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REVIEW

Non-caveolar caveolins – duties outside the caves

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ABSTRACT

Caveolae are invaginations of the plasma membrane that are remarkably abundant in adipocytes, endothelial cells and muscle. Caveolae provide cells with resources for mechanoprotection, can undergo fission from the plasma membrane and can regulate a variety of signaling pathways. Caveolins are fundamental components of caveolae, but many cells, such as hepatocytes and many neurons, express caveolins without forming distinguishable caveolae. Thus, the function of caveolins goes beyond their roles as caveolar components. The membrane-organizing and -sculpting capacities of caveolins, in combination with their complex intracellular trafficking, might contribute to these additional roles. Furthermore, non-caveolar caveolins can potentially interact with proteins normally excluded from caveolae. Here, we revisit the non-canonical roles of caveolins in a variety of cellular contexts including liver, brain, lymphocytes, cilia and cancer cells, as well as consider insights from invertebrate systems. Non-caveolar caveolins can determine the intracellular fluxes of active lipids, including cholesterol and sphingolipids. Accordingly, caveolins directly or remotely control a plethora of lipid-dependent processes such as the endocytosis of specific cargoes, sorting and transport in endocytic compartments, or different signaling pathways. Indeed, loss-of-function of non-caveolar caveolins might contribute to the common phenotypes and pathologies of caveolin-deficient cells and animals.

KEY WORDS: Caveolae, Caveolin, Cholesterol, Membrane nanodomain

Introduction

Caveolae are a striking feature of the plasma membrane (PM) of many vertebrate cell types (Parton, 2018). Caveolae have been linked to endocytosis and transcytosis, lipid regulation, mechanoprotection, and with a variety of signaling and enzymatic pathways (Cheng and Nichols, 2016). Caveolin-1 (CAV1) in most cells and CAV3 in striated muscle cells are fundamental caveolar components. Caveolins are, in many aspects, atypical proteins: they are embedded in the membrane in a distinctive hairpin form (Dupree et al., 1993); they contain several domains with a plethora of protein- and lipid-interacting and -ordering capacities (Wanaski et al., 2003); and they shape bilayers in a unique way that initiates, for example, caveola formation (Walser et al., 2012) (Fig. 1A,B).

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In striking contrast to clathrin-coated pits, caveola biogenesis begins in the endoplasmic reticulum (ER) (Monier et al., 1995) (Fig. 2). Assembling a caveola requires progressive caveolin oligomerization, and gradual binding and ordering of different lipid species within the ER, Golgi and PM bilayers (Hayer et al., 2010a). Lipids such as cholesterol, glycosphingolipids, phosphatidylserine (PtdSer) and phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] have been suggested to be enriched in caveolae, when compared with the bulk of the PM, and/or be required for caveola formation (Ortegren et al., 2004; Fairn et al., 2011; Fujita et al., 2009; Pike and Casey, 1996). After arriving at the PM, proteins such as cavins (Hill et al., 2008; Liu et al., 2008), pacsin 2 (Hansen et al., 2011; Senju et al., 2011) and those of the EHD family (Morén et al., 2012; Stoeber et al., 2012) complete caveola formation and provide to the lipid–protein assemblies a state of 'metastability' (Kovtun et al., 2015). In other words, although relatively stable, caveolae can efficiently bud as endocytic vesicles, reducing the amount of caveolar components on the PM (Boucrot et al., 2011) or can rapidly flatten into the PM in response to mechanical stresses, releasing caveolar components into the PM (Sinha et al., 2011).

Nevertheless, organisms including *Caenorhabditis elegans* and specific mammalian cells, such as hepatocytes, neurons, lymphocytes and some tumor cells, seem to express caveolins without forming distinguishable caveolae, suggesting that there are caveola-independent functions of caveolins. These roles have been appreciated for some time, although the relevance and mechanisms remain poorly understood (Head and Insel, 2007). The complex nature of caveolin intracellular trafficking (Fig. 2), in combination with their unique membrane-organizing capacities, might contribute to the functions of caveolins in these compartments. Here, by highlighting some selected publications, we revisit the non-canonical caveolin roles in a variety of processes, including cilia compartmentalization, metabolism, neuron pruning and migration, cancer progression and the assembly of immune synapses (Box 1). We also speculate that these functions can be operative in cells with abundant caveolae, when those caveolae are disassembled and caveolins are released into the bulk PM or are transported into endosomes. In addition, these aspects of caveolin function should be considered in experiments in which caveolins are overexpressed, a condition in which caveolins can flood the PM and uptake occurs through multiple pathways (Box 2). Finally, a working model of the role of non-caveolar caveolins will be proposed (Box 3).

Caveolins outside caveolae – initial considerations

How does caveolin organize outside caveolae?

Caveolins oligomerize immediately after synthesis in the ER (Monier et al., 1995). Accordingly, oligomers seem to be an energetically favorable state for caveolins in any membrane compartment. The caveolin oligomers detected in the ER are formed by ∼15 proteins and partition as 8S complexes in sucrose gradients (Hayer et al., 2010a). In the Golgi complex, assisted by

Journal of Cell Science (2020) 133, jcs241562. doi:10.1242/jcs.241562

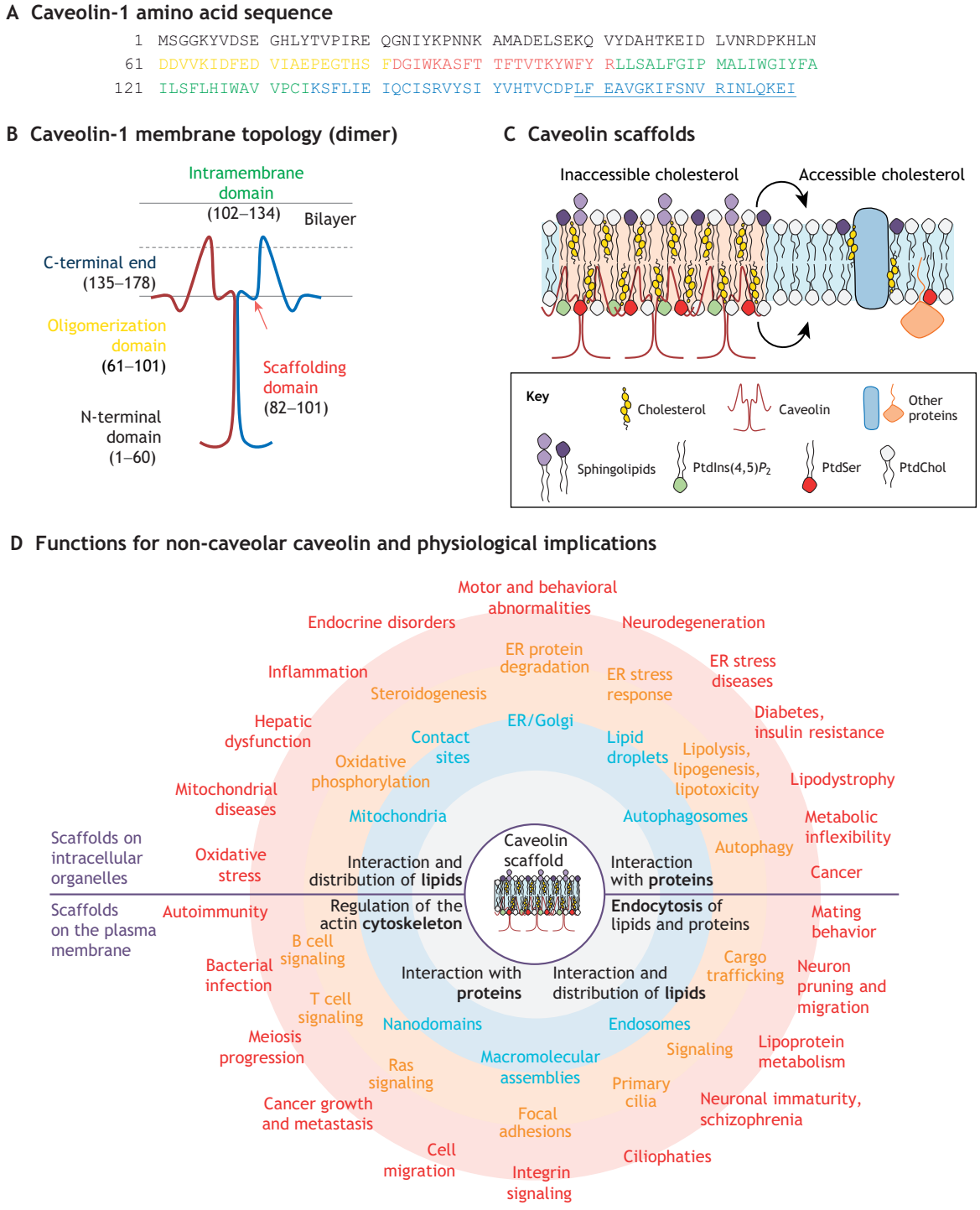

Fig. 1. See next page for legend.

