Caveolae: mechanosensing and mechanotransduction devices linking membrane trafficking to mechanoadaptation

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ABSTRACT

Mechanical forces (ECM stiffness, vascular shear stress, muscle stretching) reaching the plasma membrane (PM) determine cell behavior. Caveolae are PM invaginated nanodomains with specific lipid and protein composition. Highly abundant in mechanically challenged tissues (muscle, lungs, vessels, adipose tissue), they protect cells from mechanical stress damage. Caveolae flatten upon increased PM tension, enabling both force sensing and accommodation, critical for cell mechanoprotection and homeostasis. Thus, caveolae are highly plastic, ranging in complexity from flattened membranes to vacuolar invaginations surrounded by caveolae—rosettes—which also contribute to mechanoprotection. Caveolar components crosstalk with mechanotransduction pathways and recent studies show that they translocate from the PM to the nucleus to convey stress information. Furthermore, caveolae components can regulate membrane traffic from/to the PM to adapt to environmental mechanical forces. The interdependence between lipids and caveolae starts to be understood, and the relevance of caveolae-dependent membrane trafficking linked to mechanoadaption to different physiopathological processes is emerging.

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Introduction

Living organisms are subjected to external and internal forces such as gravity and tension from osmotic pressure. In complex organisms, cells are exposed to additional mechanical stimuli (e.g., vascular shear forces, extracellular matrix rigidity, lung and muscle stretching, volume expansion in adipocytes). Integration of these cues with biochemical signaling pathways is pivotal to cell and tissue homeostasis, development or cell proliferation control, and specific devices have evolved to sense and adapt to mechanical force [1].

Many signal transduction pathways are sensitive to mechanical forces reaching the PM [2]. The PM itself acts as a scaffold platform that organizes signaling [3]. Thus, lipid composition and concentration and physical architecture of the PM are important parameters controlling signal outputs [3]. In addition to foster appropriate environments for signaling molecules, the PM must ensure cell integrity in the face of external stress [4].

Caveolae are small PM invaginations (50-80 nm in diameter), often covering a substantial fraction of the total PM surface (up to 50 % in muscle cells [5]). Four features make caveolae unique. First, they are highly abundant in cells whose PM experiences changes in tension. Second, they are enriched in cholesterol and sphingolipids, creating a distinct nanodomain. Third, they are intimately linked to the actin cytoskeleton [4,6]. Fourth, they are highly plastic in terms of shape and organizational properties. They flatten out upon high cell tension, and cluster into rosettes (groups of caveolae around a common invagination/neck) under low tension [4,7] (Figure 1).

Here, we summarize recent findings on mechanisms underlying caveolae-dependent mechanotransduction and membrane trafficking, and lipid-caveolae interplay, as well as their physiological relevance. Recent in-depth reviews on related topics in the field are available [4,8-12].

Caveolar core components

Two major curvature-generating families stabilize the shape of caveolae in mammalian cells: caveolins and cavins (Figure 1). Three paralogs of the integral membrane protein caveolin, *CAV1-3*, exist. CAV1 is expressed in most tissues, except in skeletal muscle; CAV2 follows a similar pattern; and CAV3 is exclusive to muscle cells [13]. Genetic deletion of *CAV1* and *CAV3* prevents caveolae formation in their respective tissues [13]. As for cavins [14], CAVIN1/PTRF (Pol 1 transcription release factor) is essential for caveolae formation and is expressed in all tissues [15,16], while CAVIN2/SDPR (serum-deprivation response protein), CAVIN3/SRBC (sdr-related

gene product that binds to c-kinase) and CAVIN4/MURC (muscle-restricted coiled–coil protein) play a regulatory role [14]. In addition to these core components, the neck of caveolae is enriched in two important molecules. A curvature generating molecule of the F-BAR family, named PACSIN (also known as syndapin), and EH domain-containing protein 2 (EHD2), an ATPase related to dynamin [17]. PACSIN2 and PACSIN3 are required for caveolae biogenesis/stability, and caveolae density is reduced in their absence [18,19]. EHD2, which localizes to the caveola neck, prevents caveolae budding, reducing caveolar motility and internalization [20,21]. Caveolae biogenesis is a multi-step process that may require additional core components in specific cell types [9]. Another F-BAR domain protein, FBP17, is enriched in caveolar rosettes (see next chapter)[22].

