

Curvature, Lipid Packing, and Electrostatics of Membrane Organelles: Defining Cellular Territories in Determining Specificity

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<http://dx.doi.org/10.1016/j.devcel.2012.10.009>

Whereas some rare lipids contribute to the identity of cell organelles, we focus on the abundant lipids that form the matrix of organelle membranes. Observations using bioprobes and peripheral proteins, notably sensors of membrane curvature, support the prediction that the cell contains two broad membrane territories: the territory of loose lipid packing, where cytosolic proteins take advantage of membrane defects, and the territory of electrostatics, where proteins are attracted by negatively charged lipids. The contrasting features of these territories provide specificity for reactions occurring along the secretory pathway, on the plasma membrane, and also on lipid droplets and autophagosomes.

Introduction

Numerous molecular processes occur at the surface of organelles through the reversible association of proteins from the cytosol. These include signal transduction cascades, nucleation of cytoskeleton structures, formation of transport vesicles and lipid metabolism pathways. In the latter three cases, the organelle membrane is remodeled either physically or chemically. Not surprisingly, cells have built detectors to monitor these changes, allowing the establishment of feedback loops to amplify or arrest the remodeling processes. Herein, we discuss recent examples of such detectors and molecular circuits. Some of these examples seem unrelated: the budding of transport vesicles, the phagocytosis of bacteria, and the growth of lipid droplets are obviously very different events. Yet despite their heterogeneity, what unifies these processes is the fact that deceptively simple physical parameters such as membrane charge density, membrane curvature, or lipid packing can be informative inputs to create sharp temporal and spatial responses.

Biochemistry on Lipid Membrane Surfaces

Let's begin with some hallmarks of biochemistry on membrane surfaces. First, lipids are small compared to proteins. In a bilayer, a lipid exposes a surface of $\approx 0.7 \text{ nm}^2$. Therefore, when sitting on a membrane, a protein generally covers several lipids: a dozen in the case of a $\approx 20\text{-kDa}$ small G protein (Liu et al., 2010); a hundred in the case of some elongated proteins (e.g., a BAR domain) (Peter et al., 2004). Second, although some proteins carry a domain specific for a lipid polar head group, peripheral proteins frequently use a combination of weak binding motifs such as lipid modifications or unfolded sequences (McLaughlin and Aderem, 1995). Thus, the adsorption of a protein to a membrane can seldom be reduced to a bimolecular scheme, and binding constants are generally apparent, reflecting multiple interactions. Third, lipids are not evenly accessible. Crossing the layers that separate the polar head group from the acyl chains is not trivial and in some cases the main issue is not lipid recognition per se, but the detection of defects in the geomet-

rical arrangements of lipids (Attard et al., 2000; Davies et al., 2001). Altogether, these considerations suggest that biochemistry on membrane surfaces is a branch of soft matter physics: collective effects arising from multiple low energy interactions have at least the same importance as bimolecular stereospecific interactions.

Recognition of Organelles by Peripheral Proteins

Cytosolic proteins are surrounded by membrane surfaces equivalent to tens of millimolar lipids. This value implies that strong affinity is not needed; instead the main issue is specificity: how to distinguish one membrane-bound compartment from others. For this aim, a few general strategies have been uncovered. The most straightforward is the specific recognition of rare lipids, most prominently phosphoinositides, which display a decorated polar head group and are restricted to specific compartments (Di Paolo and De Camilli, 2006). The second strategy is kinetic trapping. The protein visits all membranes in a reversible manner through a weak anchor. On the proper compartment, a molecular event occurs that dramatically decreases the protein desorption rate. Two well-known examples are the addition of a second lipid modification (Rocks et al., 2005; Shahinian and Silvius, 1995) and the conformational changes undergone by Arf family G proteins (Liu et al., 2010). The third strategy, which is the topic of this review, is the recognition of a physicochemical parameter of the membrane such as its curvature, its electrostatics, or lipid packing (Figure 1).

Membrane Electrostatics

In eukaryotic cells, membrane electrostatics largely depends on phosphatidylserine (PS), a negatively charged lipid, the amount of which ranges from a few percent in the endoplasmic reticulum (ER) to more than 10% at the plasma membrane (PM) (Holthuis and Levine, 2005). Remarkably, results of recent experiments suggest that PS is mostly present on the luminal side of the ER, an asymmetric distribution opposite to that of the PM where PS faces the cytosol (Fairn et al., 2011; Kay et al., 2012). Consequently, the gradient of accessible PS seen by cytosolic

