

# A lipid-centric view of endocytosis by caveolae

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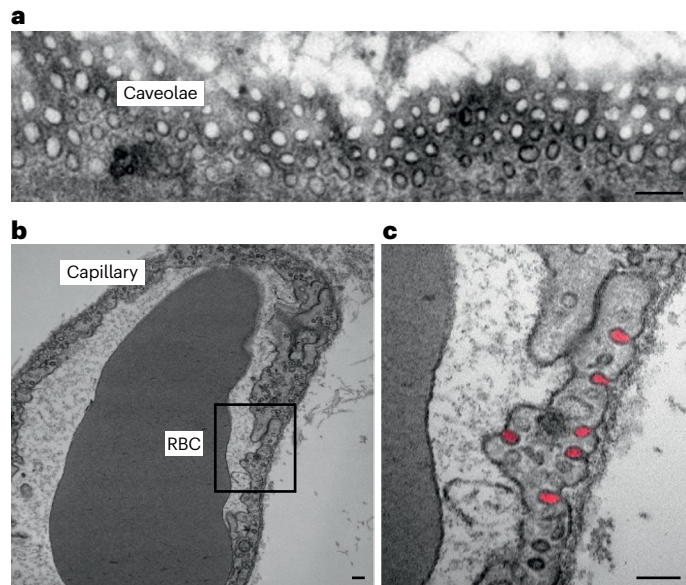
Caveolae have long been considered to be an alternative endocytic pathway, with distinct cargoes, but generally similar functions, to clathrin-coated pits. Here we suggest that the mechanisms of caveola formation and their scission are tightly interlinked and rely on specific lipids. These mechanisms are fundamentally different to those driving the formation and fission of coated pits. Both formation and scission of caveolae are driven by lipid-induced shaping of the caveolar domain, and we present biophysical models for lipid-driven curvature generation and its coupling with scission. In addition, we propose that these new insights have important implications for understanding the function of endocytosis mediated by caveolae. Rather than a parallel endocytic pathway for protein cargo, we argue that caveolae are a lipid-sensitive mobilized multifunctional surface domain.

The cells of many mammalian tissues are covered in numerous plasma-membrane invaginations (Fig. 1). These structures, known as caveolae, show similarities in morphology to clathrin-coated pits, which are well characterized as highly dynamic endocytic structures (Fig. 2). Despite these similarities, the mechanisms underlying endocytosis by caveolae and their cellular roles have proved elusive. For example, although we now understand with precise submolecular detail the molecules and steps in the clathrin-coated pit life cycle, involving nucleation, cargo concentration, interaction of cytoplasmic signals in plasma-membrane receptors with machinery proteins, invagination, scission and fusion with early endosomes, each of these steps is less-well-defined for caveolae (Fig. 2).

It is now clear that, in cultured cells, caveolae can undergo scission from the plasma membrane and fuse with early endosomes (see ref. 1 for a discussion). The dynamics of caveolae have been examined using a range of techniques (Box 1), and these point towards a somewhat variable residence time on the plasma membrane. Caveolae that undergo scission can either fuse back with the plasma membrane or with early endosomes<sup>2–5</sup>. The magnitude of caveolar traffic to the latter has been quantitated, with ~5% of cellular caveolae being detected in association with early endosomes at any time<sup>6</sup>. This is in agreement with caveolar proteins being detected in proximity to specific early endosomal recycling proteins<sup>7</sup>.

The endocytic dynamics of caveolae *in vivo* are less clear. Caveolar density is highly variable in different cell types (and even within the same cell type in different tissues; for example, brain versus cardiac endothelia<sup>8</sup>), pointing to considerable cell-type-specific functions, in contrast to the ubiquitous clathrin-coated pits<sup>9,10</sup> (Fig. 1). Budding of caveolae in endothelia has been extensively characterized<sup>8,11</sup>, whereas in other cells with abundant caveolae, such as skeletal muscle, this is far less clear. The magnitude and roles of caveolar endocytosis in these different cell types and tissues are also largely unknown. In endothelia, caveolae have been implicated in transcellular transport, but this remains controversial<sup>12</sup> (reviewed in ref. 10). Endocytosis through caveolae has also been suggested to mediate the uptake of a long list of transmembrane proteins, such as TGFbetaR<sup>13</sup>, integrins<sup>14</sup>, LRP1<sup>15</sup>, CD36<sup>16</sup>, junction proteins (in response to tumour necrosis factor (TNF) or epidermal growth factor (EGF) treatment)<sup>17,18</sup>, as well as uptake of albumin via an as yet uncharacterized putative membrane protein, albondin (see ref. 19 for a discussion). However, by using light and electron microscopy (EM), model transmembrane proteins have also been found to be excluded from caveolae<sup>6</sup>. If this is a general feature of the caveolar domain, then this raises questions regarding any involvement of caveolae in the uptake of transmembrane proteins. For example, is there regulated flexibility in the ability of individual caveolae to incorporate cargo in particular cellular contexts?

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**Fig. 1 | Electron micrographs of caveolae.** **a–c**, Caveolae in a cultured mouse embryonic fibroblast (**a**) and in the endothelial cells of a capillary in mouse skeletal muscle (**b,c**). The image in **c** is a higher-magnification view of the boxed area in **b**. The uniform shape and size of the caveolae can be seen in the section across the surface of a cultured fibroblast (**a**), whereas the characteristic bulb-like morphology of caveolae can be appreciated (red) in the cross-section of the endothelial cell (**c**). RBC, red blood cell within the capillary lumen. Scale bars, 200 nm.

The density of caveolae (as well as their apparent involvement in endocytosis) has been shown to vary in different cellular states and disease conditions, particularly in cancer<sup>20,21</sup>. For example, a recent study showed that in chemoresistant gastric cancer cells, caveolar components are upregulated, and this appears to be essential for their survival<sup>22</sup>. Experimental loss of key caveolar proteins reduces endocytosis of proteins from the extracellular medium and compromises cell survival, suggesting a role of caveolar endocytosis in the adaptation of these cancer cells<sup>22</sup>.

As illustrated by the examples described above, many studies have suggested that the role of scission of caveolae is to form an endocytic carrier, constituting an alternative endocytic pathway with distinct cargoes that operates alongside clathrin-coated pits. However, the dynamic cycle of scission of endocytic caveolae and recycling back to the plasma membrane need not necessarily reflect a role in the endocytosis of cargoes. Caveolar endocytosis may relate to the need to tightly regulate surface caveolar density (for other functions such as mechanosensation<sup>23,24</sup>) or to provide a way of transiently regulating plasma-membrane composition or its properties under specific cellular conditions.

This short summary highlights that, in comparison to endocytosis via clathrin-coated pits, the understanding of caveola endocytosis is still lacking. Numerous questions remain, including fundamental aspects of the mechanisms involved in scission of caveolae from the plasma membrane. Are there a set of cargoes that are dependent on caveolae for entry into cells? And, if so, are there well-defined signals for their concentration in caveolae, as for clathrin-coated pits? Do caveolae differ in their dynamics in different tissues and in different local regions of the same cells? And, importantly, what is the function of caveola scission from the plasma membrane? Here we will consider the mechanisms of formation and scission of caveolae, and discuss how these two processes are intricately coupled and regulated by lipids, providing clues into the role of caveola endocytosis.