While this is the basic caveolae configuration in mammalian cells, invertebrates lack cavin genes, despite having caveolin orthologs [14]. Strikingly, caveolae-like invaginations were recently described in *C. elegans*. These invaginations were reduced upon insulin receptor depletion, which also led to a significant reduction in caveolin levels [23]. It will be interesting to test whether depletion of caveolin alone produces the same phenotype [23]. Similarly, the ascidian *Ciona* expresses a caveolin ortholog that forms caveolae-like invaginations [24], consistent with the ability of CAV1 to form invaginations *per se* in heterologous systems devoid of caveolae [25]. Interestingly, CAV1 forms scaffolds of varied sizes smaller than caveolae at the PM in mammalian cells [26], whose functional role remains unclear. Similarly, the exact role of non-caveolar CAV1 scaffolds in cellular organelles is not fully understood [10,27].

Caveolar plasticity, mechanoprotection and mechanotransduction

Electron microscopy images of mammalian tissues show that caveolae frequently form clusters, named rosettes, which in some cases represent the majority of caveolae [5] (Figure 1). *Ex vivo*, these structures are formed by reducing tension in the cell, such as cell detachment [28-31]. While rosette formation is dependent on the F-BAR family member FBP17 [22], EHD proteins increase the number of caveolae per rosette [32]. Almost a decade ago, a seminal study demonstrated that caveolar shape and organization can change as a function of membrane tension [7]. When tension is increased caveolae flatten out, to be reformed when lower tension is restored (Figure 1) [7,33]. Interestingly, uncontrolled actin polymerization also induces caveolae flattening [34]. The flattening of caveolae is important to buffer the increase in tension at the PM upon osmotic swelling or mechanical stretching, protecting cells from PM rupture [7], and conferring cells and tissues resistance to physical activity in several settings, as detailed in

Table 1 [5,7,22,35-38]. Of note, caveolae in rosettes disassemble faster than caveolae outside rosettes in response to osmotic swelling [5,22]; thus, rosettes have intrinsic buffering capacity, distinct from single caveolae [22]. Additional local cues likely contribute to regulate caveolae flattening, because flattened caveolae can be observed in close proximity to curved caveolae [6]. Global actin polymerization induced by a constitutively active mDia1 mutant induces caveolae flattening [34], but it is unclear whether the local, physiological activation of this actin polymerizing factor or other actin fibers regulator, such as filamin A [34], can locally regulate caveolae flattening. Recent studies suggest that caveolae flattening not only buffer PM tension changes, but also functions as a mechanotransduction signal (Figure 1).

Signaling to the nucleus

Recent studies show that upon caveolae flattening, some of its components are released, resulting in signaling events. When tension is increased by osmotic swelling or mechanical stretching, EHD2 is released from caveolae concomitant to caveolae flattening [39] (Figure 1). Brief mechanical stretching leads to EHD2-dependent transcriptional repression of caveolar genes, providing an autoregulatory mechanism by which caveolae mechanosensing controls their own biogenesis [39,40].

Cavin family members are also released from flattening caveolae [7,41]. Interestingly, other cell stress sources such as ultraviolet light exposure can also trigger cavin release from caveolae [42]. UV-induced stress also results in caveolae disassembly, albeit with slower kinetics than osmotic swelling-induced caveolae flattening [22,42]. UV treatment releases CAVIN3 from caveolae, which relocates to cytosol and nucleus, where it interacts with and inhibits phosphatase $PP1\alpha$, favoring apoptosis [42]. Interestingly, CAVIN1 PM/cytosol ratio is sensitive to regulation by FGF13, a factor involved in susceptibility to cardiac arrhythmias [43]. Similarly, nuclear CAVIN1 is stimulated by insulin in adipocytes [44], indicating that multiple factors control the non-caveolar pools of cavins.

