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Anionic lipids and the maintenance of membrane electrostatics in eukaryotes

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ABSTRACT

A wide range of signaling processes occurs at the cell surface through the reversible association of proteins from the cytosol to the plasma membrane. Some low abundant lipids are enriched at the membrane of specific compartments and thereby contribute to the identity of cell organelles by acting as biochemical landmarks. Lipids also influence membrane biophysical properties, which emerge as an important feature in specifying cellular territories. Such parameters are crucial for signal transduction and include lipid packing, membrane curvature and electrostatics. In particular, membrane electrostatics specifies the identity of the plasma membrane inner leaflet. Membrane surface charges are carried by anionic phospholipids, however the exact nature of the lipid(s) that powers the plasma membrane electrostatic field varies among eukaryotes and has been hotly debated during the last decade. Herein, we discuss the role of anionic lipids in setting up plasma membrane electrostatics and we compare similarities and differences that were found in different eukaryotic cells.

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The inner leaflet of the plasma membrane (PM) of animal cells is composed of about 20% of anionic lipids that provide negative charges (electric field estimated at 5V/cm) giving the potential to permanently or transiently attract cytosolic cationic molecules, including peripheral membrane proteins.¹ The concept of an electrostatic potential driven by membrane surface charges (MSC) was postulated long ago by biophysicists.² However tools to sense this predicted feature were only developed during the last decade via the generation of genetically encoded biosensors (Fig. 1).³⁻⁷ These biosensors, which will be referred as MSC-probes thereafter, consist of cationic peptides or folded protein domains that transiently associate with anionic phospholipids based on their negative charges and irrespective of their head group (Fig. 1B).^{3,4,7} When fused to a fluorescent protein, these MSC-probes label strictly the cytosolic face of the plasma membrane in all eukaryotic cell type analyzed including yeast, plant and mammalian cells^{3,4,8-10} (Fig. 2C). This common feature highlights a unique signature of the plasma membrane as the most anionic membrane in cells. This particular plasma membrane property is paramount to localize signaling proteins, including for example small GTPases and kinases.³⁻¹⁰ However, in each eukaryotic kingdom, different anionic lipids are used to power this high plasma membrane electrostatic field (Fig. 2B).

Phosphoinositides cooperativity powers membrane electrostatics in mammals

In mammals, phosphatidylserine [PS], phosphatidylinositol-4-phosphate PtdIns(4)P, phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂], and phosphatidylinositol-3,4,5-triphosphate [PtdIns(3,4,5)P₃] are localized at the cell surface (Fig. 2A).^{11,12}

These lipids are candidates to power the PM electrostatic field. Phosphoinositides are low abundant lipids but highly anionic, with PtdIns(4)P, PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ containing respectively 3, 5 and 7 net negative charges.¹³ Since PtdIns(4,5)P₂ is a distinctive lipid of the plasma membrane and relatively abundant compared with other plasma membrane-localized phosphoinositides, it was a prime candidate to drive plasma membrane MSC. However, inducible PtdIns(4,5)P₂ depletion at the plasma membrane has no effect on the localization of MSC-probes, suggesting that this lipid does not specify the plasma membrane electrostatic field on its own.^{4,9} Interestingly, inhibition of PtdIns(3,4,5)P₃ synthesis by type-I PI3-Kinase inhibitors together with inducible depletion of PM PtdIns(4,5)P₂ delocalized MSC-probes to intracellular compartments, showing that these lipids are redundantly required for PM MSC.⁴ Later on, concomitant inducible depletion of plasma membrane-associated PtdIns(4)P and PtdIns(4,5)P₂ also demonstrated a role for PtdIns(4)P in plasma membrane surface charges together with PtdIns(4,5)P₂⁹ (Fig. 2C). Altogether, PtdIns(4,5)P₂ seems to be critical in defining plasma membrane MSC in human cells but acts redundantly with PtdIns(3,4,5)P₃ and/or PtdIns(4)P (Fig. 2B).

PtdInsPs are highly anionic but represent only 1–2% of total phospholipids in living cells.¹³ Other less anionic lipids might also contribute to MSC notably due to their higher abundance. In animals, PS represents about 10 to 20% of plasma membrane phospholipids but PS is less anionic than phosphoinositides (net charge -1).^{1,14} Inhibition of ATP synthesis prevents phosphorylation of PtdInsPs by kinases while lipid phosphatases are still active, triggering the rapid

