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Caveolae and the cardiac myocyte

Short title: Cardiac caveolae

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Abstract

Caveolae, invaginated lipid rafts, orchestrate signalling in the cardiac myocyte. Here we highlight advances in the field which are relevant to the role of caveolae in these cells. Recent analysis of the molecular organisation and structure of the coat complex, which lines the internal surface of caveolae, suggest a stable inner caveolin layer covered with a filamentous cavin network. Ubiquitous caveolin 1 (Cav1) and muscle-specific Cav3 are expressed, and functionally relevant, in the cardiac cell. Caveolin acts as a constitutive brake on a number of signalling proteins (e.g. endothelial nitric oxide synthase, eNOS) in the myocyte, but the molecular basis of these interactions has been questioned. The position of cavins within the coat confers a lability to these proteins and this is supported by data showing cavin detachment from caveolae with osmotic stress in non-cardiac cells, and cavin translocation to caveolae in response to β-adrenoceptor signalling in cardiac cells. The protection that caveolae and/or caveolin provide against ischaemia-reperfusion injury, together with evidence that Cav3 overexpression can reverse some characteristics of the failing myocyte, suggest that these structures are a potential target of cardiac disease treatments in the future.

Keywords: Caveolin, cavin, NO, β-adrenoceptor, mechanotransduction, ischaemia, heart failure

1. Introduction

Caveolae are small membrane invaginations, 50-100 nm in diameter, found in almost every cell in the body. They are a specialised form of lipid raft, liquid-ordered domains enriched in cholesterol and sphingolipids, characterised by the presence of caveolin and cavin proteins. Caveolae have been assigned many roles: as signalling platforms, in endocytosis and transcytosis, in membrane lipid homeostasis and in the sensing and buffering of mechanical membrane stress (see [1] for a recent review).

Within the heart, caveolae are present within all cells that make up the myocardium - the cardiac myocyte, fibroblast, vascular smooth muscle cell and endothelial cell. The contribution of caveolae to a myriad of cellular processes within all these cells has been clearly demonstrated. In this review, we will focus on the role of caveolae in the cardiac myocyte; our aim is to highlight some of the recent developments and areas of controversy which are relevant to these cells.

2. Caveolar proteins

2.1 Caveolins

In 1992, the identification of caveolin as a component of the cytoplasmic coat of caveolae [2] marked the start of intense research in the field. Caveolins are small integral membrane proteins (Fig. 1) which oligomerise within the endoplasmic reticulum before being trafficked to the plasma membrane, see [3]. Ubiquitously-expressed Cav1 and muscle-specific Cav3 are essential for caveolae formation in non-muscle and muscle cells respectively. Understandably, the muscle-specific Cav3 has been the focus of work in the cardiac myocyte. However Cav1 is also expressed in these cells [4-7] and a defined role for this isoform is emerging (see Section 5.1).

Caveolin has three C-terminal palmitoylation sites which are required to direct caveolin to rafts and stabilise caveolin oligomers [8;9]. At the N-terminus, a membrane-proximal sequence of 20 residues, the caveolin scaffolding domain (CSD), has been assigned a crucial role in caveolin-protein interactions (see Fig. 1A). In the seminal work of Couet et al. [10] a fusion protein containing the CSD was used to select interacting peptide ligands from a phage display library. The peptides identified were enriched in aromatic amino acids with a characteristic spacing (ΦXΦXXXXΦ or ΦXXXXΦXXΦ, where Φ is an aromatic amino acid and X any amino acid), subsequently named the caveolin binding motif (CBM). This work formed the basis of a concept which dominated the field for 2 decades – that caveolin binds via its CSD to a corresponding CBM in its protein partners. The interaction was thought to exert inhibitory influences on protein activity (to use eNOS as an example, see Section 3.2), meaning that caveolin acts as a constitutive brake. However the tenet of CSD-CBM interaction has recently been questioned (see Section 3.1).

Given that the cardiac myocyte expresses both Cav1 and Cav3, and that both have been assigned functional roles, it is worth noting similarities and differences between these isoforms. The CSD of Cav1 and Cav3 (but not Cav2) show comparable interactions with CBM sequences [10], however Cav3 lacks the N terminal Tyr^{14} phosphorylation site of Cav1 which is important in cardioprotection [6], see Section 5.2.

