MEMBRANES

Shaping biological matter

Biological membranes form an extremely complex and dynamic network in cells, guided by specialized protein machinery. A new algorithm analyses membrane shape to extract forces applied by proteins controlling the membranes.

Vadim A. Frolov and Joshua Zimmerberg

The space occupied by living organisms is determined by membranes. Each cell is delimited by a membrane, and additional membranes form the functional hierarchy of compartments packed within it. The intracellular compartments, for example vesicles and organelles, define the spatial organization of metabolic pathways, thus providing the topological framework for cellular life. Recent advances in structural techniques and super-resolution microscopy now allow imaging of intracellular membrane structures with unprecedented resolution. Paul Wiggins and co-workers[1] are, perhaps, first to notice that the wealth of membrane structures obtained awaits detailed elastic modelling.

The core of each membrane is the lipid bilayer, the bimolecular film consisting of two monolayers of amphiphilic molecules, the lipids. This bilayer is primarily responsible for the barrier function of membranes. However, the lipids are also deeply involved in regulation of membrane shape and dynamics: both depend on the material properties of the lipid bilayer[2,3]. One property is the elasticity, the resistance to stretching and compression that accompanies most of the deformations of lipid bilayers imposed by the specialized proteins that determine the shape of cellular membranes. The lipid bilayer can be approximated as a continuous elastic surface whose behaviour is characterized by a limited set of bulk material parameters[4]. Despite its relative simplicity, the elastic approximation has been successfully used to explain a wide variety of membrane phenomena, from the multitude of shapes of red blood cells[5] to the structure of metastable intermediates of membrane fission and fusion[6].

How can continuum elastic modelling help us to understand the action of specialized proteins creating cellular shape (and thus how the shape is encoded) at small, almost molecular scales? Proteins can be simply considered as a new bulk component effectively altering

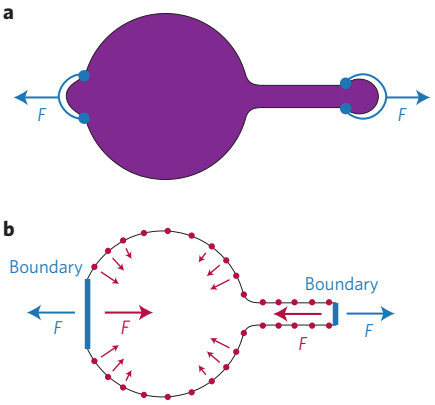

Figure 1 | Calculation of forces acting on a membrane boundary from the membrane shape. **a**, Illustration of a spherical membrane vesicle with a cylindrical tether pulled from it by application of an external force (*F*). **b**, The membrane shape obtained from images is parameterized by control vertices (red dots); an effective boundary on which the external forces act is introduced (blue); small perturbations of the vertices from their stationary positions (red arrows) yield local internal forces due to elastic response of the membrane. The total internal force (*F*, blue) projected to the tether axis is equal to the external force (*F*, red) acting on the boundary.

averaged elastic parameters of the lipid bilayer. Different algorithms linking protein embedment into a membrane to elastic deformations have recently been developed[2,7,8], yielding phenomenological descriptions of membrane morphogenesis in cells.

The Wiggins group take a different approach. They assume that proteins act externally, by applying forces required to support a membrane shape from its boundary. The force distribution can be calculated by analysing the membrane shape within this boundary, by the established algorithms of shape parameterization and localized force balancing[5,7,9]. The researchers tested this approach on a simple experimental system in which a thin membrane cylinder (tether) was pulled from a giant vesicle (Fig. 1a). The pulling force, controlled experimentally, was compared with those obtained by membrane shape analysis. For that, the membrane was parameterized by an arbitrary set of control vertices, excluding the areas of the external force application (Fig. 1b). Then the force balance at each vertex was obtained using a proximal equilibrium approximation[6], that is, by making the virtual work required to displace the vertices equal to the corresponding energy of elastic deformations. The minimization was conducted by conventional procedures[5,7], taking into account elastic deformations and the work of hydrostatic pressure and lateral tension. Finally, they obtained the external force acting at the membrane boundary to cancel the internal ones exactly (Fig. 1b). This force correctly estimated the pulling force, as expected given the decades of studies on membrane shapes that have used the giant vesicle prototype (see, for example, refs 9, 10). However, Wiggins and colleagues emphasize that the proximal equilibrium approximation[1] is tractable for shapes of greater complexity because, as is common for algorithms based on local balancing of forces and moments[5,7], it does not explicitly rely on assumptions of shape symmetries generally used to analyse simple membrane vesicles.