cholesterol, caveolins organize into 70S complexes, containing ∼150 CAV1 molecules. These complexes have been suggested to be transported in caveolar carriers to the PM where cavin-1 is recruited and caveolae form (Tagawa et al., 2005; Hayer et al., 2010a). In apparent contrast to this model, super-resolution microscopic analysis of prostate cancer PC3 cells, which express CAV1, but not cavin-1 (Hill et al., 2008), shows that CAV1 can be delivered to the PM as smaller complexes of between approximately five and eight molecules (Khater et al., 2019). These scaffolds, termed S1A (and could be similar to the 8S CAV1 oligomers), can dimerize to form S2 scaffolds and then further co-assemble into curved domains, all in the absence of cavin-1 (Khater et al., 2019). These studies suggest that in the absence of cavin-1 and morphological caveolae, caveolins exist at the PM as oligomeric structures (Fig. 1C). In contrast to caveolins that are immobilized within caveolae, these oligomers can diffuse rapidly, become internalized relatively quickly through non-caveolar pathways, and are then degraded (Hill et al., 2008). Whether S1A or other caveolin scaffolds form and function in other cell types or in organelles, such as the ER, Golgi, lipid droplets (LDs) or endosomes (Fig. 2), has yet to be determined.

Fig. 1. Caveolin and caveolin scaffolds. (A) Amino acid sequence of human caveolin-1 (CAV1). Different regions of the protein are indicated by a color code: black, the N-terminal region; orange, the oligomerization domain [including the caveolin scaffolding domain (CSD) in maroon]; green, the intramembrane section; and blue, the C-terminal region. (B) Simplified representation of CAV1 membrane topology (oligomer of two caveolins). Caveolins oligomerize through the oligomerization domain and could interact with adjacent dimers via the C-terminal domain (Schlegel and Lisanti, 2000). Caveolins are embedded in the membrane in a distinctive hairpin form (Monier et al., 1995). The bilayer is indicated with gray lines. The putative cholesterol-binding domain of the protein (CRAC motif, 94–101) (Epand et al., 2005) is found within the caveolin CSD (maroon). Notice that, as indicated by the red arrow, the CSD may be partially embedded in the membrane (Parton, 2018). The last 20 amino acids of caveolin at the C-terminal end (underlined residues in A) are sufficient to mediate the transport of caveolin into lipid droplets (LDs) and are therefore likely placed in the proximity of the bilayer (Kassan et al., 2013; Ingelmo-Torres et al., 2009). (C) Hypothetical representation and function of caveolin scaffolds. Caveolins oligomerize immediately after synthesis in the ER (Monier et al., 1995). Accordingly, oligomers seem to be an energetically favorable state for caveolins in any membrane compartment. The caveolin oligomers detected in the ER are formed by ∼15 proteins (Hayer et al., 2010a). Super-resolution microscopic analysis of prostate cancer PC3 cells suggest that, in the absence of cavins, CAV1 organizes on the PM as smaller complexes of just 5–8 molecules (Khater et al., 2019). Because CAV1 has the capacity to generate domains enriched in both sphingomyelin and cholesterol, it is possible to speculate that caveolin scaffolds could determine the balance between inaccessible and accessible cholesterol at the plasma membrane (Box 3). Signals to trigger the conversion of inaccessible cholesterol (sequestered within caveolin-enriched domains) into accessible cholesterol (freely diffusing) must be in place. Distributions of other lipids such as phosphatidylserine (PtdSer) could be identically regulated (Ariotti et al., 2014). PtdChol, phosphatidylcholine. (D) An illustration of the complexity of functions, mechanisms of action, targeted organelles and physiological implications of non-caveolar caveolins. The color code indicates the different levels of action and influence of these caveolins. The primary function of non-caveolar caveolins is indicated by the gray circle (black text). The proposed roles for non-caveolar caveolins on the plasma membrane are depicted in the bottom half of the diagram, and the role of caveolins described in intracellular organelles is detailed in the upper half. The blue circle includes those organelles or organelle domains directly affected by non-caveolar caveolins. The biological processes occurring in these organelles and affected by non-caveolar caveolins is highlighted in orange. Finally, the red circle includes disease conditions connected to the lack of function of non-caveolar caveolins. Notice that all these processes are likely mechanistically connected, and that one process occurring in each individual circle is likely to affect different processes in the next circle.

How does caveolin mediate the formation of ordered lipid domains?

CAV1 directly binds to lipids such as cholesterol and fatty acids (Trigatti et al., 1999; Hulce et al., 2013). The putative cholesterol-binding domain might involve a CRAC motif ($V^{94}TKYWFYR^{101}$) situated in the second half of the caveolin scaffolding domain (CSD) (Epand et al., 2005) (Fig. 1). The CAV1 CRAC motif not only binds cholesterol but, in liposome experiments, changes the solubility limit of the membrane, induces formation of cholesterol-enriched domains and causes depletion of cholesterol from other domains (Wanaski et al., 2003). Other caveolin domains may cooperate to enhance CRAC-mediated domain formation, including the rest of the CSD (Epand et al., 2005), the hydrophobic hairpin domain (Yang et al., 2014) and the C-terminal caveolin region (Krishna and Sengupta, 2019). These caveolin domains bind membranes by themselves when they are independently expressed (Schlegel and Lisanti, 2000; Luetterforst et al., 1999; Woodman et al., 2002). In addition, the CSD promotes the *in vitro* formation of domains enriched in PtdSer and PtdIns(4,5)P_2, abundant caveolar components (Wanaski et al., 2003). The CSD also reorganizes membranes *in vivo*; for example, expression of the CSD promotes focal adhesion traction but inhibits clathrin-independent endocytosis and bacterial invasion (Lim et al., 2017; Hoffmann et al., 2010; Chaudhary et al., 2014; Meng et al., 2017). These studies demonstrate the potential for CAV1 oligomers to dramatically remodel the lipid environment of membranes and the capacity of caveolins to generate lipid-ordered domains (Fig. 1C).

Are non-caveolar caveolins lipid sensors, transporters and organizers?

CAV1 expression is sensitive to lipids being downregulated by chronic cholesterol depletion induced by incubating MDCK cells in the cholesterol-lowering medication simvastatin (Hailstones et al., 1998), but is upregulated when fibroblasts are incubated with low-density lipoprotein (LDL) cholesterol (Bist et al., 1997). The *CAV1* gene seems to be under the control of two sterol regulatory element-like sequences, being activated by cholesterol and inhibited by sterol regulatory-element binding proteins (SREBPs) (Bist et al., 1997). CAV1 is also remotely upregulated at the transcriptional level by other lipids such as the ganglioside GM3 (Prinetti et al., 2010). Functionally, CAV1 regulates the intracellular distribution of cholesterol, and the lack of CAV1 causes an intracellular cholesterol imbalance (Fielding and Fielding, 2000). Conversely, the intracellular distribution of CAV1 is regulated by cholesterol; caveolin trafficking into the PM is more rapid after cholesterol loading and slower when cells are depleted of cholesterol or glycosphingolipids (Pol et al., 2005; Cheng et al., 2006). Caveolins could be also involved in the transport of cholesterol and fatty acids from the PM into LDs (Le Lay et al., 2006; Pol et al., 2005). Thus, CAV1 deficiency results in altered cellular lipid composition and, for example, altered distribution of PtdSer in the PM (Ariotti et al., 2014) or cholesterol in the ER (Bosch et al., 2011a).

