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Review

Modeling membrane shaping by proteins: Focus on EHD2 and N-BAR domains

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ABSTRACT

Cellular membranes are highly dynamic, undergoing both persistent and dynamic shape changes driven by specialized proteins. The observed membrane shaping can be simple deformations of existing shapes or membrane remodeling involving fission or fusion. Here we describe several mechanistic principles by which membrane shaping proteins act. We especially consider models for membrane bending and fission by EHD2 proteins and membrane bending by N-BAR domains. There are major challenges ahead to understand the general principles by which diverse membrane bending proteins act and to understand how some proteins appear to span multiple modes of action from driving curvature to inducing membrane remodeling.

1. Introduction

The ability of intracellular membranes to adopt a large spectrum of various and dynamic shapes is vital for cell physiology. Nearly flat plasma membranes undergo persistent budding in the course of different kinds of endocytosis giving rise to closed membrane vesicles of dimensions varying from 50 to 70 nm outer diameter for clathrin-mediated endocytosis to about a micron for macropinocytosis (see for review [1,2]). Endoplasmic reticulum (ER) consists of membrane tubules and sheets with thicknesses of several tens of nanometers which continually merge, divide and bud off into small separate nano-compartments traveling to the Golgi Complex (GC) (see for review [3]). The GC itself is composed of stacks of disc-like perforated cisternae of tens of nanometer thicknesses. The GC generates spherical, tubular and pleiomorphic membrane nano-structures serving for the GC-ER communication and mediating protein and lipid transport from GC to the plasma membrane and diverse cellular organelles (see for review [4–6]). The inner membranes of mitochondria fold and undulate to form deep invaginations, called cristae. Mitochondria themselves form an interconnected tubular network that continuously fuses and divides, the balance of which determines the overall network morphology [7–9]. The extended phenomenological observations accumulated by cell biologists on the intracellular diversity of membrane shapes and transitions between them require a focus on understanding of the underlying molecular mechanisms and their regulation. Here, we overview the current ideas on these mechanisms based on physical models of the cell membranes.

1.1. Two classes of membrane shaping

Shape transformations of closed membranes can be subdivided into two classes, which are essentially different from the geometrical point of view and require different physical mechanisms for their realizations.

The first class includes membrane shape changes which result from bending of the membrane surface but do not require any major transient disruption and re-connection of the membrane. In mathematical language, deformations of this class do not change the topological characteristics of the membrane surface which are characterized by a number called the surface genus (see e.g. Spivak [10]). Examples of such deformations are flattening of closed spherical membranes into disc- or sheet-like membrane compartments such as GC cisternae and ER sheets; squeezing of spherical membranes into tubules with closed ends such as intracellular tubular transport intermediates or tubular elements of ER; and tug-of-war like transitions between the ER tubules and sheets [3,11]. In the current biological literature the membrane deformations of this class are often referred to, somewhat ambiguously, as

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the generation of membrane curvature or membrane bending and we use this terminology here.

Processes of membrane shaping belonging to the second class include transient distortions of the membrane continuity and reconnections of the membrane surface in a new way. They result, geometrically, in the membrane topological transition expressed by the variation of the surface genus. The common examples are membrane fusion leading to merger of two separate membranes into one, and membrane fission resulting in splitting of one continuous closed membrane into two disconnected ones (for review see e.g. [12]). Another example is self-fusion of a closed disc-like membrane leading to formation of perforations such as those existing in GC cisternae (see e.g. [13]). We will refer to this type of membrane shaping as the membrane remodeling.

Lipid bilayer that forms a basis of every biological membrane provides the membrane with a resistance to the both kinds of shaping. The energy required to overcome this resistance and guarantee the generation, maintenance and dynamics of the intracellular membrane shapes must be provided by specialized proteins. Below, we overview the major notions of physics of lipid bilayers, which are necessary to quantify the action of the membrane shaping proteins and survey the current state of ideas about the specific mechanisms of action of these proteins.

2. Proteins in membrane shaping

2.1. Proteins in membrane curvature generation

A constantly increasing number of proteins capable of bending membranes are being discovered and characterized in terms of their ability to bend pure lipid bilayers, their effects on generation of curved intracellular membranes in vivo and the specific features of the protein structure relevant for the membrane bending [14–16]. The major mechanistic principles of the membrane bending function of these proteins have been suggested and classified into two groups – the hydrophobic insertion mechanisms and the scaffolding mechanisms [15,17].

The common element of the scaffolding mechanisms is the binding of a hydrophilic protein domain characterized by an intrinsically curved shape to the lipid bilayer surface. In order to match the protein shape, the membrane molds to a similar shape underneath and in the vicinity of the protein–lipid interface. Membrane bending by the scaffolding mechanism has been attributed to the dynamin family of proteins (see for reviews e.g. [18–20]), the BAR domain containing proteins [14,21–24], EHD2 [25], the complexes of clathrin with adaptor and accessory proteins (see for reviews e.g. [26–28]), the COPI and COPII complexes [29–31], and the proteins of the reticulon and DP1/Yop1 families [3,11,32]. The ability of a protein to be a scaffold assumes that the protein domain is sufficiently rigid compared to the lipid bilayer and the energy of the protein–lipid interaction released as a result of the protein attachment is larger than the energy cost of the bilayer deformation. While the membrane deformation energies can be reliably estimated based on studies of the elastic properties of lipid bilayers (see below), the quantitative characterization of the elasticity and the membrane binding energy of the protein domains, which are supposed to scaffold the membranes, is the matter for future experimental work.

