Biochimie 93 (2011) 71–77

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

Cavin proteins: New players in the caveolae field

Nolwenn Briand [a,b,c], Isabelle Dugail [a,b,c], Soazig Le Lay [a,b,c,*]

[a] Centre de Recherche des Cordeliers, INSERM, U872, 15 rue de l'école de médecine, Paris F-75006, France
[b] Université Pierre et Marie Curie – Paris 6, UMR S 872, Paris F-75006, France
[c] Université Paris Descartes, UMR S 872, Paris F-75006, France

ARTICLE INFO

Article history:
Received 8 January 2010
Accepted 29 March 2010
Available online 2 April 2010

Keywords:
Caveolin
Cavin
Caveolae
PTRF
SDPR

ABSTRACT

Caveolae are specialized lipid microdomains, forming small invaginations in the plasma membrane, known to be implicated in multiple functions including lipid storage, cell signaling and endocytosis. Formation of these wide flask-shaped invaginations is dependent on the expression of a caveolar coat-protein, namely caveolin. Until now, the accepted paradigm was that caveolin was the sole and only structural protein of caveolae since its expression was necessary and sufficient to drive caveolae biogenesis. The recent characterizations of PTRF/cavin-1 and subsequently other cavin family members in caveolae formation have highlighted additional levels of complexity in the biogenesis of these plasma membrane invaginations. In this review, recent advances on the role of the different cavin family members in the regulation of caveolae structures as well as potential new functions will be discussed.

Caveolae are specialized lipid microdomains that were first described by electron microscopists in the early 1950s [1,2] as (little caves) corresponding to invaginations of the plasma membrane with a diameter in the range of 50–100 nm [3]. Caveolae structures are particularly enriched in cholesterol, glycosphingolipids and lipid-anchored proteins relative to the bulk of plasma membrane and considered as a subclass of lipid-rafts microdomains, reviewed in [4]. Due their specific lipid composition, they concentrate a number of signaling molecules modulating multiple cellular processes or trafficking events from the cell surface.

Although largely described from a morphological point of view, the structural components of these plasma membrane invaginations remained unknown until two independent groups cloned simultaneously caveolin-1/VIP21 and identified this 21 kDa protein as the first true protein marker of caveolae structures [5,6]. Until now, the accepted paradigm was that caveolin was the sole and only structural protein of caveolae since its expression was necessary and sufficient to drive caveolae biogenesis in lymphocytes [7]. Despite intense research on caveolin and caveolae functions, the presence of such membrane invaginations particularly abundant in endothelial cells and in adipocytes still remained enigmatic [8,9].

Recent publications have highlighted the role of the family related-proteins cavins in caveolae formation therefore providing additional data on molecular aspects of caveolae formation and potentially new functions [10,11].

In this respect, the present paper aims at reviewing new aspects of cavins in the regulation of caveolae structures and their potential interest in elucidating the functions of these original invaginations.

1. Cavin proteins: new regulators of caveolae biogenesis

Although some proteins like PTRF (Polymerase I and transcript release factor) originally identified as an RNA Pol I transcription factor (also called Cav-P60 and now termed cavin-1) were previously shown to be enriched in caveolae, it's only recently that its crucial role in caveolae formation has been unraveled [12,13]. Following this exciting discovery, three other proteins, named cavin-2 (or SDPR for serum deprivation protein response) [14], cavin-3 (or SRBC for sdr-related gene product that binds to-c-kinase) [15] and cavin-4 (or MURC for muscle restricted coiled–coiled protein or cavin-4) [16], all sharing homology with cavin-1, were shown to be critical regulators for caveolae dynamics. All these cavins have been given several different names (alternate names of cavin family members are reviewed in [17]), but cavin nomenclature will be further used in this review.

1.1. The cavin family protein

Based on sequence homology with cavin-1, three other proteins renamed cavin-2 to 4 have been described as being part of the cavin family. They have all been shown to be present or isolated in

* Corresponding author at: Centre de Recherche des Cordeliers, INSERM, U872 Equipe 8, 15 rue de l'école de médecine, Paris F-75006, France. Tel.: +33 142346901; fax: +33 140518586.
E-mail address: soazig.lelay@crc.jussieu.fr (S. Le Lay).

0300-9084/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2010.03.022

caveolae preparations and to share similar features in terms of molecular organization [12–16]. Indeed, they all contain putative leucine zipper-like domains normally involved in protein–protein interactions and PEST domains (proline, glutamic acid, serine and threonine-rich domains), which may play a role in targeting proteins towards proteolytic degradation [18]. Cavin-1 can indeed be found under different proteolytic forms still associated with caveolae. However, cavin-1 is the only member containing poly-basic signals, possibly nuclear localization signals, in line with its initial described function as a transcription factor [19]. Other common features between cavins are their ability to bind to phosphatidylserine and to be phosphorylated on multiple sites [18]. In addition, studies have linked cavin-2 and cavin-3 to protein kinase-C delta [20] or alpha [21]. These different properties of cavin proteins raise the possibility that they would be able to regulate some aspects of caveolae functions.

1.2. Cavin-1: a new scaffold for caveolae

Cavin-1 was originally identified by two independent groups using yeast two-hybrid systems [19,22] as a soluble nuclear factor that regulates transcription process. Recombinant cavin-1 protein is able to dissociate ternary Pol I transcription complexes *in vitro* as revealed by the release of both Pol I and nascent transcripts from the template [19] and facilitates the reinitiation of RNA polymerase I [23]. It's only recently that this factor was also abundantly found in caveolae membranes derived from adipocytes by immunological and morphological procedures [18,24] leading to consider it as a caveolae marker.