The complex role of caveolins as intracellular cholesterol distributors is illustrated when caveolin mutants are expressed in cells. The H-Ras-mediated Raf-1 activation that occurs on the PM is highly sensitive to cholesterol levels, and is thus a good sensor of alterations in cholesterol distributions. The $CAV3^{DGV}$ mutant (truncation of 53 amino acids) lacks the oligomerization domain and irreversibly accumulates in the ER and LDs (Roy et al., 1999). Intriguingly, $CAV3^{DGV}$ localized in LDs inhibits the H-Ras-mediated activation of Raf-1 occurring on the PM. The inhibition is completely reversed by cholesterol addition and mimicked by cholesterol depletion. Indeed, $CAV3^{DGV}$ reduces the influx of cholesterol into the PM and also promotes accumulation of cholesterol in late endosomes (Pol et al., 2001). In contrast to what is seen with $CAV3^{DGV}$, the $CAV3^{C71W}$ mutant (a naturally occurring dystrophy-associated mutation or polymorphism) reaches the PM and generates caveolae in a similar fashion to wild-type caveolins. However, $CAV3^{C71W}$ also inhibits H-Ras, and this is rescued simply by cholesterol addition (Carozzi et al., 2002). Therefore, by mediating lipid fluxes, caveolins can remotely regulate processes occurring in virtually all the cellular membranes.

In summary, outside caveolae, caveolins are likely organized as oligomers or scaffolds that potentially generate lipid-ordered domains, with the capacity to transport and distribute these lipids to different cellular destinations. However, fundamental questions of this hypothesis remain to be experimentally corroborated. Do caveolin scaffolds generate lipid domains in complex bilayers? Are these caveolin-lipid domains dynamic assemblies? If so, what are the signals that trigger the lipid exchange with other domains? Is caveolin within scaffolds able to interact with proteins excluded from caveolae? And, do caveolin scaffolds form and function on other organelles such as endosomes or LDs? To at least partially

Journal of Cell Science (2020) 133, jcs241562. doi:10.1242/jcs.241562

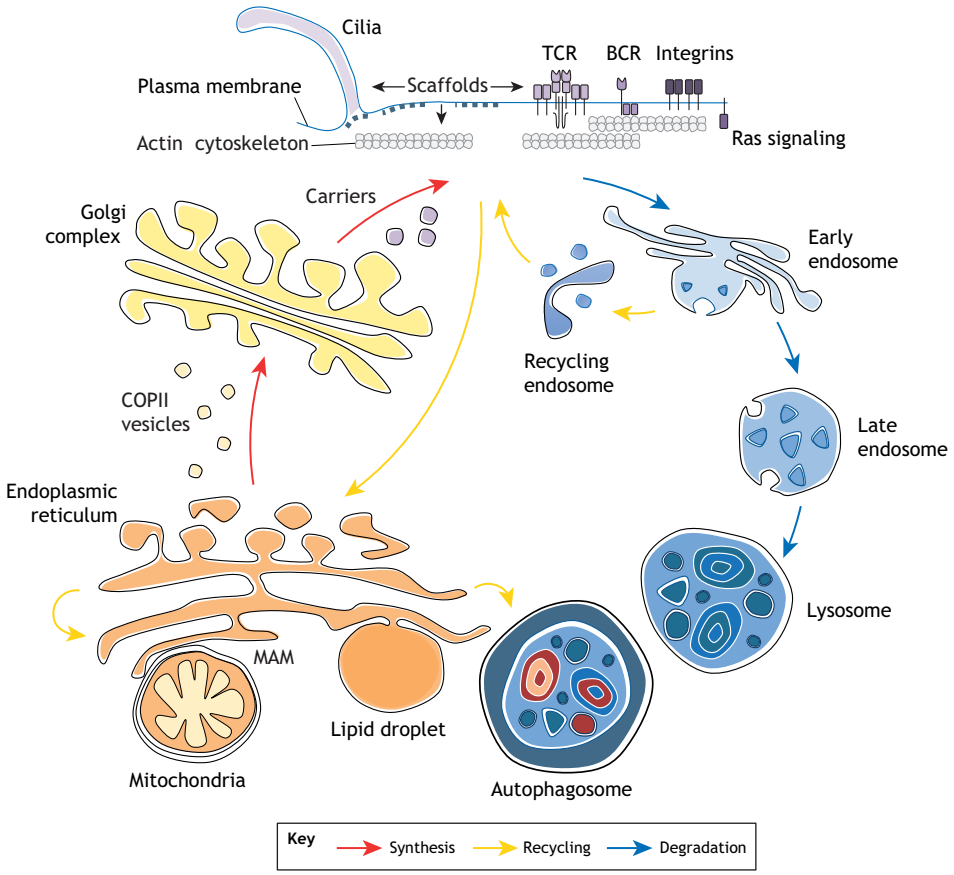

Fig. 2. Non-caveolar caveolins and intracellular traffic. Caveolins have complex intracellular trafficking. They have been observed in many organelles, and could be involved in the crucial transport of lipids occurring between these compartments. Caveolin is synthesized in the endoplasmic reticulum (ER) and rapidly assembles into low molecular mass oligomers (Hayer et al., 2010a; Monier et al., 1995). These oligomers are transported into the Golgi complex by coat protein complex II (COPII) vesicles (Hayer et al., 2010a). In the Golgi complex, caveolins bind cholesterol, forming high molecular mass oligomers (Hayer et al., 2010a; Tagawa et al., 2005). These complexes have been suggested to be transported in caveolar carriers containing ∼150 CAV1 molecules to the PM where cavin-1 is recruited and caveolae form (Tagawa et al., 2005; Hayer et al., 2010a). Other studies suggest that CAV1 can be also delivered to the PM as smaller complexes of just 14 or 15 molecules (Khater et al., 2019). In contrast to caveolins immobilized within caveolae, studies in PC3 cells demonstrate that caveolin scaffolds diffuse rapidly, being internalized through diverse non-caveolar pathways into early endosomes (Hill et al., 2008; Moon et al., 2014). Endocytosis of caveolins is active in other systems with low cavin levels, such as in neurons and *C. elegans*, regulating trafficking of specific lipids and proteins (Scheidel et al., 2018; Shikanai et al., 2018). On endosomes, caveolin could organize signaling platforms (Jung et al., 2018). Endolysosomal sorting of CAV1 and subsequent degradation in lysosomes depends on ubiquitylation (Hayer et al., 2010b; Kirchner et al., 2013; Ritz et al., 2011). Caveolins are also found in recycling endosomes, for example, when ubiquitination is inhibited (Gagescu et al., 2000; Lapierre et al., 2012; Pol et al., 1999). Recycling of caveolins from endosomes back to the PM has been described in the oocytes of *C. elegans* (Sato et al., 2014). In addition, caveolins traffic from the PM into the ER, for example, to lipid droplets (LDs) after lipid loading (Le Lay et al., 2006; Pol et al., 2005). Indeed, caveolins are LD-resident proteins (Bersuker et al., 2018; Turr; et al., 2006) but are also functioning in other ER domains such as mitochondrial-associated membranes (MAMs) (Bosch et al., 2011b; Bravo-Sagua et al., 2019; Sala-Vila et al., 2016). Caveolins have been observed in autophagosomes, but the origin of this pool of protein is unclear (Luo et al., 2017). The caveolin scaffolds on the PM could regulate different processes. Caveolins are involved in the activation of T cell antigen receptor (TCR) and B cell antigen receptor (BCR) by supplying cholesterol to the synapse or by reorganizing the actin cytoskeleton under the PM (Minguet et al., 2017; Schonle et al., 2016; Tomassian et al., 2011). Caveolins also regulate signaling pathways such as Ras by supplying lipids such as cholesterol and phosphatidylserine (PtdSer) (Ariotti et al., 2014; Scheel et al., 1999). Finally, caveolins could be also regulating different processes occurring at the base of cilia, such as the organization of the actin cytoskeleton, endosomal trafficking, or the cholesterol-dependent formation of microdomains in the ciliary transition zone (Rangel et al., 2019; Scheidel et al., 2018; Schou et al., 2017). Defects in all these non-caveolar pools of caveolins promote the cellular and systemic deficiencies detailed in Tables 1 and 2. Red arrows indicate the synthetic transport of caveolins; blue arrows indicate the degradation pathways; and orange arrows indicate other routes followed by caveolins. Purple regions of the plasma membrane indicate the signaling pathways regulated by caveolins and mediated by TCR, BCR, integrins and Ras.

address some of these questions, we will revisit the roles of caveolin in systems that lack caveolae; that is, when caveolins organize in scaffolds. Some common features emerge recurrently from these studies (Fig. 1D). First, outside caveolae, caveolins determine the fate of intracellular cholesterol with respect to different trafficking, signaling and metabolic pathways. Second, caveolins sustain membrane nanodomains in different membranes to remotely regulate protein–protein and protein–lipid interactions and signaling. Third, caveolins participate in endocytosis and the subsequent endocytic transport of specific proteins and lipids, and, finally, caveolins control the actin cytoskeleton organization, at least beneath the PM (Box 1). All of these functions are likely to be mechanistically interconnected and could explain the plethora of apparently disconnected phenotypes described in caveolin-deficient cells and animals (Fig. 1D). We also speculate that these functions can be operative in cells with abundant caveolae, when those caveolae are disassembled and caveolins are released into the bulk PM.