The hydrophobic insertion mechanism assumes the partial embedding into the membrane matrix of hydrophobic or amphipathic protein domains. An integral trans-membrane domains spanning the whole membrane would also bend membrane, if it had a asymmetric cone- or inverted cone-like shape [33,34] or an oblique intra-membrane orientation [35]. More biologically relevant appear to be small protein domains embedding only shallowly into the upper part of a lipid monolayer. Most frequently, such domains are represented by amphipathic α-helices, penetrating the membrane to the depth of about 40% of a monolayer thickness [14]. The group of proteins which bend the membranes by inserting amphipathic helices includes epsins binding phosphatidylinositol-4,5-biphosphate polar groups [36]; small G-proteins Arf1 and Sar1 exposing the hydrophobic helices upon exchange of GDP to GTP [37–43]; and N-BAR domains (see below for more discussion) [14,17,23,36,44]. Other small hydrophobic protein domains bending the membranes by the insertion mechanism are the C2A and C2B domains of synaptotagmin-1, which interact in a Ca^{2+} dependent manner with the polar groups of negatively charged phospholipids and embed their hydrophobic loops up to about the level of the glycerol backbones [45–48].

It has to be emphasized that many of the membrane bending proteins have a potential to act according to both scaffolding and hydrophobic insertion mechanisms. Some of the loops of dynamin PH domain (VL1 loop) interact with the lipid headgroups and get embedded into the monolayer matrix [42,49,50]. Also, the N-BAR domains insert into membranes their amphipathic helices [14,17]. Recruitment to the membranes of the clathrin adaptor proteins, COPI and COPII is due to the amphipathic helices of the small G-proteins (Arf1p for APs and COPI, and Sar1p for COPII) [44]. Membrane attachment of the reticulons and DP1/Yop1 scaffolds is mediated by long hydrophobic hairpin segments, which are, probably, shallowly inserted into the lipid matrix [3,32]. Which of the two mechanisms is more important for a given membrane bending protein, or what is the possible interplay between them are questions to be addressed by experimental but also by theoretical and computational methods.

2.2. Proteins in membrane remodeling

Many observations have also been made on proteins driving the membrane topological transformations. Numerous proteins and protein complexes have been proven to control and drive membrane fusion of the major cell membrane systems: viral fusion (see for some reviews [51–56]), fusion of intracellular membranes (see for some recent reviews [47,57,58]) and fusion of plasma membranes (see for some reviews [59–63]). A description of the current state-of-the-art in the field of membrane fusion mechanisms can be found in the recent reviews [64,65].

For membrane fission a few protein families have been implicated: the dynamin family (see for reviews [18–20,66] and the recent progress [67–69]), CtBP1/BARS [70], PKD [71,72] and ESCRTIII [73–76]. One of these proteins, dynamin 1, was unambiguously demonstrated to drive membrane division [67,68].

In spite of a large number of identified proteins, the mechanisms of the protein driven changes of membrane topology remain elusive and subject to speculations.

2.3. Multi-functionality of membrane shaping proteins

Two essential questions arise:

(i) whether the ability of a protein to generate membrane curvature assumes also its potential to drive membrane remodeling or does the latter requires additional protein properties;

(ii) whether the same protein (or protein complex) can drive both membrane fusion and membrane fission in spite of the topologically opposite characters of these two types of membrane remodeling, or if different sets of proteins are needed for membrane division and merger.

Currently, there are three proteins that have been demonstrated to be able to perform both membrane curvature generation and either membrane fusion or fission. The first is the C2 domain of

synaptotagmin, a protein playing an indispensible role in SNARE-mediated fusion of intracellular membranes. C2 domains bend lipid bilayers made of Folch extract into 17 nm thin tubules which likely fuse with each other by their strongly curved end-caps [46,47]. Also lipid bilayers with lower phosphatidylserine (PS) content can be tubulated by C2 domains albeit the membrane curvature is lower in these cases (tubule diameter about 50 nm for 15% PS) [48]. In the context of biological fusion, it was suggested that synaptotagmin C2 domains mediate, in Ca^{2+} dependent manner, a local membrane deformation into a dimple-like shape with a strongly curved lipidic end-cap. The elastic stresses accumulated within these end-caps are released in the course of their fusion with a target membrane, and, hence, promote the membrane merger [46]. It has to be noted that the idea of a membrane dimple formation necessary for bridging the inter-membrane distance and generation of a focal point for fusion was suggested earlier for exocytotic fusion [77]. A mechanism of membrane fusion based of relaxation of membrane stresses accumulated in a membrane dimple was first proposed within the context of fusion mediated by the influenza virus fusion protein hemagglutinin (HA) [78] and then further elaborated [79].

The second is dynamin 1 which converts flat membranes into tubules of a few tens of nanometers diameter and, at the same time, is able to drive membrane division upon GTP hydrolysis (see for latest [67,68]). All suggestions about a mechanism by which dynamin 1 drives membrane fission are based on the idea that the elastic stresses and the corresponding energy produced within the membrane in the course of this tubulation relax as a result of fission [68,80,81].

Finally, N-BAR domains of endophilin seem to be able to drive membrane remodeling in addition to curvature generation. The major common property established for various BAR domain proteins is their ability to bend initially flat membranes into tubules [22]. The N-BAR domains of endophilin generate highly curved tubules of 35–50 nm diameter and, at high concentrations of protein, formation of many small vesicles of 35–50 nm diameter has also been observed [14]. The latter implies that an increase of the N-BAR concentration on the membrane drives fission of the membrane tubule into spherical vesicles. While many efforts have been devoted to trying to understand the mechanisms of membrane bending by N-BAR domains [82–85], the physical reasons for membrane fission by the same protein have not been addressed yet.

In addition to the three specific proteins above, for which the ability to bend and remodel membranes has been supported by direct experiments on lipid bilayers, the dynamin super-family of proteins appears to be a "multi-tasking family" involved in membrane bending, fusion and fission. This proposal is based, mainly, on the phenomenology accumulated around the dynamics of mitochondrial membranes where bending, fusion and fission of the outer (OM) and inner (IM) membranes involve several dynamin-related proteins (see for reviews [3,8,86]). Examples of such proteins are Drp1/Dnm1p, which has been implicated in shaping and fission of both the OM and IM [87,88], mitofusin/Fzo1p involved in mediating fusion of the OM [89], and OPA1/Mgm1p involved in fusion of the IM [90,91]. Another dynamin-like protein, atlastin, has been demonstrated to be involved in formation of an interconnected tubular network within the ER [92]. It has been suggested that atlastins directly affect branching of the ER tubules, likely, by promoting their fusion or fission [92,93]. Finally, dynamin has been suggested to control HIV fusion with intracellular compartments and, specifically, with endosomal membrane [94].