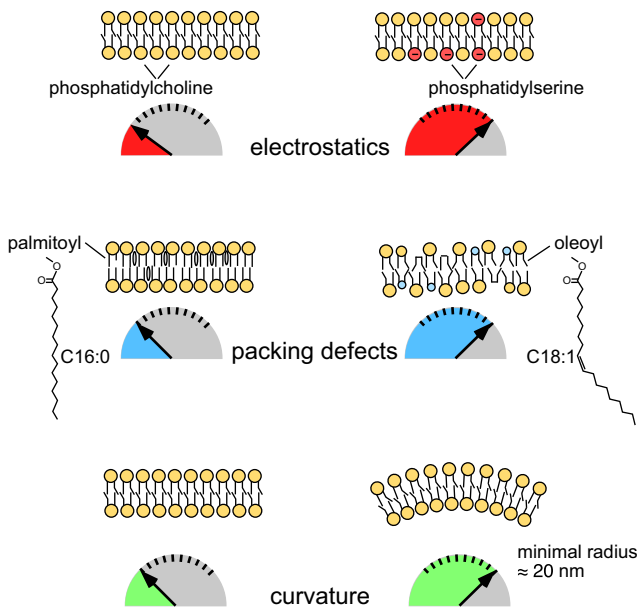


Figure 1. Three Physicochemical Parameters for Lipid Membranes
Membrane electrostatics depends on the fraction of negatively charged lipids such as phosphatidylserine (PS) and phosphoinositides. Packing defects are promoted by lipids with unsaturated acyl chains and/or small head group. Membrane curvature, which also results in lipid-packing defects, can reach values of 20 nm (radius) compared to 4 nm for membrane thickness.

proteins along membranes of the secretory pathway is probably sharper than what is suggested by bulk measurements (Figure 2A). The highly charged phosphoinositide phosphatidylinositol(4,5)bisphosphate (PIP₂) accentuates this trend because it is restricted to the inner leaflet of the PM (Di Paolo and De Camilli, 2006). The localization of PS and phosphoinositides probably explains the remarkable localization of simple cytosolic bioprobes carrying a defined number of positively charged residues: they label only membranes of the late secretory pathway and in a manner that correlates with their charge density (Yeung et al., 2006).

As rudimentary as it may seem, electrostatics stringently governs the localization of peripheral proteins. Variants of G proteins are good examples of the potency of electrostatics for precise localization. Rac1 and Rac2 are attached to the PM and to endosomes, respectively, owing to the different number of basic residues in their C-terminal region (Magalhaes and Glogauer, 2010; Ueyama et al., 2005; Yeung et al., 2006). K-Ras, the sole Ras variant to harbor a polybasic tail, is restricted to the PM, whereas H-Ras and N-Ras also explore membranes of the early secretory pathway owing to the addition of a second lipid modification (Ahearn et al., 2012). Bacterial toxins targeting these small G proteins also use electrostatics (Mesmin et al., 2004).

In liposome reconstitution assays, the effect of electrostatics can be very steep. WASP, a nucleator of actin filaments, promotes actin polymerization in a narrow range of PIP₂ (Papayanopoulos et al., 2005) because it does not probe PIP₂ through a specific site but via a cryptic polybasic region, which, like Velcro, binds firmly only above a threshold of negative charges. Finally, and this is the hallmark of nonspecific electrostatic

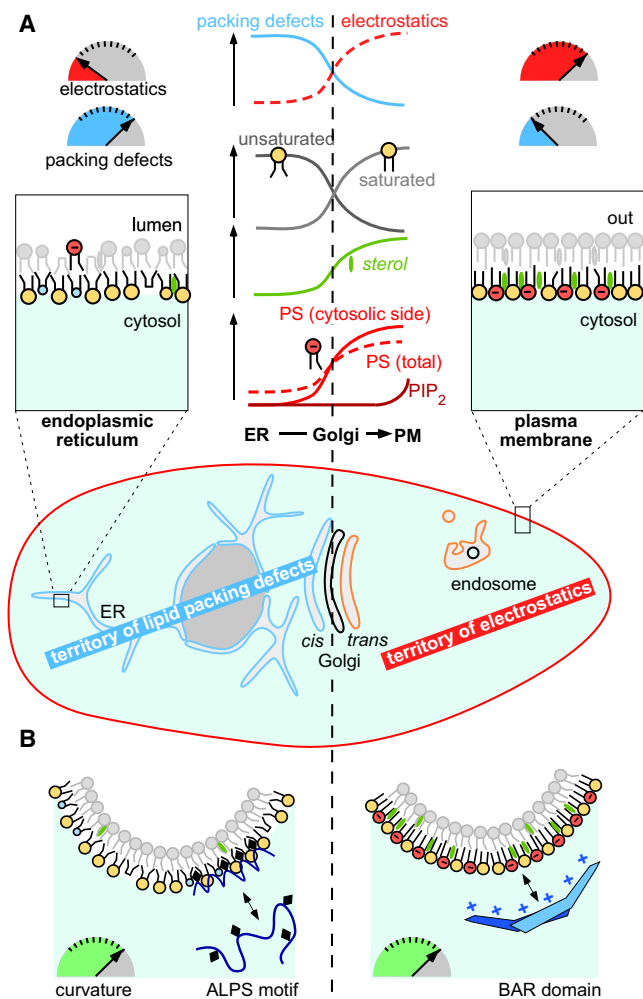


Figure 2. Two Membrane Territories

(A) Lipidomic analysis and bioprobe distribution suggest two main membrane territories in the cell. ER and *cis*-Golgi membranes are poorly charged on their cytosolic leaflet but display lipid-packing defects owing to the presence of lipids with monounsaturated chains. Membranes from the *trans*-Golgi to the plasma membrane (PM) harbor negative lipids on their cytosolic face but are tightly packed as their lipids are more saturated.