Gene expression regulated by IL6/STAT3 pathway is repressed by osmotic swelling in a CAV3-dependent manner [45]. Interestingly, *CAV3* mutations found in muscular dystrophy patients lose both this repressive activity and PM tension buffering capacity [45]. Taken together, these studies suggest that mechanical forces are transduced to caveolae and its components respond to these forces. Therefore, caveolae are PM structures capable of transducing PM tension changes into downstream consequences. However, how caveolar components change their curvature generating properties upon tension increase remains to be determined (see box 1).

BOX 1

Disassembly of caveolae by mechanical stress

A fraction of caveolae can quickly disassemble in response to tension increase [7], as soon as 2 minutes after osmotic swelling [22]. All caveolar core components have an intrinsic membrane bending property [14,17,25,46], which is disabled upon caveolae flattening. While EHD2 and cavins are released from caveolae simultaneously with flattening, CAV1 remains bound to the PM unable to induce curvature—despite displaying membrane bending capacity when expressed in CAVIN1-null cells in the absence of PM tension [25] (Figure 1). These observations suggest that tension increase induces changes in lipids and/or proteins of flattening caveolae that prevent membrane bending by caveolar components. Studies conducted on curvature-generating BAR proteins suggest that their curvature activity is modulated through different mechanisms. FBP17 is an F-BAR family member that generates membrane curvature in cells and in vitro. FBP17 is recruited to caveolae in rosettes and upon increased tension, which flattens caveolar rosettes, its membrane bending activity is severely inhibited [22,47]. Two non-mutually exclusive mechanisms of inhibition have been proposed. 1) A triple phosphorylation on the F-BAR domain prevents oligomerization and membrane bending activity [22]; and 2) its intrinsic sensitivity to osmotic variations inhibits its membrane bending activity [47]. Molecular simulations have shown that tension at the PM could be critical to determine the oligomerization capacity of BAR protein [48]. Thus, oligomerization capacity regulation may be critical to bypass curvature in caveolae. Phosphorylation on Cav1 and PACSIN2, has been shown to regulate oligomerization and membrane binding, respectively, but these modifications have been related to endocytosis so far [49,50]. In addition, caveolae flattening is an ATP-independent process [7], suggesting that other mechanisms, independent on phosphorylation, bypass the intrinsic curvature generating activity of caveolar components when tension is increased. Biophysical and biochemical studies in combination with super-resolution imaging on PM lipids will likely provide additional cues to understand the mechanism by which caveolar components are adapted to tension.

Crosstalk between caveolae and mechanotransduction pathways

The literature supports that caveolae interplay with mechanotransduction pathways and the actin cytoskeleton (reviewed in [4]). Recent evidence reinforces this notion by showing

164 additional ties with pathways that regulate the actin cytoskeleton, specifically stress fibers, or
165 that are highly dependent on tension generated by the actomyosin system.

166   A major pathway regulated by mechanical cues is the Hippo pathway, which regulates
167 tissue architecture and organ size [51]. Increased tension inhibits Hippo signaling pathway
168 leading to nuclear translocation of YAP and TAZ [51]. YAP/TAZ are the archetypal transcriptional
169 regulators sensitive to mechanical cues, and regulate the expression of gene subsets driving cell
170 proliferation, migration, survival and differentiation [51]. Interestingly, the actin cytoskeleton
171 dysregulation observed in MEFs deficient for CAV1 was responsible for reduced YAP/TAZ activity
172 [52]. In contrast, CAV1 depletion in osteosarcoma cells and in vivo stimulates YAP/TAZ
173 translocation [53], and increase the expression of YAP/TAZ target genes in mesothelial cells [54].
174 Interestingly, YAP itself can regulate the expression of caveolar components, and cells without
175 YAP/TAZ have reduced caveolar density [53], further supporting that caveolae and the Hippo
176 pathway regulate each other (Figure 2).