While some ideas on how the dynamin-related proteins may promote membrane fusion in addition to fission and bending have been suggested based on the mechanisms proposed for the action of other membrane restructuring proteins [3], the experimental proof and elucidation of the mechanistic details of these proposals remain the matter of future work.

In the following, we consider the theoretical approaches used to model and analyze membrane shaping by the scaffolding and hydrophobic insertion mechanisms and emphasize the specific models for membrane bending and remodeling by EHD2 domains and amphipathic helices.

3. Scaffolding mechanism of membrane bending: membrane tubulation and fission by EHD2 domains

3.1. Modeling membrane as an elastic surface

A lipid bilayer whose two monolayers have similar lipid compositions tends to adopt a flat conformation. The bilayer opposes bending with respect to this spontaneously adopted flat shape which means that the bilayer curving requires application of forces to its surface and related investments of energy. The ability of proteins to shape membranes and the mechanisms by which this shaping occurs crucially depend on the resistance of the membrane lipid bilayer to deformations. This resistance boils down to stresses and strains, which are generated within the bilayer interior as a result of deformation of its surface. The membrane stresses, strains and their dependence on external forces are described by physics of membrane elasticity.

The protein complexes such as a dynamin tetramers [18–20,66], BAR dimers [17,22] or EHD2 oligomers [25], which are capable of bending membranes by the scaffolding mechanism have characteristic dimensions of more than 10 nm meaning that they are large compared to the ~1.5 nm thickness of a lipid monolayer. In these cases the lipid membrane can be considered as an elastic surface and described by the well-known Helfrich model of membrane bending (see [95] for the original and [96,97] for a simpler presentation of the theory).

To quantify the amount of membrane bending at every point of the membrane surface the Helfrich model uses the notion of curvature. According to the fundamental geometry (see e.g. [10]), the shape of a small surface element is fully characterized by two lines, which lie on the surface, are mutually perpendicular and have shapes of circular arcs with radii R_1 and R_2 (Fig. 1a). The values inverse to the principal radii, $c_1 = 1/R_1$ and $c_2 = 1/R_2$, are called the principal curvatures of the surface. The curvatures can be positive or negative. Conventionally, the curvature of a lipid monolayer is defined as positive, if the monolayer bulges in the direction of polar heads, and negative in the opposite case of bulging towards the hydrocarbon chains. For a closed membrane of an intracellular organelle or transport intermediate, the positive curvature corresponds to membrane bulging towards the cytoplasm. According to the Helfrich model, the membrane energy must depend on particular combinations of the principal curvatures, namely, the sum $J = c_1 + c_2$ and the product $K = c_1 \cdot c_2$ called the total (mean) and the Gaussian curvature, respectively [10]. It is important to emphasize that membrane deformations corresponding to generation of the total, J, and Gaussian, K, curvatures are independent, meaning that J can be changed by keeping K to equal zero (e.g. bending of a flat membrane into a cylindrical one) and, vice versa, the Gaussian curvature K can be changed by keeping a zero total curvature J (transformation of a flat membrane into a saddle-like one).

To quantify the membrane resistance to bending and the bending energy accumulated by the membrane, the Helfrich model uses the notion of the membrane elastic characteristics: the spontaneous curvature J_S, the bending modulus κ_B, and the modulus of the Gaussian curvature $\bar{\kappa}$. A non-vanishing spontaneous curvature, $J_S \neq 0$, means that the membrane has an intrinsic tendency to deviate from the flat state, the strength and the direction of this

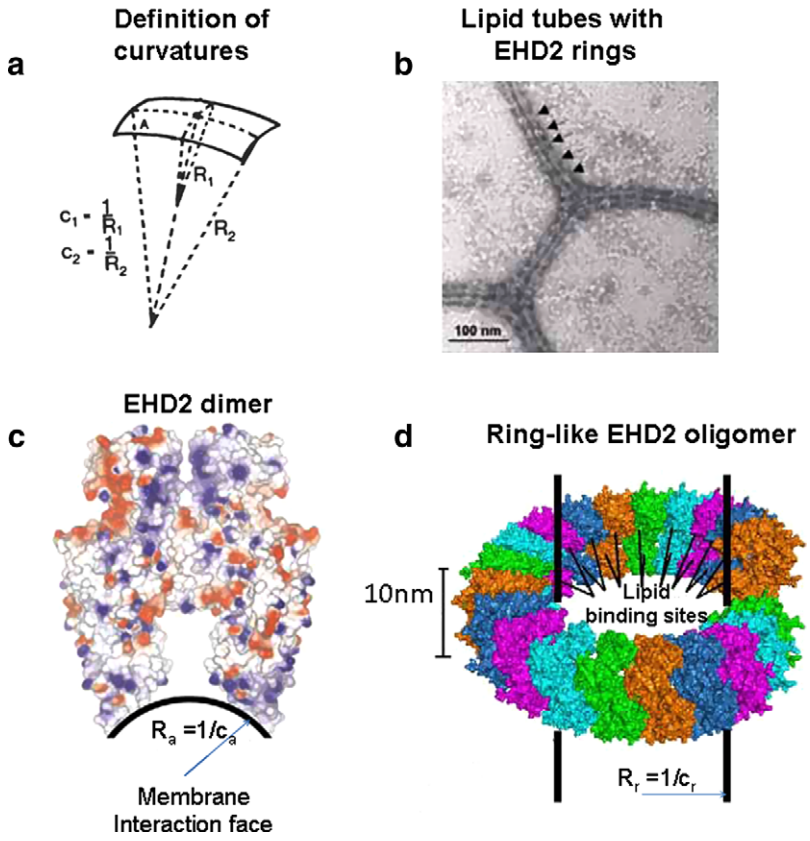

Fig. 1. Membrane bending by EHD2 proteins. (a) Definition of membrane curvature. (b) Membrane tubules decorated by ring-like EHD2 oligomers. (c) EHD2 dimer. (d) Ring-like EHD2 oligomer. The figures (b) and (d) were published in Supplementary information of [25].

deviation determined by the value of J_S. Within a reasonable approximation, the spontaneous curvature J_S can be regarded as the total curvature of the membrane shape corresponding to a complete relaxation of the membrane stresses and a minimal elastic energy.

