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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