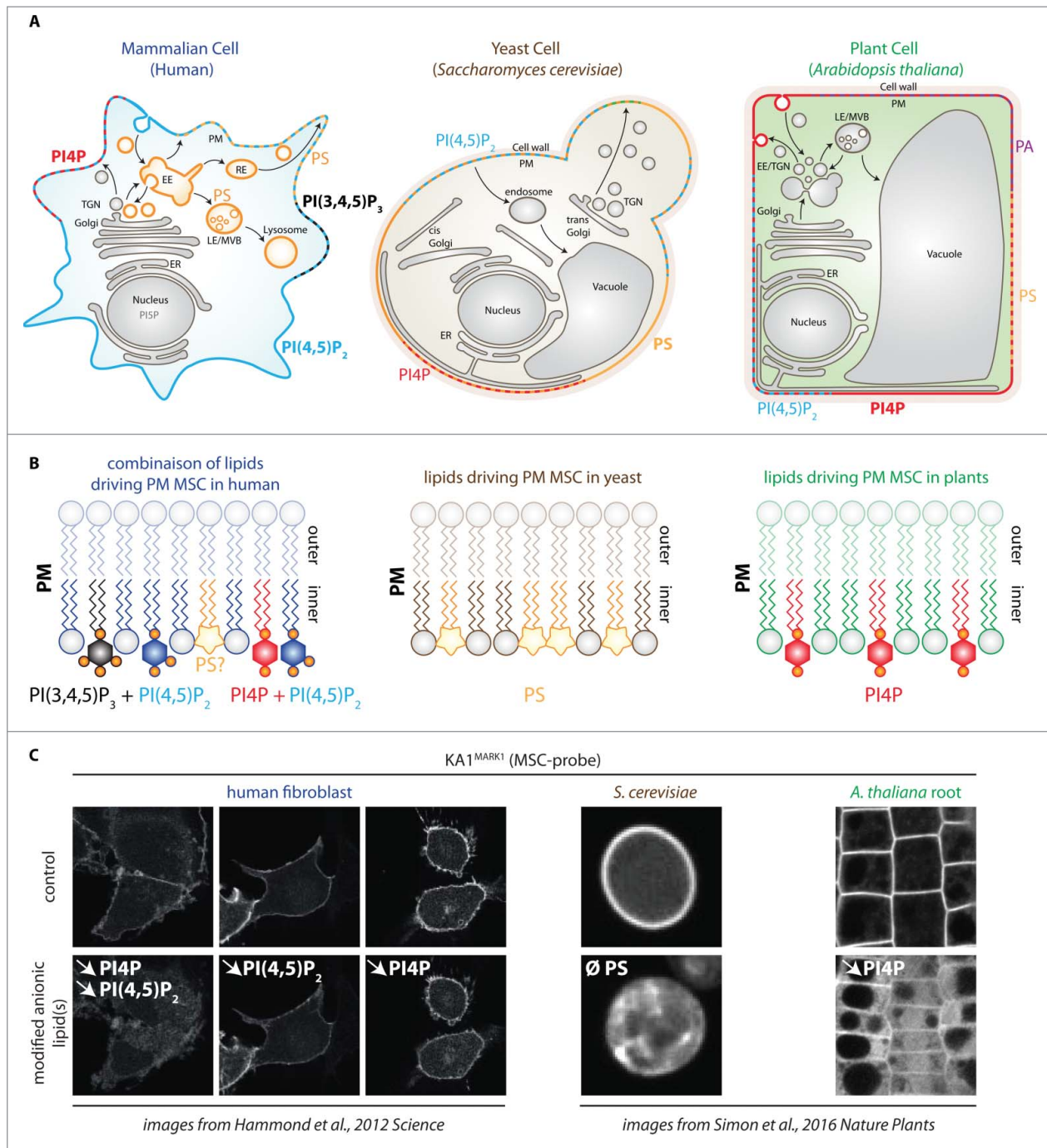


Figure 2. Contribution of different anionic phospholipids in plasma membrane surface charge. (A) schematic representation of human, yeast and plant cells. Anionic phospholipids that localize at the cell surface are indicated for each cell type. For clarity, PI3P, PI5P and PtdIns(3,4)P₂ have been omitted, although they have been shown to localize at the plasma membrane in animal cells at very low quantity and/or upon specific stimuli.¹¹ The localization of PS in plasma membrane-derived organelles is indicated by the orange color. Note that for practical purposes, dashes indicate the presence of several lipid species on the same membrane, however, this does not mean that they are necessarily organized in discrete domains. (B) schematic representation of the anionic lipids required for plasma membrane MSC in mammals (left), yeast (middle) and plants (right). Note that in human, PtdIns(4,5)P₂ acts redundantly with either PtdIns(4)P or PtdIns(3,4,5)P₃. (C) confocal pictures showing the localization of the KA1 domain of MARK1 in human fibroblast cells (left), *S. cerevisiae* (middle) and *A. thaliana* root epidermis (right). KA1 is a domain that interacts with all negatively charged lipids and therefore acts as a sensor of membrane electrostatics (so called MSC-probe). Top panels are control cells and bottom panels show conditions in which anionic phospholipids have been genetically or chemically perturbed. The targeted lipid(s) is indicated in white (downward pointing arrows indicate the reduction in the given lipid content and ∅ total absence in the lipid in the $\Delta cho1$ yeast mutant). Note that KA1^{MARK1} localizes at the cell surface in mammals, yeasts and plants, but that this strict plasma membrane localization relies on different anionic phospholipid in these cells. EE, early endosome; LE, late endosome; RE, recycling endosomes; TGN, trans-golgi network; ER, endoplasmic reticulum; MSC, membrane surface charge. Pictures of fibroblasts are from Hammond et al.⁹ and pictures from yeast and plants are from Simon et al.⁸ The cartoon representing the cell from the top left corner is inspired from Jean and Kiger 2012 and adapted by permission from Macmillan Publisher Ltd: [NATURE REVIEW MOLECULAR CELL BIOLOGY], ref. 12 copyright (2012).

