Caveolae: The FAQs

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Synopsis

Caveolae are an abundant, but enigmatic, plasma membrane feature of vertebrate cells. In this brief commentary, the authors attempt to answer some key questions related to the formation and function of caveolae based on round-table discussions at the first EMBO Workshop on Caveolae held in France in May 2019.

At the first EMBO Workshop dedicated to caveolae held in Le Pouliguen, France, May 12-16 2019 (http://meetings.embo.org/event/19-caveolae), round-table discussions were used to address some of the long-standing issues in the field and to decide upon a consensus view regarding key aspects of caveola biology. Here we address some of the frequently asked questions (FAQs) posed by cell biologists about caveolae. We provide some brief consensual answers based on these discussions and some suggestions for further reading.

FAQs

What are the protein components of caveolae?

In mammalian cells, integral membrane proteins termed caveolins (caveolin-1 in non-muscle cells and caveolin-3 in muscle) work together with cavins (particularly cavin-1) to generate caveolae. These proteins can be considered universal structural elements of caveolae and their knockout leads to loss of caveolae. In the absence of cavin-1, caveolin-1 can form functional non-caveolar domains called 'scaffolds' that have been characterized biochemically and, more recently, by single molecule super resolution microscopy. Other proteins work together with these components including caveolin-2, cavin-2, cavin-3, cavin-4, EHD (Eps-15 Homology Domain) 2, Pacsin2/Syndapin II and Pacsin3/Syndapin III, as well as ROR1 (Receptor tyrosine kinase-like Orphan Receptor 1) in specific cell types (for example cavin-4 in striated muscle, ROR1 in embryonic tissues and

tumors). In addition to their plasma membrane association, it is also important to note that many of these components can have other cellular locations. For example, caveolins can also be detected in association with the Golgi complex, early endosomes, and lipid droplets.

Are caveolae morphologically similar to clathrin coated pits? *How can these be distinguished?*

Caveolae have a defined diameter of 60 to 80 nm and an omega or U shape depending on the EM technique used to visualize them. Clathrin coated pits usually have wider diameter (around 100 nm) and the coat is more evident. Using platinum-replica EM (PREM), the coat of clathrin coated pits is visualized as a network of hexagons, while the caveolar coat shows a striped structure (Figure 1), at times forming spirals. The organization of clathrin coated pits is relatively uniform, while caveolae can organize into clusters of different sizes (known as caveolar 'rosettes', or 'clusters') and can flatten and remain at the plasma membrane (see below).

How do caveolae compare to clathrin coated pits in terms of abundance and density in different tissues?

Caveolae can occupy over 30% of the surface of some cell types (such as skeletal muscle, endothelial cells and adipocytes) but in other cell types, such as red blood cells, immune cells, and neurons, caveolae are not detectable. This contrasts with clathrin coated pits, which show a similar density in all nucleated mammalian cells. Caveolins, the major membrane proteins of caveolae, may

have significant roles other than caveola formation in cells with very low levels of caveolins (for example in liver, neurons, and B and T lymphocytes).

Are caveolae endocytic? If so, what is the destination of budded caveolae and do they carry specific cargo?

Caveolae can bud from the plasma membrane and fuse with early endosomes. However, there appear to be few cargoes, if any, which are strictly dependent on caveolae for their uptake into cells. In fact, transmembrane proteins may be largely excluded from caveolae. The role of caveola budding may be to regulate the density of surface caveolae, rather than caveolae acting as an endocytic carrier analogous to a clathrin coated pit. An exception to this view is the proposed role of caveolae in endothelia where caveolae have been implicated in transendothelial transport.

What is the link between caveolae and the actin cytoskeleton?

Immunofluorescence images of caveolar markers, such as caveolin-1 or cavin-1 in tissue culture cells, frequently show a linear pattern that co-aligns with actin stress fibers. This pattern is not often observed with other membrane invaginations. The reasons for this co-alignment are still not completely clear, but a coordination between two tension-controlling systems has been proposed as an explanation. When visualized by PREM, caveolae are invariantly surrounded by cortical actin filaments although the exact function of these filaments on caveolae and their composition are still unknown.

Actin cytoskeleton disruption leads to caveolar clustering and prevents their endocytosis. Caveolin-1 binds actin indirectly via filamin A and another, yet unknown, linker. Caveolin-1 regulates focal adhesions, stress fibers and acto-myosin contraction via Rho GTP loading through regulation of the localization of p190RhoGAP, an endogenous inhibitor of Rho. Cells devoid of caveolin-1 lose their intrinsic sense of directional cell migration and show a distorted actin cytoskeleton. Tyrosine phosphorylated caveolin-1 promotes actin cytoskeleton stabilization, focal adhesion tension, and tumor cell migration.

What is the evidence for the flattening and membrane buffering capabilities of caveolae?

Cells exposed to stimuli that increase plasma membrane tension, such as osmotic swelling, cell stretching, and excessive actin polymerization on stress fibers, cause a reduction of caveolae, which is a consequence of their flattening. This flattening reduces cortical tension, which acts as a buffer system to prevent plasma membrane rupture upon increased tension. This function could explain the abundance of caveolae in mechanically challenged tissues, such as muscle, the endothelia and adipose tissue. This plasticity has been observed in a variety of cell lines and at the organismal level in mice and zebrafish. By contrast, a reduction in tension at the plasma membrane favours the clustering of caveolae and, eventually, can give rise to internalization of caveolae.

What are the major functions of caveolae and how do these relate to the abundance of caveolae in different cell types?

