Review

BAR domains, amphipathic helices and membrane-anchored proteins use the same mechanism to sense membrane curvature

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ARTICLE INFO

Article history:
Received 26 January 2010
Accepted 26 January 2010
Available online 31 January 2010
Edited by Wilhelm Just

Keywords:
Membrane curvature sensing
Lipid packing defect
Hydrophobic insertion
Membrane-anchored protein
Single vesicle assay

ABSTRACT

The internal membranes of eukaryotic cells are all twists and bends characterized by high curvature. During recent years it has become clear that specific proteins sustain these curvatures while others simply recognize membrane shape and use it as “molecular information” to organize cellular processes in space and time. Here we discuss this new important recognition process termed membrane curvature sensing (MCS). First, we review a new fluorescence-based experimental method that allows characterization of MCS using measurements on single vesicles and compare it to sensing assays that use bulk/ensemble liposome samples of different mean diameter. Next, we describe two different MCS protein motifs (amphipathic helices and BAR domains) and suggest that in both cases curvature sensitive membrane binding results from asymmetric insertion of hydrophobic amino acids in the lipid membrane. This mechanism can be extended to include the insertion of alkyl chain in the lipid membrane and consequently palmitoylated and myristoylated proteins are predicted to display similar curvature sensitive binding. Surprisingly, in all the aforementioned cases, MCS is predominantly mediated by a higher density of binding sites on curved membranes instead of higher affinity as assumed so far. Finally, we integrate these new insights into the debate about which motifs are involved in sensing versus induction of membrane curvature and what role MCS proteins may play in biology.

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1. Introduction

Since electron microscopy allowed us to appreciate the delicate architecture of the internal membranes in eukaryotic cells, scientists have tried to rationalize the twists and bends of the undulating networks that make up the endoplasmic reticulum, the Golgi complex and the endocytic pathways. Clearly energy needs to be spent to sustain these shapes, since lipid membranes in general are identified\cite{2–4}. One obvious reason for the contortions of the lipid membrane is that you can fit more membrane into the confined space of the cells, much like DNA is wound up in the nucleus. This is likely the rationale for the tight packing of the inner membranes of the mitochondria, the thylakoid membranes in plants and the rhabdomere membranes in the eye of fruit flies all having high concentration of membrane proteins involved in energy absorption or production. The tubular network architecture of organelles involved in protein synthesis, processing and sorting probably serve additional purposes. Beyond the apparent need to create high curvature areas for budding of the vesicles that transport lipids and proteins between these compartments and the plasma membrane, it seems that the tubular networks themselves are involved in sorting of membrane proteins in, e.g. the Golgi network and the recycling endosomes\cite{5}. Moreover, the high curvature itself serves as recognition motif for binding of specific membrane-anchored proteins by a mechanism referred to as membranes curvature sensing (MCS). The importance of membrane bend as a biological signal was captured in essence in a study of the small GTPase Arf1\cite{6}. In its GTP-bound form Arf1 stabilizes the COPII protein coat which is involved in formation of vesicles travelling from Golgi to target membranes. Arf1 displays increased GTPase activity on small vesicles thereby concentrating its activity on highly curved vesicles that have budded off and need to shed their coats. This reveals one way in which membrane shape can serve as information thereby organizing cellular processes in space and time.
The term sensing or recognition of membrane curvature can be associated with two different types of processes. On one hand, sensing may refer to spatial localization or redistribution, resulting from curvature-dependent binding. The curvature dependent function of Arf1 described above is actually an example of curvature-sensitive binding, since the increase in GTPase activity is due to the curvature-sensitive binding of its GTPase-activating protein, ArfGAP1, to vesicles of smaller diameter via two amphiphatic helices [6–8]. Indeed three structurally unrelated types of membrane associated molecules (proteins containing amphiphatic helices (AHs) [9,10], proteins containing Bin/Amphiphysin/Rvs (BAR) domains [11,12] and most recently membrane-anchored proteins [13] have been observed to bind with increased densities in areas of high membrane curvature [6,7,11,13–19]. On the other hand, molecules whose equilibrium location does not depend on membrane curvature can be subject to a modulation of structural and consequently functional properties imposed by membrane-curve, and hence “sense” or respond to its presence. Examples of the latter case are enzymes like protein kinase C (PKC) [20] and Thermomyces lanuginosus lipase (TLL) [21], whose intrinsic activity is dramatically increased by increasing membrane curvature, though it is not clear how. In the following we will use the term sensing to denote solely upconcentration in areas of high membrane curvature. It is noteworthy that in both of the above cases, so far, there is no experimental evidence of preference for sensing of a specific range of curvatures. Thus at present sensing of membrane curvature does not describe selection of an optimum curvature but rather a monotonic preference for the smallest available curvature in a sample.