## Shaping of the caveolar membrane by proteins and lipids

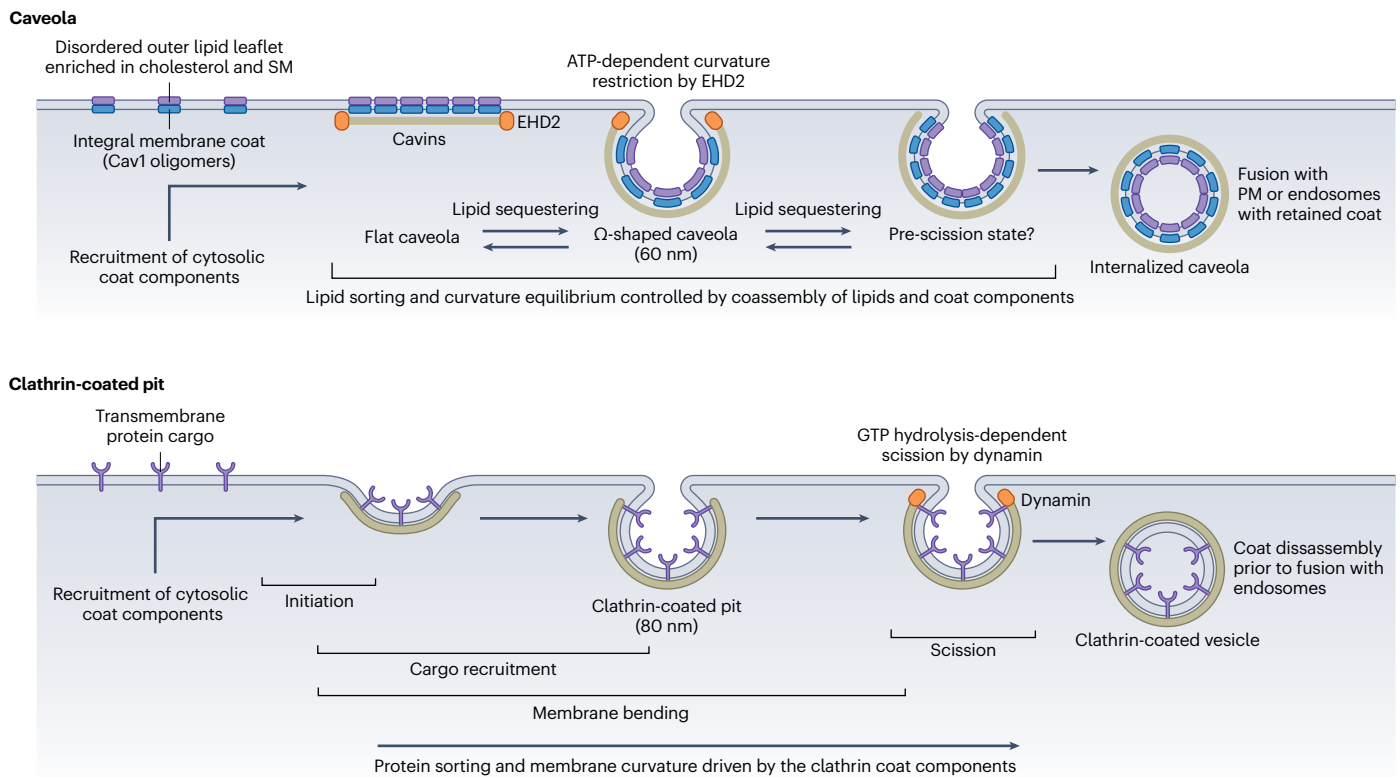
Caveolae in non-muscle cells are characterized by ~140–150 molecules of the oligomeric integral membrane protein caveolin-1, 30–70

molecules of caveolin-2, and an oligomeric complex of cavin peripheral membrane proteins<sup>25–34</sup>. Cavins 1, 2 and 3 associate with the caveolae of mammalian non-muscle cells. The caveolae of skeletal muscle cells are generated by caveolin-3<sup>35,36</sup> and have an additional cavin family member, cavin4 (also known as MURC)<sup>31,37</sup>. Cavin1 can bind with low affinity to caveolin-1 through electrostatic ‘fuzzy’ interactions<sup>38</sup>. Together with hydrophobic insertion of cavin1 into the membrane, this helps to generate caveolae<sup>39</sup>. EH-domain-containing protein 2 (EHD2), which associates with the neck of caveolae, is a key negative regulator of caveola budding<sup>40,41</sup>. Loss of EHD2 increases caveolar budding, as well as recycling, suggesting that it plays a crucial role in regulation of the caveolar endocytosis and/or recycling pathway<sup>41</sup>. EHD2 also interacts with Pascin2, another key regulator of caveolar formation<sup>42,43</sup>. In addition to these core components, caveolae are associated with Pascin2 (PKC and casein kinase substrate in neurons 2), dynamin2, EHD2 and, in some cell types, ROR1 (receptor tyrosine kinase-like orphan receptor 1). Each of these proteins can have context-dependent roles in caveola function. For example, in some cell types, ROR1 is essential to link cavin1 and caveolin-1<sup>44</sup>, and pascin2 can also contribute to the formation of caveolae<sup>42,43,45</sup>. Dynamin was initially proposed to mediate scission of caveolae in a fashion analogous to its role at the neck of clathrin-coated pits<sup>46,47</sup>, but this has since been questioned<sup>48–50</sup>. Instead, dynamin appears to stabilize a population of caveolae and reduce their scission<sup>48</sup>. Furthermore, studies of endothelial cells have shown various components of the cellular membrane fusion machinery, including NSF (*N*-ethylmaleimide-sensitive factor), SNAP (soluble NSF-attachment protein) and VAMP2 (vesicle-associated membrane protein), to be associated with caveolae<sup>51,52</sup>. VAMP2 appears to be a major component of isolated caveolae and has been shown to be required for endothelial uptake of cholera toxin by caveolae<sup>51,52</sup>.

In addition to these protein components, purified caveolae are enriched in specific lipids, particularly cholesterol and sphingomyelin<sup>53</sup>. Nanoscale mapping of the lipid components of caveolae has revealed a synergistic role for caveolar proteins in generating a lipid domain enriched in cholesterol, phosphatidic acid and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>)<sup>54</sup>. This involves the caveolin oligomer, embedded in the membrane, and the peripheral membrane-associated oligomeric cavin complex<sup>55</sup>. Furthermore, groundbreaking cryoelectron microscopy (cryoEM) studies have shown that caveolin forms a unique oligomeric disc within the cytoplasmic leaflet of the caveolar membrane<sup>56</sup> (Fig. 3). Simulations, reconstitution studies and biophysical modelling suggest that high levels of cholesterol are required to generate the caveolar domain in mammalian cells<sup>57,58</sup>. Accordingly, perturbation of cholesterol, either through depletion, chelation or by inhibiting its synthesis, causes a flattening of caveolae<sup>26,59,60</sup>. The cavin proteins also have lipid-binding capabilities. Cavin1 binds both phosphatidylserine<sup>61</sup>, shown to be important for caveola formation and dynamics<sup>62</sup>, and PI(4,5)P<sub>2</sub><sup>63</sup>, which acts as a sensor for membrane association<sup>39,64</sup>. Cavin1 can associate with the plasma membrane in the absence of caveolin if cholesterol levels are increased<sup>38</sup>. These observations point to a model in which multiple low-affinity charge interactions between caveolins and cavins, as well as with membrane lipids<sup>65</sup>, help generate caveolae<sup>38</sup>. Most importantly, lipids such as cholesterol play a crucial role in curvature generation through modulation of the caveolin-rich domain.

