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Minireview

VIP21-Caveolin, a protein of the *trans*-Golgi network and caveolae

Teymuras V. Kurzchalia[a,*], Paul Dupree[b], Solange Monier[a]

[a]*Department of Cell Biology, Max-Delbrück Centre for Molecular Medicine, Robert-Rössle-Str. 10, 13 122 Berlin-Buch, Germany*
[b]*Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK*

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Abstract

VIP21-Caveolin is a component of the filamentous coat surrounding the invaginations of the plasma membrane called caveolae. Unlike the vesicular coat proteins identified so far, VIP21-Caveolin can be classified as an integral membrane protein. Furthermore, it is found in high molecular mass oligomers. Based on its localisation in specialised membrane subdomains, a role for VIP21-Caveolin in membrane protein sorting has been proposed.

Key words: Caveolae; Glycosylphosphatidylinositol-anchored protein; Glycolipid; Protein sorting; VIP21-Caveolin

1. Introduction

A protein with two names in cell biology is often the manifestation of the merging of two or more lines of research, and this is also the case for VIP21-Caveolin. In this minireview we will firstly consider the origins of both parts of the composite name, reflecting both research directions that led to the discovery of the protein, and secondly the role of VIP21-Caveolin in the formation of caveolar filaments and in the sorting of glycosylphosphatidylinositol (GPI)- anchored proteins.

2. VIP21

VIP21 (Vesicular integral membrane protein of 21 kDa) was identified through research into protein sorting in polarised epithelial cells [1]. These cells have apical and basolateral membrane subdomains that differ in their protein and lipid content. The fundamental questions of the field are: how does the polarised distribution of proteins occur, what are the signals for sorting and what is the molecular machinery involved in the sorting (for reviews see [2,3]). It is widely accepted that the sorting of apical and basolateral plasma membrane proteins occurs in the *trans*-Golgi network (TGN) by inclusion into separate vesicular carriers that transport the proteins to the correct destination [4].

Our experimental approach for the identification of components of the sorting machinery was based on a hypothesis postulated by Simons and his co-workers [5,6], predicting that glycolipids, which are enriched in the apical membrane are co-sorted with apical proteins in the TGN. Glycolipid microdomains or 'rafts' would be the framework of budding apical vesicle: apical proteins would associate directly with these glycolipids or with a component of the sorting machinery. The main question we addressed was whether the apically destined influenza haemagglutinin (HA) forms specific complexes with specific constituents of the TGN-derived vesicles that had been isolated according to Bennett et al. [7,8]. We used the property of some membrane subdomains to be insoluble in particular detergents [1]. This approach has become a powerful tool for studying protein sorting in the last few years. The apical marker HA becomes insoluble in TX-100 in the late Golgi, before its arrival at the cell surface [9]. Similarly, the apically targeted GPI-anchored proteins of MDCK-cells become detergent-insoluble during their transit through the Golgi apparatus [10]. Treatment the TGN-derived vesicles with the detergent CHAPS yields a high-molecular-weight complex, containing influenza HA associated with a subset of the vesicular proteins, including presumably those associated with glycolipid rafts. A cDNA encoding one of these proteins, VIP21, was isolated [1]. VIP21, bearing a c-*myc* tag, was localised to the Golgi apparatus, to vesicular structures and to the plasma membrane. In further work using antibodies raised against the N- and C-terminal domains, VIP21 was found in the Golgi apparatus but was also concentrated in the plasma membrane domains termed caveolae (see below) [11]. The presence of VIP21 in both locations suggested that the mechanisms governing inclusion into caveolar plasma membrane domains are related to the processes of protein and lipid sorting at the TGN.

3. Caveolin

A 22 kDa protein was originally identified as one of the main tyrosine-phosphorylated substrates upon v-*src*-

*Corresponding author. Fax: (49) (30) 949 4161.

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transformation of chick embryo fibroblasts [12]. Antibodies raised against this protein [13] decorated caveolar filaments [14]. Based on this localisation, the 22 kDa protein was termed Caveolin and has been suggested to be a component of the caveolar coat. The sequence of chicken Caveolin is almost identical to the canine VIP21 and the human homologue [15,16].

Caveolae, or plasmalemmal vesicles [17,18], are non-clathrin-coated membrane invaginations of about 50 nm, with a very characteristic striated appearance (for reviews see [19,20] and references therein). They are found on the plasma membrane of almost all types of cells. Although the function of caveolae has not been yet clarified, several possibilities have been postulated, including an alternative pathway of endocytosis, a role in receptor-mediated uptake of small molecules (potocytosis), in the regulation of intracellular calcium concentration and in signal transduction.

4. Structure and properties of VIP21-Caveolin

VIP21-Caveolin is a protein of 178 amino acids, with a molecular mass of 21 kDa. VIP21-Caveolin fulfils all the criteria required for classification as a membrane protein: it cannot be extracted from membranes by either high salt or carbonate treatment [11] and partitions into the detergent phase of the TX-114 extract [1]. The amino acid sequence predicts no cleavable signal peptide but a single, long stretch of 33 hydrophobic residues found between amino acids 102 and 134. It is likely that the hydrophobic stretch serves as membrane anchor [1]. We suggested that VIP21-Caveolin is a type II molecule with its C-terminus in the lumen of the Golgi. However, using antibodies produced against N- and C-terminal peptides, it has been demonstrated that both domains of the molecule are accessible to the antibodies only from the cytosolic side of the membrane [11]. Furthermore, using an in vitro translation system we have shown that VIP21-Caveolin associates specifically with dog pancreas membranes in a classical, SRP-dependent manner and remains accessible to protease digestion (Monier et al., unpublished data). Taken together these data indicate that VIP21-Caveolin could form an unusual loop in the membrane bilayer. Although the hydrophobic stretch is long enough to cross the membrane twice, it does not contain a hydrophilic turn which usually connects two membrane spanning segments. It is possible that the formation of the loop structure results from the interaction between the regions flanking the hydrophobic stretch. It is also not excluded that VIP21-Caveolin does not traverse the entire bilayer and is a monotopic membrane protein (according to G. Blobels classification [21]). An example of such an integral membrane protein prostaglandin H2 synthase-1 which lies on the surface of the membrane was recently documented [22]. Three amphipathic helices form a hydrophobic patch which anchors the dimer in the membrane. Our model of VIP21-Caveolin membrane topology is in contradiction with the data published by Sargiacomo et al. [23]. The authors claim to detect surface biotinylation of VIP21-Caveolin in MDCK-cells using a membrane-impermeable reagent. To what extent a bona fide cytoplasmic protein (e.g. actin) was not biotinylated was not clarified.