In cells with abundant caveolae, such as skeletal muscle, adipocytes and endothelial cells, caveolae mediate mechanoprotection and control of lipid homeostasis. These roles are intimately coupled to the properties and components of the caveolae and further transduced via signalling and transcriptional regulation. For example, caveolar disassembly is associated with changes in caveolin phosphorylation and release of cavin and EHD2 proteins from the plasma membrane; EHD2 translocates to the nucleus, where it can regulate gene transcription. Caveolae regulate fatty acid transport, lipid metabolism and, in the case of adipocytes, lipid storage. Consistent with this, caveolin or cavin dysfunction can lead to lipoatrophy in which adipocyte lipid stores are depleted. In endothelial cells, caveolae have been linked to transcellular transport and are crucial regulators of nitric oxide synthase (eNOS/NOS3).

What diseases are associated with caveolar dysfunction?

Patients lacking caveolin-1 show a severe lipodystrophy while mutations in caveolin-1 are associated with pulmonary arterial hypertension. Mutations in muscle-specific caveolin-3 cause a number of muscle diseases including Limb Girdle Muscular Dystrophies and Rippling Muscle disease. Loss of cavin-1 is associated with lipodystrophy and muscle disease whereas mutations in muscle-specific cavin-4 are associated with cardiomyopathies. In addition to these specific effects of loss or mutations of key caveolar proteins, there are numerous studies linking caveolins and cavins to cancer. This literature is currently difficult to summarise because striking examples exist of caveolar proteins linked to tumor promoting or tumor suppressive roles depending on the tumor type. Caveolin-1 expression in the tumor stroma also modifies tumor cell growth and invasion capabilities. Undoubtedly, the role of caveola proteins in cancer is highly complex and it is likely that caveolae will have tumor type-specific roles at multiple stages of cancer progression.

What is the evolutionary conservation of caveolae and caveolar components?

Caveolae are well-characterized in vertebrate cells, where they show the classical bulb-shaped architecture and are generated by caveolins and cavins. Cavins are exclusively found in vertebrates but caveolins are expressed in some invertebrates. These include *Caenorhabditis elegans* and the honey bee *Apis mellifera* but not *Drosophila melanogaster* or yeast.

If caveolae are so abundant why does a loss of caveolae not cause embryonic lethality? Is there compensation?

Mice lacking caveolin-1 or cavin-1 can survive to adulthood but fertility and lifespan are reduced and they are more prone to develop tumors and other conditions. These mice lack caveolae in either a tissue-specific manner (caveolin-1 or caveolin-3) or in all tissues (cavin-1). Undoubtedly, compensatory mechanisms are involved in coping without this abundant surface feature but

interestingly, this occurs without formation of recognisable pits with similar morphology. The consequence of caveolar dysfunction can be late onset human diseases, such as muscle disease conditions associated with caveolin-3 mutations. Loss of caveolae can have prominent effects in specific tissues as shown by the lipodystrophy associated with loss of caveolin-1 or cavin-1, or only revealed in response to specific stresses, such as defective liver regeneration after damage. Caveolar dysfunction has been also linked to age-related diseases with caveolin-1 being an important regulator of cellular senescence and caveolin-1 loss linked to neurodegeneration.

Outlook

The number of disease conditions associated with dysfunction of caveolae emphasizes the need to understand this enigmatic structure. This article hopefully represents the current view of the majority of researchers in the field, but clearly the consensus view can evolve as the scientific process runs its course. We hope that this article will stimulate further work to test these ideas, particularly to test some of the key unresolved questions (Box 1).
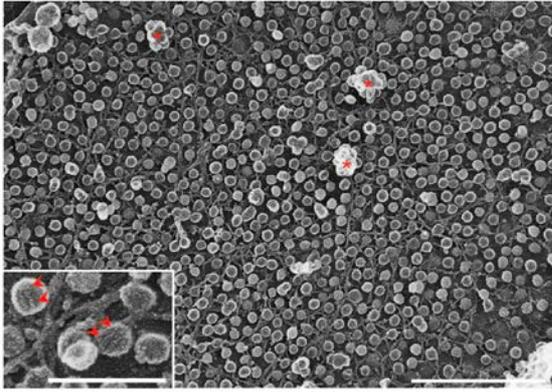
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• What is the relationship between caveolae and exosomes/extracellular vesicles?

Box 1 Key questions

• What is the structural architecture of the caveolar complex formed by caveolins, cavins, and membrane lipids?

• What happens to caveolar components upon caveolar disassembly?

• What is the structural state of these components after release/during reassembly?

• How does caveola disassembly/reassembly regulate or impact cellular responses?

• What are the dynamics of caveolae (including caveolar formation, trafficking and flattening) in different tissues *in vivo*?

• What is the role of non-caveolar caveolin and what fraction of total cellular caveolin exists outside caveolae?

• What is the function of lipid droplet-associated caveolin?

• What lipid species are required for caveola formation/function and how does caveolar disassembly impact lipids?

• How is fission of caveolae from the plasma membrane achieved?

• Are there subpopulations of caveolae that differ in composition and dynamics?

• What is the role of caveolin Tyr-phosphorylation *in vivo*?

• What is the role of the "caveolin scaffolding domain" *in vivo*?

• What is the relationship with other endocytic pathways, particularly the CLIC/GEEC pathway, in both trafficking and mechanoadaption?

• How is the expression of caveolar components and the dynamics of caveolae regulated in disease?

• What role do caveolae play in integrin endocytosis and recycling?

Figure 1
Platinum-replica EM of the plasma membrane of cultured myotubes showing abundant caveolae; both single caveolae and caveolar clusters or rosettes (asterisks) are evident. The characteristic striped coats of caveolae can be observed at higher magnification (inset; arrowheads). Bars, main figure 1μm; inset 0.25μm.