## Lipids as both cargo and key regulators of caveolar endocytosis

Early studies showed that glycosphingolipid-binding toxins<sup>66–68</sup> and proteins anchored to the plasma membrane through a glycosylphosphatidylinositol moiety after clustering using antibodies<sup>69,70</sup> were concentrated in caveolae and delivered to endosomes. These studies suggested that lipids and lipid-anchored proteins could be important cargoes for caveolae. Subsequent studies showed that lipids were more than just cargo for endocytic caveolae. Treatment of cells with a range of lipids, including glycosphingolipids and cholesterol, were shown to



**Fig. 2 | Comparison of caveolae and clathrin-coated pits.** Schematic illustration of the budding process, as well as the key lipid and protein components, associated with caveolae and clathrin-coated vesicles. Clathrin-coated vesicle formation includes well-described sequential steps that involve receptor-dependent recruitment of a peripheral coat, which drives membrane curvature to the stage where the vesicle is released by dynamin-mediated scission. In comparison, the caveolae membrane unit consists of

an intramembrane lipid–protein core held together by a peripheral cavin coat. Membrane curvature is influenced by lipid composition and membrane tension; this generates a specialized domain with a unique lipid composition that can adopt different levels of curvature. Scission of caveolae is restricted by EHD2 rings at the neck region. Far less is known regarding the scission and subsequent endocytosis of caveolae compared to clathrin-coated vesicles. SM, sphingomyelin.

induce scission of caveolae from the plasma membrane<sup>5,14,71–73</sup>. Importantly, these studies involved a number of different groups, used a variety of techniques and cell systems, and came to similar conclusions, suggesting that specific lipids may have universal effects in inducing the scission of caveolae.

The possible mechanisms underlying this lipid-stimulated endocytosis have been revealed using a liposome-fusion protocol to rapidly introduce lipids into the plasma membrane, thereby altering concentration ratios of specific lipids<sup>73</sup>. The concentration change of lipids not only caused increased curvature of the caveolae, but also induced their scission from the plasma membrane. Exogenously added cholesterol and glycosphingolipids were proposed to induce curvature, leading to scission from the plasma membrane—a biophysical process in which the interactions between the caveolin oligomer and lipids drive the curvature to eventually cause scission<sup>73</sup>. There is support for the proposed mechanism from a model system, where expression of caveolin in a bacterial system has been shown to induce curvature driven by caveolin oligomers embedded in the membrane that give rise to scission without involvement of classical ‘pinches’ such as dynamin<sup>74</sup>. In the following sections, we consider such a biophysical mechanism as the driving force for caveolar formation and scission from the plasma membrane, then discuss implications for the cellular roles of caveolar endocytosis.

### A model for lipid-induced membrane shaping and scission of the caveolin-rich membrane

At this point, it is of interest to compare what we know about clathrin-coated pits and caveolae in terms of their formation and

scission (Fig. 2). As we explain in the following, fundamental distinctions suggest that basic differences in the physical principles underlie the mechanisms of the two processes.

Clathrin-coated pit formation and endocytosis are driven by a highly complex choreography of more than 50 proteins whose action is subject to tight coordination in space and time<sup>75</sup>. The two major events constituting clathrin-mediated endocytosis—vesicle shaping and cargo recruitment—are mutually dependent, and their temporal interplay has been a subject of extensive discussion (a review is provided in ref. 76). The curvature of clathrin-coated vesicles appears to be generated in parallel by several mechanisms, all of which have been previously proposed and elaborated for individual proteins involved in the endocytic machinery<sup>77</sup>. The common principle underlying all these mechanisms is the creation of structural trans-thickness (‘up–down’) asymmetry of the cytoplasmic leaflet of the plasma membrane. The difference between these mechanisms lies in the specific mode of asymmetry generation, which can be grouped into three classes: hydrophobic insertion, scaffolding by bent hydrophilic macromolecular complexes, and protein crowding<sup>77</sup>. However, these mechanisms are not sufficient to cause scission by themselves. Indeed, dynamin, which uses guanosine-5′-triphosphate (GTP)-powered oligomerization, is required to constrict the neck and complete scission.

In contrast, the number of different types of protein responsible for caveola formation and scission in mammalian cells appears to be limited to just three. Caveolins and cavins form a complex covering the surfaces of caveolar bulbs, and oligomers of the dynamin family protein EHD2 are found at bulb necks<sup>41,78</sup>. Moreover, expression of caveolin-1 in bacterial membranes, which lack endogenous caveolae, has revealed

## BOX 1

## Methods to study caveola endocytosis

The lack of extracellular markers that are completely specific to caveolae and that could be used to track their endocytosis has necessitated the development and application of a range of complementary techniques, each with advantages but also caveats. This includes tracking of caveolin fused to fluorescent proteins using live fluorescence microscopy. Note that the fluorescent proteins are larger than the caveolin protein, so researchers have endeavoured to ensure that the fusions are non-perturbing (see, for example, careful quantitative characterization in ref. 6). It has also been important to avoid high expression levels of these markers, as they are linked to organelle perturbation<sup>2,96</sup> and toxicity<sup>97</sup>. Therefore, genome-edited cells have provided a powerful tool. Tracking of cavin1, which associates with endocytic caveolae that fuse with the early endosome<sup>4</sup>, provides an additional tool to ensure that caveolae, rather than caveolin-1, is being followed. Note that caveolin-1 can be endocytosed through an alternative dynamin-independent CLIC/GEIC (clathrin-independent carriers/glycosylphosphatidylinositol-attached protein-enriched endosomal compartments) pathway in the absence of cavin1<sup>98</sup>. Mutant proteins that perturb the disassembly of the caveolar coat provide additional tools for dissection of the process<sup>38</sup>. Furthermore, although EM provides a snapshot of the cell rather than a live view, it has served as an important validation of other techniques. Finally, use of a surface marker, which can be rapidly endocytosed and then localized under conditions where the remaining pool at the surface has been removed, has been an important step in directly identifying endocytic caveolae that have separated from the plasma membrane<sup>5,41,72,99</sup>, particularly when combined with immunogold labelling of the caveolar proteins<sup>99</sup>.

the formation of caveola-like membrane structures called heterologous caveolae (h-caveolae) that have a similar degree of curvature to regular caveolae<sup>74</sup>. This suggests that caveolin alone is sufficient to induce this range of curvature. Remarkably, knowledge regarding the mechanisms driving the curvature of clathrin-coated vesicles does not help us understand the caveolar curvature. The challenge originates from multimers of the integral membrane protein caveolin, termed 8S complexes, for which there is no analogous complex in clathrin-mediated endocytosis. The 8S complex comprises 11 monomers forming a disc (-14 nm in diameter) with one hydrophobic and one hydrophilic flat surface and a central  $\beta$ -barrel<sup>56</sup>. The thickness of the hydrophobic rim of the disc (a few nanometres) suggests that it embeds into the cytosolic membrane leaflet spanning the whole leaflet thickness, displacing ~250 lipid molecules, such that its flat hydrophobic face touches the bottom surface of the extracellular membrane leaflet (Fig. 1). This predicted mode of association of a caveolin disc with the plasma membrane<sup>56</sup> has been supported by the results of molecular dynamic simulations<sup>58</sup>.