In this review, we will discuss this new important recognition process termed MCS. First, we will review a new fluorescence-based, single vesicle experimental method allowing characterization of MCS and will compare it to bulk/ensemble sensing assays that use liposome samples of different mean diameter. Understanding the pros and cons of the existing assays is necessary for evaluating limitations in the collected data and the conclusions drawn from it. Next, we will describe the different curvature-sensitive protein motifs including AHs and BAR domains, and suggest that curvature-sensitive binding on membranes results solely from the asymmetric insertion of hydrophobic amino acids in the lipid membrane. This mechanism extends to the insertion of alkyl chains in the lipid membrane, and consequently palmitoylated, myristoylated and GPI-anchored protein are predicted to display significant MCS properties [13]. We then discuss a model based on curvature-induced defects in lipid packing that accurately describes curvature-sensitive lipid binding and suggest that it surprisingly arises from higher binding capacity on curved membranes rather than higher affinity. Finally, we will try and integrate these new insights into the debate about which motifs are involved in sensing and induction of membrane curvature and into the proposed biological role of curvature sensitive protein.

2. Assays for MCS

Recent methodological progress has been critical in order to advance the understanding of how and why various motifs bind preferentially to certain curvatures in vitro. In this section we will briefly review two different types of assays and try to summarize their advantages and disadvantages. Understanding the pros and cons of the existing assays is necessary for evaluating limitations in the collected data and the conclusions drawn from it.

2.1. Comparative protein binding on liposome ensembles of different mean diameter

The simplest and most widely employed way to perform systematic experiments on membranes of different curvature is to use liposome samples that are extruded through pore sizes of different diameters, e.g. 50, 100, 200 and 800 nm, since the smaller the diameter, the higher the curvature [22–26]. Even though such extruded samples do not contain a unique size/curvature but a range of sizes that usually overlap significantly between samples [13], they still have a mean size that differs between samples. Protein binding on the samples of different mean curvature has been assessed originally by a variety of methods (e.g., calorimetry, spectroscopy) though lately centrifugation has been used quite extensively (Fig. 1A) [7,11,14,17]. The centrifugation assay has slight variations. In the simplest version protein and liposomes are mixed, liposomes are pelleted with ultracentrifugation and amount of bound protein is evaluated, e.g. with SDS–PAGE [11]. The amount of protein pulled down by each different liposome population is then plotted as a function of extrusion pore size (Fig. 1A). Another version with improved vesicle retrieval [7], centrifuges vesicles and proteins against a sucrose gradient in a flotation assay, thus avoiding pelleting of protein aggregates together with the vesicles. Charactering directly the average vesicle diameter by DLS gives more precise results on MCS [7,8,16] rather than reporting protein binding as a function of the extrusion pore size [11,14,17] which does not always correlate with liposome size distribution.

Although these bulk ensemble assays initially proved an efficient way to determine membrane curvature dependent interactions, they do not account for size polydispersity of the different extruded liposome samples [27]. The extensive overlap in size distribution (~70%) will reduce to a significant extent the magnitude of curvature dependant observations made in bulk experiments, in certain cases below the detection limit [15]. Another inherent limitation of an ensemble-based assay is the fact it averages over all liposomes in the sample. Under the assumption that all vesicles (of a given size) bind in the same manner, averaging would not be a critical issue. However recent data that is discussed in Section 3, demonstrate that MCS motifs exhibit strongly heterogeneous and asynchronous binding that if not taken into account will lead to a false interpretation of MCS data obtained through measurements in bulk.

