PUBLISHED: 1 JULY 2016 | ARTICLE NUMBER: 16102 | DOI: 10.1038/NPLANTS.2016.102

PLASMA MEMBRANE

Negative attraction

The electrostatic charge at the inner surface of the plasma membrane is strongly negative in higher organisms. A new study shows that phosphatidylinositol-4-phosphate plays a critical role in establishing plasma membrane surface charge in *Arabidopsis*, which regulates the correct localization of signalling components.

Gergely Molnár, Matyáš Fendrych and Jiří Friml

Lipid composition is crucial for membrane identity and trafficking, and for determining membrane structural and biophysical characteristics. Structural phospholipids can recruit proteins to specific membrane domains, and they also participate in exocytosis, endocytosis and vesicle trafficking through the endomembrane system[1]. Signalling phospholipids and their metabolic enzymes have been visualized in living plant cells, hinting at their role in compartment/membrane identity establishment[2,3]. In this issue of *Nature Plants*, Simon and colleagues[4] show that the plasma membrane (PM) is unique among other membrane compartments because of its strongly negative membrane surface charge (MSC), how this charge is achieved, and, finally, how it is used by plant cell signalling machineries.

In eukaryotes, several proteins are known to be attached to the PM through electrostatic interactions between their positively charged polycationic domains and the anionic phospholipids of the PM[5]. The authors decided to test this notion in *Arabidopsis* with a set of biosensors consisting of a membrane-anchored fluorescent protein attached to a lysine-rich stretch; the biosensors covered a range of net positive charges, depending on the number of lysine residues. Increasing this charge gradually shifted the localization of the sensor from endomembranes towards the PM exclusively (Fig. 1a). The nature of cationic residues was not important; only the charge determined the balance between endomembrane and PM localization. This led to the conclusion that, at least in *Arabidopsis* and tobacco, the PM has a strong negative electrostatic field, stronger than that of endomembranes. This negative charge signature may be sufficient to specifically target proteins with a highly positive domain to the PM.

In animals, MSC is mainly determined by phosphatidylinositol-4-phosphate (PtdIns(4)P) and phosphatidylinositol-4,5-biphosphate (PtdIns(4,5)P_2), together with phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P_3)[6]; the latter, however, is apparently absent in plants. With the help of pharmacological treatments, genetically encoded biosensors with various affinities for the phospholipids, and a system to locally deplete PtdIns(4)P, the authors showed that PtdIns(4)P is highly enriched in the PM and is the main source of its negative electrostatic field in plants. To confirm these observations in a physiological context, the authors took advantage of the fact that during cell plate formation in cytokinesis, accumulation of PtdIns(4)P precedes PtdIns(4,5)P_2 (ref. 7). Biosensors for phospholipids and MSC indicated that in *Arabidopsis* root epidermal cells, PtdIns(4)P is recruited much earlier than PtdIns(4,5)P_2. Similarly, the cell plate was electronegative from the very beginning, so its MSC correlates neatly with PtdIns(4)P recruitment (Fig. 1b). These phospholipid dynamics at the cell plate resemble and possibly explain the sequential recruitment of the TRAPPII and exocyst tethering complexes[8].

Although PtdIns(4)P previously appeared to be present in both the PM and endomembranes[7], the negative MSC is present only at the PM. The authors solved