This notion is yet to be tested. Conceivably, it is even more important that the work of Wiggins's group reiterates the importance of membrane boundary in supporting a bulk elastic stress[11]. This conceptualization could be incisive when studying how particular protein complexes control the shape of cellular membranes.

Such proteins often have to restrain large membrane areas and/or create a substantial membrane stress, examples being the protein rings supporting the large structure of the tubular endoplasmic reticulum. This membrane system makes up the bulk of the internal membranes

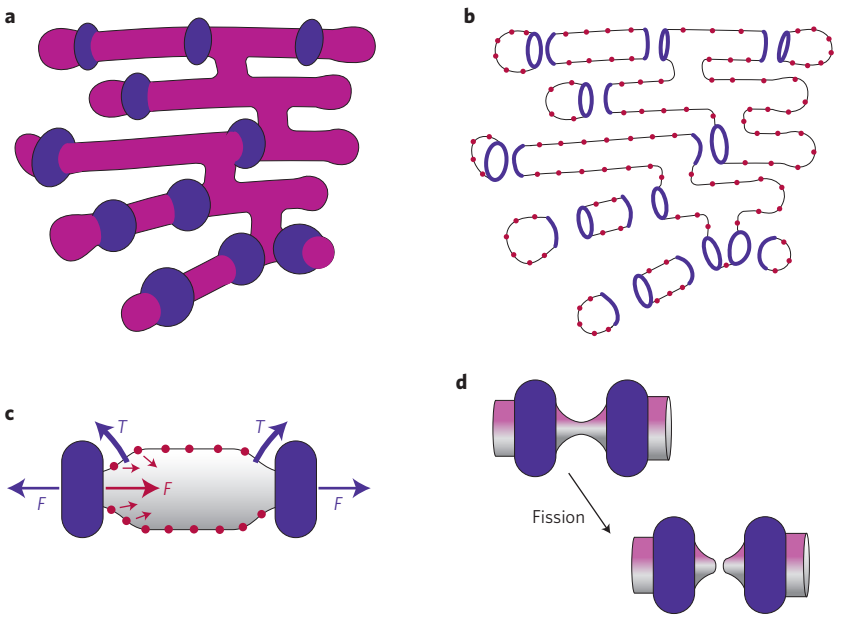

Figure 2 | Hypothetical algorithm of calculation of forces shaping an organelle. **a**, Cartoon illustrating the endoplasmic reticulum shape controlled by rings of specialized proteins (blue). **b**, The shape is parameterized by a set of vertices labelling 'free' membrane pieces. **c**, From the shape analysis, the forces (*F*) and torques (*T*) exerted by the rings are calculated[1,9]. **d**, Protein rings can also enforce membrane narrowing, leading to shape instability and, ultimately, membrane fission[13].

of a cell, and a substantial part of it needs to remain freely accessible for the endoplasmic reticulum to communicate with other organelles[12]. The forces applied by these boundary rings can be revealed by analysing the shape of the 'free' pieces of the membrane (Fig. 2a–c). Protein assembly into ring-like structures is also responsible for shaping the intermediates of membrane fusion and fission[6]. In these cases the need for multiple proteins is stipulated by the high energy requirements of the processes, and because of the large size of the proteins involved, the proteins' assembly can be seen as controlling membrane remodelling through a ring-like boundary[6,13] (Fig. 2d).

The recent reconstitution of endoplasmic reticulum morphogenesis and membrane remodelling in simple systems containing a few proteins and lipid matrix[12,13] is expected to make the membrane shape analysis proposed by Wiggins and co-workers more straightforward. If proven successful in model systems, their algorithm may provide unique information about the forces applied by proteins in their physiological environment, where direct mechanical measurements of forces are not possible. Such measurements can be particularly useful in the case of cell and tissue damage associated with the alterations of lipid metabolism. Perhaps slight changes in the bulk material properties of the membrane will be revealed by organelles that are distorted from their equilibrium shapes. These could then be related to specific lipid species to reveal the chemical basis of the changes in membrane material properties. ❐

Vadim A. Frolov and Joshua Zimmerberg are part of the Program on Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland 20892-1855, USA.
e-mail: zimmerbj@mail.nih.gov

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