The bending modulus κ_B determines the strength with which the membrane tends to adopt the spontaneous curvature J_S. In case the membrane prefers a flat shape, $J_S = 0$, the bending modulus sets the energy needed to bend the membrane into a shape with a non-vanishing total curvature J.

The energy for generation of the Gaussian curvature K is determined by another elastic characteristic of the membrane called the modulus of the Gaussian curvature, $\bar{\kappa}$.

Based on above elastic characteristics, the energy per unit area of the membrane surface associated with membrane bending from the flat state to a state with the curvature values J and K is determined by the famous Helfrich formula $f = \frac{1}{2}\kappa_B J(J - 2J_S) + \bar{\kappa} \cdot K$. The bending modulus κ_B has been measured by a different experimental approaches (see e.g. [98]) and its characteristic value is about 10 kT for a monolayer and 20 kT for a bilayer where the monolayers of the latter are free to slide with respect to each other ($kT \approx 4 \times 10^{-21}$ J ≈ 0.6 kcal/mol). The modulus of Gaussian curvature $\bar{\kappa}$ is difficult to measure and can depend on the measurement conditions and lipid compositions. Its value for a lipid monolayer was estimated to be negative and to constitute a decimal of the bending modulus (see e.g. [99] and refs therein). For a bilayer, $\bar{\kappa}$ is the sum of the monolayer contributions and an addition related to the monolayer spontaneous curvature [99]. More recently, the Helfrich model was extended by taking into account the membrane elasticity related to tilting of the hydrocarbon chains of lipid molecules with respect to the membrane plane [100–102]. The elastic modulus, κ_t, determining the amount of energy needed to tilt the lipid chains has not been measured but was estimated theoretically to be equal to 30–40 mN/m [100–102].

The Helfrich model was recently used to analyze the membrane scaffolding into tubular structures by arc-, ring- or helix-like proteins such as reticulons and Yop1 family proteins [32]. The results of this work demonstrated the efficiency of the Helfrich model for understanding the intracellular membrane shapes such as tubules constituting a large part of the smooth endoplasmic reticulum (ER). The computations showed that membranes must exhibit shapes of almost perfect cylindrical tubes even for fairly large distances between the protein rings and recovered the membrane shapes observed upon addition of Yop1p to lipid bilayers. The calculations predicted that formation of the shapes of the ER shaped tubules requires the biologically feasible amounts of reticulons or Yop1p corresponding to about 10% fraction of the membrane surface area covered by the proteins.

3.2. Membrane tubulation and fission by EHD2 proteins

An extension of the analysis of membrane tubules generated by ring- or arc-like protein scaffolds enables an understanding of the recently discovered membrane shaping by EHD2, a member of a highly conserved family of Eps15 homology (EH)-domain-containing proteins (EHDs) [25]. These proteins are eukaryotic ATPases implicated in clathrin-independent endocytosis, and recycling from endosomes [103–105]. It has been suggested that in vivo these proteins may serve as analogs of dynamin and generate membrane deformation and scission [25].

Recent structural work demonstrated that EHD2 binds acidic liposomes, deforms them in a nucleotide-independent manner into 20-nm diameter tubules and the protein oligomerizes into ring-like structures around these tubules [25] (Fig. 1b). A structural unit

of these oligomers is an EHD2 dimer which interacts with a membrane surface along a highly curved concave interface (Fig. 1c). An EHD2 ring-like oligomer composed of about 20 dimers (Fig. 1d) imposes on the membrane two different curvatures in two perpendicular directions – the radial curvature, C_r, which determines the radius of the membrane tube, $R \approx 10$ nm, and is relatively small, $C_r = \frac{1}{R} \approx 0.1\ \text{nm}^{-1}$ (Fig. 1d), and the axial curvature, C_a, corresponding to the large curvature of the concave membrane binding face of the EHD2 dimer (Fig. 1c). Most of the EHD rings are equally spaced on top of the membrane tube. The distance between consecutive rings can be roughly estimated to be about 14 nm (Fig. 1b).

The elastic approach can be used to understand the observed features of membrane shaping by EHD2 oligomers such as the regular partitioning of the rings along the axis of the membrane tubule with a given spacing between them (Fig. 1b), describe the detailed shape of the membrane between the rings, analyze the membrane-mediated forces acting between the protein rings, and, finally, predict how the EHD2 oligomers could be involved in the membrane fission.

To perform the analysis, the model of the EHD2-membrane system has to be formulated in mathematical terms. To this end, we consider a smallest sub-system of the whole system consisting of only two protein rings with an element of the membrane tubule between them (Fig. 2a). The rest of the system serves then as a reservoir with which the membrane between the rings can exchange lipid material. Based on the membrane-EHD2 interaction data [25], we assume that the membrane attachment is only via the concave face of the EHD2 ring. This determines a certain angle θ, referred below to as the attachment angle, between the membrane profile and the tube axis determined at the ring boundary (Fig. 2a). At the same time, we assume that the membrane is free to slip along the protein–lipid interface and, hence, exchange the material with the reservoir. The parameters determining the membrane configuration are the ring radius R, the distance between the rings, L, and the attachment angle, θ.

