Domains are present in every natural membrane. They are characterized by a distinctive protein and/or lipid composition. Their size is highly variable from the nano- to the micrometer scale. The domains confer specific properties to the membrane leading to original structure and function. The determinants leading to domain organization are therefore important but remain obscure. This review presents how the ability of lipids to organize into hexagonal II or lamellar phases can promote particular local structures within membranes. Since biological membranes are composed of a mixture of lipids, each with distinctive biophysical properties, lateral and transversal sorting of lipids can promote creation of domains inside the membrane through local modulation of the lipid phase. Lipid biophysical properties have been characterized for long based on in vitro analyses using non-natural lipid molecules; their re-examinations using natural lipids might open interesting perspectives on membrane architecture occurring in vivo in various cellular and physiological contexts.

Keywords: glycerolipid, lipid bilayers, hexagonal phase, membrane domains, lipid phase
The raft domains that involve also sterols and sphingolipids and correspond to patches of lipids, in a liquid ordered phase, within a matrix in a liquid disordered phase, have been referenced in depth in different reviews (Bagatolli et al., 2010; Simon and Sampaio, 2011) and will not be described further here. This review will consider more specifically domains resulting from modification of glycerolipid biophysical organization since glycerolipids represent the main constituent of the membrane lipid matrix. We shall focus here on the non-bilayer organization that can adopt glycerolipids, their biophysical properties, and their impact on membrane biology, since this topic is rarely raised in recent biological literature.

Membrane glycerolipids are a category of amphiphilic molecules having a 3-carbon glycerol scaffold (each carbon is numbered following the stereospecific numbering nomenclature sn-1, sn-2, sn-3), harboring one or two hydrophobic acyl chains esterified at positions sn-1 and sn-2, and a hydrophilic polar head at position sn-3. Glycerolipids can be separated into two classes in function of their polar head: phospholipids that contain a phosphorous atom and non-phosphorous glycolipids. Major membrane phospholipids found in prokaryotes and eukaryotes are PC, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) also called cardiolipin, phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA). Major glycolipids are monogalactosyldiacylglycerol (MGDG), monoglucosyldiacylglycerol (MGlcDG), digalactosyldiacylglycerol (DGDG), diglucosyldiacylglycerol (DGlcDG), and sulfoquinovosyldiacylglycerol (SQDG). Physical studies showed that the aqueous dispersions of glycerolipid do not always spontaneously form lipid bilayers as it was guessed at first (Gorter and Grendel, 1925). Indeed, the size of the polar head by comparison of the hydrophobic acyl-glycerol backbone affects lipid behavior in aqueous dispersions (Figure 1). By convention, large negative curvature lipids such as MGDG, MGlcDG, PE, DPG, PS, and PA tend to form HII phase or cubic phase, large positive curvature lipids such as lysolipids form hexagonal I (HI) phase whereas small curvature lipids such as DGDG, DGlcDG, SQDG, PC, PG, and PI form lamellar phase, corresponding to the classical bilayer (Shipley et al., 1973; Seddon, 1998; Hansbro et al., 1992; Vikstrom et al., 1999). Bilayers create a planar structure whereas HI phase forms micellar tubules with the polar head on the outside of the tubules and HII phase forms inverted tubules, with the fatty acyl chains pointing toward the outside of tubules and the polar head groups toward the center establishing an aqueous channel (Figure 1).

However, within a class of lipids, fatty acids can also influence the lipid architecture; the effect of increasing chain length and of unsaturation number is expected to favor in general the

| FIGURE 1 | Shape structure concept of lipid polymorphism. Lipids with a small polar head have a molecular shape that resembles a truncated cone. They induce a negative curvature strain and favor the organization of membranes into inverted micelles (HII phases) or cubic (bicontinuous) structures. Lipids with a bulky polar head and only one acyl chain have a molecular shape similar to an inverted cone and induce a positive curvature strain in membranes. They favor the formation of tubular (HI) or spheric micelles. Lipids that have similar cross-sectional areas for the polar head and hydrophobic region look like cylinders. They form lamellar phases, with no curvature strain. |
formation of HII phase. For instance, saturated PE form a lamellar phase whereas unsaturated PE form an HII phase (for a review, see Seddon, 1998). Furthermore, HII forming lipids are able to switch from a HII phase to a lamellar phase sometimes through an intermediate cubic phase (Figure 1) by lowering the temperature (Tenchov and Kuyanova, 2012). Proteins and pigments might also be involved in cubic phase formation (Wang and Quinn, 1999; Almberg et al., 2006; Tenchov et al., 2013). All these phase transitions are spontaneous and reversible (Siegel and Tenchov, 2008).

Biological membranes of course contain complex mixtures of lipids, and so it is of great importance to understand the polymorphic phase behavior of such mixtures in well-defined model systems. The use of synthetic lipid mixtures and the development of techniques, such as electron microscopy, nuclear magnetic resonance (NMR), X-ray, and neutron diffraction, helped a lot to characterize the parameters that trigger the transition from lamellar phase toward HII phase. Lipid membrane composition, hydration, pH, and presence of cations contribute to lipid organization. For example, an equimolar mixture of PE and PC at low hydration pressure is organized in HII phase whereas at high hydration pressure it adopts a bilayer conformation (Ding et al., 2005). Lowering the pH induces lamellar toward HII transition phase in charged phospholipid system such as PS and PA (Seddon, 1990). Furthermore, transition of DPG from lamellar phase to HII phase is induced either upon lowering pH to below 2.8, or upon increasing NaCl concentration to above 1.6 M at pH 7 (Seddon et al., 1983).