(B) Curved membranes are present everywhere in the cell, but curvature sensors are adapted to each territory: ALPS motifs to early membranes as they insert hydrophobic residues into lipid-packing defects; BAR domains to late membranes as their positive concave face fits with negatively charged membranes.

interactions, binding depends on the number of positive residues, but is poorly sensitive to the exact amino acid sequence.

In conclusion, electrostatics seems to define two territories (Figure 2A): membranes of the early secretory pathway whose cytosolic leaflet is weakly charged (ER, *cis*-Golgi), and membranes of the late secretory pathway whose cytosolic leaflet is highly charged (endosomes, PM).

Lipid-Packing Defects

The concept of lipid-packing defects pertains to the idea that biological membranes display imperfections in the geometrical arrangement of their lipids because they contain substantial levels of lipids whose shape departs from the canonical

cylindrical shape (Janmey and Kinnunen, 2006; van den Brink-van der Laan et al., 2004). For example, phosphatidylethanolamine (PE) and diacylglycerol are defined as conical since their polar heads are smaller than that of phosphatidylcholine (PC). Lipid geometry depends also on the acyl chains. An oleyl chain (C18:1) occupies a larger volume than a palmitoyl chain (C16:0) because the double bond induces a “kink” in the middle of the chain (Figure 1). Therefore, lipid packing depends on two ratios: the ratio between small and large polar heads and the ratio between unsaturated and saturated acyl chains. In addition, sterols, which pack preferentially along lipids with saturated chains, notably sphingolipids, improve lipid packing. Under extreme conditions, tight lipid packing leads to the formation of a liquid-ordered phase. However, even within the classical liquid disordered phase, variations in the packing of lipids can be large enough to dramatically influence the binding of peripheral proteins (Antonny et al., 1997; Attard et al., 2000; Davies et al., 2001; Matsuoka et al., 1998).

Our knowledge of the lipidome of most cells remains rudimentary. Nevertheless, several important features are emerging thanks to recent progress in lipid mass spectrometry. First, when a single phospholipid type is considered, its acyl chain composition can display significant differences depending on the organelle. In yeast, PS and PE are more saturated at the PM than at the ER (Schneiter et al., 1999; Tuller et al., 1999). In neurons, polyunsaturated lipids are more abundant in the axon than in the cell body (Yang et al., 2012). Second, manipulations aimed at altering the ratio between conical and nonconical lipids suggest that this ratio is tightly regulated through compensatory mechanisms (Boumann et al., 2006). Overall lipid packing probably increases along the secretory pathway (Figure 2A) (Brügger et al., 2000; Holthuis and Levine, 2005; Klemm et al., 2009). At one extreme, the ER is characterized by loose lipid packing owing to the abundance of unsaturated phospholipids and to the scarcity of cholesterol, which is tightly regulated (Bretscher and Munro, 1993; Radhakrishnan et al., 2008). At the other extreme, the PM is characterized by tight lipid packing due to the presence of saturated lipid species and a high sterol level.

The importance of loose lipid packing at the ER is beginning to emerge. In an extensive screening, 65 fatty acids of varying length and saturation have been tested for their ability to alleviate ER stress in a yeast strain deficient in unsaturated phospholipid synthesis (Deguil et al., 2011). All fatty acids could be incorporated into phospholipids, but only unsaturated fatty acids restored growth. Interestingly, oleate, which bears a single *cis* double bond, was more beneficial than fatty acids bearing multiple *cis* unsaturations or a single *trans* unsaturation. Because the central kink in the oleate chain creates more distortion than any other unsaturations, deviation from the straight conformation and consequently defects in lipid packing seem critical for some functions of the ER.

The small G protein Sar1 is a nice example of the adaptation of a peripheral protein to loose lipid packing. Sar1 is the house-keeping G protein of the ER. Among all small G proteins, Sar1 displays the longest and most hydrophobic sequence for membrane attachment (Huang et al., 2001; Lee et al., 2005). Sar1 therefore strongly contrasts with Rac1 or K-Ras, whose binding is governed by electrostatics. Sar1 binds better to C18:1-C18:1 than to C16:0-C18:1 phospholipid membranes,

suggesting that its hydrophobic amino terminal residues insert preferentially into a bilayer with packing defects (Matsuoka et al., 1998).

In conclusion, the territories governed by lipid-packing defects might mirror those defined by electrostatics: membranes of the early secretory pathway seem to combine loose lipid packing and low electrostatic, whereas membranes of the late secretory pathway seem to combine tight lipid packing and high electrostatics (Figure 2A).

Membrane Curvature

The ER is composed of a network of tubules and sheets. The Golgi apparatus combines flat cisternae, fenestrations, tubules, and vesicles. In endocytic organelles, outward tubulations permit cargo protein recycling whereas inward invaginations engage cargo proteins in a degradation pathway. At the PM, flat regions coexist with invaginations and protrusions of different sizes, shapes, and dynamics (Shibata et al., 2009). Considering the two broad territories defined above, the question then arises as to whether membrane curvature should be considered independently from membrane electrostatics and lipid-packing defects or whether these parameters combine, at least to some extent. The two major classes of membrane curvature sensors, the BAR domains and the ALPS motifs, suggest that the division of territories between early and late membranes also applies to membrane curvature detection (Figure 2B).