The first goal of the analysis is to compute the dependence of the membrane energy, F, on the inter-ring distance L. Once the function $F(L)$ is obtained, we can check whether a specific distance L^* corresponding to the minimum of the energy F is expected to be established spontaneously between the protein rings, hence explaining the experimentally observed arrangement of the EHD2 rings (Fig. 1b). This distance L^* referred below to as the equilibrium distance would correspond to a vanishing force between the protein rings. Any deviation from the equilibrium distance will increase the elastic energy of the system and require action of axial forces f on the rings (Fig. 2b). If a certain distance L is reached due to the force application, the elastic energy accumulated within the membrane tubule may become large enough to drive the tube fis-

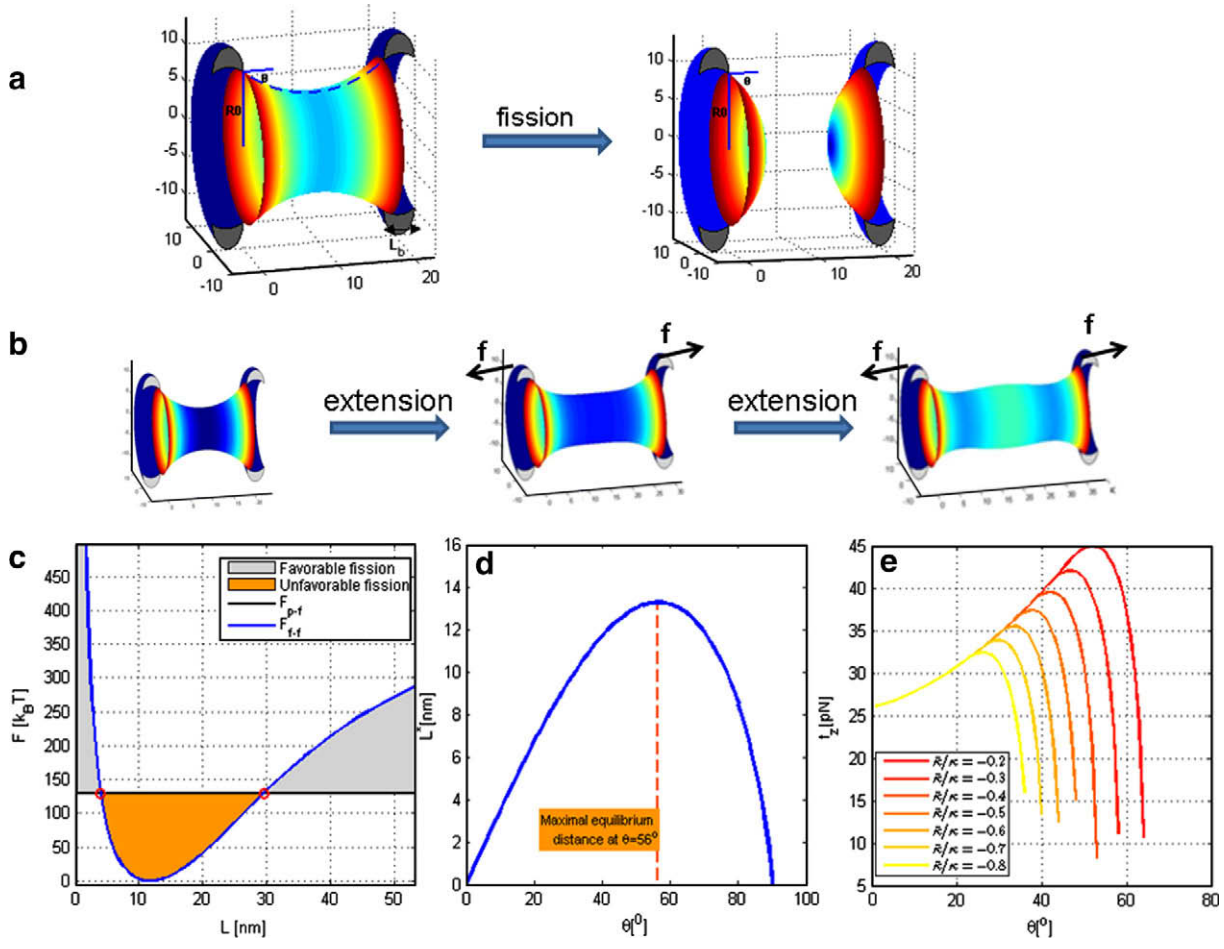

Fig. 2. Computational results of a physical model of membrane shaping by EHD2 oligomers. The color code indicates the elastic deformation of the membrane. Computations were performed numerically using the COMSOL Multiphysics software. (a) The pre-fission (left panel) and post-fission (right panel) states of the system. Definitions of the attachment angle θ and the ring radius R_0. (b) Evolution of the membrane shape upon increase of the inter-ring distance by applying a pulling force f to the rings. (c) Membrane elastic energy in the pre-fission, F_{f-f}, and post-fission, F_{p-f}, states as functions of the inter-ring distance for $\theta = 40°$ and $\frac{\tilde{R}}{\kappa} = -0.2$. In the regions where $F_{p-f} < F_{f-f}$, the membrane fission can occur. Minimum of F_{f-f} corresponds to an equilibrium distance between the rings. (d) The equilibrium inter-ring distance as a function of the attachment angle θ for $R_0 = 11$ nm. (e) The pulling force needed to generate membrane fission for different values of the ratio $\frac{\tilde{R}}{\kappa}$.

sion. After fission occurs, the membrane will form two hemispherical caps attached to the EHD rings (Fig. 2a), which implies an exchange of the lipid material with the reservoir. Two requirements have to be satisfied for fission to happen. First, the membrane scission event has to be overall energetically favorable, meaning that the total energy of the system before fission must exceed the energy of the post-fission state. This is a necessary condition for the fission reaction, whose fulfillment ensures the general feasibility of the process, but does not guarantee that its rate will be sufficiently fast to make it biologically relevant. The rate of the fission reaction can be limited by the energy barriers related to the strongly deformed intermediate structures forming transiently in the course of the process. By analogy to the well understood process of membrane fusion (see for review [64,106,107]), we assume that the major energy barrier is associated with the hemi-fission intermediate, which is a structure where the internal monolayer of the membrane neck is already split, while the second monolayer is still intact [108]. To assure a feasible rate of fission, this energy barrier must be relatively low or vanishing.