Cav3 is found throughout surface and t-tubular membranes in the cardiac cell [11;12], but whether t-tubular Cav3 is associated with morphologically identifiable caveolae or simply serves as a protein scaffold is a point of debate. One reason for this uncertainty is the difficulty in imaging t-tubular caveolae using 2D methods. However, the recent advent of high resolution 3D imaging modalities such as STED (Z-stack) and EM tomography has enabled the visualisation of caveolae-like structures within t-tubular membranes in both mouse and rabbit myocytes [13;14]. Where present in t-tubules, it makes sense that caveolae are excluded

from dyadic couplings in order to retain the close proximity between the L-type Ca^{2+} channel (LTCC) and RyR2. This is supported by super-resolution imaging (dSTORM) showing minimal co-localisation of Cav3 and RyR2 in surface or t-tubular membranes [12]. Nevertheless, some recent models of Cav3 regulation of LTCC place a proportion of Cav3 within the dyad in a scaffolding role [15], see Section 3.2.

2.2 Cavins

The discovery of cavins as additional caveolar resident proteins in 2005 [16] has transformed the field in recent years. Cavins 1-3 are broadly expressed, whereas cavin 4 is the muscle-specific isoform. All 4 cavin isoforms are present in caveolae-containing fractions of the cardiac myocyte [17]. The importance of cavins for normal cardiac function is highlighted by data showing diseases associated with cavin mutations. For example, a group of 12 patients homozygous for mutations in cavin 1 with generalised lipodystrophy and muscle weakness displayed arrhythmia that resulted in sudden cardiac death in teenage years in two of the cohort [18]. Mutations in cavin 4 are associated with dilated cardiomyopathy characterised by progressive heart failure and cardiac arrhythmia, see Section 5.2 [19;20].

Cavin 1 plays a pivotal role in caveolae formation throughout the body. In the heart, the absence of cavin 1 leads to reduced myocardial expression of all caveolins [21;22], cavin 2 [21;22] and cavins 3/4 [22] and a loss of myocyte caveolae [22]. By contrast, cavin 2, 3 and 4 are not essential for caveolae formation or expression of caveolins/cavins [23;24], although cavin 4 stabilises Cav3 at the membrane [25]. The profound impact of cavin 1 deficiency places this isoform centre-stage in the field, and explains why mutations in cavin 1 (see above) mimic the impact of mutations in Cav1 (lipodystrophy) and Cav3 (myopathy). However, these findings also highlight the limitations of the cavin 1 KO mouse as a model for selectively determining the cavin 1 role within the caveola.

All cavins share common structural characteristics: 2 basic (coiled-coil) helical regions (HR) flanked by acidic disordered regions (Fig. 1D) [26]. Cavins require caveolin expression to direct them to the membrane [21]. However, with the exception of cavin 3 [27], they do not bind directly to caveolin and are tethered to the membrane through interaction with membrane lipids. The basic patches within HR1 and HR2 mediate interaction with phosphatidylinositol bisphosphate (PIP_2) and phosphatidylserine (PS) respectively [26]. All cavin isoforms interact with each other (apart from cavin 2 and 3) via the HR1 [24;26]. For cavins 1-3, this interaction has been shown to form the basic homo-or heterotrimeric cavin unit [28] (as depicted in Fig. 1D).

2.3 The caveolar coat complex

Caveolae are lined with caveolin and cavin proteins. Last year the architecture of this caveolar coat complex, was described in some detail by two independent groups, using human (non-cardiac) cell lines [29;30](see Fig. 1). Ludwig *et al.* crosslinked the 80S caveolar coat complex *in situ* to preserve its integrity, and visualised this by negative-stain EM and 3D cryo-electron tomography. The complex appeared as a hollow sphere of the same size and shape of a caveola, with an inner (caveolin) and outer (cavin) layer, and often adopted a polyhedral (rather than rounded) profile [29]. Stoeber *et al.* visualised 8S Cav1 oligomers as discs around 15-17 nm wide and 5 nm thick, each of which could occupy a face of the polyhedral structure [30]. Interestingly, these 8S complexes are of similar dimensions to the flat 'doughnut-like' structure of the Cav3 nonomer isolated from insect cells by the Kitmitto group [31] (Fig. 1B).