All BAR domains are built on the same fold resulting in a crescent shape (Frost et al., 2009; Peter et al., 2004). BAR domains sense, stabilize, or induce membrane curvature in a manner that depends on protein concentration, protein self-assembly, and additional membrane-interacting regions such as amphipathic helices (Frost et al., 2009; Galic et al., 2012; Peter et al., 2004). Nevertheless, what unifies all BAR domains is their association with late membranes, such as the PM or endosomes. Put differently, a recurrent observation is that BAR domains, even when overexpressed, do not associate with the ER (Peter et al., 2004). This negative observation is informative since ER tubules are abundant and have a diameter of about 50 nm that should fit well with the concave face of many BAR domains (Shibata et al., 2009). However as pointed out above, the electrostatic of the ER is probably kept at minimum due to PS orientation toward the lumen (Fairn et al., 2011; Kay et al., 2012). Because BAR domains interact with membranes through a basic surface, the early secretory pathway is probably not adapted to this family of peripheral proteins.

ALPS motifs form a family of membrane-associated amphipathic helices that are defined by the abundance of serine, glycine, and threonine residues in their polar face (Antonny, 2011; Bigay et al., 2005; Drin et al., 2007). Three salient features characterize the binding of ALPS motifs to lipid membranes: a sharp dependency on membrane curvature, a high sensitivity to lipid shape (C16:0-C16:0 PC << C18:1-C18:1 PC), and no sensitivity to lipid charge (PC = PS). Mutagenesis studies suggest that ALPS motifs use their bulky hydrophobic residues to detect large lipid-packing defects that arise from the conjunction of positive curvature and the presence of lipids with conical shape but essentially ignore membrane surface charge (Drin et al., 2007). Thus, ALPS motifs do not sense membrane geometry per se but the stress corresponding to the mismatch

between the actual curvature of the membrane and the spontaneous curvature of its cytosolic leaflet (the curvature that this leaflet would adopt at equilibrium according to its composition and without the constraint of being associated with another leaflet; for discussion on the links between spontaneous curvature, curvature stress, lateral pressure profile, and lipid-packing defects see Attard et al., 2000; Davies et al., 2001; Janmey and Kinnunen, 2006; van den Brink-van der Laan et al., 2004; Antony, 2011). ALPS motifs have been found in proteins associated with the nuclear envelope (Nup133), the ER (Atg14L/BARKOR), and the *cis*-Golgi (ArfGAP1, GMAP-210), and thus seem adapted to membranes of the early secretory pathway (Cardenas et al., 2009; Doucet et al., 2010; Fan et al., 2011; Levi et al., 2008).

In conclusion, examination of the general features of the two main classes of membrane curvature sensors, the BAR domains and the ALPS motifs, further underlines the general division of territories between membranes of the early secretory pathway and membranes of the late secretory pathway. The code used in early membranes seems to be curvature and lipid-packing defects whereas the code used in late membranes is rather curvature and electrostatics.

Changing Membrane Properties

So far, we have considered three parameters and argued that they are tuned in such a way that they contribute to the identity of cellular organelles. However, cellular membranes are not fixed entities because they exchange materials and can mature (Bonifacino and Glick, 2004). Necessarily, these processes are accompanied by changes in the bulk properties of the lipid bilayer. On the one hand, these changes can be interpreted from the point of view of homeostasis: a way to maintain compartment identity, which otherwise would vanish due to membrane budding and fusion events. On the other hand, the examples presented below suggest that membrane electrostatics, lipid packing, and membrane curvature are active parameters in the sense that they contribute to the self-organization of reactions at the surface of organelles.

Phagocytosis: Decrease in Electrostatics Drives Membrane Maturation

Phagocytosis is the way specialized cells capture pathogens to eliminate them. The membrane surrounding the pathogen derives from the PM and initially displays its bulk features, with charged lipids (PS and PIP₂) enriched in the cytoplasmic leaflet promoting actin polymerization for pathogen engulfment (Yeung et al., 2009). Then the phagosome undergoes a maturation process that leads to its fusion with endosomal and lysosomal compartments and finally pathogen degradation.

Owing to their large size, phagosomes are ideal structures for following membrane maturation under a light microscope. With the parallel development of lipid probes (some specific and some adapted to membrane electrostatics), a clear picture is now emerging (Roy et al., 2000; Yeung et al., 2006, 2009). Once the phagosome membrane is detached from the PM, an abrupt decrease in PIP₂ is observed, allowing actin depolymerization (Figure 3A). Yet, the phagosome remains partially charged due to the persistence of PS. This sequential decrease in electrostatics favors a well-ordered change in the repertoire

of associated small G proteins and correct delivery to the lysosome (Magalhaes and Glogauer, 2010; Ueyama et al., 2005; Yeung et al., 2009). Not surprisingly, pathogens have developed survival strategies to resist degradation. *Salmonella typhimurium* injects a phosphoinositide phosphatase (SopB) whose action reduces both the PIP₂ and PS levels. Consequently, the proper sequence of small G protein recruitment cannot occur and the *Salmonella*-containing vacuole escapes lysosomal degradation (Bakowski et al., 2010).