Importantly, because, according to the originally published structure<sup>56</sup>, a caveolin disc is flat and spans the entire thickness of the cytoplasmic leaflet, it does not create any up-down asymmetry of the leaflet structure. Therefore, the physical mechanism by which caveolin discs generate membrane curvature must be fundamentally different from those discussed above in the context of clathrin-mediated endocytosis. A mechanism has been proposed assuming that the forces driving the membrane curvature originate from the energy of the contact interaction between the caveolin disc and the lipid molecules of the external membrane leaflet<sup>57</sup>. It has been assumed that contact

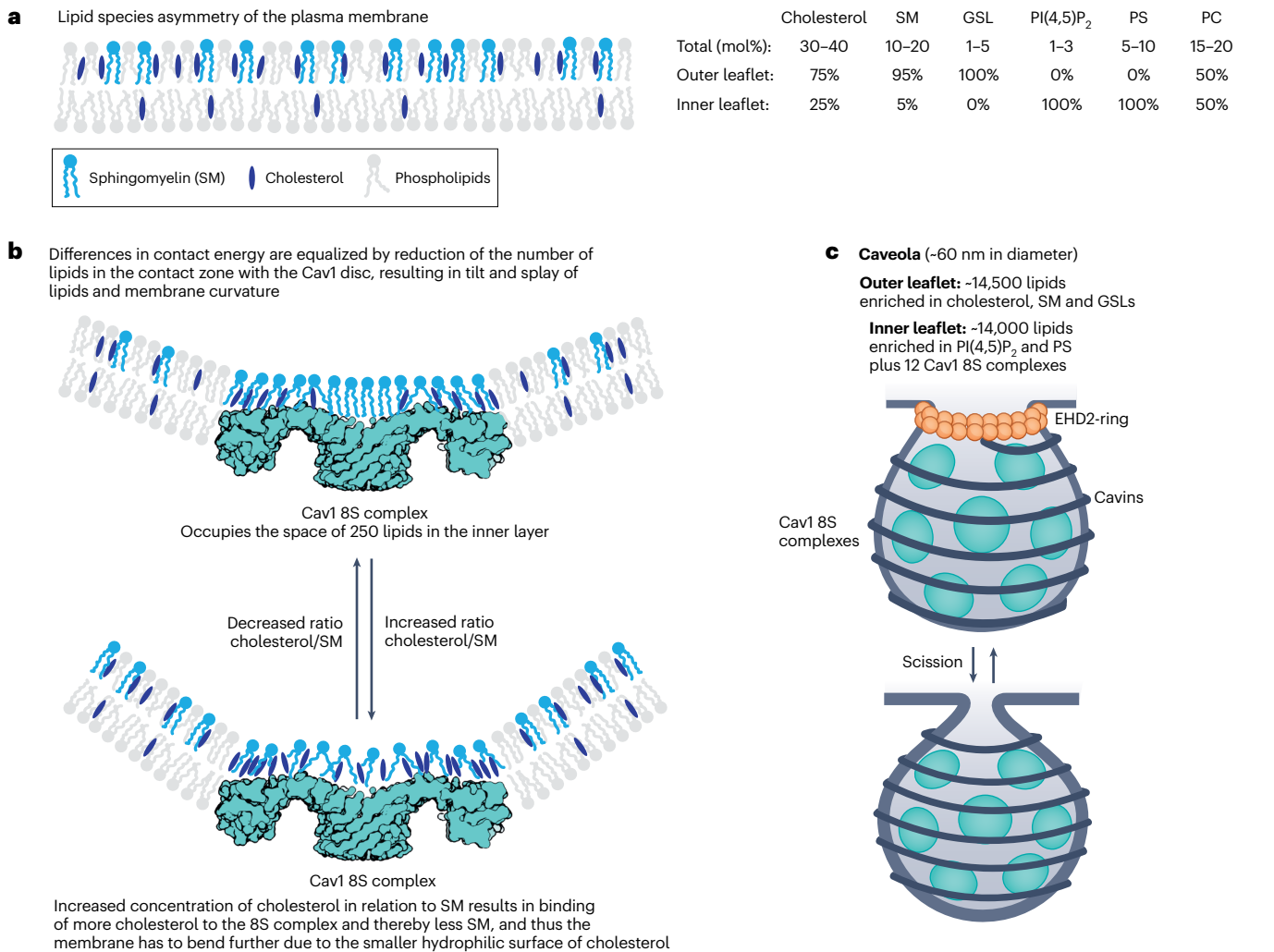
between the lipid molecules of the external leaflet of the plasma membrane and the hydrophobic face of the caveolin disc is energetically unfavourable compared to their contact with the lipidic regions of the cytoplasmic leaflet. Due to this differential contact energy, the system tends to minimize the number of lipid molecules of the external leaflet, which cover the hydrophobic face of the caveolin disc embedded in the cytosolic leaflet. This leads to tilting, with respect to the disc plane, of the lipid molecules that face the disc boundary in the external leaflet, and the subsequent emergence of the intramembrane strains and elastic stresses of lipid tilt and splay. These stresses drive overall membrane kinking along the edges of the caveolin discs<sup>57</sup>. As a result of this kinking, the membrane adopts an overall bent shape, whose curvature is set by the differential contact energy and the distance between the caveolin discs along the membrane plane<sup>57</sup> (Fig. 3). The intramembrane strains predicted by this model were confirmed by numerical simulations<sup>58</sup>.

This model can be extended to account for the model proposed in a recent preprint that describes an effect on the curvature generation of the patterning of the hydrophobic residues along the outer rim of a caveolin disc<sup>79</sup>. Because the hydrophobic residues can be seen as effectively attracting the hydrocarbon chains of lipid molecules, while the hydrophilic residues are effectively repelling, the hydrophobicity distribution across the rim of a caveolin disc can be described by an effective profile of the rim. If the hydrophobicity changes across the rim thickness, the effective profile of the rim is tilted with respect to the caveolin disc plane. This imposes a tilt on the lipid molecules of the cytoplasmic leaflet adjacent to the rim<sup>79</sup>, which, in turn, induces the intramembrane distribution of tilt and splay strains and stresses. The transmembrane interplay between the stresses induced by the differential contact energy and those generated by the tilted rim profile can either enhance or inhibit—or even redirect—the membrane curvature, depending on the tilt direction of the disc rim profile.

This model for curvature generation also predicts that if several caveolin discs are clustered in close proximity, this would result in the generation of a membrane bulb. Each caveolae is estimated to contain 144 caveolin-1 proteins, equivalent to ~12 caveolin-1 discs, which would account for curvature generation in the range of 50–60 nm if the inter-disc distance is in the range of a few nanometres. We propose that clustering and spacing of these caveolin discs is facilitated by cavin proteins. Recruitment of cavins is dependent on the presence of PI(4,5)P<sub>2</sub> and phosphatidylserine at the inner lipid leaflet, and makes up the actual protein coat of caveolae<sup>55,61–63</sup>. In addition to clustering caveolin discs, cavins are also proposed to bend membranes via both hydrophobic insertion and protein crowding<sup>38,39</sup>. This can contribute to the high curvature of caveolar domains in vertebrate cells that express cavins as compared to caveolin domains lacking cavins<sup>80,81</sup>.