The question now is: Do these structures occur in vivo? Most natural membranes are composed of two main glycerolipids: a bilayer forming lipid and a HII forming lipid, respectively the couple PC/PE in yeast and animal cells, PG/PE in Escherichia coli and Bacillus subtilis, DGdG/DMGdG in Acholeplasma laidlawii, and DGDG/MGDG in plants. At a microscopic level, HII structure might be a key factor for this kind of adaptability like in plasmodesmata. This might comfort the theory that cells are open interesting perspectives how membrane structure organization and, consequently, the membrane properties of lipids have been characterized for long based on in vitro analyses using non-natural lipid molecules; their re-examinations using natural lipids might be of high importance for some enzyme activities. Enzymes like the calcium pump in the sarcoplasmic reticulum (Yagil, 1988), the CTP:phosphocholine cytidylyltransferase (Attard et al., 2000) or the viologansulfenyl-epoxide in the thylakoids (Latosinski et al., 2004) have an activity dependent of HII structures. Membrane anchoring of some proteins like G protein and phosphokinasases C is enhanced by HII phase (Escriva et al., 1997; Vogler et al., 2004). Membrane fusion and fission events seem also to be dependent on the presence of non-bilayer forming lipids (for a review, see Burger, 2000). It has been shown that lipid bilayers can fuse in the complete absence of proteins even if membrane fusion is regulated in vivo by specialized proteins. Membrane fusion between phospholipid bilayers can be induced by the HII lipids, PA, and PS, in conjunction with Ca2+ (for a review see Papahadjopoulos et al., 1990) or by dehydration, that drives bilayers into very close contact (Yang and Huang, 2003). All actual models (Chakraborty et al., 2012; Hamilton et al., 2012; Colpi et al., 2013) for the membrane fusion process share at least one intermediate structure called the fusion stalk (Figure 2). Marklin et al. (1998). Stalk formation is promoted by an HII forming lipid like PE whereas it is inhibited by an HI forming lipid like lysoPC (Chernomordik and Kozlov, 2008). The stalk structure is also an intermediate in the lamellar/HII phase transition (Figure 2) and was observed for the first time in a mixture of PE/PC upon dehydration (Yang and Huang, 2002). Parameters affecting the lamellar/HII phase transition can probably be considered also as fusion parameters and biophysical studies of this phase transition, such as calculation of the energy needed for the process, is starting to give a lot of insights on membrane fusion mechanism (Kozlovsky et al., 2004; Pan et al., 2006).

For the preservation of cell structure and compartmentalization, the membrane needs to be in a lamellar phase but, for membrane architecture and for some enzyme activities, HII phase domains must be present. It was shown that bacteria cells are able to keep the membrane lipids in a “window” between lamellar phases and HII phases. For example, E. coli or A. laidlawii maintain a balance between HII forming lipids and bilayer forming lipids by adjusting the composition of the polar head group (A. laidlawii) or the acyl chains (E. coli; Lindblom et al., 2002). This lead to the hypothesis that biomembranes heteronomously adjust their intrinsic curvatures to maintain a constant net spontaneous curvature in each leaflet of the bilayer (Gruner, 1985). Activation of the CTP:phosphocholine cytidylyltransferase by HII phase might be a key factor for this kind of adaptability (Attard et al., 2000). On this model, it was postulated that several enzymes involved in lipid biosynthesis could also be regulated by membrane stored curvature elastic energy. Kinetic simulations of the eukaryotic lipid biosynthetic pathway were used to show how this elastic energy was homeostatically maintained through a HII/bilayer ratio control mechanism (Alley et al., 2008; Beard et al., 2008) similarly to what was proposed for A. laidlawii (Vikstrom et al., 2000).

In conclusion, the biophysical properties of lipids have been characterized for long based on in vitro analyses using non-natural lipid molecules; their re-examinations using natural lipids might open interesting perspectives how membrane structure organization occurs in vivo in various cellular and physiological contexts, like in plasmodesmata. This might comfort the theory that cells adjust their membrane lipid composition in response to perturbations in order to maintain bilayer stability, but keeping the bilayer close to a point of instability, where a confined transformation to some non-bilayer structure would tend to occur. The mechanisms sensing the physical state of lipids and regulating the lipid biosynthetic pathways accordingly are unknown.
**FIGURE 2** | HII phase in bilayers. (A) Lipidic particle as described in (Siegel, 1984). (B) Mechanisms of membrane fusion involving HII via the stalk intermediate. (1) Apposition of two bilayers. (2) Stalk. The stalk is cylindrically symmetrical. (3) Hemifusion intermediate. It can form two different types of structures. If the bilayer diaphragm in the middle of the hemifusion intermediate ruptures, it forms a fusion pore (4). If fusion pores accumulate in sufficient numbers, they can rearrange to form a cubic phase (5). For systems close to the lamellar/HII phase boundary, hemifusion intermediates can also aggregate to form HII phase (6). Figure adapted from Siegel (1999).

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