When isolated separately, the 60S cavin coat was detected as a filamentous mesh which formed a polyhedral lattice when incubated with liposomes containing PS [30]. In the intact coat, cavin filaments aligned with the edges of the caveolin polyhedral cage [29] (Fig. 1C). Details of the molecular arrangement of cavins within the outer coat have been gleaned, in part, by its dissociation by osmotic stress (see Section 4.2)[28]. Each

cavin trimer contains two cavin 1 molecules with one of either cavin 1, 2 or 3 (Fig. 1D). The cavin net is formed from lateral and end-to-end connections between cavin trimers; the HR2 domain is important for this [26;30].

This concept of the integral membrane caveolin coat, surrounded by a cavin 'nano-net' held in place by interaction with membrane lipids, is consistent with the relative stability of caveolins and lability of cavins within caveolae (see Section 4.3). Of note, although some structural information is available for Cav3 complexes, none of the work on the caveolar coat has been done in muscle cells and integration of cavin 4 in the coat complex has yet to be addressed.

2.4. Caveolar neck proteins

Caveolins and cavins surround the caveolar bulb, but an entirely different group of proteins cluster in the caveolar neck region. Neck proteins, including the ATPases dynamin and EH domain containing 2 (EHD2), control the dynamics of membrane invagination [32;33] (Fig. 1).

3. Caveolae as cardiac signalling platforms

3.1 Models of caveolar control of signalling

For more than 2 decades, the concept that compartmentalisation of signalling molecules and their targets within caveolae could promote the efficiency and fidelity of signalling has underpinned research in this field. An important element of this is the regulatory interaction between the CSD of caveolin and the complementary CBM of its binding partners (see Section 2.1). However, in 2012, two papers were published which capitalised on developments in bioinformatics and protein structure to cast doubt on the importance of CSD-CBM interactions. These concerns are based on the diverse structural characteristics of the CBM and its inaccessibility within the 3D protein structure [34;35]. In support of this, we have recently shown a poor enrichment of CBM-containing proteins in the caveolar fraction of the cardiac cell: only 45% of caveolar proteins contain a CBM versus 37% of proteins in the human proteome [17]. The concept that caveolin may interact with proteins indirectly, or through domains other than the CSD, is an important consideration for future work. Furthermore, it has implications for some of the commonly used tools in this field which are based on targeting the CSD or CBM (see Section 3.2).

Given uncertainties about the nature of caveolin-protein interactions, it is interesting to consider a totally different perspective on caveolar control: that caveolae (as a lipid storage centre) regulate signalling through modulation of membrane lipid composition and distribution [36]. Using mouse embryonic fibroblasts and baby hamster kidney (BHK) cells, Parton and co-workers showed that Cav1 knockdown changed membrane lipid composition and PS organisation in the inner leaflet of the membrane and had disparate consequences for clustering of H- and K-Ras proteins. Other means of disrupting caveolae (depletion of cavin 1, osmotic stress) had identical effects on Ras clustering, suggesting that it was the loss of caveolae that was responsible for altered Ras organisation in the membrane. This lipid-centric view of caveolar control has implications for cavin interaction with the membrane, which is mediated by lipids including PS (see Section 2.2), and is relevant to the response of caveolae to mechanical stimuli which can disrupt the caveolar structure (see Section 4).