Besides identifying the differential contact energy as the major driving force for membrane bending by caveolin discs, the mechanistic model addresses the role of lipids in modulating the amount of emerging curvature. Lipids such as cholesterol and diacylglycerol have effective molecular shapes of truncated cones<sup>82</sup>. They can thus facilitate caveolin-driven curvature generation by repartitioning into the regions of membrane kinking in the vicinity of the edges of the caveolin discs and enabling a partial relaxation of the membrane stresses<sup>57</sup>. This would explain why lowering of cholesterol concentration in cells results in caveolae flattening<sup>26</sup>, and why increased concentrations of cholesterol give rise to caveolae with smaller diameters and thinner necks<sup>73</sup>. The model also indicates that the role of cholesterol can be played by other molecules that have an effective molecular shape of a truncated cone. For example, diacylglycerol may substitute for cholesterol during the formation of h-caveolae in sterol-free bacterial membranes<sup>74</sup>.

It is important to emphasize that the amounts of cholesterol needed for the membrane curvature modulation predicted by our model are substantially smaller than those predicted by simulations and resulting from direct atomistic caveolin-cholesterol



**Fig. 3 | Model of membrane bending and scission caused by the intramembrane protein–lipid core of caveolae, with EHD2 preventing scission.** **a**, Schematic representation of a cross-sectional slice of the plasma membrane without curvature. Asymmetry is highlighted by specific lipids colour-coded as indicated (left) with their relative distribution highlighted (right)<sup>87</sup>. SM, sphingomyelin; GSL, glycosphingolipid; PI(4,5)P<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; PS, phosphatidylserine; PC, phosphatidylcholine. **b**, Schematic representation of cross-sectional slices of the plasma membrane containing one caveolin-1 (Cav1) 8S complex (caveolin disc) after relaxation leading to curvature (top) and after relaxation in the presence of an increased concentration of cholesterol leading to higher curvature (bottom). The Cav1 8S complex is shown as a 10-nm central slice of the surface representation of the atomic model (PDB 7SC0), and phospholipids are colour-coded as described in **a**. The leaflet opposing the caveolin disc is predicted to

primarily contain cholesterol and sphingomyelin due to the high affinity of cholesterol towards both caveolin and sphingomyelin and due to the cholesterol repartitioning driven by relaxation of the membrane elastic energy. In this way, the ratio between cholesterol and sphingomyelin in the outer leaflet is predicted to influence the degree of caveolar curvature and scission. **c**, Approximate number of lipids in the inner and outer leaflets of a caveola based on the geometry of a 60-nm sphere and incorporation of 13 Cav1 8S complexes in the inner leaflet. Lipids that are enriched in comparison to the surrounding plasma membrane are indicated. A schematic illustration is shown of the proposed curvature change, leading to scission of a caveola in response to an altered cholesterol:sphingomyelin ratio in the outer leaflet. The membrane is shown in light grey, cavins (cavin oligomers) in grey, EHD2 oligomers (EHD2-ring) in dark grey and Cav1 8S complexes in turquoise.

interactions<sup>58,83</sup>. Therefore, our model accounts only partially for the cholesterol involvement in caveola formation.

In our model for scission, the cavin-driven clustering of caveolin discs, assisted by the local concentration of cholesterol, drives membrane bending towards formation of a closed spherical shape, which is accompanied by formation of a progressively narrowing hourglass-like caveolar neck (Fig. 3). Such a narrowing membrane neck will be intrinsically unstable due to the accumulating membrane bending stress, which could lead to membrane fission when the neck waist reaches the predicted cross-sectional diameter of a few nanometres<sup>84</sup>. Therefore, oligomerization of EHD2 into rings of a certain radius around the waist of the neck must stop the neck narrowing, progression of the clustering of caveolin discs and, hence, the budding. Without the restriction

of this curvature-generating mechanism by EHD2 ring formation, scission would occur in agreement with the results from cells<sup>41</sup>. Thus, the balance between curvature-driving clustering of caveolin and the constraining effect of EHD2 rings controls the fission of caveolae. The proposed fission mechanism is distinct from that of clathrin-coated pits and is made possible by the unique protein–lipid core of caveolae, whereby increasing the curvature angles between caveolin discs is sufficient to induce scission without insertion of extra proteins. EHD2 is a key factor in this model and provides control over the budding process. Membrane binding and oligomerization of EHD2 is controlled by an adenosine triphosphate (ATP)-dependent mechanism that facilitates the assembly of EHD2 rings of varying diameters<sup>85,86</sup>. EHD2 is also associated with flat caveolae, where it appears to form a ring around

**BOX 2****Key outstanding questions**

- How does a caveola undergo scission and how is this regulated *in vivo*?
- Are there tissue-specific differences in caveola endocytosis in different cells and tissues, and do some populations of caveolae show stable association with the plasma membrane?
- Do lipids transiently trigger scission of caveolae in cells and tissues and, if so, under what conditions does this occur and what is the function of this lipid-controlled pathway?
- Are there a set of cargoes that are dependent on caveolae for entry into cells, or is the major function related to control of the plasma-membrane lipid balance?
- Are transmembrane proteins excluded from caveolae or is there flexibility in the caveolar domain that allows incorporation of membrane proteins?

the edge of a caveola<sup>50</sup>. Taken together, this suggests that EHD2 might serve as an adjustable outer rim that limits the number of caveolin discs and prevents the scission of caveolar buds.

So why do caveolae undergo scission in a mammalian cell? Based on current knowledge, we predict that lipid composition is the main modulator of caveola endocytosis. The plasma membrane has a distinct asymmetric lipid composition, with the outer layer enriched in sphingomyelins (10–20%), cholesterol (40%) and glycosphingolipids (1–5 mol%), and glycerophospholipids being more abundant in the inner leaflet<sup>57</sup> (Fig. 3). Outer-leaflet cholesterol is found in a dynamic equilibrium between a 'free' pool and a pool complexed to sphingomyelin due to the high affinity between these lipids<sup>88</sup>. The concentration ratio between cholesterol and sphingomyelin appears to have a major influence on caveola scission<sup>73</sup>. This is in agreement with our mechanistic model for scission, whereby increased plasma-membrane cholesterol levels would result in increased bending and fission, whereas increased levels of sphingomyelins would reduce membrane bending by limiting the enrichment of cholesterol in the leaflet opposing the caveolin disc. This mechanism is facilitated by the high affinity between cholesterol and sphingomyelin and the difference in the contact energy that limits the number of lipids opposing the caveolin disc (Fig. 3). Further evidence that cholesterol influences caveola scission comes from studies of the endocytosis inhibitory compound Dyngo-4a<sup>89</sup>. Membrane insertion of Dyngo-4a at the position of cholesterol has been found to prevent caveola budding and lateral diffusion, and could be counteracted by artificially increasing plasma-membrane cholesterol levels<sup>89</sup>.

