cavins total	45,539	60–80	569–759
Pacsin2	256,595	–	–
EHD2	17,104	40#	428

*Based on ratio of CAV1/CAV2 copy numbers. #Approximation based on preference of EHD2 for 18-nm diameter tubules *in vitro* and predicted number of dimers predicted to form a ring of this size [35].

[a] From [72].

[b] From [32,35–37].

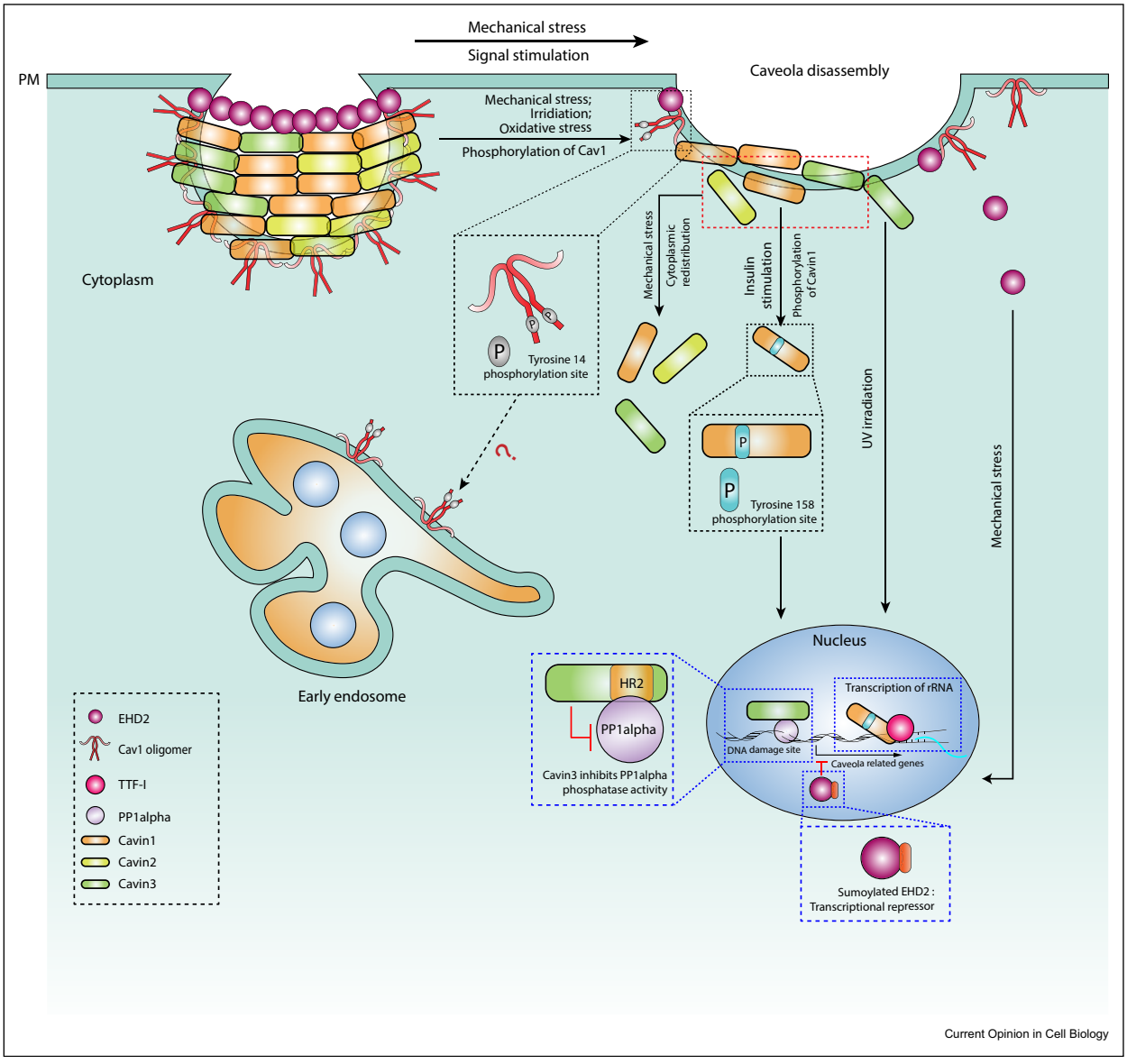
[c] Note that the estimated number of caveolae per cell assumes that all of that particular protein is associated with surface caveolae; a simplification for the purpose of this comparison. The surface area of the PM occupied by one caveolae is approximately 0.008 μm^2 (8000 nm^2); 500 caveolae equates to approximately 4 μm^2 or 0.14% of the surface of a HeLa cell (surface area approximately 2800 μm^2).