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Box 1. Non-caveolar caveolins

- Caveolins work together with cavins, other proteins, and lipids to generate caveolae – 'metastable' assemblies that efficiently flatten or bud in response to specific stimuli.
- Caveolins have important non-caveolar roles in liver, neurons and lymphocytes; absence of caveolin in these tissues promotes diseases.
- Non-caveolar caveolins organize in low-molecular-weight oligomers or 'scaffolds' – dynamic assemblies that interact with proteins and organize and/or transport lipids, such as cholesterol.
- Non-caveolar caveolin 'scaffolds' also exist in cells with endogenous caveolae when the caveola flattens.
- Non-caveolar caveolin roles occur at the PM and likely in other intracellular locations, such as endosomes.
- Caveolins regulate intracellular lipid distributions both directly and remotely.
- Non-caveolar roles of caveolins may be evolutionarily conserved; invertebrate species with caveolins lack cavin proteins.

Roles for non-caveolar caveolins

Liver

The liver expresses detectable CAV1 levels (Pol et al., 1998) and, although some curvature has been described in the sinusoidal PM, hepatocytes do not form abundant caveolae (Calvo et al., 2001). Cavin-1 is not detectable with antibodies in liver homogenates (Hansen et al., 2013; Bastiani et al., 2009). Intriguingly, strong CAV1 re-expression is observed during the de-differentiation process that occurs in cultured primary hepatocytes (Meyer et al., 2013), followed by the formation of caveolae (Calvo et al., 2001). The liver of *Cav1*$^{-/-}$ mice appears normal, at least in young individuals. However, these animals are more vulnerable to experimental hepatic insults that trigger non-alcoholic fatty liver disease (NAFLD) (Li et al., 2017) and non-alcoholic steatohepatitis (NASH) (Bosch et al., 2011b), the most common human hepatic dysfunctions (Tables 1 and 2). In addition, the liver of *Cav1*$^{-/-}$ mice is unable to regenerate after partial hepatectomy unless the animal is supplemented with glucose (Fernández et al., 2006), suggesting metabolic inflexibility – the inability to switch from glycolytic to lipid oxidative metabolism, for example, during fasting (Bosch et al., 2020). This is probably a consequence of mitochondrial dysfunction and impaired lipid metabolism, and thus, a high dependence on glucose metabolism (Fernández-Rojo et al., 2013). In addition, the *Cav1*$^{-/-}$ liver produces low levels of bile acids, ketone bodies and very-low-density lipoprotein (VLDL) (Fernández-Rojo et al., 2013; Frank et al., 2008). This likely has important systemic consequences, as bile acids and ketone bodies are crucial metabolic and signaling mediators, and VLDL distributes cholesterol from the liver to the rest of tissues.

Box 2. Practical aspects for the study of caveolins

- Overexpressed caveolin levels must be tightly controlled to avoid excess levels of non-caveolar caveolins (Parton and Howes, 2010; Hayer et al., 2010b).
- Fluorescent protein tagging of caveolins at the C- or N-termini have both been reported to perturb cellular processes (Pelkmans et al., 2001; Hanson et al., 2013; Han et al., 2015).
- Depending on experimental conditions and cell type, caveolins cannot be assumed to be a marker of caveolae, and can associate with diverse cellular compartments.
- Recognition of endogenous caveolins by antibodies is specific to their cellular location (Pol et al., 2005; Luetterforst et al., 1999; Bush et al., 2006; Dupree et al., 1993; Hayer et al., 2010a).

Box 3. Caveolin and the inaccessible/accessible cholesterol equilibrium – a working model

- The existence of different PM pools of cholesterol with different biological properties is increasingly evident. Sphingomyelin sequesters cholesterol in complexes and decreases cholesterol availability for other PM domains. For example, at the ciliary membrane cholesterol availability determines Hedgehog signaling. Experimental depletion of sphingomyelin increases the PM pool of accessible cholesterol to amplify the Hedgehog pathway (Kinnebrew et al., 2019).
- Free cholesterol regulates PM fluidity, integrity and signaling. Accumulation of free cholesterol in bilayers has many deleterious effects (Bosch et al., 2011a; Bosch et al., 2011b). The equilibrium between both pools must be tightly regulated.
- CAV1 has the capacity to generate domains enriched in sphingomyelin and cholesterol (Wanaski et al., 2003). Thus, scaffolds could regulate the balance between inaccessible and accessible cholesterol (Fig. 1C). Other lipids such as phosphatidylserine could be similarly regulated (Ariotti et al., 2014).
- Signals to trigger conversion of inaccessible cholesterol (sequestered within caveolin-enriched domains) into accessible cholesterol (freely diffusing) must be in place. Phosphorylation of CAV1 could be one of the signals (Fielding et al., 2004; Minguet et al., 2017; Meng et al., 2017; del Pozo et al., 2005).
- These mechanisms would explain how caveolin remotely regulates the formation of cholesterol-enriched immune synapses in B and T cells, or why LD-resident caveolin mutants inhibit processes occurring on the PM and endosomes (Pol et al., 2001).
- Scaffolds are highly mobile entities (Hill et al., 2008) able to regulate the cytoskeleton (Meng et al., 2017) and endocytosis of PM cholesterol-enriched domains (Gaus et al., 2006; del Pozo et al., 2005).
- In this context, caveolae could be defined as 'large metastable pools of inaccessible cholesterol at the PM'. This cholesterol pool could be downregulated by caveolar endocytosis (Boucrot et al., 2011) or rapidly converted into accessible cholesterol when caveolae flatten into the PM (Sinha et al., 2011).
- CAV1 could, in addition, be mediating cholesterol transport between organelles. For example, after synthesis, CAV1 rapidly organizes into scaffolds and could generate blocks of inaccessible cholesterol to mediate its transport from the sites of synthesis (ER) to the sites of use (PM or endosomes). This transport could explain why, in the absence of caveolins, cholesterol accumulates in different intracellular membranes such as the ER, MAMs and late endosomes (Sala-Vila et al., 2016; Bosch et al., 2011b; Pol et al., 2001).
- In conclusion, caveolins may have evolved to be lipid sensors, organizers, transporters and suppliers – as illustrated by studies in *C. elegans* (Scheel et al., 1999) – with their role in caveola formation a later adaptation concomitant with the evolution of the cavin proteins.
- Cavin-1 and EHD2 also regulate lipolysis and thus, the pool of intracellular lipids that can potentially follow these pathways (Ding et al., 2014; Moren et al., 2019).

Owing to the low levels of hepatic CAV1, the relative distribution of intracellular caveolins in liver has been difficult to define but has been studied using purified organelles. When the relative distribution of CAV1 is compared between different populations of purified rat liver endosomes, CAV1 is found to be enriched in endosomal recycling compartments (Pol et al., 1999). In mouse liver, CAV1 is enriched in Golgi preparations, and is also present in the ER and LDs (Bosch et al., 2011b; Pol et al., 2004). Within the ER, CAV1 is enriched in mitochondrial-associated membranes (MAMs) (Sala-Vila et al., 2016), which, similar to caveolae, are enriched in cholesterol, glycosphingolipids and PtdSer (Kannan et al., 2017). CAV1 is also found in MAMs purified from brain and HeLa cells (Bravo-Sagua et al., 2019; Sano et al., 2009). Caveolins determine the stability and composition of hepatic MAMs (Sala-Vila et al., 2016), and it