Our model predicts that caveolae scission is sensitive to fluctuations in lipid concentrations that can outcompete the constraining force of EHD2. In this way, the scission of caveola provides a means to monitor the concentration of outer-leaflet lipids of the plasma membrane, which cannot be sensed and endocytosed via an intracellular protein coat. This raises the question of how much lipid concentrations fluctuate within the plasma membrane over time. Taking into account the constant *de novo* synthesis and trafficking of lipids, the concentrations are likely to vary, but currently there are no experimental studies addressing this due to methodological challenges. Interestingly, acute cholesterol concentration changes, in the range of 0.1 mol% for the total plasma membrane, have been found to influence the frequency of caveolae scission<sup>73</sup>. This suggests that even small concentration fluctuations can indeed be reflected in the degree of caveolae curvature and scission. As an extension of this model, the functional role of caveolae endocytosis might be more related to the remaining lipid concentrations in the plasma membrane (that is, to preserve integrity)

**BOX 3****Key points of the proposed model**

- The model proposes a fundamentally different mechanism for the formation and scission of clathrin-coated vesicles and caveolae.
- Transmembrane protein exclusion from surface caveolae argues against a role as an alternative endocytic pathway for protein receptors and ligands.
- Lipids drive curvature and scission of caveolae.
- The ratio between cholesterol and sphingomyelin is crucial.

than the actual internalized lipids themselves. This also provides a plausible explanation as to why caveolae so frequently are found as highly invaginated structures and that scission appears temporally random and of varying frequency in different cell types. Rather, caveolae are preformed entities that respond to lipid-concentration differences by changes in curvature leading to either flattening or scission. This is analogous to and in agreement with the observation that caveolae have been shown to respond to mechanical stretching by flattening<sup>23</sup>. Our model does not explicitly address the effects of alterations in membrane tension on caveola formation and scission. However, the model predicts that caveolae would function as entities that can sense properties of the plasma membrane and buffer mechanical and chemical stresses by either flattening or scission. In summary, we propose a lipid-centric equilibrium model for the curvature regulation and scission of caveolae that could serve as means to monitor and control the concentration ratio of outer-leaflet lipids<sup>89</sup>.

**Future perspectives**

A lipid-driven model for caveola endocytosis raises new questions regarding the regulation, dynamics and function of this pathway (Box 2). First, do lipids transiently trigger scission of caveolae in cells and tissues, and, if so, under what conditions does this occur. Second, what is the function of this lipid-controlled pathway? Observations of cells in culture show that caveola endocytosis occurs spontaneously over the entire cell surface. Does this represent sites of lipid fluctuation, which cause an imbalance in bilayer lipids and scission, or reflect regulation of the lipid-driven process by accessory proteins such as EHD2 (Box 3)?

Regarding the functions of caveolae in lipid regulation, an attractive idea is that a plasma domain that excludes transmembrane proteins but is enriched in specific lipids would provide a distinct system for sensitive control of lipid composition without altering levels of transmembrane proteins.

Loss of caveolae due to genetic disorders is known to cause dystrophy of cells and tissues where caveolae are normally very abundant (for example, lipodystrophy in adipose tissue<sup>90</sup>). The cause of this is currently not known, but lack of surveillance of the outer-leaflet lipid composition might contribute to the reduced resilience of the plasma membrane. Dysregulation of cholesterol has also been observed in a number of different cellular systems in response to modulation of caveolae<sup>91–93</sup>. Loss of caveolae or overexpression of mutant caveolin proteins disrupts specific plasma-membrane microdomains in a cholesterol-dependent manner<sup>94,95</sup> and causes changes in phosphatidylserine clustering in the inner leaflet of the plasma membrane<sup>94</sup>. Interestingly, these effects are also observed upon the acute disruption of caveolae upon osmotic stress-induced changes in membrane tension<sup>94</sup>, pointing towards the importance of a functional caveolar domain, not simply its protein components, in regulating plasma-membrane

lipid distribution. Furthermore, loss of caveolae has been shown to have widespread effects on the levels of specific glycosphingolipids, as revealed by lipidomic analyses<sup>94</sup>. In addition, cells lacking caveolae show differences in the trafficking of glycosphingolipids; here, excess plasma-membrane-incorporated gangliosides were found to be sorted to the endoplasmic reticulum and Golgi, where they can be metabolized in wild-type cells but missorted to lysosomes in cells lacking caveolae<sup>6</sup>. This suggests a role of caveolae in the trafficking of glycosphingolipids to specific cellular destinations. Dysregulation of cholesterol has also been observed in a number of different cellular systems in response to modulation of caveolae<sup>91–93</sup>. We propose that considering the role of caveolae in responding to, and regulating, membrane lipids will prove crucial in understanding their complex roles in physiological cellular processes, as well as their dysfunction in disease.

## References

1. Mayor, S., Parton, R. G. & Donaldson, J. G. Clathrin-independent pathways of endocytosis. *Cold Spring Harb. Perspect. Biol.* **6**, a016758 (2014).
2. Hayer, A. et al. Caveolin-1 is ubiquitinated and targeted to intraluminal vesicles in endolysosomes for degradation. *J. Cell Biol.* **191**, 615–629 (2010).
3. Pelkmans, L., Burli, T., Zerial, M. & Helenius, A. Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. *Cell* **118**, 767–780 (2004).
4. Boucrot, E., Howes, M. T., Kirchhausen, T. & Parton, R. G. Redistribution of caveolae during mitosis. *J. Cell Sci.* **124**, 1965–1972 (2011).
5. Sharma, D. K. et al. Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol. *Mol. Biol. Cell* **15**, 3114–3122 (2004).
6. Shvets, E., Bitsikas, V., Howard, G., Hansen, C. G. & Nichols, B. J. Dynamic caveolae exclude bulk membrane proteins and are required for sorting of excess glycosphingolipids. *Nat. Commun.* **6**, 6867 (2015).
7. Anton-Plagaro, C. et al. Mapping of endosomal proximity proteomes reveals Retromer as a hub for RAB GTPase regulation. *Nat. Commun.* **16**, 6990 (2025).
8. Simionescu, M., Gafencu, A. & Antohe, F. Transcytosis of plasma macromolecules in endothelial cells: a cell biological survey. *Microsc. Res. Tech.* **57**, 269–288 (2002).
9. Ariotti, N. & Parton, R. G. SnapShot: caveolae, caveolins and cavins. *Cell* **154**, 704–e701 (2013).
10. Parton, R. G. & del Pozo, M. A. Caveolae as plasma membrane sensors, protectors and organizers. *Nat. Rev. Mol. Cell Biol.* **14**, 98–112 (2013).
11. Oh, P. et al. Live dynamic imaging of caveolae pumping targeted antibody rapidly and specifically across endothelium in the lung. *Nat. Biotechnol.* **25**, 327–337 (2007).
12. Rosengren, B. I. et al. Transvascular protein transport in mice lacking endothelial caveolae. *Am. J. Physiol. Heart Circ. Physiol.* **291**, H1371–H1377 (2006).
13. Di Guglielmo, G. M., Le Roy, C., Goodfellow, A. F. & Wrana, J. L. Distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover. *Nat. Cell Biol.* **5**, 410–421 (2003).
14. Sharma, D. K. et al. The glycosphingolipid, lactosylceramide, regulates  $\beta$ 1-integrin clustering and endocytosis. *Cancer Res.* **65**, 8233–8241 (2005).
15. Kanai, Y., Wang, D. & Hirokawa, N. KIF13B enhances the endocytosis of LRP1 by recruiting LRP1 to caveolae. *J. Cell Biol.* **204**, 395–408 (2014).
16. Hao, J. W. et al. CD36 facilitates fatty acid uptake by dynamic palmitoylation-regulated endocytosis. *Nat. Commun.* **11**, 4765 (2020).
17. Marchiando, A. M. et al. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *J. Cell Biol.* **189**, 111–126 (2010).
18. Orlichenko, L. et al. Caveolae mediate growth factor-induced disassembly of adherens junctions to support tumor cell dissociation. *Mol. Biol. Cell* **20**, 4140–4152 (2009).
19. Stukan, I. et al. Wolf in sheep's clothing: taming cancer's resistance with human serum albumin?. *Int. J. Nanomedicine* **20**, 3493–3525 (2025).
20. Koleske, A. J., Baltimore, D. & Lisanti, M. P. Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc. Natl Acad. Sci. USA* **92**, 1381–1385 (1995).
21. Elkin, S. R. et al. A systematic analysis reveals heterogeneous changes in the endocytic activities of cancer cells. *Cancer Res.* **75**, 4640–4650 (2015).
22. Hwang, N. et al. Caveolin-1 mediates the utilization of extracellular proteins for survival in refractory gastric cancer. *Exp. Mol. Med.* **55**, 2461–2472 (2023).
23. Sinha, B. et al. Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell* **144**, 402–413 (2011).
24. Parton, R. G. Caveolae: structure, function and relationship to disease. *Annu. Rev. Cell Dev. Biol.* **34**, 111–136 (2018).
25. Engelman, J. A. et al. Molecular genetics of the caveolin gene family: implications for human cancers, diabetes, Alzheimer disease and muscular dystrophy. *Am. J. Human Genet.* **63**, 1578–1587 (1998).
26. Rothberg, K. G. et al. Caveolin, a protein component of caveolae membrane coats. *Cell* **68**, 673–682 (1992).
27. Kurzchalia, T. V., Dupree, P. & Monier, S. VIP21-caveolin, a protein of the trans-Golgi network and caveolae. *FEBS Lett.* **346**, 88–91 (1994).
28. Scherer, P. E. et al. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc. Natl Acad. Sci. USA* **93**, 131–135 (1996).
29. Hansen, C. G. & Nichols, B. J. Exploring the caves: cavins, caveolins and caveolae. *Trends Cell Biol.* **20**, 177–186 (2010).
30. Hansen, C. G., Bright, N. A., Howard, G. & Nichols, B. J. SDPR induces membrane curvature and functions in the formation of caveolae. *Nat. Cell Biol.* **11**, 807–814 (2009).
31. Bastiani, M. et al. MURC/Cavin-4 and cavin family members form tissue-specific caveolar complexes. *J. Cell Biol.* **185**, 1259–1273 (2009).
32. Liu, L. & Pilch, P. F. A critical role of cavin (polymerase I and transcript release factor) in caveolae formation and organization. *J. Biol. Chem.* **283**, 4314–4322 (2008).
33. Hill, M. M. et al. PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. *Cell* **132**, 113–124 (2008).
34. McMahon, K. A. et al. SRBC/cavin-3 is a caveolin adapter protein that regulates caveolae function. *EMBO J.* **28**, 1001–1015 (2009).
35. Way, M. & Parton, R. G. M-caveolin, a muscle-specific caveolin-related protein. *FEBS Lett.* **376**, 108–112 (1995).
36. McNally, E. M. et al. Caveolin-3 in muscular dystrophy. *Hum. Mol. Genet.* **7**, 871–877 (1998).
37. Tagawa, M. et al. MURC, a muscle-restricted coiled-coil protein, is involved in the regulation of skeletal myogenesis. *Am. J. Physiol. Cell Physiol.* **295**, C490–498 (2008).
38. Tillu, V. A. et al. Cavin1 intrinsically disordered domains are essential for fuzzy electrostatic interactions and caveola formation. *Nat. Commun.* **12**, 931 (2021).
39. Liu, K. C. et al. Membrane insertion mechanism of the caveola coat protein Cavin1. *Proc. Natl Acad. Sci. USA* **119**, e2202295119 (2022).