Caveolar formation and dynamics. The caveola-forming protein CAV1 is synthesized in the ER. The hydrophobic domain allows the accumulation of CAV1 on lipid droplets budding from the ER. Synthesized CAV1 undergoes transport to the Golgi complex, where CAV1 assembles into a cholesterol-rich complex and is then transferred to the PM. The insertion of CAV1 into the PM results in lipid clustering and the recruitment of other caveolar components such as the cavin coat proteins and EHD2. CAV1 can be internalized through caveolar endocytosis and delivered to early and late endosomes. In response to mechanical force or signal stimulation, caveolae can flatten and release cavins and EHD2 for intracellular targeting, whereas CAV1 is redistributed into the bulk PM. ER, endoplasmic reticulum; PM, plasma membrane.

interaction between the caveolin membrane protein and specific signaling proteins. Here, we will consider the emerging evidence that a specific set of signaling pathways, specifically related to stress signaling, are linked to caveolae and, particularly, through the cavin proteins.

It has long been appreciated that caveolae are sensitive to a number of stresses, as indicated by the phosphorylation of CAV1 on a key tyrosine residue close to the N-terminus, Tyr14. These include mechanical stress [54], UV, oxidative stress [21*,55], and microgravity [56]. In recent years, it has become increasingly apparent that

Figure 2



Caveolar signaling under stress conditions. Caveola-related signal transduction under stress conditions is mediated by the intracellular redistribution of caveolar components involving CAV1, EHD2 and the cavin proteins. Mechanical stress-induced caveola disassembly is responsible for the nuclear translocation of EHD2 and the subsequent transcriptional inhibition on caveola-related genes. Phosphorylation of CAV1 at tyrosine 14 is required for the redistribution of CAV1 from caveolae to the early endosome in response to mechanical stress, as well as nonmechanical stimuli including irradiation and oxidative stress. However, the mechanisms for the redistribution of CAV1 to the endosome under these stress conditions is still unclear. The molecular roles of the cavin proteins have been mainly linked to nonmechanical stress-induced signaling. Cavin1, in response to insulin stimulation, translocates to the nucleus to regulate the transcription of ribosomal RNA (rRNA) through interaction with TTF-I. Tyrosine 158 phosphorylation may be required for the nuclear import of cavin1. Upon UV irradiation, cavin3 can be released into the nucleus where it inhibits PP1 alpha phosphatase activity to promote apoptosis.

many, if not all, of these stresses may cause caveola disassembly and release of caveolar proteins, particularly cavins, into the cell (Figure 2). An example of such a pathway from recent work is the response to UV. A brief UV treatment causes redistribution of cavins from surface caveolae to the cytosol and partial loss of morphological caveolae, as judged by electron microscopy, indicative of partial disassembly of caveolae [44**]. The released cavin3 protein then directly interacts with the phosphatase, PP1 alpha, inhibiting its catalytic activity and increasing phosphorylation of H2AX, a key DNA damage indicator, triggering apoptosis. In this instance, the caveolae appear to act as a safety mechanism to indicate severe damage, triggering cell suicide, and preventing survival of cells harboring deleterious DNA damage. Interestingly, loss of cavin3 expression through promoter methylation is tightly linked to cancer progression [57–63].