Based on the Helfrich model of membrane elasticity, the energy of the membrane tube in the pre-fission state is calculated according to the formula $F_{f-f} = \frac{1}{2}\kappa_B \oint\!\!\oint_{A_{tube}} J^2 dA$, where the integration is performed along the membrane area between the two protein rings.

In the post-fission state, the system energy F_{p-f} includes also a contribution of the Gaussian curvature term and is given by $F_{p-f} = 8\pi\kappa(1 - \sin(\theta) - \frac{1}{2}|\frac{\bar{\kappa}}{\kappa}|)$.

The computed shapes of the membrane tube for different inter-ring distances and a fixed value of the attachment angle $\theta = 56^\circ$ are presented in (Fig. 2b). Dependence of the tube energy on the inter-ring distance is illustrated in (Fig. 2c) for a specific value of the attachment angle $\theta = 40^\circ$. This dependence is non-monotoneous and characterized by a distance L^* at which the energy is minimal and equals zero. L^* is the preferred equilibrium distance between the rings, which is established spontaneously and corresponds to a vanishing force acting between the protein oligomers. The dependence of L^* on the attachment angle θ is presented in Fig. 2d according to which a maximal equilibrium spacing between the rings, L^*_{max}, has to be observed if $\theta \approx 56^\circ$. The value of this maximal spacing, L^*_{max}, is related to the ring radius, R_0, by $L^*_{max} = 1.326R_0$. Comparing this prediction with the experimentally determined inter-ring spacing of about 14 nm (Fig. 1b) and taking into account that the ring radius is $R_0 \approx 11$ nm, we conclude that the observed ring arrangement is, indeed, in agreement with that predicted by the membrane elasticity model, and that the membrane-protein attachment angle must be close to 56°.

Now, we analyze the conditions for fission of the membrane tube between the protein rings. To reach fission conditions, axial forces have to be applied to the protein rings bringing them to distances different from the equilibrium distance L^* and resulting in accumulation of the membrane elastic energy. If this energy F_{f-f}, corresponding to the pre-fission state (Fig. 2a, left panel) exceeds the energy F_{p-f} of the post-fission state (Fig. 2b, right panel), the fission process becomes energetically favorable. This may happen if relatively large or short inter-ring distances are obtained as illustrated in (Fig. 2c) for a specific value of the attachment angle $\theta = 40^\circ$ and the ratio between the modulus of Gaussian curvature and the bending modulus $\frac{\bar{\kappa}}{\kappa} = -0.2$. Formally, according to Fig. 2c, membrane fission becomes favorable energetically if the inter-ring distance L exceeds 30 nm or becomes smaller than 4 nm. However, in practical terms, fission at small distances is unfeasible since the resulting separate membranes would overlap. Therefore, to generate conditions for membrane fission, the protein rings have to be brought to a sufficiently large distance, which requires application of forces, f, pulling the rings from each other (Fig. 2b). The inter-ring distances which have to be reached and the values of the pulling forces which have to be applied for fission to become possible are presented in (Fig. 2d and e). The predicted forces do not exceed a few tens of pN, meaning that they can be developed by an ensemble of molecular motors or self-assembly of several cytoskeletal polymers such as actin filaments or microtubules.

Altogether, the results of the analysis above demonstrate that membrane shaping by the ring-like oligomers of EHD2 can be successfully described by the Helfrich model of membrane elasticity. More importantly, the modeling predicts that the EHD2 rings can serve as a key element of a membrane fission machinery which must involve, however, the force-producing elements generating forces in a range of few tens of piconewtons. In addition, according to the calculation based on the model above (not shown) the fission reaction may be slow since vanishing of the hemi-fusion energy barrier required unfeasibly large values of the attachment angle, $\theta > 70^\circ$. Hence additional protein players may be required to accelerate the fission reaction by reducing its energy barrier.

4. Hydrophobic insertion mechanism: membrane bending by amphipathic helices

4.1. Modeling membranes as thick elastic layers

Generation of large membrane curvatures by N-BAR domains, epsins, and small GTPases is mediated by shallow insertions into the lipid monolayer matrix of small amphipathic helical domains having an effective rod shape with dimensions between one and two nanometers (see e.g. [14]). Also the synaptotagmin C2 domains bend membranes by embedding into the membrane interior their hydrophobic loops of about a half nanometer length [45,46,48]. In all these cases the inserting protein domains are smaller than or comparable with the lipid monolayer thickness. Analysis of membrane deformations produced by such protein insertions cannot be performed by considering the membrane as a thickness-free elastic surface and, hence, the Helfrich model of membrane bending cannot be applied. Indeed, small protein insertions generate a complex array of strains and stresses within the membrane matrix directed both along and transverse to the membrane plane and changing both throughout the membrane thickness and along the membrane surface [84]. These strains determine the overall membrane deformation, which can be described by an effective curvature. To account for these strains and stresses, determine their distributions and find the resulting membrane shape, the lipid monolayer has to be considered as a thick elastic layer.

Treatment of membranes as thick layers has been performed according to two different strategies. The one, referred to in the following as the macroscopic approach, is based on consideration of the monolayer interior as an elastic three-dimensional continuum described by the traditional theory of elasticity. Within this approach, a lipid monolayer is characterized by two sets of physical factors – the trans-membrane stress profile and the trans-membrane elastic moduli profile. The trans-membrane *stress profile* was introduced in the beginning of the era of modeling membrane elasticity [96,109,110] to account for the hypothetical local stresses existing at every point of the membrane matrix and changing throughout the lipid monolayer thickness. Since then, the notion of the trans-membrane stress profile has been considerably developed and used for the treatment of different membrane phenomena such as the origin of the monolayer spontaneous curvature, the influence of the membrane lipid matrix on protein binding to membranes, and the conformational transitions of membrane inserted proteins (see e.g. [111–115]).