As an integral membrane protein, VIP21-Caveolin has the unusual property of forming high-molecular-mass oligomers (Monier et al., submitted for publication). Discrete VIP21-Caveolin-containing complexes of 200 kDa, 400 kDa and 600 kDa have been detected. The 400 kDa complex was purified to homogeneity and shown to be an oligomer consisting of the 21 kDa monomer exclusively. Examples of membrane proteins which form oligomers containing such a high number of monomeric units are quite rare and poorly understood [24–26]. The predicted helices of the hydrophobic stretch are amphipathic, which could lead to the interaction between two or more molecules of VIP21-Caveolin and hiding the hydrophilic residues from the lipid.

We still do not know in what cellular compartment the VIP21-Caveolin oligomerisation occurs and whether it is a regulated or reversible process. It it tempting to speculate that the organisation of the glycolipid-protein microdomains that takes place in the Golgi apparatus, and the VIP21-Caveolin oligomerisation could be synchronised processes. Alternatively, the oligomers could be found exclusively in the plasma membrane caveolae and the caveolar coat might then be constituted largely by these VIP21-Caveolin oligomers organised into filaments. Other proteins could hold the oligomers together in the filaments or be loosely associated with them, as suggested by the co-immunoprecipitation of VIP21-Caveolin into a complex with several other proteins [27].

5. A function of VIP21-Caveolin in the sorting of GPI-anchored proteins

GPI-anchored proteins have no membrane spanning domain and are held in the bilayer via a glycolipid moiety (for a review see [28]). In MDCK cells, GPI-anchored proteins are sorted exclusively apically [29] and the GPI-anchor itself can direct reporter proteins to the apical domain [30,31]. There are several observations linking caveolae with GPI-anchored proteins. One comes from immunofluorescence studies of the folate receptor, a GPI-anchored protein [32,33]. Treatment of cells with antibodies against this protein led to the receptors enrichment in caveolae. Other GPI-anchored proteins have also been localised to caveolae [34], although it is not clear yet to what extent GPI-anchored proteins are localised in caveolae in the absence of antibody treat-

ment. Indeed, using photobleaching and fluorescence resonance energy transfer measurements, Hannan et al. have shown that, in contrast to the newly arrived molecules, the resident pool of a GPI-anchored protein gl-DAF on the surface of MDCK cells is not clustered [35].

Like VIP21-Caveolin, GPI-anchored proteins are detergent-insoluble [10,36]. The protein content of the CHAPS-insoluble pellet, which was used for the isolation of VIP21-Caveolin, and the TX-114-insoluble material seem to be quite similar [37]. Lisanti and co-workers have also demonstrated that VIP21-Caveolin is enriched in the TX-100-insoluble fraction together with a GPI-anchored protein gl-DAF [23]. Moreover, the TX-100-insoluble fraction containing GPI-anchored proteins is enriched in glycosphingolipids and cholesterol. Bearing in mind that VIP21-Caveolin and GPI-anchored proteins are to some extent localised in caveolae, the caveolae seem to be a manifestation of the glycolipid 'rafts' proposed to be involved in protein sorting [5,6,11]. However it is clear that the Golgi membranes contribute considerably to the detergent-insoluble material. Indeed an essential feature of the protein sorting hypothesis requires that the interaction between GPI-anchored proteins, glycolipids and VIP21-Caveolin occurs first in the Golgi apparatus.

A further observation correlates the sorting of GPI-anchored proteins to the apical surface with the presence of VIP21-Caveolin. The Fisher rat thyroid (FRT) cells which, in contrast to MDCK cells, sort GPI-anchored proteins to be basolateral membrane domain [38], do not express VIP21-Caveolin in detectable quantities [39]. However, VIP21-Caveolin cannot alone be responsible for sorting of GPI-anchored proteins. In the mutant MDCK-ConA^r cells that express VIP21-Caveolin in high amounts, GPI-anchored proteins are also missorted [39]. Thus VIP21-Caveolin could be a necessary but insufficient component for the sorting of GPI-anchored proteins.

How is VIP21-Caveolin involved in the process of GPI-anchored protein sorting? Is there a direct physical association between VIP21-Caveolin and GPI-anchored proteins? The membrane topology of VIP21-Caveolin discussed above makes a direct protein-protein interaction unlikely, but the interaction may occur between the lipid anchor of GPI-anchored proteins and the hydrophobic stretch of VIP21-Caveolin. Oligomerisation of VIP21-Caveolin and the formation of glycolipid rafts may serve as a platform for the clustering of GPI-anchored proteins. We believe that other, still unidentified protein(s) that connect VIP21-Caveolin and GPI-anchored proteins may exist.

In summary, VIP21-Caveolin is an integral membrane protein with an unusual membrane topology. It forms high-molecular-mass oligomers and participates in the formation of the caveolar coat.

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