at restrictive temperature, KINASE ASSOCIATED1 (KA1) domains, which are domains that bind to all anionic phospholipids and therefore act as MSC-probes, remain strictly

localized at the PM in all these yeast mutant strains.⁷ These results suggest that unlike in animals, PtdInsPs do not play a major role in PM MSC.⁷

By contrast to mammals in which PS is spread all along the endocytic pathway,^{10,14,15} PS is highly enriched at the PM in yeast (Fig. 2A).^{7,10} Therefore, PS is a good candidate to specify plasma membrane electrostatics in yeast cells. Cho1p is the only PS synthase in yeast, and the *cho1* mutant does not produce any PS.^{10,16} Mislocalization of the KA1 MSC-probes in *cho1* shows a prominent contribution of PS in plasma membrane surface charge (Fig. 2C).^{7,8} Altogether, these results suggest either no or minor roles of PtdInsPs in plasma membrane surface charge in yeast, while PS is the main anionic lipid regulating the plasma membrane electrostatic potential.⁷

PtdIns(4)P massively accumulates at the plasma membrane in plants and drives its electrostatic field

By contrast to yeast and animals, PtdIns(4)P massively accumulates at the plasma membrane in plants.^{8,17} In Arabidopsis root cells, short-term (up to 30 min) pharmacological inhibition of PI4-Kinase (PI4K) rapidly depletes the cellular PtdIns(4)P pool but has no effect on PtdIns(4,5)P₂.⁸ This result is surprising since PtdIns(4)P is the precursor of PtdIns(4,5)P₂. However, short-term depletion of PtdIns(4)P has also no effect on PtdIns(4,5)P₂ in human fibroblast cells, suggesting that in both kingdoms the metabolism of these two lipids are largely independent within this short time frame.^{8,9} In addition, PtdIns(4)P is substantially more abundant than PtdIns(4,5)P₂ in plant tissues,^{17,18} therefore the residual PtdIns(4)P molecules might be sufficient to sustain PtdIns(4,5)P₂ synthesis. The relative abundance of PtdIns(4)P over PtdIns(4,5)P₂ and its accumulation at the cell surface suggest that it might be involved in plasma membrane electrostatics. Indeed, inhibition of PI4K largely delocalized MSC-probes from the plasma membrane.⁸ In addition, genetic depletion of PtdIns(4)P specifically at the plasma membrane induced the ectopic localization of MSC-probes in less anionic endomembrane compartments.⁸ Together, these results indicate that PtdIns(4)P is important for plasma membrane electrostatics and that, by contrast to mammals, it does not act redundantly with PtdIns(4,5)P₂.

However, it is worth noting that MSC-probes retain a certain degree of plasma membrane localization upon PtdIns(4)P depletion (Fig. 2C), and that therefore other anionic lipids might contribute to the plant plasma membrane electrostatic field.⁸ Candidate lipids include PtdIns(4,5)P₂, PS and/or phosphatidic acid (PA) that all localized at the plasma membrane at least in some plant cell types (Fig. 2A).^{8,17,19} Pharmacological and/or genetic perturbation of PtdIns(4,5)P₂ and PA indeed suggest that these lipids are involved in the plasma membrane localization of proteins with cationic stretches.²⁰ Therefore, as seen for mammals, lipid cooperativity might also be important for membrane electrostatics in plants. Nevertheless, unlike in animals, depletion of PtdIns(4)P alone is sufficient to perturb PM electrostatics in plants (Fig. 2C), highlighting the unusual importance of PtdIns(4)P in specifying the identity of the plant plasma membrane.

Concluding remarks

To conclude, the plasma membrane is highly electronegative across eukaryotes, but differences exist concerning the lipids

involved in the maintenance of plasma membrane electrostatics (Fig. 2B). The main difference comes from yeast where PS is the major anionic lipid that drives plasma membrane surface charge, while PtdInsPs are not required.⁷ This striking contrast brings the question of the role of PS in membrane electrostatics in multicellular eukaryotes such as plants or mammals. Indeed, while PS has been postulated to control electrostatic properties of plasma membrane-derived organelles,^{5,6} this has not been fully addressed experimentally. Future researches are therefore awaited to tackle this question. Similarly, it would be interesting to explore the contribution of PA in membrane electrostatics.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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