Feedback Loops Linking Membrane Curvature and Vesicle Biogenesis

The budding of coated vesicles is one of the best-studied processes of membrane traffic (Bonifacino and Glick, 2004). In contrast to phagocytosis, it involves huge changes in curvature because the membrane ultimately forms a vesicle of ≈ 30 nm in radius compared to μm for phagosomes. This shape is the result of the mechanical actions of cytosolic proteins that together form a polymerized spherical coat. After vesicle formation, the coat starts to detach, at least partially, and subsequent steps proceed up to membrane fusion.

The COPI coat is attached to Golgi membranes by Arf1-GTP (Yu et al., 2012); the AP2-clathrin coat is attached to the PM by PIP₂ (Jackson et al., 2010). Although a small G protein and a phosphoinositide are anything but similar, a common basis unites these modes of attachment. The Arf1-binding sites on the COPI coat are spatially related to the PIP₂-binding sites on the AP2 complex (Jackson et al., 2010; Yu et al., 2012). Furthermore, the parallel between the two systems extends to their regulation (Figure 3B). Elimination of Arf1-GTP by the GTPase activating protein ArfGAP1 and PIP₂ hydrolysis by the phosphatase synaptojanin are both stimulated by membrane curvature (Bigay et al., 2003; Chang-Ileto et al., 2011). However, the analogy here is purely functional: the response of ArfGAP1 to membrane curvature relies on its ALPS motifs, whereas synaptojanin seems to respond to membrane curvature by interacting with the BAR-containing protein endophilin (Bigay et al., 2005; Chang-Ileto et al., 2011; Milosevic et al., 2011). These different mechanisms reflect the contrasting properties of the membranes on which the two coats act.

Although the reported effect of membrane curvature on the synaptojanin-endophilin tandem is modest compared to that observed on ArfGAP1, recent studies suggest possibilities for sharper regulation. Structural analysis of endophilin bound to membrane tubes indicates that its SH3 domain undergoes a monomer to dimer transition, which depends very precisely on the tube radius (Mim et al., 2012). Because endophilin recruits synaptojanin through its SH3 domain, the accessibility of this domain could serve to communicate the curvature state of the underlying membrane in a precise manner (Mim et al., 2012). Testing this hypothesis seems possible thanks to the development of micromanipulation assays allowing fine adjustment of membrane tube radius (Roux et al., 2010; Zhu et al., 2012).

Barkor: Interplay between Curvature and PI(3)P Synthesis for Autophagosome Formation

The autophagosome is a cup-shaped membrane compartment that engulfs organelles and part of the cytosol in a nonselective manner, thereby allowing cells to reduce their volume under

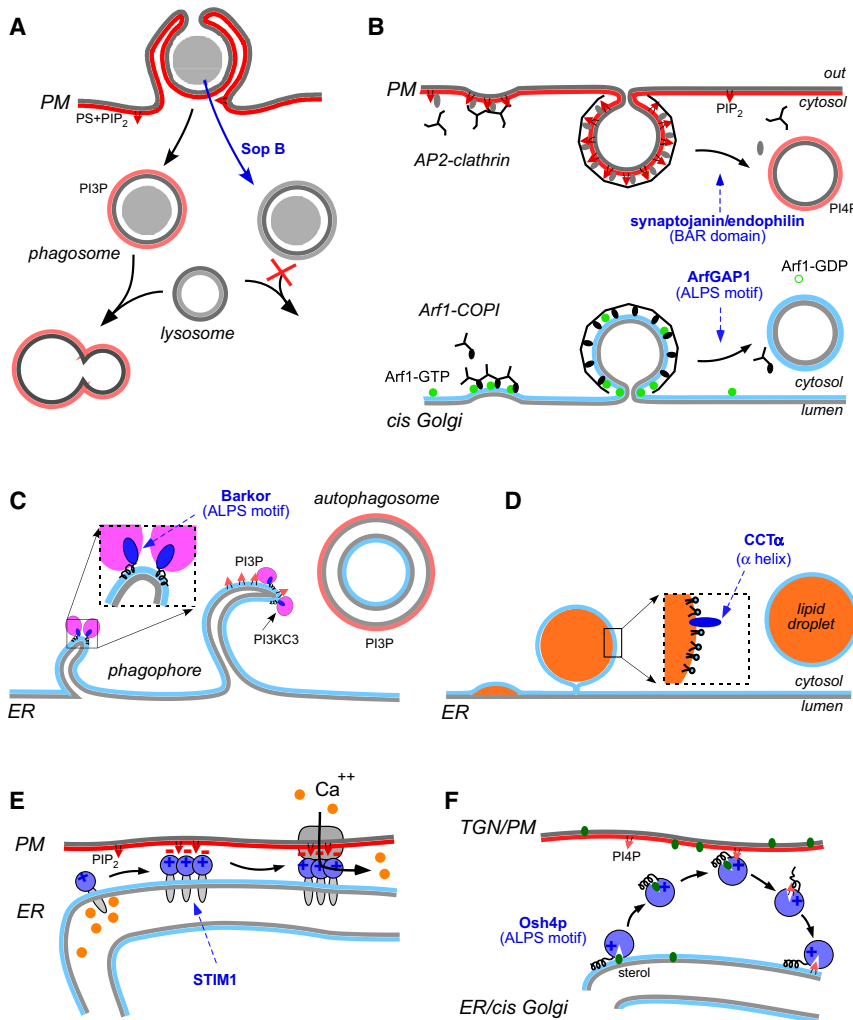


Figure 3. Cellular Events Controlled by Changes in the Physical Chemistry of Membranes

(A) During phagocytosis, charged lipids are initially mobilized, but once the phagosome detaches, membrane charge steadily decreases up to degradation by lysosomes. The pathogen effector SopB eliminates negatively charged lipids, preventing phagosome maturation.