2.2. Comparative protein binding on single liposomes of different diameter

The most recent development in in-vitro MCS assays is a platform we introduced recently based on single immobilized liposomes of various size (Ø 50–700 nm) and therefore curvature (Fig. 1B i) [13,15]. To ensure that immobilization does not deform the liposomes we measured the size of the contact area between liposome and surface using FRET [28]. We then screened for and found, immobilization conditions that minimized FRET, i.e. contact area, and hence deformation, so as to ensure the liposomes retained a spherical shape. The single liposome curvature assay (SLiC assay) was developed as an extension and refinement of the most commonly used ensemble/bulk assays on extruded vesicle populations of different average diameters. The use of surfaces for immobilization and of sensitive fluorescence microscopy techniques for improving sensitivity allowed down-scaling the assay to the single liposome level. The primary advantage of the SLiC assay is that each data point is not anymore a polydisperse ensemble of curvatures but a single liposome of a unique, well-defined, curvature. The theoretical size of each individual fluorescent nano-scopic liposome can be accurately determined (±5 nm) by integrating its fluorescence intensity and calibrating it to a reference sample [27]. In the SLiC assay the natural size polydispersity of a liposome ensemble becomes an advantage, since hundreds of distinct individual curvatures can be imaged and analyzed simultaneously in a few seconds. In this manner one can record protein binding on
a continuum of isolated membrane curvatures (corresponding to liposome diameters from 20 to 1 μm) in a high-throughput fashion (Fig. 1B ii).

2.3. Single curvature experiments versus ensemble curvature experiments

Under the assumption that all vesicles bind protein in the same manner, bulk assay-averaging would not be a critical issue. However recent data demonstrated that several MCS motifs exhibit strongly heterogeneous and asynchronous binding that if not taken into account can lead to a false interpretation of bulk MCS data [13,15]. By recording protein binding kinetics on single liposomes it was demonstrated that binding of both several BAR domains and AHs were not synchronized on all liposomes, i.e. binding kinetics on different liposomes exhibited a lag phase that varied from minutes to hours depending on the protein concentration. Thus even after hours of incubation at micromolar protein concentrations not all liposomes bound MCS motifs a property termed fractional binding (Fig. 1B iii). These slow kinetics suggest that the activation energy for binding is very high and that binding once initiated is highly cooperative. It remains to be determined what is the biological relevance of this binding behavior. However, since fractional binding depended significantly on protein concentration and importantly scaled linearly with liposome membrane area, it has severe consequences for ensemble binding experiments designed to monitor size depended binding [15].

Though fractional binding is a quantity inherently different from the density of bound protein, in a bulk experiment these two parameters cannot be distinguished since they would both contribute equally to the total amount of protein bound to an ensemble of liposomes. Furthermore, fractional binding has a reverse dependency on liposome size than the one of protein density for curvature sensors, thus when averaged out in an ensemble experiment it will decrease the apparent bulk MCS efficiency. This reduction is illustrated in Fig. 1B iv, showing a bulk MCS graph that is simulated from single liposome data. From single liposomes experiments we know the “real” MCS efficiency (i.e. protein density of small over
3. Proteins motifs responsible for MCS

During recent years several lines of experiments demonstrated that certain protein motifs involved in sorting and trafficking of proteins and lipids are endowed with the ability to localize functional properties precisely at the curved regions of membranes [7,11,13,18]. Two different types of such membrane MCS protein motifs have been described, namely AHs and BAR domains, and interestingly they have been proposed to function in two very different ways. Whereas AHs are thought to insert their hydrophobic part into the membrane thereby seeking out and filling up lipid packing defects [8,13], BAR domains have been proposed to use electrostatic interactions to sense the actual overall curvature of the membrane using a crescent shaped quaternary structure [11]. Here we will review the data describing the two modes of interaction as well as recent data that demonstrate that N-BAR domains actually use their associated AHs (or other hydrophobic residues) to sense membrane curvature. Thus the two different hypotheses on how protein motifs might be able to sense membrane curvature are reduced to a single one which is based on the insertion of hydrophobic motifs into the lipid membrane.