177   The epithelium is highly sensitive to tension, and the actin cytoskeleton is vital to control
178 this tension [55]. A recent study has shown that CAV1 is important to downregulate tension in
179 epithelial sheets, as CAV1 regulates the activity of actin polymerization factor FMNL2, a formin
180 family member. CAV1 deficiency favors recruitment of FNML2 to cell-cell junctions, increasing
181 tension on the cell monolayer [55]. Similarly, the F-BAR protein FBP17, which localizes to
182 caveolar rosettes, inhibits formin mDia1. Increases in tension induce c-Abl kinase-mediated
183 phosphorylation of FBP17, abolishing FBP17-dependent inhibition of mDia1 and upregulating
184 stress fibers [22] (Figure 2). Interestingly, non-caveolar Cav1-mediated mDia1 regulation has
185 also been recently observed in the context of cilia stability [56]. Collectively, these studies
186 suggest that caveolar components regulate formins in the context of mechanotransduction
187 pathways.

188   The actin cytoskeleton is regulated by multiple pathways, including ephrin (Eph) receptor
189 tyrosine kinases, which play a major role in cell-cell communication [57]. Supporting the role of
190 CAV1 in modulating signaling, recent studies have shown that CAV1 is downstream of Eph
191 receptors. EPHB4 regulates CAV1 tyrosine 14 phosphorylation, cell stiffness, and mechanical
192 stability of endothelial cells, determining heart vasculature integrity [58]. EPHB4-CAV1 axis is
193 also important for arteriovenous fistulae maturation [59]. Similarly, CAV1 is also linked to EPHB2
194 kinase [60], which regulates CAV1 stability and caveolae density [61].

195   CAV1 promotes stress fibers-driven biomechanical remodeling of the extracellular matrix
196 (ECM) via RhoA [62,63] and YAP [52]. Interestingly, CAV1 also regulates the amount of ECM

197   components [64] by driving exosome biogenesis and cargo sorting for ECM deposition [65].
198   Thus, CAV1 is a central regulatory hub for ECM remodeling, by both mechanical and chemical
199   means; whether both mechanisms are coupled remains to be determined.

200   Collectively, these and other studies showing the association of caveolae with stress fibers
201   (reviewed in [4]) strongly suggest that the crosstalk between caveolae and actin cytoskeleton-
202   regulating networks contribute to balance the cell tensional status.

203

204   Functional interplay between caveolae and lipid biology

205   Caveolae as lipid organizing centers

206   The literature strongly suggests an active interplay between caveolae and the lipids within
207   (recently reviewed [8]). There are two properties shared by all caveolar core protein
208   components: they all have membrane bending capacity [14,17,66] and they all bind lipids. CAV1
209   binds cholesterol [67] while cavins, PACSIN and FBP17 bind preferentially phosphatidylinositol
210   4,5-bisphosphate (PIP2) [14,66,68], and EHD2 binds PIP2- and phosphatidyl-serine (PtdSer)-
211   containing liposomes [17,68]. These properties allow for retaining certain specific lipid species
212   within a mechanosensitive PM nano-domain [69,70]. Indeed, trafficking, distribution and
213   abundance of certain lipids is altered in CAV1-depleted cells [70,71]. Similarly, CAV1-dependent
214   PIP2 localization regulates signaling important for epithelial monolayer tensional status [55], and
215   CAVIN1 has been shown to regulate the amount of lipids in prostate cancer stroma [72]. The
216   effect of caveolar components in lipid biology is not restricted to the localization of lipids, as the
217   amount of peroxidated lipids is also increased in cells silenced for CAV1 [73], indicating the
218   complex nature of the interplay between lipids and caveolae.

