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Molecular composition of functional microdomains in bacterial membranes

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ABSTRACT

Membranes of eukaryotic cells organize a number of proteins related to signal transduction and membrane trafficking into microdomains, which are enriched in particular lipids, like cholesterol and sphingolipids and are commonly referred as to lipid rafts or membrane rafts. The existence of this type of signaling platforms was traditionally associated with eukaryotic membranes because prokaryotic cells were considered too simple organisms to require a sophisticated organization of their signaling networks. However, the research that have been performed during last years have shown that bacteria organize many signaling transduction processes in Functional Membrane Microdomains (FMMs), which are similar to the lipid rafts that are found in eukaryotic cells. The current knowledge of the existence of FMMs in bacteria is described in this review and the specific structural and biological properties of these membrane microdomains are introduced. The organization of FMMs in bacterial membranes reveals an unexpected level of sophistication in signaling transduction and membrane organization that is unprecedented in bacteria, suggesting that bacteria as more complex organisms than previously considered.

1. Introduction

Cellular membranes are constituted by a specific composition of lipids and proteins and their correct organization is essential for the correct functionality of different cellular processes, such as cell adhesion, ion conductivity, transport of solutes and cell signaling (Grecco et al., 2011). Consequently, the molecular composition and organization of membranes is an important research area in the field of cellular and molecular biology. In this field of research, a number of advances in the molecular composition of membranes have revealed that the organization of membrane components is crucial to define the biological significance of cellular membranes. Because of this, many laboratories have focused their attention to study this issue and and how the molecular organization of membranes is linked to the correct functionality of specific cellular processes (Lindner and Naim, 2009; Schroeder et al., 1995; Silvius, 2012).

One of the most important contributions to understanding the organization of cellular membrane was reported in 1972 by Singer and Nicolson with the pioneering *fluid mosaic model* (Singer and Nicolson, 1972). This model proposes that cell membranes are two-dimensional liquid organelles in which phospholipids and proteins diffuse easily and therefore, distribute randomly. The diffusion properties of proteins and the dynamic capabilities of membranes suggest that constituent lipids and proteins distribute homogeneously across the membrane hence, they exhibit no particular organization pattern. The hypothesis of the fluid mosaic model has been updated to account for recent information regarding the existence of different lipid species within cellular membranes. Those different lipids show distinct physico-chemical properties, which results in their lateral segregation into membrane microdomains, attending simply to the physico-chemical affinities that exist within them. (Coskun and Simons, 2011; Cronan, 2003; van Meer et al., 2008). The heterogeneous organization of distinct type of lipids in cellular membranes occurs concomitantly to a heterogeneous distribution of the embedded membrane proteins and therefore, the generation of membrane domains (Kraft, 2013; Neumann et al., 2010; Simons and Sampaio, 2011).

The membrane of essentially any cell contains different membrane domains, which seem to play an important role in the functionality of many cellular processes. One of the best-characterized examples is the polarization of the epithelial cells of the intestines These epithelial cells form a monolayer that displays

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apical and basolateral surfaces, which are in contact with the outside environment and the lumen of the intestines, respectively. Cells that constitute the monolayer show a very different protein and lipid compositions in the apical and the basolateral surfaces (Brown and Rose, 1992, Simons and van Meer, 1988; Van 'T Hof and van Meer, 1990; van Meer and Simons, 1988). The basolateral surface concentrates receptor proteins that are responsible for adhering the epithelial cells to other cells and for the uptake of nutrients from blood vessels. In contrast, the apical surface contains a higher concentration of transporters that are responsible for exchanging different types of substrates, signaling molecules and other small molecules with the environment (Schuck and Simons, 2004; Simons and van Meer, 1988; van Meer and Simons, 1988). Regarding the lipid composition, it has been shown that there are no qualitative differences between the basolateral and apical surfaces of epithelial cells but substantial quantitative differences. Apical membranes contain a glycosphingolipid:phospholipid:cholesterol ratio of 1:1:1 and a low percentage of phosphatidylcholine. In contrast, phosphatidylcholine is the most abundant lipid in the basolateral membrane shows and glycosphingolipids constitute only a small fraction of the total lipid content. The concentration of cholesterol is similar between apical and basolateral surfaces (Brown and Rose, 1992; Simons and van Meer, 1988; Van 'T Hof and van Meer, 1990; van Meer and Simons, 1988).