3.1. AHs as membrane curvature sensors

Evidence for curvature-dependent lipid binding by AHs originates from studies on serum apolipoproteins [25,26], and has subsequently been identified in other classes of membrane associated proteins including the enzyme CTP: phosphocholine cytidylyltransferase (CCT) and the scaffolding protein (α-synuclein) (reviewed in [9]). In general, such helices have several charges on the hydrophilic side and a small net positive charge. They have a high hydrophobic moment, as expected for surface-localized helices and their hydrophobicity, normalized over their length, varies only slightly. The major difference is the length of the helices [33]. AHs are thought to respond to membrane curvature by sensing lipid packing defects [7,16,18]. By shallowly inserting the hydrophobic wedge of the helix into membrane defects, packing stress and curvature strain is alleviated (Fig. 2). The ability to sense curvature-induced defects is affected by the membrane composition, thus as shown in-vitro, the curvature strain is significantly diminished in a curved membrane containing lyso-lipids which therefore antagonized the activation of AH-containing enzyme CCT [34].

More recently two MCS AHs motifs were identified in ArfGAP and were termed ALPS motifs (Amphipathic Lipid Packing Sensor) [7]. These ALPS motifs differ from many AHs believed primarily to be involved in generation of curvature, such as the ones in epsin and amphiphysin by having non-charged serines and threonines on the interface between the non-polar and polar region. Furthermore, it has been shown that introducing charges in this interface of the amphipathic helix increases binding on flat membranes and thereby suppress curvature sensing by ALPS [16]. This encouraged the idea that MCS in general depends on non-charged residues at the interface, whereas positively charged residues would promote deformation. This proposal however is in conflict with data on both large liposomes (is ~50 fold. The first reconstruction (blue) assumes 100% fractional binding and illustrates that due to ensemble-averaging the apparent MCS efficiency is decreased to ~10-fold. Including the experimentally measured fractional binding in the reconstruction of bulk data, decreased further the MCS efficiency to ~2-fold at low concentrations. Notably, if the true MCS efficiency was not so high for a given MCS motif, fractional binding could completely inverse the tendency and indicate an apparent preference for larger diameters. In conclusion, bulk measurements should be interpreted with great caution. A bulk MCS graph will typically greatly underestimate the true MCS ability of a molecule and thus may potentially falsely report a molecule not to be a sensor. Even more critical are comparative bulk MCS studies between hydrophobic or charge mutations. Since such mutations strongly affect fractional binding [15], it becomes clear that, it is not possible to interpret from bulk experiments their influence on the MCS properties of a protein [7,14,17,29–32].

Fig. 2. Molecular details of three unrelated protein motifs known to sense membrane curvature – BAR domains, AHs and alkyl-chain anchored proteins. (A) BAR domains are uniquely shaped dimeric scaffold proteins that often contain additional lipid binding domains, such as an N-terminal AH (making it an N-BAR). They bind preferentially to high membrane curvature areas, indicated schematically with thick arrows. (B) An AH motif is a peptide stretch that upon interaction with membranes forms an α-helix with a polar (blue) and a hydrophobic face (yellow). AHs respond to membrane curvature by sensing lipid packing defects, present in higher densities on curved membranes. (C) Membrane anchored proteins sense membrane curvature by the same mechanism as AHs, by inserting the hydrophobic alkyl chain (yellow) into lipid packing defects present in curved membranes.
CTT and α-synuclein that both demonstrate curvature selectivity although they have charges at the interface [18,34]. Moreover, recent data showing also the N-terminal helix of amphiphysin to be a bona fide curvature sensor [13,15], clearly demonstrate that not only positive charges at the interface are tolerated for MCS, but high overall positive charge is as well.

Thus in view of the many different types of sequences shown to sense curvature, the AH appears to be a quite general and flexible motif that proteins use to mediate weak reversible interaction with membranes and sense lipid-packing defects, thus upconcentrating to highly curved membranes.