3.2 Key signalling pathways regulated by caveolae

Despite some controversy regarding the mechanisms by which caveolae regulate signalling, there is clear evidence for their involvement in a number of key pathways relevant to cardiac function and disease. Here we will focus on recent developments regarding nitric oxide (NO) and β-adrenoceptors (βAR).

eNOS is the main constitutively expressed NOS in the cardiac cell and its activity is exquisitely dependent on caveolae. It is directed to the caveolar domain through acylation, and binding of either Cav1 or Cav3 isoforms maintains the enzyme in an inactive state, see [37]. Recent work revealed NO-dependent enhancement of diastolic stiffness and the Frank-Starling response in *ex-vivo* hearts from cavin 1 null mice [38]. Consistent with other work [21;22], these mice showed reduced expression of Cav1 and Cav3 and a loss of myocyte caveolae, and the impact on function was ascribed to loss of NOS-inhibitory Cav1/3 [38]. This study highlights an additional limitation of the global cavin 1 KO mouse for the study of the role of cavin 1 in the heart (see Section 2.2): reduced expression of Cav in endothelial cells will also have NO-dependent consequences for vascular function (e.g. vasodilation) which may impact on cardiac function indirectly.

Caveolar control of βAR signalling is well established, see Harvey & Calaghan for a recent review [39]. In general, the intact caveolar domain restrains inotropic and lusitropic responses to sub-maximal β1AR stimulation by inhibitory influences (perhaps through adenylyl cyclase) on both the LTCC and phospholamban [40]. For β2AR, caveolae and/or Cav3 are important for confining the β2AR signal to the membrane and restricting its impact on sarcoplasmic reticulum [41] and myofilament [42] proteins through Gαi- and phosphodiesterase-dependent mechanisms respectively.

If we look in more detail at the impact of Cav3 on LTCCs, there is clear evidence for its role in β2AR, but not β1AR, regulation of the channel. A proportion of LTCC are found in Cav3-containing domains which may include surface caveolae [43] and Cav3 scaffolds within, or close to, junctional dyads [15]. The LTCC-Cav3 interaction is supported by immunoprecipitation data, which suggest that 25-50% of LTCCs are bound to Cav3 [15;44]. Although this subpopulation of Cav3-associated channels have been shown to be selectively phosphorylated at Ser^{1928} following β1AR stimulation [15], the functional relevance of phosphorylation at this site has been questioned [45]. Other work has shown that an LTCC blocker directed to caveolae-containing fractions (Rem-CBM) has no impact on β1AR stimulation of the L type Ca^{2+} current [44]. For the β2AR response, there is more agreement. The Rem-CBM molecule inhibits β2AR stimulation of the Ca^{2+} current [44;46]. This fits nicely with data from Bryant *et al.* showing that a CSD peptide used to disrupt Cav binding limits protein kinase A-dependent activation of the Ca^{2+} current by β2AR [47]. Together these data suggest that β2AR stimulation of LTCC within the t-tubules requires Cav3. Cav3 also dictates the normal spatial restriction of the β2AR signal in the t-tubule, as demonstrated by adenoviral overexpression of a dominant negative Cav3 [48].

Caveolae regulate βAR signalling and, in turn, βAR signalling can affect caveolae through cavin proteins. All cavins have multiple phosphorylation sites which map primarily, but not exclusively, to the disordered regions [26]. In the murine heart, a phosphoproteomic screen showed that βAR stimulation results in phosphorylation of all cavin isoforms. Sites include Ser^{118}, Ser^{122}, Ser^{97} in cavin 1, 2 and 4 respectively, which lie within PKA consensus phosphorylation sites in HR1. Phosphorylation of cavins within the HR1 region responsible for cavin trimerisation may alter the formation of cavin trimers and the cavin coat, with implications for caveolar structure and signalling. Indeed, we recently showed that β1 and β2AR stimulation promote translocation of cavin 1 to caveolae in the cardiac cell [17]; it is tempting to speculate that cavin phosphorylation could mediate this redistribution. In broad terms these data fit with the established lability of the cavin proteins within the caveolar coat (Section 4.3).

3.3 Caveolar subpopulations

Evidence from non-cardiac cells suggests that caveolae exist as multiple subpopulations defined by cholesterol, Cav isoform, signal effector and target content [49-51]. Whether cavin isoforms characterise

separate caveolar populations is unclear; cavin 2 and 3 are excluded from the same trimer but they are still found within the same caveola [28]. We have recently identified 249 high-confidence caveolar resident proteins within the cardiac myocyte which supports the concept of caveolae subpopulations; each caveola is not large enough to accommodate all caveolar proteins [17].