Journal of Cell Science (2020) 133, jcs241562. doi:10.1242/jcs.241562

Table 1. Roles for non-caveolar caveolins

Cell type	Organelles	Molecular processes	Defects in absence of CAV1	Reference
Hepatocytes	Golgi and ER	Regulation of intracellular trafficking of cholesterol	Accumulation of cholesterol in the ER	Bosch et al., 2011a,b
	MAMs	Regulation of MAM composition and function	Accumulation of cholesterol and impaired MAM formation	Sala-Vila et al., 2016
		Regulation of PKA-mediated signaling in MAM	Decreased ER stress response	Bravo-Sagua et al., 2019*
	LDs	LD biogenesis	Impaired LD formation and lipid oxidation	Fernandez et al., 2006
	Mitochondria	Control of mitochondrial membrane cholesterol content	Defective oxidative phosphorylation Decreased antioxidant uptake (GSH) Accumulation of ROS Increased of mitochondrial susceptibility to apoptosis	Bosch et al., 2011a,b
Cortical and hippocampal neurons	PM and endosomes	Lipids (LacCer) and adhesion molecules (N-cadherin and L1 cam) internalization	Defects in neurite pruning Impaired neuronal migration and maturation	Shikanai et al., 2018*
	ER/Golgi	Neurotransmitter and tyrosin-kinase receptors signaling regulation	Increased susceptibility to psychomimetic compounds	Allen et al., 2011
		Cholesterol imbalance due to cooperation with mutated Htt	Reduced transport of intracellular cholesterol into the Golgi complex	Trushina et al., 2014
	Mitochondria and MAM	Avoid cholesterol accumulation	Accumulation of mitochondrial cholesterol	Bosch et al., 2011a,b
T and B cells	PM	Regulation of immunological synapse (T-APC junction) and Regulation of TCR signaling	Decrease of cholesterol and glycosphingolipid enriched nanodomains	Tomassian et al., 2011
			Uncontrolled TCR-Lck proximity (decreased TCR activation)	Schonle et al., 2016
		Regulation of BCR downstream signaling	Decreased immunoglobulin receptor organization in BCR Decreased actin organization to maintain BCR signaling	Minguet et al., 2017
Most cells	Primary cilia	Regulation of PKD-2 endocytosis	Increased PKD-2 levels	Scheidel et al., 2018**
		Regulation of the ciliary transition zone	Decreased Hedgehog signaling (Increase in Patched and decrease in Smoothened levels)	Schou et al., 2017*
		Regulation of the actin cytoskeleton	Reduced RhoA activation Impaired cytoskeleton formation Increased endosomal trafficking Elongated cilia	Rangel et al., 2019

Most of these studies were performed using different backgrounds of *Cav1*-knockout mice unless indicated with * (knock-down in cell lines) or ** (*C. elegans* model). Importantly, it is likely that these caveolin functions are also operative in cells with abundant caveolae, when those caveolae are disassembled and caveolin is released into the bulk PM.

has been suggested that they mediate the ER stress response of HeLa cells by reducing protein kinase A-mediated signaling (Bravo-Sagua et al., 2019).

The liver is central to the cholesterol metabolism of the body. Cholesterol biosynthesis, excretion into bile, secretion into blood (VLDL), uptake (LDL), esterification and storage all occur simultaneously in hepatocytes (Zhao and Dahlman-Wright, 2010). Thus, regulation of hepatic cholesterol fluxes is not only critical within hepatocytes but also for the whole animal. Primary hepatocytes purified from *Cav1*$^{-/-}$ liver show reduced cholesterol efflux into extracellular acceptors such as cyclodextrin, suggesting intracellular defects in these fluxes. In contrast, cholesterol accumulates in purified Golgi and ER in these cells (Bosch et al., 2011b). This is similar to the cholesterol imbalance observed in cells expressing caveolin mutants. Although the synthesis of cholesterol occurs in the ER, the cholesterol concentration in this organelle is very low, comprising only ∼0.5–1% of cellular cholesterol (Maxfield and Wüstner, 2002). However, regulation of the ER cholesterol levels is critical for cells because the cellular cholesterol sensors are in the ER. Thus, by regulating the levels of ER cholesterol, CAV1 could be functioning as an important cellular cholesterol sensor.

In the absence of caveolins, cholesterol accumulates in hepatic MAMs and mitochondria (Bosch et al., 2011b; Sala-Vila et al., 2016). Cholesterol likely reaches the mitochondria through MAMs (Hayashi et al., 2009). In steroidogenic cells, after synthesis in the ER or arriving from LDs, cholesterol is transported into the mitochondria and the P450 side-chain cleavage enzyme (CYP11A1) converts it into pregnenolone, the steroid precursor. Mitochondrial cholesterol availability is the rate-determining step in steroid biosynthesis (Jefcoate, 2002), so pregnenolone levels indicate the rate of mitochondrial cholesterol influx. Reduction of CAV1 levels in steroidogenic F2-CHO cells causes a significant increase in pregnenolone biosynthesis (Bosch et al., 2011b). Similarly, serum concentrations of pregnenolone, corticosterone and testosterone are significantly higher in *Cav1*$^{-/-}$ mice (Bosch et al., 2011b), confirming, at the systemic level, that CAV1 deficiency promotes a higher mitochondrial cholesterol influx. Since these hormones play key roles in carbohydrate, lipid and protein metabolisms, important systemic consequences could again be anticipated.

Mitochondrial dysfunction, characterized by defects in oxidative phosphorylation, underlies common diseases including hepatic and neurological disorders (Gorman et al., 2016). For example,

Table 2. Implications of non-caveolar caveolins in disease

Organ	Pathological conditions in the absence of Caveolin	Reference
Liver	Impaired liver regeneration	Fernandez et al., 2006
	Sensitization to NAFLD[1] and NASH[2]	[1]Li et al., 2017
		[2]Bosch et al., 2011a,b
	Impaired lipid metabolism and reduced metabolic flexibility	Fernandez-Rojo et al., 2012; 2013
	Inflammation and metabolic syndrome	Briand et al., 2011
	Reduced synthesis of bile acids, ketone bodies and VLDL	Fernandez-Rojo et al., 2013
		Frank et al., 2008
	Increased pregnenolone, testosterone and corticosterone synthesis	Bosch et al., 2011a,b
Brain (cortex and hippocampus)	10% reduction in brain weight	Trushina et al., 2006a
	Motor and behavioral abnormalities	Gioiosa et al., 2008
	Reduced synaptic plasticity, premature neuronal aging and neuron degeneration	Head et al., 2010
		Kassan et al., 2017
	Rare structural variant of schizophrenia in humans	Walsh et al., 2008*
	Increased susceptibility to Huntington's and Alzheimer's disease	Bosch et al., 2011a,b
Immune system	Increased mortality during polymicrobial sepsis	Feng et al., 2010
	Susceptibility to some bacterial, viral and parasitic infections	Medina et al., 2006a,b and 2007
		Tomassian et al., 2011
	Autoimmunity (large spleens, spontaneous B cell activation, elevated autoantibody titers and IgG deposits in the kidneys)	Minguet et al., 2017
Primary cilia (most non-dividing cells)	Ciliopathies: Inefficient male mating behavior in *C. elegans*	Scheidel et al., 2018**

Most of these studies were performed using different backgrounds of *Cav1*-knockout mice unless indicated with * (knockdown in cell lines) or ** (*C. elegans* model).

mitochondrial cholesterol loading sensitizes hepatocytes to tumor necrosis factor (TNF) and Fas-mediated steatohepatitis (Marí et al., 2006). Cholesterol loading causes mitochondrial dysfunction at different and cooperative levels, such as reducing the efficiency of oxidative phosphorylation, increasing production of reactive oxygen species, and reducing membrane permeability and the uptake of antioxidant molecules (Bosch et al., 2011b). CAV1 deficiency also causes mitochondrial dysfunction in tissues that normally contain abundant caveolae, such as white adipose tissue (Asterholm et al., 2012), brown adipose tissue (Cohen et al., 2005) and cardiac muscle (Fridolfsson et al., 2012). Whether caveolins regulate mitochondrial functioning remotely (Bosch et al., 2011b; Sala-Vila et al., 2016), locally (Fridolfsson et al., 2012; Li et al., 2001) or even in both ways, needs to be clarified. In any case, a primary effect of this malfunctioning is a net boost in the production of reactive oxygen species (Asterholm et al., 2012; Bosch et al., 2011b). This could partially manifest in some long-term phenotypes described for *Cav1*$^{-/-}$ animals such as inflammation, metabolic syndrome, neurodegeneration, aging and even cell transformation (Head et al., 2010; Pavlides et al., 2010; Briand et al., 2011).