(B) Clathrin and COPI coated vesicles display high curvature, which favors the recruitment of synaptotagmin via endophilin, which contains a BAR domain, or of ArfGAP1, which contains ALPS motifs. Synaptotagmin and ArfGAP1 eliminate PIP₂ and Arf1-GTP, respectively, contributing to coat destabilization.

(C) Autophagosome formation depends on the recruitment of a PI3-kinase. Via an ALPS C-terminal motif, Barkor/Atg14(L) specifically targets the PI3 kinase complex to curved ER, promoting elongation of the phagophore and accumulation of PI3P.

(D) During lipid droplet expansion, CCT α , a key enzyme for phosphatidylcholine (PC) synthesis, detects the deficit in phospholipid in the droplet monolayer.

(E) Ca²⁺ decrease in the ER induces STIM1 oligomerization. The polybasic end of STIM1 interacts with negative charges at the PM promoting ER-PM bridges and activation of a Ca²⁺ channel.

(F) The lipid transfer protein Osh4 might interact alternatively with loosely packed membranes to extract sterols, and with negatively charged membranes, to deliver sterol and extract PI(4)P.

Red and blue lines represent inner leaflets rich in negative lipids or in lipid-packing defects, respectively.

starvation conditions (Mizushima et al., 2011). The recent identification of an ALPS motif in a protein complex involved at early stages of autophagy suggests an interesting feedback loop to initiate autophagosome formation (Fan et al., 2011). The mammalian protein Atg14L/Barkor forms a complex with three other proteins including a PI3-kinase (Matsunaga et al., 2010). Through its C-terminal ALPS motif, Barkor seems to recognize curved regions of the ER, hence triggering the unusual synthesis of PI(3)P in this compartment (Fan et al., 2011). Because Barkor also binds PI(3)P, the dual detection of positive curvature and PI(3)P may create a positive feedback loop for PI(3)P synthesis and contribute to the emergence of an atypical compartment, both in shape and in lipid composition, from the ER (Figure 3C). Note that here electrostatics increases and thus follows the opposite trend as in the case of phagocytosis. These findings should help to clarify the origin of the autophagosome membrane, which has been heavily discussed (Mizushima et al., 2011).

Lipid-Packing Defects as an Index of Lipid Droplet Expansion

To store carbon sources in the densest way, cells use lipid droplets: a core of triglycerides and sterol esters surrounded

by a monolayer of phospholipids and specific peripheral proteins (Wolins et al., 2006). One interesting feature of the lipid droplet monolayer is its composition: it contains mostly PC and PE, whereas PS is barely detectable (Bartz et al., 2007). This composition fits with the hypothesis that lipid droplets emerge from the ER. In this scenario, a lens of triglycerides and sterol esters forms within the ER bilayer and bulges toward the cytoplasm (Figure 3D). Consequently, the droplet monolayer arises from the cytoplasmic leaflet of the ER, which is poor in PS (Fairn et al., 2011; Fan et al., 2011).

Lipid droplets change their volume depending on triglyceride synthesis or consumption, hence requiring adjustment of the monolayer surface. A recent study suggests an elegant feedback mechanism for such an adjustment (Krahmer et al., 2011). Phosphocholine synthetase (CCT α), the rate-limiting enzyme of phosphatidylcholine synthesis, is recruited to lipid droplets in the growing phase, thus allowing the production of more PC molecules to surround the oil core. CCT α contains a C-terminal amphipathic helix, which seems to sense the decrease in packing in the phospholipid monolayer that accompanies droplet expansion. Because this helix was shown to bind preferentially to lipid bilayers displaying a high PE/PC ratio (Attard et al., 2000; Davies et al., 2001), CCT α seems to act as a general sensor of PC deficiency, either in a droplet monolayer or in a lipid bilayer. However, the conformation of phospholipids above an oil core may be quite different from that of

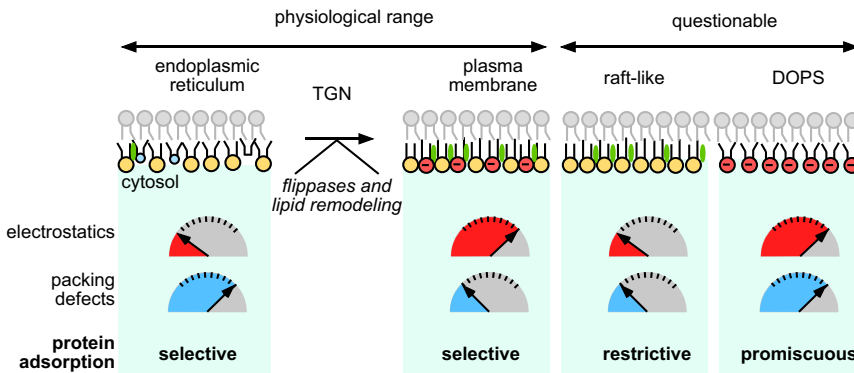


Figure 4. Membrane Electrostatics or Lipid-Packing Defects

The inner leaflet of cellular organelles can be rich in negatively charged lipids or in lipid-packing defects. Cells may avoid mixing these two features as this would lead to promiscuous binding of many peripheral proteins. At the other extreme, a highly packed and poorly charged leaflet seems not adapted to most cytosolic proteins.

phospholipids in a bilayer and the mechanisms by which peripheral proteins selectively bind to lipid droplets remain largely mysterious.