4. Caveolae in response to mechanical stimuli

The cardiac myocyte is repeatedly exposed to both internal and external forces and must be able to withstand and respond to these stimuli. One structure which contributes to both of these roles is the caveola.

4.1 Membrane protection

The lipid bilayer can only increase by around 3% in area before rupture [52], therefore membrane reserves are essential in the cardiac myocyte which is subjected to significant degrees of stretch in the physiological and pathological setting. Caveolae in the cardiac myocyte flatten in response to hyposmotic swelling and mechanical stretch [4;53] and this can offer a significant degree of protection from rupture, see [54].

4.2 Mechanosensation and mechanotransduction

There are several ways in which caveolae can contribute to the sensing and transduction of mechanical stimuli in the cardiac cell: as buffers of membrane tension, regulators of membrane lipid composition and as signalling platforms.

As a membrane reserve, caveolae buffer increases in membrane tension in response to mechanical stimuli, as demonstrated directly in endothelial cells [54]. This, in turn, has consequences for mechanosensitive proteins (particularly ion channels) which are gated by membrane tension. Indeed, in cardiac cells, disruption of caveolae enhances indices of the mechanosensitive swelling-activated chloride current ($I_{Cl,swell}$) activation by hyposmotic stress [4]. The flattening of caveolae may also transduce mechanical stimuli through changes in membrane lipids, see Section 3.1.

Integrins are mechanotransductive proteins which are found in caveolae [17] and bind to Cav3 [55]. Cav3 is required for the activation of integrin β1D in caveolar fractions, and (neonatal) myocytes depleted of Cav3 show impaired downstream integrin signalling [55]. Together this evidence supports a role for caveolae as a platform for integrin-dependent mechanotransduction. In this context it is interesting to consider how integrins within the caveolar bulb may have restricted access to their extracellular matrix ligands (see [17]). Another recent example of caveolae as a platform that integrates mechanotransductive signalling comes from proteomic approaches to identify targets of protein kinase G1α following stretch of the mouse heart. One target of relevance here is cavin 1 which is phosphorylated at two sites, Ser^{302}, Thr^{304} [56]. Both sites lie within disordered regions thus the impact on cavin coat formation is more difficult to predict than for PKA-dependent phosphorylation discussed above (Section 3.2).

4.3 Stretch and the caveolar coat complex

Recent descriptions of the caveolar coat complex suggest that the outer filamentous cavin coat may be more labile than the inner caveolin complex, and this concept is supported by data showing the impact of stretch. Cavins 1-3 dissociate from caveolae in Madin Darby canine kidney (MDCK) and HeLa cells in response to severe hyposmotic stress (0.1 tonicity, T) [28;54] but the loss of cavin 1 was greater than that of Cav1 [54]. There are no data on cavin dissociation in the cardiac cell with swelling, but neither Cav1 nor Cav3 membrane distribution changes in the myocyte in response to a moderate hyposmotic challenge (0.64T) [4]. This year, super-resolution (STORM) microscopy was used to show that hyposmotic stress (0.5T) flattens the caveolar domain *without* release of Cav1 or other signalling molecules (Gαq) in vascular smooth muscle cells [57].

Yang & Scarlata concluded that caveolar domains are very strong, and that functional consequences of hyposmotic stress for β2AR-Gαq induced Ca^{2+} release from intracellular stores arose from loss of protein-protein interaction through flattening of the caveolar domain.

In the cardiac cell, swelling occurs during ischaemia when metabolites accumulate within the myocyte, and is exacerbated on reperfusion when the hyperosmotic extracellular milieu is exchanged for blood with normal osmolarity. The degree of osmotic stress (which varies from 0.64T to 0.1T in the studies described above) is an important factor to be taken into account when considering the physiological relevance of findings and the relative lability of cavin/Cav proteins between cell types.

5. Caveolae and cardiac disease

Caveolae have been linked with a range of cardiovascular diseases including arrhythmia, ischaemic heart disease, hypertrophy and heart failure. Here we will focus on recent developments regarding the role of caveolae in ischaemia and heart failure.

