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1 **Metabolic cellular communications: feedback mechanisms between membrane lipid**  
2 **homeostasis and plant development**

3

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12

13 **Abstract:**

14 Membrane lipids are often viewed as passive building block of the endomembrane system.  
15 However, mounting evidences suggest that sphingolipids, sterols and phospholipids are  
16 specifically targeted by developmental pathways, notably hormones, in a cell or tissue specific  
17 manner to regulate plant growth and development. Targeted modifications of lipid homeostasis  
18 may act as a way to execute a defined developmental program, for example by regulating other  
19 signaling pathways or participating in cell differentiation. Furthermore, these regulations often  
20 feedback on the very signaling pathway that initiated the lipid metabolic changes. Here, we  
21 review several recent examples highlighting the intricate feedbacks between membrane lipid  
22 homeostasis and plant development. In particular, these examples illustrate how all aspects of  
23 membrane lipid metabolic pathways are targeted by these feedback regulations. We propose  
24 that the time has come to consider membrane lipids and lipid metabolism as an integral part of  
25 the developmental program needed to build a plant.

26

27 **Blurb:** Boutté and Jaillais present a Review discussing the importance of membrane lipids in  
28 regulating plant growth and development. They consider the intricate feedbacks between  
29 membrane lipid homeostasis and plant development and propose that membrane lipid  
30 metabolism is an integral component of the developmental program needed to build a plant.

31

## 32 Introduction

33 Lipids are essential building blocks of all biological membranes. They have strong structural  
34 roles to maintain the integrity and function of these membranes. This includes for example the  
35 ability of membranes to act as impermeable barriers, but also the modulation of their  
36 physicochemical properties, including elasticity, fluidity, deformability or thickness (Bigay and  
37 Antonny, 2012; Boutte and Moreau, 2014; Holthuis and Menon, 2014). The endomembrane  
38 network is a structurally-linked membrane system in eukaryotic cells—either by direct  
39 membrane connections or vesicular trafficking. As such, the endomembrane system includes  
40 the endoplasmic reticulum and connected nuclear envelop, the Golgi apparatus and the *trans*-  
41 Golgi Network (TGN), endosomes, vacuoles/lysosomes and the plasma membrane. The  
42 endomembrane system is composed of three main lipid classes: sphingolipids, sterols and  
43 phospholipids (see Box 1). While the same three lipid classes are present in the endomembrane  
44 system of all eukaryotes, plants present some specificity, with for example the existence of  
45 phytosterols, specific headgroups in sphingolipids, or the incorporation of very-long-chain fatty  
46 acids (VLCFAs) in the phospholipid phosphatidylserine in addition to sphingolipids (Box 1)  
47 (Jaillais and Ott, 2020; Mamode Cassim et al., 2019; Murata et al., 1984). Additionally, there is a  
48 high degree of variability in the lipid composition of each compartment of the endomembrane  
49 network (Holthuis and Levine, 2005; Noack and Jaillais, 2017). This diversity is critical to  
50 establish the respective identity of each organelle, but also to ensure their specific function. For  
51 example, the lipids in the endoplasmic reticulum tends to be unsaturated and cone-shaped  
52 while the level of cholesterol is relatively low; this lipid composition creates a loose lipid  
53 packing (Bigay and Antonny, 2012; Boutte and Moreau, 2014; Holthuis and Menon, 2014).  
54 Oppositely, the plasma membrane contains more saturated cylinder-shaped lipids while the  
55 level of cholesterol is relatively high; this creates a tight lipid packing. Moreover, the ER is  
56 enriched in highly curved tubules which induce lipid packing defects favorable to translocation  
57 of proteins across the membrane, consistent with the function of this endomembrane  
58 compartment as a protein production center (Bigay and Antonny, 2012; Holthuis and Menon,  
59 2014). By contrast, the membrane curvature of the plasma membrane is lower which limits lipid  
60 packing defects and creates a more impermeable membrane, consistent with the function of  
61 this endomembrane compartment as a barrier to the outside cellular world (Bigay and Antonny,  
62 2012; Holthuis and Menon, 2014).

63 Lipid variability within membrane is enormous and comes not only from variations in the  
64 relative amount in each lipid class, sphingolipids, sterols and phospholipids but also from  
65 differential composition in their carbon chains and head groups. Indeed, they can harbor many  
66 different head groups with sometime drastically distinct properties, they can have acyl-chains  
67 of different sizes and with varied degree of unsaturation and hydroxylation (Box 1) (Holthuis  
68 and Menon, 2014; Mamode Cassim et al., 2019). Because of the importance of lipids for the

69 organization of the endomembrane system, changes in the equilibrium between, or within, lipid  
70 classes have strong impact on cellular functions. As such, the underlying metabolic pathways  
71 that drives the variations in membrane lipid composition are highly regulated. Importantly,  
72 these regulations are not only happening at the cell level but also within the context of an  
73 entire plant. Indeed, in addition to the lipid variability observed at the cellular scale, the  
74 membrane lipid composition also varies at the developmental scale, for example according to  
75 the organs, tissues and even cell types (Colin and Jaillais, 2019; Li et al., 2019; Stanislas et al.,  
76 2018). This suggest that membrane lipid metabolism is regulated by developmental pathways  
77 and that variations in membrane lipid homeostasis may impact plant development in turn.  
78 Here, we review the recent evidences that support the importance of membrane lipids in plant  
79 development with a focus on phytohormone responses. In particular, we will illustrate, using  
80 chosen examples mainly from *Arabidopsis* root, how developmental pathways target lipid  
81 metabolism to control growth and morphogenesis and in turn how modifications of membrane  
82 lipids feedback on these signaling pathways. These examples illustrate how all aspects of  
83 membrane lipid metabolic pathways are targeted by these feedback regulations, including:  
84 fatty acyl chain length and saturation degrees, lipid synthesis and breakdown, the role of  
85 metabolic intermediate or the rapid interconversion of lipid head groups.