Recently, the notion of the trans-membrane *elastic moduli profile* was introduced within the context of lipid monolayer/bilayer deformations generated by small protein insertions [84]. It is

important to emphasize that elastic stresses and elastic moduli are fundamentally different characteristics of membranes. The elastic moduli characterize the material properties of the membranes and depend only on the membrane structure and the intra-membrane interactions. In contrast, the elastic stresses are the membrane's reply to application of external forces and, therefore, depend also on these forces in addition to the properties of the membrane itself. The local *elastic moduli* distributed throughout the membrane thickness determine the changes of the local *elastic stresses* as a result of generation of local strains within the membrane matrix. A specific trans-membrane profile of the local elastic moduli was suggested in [84] based on the known values of the overall elastic moduli of a lipid monolayer as a whole. It is to be expected that a more exact character of distributions of the elastic moduli through the lipid monolayers will be elaborated in the near future.

Taken together, the macroscopic approach does not use any explicit information about the interactions between the elements of lipid molecules and their dynamics. It uses effective distributions through the monolayer thickness of the local stresses and elastic moduli, which can be justified based on experimental knowledge of the overall elastic properties of lipid monolayers such as their spontaneous curvature, bending, stretching and tilt elasticities [84].

An alternative group of approaches, referred below to as the microscopic approaches, is based on consideration, at some level of approximation, of the inter-monolayer interactions and the statistical–dynamical properties of the lipid molecules and their parts. One of the most efficient models of this kind employs the numerical methods of statistical physics of the hydrocarbon chains of lipid molecules, which is based on the so-called mean-field approximation (see for review [111]). The essence of other powerful microscopic approaches is simulation of the lipid bilayer interior by the methods of molecular dynamics or Monte Carlo using a coarse-grain or atomistic detailed representation of lipid molecules (see for recent reviews [116,117]). These methods have been used to simulate self-assembly of lipid molecules into bilayers, to recover the membrane elastic properties, and to simulate structural rearrangements of the bilayers during their fusion and other membrane phenomena (see for review [116]). The general feature of microscopic approaches is the use of a number of parameters and functions such as the "force field", which characterize the intra-membrane interactions and are not accessible to a direct experimental verification. These unknown parameters have been commonly found by fitting the computed physical characteristics of the membranes such as the overall elastic moduli or the temperatures of the lipid phase transitions to the corresponding values measured experimentally.

4.2. Membrane bending by N-BAR domains

Approaches considering the lipid monolayers as thick elastic layers were recently applied to analyze generation of membrane curvature by the whole N-BAR domains [82,83] and, separately, by the BAR scaffolds [83,85] and small membrane insertions such as amphipathic helices [82,84,118]. One of the major goals of these studies was to find out whether the membrane scaffolding by the crescent-like BAR dimer alone is responsible for the membrane shaping by the N-BAR domains, or if insertion of the terminal amphipathic helices is crucial for membrane bending, or if the interplay between these two factors is indispensable for generation of the experimentally observed membrane shapes. The molecular dynamic simulations [83] showed that the scaffolding effect of N-BAR might be responsible for generation of the observed membrane curvatures, but this required a sufficiently strong binding of the protein module to the membrane surface. This membrane-protein interaction responsible for this binding must exist in addition to the electrostatic interaction between the positively charged concave surface of the protein and the negatively charged polar heads of the acidic lipids such as PIP2 [82,83]. According to these works, the role of the amphipathic helices of the N-BAR domains consists, mostly, in generation of this tight BAR-membrane binding, although a direct contribution of the amphipathic helices to the curvature generation is also possible for large amounts of the latter.

The works [84,118] analyzed the separate effect of insertions into the membrane matrix of small hydrophobic inclusions such as amphipathic helices or hydrophobic peptides. The model presented in [118] did not include the elastic contribution of the lipid polar heads, which appears to be essential for the effects of amphipathic helices, which embed into the lipid monolayer to a shallow depth and disturb mainly the region of the polar groups [14]. The macroscopic approach employed in [84] accounts for the elasticity of all parts of the lipid membranes including that of the polar heads and, hence, should describe the realistic membrane shapes generated by the small insertions. In this work, the amphipathic helices with their side chains were modeled as cylindrical rods of 1.2 nm diameter and 2.5 nm length which, according to [14], embed into the monolayer matrix such that the rod axes are oriented along the membrane surface. Simplistically, such a rod can be viewed as a wedge inserted into the lipid monolayer (Fig. 3a). For the case where the rods are ordered in rows on the membrane surface, and the initially flat membrane becomes tubular, the typical calculated shape of the tube cross-section is presented in (Fig. 3b) [84]. While this cross-section is not ideally circular, it can be characterized by an effective curvature (Fig. 3b). For the sake of the present review, we extended the computations of [84] to a more general case of unordered inclusions, where each rod, independently of the others, deforms the membrane in its vicinity. A characteristic computational result for deformation of a membrane patch by one rod-like inclusion mimicking an amphipathic helix is presented in (Fig. 3c). The color code in (Fig. 3c) shows the distribution of the effective curvature of the monolayer surface as a function of the angle determined with respect to the axis of the rod. The computation shows that very close to the rod, at distances comparable with the rod length, the membrane curvature is anisotropic, i.e. depends on the angle, as could be expected for an anisotropic wedge-like inclusion. However already beginning from the distances equal, approximately, to the rod length, the membrane curvature becomes, practically, axially symmetric, as if the inclusion would have a conical rather than the wedge-like shape. This averaging of the membrane curvature over the angles making the membrane deformation isotropic in spite of the inclusion asymmetry is due to the lateral fluidity of the lipid bilayer. As a result, a membrane fragment with an amphipathic helix in the middle has an effective funnel-like shape, which can fit to a sphere with a certain radius and the corresponding total curvature. The curvature of this sphere quantifies membrane bending by the inclusion.