A critical role for cavin-1 at the plasma membrane has emerged from recent publications reporting that this protein was required for caveolae formation [12,13]. Accordingly, cavin-1 expression was strictly parallel to that of caveolin-1 in mice tissues with its highest expression found in fat and lung, predicting a functional link between both proteins [13,22]. Cavin-1 is recruited by caveolins to plasma membrane caveolar domains and is necessary for caveolae formation [12,13]. Indeed, absence of cavin-1 leads to the loss of morphologically identifiable caveolae and to diminished protein expression of all three caveolin isoforms whereas no alterations at the level of mRNA of caveolins were observed. Cavin-1 downregulation is also accompanied by an increase of caveolin-1 mobility, which is released from the cell surface and rapidly internalized and degraded [13].

Caveolin and cavin-1 were found to be in close proximity by FRET experiments when situated at the plasma membrane [13] but a direct interaction between these two proteins is still controversial (see Section 2). Experimental results tend to prove that cavin-1 will contribute to the last steps of caveolae biogenesis. Indeed, it only associates with plasma membrane caveolae but not with non-caveolar caveolins (like caveolins at the Golgi level) [13,25]. Therefore, cavin-1 has to be considered as a soluble protein, which would be recruited to caveolae operating like a new scaffold stabilizing the caveolae unit.

It has been estimated that cavin-1 and caveolin were present in an approximate ratio 1:1 within caveolae [13] and that lipid-binding was likely to play a role in cavin association with these invaginations. In agreement, cavin-1 and caveolin-1 associate in a cholesterol-dependent manner and can both bind to phosphatidylserine [12,13], consistent with the fact that caveolae are particularly enriched in these lipid species [26]. However, further investigations will be needed to understand lipid–protein interaction that may be required for the structural stability of the nascent caveolae structures.

1.3. Cavin-2: generating caveolae curvature?

The breach opened by studies of cavin-1 in caveolae formation leads to question about the respective roles of other protein members of the cavin family. Cavin-2 is sharing more than 20% similarities with cavin-1 [14,18]. This 2nd member of the cavin family was found to be enriched in caveolae preparations and to co-localize with caveolin [21] filling the requirements for being a modulator of caveolae formation as cavin-1. Indeed, cavin-2 downregulation induces loss of cavin-1 and caveolin expression and therefore limits caveolae formation meaning that cavin-1, cavin-2 and caveolin-1 are functionally inter-dependent [14]. Co-immunoprecipitations experiments showed that cavin-2 and cavin-1 do indeed bind to each other, but that this interaction does not require caveolin-1 and that cavin-2 promotes recruitment of cavin-1 to caveolae [14]. An interesting observation is that, unlike caveolin-1 or cavin-1 respectively, cavin-2 alone does not increase caveolae number but induces changes in caveolae morphology, which turns to appear distended and induces caveolae-associated tubule formation [14]. These membrane tubulations closely resembling those induced by B-subunit of Shiga toxin (STB) [27] can originate from caveolae [14], even if these structures are not required for their formation [27]. Therefore, cavin-2 could be seen as the membrane-curvature component of caveolae although no sequence similarity has been found so far between cavin-2 and known curvature-inducing protein domain [28].

1.4. Cavin-3: regulator of the caveolar endocytic pathway?

Cavin-3 was originally identified in screens looking for PKCdelta-binding protein [20] but also in yeast two-hybrid screening using BRCA1 as bait [29]. The human CAVIN3 gene (maps to 11q15.5-15.4) is situated in a tumor suppressor region and is inactivated in breast and lungs cancers. Considering its homology with cavin-1, this has recently led to consider its role in caveolae structures. Alternative splicing of cavin-3 mRNA can potentially produce five isoforms with size ranking from 14.3 kDa to 31.1 kDa [15]. Although all cavin-3 isoforms were predicted to be soluble, this protein was previously identified in proteomic screens enriched in detergent-free caveolae [18,30]. This localization was recently confirmed by immunofluorescence and shown to be dependent on leucine-zipper domain and expression of caveolin-1 [15]. Interestingly, cavin-3 still associates with caveolin upon caveolae budding to form vesicles and intracellular caveolin-1 traffic is markedly impaired in the absence of cavin-3 [15]. Altogether, these results suggest that one potential role for cavin-3 might be to couple caveolae to the intracellular transport machinery.

1.5. Cavin-4: the muscle-restricted cavin

Through sequence homology searches, a fourth member of the cavin family was reported and termed cavin-4 [16]. This protein was previously described as purely cytosolic and able to interact with cavin-2 [31]. Cavin-4 was shown to be associated with cardiac dysfunction through the modulation of the Rho/ROCK pathway and to be important in muscle biogenesis [32]. This is in agreement with the specific expression of cavin-4 in cardiac and muscle tissues, which parallels caveolin-3 expression [16]. Cavin-4 was also found to associate with sarcolemmal caveolae of muscle cells and its expression is perturbed in human muscle diseases associated with caveolin-3 dysfunction. This clearly identifies cavin-4 as a new potential candidate for muscle-related caveolinopathies.

2. The cavin oligomeric complex

Since the individual characterization of each cavin member revealed many common features, this questioned about the way they may interact. Recent data revealed the existence of a multimeric protein complex containing all cavin members, which will associate with caveolins at the plasma membrane [16]. The stepwise