40. Matthaeus, C. et al. EHD2-mediated restriction of caveolar dynamics regulates cellular fatty acid uptake. *Proc. Natl Acad. Sci. USA* **117**, 7471–7481 (2020).
41. Moren, B. et al. EHD2 regulates caveolar dynamics via ATP-driven targeting and oligomerization. *Mol. Biol. Cell* **23**, 1316–1329 (2012).
42. Hansen, C. G., Howard, G. & Nichols, B. J. Pacsin 2 is recruited to caveolae and functions in caveolar biogenesis. *J. Cell Sci.* **124**, 2777–2785 (2011).
43. Senju, Y., Itoh, Y., Takano, K., Hamada, S. & Suetsugu, S. Essential role of PACSIN2/syndapin-II in caveolae membrane sculpting. *J. Cell Sci.* **124**, 2032–2040 (2011).
44. Yamaguchi, T. et al. ROR1 sustains caveolae and survival signalling as a scaffold of cavin-1 and caveolin-1. *Nat. Commun.* **7**, 10060 (2016).
45. Koch, D., Westermann, M., Kessels, M. M. & Qualmann, B. Ultrastructural freeze-fracture immunolabeling identifies plasma membrane-localized syndapin II as a crucial factor in shaping caveolae. *Histochem. Cell Biol.* **138**, 215–230 (2012).
46. Henley, J. R., Krueger, E. W., Oswald, B. J. & McNiven, M. A. Dynamin-mediated internalization of caveolae. *J. Cell Biol.* **141**, 85–99 (1998).
47. Oh, P., McIntosh, D. P. & Schnitzer, J. E. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. *J. Cell Biol.* **141**, 101–114 (1998).
48. Larsson, E., Moren, B., McMahon, K. A., Parton, R. G. & Lundmark, R. Dynamin2 functions as an accessory protein to reduce the rate of caveola internalization. *J. Cell Biol.* **222**, e202205122 (2023).
49. Parton, R. G., Taraska, J. W. & Lundmark, R. Is endocytosis by caveolae dependent on dynamin? *Nat. Rev. Mol. Cell Biol.* **25**, 511–512 (2024).
50. Matthaeus, C. et al. The molecular organization of differentially curved caveolae indicates bendable structural units at the plasma membrane. *Nat. Commun.* **13**, 7234 (2022).
51. McIntosh, D. P. & Schnitzer, J. E. Caveolae require intact VAMP for targeted transport in vascular endothelium. *Am. J. Physiol.* **277**, H2222–2232 (1999).
52. Schnitzer, J. E., McIntosh, D. P., Dvorak, A. M., Liu, J. & Oh, P. Separation of caveolae from associated microdomains of GPI-anchored proteins. *Science* **269**, 1435–1439 (1995).
53. Ortegren, U. et al. Lipids and glycosphingolipids in caveolae and surrounding plasma membrane of primary rat adipocytes. *Eur. J. Biochem.* **271**, 2028–2036 (2004).
54. Zhou, Y. et al. Caveolin-1 and cavin1 act synergistically to generate a unique lipid environment in caveolae. *J. Cell Biol.* **220**, e202005138 (2021).
55. Kenworthy, A. K., Han, B., Ariotti, N. & Parton, R. G. The role of membrane lipids in the formation and function of caveolae. *Cold Spring Harb. Perspect. Biol.* **15**, a041413 (2023).
56. Porta, J. C. et al. Molecular architecture of the human caveolin-1 complex. *Sci. Adv.* **8**, eabn7232 (2022).
57. Barnoy, A., Ariotti, N., Parton, R. G. & Kozlov, M. M. A model for membrane curvature generation by caveolin discs driven by differential contact interaction. *Nat. Commun.* **16**, 9030 (2025).
58. Doktorova, M. et al. Caveolin assemblies displace one bilayer leaflet to organize and bend membranes. *Proc. Natl Acad. Sci. USA* **122**, e2417024122 (2025).
59. Carozzi, A. J., Ikonen, E., Lindsay, M. R. & Parton, R. G. Role of cholesterol in developing T-tubules: analogous mechanisms for T-tubule and caveolae biogenesis. *Traffic* **1**, 326–341 (2000).
60. Hailstones, D., Sleer, L. S., Parton, R. G. & Stanley, K. K. Regulation of caveolin and caveolae by cholesterol in MDCK cells. *J. Lipid Res.* **39**, 369–379 (1998).
61. Tillu, V. A. et al. A variable undecad repeat domain in cavin1 regulates caveola formation and stability. *EMBO Rep.* **19**, e45775 (2018).
62. Hirama, T. et al. Phosphatidylserine dictates the assembly and dynamics of caveolae in the plasma membrane. *J. Biol. Chem.* **292**, 14292–14307 (2017).
63. Kovtun, O. et al. Structural insights into the organization of the cavin membrane coat complex. *Dev. Cell* **31**, 405–419 (2014).
64. Tillu, V. A., Kovtun, O., McMahon, K. A., Collins, B. M. & Parton, R. G. A phosphoinositide-binding cluster in cavin1 acts as a molecular sensor for cavin1 degradation. *Mol. Biol. Cell* **26**, 3561–3569 (2015).
65. Wu, Y. et al. Pro-ferroptotic lipids as key control points for caveola formation and disassembly. *Cell Rep.* **44**, 115789 (2025).
66. Tran, D., Carpentier, J. L., Sawano, F., Gorden, P. & Orci, L. Ligands internalized through coated or noncoated invaginations follow a common intracellular pathway. *Proc. Natl Acad. Sci. USA* **84**, 7957–7961 (1987).
67. Montesano, R., Roth, J., Robert, A. & Orci, L. Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins. *Nature* **296**, 651–653 (1982).
68. Parton, R. G. Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae. *J. Histochem. Cytochem.* **42**, 155–166 (1994).
69. Mayor, S., Rothberg, K. G. & Maxfield, F. R. Sequestration of GPI-anchored proteins in caveolae triggered by cross-linking. *Science* **264**, 1948–1951 (1994).
70. Parton, R. G., Joggerst, B. & Simons, K. Regulated internalization of caveolae. *J. Cell Biol.* **127**, 1199–1215 (1994).
71. Singh, R. D. et al. Selective caveolin-1-dependent endocytosis of glycosphingolipids. *Mol. Biol. Cell* **14**, 3254–3265 (2003).
72. Le Lay, S. et al. Cholesterol-induced caveolin targeting to lipid droplets in adipocytes: a role for caveolar endocytosis. *Traffic* **7**, 549–561 (2006).
73. Hubert, M. et al. Lipid accumulation controls the balance between surface connection and scission of caveolae. *eLife* **9**, e55038 (2020).
74. Walser, P. J. et al. Constitutive formation of caveolae in a bacterium. *Cell* **150**, 752–763 (2012).
75. Kaksonen, M. & Roux, A. Mechanisms of clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* **19**, 313–326 (2018).
76. Haucke, V. & Kozlov, M. M. Membrane remodeling in clathrin-mediated endocytosis. *J. Cell Sci.* **131**, jcs216812 (2018).
77. Kozlov, M. M. & Taraska, J. W. Generation of nanoscopic membrane curvature for membrane trafficking. *Nat. Rev. Mol. Cell Biol.* **24**, 63–78 (2023).
78. Stoeber, M. et al. Oligomers of the ATPase EHD2 confine caveolae to the plasma membrane through association with actin. *EMBO J.* **31**, 2350–2364 (2012).
79. Connolly, S. M. et al. Structural basis of caveolin-driven membrane bending. Preprint at <https://doi.org/10.64898/2026.02.05.703862> (2025).
80. Lolo, F. N. et al. Caveolin-1 dolines form a distinct and rapid caveolae-independent mechanoadaptation system. *Nat. Cell Biol.* **25**, 120–133 (2023).
81. Bhattachan, P. et al. Ascidian caveolin induces membrane curvature and protects tissue integrity and morphology during embryogenesis. *FASEB J.* **34**, 1345–1361 (2020).
82. Zimmerberg, J. & Kozlov, M. M. How proteins produce cellular membrane curvature. *Nat. Rev. Mol. Cell Biol.* **7**, 9–19 (2006).
83. Liebl, K. & Voth, G. A. Lipid organization by the Caveolin-1 complex. *Biophys. J.* **123**, 3688–3697 (2024).
84. Kozlovsky, Y. & Kozlov, M. M. Membrane fission: model for intermediate structures. *Biophys. J.* **85**, 85–96 (2003).