5.1 Ischaemia

Ischaemia-reperfusion (IR) injury is a major cause of cardiac morbidity and mortality [58]. Caveolae protect against membrane rupture during cell swelling associated with reperfusion (Section 4.1), and caveolae/caveolin play important roles in pre- and post-conditioning (preC, postC) which limit IR damage. In broad terms, Cav1 and 3 are lost from caveolae following IR [59] and preC and postC stimuli enhance the number of myocyte caveolae [6;60;61]. This area of cardiac research is one where evidence supports a significant role for both Cav1 and Cav3 isoforms. For example, Cav1 restrains the intracellular activity of matrix metalloproteases (MMP2), and Cav1 loss following IR may contribute to MMP2-dependent proteolysis [62]. Membrane Cav1 expression increases following helium treatment, used to mimic the anaesthetic preC stimulus [63], and anaesthetic preC activates the cardioprotective tyrosine kinase src which phosphorylates Cav1 at Tyr^{14} [6]. $Cav1^{-/-}$ mice are resistant to anaesthetic preC [6]. These are exciting data but, as much of the biochemistry from global Cav1 KO mice is performed on myocardial preparations (which contain cells besides the cardiac myocyte), effects on cells other than the cardiac myocyte must be considered in some contexts.

Cav3 is also required for preC and its overexpression in isolated myocytes mimics ischaemic preC [60]. Elegant work from the Patel group has shown that Cav3 translocates from sarcolemmal caveolae to the inner membrane of closely apposed mitochondria following preC. Cardiac myocyte-specific Cav3 overexpression evokes protection from IR damage and this effect could be replicated using an adeno-associated virus engineered to express Cav3 selectively in the inner mitochondrial membrane [64]. Mitochondrial-targeted Cav3 overexpression improves mitochondrial Ca^{2+} tolerance and promotes more efficient function of complexes I, II and IV [64]. Furthermore, myocyte-specific Cav3 overexpression limits mitochondrial superoxide generation consistent with a tighter coupling of complex I [64]. Electron paramagnetic resonance with phospholipid spin probes indicated a reduction in membrane fluidity in mitochondria with higher levels of Cav3 which may contribute to improved mitochondrial function [64]. Recently cardioprotective ERK1/2 signalling has also been shown to be directed from caveolae to mitochondria as part of a postC response, and this process was lost when caveolae were disrupted [61].

Another element of protection from IR damage relates to autophagy, a process whereby misfolded proteins and damaged organelles are broken down, providing nutrients and energy for cellular repair. In the HL-1 cardiac cell line, Cav3 knockdown inhibits autophagy and worsens cell survival in response to IR, whereas

Cav3 overexpression stimulates the process and improves survival. Association between Cav3 and autophagy mediators has been shown [65]. Thus together there is a consensus that caveolae, Cav1 and Cav3 play protective roles against IR injury in the cardiac cell.

5.2 Heart Failure

Caveolae and caveolar protein expression change during heart failure (HF) progression but there is no coherent picture of these changes; this may be due in part to the range of experimental models used and time-points studied. Caveolae increase in a canine model of pacing-induced heart failure [66] and cluster in the peri-infarct zone of a pig myocardial infarction (MI) model [67]. By contrast, in mice 4 weeks after trans-aortic constriction (TAC), surface membrane caveolar density is reduced [68] but Cav3-positive longitudinal elements of the t-tubular structure are increased [13]. In MI models, Cav3 expression (in both myocardial and isolated myocyte preparations) remains stable from 4-12 weeks, but increases at 16 weeks in the rat [69;70], whereas in the mouse Cav3 is elevated at 4 weeks, but returns to control levels by 8 weeks [13]. By contrast, in a rabbit model with aortic insufficiency, Cav3 expression is markedly reduced [42]. In human heart failure, a positive correlation between Cav3 and SERCA2A expression implies that heart failure severity is linked to loss of Cav3 expression [71]. Most studies have documented changes in Cav3 expression in HF, but a few have also assessed the subcellular distribution of the protein. For the rat MI model, an increase in Cav3 in Triton-soluble (i.e. non-caveolar) fractions at 4-16 weeks post-MI suggests dissociation of Cav3 from the membrane [69;70], although higher overall Cav3 expression at the later time-point ensures that caveolar Cav3 levels are preserved [69].