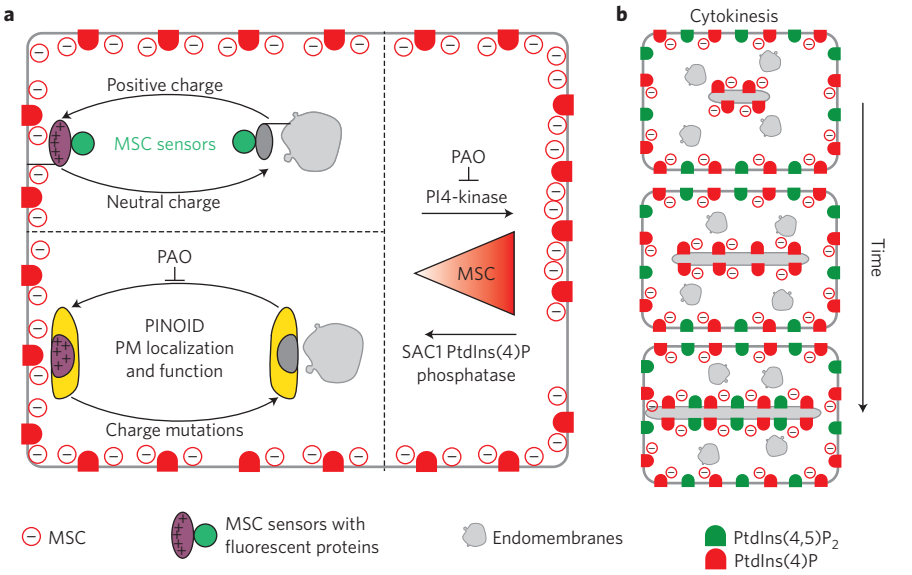

Figure 1 | Negative surface charge of the plasma membrane is generated by PtdIns(4)P. **a**, Top left: positively charged MSC fluorescent sensors localize to the PM; decreasing their charge shifts their localization towards the endomembrane system. Bottom left: PINOID (PID) localization to the PM depends on a stretch of positively charged amino acids. This localization is disrupted by replacing the charged residues to neutral ones, or by PtdIns(4)P depletion from the PM, which renders PID incapable of regulating auxin transport. Right: the negative surface charge is dependent on the levels of PtdIns(4)P in the PM. PAO (phenylarsine oxide), an inhibitor of PI4-kinase, depletes PtdIns(4)P from the PM and disrupts the negative MSC. Similarly, PtdIns(4)P phosphatase (SAC1) activity leads to the depletion of PtdIns(4)P from the PM and to the reduction of the negative MSC. For clarity, PtdIns(4,5)P_2 is not shown. **b**, During cell division, PtdIns(4)P and the negative MSC are present on the cell plate from its initial stages. PtdIns(4,5)P_2 appears only in the later stages of cytokinesis (bottom panel), confirming that the MSC is largely dependent on PtdIns(4)P but not PtdIns(4,5)P_2.

this apparent controversy by demonstrating that the previously used PtdIns(4)P sensor FAPP1 can recognize both PtdIns(4)P and an ARF1 GTPase. By constructing a more specific new sensor incapable of GTPase binding, the signal became present only at the PM, which was also confirmed by introducing other PtdIns(4)P-specific sensors. These observations and other detailed examinations established that the amount of accessible PtdIns(4)P is incomparably higher at the PM than in endomembranes, which is in striking contrast to animal systems[6].

To lend further support to their findings, the authors described well-known hormone signalling proteins as examples of the MSC as a targeting determinant. PINOID (PID) is a protein kinase involved in the regulation of auxin transport polarity[9], which uses a polybasic stretch for PM attachment and for binding phospholipids[10]. Attachment to the PM is dependent on charge rather than on PID protein structure, and charge-driven proper localization of the protein is essential for its biological function (Fig. 1a). A similar mode of PM association was shown for the brassinosteroid signalling protein BKI1 and its homologues[11], and it is expected that more proteins will be discovered to be part of this group.

Proteins have to be basic enough for the charge-dependent PM attachment, but is it again just the PtdIns(4)P-mediated negative charge that is needed at the PM or does PtdIns(4)P itself have a more specific role? To answer this, the authors moved over to yeast, in which (unlike in plants) PM MSC is mainly determined by phosphatidylserine. They demonstrated that an MSC biosensor, PID and members of the BKI1 family were attached to the PM in a phosphatidylserine-dependent manner. Thus, charge itself (from both sides), and not necessarily its origin, is the most important factor for attaching proteins of interest specifically to the PM.

This study clearly demonstrates that the highly negative surface charge of the PM is mostly generated by PtdIns(4)P and is a crucial part of the membrane's identity. The correct localization of proteins needed for plant growth and development relies on charge-dependent interactions, which can be dynamically modified by several mechanisms. Some questions still remain, however. For example, if the surface charge is mostly uniform around the cell, is the MSC and/or PtdIns(4)P exploited in any way for polar protein targeting[3]? Nevertheless, this work not only crucially advances our understanding on the protein–PM association and its regulation in plant cells, it also introduces a new set of tools to visualize and manipulate membrane charges in plants, and thus will allow the role of membrane electrostatic properties to be addressed in other cellular processes. ❐

Gergely Molnár, Matyáš Fendrych and Jiří Friml are at the Institute of Science of Technology Austria, Klosterneuburg, Austria.
e-mail: jiri.friml@ist.ac.at

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