85. Daumke, O. et al. Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. *Nature* **449**, 923–927 (2007).
86. Hoernke, M. et al. EHD2 restrains dynamics of caveolae by an ATP-dependent, membrane-bound, open conformation. *Proc. Natl Acad. Sci. USA* **114**, E4360–E4369 (2017).
87. Doktorova, M. et al. Cell membranes sustain phospholipid imbalance via cholesterol asymmetry. *Cell* **188**, 2586–2602 e2524 (2025).
88. Das, A., Brown, M. S., Anderson, D. D., Goldstein, J. L. & Radhakrishnan, A. Three pools of plasma membrane cholesterol and their relation to cholesterol homeostasis. *eLife* **3**, e02882 (2014).
89. Larsson, E. et al. Lipid packing contributes to the confinement of caveolae to the plasma membrane. Preprint at <https://doi.org/10.1101/2025.03.13.643064> (2025).
90. Garg, A. & Agarwal, A. K. Caveolin-1: a new locus for human lipodystrophy. *J. Clin. Endocrinol. Metab.* **93**, 1183–1185 (2008).
91. Inder, K. L. et al. Expression of PTRF in PC-3 cells modulates cholesterol dynamics and the actin cytoskeleton impacting secretion pathways. *Mol. Cell Proteomics* **11**, M111.012245 (2012).
92. Fu, Y. et al. Expression of caveolin-1 enhances cholesterol efflux in hepatic cells. *J. Biol. Chem.* **279**, 14140–14146 (2004).
93. Frank, P. G. et al. Influence of caveolin-1 on cellular cholesterol efflux mediated by high-density lipoproteins. *Am. J. Physiol. Cell Physiol.* **280**, C1204–1214 (2001).
94. Ariotti, N. et al. Caveolae regulate the nanoscale organization of the plasma membrane to remotely control Ras signaling. *J. Cell Biol.* **204**, 777–792 (2014).
95. Roy, S. et al. Dominant-negative caveolin inhibits H-Ras function by disrupting cholesterol-rich plasma membrane domains. *Nat. Cell Biol.* **1**, 98–105 (1999).
96. Parton, R. G. & Howes, M. T. Revisiting caveolin trafficking: the end of the caveosome. *J. Cell Biol.* **191**, 439–441 (2010).
97. Hanson, C. A. et al. Overexpression of caveolin-1 is sufficient to phenocopy the behavior of a disease-associated mutant. *Traffic* **14**, 663–677 (2013).
98. Chaudhary, N. et al. Endocytic crosstalk: cavins, caveolins, and caveolae regulate clathrin-independent endocytosis. *PLoS Biol.* **12**, e1001832 (2014).
99. Kirkham, M. et al. Ultrastructural identification of uncoated caveolin-independent early endocytic vehicles. *J. Cell Biol.* **168**, 465–476 (2005).

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## Author contributions

R.G.P., M.M.K. and R.L. all contributed to conceptualization, writing, reviewing and editing of the manuscript.

## Competing interests

The authors declare no competing interests.

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