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Minireview

And still they are moving...

Dynamic properties of caveolae

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Abstract Caveolae are structures found on the surface of many mammalian cells. In the last few years the biogenesis and the function of these organelles have been intensively investigated but many challenging questions remain. One of these is whether caveolae are statically attached to the cytoplasmic surface of the plasma membrane or are moving to other intracellular organelles. Also the cycling of the caveolar coat component, VIP21-caveolin, is a subject of intensive discussion. The solution to these problems could give an insight into the understanding of caveolar function.

Key words: Caveolae; VIP21-caveolin; Detergent insolubility; Cholesterol

1. Introduction

While the function of many proteins is still unknown, there are very few cellular organelles today which have not been assigned a defined function. Caveolae belong to this group of organelles, although they were among the first cellular structures identified by electron microscopic studies [1,2]. As often happens, the lack of a clear-cut function led to the suggestion of many, sometimes mutually exclusive, possibilities. These include: an alternative endocytic pathway [3–5]; transcytosis [6,7]; receptor-mediated uptake of small molecules (potocytosis; [8,9]); regulation of intracellular calcium concentration [10,11]; and signal transduction [12–14].

There are many interesting, still unsolved problems in the caveolar field. These include the biogenesis, the biochemical constitution and the function of this organelle, just to mention some of the major ones. However, the main issue of our minireview will be the dynamic properties of caveolae and their constituent VIP21-caveolin.

2. Caveolae and VIP21-caveolin

The original definition of caveolae was exclusively structural. The plasma membrane of many mammalian cells contains non-clathrin-coated membrane invaginations with diameter of 40–60 nm (see for reviews [15,16]). The cytoplasmic surface of these structures is covered by very characteristic multiple filaments [4,16–18], which are oriented in a parallel fashion or form spirals. The investigation of caveolae was significantly facilitated by the identification of VIP21-caveolin, a protein of 21–22 kDa, which is so far the best biochemical or structural marker for caveolae (for review see [19]). In their pioneering study Rothberg et al. have shown that antibodies against this protein decorate the caveolar coat filaments [18]. The surprise came when it became clear that in contrast to other vesicular coat constituents, VIP21-caveolin is an integral membrane protein [20]. The protein forms an unusual hairpin loop structure in the membrane, with two cytoplasmically exposed regions flanking a very long hydrophobic segment [21–23]. In addition, the protein has two quite remarkable properties which are possibly connected to the structure of caveolae: it is insoluble in non-ionic detergents such as Triton X-100 or CHAPS [13,20] and it can associate with itself to form high molecular mass homo-oligomers in vivo as well as in vitro [23,24]. These oligomers could be structural elements of the caveolar coat.

There is a link between caveolae (or VIP21-caveolin) and lipids. First, the addition of cholesterol-binding drugs like filipin or nystatin changes the morphology of caveolae, making them flat [18,25]. Second, caveolae are found in a Triton X-114-insoluble floating fraction (TIFF) [23], which is enriched in glycosphingolipids as well as in cholesterol [26,27]. Moreover, as was recently shown, VIP21-caveolin itself could be a cholesterol-binding protein [28].

VIP21-caveolin appears to be a member of an emerging protein family. Recently two homologues of the protein have been identified [29–31], with one of these homologues being expressed exclusively in muscle [29,31]. In this review for the sake of simplicity, caveolin-1 will be used instead of VIP21-caveolin (see [41]).

3. Static vs. mobile

The discussion of whether caveolae are static invaginations on the cell surface or dynamic structures has a long history. In the beginning it was more or less accepted that they are pinocytic vesicles fixed in the process of budding from (or fusion with) the plasma membrane. In endothelial cells caveolae were ascribed the major role in the process of transcytosis (transendothelial transport) [6,32]. However, a number of investigations in the mid-eighties questioned this assumption and an alternative view that caveolae are statically attached to the surface of endothelial cells was suggested (see for review [33]). Also the role of caveolae in the process of endocytosis remains unclarified. On the one hand studies on the internalization of cholera and tetanus toxins demonstrated the role of uncoated invaginations in this process [4,5] and these invaginations were subsequently shown to be identical to caveolae [34]. On the other hand van Deurs and colleagues categori-

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cally argued against the involvement of caveolae in endocytosis [35].

Several recent papers shed new light on the dynamic properties of caveolae. Recently two phenomena, dynamics of caveolae as an entity and the cycling of caveolin-1, were described. What are the new lines of evidence for these dynamic properties?