This pathway (Figure 2) may be illustrative of just one of a series of interactions that can occur following release of proteins from caveolae. Proximity interaction analyses using a model cell system that mimics release of cavins from caveolae and direct *in vitro* interaction studies suggest that cavins can interact with a host of different cellular proteins in a range of pathways, including Period circadian regulator 2 (PER2) and Cytochrome circadian regulator 2 (CRY2), two key components of circadian rhythm regulation [64,65], the guardian of the genome p53, and several ribosomal RNA binding proteins such as DExD-Box helicase 21 (DDX21), ELAV like RNA binding protein 1 (ELAVL1) and Myb binding protein 1a (Mybbp1a) [44**]. Whether these interactions with specific subsets of proteins only occur in response to particular upstream signals is not clear but the release of different oligomers of cavins (eg. cavin1/cavin2 or cavin1/cavin3; [32]) certainly expands the number of possible interactions. It is also likely that the phosphorylation of the cavins themselves will further modulate these interactions further tuning the specificity. In fact, phosphorylation of cavin1 may be sufficient to trigger dissociation from caveolae. Insulin stimulation of differentiating adipocytes causes cavin1 phosphorylation, cavin1 release from caveolae, and translocation to the nucleus [66]. In this pathway, nuclear cavin1 upregulates ribosomal DNA transcription in response to metabolic challenges to increase ribosomal biosynthesis essential for the adipocyte differentiation process. This pathway may be linked to the lipodystrophic phenotype associated with loss of cavin1. In other pathways, phosphorylation may further modulate the specificity/activity of released cavins.

These pathways highlight the emerging role of cavins in regulated interactions with intracellular proteins. Caveolae can be envisaged as a reservoir of these signaling proteins that can be released into the cell when caveolae are disrupted or in response to post-translational modifications. In parallel to these events, Tyr14 phosphorylated CAV1 generated in response to growth factors or stress stimuli, can recruit specific proteins. For example, oxidative stress causes CAV1 phosphorylation and interaction with the adapter protein, TRAF2 [21,67,68]). Phosphorylation of CAV1 in response to cyclic stretch is required for transcriptional upregulation of caveolar proteins via Egr1 [54]. In addition, caveolar disassembly can impact upon specific lipid-anchored signaling pathways through changes in the nanoscale organization of lipids at the PM [69]. Thus, at least three different effects of caveolar modification, involving cavins, caveolin, and lipids, result in downstream effects on signaling processes.

Perspectives

We speculate that caveolar assembly, built upon multiple low affinity interactions between proteins and lipids and dependent on the curvature of the domain, is poised to disassemble in response to changes in curvature, changes in lipid composition, stress conditions and/or phosphorylation of key components. This can release caveolar proteins into the cell to mediate their biological effects, as exemplified by transcriptional regulation by EHD2, regulation of protein phosphorylation networks by cavin3, or regulation of ribosomal biogenesis by cavin1.

Despite the extensive literature linking caveolae to stress, the upstream mechanisms involved in this response are unclear. For example, it remains to be elucidated how UV or other nonmechanical stresses cause cavin release and caveola disassembly and whether this might be linked to Caveolin-1 phosphorylation (and, if so, how). One interesting possibility, in view of the proposed unique lipid composition of caveolae, is that lipid peroxidation by reactive oxygen species might trigger the disassembly process. A further question relates to how the complement of released cavins allow the correct physiological response, such as the different responses to disassembly of caveolae in muscle as compared to severe stress leading to apoptosis in other cell types. In view of the phosphorylation of cavin proteins [70], it is likely that stress-specific post-translational modifications might further tune the specific responses to intracellular cavins.

Finally, how are these new concepts related to the multiple signaling pathways previously linked to caveolae? For example, are membrane proteins actually excluded from caveolae, as suggested by studies of model membrane proteins [30], or can they be incorporated during formation or only after disassembly? Interestingly, insulin receptors have been shown to associate specifically with the neck of caveolae, rather than the bulb [71], yet insulin causes phosphorylation of cavin1 [66]. If membrane proteins are generally excluded from caveolae, does their disassembly allow

caveolins to interact with membrane proteins, explaining some of the caveolin membrane protein interactions described in the literature?

Dysfunction of caveolae has been linked to a number of diseases so there remains a need to understand these enigmatic structures, from their role in endocytosis to their ability to flatten in response to stress. The extensive literature connecting stress and caveolae has led to a new hypothesis that caveolae, through release of cavins, act as general transducers of cellular stress, allowing interaction with intracellular proteins. This new concept for cell signaling and for caveolae will serve as a paradigm for understanding caveolar function and will hopefully ignite further work to address these questions.

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Conflict of interest statement

Nothing declared.

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** of outstanding interest

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