219   Accordingly, certain lipids are enriched in caveolae. Cholesterol, sphingolipids,
220   sphingomyelin and gangliosides are enriched in caveolae as compared with the surrounding PM
221   [74]. In addition, PtdSer and PIP2 localize to caveolae [75,76]. Changes in the availability of these
222   lipids have profound effects in caveolae organization, shape and dynamics. PtdSer is important
223   to regulate caveolae stability and formation, while phosphatidylinositol 4-phosphate (PI4P) and
224   PIP2 increases caveolae confinement [77]. Cholesterol is essential for caveolae formation [6] and
225   addition of extra cholesterol favors endocytosis [78] and caveolae shape changes, decreasing
226   neck width and bulb diameter [21]. Recent computational analysis based on coarse-grain
227   simulations has advanced our understanding of how CAV1 determines membrane curvature
228   through its interaction with lipids, especially cholesterol and sphingomyelin [79,80]. Upon

229 binding the inner membrane leaflet, CAV1 induces membrane curvature and cholesterol
230 clustering in both leaflets, suggesting that these processes could be functionally linked through
231 both direct and indirect interactions [79,80]. This interplay may suggest a self-assembly
232 molecular mechanism as proposed elsewhere [81], as it seems to be CAV1 concentration-
233 dependent. Sphingomyelin clustering also seems to occur in a curvature-dependent manner,
234 following CAV1 induced-membrane bending [79]. Therefore, the ability of CAV1 to induce liquid-
235 ordered domains leading to lipid clustering may constitute a key property of caveolae and CAV1
236 scaffolds with functional consequences [10].

237

238 **Caveolae as mechanosensing devices linking membrane trafficking to**
239 **mechanoadaptation: physiopathological implications**

240   Caveolae trafficking, its impact on lipid homeostasis, and their relevance to different
241 physiological processes, such as adipose tissue homeostasis, endothelial permeability and
242 vascular biology, is emerging (Figure 2). Upon cell/tissue mechanical challenge, membrane
243 traffics from/to the PM regulated by caveolae components to adapt to such environmental
244 forces (Figure 1 and 2), including PM tension reduction upon cell detachment [28,29,31,34],
245 substrate stiffness [22,52], cell stretching [22,52,54], shear stress [82] (Lolo and Del Pozo,
246 unpublished observations) or lipid storage [83]. This mechanoadaptive caveolae-mediated
247 membrane trafficking occurs in the absence of cargo in most cases, consistent with the concept
248 that cargoes reported to internalize via caveolae can also use the CLIC/GEEC pathway [12,13,29].
249 Therefore, with few exceptions such as endothelial transport, caveolae-membrane trafficking
250 could serve primarily to buffer changes in PM tension, rather than endocytosis, as was
251 consensued in round-table discussions at the first EMBO Workshop on Caveolae held in Le
252 Pouliguen in May 2019 (http://meetings.embo.org/event/19-caveolae)[12].

253   Caveolae are essential for the expansion of the main lipid reservoir in mammals, the
254 adipose tissue, highlighting the importance of the caveolae-lipid interplay. Genetic depletion of
255 caveolae upon deletion of either *CAV1* or *CAVIN1* leads to lipodystrophy in mice, and several
256 mutations in *CAV1* and *CAVIN1* have been identified in human patients with lipodystrophy [13].
257 As a consequence, metabolism is severely disrupted in caveolae-deficient animal models [13].
258 Interestingly, genetic ablation of *EHD2* leads to increased adipocyte lipid droplet size in mice
259 [83]. Increased adipocyte size and lipid droplet area was suggested to derive from increased
260 fatty acid uptake via caveolae [83]. Using EM tomography, Matthaeus et al., showed that at least
261 a fraction of caveolae are detached from the PM in adipose tissue in the absence of EHD2, which

262 stabilizes caveolae *ex vivo* [21]. Although in the presence of EHD2 this pool of detached CAV1-
263 positive vesicles may be residual [84] or highly dynamic and therefore difficult to image [85],
264 trafficking of caveolae in adipocytes is likely physiologically relevant [83].