Cav 3 has been highlighted as potential target for treating heart failure and there is general support for this view. Cav3 over-expression attenuates hypertrophy and prevents the fall in ejection fraction following TAC in the mouse [68]. In rat myocytes at 16 weeks post-MI, Cav3 overexpression returns the diffuse β2AR response to a confined response [48]. Restoring Cav3 expression in myocytes from a rabbit model of failure also partially reversed the diffuse β2AR signalling characteristic of the failing myocyte [42]. What is particularly interesting about these data is that, even in experimental models when total Cav3 expression is increased (and caveolar Cav3 expression unchanged)[69], Cav3 overexpression partially reverses the changes characteristic of the failing state [48].

Less is known about the role of the cavin proteins in heart failure. Cardiomyopathy in the cavin 1 null mouse cannot be ascribed directly to loss of cavin 1 protein because of parallel loss of Cav1/3. Cavin 4 was recently highlighted as a gene associated with dilated cardiomyopathy (DCM) [20], which fits with other data showing that those carrying mutations in cavin 4 develop heart failure at a relatively young age [19]. Conversely, although cavin 4 null mice have structurally and functionally normal hearts at baseline, they are protected from the hypertrophic response to α1AR stimulation [24]. Little is known about changes in cavin expression during the progression of heart failure.

6. Summary and future directions

Within this field, some of the most exciting research to be published in recent years relates to the structure of the caveolar coat, particularly the outer filamentous cavin net. Although the vast majority of this work has been performed in non-cardiac cells, it has raised the profile of cavins as the labile component of caveolae, and suggests how mechanical stimuli and post-translational modifications may modify the cavin complex. There is also strong evidence that caveolae orchestrate NO and β2AR signalling in the cardiac cell and these may, in turn, contribute to the phenotype of cardiac disease (e.g. heart failure) where Cav3 expression or distribution is perturbed. Given the size and shape of a caveola it should be noted how important recent

improvements in microscopy, which permit 3D imaging with high resolution, have been for the latest discoveries, and how these methods will play a significant role in future developments in the field. However, despite major advances, there are still many questions which remain unanswered. These include:

- How important is the CSD-CBM interaction for caveolar control of signalling? Understanding caveolin-protein interactions is essential for the design of therapeutic agents targeted specifically to the caveolar domain, see [44;46].
- What is the relative expression of Cav1 and Cav3 in the cardiac cell; is there a degree of redundancy in the Cav1 role?
- How are cavins arranged in the cardiac myocyte coat and what is the functional significance of the four isoforms in this setting?
- How dynamic are caveolae with physiologically relevant stimuli?