3.2. BAR domains as membrane curvature sensors

The BAR domain was originally identified as an evolutionary conserved region shared by the yeast proteins Rvs161 and 167 and the metazoan amphiphysins (amphiphysin and its splice variant Bin1) [35–38], and they were initially characterized by their ability to deform lipid membranes in-vitro and cause tubulation in cells [39,40]. BAR domains are now recognized in many different proteins which are implicated in an extraordinary diversity of cellular processes, including fission of synaptic vesicles, endocytosis, maintenance of cell polarity, regulation of the actin cytoskeleton, cell–cell fusion, secretory vesicle fusion, excitation–contraction coupling, apoptosis and tumour suppression. Significant contributions on the mechanism by which BAR domains impose membrane curvature have appeared recently [32,41,42] however scarce data exist to explain how these protein motifs sense membrane curvature.

Until recently the MCS mechanism by BAR domains was deduced from the crystal structure of the amphiphysin BAR domain [11] which revealed a crescent-shaped homodimer with clusters of positive residues on the concave surface (Fig. 2A). The intuitively appealing idea that the overall shape of the quaternary structure of the protein through electrostatic interactions would fit given curvatures of membrane, much like the shape of the clathrin coat encapsulates budding vesicles, was brought forward [11]. Thus MCS was thought to rely on two features which would maximize the interaction energy of a BAR domain and a negatively charged membrane of a given curvature: (i) the overall curved structure of the dimer and (ii) the net positive charge along its concave surface [11,43]. However, once MCS by BAR domains was demonstrated, the curvature-sensing mechanism was not validated using curvature sensing assays, but rather by testing the ability of various mutations to deform membranes. Thus charged residues found to be critical for deformation in-vivo as well as in-vitro, were presumed important also for sensing though they were never explicitly tested [11,14,29,44]. Similarly, mainly on the basis of deformation data, the mechanism of MCS was generalized to the whole BAR family including (i) F-BARs which exhibit a less curved crescent shape [29,30,32,41,45] and (ii) I-BARs which are inversely shaped and, once shown to induce inverse tubules [42,45,46], were speculated to have inverse preference for membrane curvature.

However, recent work based on the SLIC assay specifically addressed the nature of the MCS mechanism of several BAR domains and revealed that neither BAR dimer shape, nor dimer charges are involved in MCS [15]. Measurements on members of the BAR superfamily (including N-BAR, F-BAR and I-BARs) revealed that all proteins bound highly curved membranes with similar preference illustrating that dimer shape does not influence MCS by BAR domains. Furthermore, BAR domain charge mutants (endophilin 3KE and amphiphysin 2KE) [11,31] with critical positive residues changed to negatively charged residues did not lose their sensing ability compared to the wt [15] as in-vivo and in-vitro deformation experiments would otherwise advocate [11,31].

If dimer curvature and charges are not important for MCS, then how do BAR domains sense membrane curvature? Previous reports have pointed out that the N-terminal AH often found in combination with BAR domains (making them N-BARs) is critical for membrane binding and deformation [39,47]. Surprisingly, recent experiments found that mutations which impair formation of the AH also critically affect MCS by BAR domains such as endophilin and amphiphysin, and the helices themselves could furthermore fully replicate the MCS of the N-BARs [15]. It was moreover shown that blocking membrane defects sites by pre-incubation with an amphiphile shaped like a lyso-lipid, almost abolished MCS, presumably by reducing the bilayer defect density. Thus, MCS by BAR domains critically depends on hydrophobic insertion of amphipathic motifs in lipid-packing defects, a mechanism that emerges as the sole mediator of MCS both for AH and BAR domains [13,15] (Fig. 2A).

4. Will any membrane insertion be sensitive to membrane curvature?

MCS simply resulting from asymmetric insertion of hydrophobic residues in lipid membranes, raises the question whether hydrophobic membrane insertion in general, and not only by AHs, will give rise to curvature sensitive membrane binding. Indeed this hypothesis would rationalize several independent reports in the literature which have confirmed that proteins such as synaptotagmin [48], dynamin [49] and cytochrome b5 [22], are able to sense membrane curvature though they associate to membranes by inserting hydrophobic amino acids in a non-helical conformation. However, if the mechanism is truly general it should also apply to other hydrophobic protein-anchoring motifs like alkyl chains that govern the spatial localization of several classes of proteins, including G-proteins and GPI-anchored proteins [50–53].