Brain

The levels of caveolins in brain homogenates are similar to those in liver (Bastiani et al., 2009; Hansen et al., 2013). CAV1 and CAV2 are mainly expressed in cortical and hippocampal neurons (Allen et al., 2007; Francesconi et al., 2009; Boulware et al., 2007; Heiman et al., 2008) but caveolins are also expressed in microvessels, astrocytes, oligodendrocytes, Schwann cells and dorsal root ganglia cells. Significant levels of CAV1 are found in striatal neurons in which no morphological caveolae are detectable by electron microscopy (Trushina et al., 2006b); as cavins are also undetectable in brain, it is therefore assumed that neurons generally do not form caveolae (Echarri and del Pozo, 2015). The brain appears normal in young *Cav1*$^{-/-}$ mice although a significant 10% reduction in brain weight has been observed in adult animals, suggesting global atrophy (Trushina et al., 2006a) (Tables 1 and 2). *Cav1*$^{-/-}$ mice display a number of motor and behavioral abnormalities (Gioiosa et al., 2008; Trushina et al., 2006a). At the cellular level, reduced synaptic plasticity, premature neuronal aging and neuron degeneration have been observed (Head et al., 2010; Kassan et al., 2017). CAV1 expression in the brain decreases with age (Head et al., 2010), and neuron-targeted CAV1 enhances signaling and promotes neuronal arborization (Head et al., 2011). In neurons, caveolins regulate the function of neurotransmitter receptors and receptor tyrosine kinases, and interact with components of the actin and tubulin cytoskeletons (Egawa et al., 2016).

Shikanai et al. have demonstrated that CAV1 is strongly expressed in immature neurons of the mouse embryonic cerebral cortex (Shikanai et al., 2018), where CAV1 distributes between the PM and endosomes. Primary cortical neurons with reduced CAV1 expression show impaired glycosphingolipid internalization. In addition, endocytosis and intracellular transport of cell adhesion molecules, such as N-cadherin (Cdh2) and neural cell adhesion molecule L1 (L1cam), are also impaired. This results in immature neurite pruning leading to process elongation, and neuronal migration and maturation. In migrating neurons, CAV1 is enriched at the tip of the leading process where it is also probably involved in endocytosis (Shieh et al., 2011). At the leading edge of moving cells, cholesterol domains regulate the actin cytoskeleton in a process controlled by Rho GTPases (Mañes and Martínez, 2004). Therefore, CAV1 determines specific steps of the early phase of neuronal maturation. Mental disorders, such as schizophrenia, can be caused by immaturity of the prefrontal cortex (Hagihara et al., 2014), and interestingly, *CAV1* gene disruption is considered to be a rare structural variant of schizophrenia (Walsh et al., 2008). Indeed, although *Cav1*$^{-/-}$ mice display no baseline behavioral disruptions, these animals show an increased sensitivity to psychotomimetic compounds (Allen et al., 2011).

Huntingtin (Htt), the protein mutated in Huntington's disease, is enriched in polarizing projection neurons, which are also important during maturation of the cerebral cortex. Similar to what is seen for CAV1, Htt mediates the trafficking of neuronal N-cadherin in a process regulated by Rab11 (Barnat et al., 2017). Neurons expressing mutated Htt exhibit an intracellular cholesterol imbalance and increased caveolin expression (del Toro et al., 2010; Trushina et al., 2006b). CAV1 and mutated Htt interact, as observed by immunoprecipitation, and reduction of CAV1 expression rescues the cholesterol phenotype in neurons expressing mutated Htt and

significantly delays the onset of motor decline in a knock-in Huntington disease mouse model (Trushina et al., 2014). Thus, it has been suggested that the deleterious effects caused by mutations in Htt are produced by interference in the CAV1-mediated cholesterol trafficking from the ER/Golgi to the PM.

Taken together, the above studies suggest that the primary effects of CAV1 loss in the brain is linked to cholesterol dysregulation. Brain cholesterol metabolism is complex, and defects in these systems seem to be at the origin of many neurodegenerative processes (Zhang and Liu, 2015). After myelination, brain cholesterol turnover is maintained at a very low level. Indeed, cholesterol half-life in the adult brain has been estimated to be between 6 months and 5 years (Bjorkhem, 2006), suggesting that defects in cholesterol transport will likely have long-term consequences. However, mitochondria purified from the brains of young *Cav1*$^{-/-}$ mice contained twice the cholesterol concentration of wild-type mitochondria (Bosch et al., 2011b). Mitochondrial cholesterol loading sensitizes *Cav1*$^{-/-}$ animals to rapid neurodegeneration when submitted to experimental models of Huntington's and Alzheimer's disease (Bosch et al., 2011b). Although determined in the presence of detergents, CAV1 seems to also be a MAM component in brain cells (Sano et al., 2009). Thus, similar mechanisms to those described in hepatocytes could be occurring in neurons to promote mitochondrial dysfunction, susceptibility to apoptosis and neurodegeneration.

Lymphocytes

As in hepatocytes and neurons, caveolins are expressed at low levels in most immune cells (Fiala and Minguet, 2018). Although caveolin expression was not initially detected in some lymphocyte cell lines (Fra et al., 1994), subsequent studies have determined that B cells and T cells express low but functional pools of caveolins without observable caveola formation. Indeed, the level of *Cav1* mRNA in B cells is 0.001% that of lung endothelial cells (Minguet et al., 2017). However, CAV1 expression in lymphocytes is regulated; B cells express more caveolin in response to lipopolysaccharide (Medina et al., 2006b), and T cells similarly express more caveolin after syngeneic and allogeneic hematopoietic cell transplantation (Schonle et al., 2016). As an indication of the systemic relevance of these caveolin pools, aged *Cav1*$^{-/-}$ mice develop features of autoimmunity such as large spleens, spontaneous B cell activation, elevated autoantibody titers, reduced lifespan and IgG deposits in the kidneys (Minguet et al., 2017). Further, after cecal ligation and puncture, a common experimental model of polymicrobial sepsis and inflammation, *Cav1*$^{-/-}$ mice show significantly increased mortality when compared with wild-type animals (67% versus 27%) (Feng et al., 2010). In addition, *Cav1*$^{-/-}$ mice are more susceptible to some bacterial, viral and parasitic infections (Medina et al., 2006a, 2007; Tomassian et al., 2011).

In lymphocytes, proper PM compartmentalization is essential for the functioning of the T cell antigen receptor (TCR) and the B cell antigen receptor (BCR). When the TCR recognizes the antigen presented by an antigen-presenting cell (APC), an immunological synapse is formed at the T cell–APC junction. Cholesterol and glycosphingolipids migrate to the synapse to form membrane nanodomains enriched in cholesterol that concentrate TCR signal transducers, exclude negative regulators, and reorganize the actin cytoskeleton. Three studies illustrate different roles for CAV1 in organizing BCRs and TCRs in basal and activated conditions (Tomassian et al., 2011; Schonle et al., 2016; Minguet et al., 2017). Tomassian et al. demonstrate that CAV1 is essential for the formation of the membrane nanodomains at the synapse and for the subsequent actin reorganization (Tomassian et al., 2011). The lack of CAV1 does not affect TCR internalization prior to or after engagement. Schonle et al. show that CAV1 determines the proximity of the TCR to Lck (a nonreceptor tyrosine kinase), facilitating TCR phosphorylation and downstream signaling (Schonle et al., 2016). Indeed, cholesterol, and probably other lipids, are essential for TCR activation and function with important implications for disease (Schamel et al., 2017). Minguet et al. demonstrate that CAV1 also regulates formation of membrane nanodomains, which have an important role in organizing immunoglobulin receptors at the PM of B cells (Minguet et al., 2017). A non-phosphorylatable CAV1 mutant was unable to mediate the nanodomain formation. In this case, the authors favor a role for CAV1 in the actin organization required to form and maintain the BCR. Taken together, these studies point towards a role for caveolins as a scaffolding agent that are independent of caveolae. The lipid-organizing properties of caveolins, the capacity to distribute these lipids and the capacity to regulate the organization of the cytoskeleton might be crucial for these roles (Fig. 1C,D).

Primary cilia

Recent publications have identified a role for non-caveolar CAV1 in cilia in both mammalian cell systems and in *C. elegans* (Rangel et al., 2019; Scheidel et al., 2018; Schou et al., 2017). Primary cilia are microtubule-based sensory organelles on the surface of most non-dividing cells that transduce signals from the environment or from other cells. Through a plethora of signaling cascades, such as the hedgehog–smoothened pathway, cilia determine correct development, sensory perception and general homeostasis. In *C. elegans*, endocytosis and intracellular trafficking of polycystin-2 (PKD-2) in ciliated neurons depends on the presence of CAV1 (Scheidel et al., 2018). CAV1 deficiency results in reduced ciliary levels of PKD-2 and inefficient male mating behavior. Interestingly, CAV1 endosomal trafficking, through the periciliary membrane compartment, strongly relies on the function of the endosome maturation factors Rabenosyn-5 (RABS-5) and VPS-45.