Membrane Contact Sites: When Lipid Territories Meet

Besides exchanging material through tubules and vesicles, cellular compartments make specific contacts by tightly apposing their membranes (Carrasco and Meyer, 2011; Lev, 2010). From the physicochemical perspective, contact sites are interesting as they join membranes displaying different bulk properties. The ER is almost systematically involved, contacting organelles as different as mitochondria, vacuoles, the *trans*-Golgi and the PM.

The yeast transmembrane protein Ist2p provides a straightforward mechanism for membrane contact site formation (Ercan et al., 2009; Lavieu et al., 2010). Although biochemical fractionation indicates that this protein is retained at the ER, it also decorates the PM when observed with light microscopy. This deceptive localization results from the engagement of its cytosolic basic domain with anionic lipids, notably PIP_2 , of the PM, which leads to the formation of bridges between the cortical ER and the PM (Ercan et al., 2009). Another related example is the STIM1-Orai1 tandem. STIM1 is an ER transmembrane protein that senses depletion of calcium in the lumen. Upon calcium drop, STIM1 oligomerizes, invades the cortical ER, and activates the PM calcium channel Orai1 in a process referred to as store-operated calcium entry (Carrasco and Meyer, 2011). The formation of the STIM1-Orai1 complex seems to take advantage of the negative charge of the PM: oligomerization of STIM1 leads to the formation of a cytosolic patch very rich in positive charges and sufficient to surpass the threshold for efficient contact with PIP_2 at the PM (Figure 3E).

Membrane contact sites are also important for lipid transport between organelles. Proteins such as CERT (ceramide trafficking protein), FAPP (four-phosphate adaptor protein), and ORPs (OSBP related proteins) contain a domain that can extract a specific lipid and additional domains or motifs to tether the ER to the *trans*-Golgi (D'Angelo et al., 2007; Hanada et al., 2003; Im et al., 2005; Lev, 2010). Although in most cases the details of the lipid exchange reaction remain to be investigated and the function of these proteins may go beyond exchanging lipids (Mousley et al., 2012), a few reconstitution experiments suggest an exquisite adaptation to membrane interfaces. In vitro, the

yeast protein Osh4 exchanges sterol for $PI(4)P$ between liposomes (de Saint-Jean et al., 2011). The rate of lipid exchange is optimal when the sterol donor liposomes are poorly packed and the acceptor liposomes are charged and more strongly packed (de Saint-Jean et al., 2011). The alternative use of an ALPS motif (Drin et al., 2007) and basic surfaces (Im et al., 2005) might allow Osh4 to rapidly land and take off from each membrane type, hence optimizing lipid transport. Along the same line, CERT extracts preferentially ceramide from poorly packed neutral liposomes (Tuuf et al., 2011).

The fine balance between electrostatics, lipid-packing defects, and curvature might also control other complex reactions involving two different membranes (Drin et al., 2008; Kunding et al., 2011; Park et al., 2012).

Dangerous Combinations

The aforementioned examples illustrate the power of combining surface charge, curvature, and defects in lipid packing to control biochemical reactions on membrane organelles. Yet, certain combinations seem to predominate. Let's examine other formal cases and discuss their relevance in a cellular context (Figure 4).

The first extreme combination is when a membrane displays a high density of anionic lipids together with large lipid-packing defects. In the test tube, this situation is mimicked by pure C18:1-C18:1 PS (DOPS) liposomes where every lipid carries a negative charge and two kinked acyl chains. Experimentally, many peripheral proteins bind avidly to such liposomes. For example, the seemingly innocent replacement of POPS by DOPS causes Osh4 to remain associated to the liposomes after sterol extraction, thereby preventing fast sterol transport (de Saint-Jean et al., 2011), and can even cause liposome aggregation when all Osh4 membrane determinants are simultaneously engaged with this too-accommodating membrane surface (Schulz et al., 2009).

The opposite case is when the bilayer contains no anionic lipids and is tightly packed. In the presence of high amounts of cholesterol, lipids with saturated chains form a liquid-ordered ("raft") phase. Although this phase is assumed to favor protein-signaling platforms (Lingwood and Simons, 2010), the majority of intracellular peripheral proteins tested to date do not readily partition into such domains in vitro (Silvius, 2005). This is evidently the case for proteins that adsorb to membranes through electrostatics, but this exclusion also applies to other peripheral proteins. The small G protein Arf partitions exclusively to the disordered phase when incubated with giant liposomes exhibiting phase separation, suggesting that tight packing in the liquid-ordered phase prohibits amphipathic helix insertion

(Manneville et al., 2008). Neutral liquid-ordered domains seem adapted to the penetration of dual lipid modifications such as those of GPI-anchored proteins (Silvius, 2005), but this situation applies only to the external leaflet of the PM.