4. What moves and how?

One of the findings which gave hints of the dynamic properties of caveolae was that treatment of cells with the phosphatase inhibitor, okadaic acid, leads to a rapid decrease in the number of cell-surface attached caveolae [36]. This internalization process was blocked by cytochalasin D and by the kinase inhibitor staurosporine and apparently involved fusion of caveolae with endosomes. The effect of okadaic acid was most pronounced in hypertonic medium as groups of caveolae accumulated in the center of the cell close to the microtubule-organizing center. Interestingly, this process was reversible and dependent on an intact microtubule network. Presently, the molecular mechanisms of the internalization are not clear. However, one can speculate that the process is kinase dependent and is connected to the actin network. The main question remains whether the effect of this drug reveals a physiological process in which caveolae move between the surface and the center of the cell, for example in response to a plasma membrane stimulus. The fact that kinase inhibitors appeared to inhibit uptake via caveolae even in the absence of okadaic acid [36] supports this idea.

The above studies suggest a model for caveolae dynamics far removed from the potocytosis model proposed by Anderson and co-workers [8]. In this model caveolae close transiently but do not move away from the plasma membrane. This process is also apparently regulated by phosphorylation as protein kinase C activators inhibit the potocytosis cycle [37]. In this scheme caveolae do not interact with endosomes or other intracellular organelles.

In such a model it might be predicted that caveolin-1, as an integral membrane protein which is apparently firmly embedded in the caveolar membrane, would not be transferred to other organelles. Interestingly, a third set of papers describe exactly such a phenomenon in which caveolin-1 cycles between intracellular organelles and the plasma membrane. Smart and co-workers have investigated the influence of the membrane-impermeable enzyme cholesterol oxidase on the structure and the distribution of caveolae [38,39]. They observed that the oxidation of cholesterol led to a quantitative disappearance of caveolin-1 from the cell surface. Surprisingly, after the cholesterol oxidase treatment the number and the structure of caveolae, as revealed by electron microscopy, was unchanged. Therefore, the authors questioned whether caveolin-1 could per se be the main constituent of the caveolar coat. Even more surprising appeared to be the fate of caveolin-1 after the enzymatic treatment. The protein was first found in the rough ER and then migrated to the Golgi. At this stage caveolin-1 became detergent soluble, resistant to protease treatment and was localized in the lumen of these compartments. Removal of cholesterol oxidase led to the return of caveolin-1 to caveolae. It must be noted that this finding raises fundamental questions concerning much more than the cycling of caveolin-1. As mentioned earlier, the protein in the membrane is oriented towards the cytoplasm and is part of a high molecular mass oligomer. The oxidation of cholesterol should lead to as yet undescribed post-translational rearrangements, even membrane-translocation, of high molecular mass complexes. At first glance it is quite difficult to arrange these findings into the accepted paradigms of membrane translocation. Obviously, the verification and subsequent clarification of the molecular mechanisms of this process are of tremendous importance.

Further investigation of the caveolin-1-cycling phenomenon led to several new observations [40]. The authors argued that caveolin-1 must cycle constitutively between the plasma membrane and the Golgi apparatus. The cycling appears to be a complex process which includes microtubule-dependent as well as microtubule-independent steps. In particular, they showed that even in the absence of cholesterol oxidase, nocadozole causes accumulation of the protein in the Golgi-ER intermediate compartment whereas the transport from Golgi to the plasma membrane is not affected by the drug. Moreover, incubation of cells at 15°C led to accumulation of caveolin-1 in the intermediate compartment. One possible proviso to these studies is that it is not yet clear how general the phenomena described in these papers are. In contrast to the human fibroblast cell-line used by the authors, MDCK, HeLa and CHO cells appear not to show detectable redistribution of caveolae upon cholesterol oxidase treatment or incubation at 15°C (T.K., unpublished observations). Also the detergent-solubility or protease-resistance of caveolin-1 remain unchanged. All in all, much work is needed to clarify these complicated problems.

5. Conclusions

According to a growing body of experimental data, caveolae are not static invaginations on the plasma membrane. Undoubtedly they are capable of being internalized in a regulated manner under special conditions. However, it is still early to conclude that caveolae are permanently on the move and thus could be involved in a process similar to endocytosis. In our opinion the cycling of caveolin-1 also needs to be scrutinized using different cell types and different experimental conditions. In any case, this phenomenon, even if it is restricted to only a small number of cell-lines, deserves close attention, and dissection of the novel underlying principles.

And still they move...

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