In mammalian cell systems, Schou et al. observe that CAV1 accumulates in the ciliary transition zone (TZ) of RPE1 cells (Schou et al., 2017). The location of CAV1 is indirectly regulated by the kinesin family member 13B (KIF13B) and nephrocystin-4 (NPHP4). The TZ is a specialized membrane domain at the base of the primary cilium, which functions as a fence to regulate a selective exchange of proteins and lipids between the cilia and the rest of the cell to maintain the cilium as a compartmentalized signaling organelle. The TZ is a membrane nanodomain, and CAV1 can be removed from the TZ by extracting cholesterol with cyclodextrin. The absence of CAV1 does not grossly perturb TZ structure or function. However, cholesterol extraction or CAV1 downregulation inhibits the sonic hedgehog (Shh)-promoted entry of smoothened into the cilia, and thus the hedgehog signaling cascade. Interestingly, the function and transport of the hedgehog receptors patched 1 (Zhang et al., 2018) and smoothened (Myers et al., 2013) strongly depend on the presence of accessible cholesterol (Kinnebrew et al., 2019).

Finally, Rangel et al. demonstrate that downregulation of CAV1 increases ciliary length in different cell types (Rangel et al., 2019). Using cysts and polarized MDCK cells, the authors show that, in the absence of CAV1, there is reduced RhoA GTPase activity in the apical PM and disorganization of the actin cytoskeleton. In addition, these cells accumulate Rab11-positive endosomes around the centrosome. Since there are negligible caveolae at the apical PM of fully polarized epithelial cells (Scheiffele et al., 1998), these

effects are likely caused by the lack of non-caveolar caveolins. Activation of RhoA and its effectors [ROCK proteins and DIA1 (also known as mDia1 formin)] restores the apical actin meshwork and the length of the cilia. The authors propose that in the absence of an organized actin meshwork at the base of the cilia, increased vesicular trafficking will promote cilia elongation. In conclusion, these publications suggest that caveolins regulate different mechanisms involved in primary cilia functioning. Although cilia defects are observed in *C. elegans*, whether they also occur in *Cav1*$^{-/-}$ mice, which appear to develop normally, is not yet clear. Whether ciliary defects can contribute to caveolinopathies is also yet unknown.

Other cellular systems showing a role for non-caveolar caveolins

Cancer cells

CAV1 has been found in some cancer cells that do not express cavin-1; for example, in cell lines of prostate adenocarcinoma such as PC-3 (Hill et al., 2008). Cavin-1 re-expression in these cells is sufficient to inhibit their anchorage-independent growth and reduces tumor growth and metastasis in mice (Moon et al., 2014). Consistent with the proposed tumorigenic role for CAV1 in the absence of cavin-1 and caveolae, CAV1 (but not cavin-1) has been shown to be upregulated in advanced prostate carcinoma samples from human patients (Moon et al., 2014). In human breast cancer cells, CAV1 deficiency promotes basal and inducible autophagy (Shi et al., 2015). Lack of CAV1 increases autophagosome–lysosome fusion, a phenotype that is not reproduced in cavin-1-deficient cells but can be mimicked by cholesterol extraction. The regulation of autophagy by non-caveolar CAV1 has been also observed in endothelial cells (Luo et al., 2017).

In addition, caveolins regulate the functioning, ordering and endocytosis of focal adhesions – highly ordered macromolecular complexes that link the extracellular matrix to the actin cytoskeleton (Gaus et al., 2006). This role is partially dependent on CAV1 phosphorylation at tyrosine 14, and mediates the internalization and clearance of cholesterol-enriched domains from the PM (del Pozo et al., 2005). The role of caveolin in regulating focal adhesions has been observed in PC3 cells (Meng et al., 2017). Interestingly, when compared with a nonphosphorylatable CAV1, the phosphomimetic CAV1 Y14D displays an enhanced CSD-mediated interaction with adapter proteins, such as vinculin, that connect integrins with the cytoskeleton (Meng et al., 2017). Expression of cavin-1 in PC3 cells reduces stabilization of focal adhesion kinases, and decreases cell motility and migration, again suggesting an active role of CAV1 scaffolds in the tumorigenic behavior of these cells (Meng et al., 2015). These studies illustrate that caveolin scaffolds regulate the endocytosis of cholesterol-enriched domains, remotely determine signaling pathways, and can potentially interact with proteins normally excluded from caveolae to, for example, organize the actin cytoskeleton. Caveolin phosphorylation could be one of the signals to trigger these functions outside caveolae.

Endosomes

Caveolins traffic to early endosomes in association with caveolae (Stoeber et al., 2012). This is a constitutive pathway, but can be modulated, for example, after glycosphingolipid loading or during the cell cycle (Shvets et al., 2015; Boucrot et al., 2011). Caveolins are also found in late endosomes en route to degradation, when assembly of caveolae is compromised (Hayer et al., 2010b). Endolysosomal sorting of CAV1 strongly depends on ubiquitylation (Hayer et al., 2010b).

However, at steady state, caveolins are also found in endocytic recycling compartments (ERCs). When compared with other endosomal populations, caveolins are found to be enriched in ERCs purified from rat liver (Pol et al., 1999). In addition, CAV1 is abundant in apical ERCs of MDCK cells (Gagescu et al., 2000) and in cortical granules of *C. elegans* (Sato et al., 2008). This is noteworthy in the context of this Review, as the apical surface of fully differentiated MDCK cells lacks caveolae but has high levels of CAV1. In *C. elegans*, caveolin sorting into ERCs increases when ubiquitylation is inhibited (Sato et al., 2014). Among endosomes, ERCs are particularly enriched in cholesterol (Maxfield and Wüstner, 2002), and the intracellular distributions of cholesterol and glycosphingolipids are regulated by Rab11-positive ERCs (Holtta-Vuori et al., 2002). Thus, a role for non-caveolar caveolins in regulating lipid fluxes through these endosomes is possible.

CAV1 colocalizes with Rab11 in MDCK cells and in oocytes of *C. elegans* (Lapierre et al., 2012; Sato et al., 2008; Gagescu et al., 2000). Furthermore, CAV1 downregulation results in the accumulation of Rab11-positive ERCs at the base of the cilia (Rangel et al., 2019). Interestingly, other caveolar lipids, such as PtdSer, are also enriched in ERCs and regulate trafficking pathways such as reverse ERC-to-Golgi transport (Uchida et al., 2011). In conclusion, a pool of non-caveolar caveolins is frequently found in endosomes. However, whether this indicates a pool of caveolins trafficking through these compartments or a functional role for caveolins within these organelles is still unclear. It is tempting to speculate that, similar to the caveolin pools functioning in exocytic compartments, these non-caveolar caveolins could be involved in the transport of specific lipids to and from endosomes. Furthermore, the possibility that phosphorylated caveolins could organize signaling platforms on early endosomes after stress stimuli has also been proposed (Jung et al., 2018).

ER and LDs

CAV1 has been implicated in crucial ER homeostatic processes, such as the response to stress and mitochondrial dynamics by regulating MAMs and the ER-associated degradation (ERAD) of proteins (Chen et al., 2013). In addition, CAV1 and CAV2 are bona fide LD-resident proteins (Bersuker et al., 2018). LDs are crucial lipid and protein storage organelles formed in the ER of eukaryotic cells (Pol et al., 2014). The presence of caveolins on LDs has been simultaneously observed by three groups. Interestingly, three different physiological explanations were proposed by each one of these studies: that the LD might represent an ‘overflow’ pathway when the caveolin concentration increases in the ER (Ostermeyer et al., 2001), that caveolins can organize membrane nanodomains on LDs (Fujimoto et al., 2001), and that caveolins on LDs could be important to regulate intracellular cholesterol distribution (Pol et al., 2001). Even today, almost two decades later, the three explanations seem to be at least partially correct.

Caveolins accumulate on LDs when export from the ER is inhibited pharmacologically with the lactone antiviral drug brefeldin A (Pol et al., 2004), or when they display naturally occurring frameshift mutations that generate a functional ER-retention signal (Copeland et al., 2017). When brefeldin A is removed, caveolins rapidly leave the LD, moving into the PM. Because accumulation of caveolins in the ER is likely toxic, as suggested by naturally occurring mutations that promote disease (Copeland et al., 2017), restricting their accumulation on LDs could prevent harmful effects.