Altogether, and as far as the cytosolic leaflet of membranes is concerned, the division of territories between loose lipid packing and electrostatics seem advantageous because it provides both a broad level of specificity and a means of regulation (Figure 4).

Limits of the Two Territories Model and Perspectives

We have put the emphasis on the separation between early and late membranes for the association of peripheral proteins from the cytosol. This separation is also important when considering the distribution of transmembrane proteins, as well as other general aspects of membrane traffic (Lippincott-Schwartz and Phair, 2010; Saraste and Goud, 2007; Sharpe et al., 2010). However, the role of membrane asymmetry in mechanisms such as organelle shaping and sorting of luminal proteins remains in most cases to be explored. In this last paragraph we will evoke the merits and limitations of the two territories model, and suggest some general lines for future works.

The development of bioprobes with a defined number of positive charges gives an illuminating picture of the electrostatics of cell membranes (Yeung et al., 2006). For lipid-packing defects, we are far from having a similar level of accuracy. First, lipid packing is a more elusive concept and only recent atomic simulations start depicting lipid-packing defects in a quantitative manner (Cui et al., 2011; van den Brink-van der Laan et al., 2004). Second, the influence of these defects on the functioning of machineries acting on early membranes, notably the ER, in most cases remains to be studied (however, see Fu et al., 2011; Matsuoka et al., 1998; Nilsson et al., 2001). Lastly, cellular approaches will require the development of bioprobes of varying hydrophobicity to map the distribution of membranes with loose versus tight lipid packing. For this aim, amphipathic helices seem promising because their elongated structure is adapted to probe the membrane interface in a repetitive manner (Antonny, 2011).

In a recent study, two amphipathic helices with the most contrasting hydrophobic and polar faces have been compared for their localization in yeast cells (Pranke et al., 2011). The helix with bulky hydrophobic residues (an ALPS motif) decorated small vesicles of the early secretory pathway, whereas the helix with small hydrophobic residues but a highly charged polar face (from α -synuclein) decorated small endocytic vesicles. This distribution seems driven by protein-lipid interactions because the two helices are heterologously expressed and the ALPS localization remains the same after sequence inversion. In the future, other amphipathic helices with peculiar features should help to evaluate the balance between surface charge and lipid-packing defects on various organelles. Among the most interesting helices are those of perilipins, which decorate lipid droplets (Bulankina et al., 2009); synapsin, which binds to synaptic vesicles (Krabben et al., 2011); Hsp12, which binds to the PM under stress conditions (Welker et al., 2010); and the yeast lipin, Pah1p, which associates with the nuclear/ER membrane (Karanasios et al., 2010).

The contrasting physical chemistry of early versus late membranes has an obvious corollary: the key role of lipid

metabolism at the Golgi apparatus (Bankaitis et al., 2012; Lippincott-Schwartz and Phair, 2010). Two reactions are particularly interesting: phospholipid flip-flop, to generate asymmetry, and phospholipid remodeling, to change acyl chain composition (Figure 4). Several P4-type ATPases have now been identified that promote PS and other phospholipid translocation from the lumen to the cytoplasmic leaflet of the TGN or the PM (Alder-Baerens et al., 2006; Hua et al., 2002). Their activities help to explain why some mechanisms of membrane shaping at the *trans*-Golgi are reminiscent of those occurring at the PM, involving not only Arf and coats but also actin, Rac, and charged lipids (Anitei et al., 2010; Koronakis et al., 2011; Wang et al., 2003). Phospholipid remodeling is a long known reaction in which a phospholipase and an acyltransferase act sequentially to replace the esterified acyl chains on the phospholipid glycerol backbone; for example, to convert a C18:1-C18:1 into a C16:0-C18:1 lipid. The recent cloning of many acyl transferases and the realization that some members reside at the Golgi suggest interesting possibilities for their role in changing membrane properties across this organelle (Schmidt and Brown, 2009; Shindou et al., 2009; Yang et al., 2011).

So far, we have avoided discussing two lipids whose subcellular distribution is difficult to integrate in a general scheme. These are phosphatidic acid (PA) and phosphatidylinositol (PI). Both are anionic and are present at the ER, thus posing the question of their contribution to the electrostatics of this organelle. PA is a key intermediate in the synthesis of most lipids but its steady state level at the ER seems very low, suggesting no major contribution to general electrostatics. Instead, the level of PA serves as an index to control many lipid metabolism pathways (Loewen, 2012). In contrast to PA, PI is an abundant lipid. However, recent studies suggest that PI is not evenly present in the ER but, instead concentrates at specific subregions (Kim et al., 2011). Together with the luminal distribution of PS (Fairn et al., 2011; Kay et al., 2012), this finding reinforces the idea that the surface of most of the ER is in fact quite neutral and, as such, strongly contrasts with the PM inner leaflet.

Reducing the membrane complexity to two main membrane territories is of course an oversimplification. It does not account for the heterogeneities that exist within a continuous membrane and which involve specific protein-lipid interactions, lipid domain formation, restricted membrane diffusion by fences, and localized enzymatic activities. However, the two territories model is flexible enough to incorporate such variations.

ACKNOWLEDGMENTS

We thank S. Vanni and A. Copic for comments on the manuscript. Work in our laboratory is supported by the ERC, the CNRS, and the ANR.

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