265 Lipid composition is a major determinant for caveolae-mediated transcytosis *in vivo*.
266 MFSD2A, a lipid transporter involved in omega-3 fatty acid docosahexaenoic acid (DHA)
267 trafficking in the central nervous system, specifically inhibits caveolae formation/stability and
268 transcytosis, contributing to blood brain barrier integrity in capillary endothelial cells (EC)
269 [86,87]. Consequently, mice lacking MFSD2A exhibit increased CAV1-positive vesicles and
270 transcytosis, a process that depends of caveolae [88,89]. This leads to reduced barrier function,
271 i.e. increased endothelial leakiness due to transcytosis [86,87]. The precise mechanisms by
272 which DHA species lead to reduced caveolae density remains to be determined [90-92]. It is
273 currently unclear why, in order to control caveolar density, these cells regulate a specific lipid
274 species as opposed to transcriptional regulation of caveolar components. Interestingly, in
275 comparison to capillary ECs, caveolae are abundant in brain arteriolar ECs, where they are
276 important to mediate neurovascular coupling [93] (Figure 2). Collectively, these studies show
277 that brain vasculature function is actively regulated by caveolae.

278 Significant differences in caveolae density are also found across different aortic regions, a
279 feature that seems to be relevant in the context of atherosclerosis. ECs lining so-called
280 atheroprone sites (such as iliac bifurcations) predominantly exhibit intracellular caveolae-like
281 vesicles, whereas those in athero-resistant sites (like the descending aorta), present a relatively
282 high numbers of surface caveolae [82]. Caveolae deficiency seem to attenuate plaque formation
283 in genetic models of hypercholesterolemia by limiting low density lipoprotein (LDL) transcytosis
284 and endothelial inflammation, through mechanisms independent from nitric oxide production
285 [82]. CAV1/caveolae deficiency-derived protection from atherogenesis is abolished upon
286 disrupting autophagy, which is in fact upregulated in CAV1-null cells and may dampen
287 endothelial inflammation and LDL transcytosis [94,95]. Interestingly, activin-like kinase 1, Alk1,
288 a TGFbeta1 receptor, supports LDL transcytosis under atherogenic conditions [96] and Alk1 is
289 localized to caveolae [97]. It is currently unclear whether and how these phenotypes are linked
290 to the sensing of flow shear forces by caveolae [35].

291

292 **Concluding remarks**

293   Caveolae constitute nanodomains with specific characteristics that are different from the
294   rest of the PM. Apart from embodying a system to buffer increase in membrane tension [7],
295   caveolae provide platforms for the regulation of cell signaling and metabolism, either by
296   controlling the activity of proteins or lipid localization [10,69,71]. However, it is still unclear how
297   these two major functions are coupled, i.e. how caveolar curvature changes affect signaling
298   locally. Here, two non-mutually exclusive possible scenarios emerge: i) mechanosensitive
299   signaling molecules in caveolae respond to caveolae-dependent curvature changes, which in
300   turn modulate their signaling capacity, and ii) lipid/protein re-distribution upon
301   flattening/reformation changes the signaling output. The technology to measure protein activity
302   and lipid distribution on caveolae, either curved or flattened, will provide important information
303   about the implications of caveolae plasticity for signaling regulation.

304   The release of specific caveolar components upon flattening is another way by which
305   caveolae mechanosensitive functions can be coupled to signaling [39,42,45]. The extent of this
306   type of distant signaling is beginning to be elucidated, as the biological meaning of many of the
307   novel caveolar components binding partners outside caveolae remains unknown [42].

308   Last, but not least, how changes in caveolae morphology and motility/trafficking occur in
309   vivo and what stimuli control them remain to be determined. Generation of knock-in animal
310   models with labeled caveolar components, or caveolae unable to fulfill some of its key
311   properties –move around, cluster or flatten-out- will undoubtedly provide convincing evidence
312   of the physiological roles of specific caveolae intrinsic properties.