Membrane domains are also present in prokaryotic cells. In these unicellular organisms, the existence of membrane domains play an important role in orchestrating deiverse cellular processes, such as cell division or signal transduction (Barak and Muchova, 2013; Barak et al., 2008; Matsumoto et al., 2006; Muchova et al., 2010). For instance, cell division in bacteria occurs by the formation of a division septum after chromosome replication and segregation, which leads to the separation of the daughter cells (Adams and Errington, 2009; Pinho et al., 2013; Rothfield et al., 2005). The membrane that constitute the division septum shows a different lipid and protein composition to the rest of the cellular membrane. Septal membrane is enriched in cardiolipin, a negatively charged diphosphatidylglycerol lipid that is found in bacterial membranes and other bacterial-derived eukaryotic organelles, such as mitochondria and chloroplasts. Cardiolipin represents approximately the 30% of the total bacterial membrane lipids (Vanderwinkel et al., 1976; Card and Trautman, 1990; Kawai et al., 2004; Schlame, 2008). Visualization of cardiolipin-enriched lipid domains in bacterial membranes has been performed using the fluorescent probe NAO (10 nonyl-acridine orange) (Mileykovskaya and Dowhan, 2000; Kawai et al., 2004) (Fig. 1). Although this dye does not show specificity for cardiolipin (Oliver et al., 2014), it preferentially concentrates in the membrane regions that are enriched in cardiolipin. When analyzing the subcellular localization of NAO in bacterial membranes, the dye usually concentrates at the poles and the septal regions of cells of *Escherichia coli* and *Bacillus subtilis* prokaryotic working models (Kawai et al., 2004; Mileykovskaya and Dowhan, 2000, 2009; Rosch et al., 2007) (Fig. 1). Moreover, the higher content of cardiolipin in the division septum seems important for the correct localization of cell division proteins at the division septum and therefore, a correct cell division process (Kawai et al., 2004; Mileykovskaya and Dowhan, 2000, 2009; Rosch et al., 2007). In fact, mutants unable to produce cardiolipin are still viable, but they show severe defects in growth, which are indicative of a deficient cell division process (Kawai et al., 2004; Mileykovskaya and Dowhan, 2000, 2009; Rosch et al., 2007). Moreover, the growth defects that show these mutants are particularly severe when they are exposed to high salt conditions, indicating the importance of cardiolipin in modulating the membrane composition to adapt to stress conditions and membrane fluidity (Mileykovskaya and Dowhan, 2000, Kawai et al., 2004; Mileykovskaya, 2007; Tsai et al., 2011).

An important contribution in understanding the organization of membrane domains was reported in 1997. Simons and Ikonen (1997) suggest that cellular membranes organize *lipid rafts* or *membrane rafts* (1997), arguing that membranes of eukaryotic cells organize a variety of proteins related to signal transduction and membrane trafficking into microdomains or rafts, which are enriched in particular lipids, like cholesterol or sphingolipids. Moreover, the integrity of these signaling platforms results essential for the correct functionality of the associated proteins. Alterations of the lipid structure of lipid rafts causes serious defects in signal transduction and membrane trafficking (Morrow and Parton, 2005; Babuke and tikkanen, 2007, Otto and Nichols, 2011, Stuermer, 2011; Zhao et al., 2011), which they have been recently related to the occurrence of severe diseases, such as Alzheimer's disease, Parkinson's disease or muscular dystrophy (Michel and Bakovic, 2007).

The notion of lipid rafts represents an important step forward in understanding the organization of cellular membranes because it suggests that certain membrane lipids do not simply act as passive solvent but could play a regulatory role in protein clustering, protein organization and signal transduction (Simons and Ikonen, 1997). The constituent lipids of lipid rafts (*e.g.* cholesterol and sphingolipids) exhibit very specific physico-chemical properties, which provide to the lipid rafts compact, tight lipid packing and highly hydrophobic properties that may contribute to reducing the molecular diffusion of membrane proteins to probably facilitate the correct environmental conditions to favor protein–protein collisions and interaction of raft-associated proteins (Nicolau et al., 2006).

Protein–protein clustering and protein interaction in lipid rafts seem to be facilitated by the activity of a scaffold protein that is called flotillin (Morrow and Parton, 2005; Babuke and tikkanen, 2007; Otto and Nichols, 2011; Stuermer, 2011; Zhao et al., 2011).

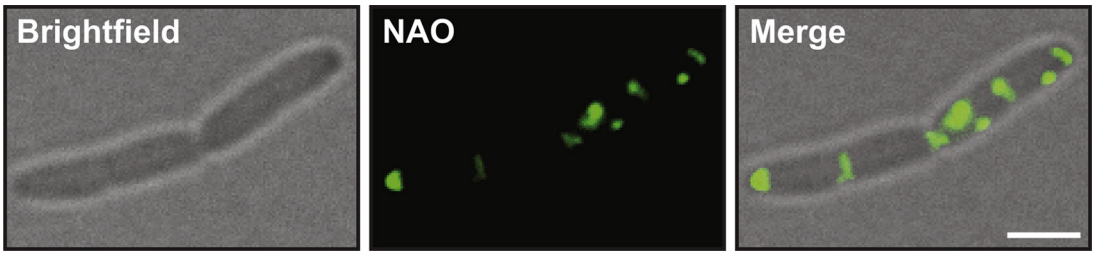

Fig. 1. Cardiolipin-enriched membrane domains in the cellular membrane of *Bacillus subtilis*. Fluorescence microscopy images of *B. subtilis* cells stained with the fluorescence dye NAO. The dye concentrates in cardiolipin-enriched membrane regions, which can be detected by the emission of fluorescence (false colored in green). Fluorescence signal concentrates in the septum of dividing cells and the cell poles. Left panel shows the bright field image and center panel shows the fluorescence signal. Fluorescence signal is colored in green. Right panel shows the overlay image of the bright field image and the fluorescence image. Scale bar is 2 μm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).