Indeed results obtained on two biochemically alkylated proteins (BSA and GST) and the geranylgeranyl-modified β subunit of a G-protein (Gβ1γ2), demonstrated a significant ability to sense membrane curvature [13] (Fig. 2C). These results were supported by a recent report that established that the membrane association of the alkylated protein HIV-1 Nef depends on target membrane curvature [54]. These findings lead to the prediction that highly curved membranes, e.g. cytosolic vesicles, will recruit membrane-anchored proteins with high efficiency. Similarly one may expect that on a cell membrane with a continuum of different curvatures (e.g. folds in the ER, or endocytic buds in the plasma membrane), anchored proteins will redistribute to the areas of highest curvature.

The finding that membrane-anchored proteins are recruited by membrane curvature broadens the scope and implications of membrane curvature for protein sorting, trafficking and signalling in cell biology. Notably, numerous of, e.g. the small GTPases involved in sorting and regulation of vesicle budding and transport in endosomal compartments have lipid modifications [50,53]. It will be very interesting to see how the MCS property conferred by these modifications affects protein localization and function. In addition, localization and sorting of membrane-anchored proteins at the plasma membrane have so far been rationalized in the context of the raft hypothesis, which is based on the spatial compositional heterogeneities in membranes [55–57]. A sorting mechanism based on membrane shape suggests that curved areas might also serve as local signalling hubs.

5. Closing in on the mechanism for MCS

Specificity and selectivity of molecular interactions in biology almost exclusively rely on affinity, and accordingly MCS was orig-
mechanism was proposed recently by Hatzakis et al. [13]. They complete range of concentrations up to saturation, an alternative on the proposed sensing mechanism. However, the limited range of concentrations in their binding curves leaves room for debate on the proposed sensing mechanism.

Based on binding curves as a function of curvature for a complete range of concentrations up to saturation, an alternative mechanism was proposed recently by Hatzakis et al. [13]. They studied two AHs and the binding curves for the full concentration range revealed that there was no significant change in the Hill coefficient as a function of curvature and only a modest (2.5-fold) increase in affinity [13] in agreement with previous quantitative studies [18,25,26,58]. Surprisingly, the binding revealed a strong increase (~40-fold) in the maximal binding density ($B_{\max}$) with decreasing vesicle diameter [13], suggesting that membranes of different curvature exhibit a different density of effective binding sites for AH.

Insertion of AHs in curved membranes is thought to be facilitated by structural lipid-packing defects induced in membranes upon bending [7,9,16,18,60]. Thus, the mechanistic conclusion is a model where curvature-selective binding is mediated by higher density of binding sites on curved membranes rather than increased affinity or cooperative interactions [13]. It remains to be investigated how these in-vitro studies translate to in-vivo conditions, however one obvious consequence of these findings is that selectivity based on binding capacity allows for MCS even at high concentrations of a given protein and not only in a narrow concentration range around the $K_D$. In this way, the high concentration of lipid binding sites at curved membrane resembles clustering of membrane receptors at specific sites such as neuronal synapses or specific tissues which endow that location or tissue with the exclusive ability to respond to an agonist, even at saturating concentrations.

6.1. Sensing versus induction for amphipathic helices

Since proteins can both generate and sense membrane curvature it seems appropriate to ask the question whether the same proteins or even the same motifs are responsible for both sensing and induction of membrane curvature. The pioneering work in the field proposed arbitrary classification of molecules as either sensors or inducers of membrane curvature [11,16,59], however evidence from the BAR domain family of proteins show that, e.g. endophilin and amphiphysin previously shown to tubulate lipid membranes at high concentrations [39,40] are indeed curvature sensors at low (nanomolar) concentrations [13,15]. These recent findings prompt the question whether other AHs that tubulate membranes such as the AHs in, e.g. epsin and Sar1 [66,67] are indeed membrane curvature sensor as well. To address this question we have now probed the MCS properties of the ENTH domain of epsin using the SLiC assay, and indeed the ENTH domain displayed highly curvature sensitive membrane binding (Fig. 3). In conclusion, the data strongly argues that for AHs the sensing and induction of membrane curvature is indeed the low and high affinity versions of the same mechanism, although the theoretical framework needs to be rephrased in terms of binding densities. Given that alkylated proteins sense membrane curvature by a mechanism similar to AHs [13] it will be very interestingly to see whether they can induce curvature as well.