313

Table 1

Mechanoprotective role of caveolae in different biological systems

Ex vivo			
Cell type	Type of stress	Targeted protein and effect in PM	Ref.
Endothelial	Hypo-osmotic shock	CAV1, no caveolae	[7]
Muscle fibers	hypo-osmotic shock	Cavin1, no caveolae	[5]
NIH 3T3	Mechanical stretching	EHD1, 2, 4, less caveolae clusters	[32]
NIH 3T3	Mechanical stretching	CAV1, loss of caveolae	[32]
Fibroblasts	Hypo-osmotic shock Mechanical stretching[#]	FBP17, reduction of rosettes	[22]
Melanocytes	Hypo-osmotic shock	CAV1, reduction in caveolae	[38]

| In vivo			
Model/tissue	Type of stress	Protein depleted and effect in PM	Ref.
Mice/Endothelium	Increased cardiac output	CAV1, no caveolae	[35]
Zebrafish/Muscle	Forced swimming	Cavin1a, no caveolae	[5]
Zebrafish/Notochord	Continuous muscle contraction	Cavin1b, no caveolae	[37]
Zebrafish/Notochord	Forced swimming	Cavin1b, severely reduced caveolae	[36]
Zebrafish/Notochord	none[*]	CAV1/CAV3, severely reduced caveolae	[36]

Mechanical stretching was performed for 24h.
*No stress was applied to CAV1/CAV3 KO zebrafish but lessions in the notochord were observed under normal growth conditions.
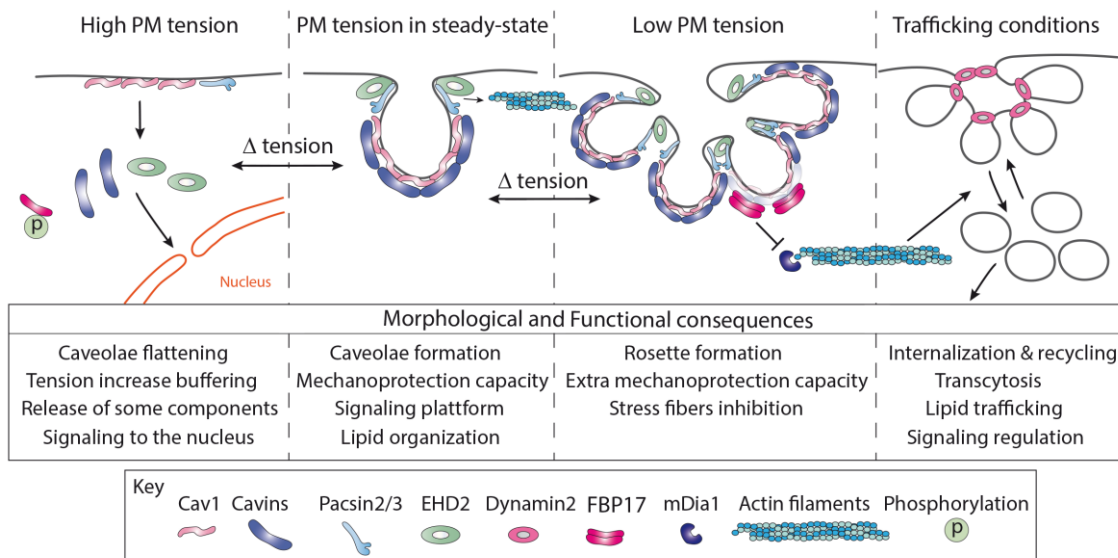
Figure 1. Different stages in which caveolae can be found and the specific functional consequences of each stage. Conditions that increase PM tension induce flattening of caveolae (left) which releases some of its components (cavins and EHD2) that reach the nucleus [14,39,42]. Low tension conditions favor the formation of rosettes [4,31]. Certain conditions, such as loss of cell adhesion [28] favor the trafficking of caveolae, which also depends on the actin cytoskeleton and microtubules [34]. The trafficking abilities of caveolae and their components linked to their mechanosensing properties specialize these devices for mechanoadaptation and mechanoprotection.