CAV1 associates preferentially with LDs of differentiated adipocytes (Blouin et al., 2008), especially in lipolytic conditions (Brasaemle et al., 2004). In adipocytes, CAV1 on LDs interacts with protein kinase A and perilipin-1 to facilitate lipolysis (Cohen et al.,

2004). In the absence of CAV1, adipocytes display deregulated lipolysis, reduced fat mass, elevation of free fatty acids and mitochondrial dysfunction (Asterholm et al., 2012). The LDs purified from the adipose tissue of *Cav1*$^{-/-}$ mice show compositional defects, including reduced levels of caveolar proteins and lipids, such as cholesterol, PtdSer and lysophospholipids (Blouin et al., 2010; Le Lay et al., 2006). CAV1 could organize these lipids in the ER or/and transport lipids from the PM. Indeed, when adipocytes or fibroblasts are loaded with cholesterol or fatty acids, respectively, CAV1 rapidly moves from the PM into LDs (Le Lay et al., 2006; Pol et al., 2005). However, abundant contact sites between caveolae and LDs are often formed in adipocytes, mediated by EHD2, for instance, to regulate lipolysis (Morén et al., 2019); the LD, therefore, is additionally regulated through contact sites with caveolae.

Cav1$^{-/-}$ mice are lipodystrophic and resistant to diet-induced obesity, clearly reflecting a complex regulation of LDs mediated by caveolins (Pilch and Liu, 2011; Razani et al., 2002). This role is not exclusively performed by non-caveolar caveolins because cavin-1-null mice are also lipodystrophic (Rajab et al., 2010), and cavin-1 regulates lipolysis (Ding et al., 2014). Furthermore, caveolins also regulate lipolysis on LDs via an autocrine prostacyclin-stimulated pathway in endothelial cells (Kuo et al., 2018). Therefore, although caveolins regulate LD metabolism, the mechanisms are complex and imply direct and remote effects of caveolar and non-caveolar caveolins. The variety and complexity of mechanisms through which caveolins control the metabolism of LDs, the main lipid reservoirs of eukaryotic cells, again clearly illustrates the fundamental role of caveolins in regulating intracellular and systemic lipid fluxes.

Invertebrate caveolins – *C. elegans* as a paradigm

Caveolins are expressed in a number of invertebrates but no proteins with significant sequence homology to cavins have been identified (www.treefam.org). *C. elegans* has two caveolin genes (CeCAVs) with six possible isoforms; CeCAV-1b and CeCAV-2c are the closest homologs to human caveolins (~70% similarity) (www.wormbase.org). CeCAV-1 oligomerizes but does not induce formation of caveolae in mouse cells (Kirkham et al., 2008; Tang et al., 1997) or highly curved membranes in model systems (Jung et al., 2018; Walser et al., 2012). However, structures with caveolar morphology have been described (Roitenberg et al., 2018) and thus, the existence of a cavin-1-like protein operative in certain cellular contexts cannot be ruled out. In any case, the functions of CeCAVs appear to be largely independent of caveolae, in most of the cell types studied.

CeCAV-2 (the most similar to CAV1) is almost exclusively expressed in the intestine, the tissue dedicated to fat storage (Branicky et al., 2010). In these cells, CeCAV-2 distributes between the apical membrane and intracellular endocytic compartments (Parker et al., 2009; Parker and Baylis, 2009). Genetic ablation of CeCAV-2, similar to what is seen with hepatic caveolins and mammalian lipoproteins, promotes abnormal trafficking of yolk proteins (evolutionary predecessors of lipoproteins) and defects in the uptake of lipophilic dyes or sphingolipids.

In adults, CeCAV1 is highly restricted to oocytes and spermatozoa (Scheel et al., 1999) – cells with an active transport of yolk proteins and highly enriched in cholesterol (Matyash et al., 2001). In oocytes, CeCAV-1 regulates cholesterol distribution, which is essential for the signaling mediated by the Ras–MAPK pathway and the progression through the meiotic cell cycle (Scheel et al., 1999). Recently, in adult neurons, CeCAV-1 has been connected to the signaling mediated by the insulin–insulin-like growth factor 1 (IGF-1) pathway, and the regulation of genes involved in aging (Roitenberg et al., 2018). Hence, these studies point to a primary role of caveolins in the control of lipid distribution that in turn regulates signaling and membrane trafficking.

Concluding remarks and perspectives

The data discussed here illustrate the active role of non-caveolar caveolins in cell biology. Caveolins may have evolved to be lipid sensors, transporters and organizers – as illustrated by studies in *C. elegans* – with their role in caveola formation a later adaptation concomitant with the evolution of the cavin proteins (Box 3). Note, however, that this may not be absolute; formation of caveola-like structures occurs in some *C. elegans* cells (Roitenberg et al., 2018) and in *Ciona* embryos (Bhattachan et al., 2020) in the absence of cavins, and CAV1 in mammalian systems may also generate membrane curvature without cavin-1 (Khater et al., 2018). A consistent feature of the non-caveolar caveolins is their ability to organize and/or transport lipids, and particularly cholesterol, but potentially also PtdSer and PtdIns(4,5)P_2. This role can be primarily at the PM, as demonstrated in lymphocytes or cilia, or in other compartments, such as early endosomes, as observed, for example, in neurons and liver. Phosphorylation of caveolins could be one of the signals for regulating non-caveolar CAV1; for example, in triggering lipid delivery to specific nanodomains (Minguet et al., 2017; Fielding et al., 2004) or in regulating signal transduction occurring in these nanodomains (Jung et al., 2018; Joshi et al., 2012). On the PM, caveolin scaffolds can also regulate the actin cytoskeleton (Rangel et al., 2019; Tomassian et al., 2011), which, in turn, regulates endocytosis and membrane nanodomains. How the balance of PM-to-endosomal caveolins is maintained in different cell types and the precise dynamics of trafficking of caveolins in the absence of caveolae is still to be established. The association of non-caveolar caveolins with LDs is a particularly striking example of an intracellular destination for caveolins, but the function of this particular pool of protein is still unclear.

Despite these unresolved questions, the importance of these pools of caveolins should not be underestimated: loss of CAV1 has profound effects on the ability of the liver to regenerate after partial hepatectomy (Fernandez et al., 2006), promotes defects in inflammatory responses mediated by immune cells (Fiala and Minguet, 2018) and causes neuronal degeneration (Trushina et al., 2006b). Thus, loss of non-caveolar caveolins in specific cell types that naturally express relatively low levels of caveolins has important pathological consequences.

Finally, the role of non-caveolar caveolins is not restricted to cells that lack caveolae. Indeed, a crucial aspect of caveolar biology is their disassembly (Lamaze et al., 2017). Caveolar disassembly can be triggered by increasing membrane tension (Sinha et al., 2011) or in response to other cellular stress conditions (McMahon et al., 2019). Analysis of the role of caveolins in cells that naturally lack caveolae, as discussed here, may therefore also provide insights into the effects of caveolins when caveolae are disassembled in other cell types. When cavins are released from the PM, caveolins become more mobile (Sinha et al., 2011) and there is a concomitant change in nanoscale lipid organization and signal transduction (Ariotti et al., 2014). Many of these effects may be due to the different properties of caveolins when they are released from caveolae. For example, transmembrane proteins are generally excluded from caveolae (Shvets et al., 2015) but may become available to interact with caveolins when caveolae disassemble. One example of the importance of the different roles of caveolar and non-caveolar

caveolins is the pro-tumorigenic properties of non-caveolar CAV1 in prostate cancer cells (Moon et al., 2014). For researchers studying caveolae, it is also important to appreciate that the effects of overexpressed caveolins (for example, in signaling pathways) will likely reflect the properties of non-caveolar caveolins and not caveolae, as caveolae become rapidly saturated upon caveolin overexpression (Box 2). Systems in which caveola formation is lost without loss of caveolins, such as in mice lacking pacsin 3, will prove extremely valuable in this respect (Seemann et al., 2017). Future studies should strive to clearly differentiate between the roles of the caveolins in the caves and to correlate the different pools of caveolins to their roles in diverse disease conditions.

Acknowledgements
We thank the members of the Parton and Pol groups for the critical reading of the manuscript.

Competing interests
The authors declare no competing or financial interests.

Funding
A.P. is supported by the Spanish Ministerio de Ciencia e Innovación (MICINN, RTI2018-098,593-B-I00), Fundació Marató de TV3 (31/U/2016), and the CERCA Programme/Generalitat de Catalunya. R.G.P. is supported by the National Health and Medical Research Council (NHMRC) of Australia (program grant, APP1037320 and Senior Principal Research Fellowship, 569452), and the Australian Research Council Centre of Excellence in Convergent Bio-Nanoscience and Technology (CE140100036). F.M.-P. is supported by the Ministerio de Economía y Competitividad FPI program (MINECO, BFU2015-66401-R).

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