The computations for the case of a cylindrical shaping of membranes by rows of inclusions (Fig. 3b) [84] and the more general case of bending by an individual inclusion (Fig. 3c) predicted, practically, the same value of the induced membrane curvature depending on the inclusion density on the membrane surface and the depth of the inclusion insertion (results not shown). Remarkably, the induced curvature is predicted to be maximal for the typical insertion depth of the amphipathic helices, constituting about 40% of the monolayer thickness, which is very close to the experimentally determined value of the helix embedding into the membrane matrix [14]. It is convenient to regard the insertion together with the adjacent portion of the deformed lipid matrix as an effective membrane particle. The spontaneous curvature of such a particle corresponding to an amphipathic helix

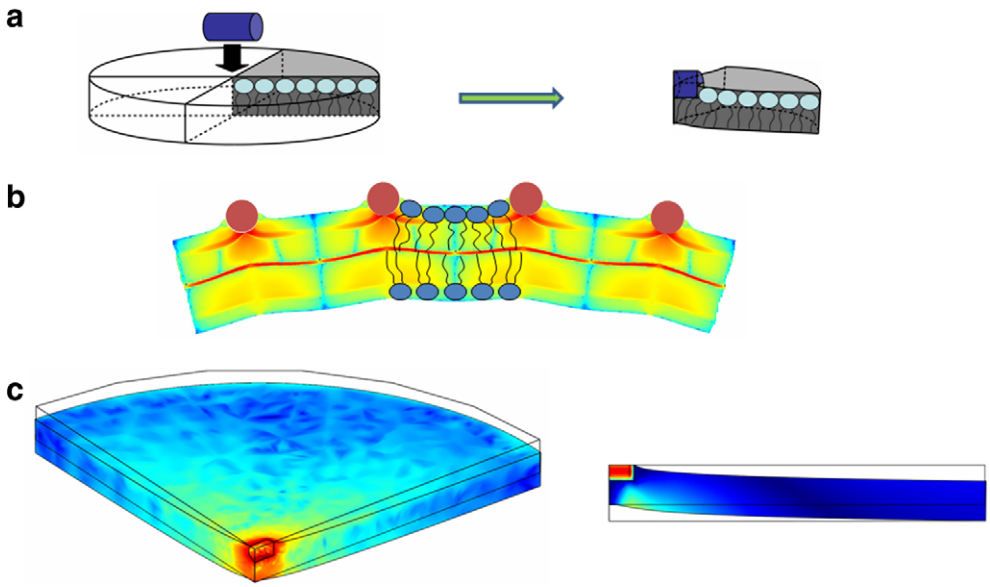

Fig. 3. Hydrophobic insertion mechanism of membrane bending. Modeling of membrane curvature generation by rod-like particles imitating amphipathic helices. The computations were performed by COMSOL Multiphysics software. (a) Insertion of a helix. (b) Computational result for membrane bending by rows of rods. The figure shows an element of cross-section of a resulting tubule-like membrane [84]. (c) Computational result for bending of a membrane element by a single insertion. Left panel – a 3D view, right panel – a cross-section element.

inserted to the preferable depth constitutes about 0.75 nm^{-1} [84]. This is more than two times larger than the spontaneous curvature of lysophosphatidylcholine, which is characterized by a maximal positive spontaneous curvature measured to date for individual lipids (see for review [15]) and belongs to the class of so-called "non-bilayer" lipids which tend to self-organize into curved micelles rather than flat bilayers. Hence, the amphipathic helices are predicted to be much more powerful agents of generation of positive curvature than the "non-bilayer" lysolipids. Finally, the computations demonstrated that the fraction of membrane surface which has to be occupied by the amphipathic helices in order to generate the experimentally observed membranes tubes of 35-50 nm diameter [14,23] varies in the range of 7–10% [84]. These are feasible concentrations of the amphipathic helices, since they are considerably smaller than the maximal possible fraction constituting about 50% for endophilin and 25% for amphiphysin BAR domains.

The conclusion of the analysis by the macroscopic approach [84] is that the sole insertion of the amphipathic α-helices into the membrane matrix can be responsible for the membrane curvature generation by the N-BAR domains. This conclusion has been supported by a recent numerical analysis of N-BAR-membrane complex performed by self-consistent mean-field theory [85] which took into account the whole set of interactions between the BAR domain and the membrane containing acidic lipids. According to these computations, insertion of the amphipathic helices must serve as the major factor of membrane bending. The likely role for the membrane scaffolding by the BAR domain mediated by the electrostatic attraction between the concave protein face and the membrane surface is to stabilize this curvature [85].

Taken together, the recent theoretical works [84,85] indicate that the amphipathic helices are necessary and sufficient to generate the membrane curvatures produced by the N-BAR domains. Clearly, separate computations are needed to analyze membrane bending by other BAR domains such as F-BAR domains, which do not possess the amphipathic anchors, bind to the membranes only due to the electrostatic interactions with the acidic lipid head groups, have, in some cases, more complicated shapes, orient on the cylindrical membrane surface obliquely to the cylinder axis and generate smaller curvatures than those produces by N-BAR domains [119,120].

5. Conclusions

Generation of membrane curvature and membrane remodeling by fusion or fission are two types of cellular membrane shaping catalyzed by specialized proteins. Currently, the list of proteins which have been shown or suggested to be involved in one or both types of membrane shaping in different cell systems is rapidly growing. The major tasks of modeling are to try revealing the common mechanistic principles of action of various proteins, which are totally diverse in terms of their biochemical and structural natures, and to predict whether the same protein or protein complex can drive several types of membrane shaping and remodeling. The key to approaching these tasks is in understanding the elastic behavior of membranes in the course of their shaping, and in quantitative determination of the forces and energies the proteins have to generate in order to drive the required membrane transformations. At the current stage, several models have been proposed for the membrane shaping action of a few specific proteins but a general understanding remains the matter of the future work.

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