6.2. Sensing versus induction for BAR domains

In contrast to AHs, BAR domains were identified as curvature sensors [11,14,31], and it was (implicitly) argued that if BAR domains are sensors at low concentration then they will be able to induce membrane curvature [11] and stabilize tubules at high concentration [44]. The reverse argument was also made frequently, and point mutations that were proven important for
deformation were assumed to be critical also for sensing. Indeed data from cryoTEM [41] and cellular studies [32,42,68] as well as molecular dynamics simulations [69] have been accumulating showing BAR domains to induce and stabilize high curvature. On the other hand, the data demonstrating MCS by BAR domains remain very scarce. Moreover, since BAR domains most likely induce and stabilize tubules as multimeric networks in a concerted fashion [32,70] it is not straightforward to predict by microscopic reversibility that they are curvature sensors. Such an inference would also predict clathrin to be a sensor of membrane curvature. Finally, as described above, recent data suggest that the BAR domains of endophilin and amphiphysin show no selectivity for vesicles of different sizes [15].

7. Conclusion

Lipid interactions are central for many proteins involved in cell signaling, shaping the membrane, and trafficking of transmembrane proteins. Proteins are known to localize to selected parts of the membrane, and traditionally this targeting has been rationalized as a result of specific recognition motifs [59,71,72], electrostatic interactions [73,74], the phase-state of membranes, nanoscale domains of distinct composition [55–57] etc. The discovery of proteins that recognize membrane curvature created a paradigm shift by suggesting that membrane shape may act as an additional cue for protein localization that is independent of lipid or protein composition.

Here we reviewed recent data suggesting that MCS simply results from insertion of hydrophobic motifs in one leaflet of the bilayer. Thus curvature-sensing does not require highly refined motifs on the protein side, but rather results from the physicochemical properties of the curved bilayer membrane itself. Adopting a “membrane-centric” point of view, rather than the conventional “protein-centric” one, permitted us to envision that the principle of curvature-mediated redistribution may be generalized to any protein associated to the membrane through hydrophobic insertions [13]. Thus, we were able to rationalize several independent reports of membrane associated proteins like synaptotagmin [48], dynamin [49] and cytochrome b5 [22] being able to sense membrane curvature. We were furthermore able to predict and demonstrate in-vitro, several examples of alkylated proteins that were preferentially recruited in areas of high curvature [13,54]. This finding widens the scope and the implications of membrane curvature for protein sorting, trafficking and signalling in cell biology. The novelty of a shape-mediated targeting mechanism, as well as the discovery that a plethora of membrane-associated proteins may be involved [51,52,71,75,76], suggest enormous potential implications for cell biology.

However, whereas proteins have thoroughly been demonstrated to affect membrane curvature in cellular systems, MCS by membrane-associated proteins has so far almost exclusively been demonstrated in-vitro, consequently two important tasks lie ahead. First, it would be pertinent to establish that curved membranes in biological systems, which are actively maintained far from equilibrium by a variety of energy consuming processes, actually display similar type of effective binding sites for hydrophobic molecules as membranes in in-vitro experiments. Second, we need to address the biological function of MCS experimentally. The limited amount of information at hand is partly due to the young age of the field and partly due to experimental difficulties that prevent the systematic investigation in-vivo of protein function and localization versus nanoscale membrane curvature. Addressing the later challenge will be one of the important next challenges for the field.

Acknowledgements

The authors would like to thank for financial support the Danish Research Councils for Independent and Strategic Research, the Lundbeck Foundation, the National Institutes of Health grant P01 DA12408, and the University of Copenhagen programs of excellence “BioCart” and “Single Molecule Nanoscience”.

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