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13
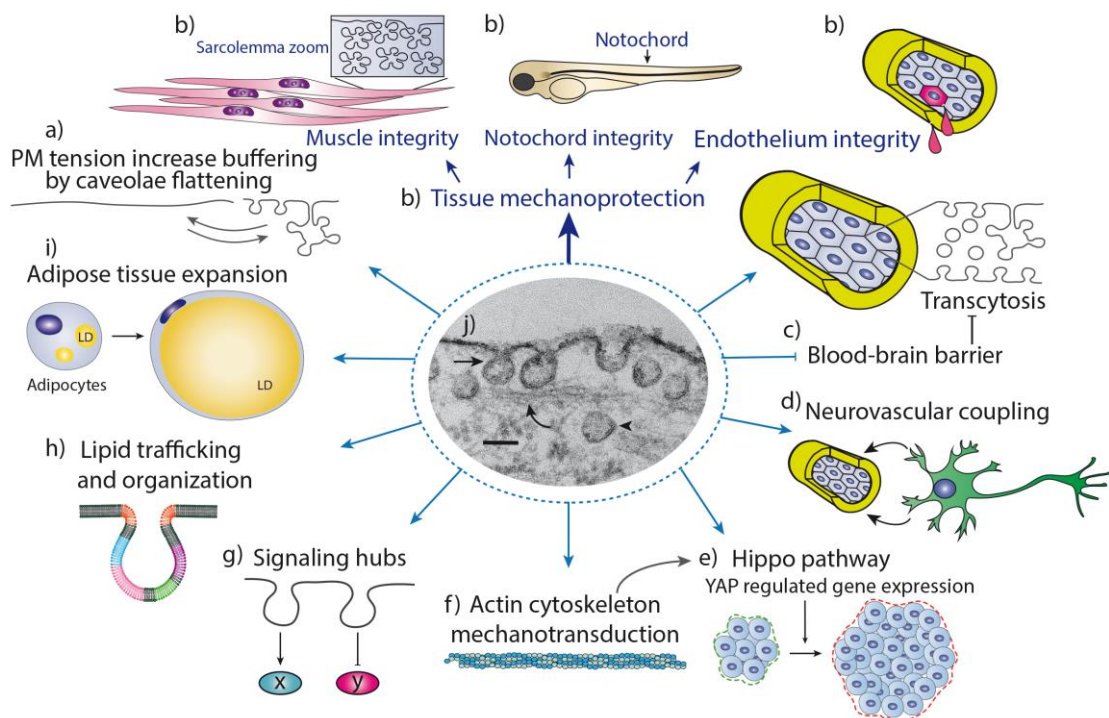
Figure 2. The main biological and physiological functions of caveolae are depicted.

(a) PM tension increase induces caveolae flattening, which buffers PM tension increase [7]. **(b)** Caveolae flattening contributes to the role of caveolae as mechanoprotective devices in several cells and tissues (muscle, notochord and endothelial cells [5,35-37,45]. **(c)** In the capillary endothelium of the central nervous system, caveolae downregulation contributes to blood-brain barrier function, which requires the downregulation of transcytosis aided by caveolae [87]. **(d)** In contrast, caveolae are abundant in arteriolar endothelial cells, where are important for neurovascular coupling [93]. **(e)** A crosstalk exists between caveolae and the Hippo pathway [53,54]. **(f)** Caveolae biology is intimately linked to the actin cytoskeleton [4]. **(g)** Multiple signaling pathways are regulated by caveolar components. **(h)** The physical and functional links between caveolae and lipids play a major role in caveolae and lipid biology [8], **(i)** which is exemplified by the lipodystrophic phenotype observed in caveolae-deficient mice and humans, which present small lipid droplets [13]. **(j)** The central image corresponds to an electron microscopy image of human fibroblast PM containing caveolae (straight arrow) and ruthenium red-labeled vesicles that have the diameter of caveolae (arrowhead). Fibers consistent with the width of actin fibers are marked with a curved arrow. It is important to note that *ex vivo* and *in vivo*, vesicles apparently detached from the PM are frequently observed (labeled with an arrowhead). These vesicles are caveolae that frequently are part of a cluster or rosette that maintains the connection with the PM [22,84] but can also correspond to independent vesicles [83]. Scale bar 100 nm.

Credit author statement

Asier Echarri: Conceptualization, Writing - Original draft; Writing - Reviewing & Editing. Fidel Lolo: Writing - Original draft; Writing - Reviewing & Editing. Miguel Angel Del Pozo: Theoretical framework, Writing – Reviewing & Editing Writing - Reviewing and Funding acquisition.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

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