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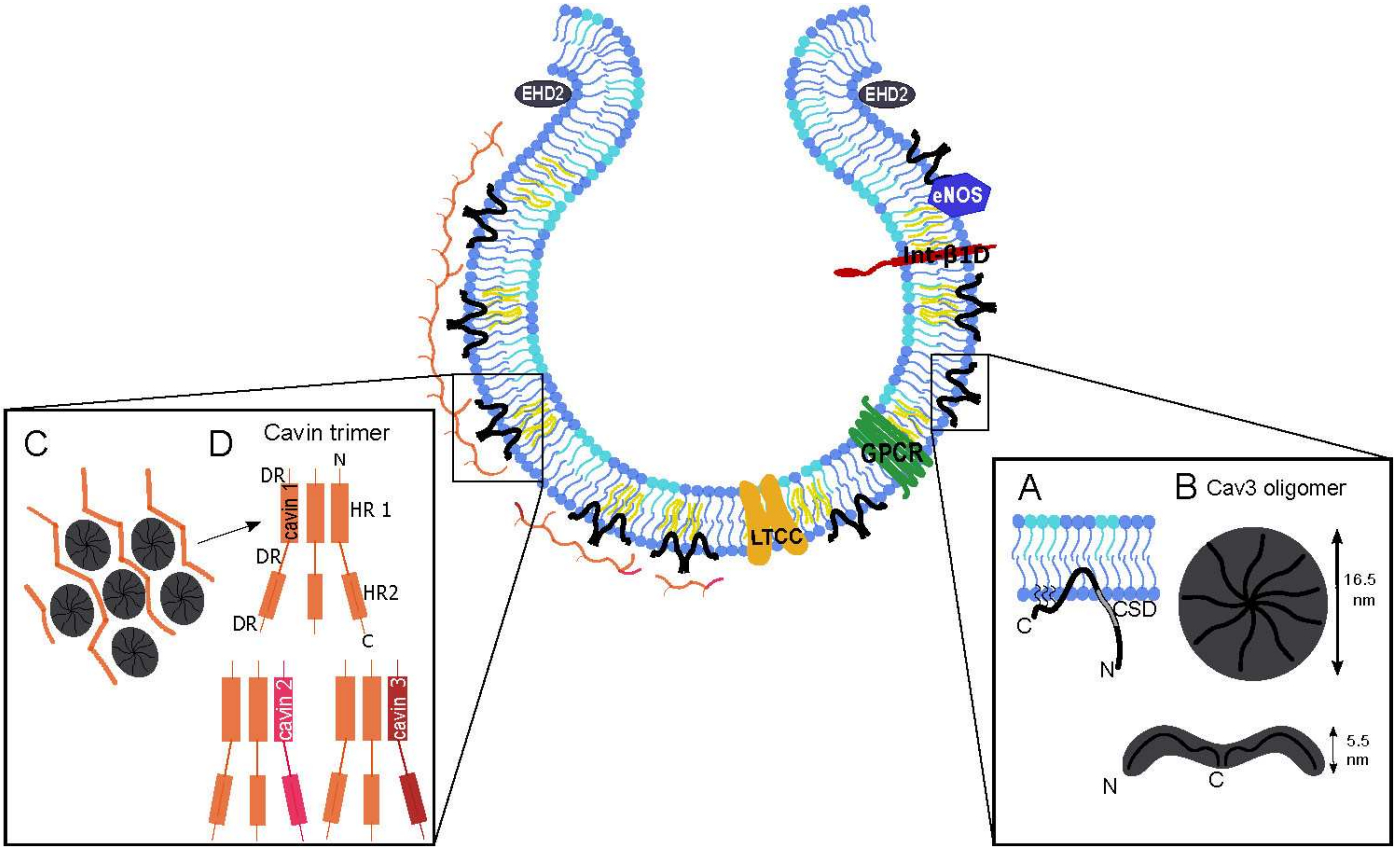

Figure 1. Caveolae and the caveolin-cavin coat complex. Caveolae are invaginated lipid rafts, enriched in cholesterol (yellow). The left side of the caveola (main image) shows the complex which lines the bulb formed of an inner caveolin and outer cavin layer. The right side of the caveolae (main image) shows some proteins enriched in the caveolar domain in the cardiac myocyte: G-protein coupled receptors (GPCR) such as the β2 adrenoceptor, endothelial nitric oxide synthesis (eNOS), L-type Ca^{2+} channels (LTCC) and integrin β1D (Int β1D). ATPases such as EHD2 are found in the neck region of the caveola and control the dynamics of membrane invagination [33]. Caveolin (right insert, **A**) is an integral membrane protein which has 3 C-terminal palmitoylation sites involved in membrane tethering and a 20-residue membrane proximal caveolin scaffolding domain (CSD) which may mediate some of its regulatory interactions. Caveolin forms oligomers with a sedimentation coefficient (S) of 8 [30]. **B**. Muscle-specific caveolin 3 is isolated as a doughnut-shaped disc of 9 caveolin molecules [31], viewed from the top (upper) and side (lower image). Cavins (left insert, **C**) form a 60S filamentous net which lines up with the edge of the polyhedral structure created by the caveolin discs [29]. **D**. Cavin trimers are the building blocks of the cavin net. Cavins have 2 helical regions (HR) flanked by disordered regions (DR). HR1 is responsible for trimer formation; trimers consist of two cavin 1 molecules with an additional cavin 1, 2 or 3 [26].

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