86

### 87 **Acyl-chain length of fatty acids is a membrane orchestrating factor of hormonal-induced** 88 **development programs**

89 Cell specification is a key process in plant development that occurs at defined spots of a tissue  
90 layer. It results in the regulation of cell proliferation, through modulation of cell division, and  
91 induction of cell pluripotency, an instrumental process in acquisition of founder cell identity  
92 and production of new organs. While transcriptional regulation networks have been well  
93 studied during *de novo* developmental processes primed by the phytohormone auxin, such as  
94 generation of new floral primordia in the shoot apical meristem or new lateral root primordia  
95 from the primary root, little attention has been given to how these transcriptional changes  
96 translate into cellular processes. Both the shoot apical meristem and lateral root primordia are  
97 excellent experimental systems to explore *de novo* generation of a new organ from founder  
98 cells. The shoot apical meristem is composed of several cell layers including the outermost  
99 epidermal L1 cell layer and more internal tissues involved in cell proliferation. In a genetic  
100 screen for *Arabidopsis* mutants displaying ectopic cell proliferation, the *pasticcino* (*pas*)  
101 mutants were first identified (Faure et al., 1998; Vittorioso et al., 1998). *PAS* genes are involved  
102 in VLCFAs synthesis. VLCFAs are included into a restricted number of membrane lipid classes,  
103 i.e. sphingolipids and phosphatidylserine (Box 1), or into extracellular lipids such as suberin and  
104 waxes (Delude et al., 2016a; Li-Beisson et al., 2013; Murata et al., 1984; Wattelet-Boyer et al.,  
105 2016). VLCFA are synthesized in the endoplasmic reticulum by the fatty acid elongase complex

106 that catalyzes rounds of 2-carbon elongation in a 4-step mechanism. The elongase complex is  
107 made of a 3-KETOACYL-COA SYNTHASE (KCS), a 3-KETOACYL-COA REDUCTASE (KCR), a 3-  
108 HYDROXYACYL-COA DEHYDRATASE (HACD), and a TRANS-2,3-ENOYL-COA REDUCTASE (ECR)  
109 (Haslam and Kunst, 2013). PASTICCINO2 (PAS2) is a HACD while PASTICCINO1 (PAS1) physically  
110 interacts with the elongase complex core members KCR1, PAS2 and ECR/CER10 to favor VLCFAs  
111 synthesis (Roudier et al., 2010). Hypertrophy of apical parts and uncoordinated cell division in  
112 the *pas* mutants were originally correlated to hypersensitivity to the phytohormone cytokinin  
113 (Faure et al., 1998; Vittorioso et al., 1998). Later on, it was shown that expression of *PAS2* is  
114 restricted to the outermost L1 epidermal cell layer of the shoot apical meristem (Nobusawa et  
115 al., 2013). Importantly, the *pas2* mutant causes decreased VLCFAs synthesis in the epidermal  
116 cell layer of the shoot meristem, increased synthesis of cytokinin and overproliferation of cells  
117 in internal tissues of the shoot apical meristem (Nobusawa et al., 2013). Hence, VLCFAs regulate  
118 auxin-induced shoot apical meristem development through repression of cytokinin synthesis in  
119 a non-cell autonomous manner. In *Arabidopsis* root, *PAS2* expression is restricted to the  
120 internal endodermis cell layer while expression of a second HACD, the *PROTEIN TYROSINE*  
121 *PHOSPHATASE-LIKE (PTPLA)*, is confined to root vascular tissue and pericycle cells (Morineau et  
122 al., 2016). Interestingly, *PAS2*-dependent fatty acyl elongation is enhanced in absence of *PTPLA*  
123 which suggests that a non-cell autonomous signal transit between the pericycle and the  
124 endodermis, similarly to what was observed in the shoot apical meristem (Morineau et al.,  
125 2016). The physiological function of this pericycle/endodermal communication through VLCFAs  
126 has been elegantly addressed during induction of cell pluripotency, which is essential for new  
127 organ generation, especially during formation of lateral root primordia which originates from  
128 auxin-induced initiation site in the pericycle (Figure 1). Interestingly, on the one hand, the  
129 condensing enzymes *KCS2*, *KCS20* and *KCS1*, important for >C20 fatty acyl elongation, are  
130 specifically expressed in root endodermis (Lee et al., 2009; Shang et al., 2016). On the other  
131 hand, *KCS1*-mediated VLCFAs production has been shown to restrict pericycle cell proliferation  
132 and lateral root primordia development (Figure 1) (Shang et al., 2016; Trinh et al., 2019). It is  
133 thought that restriction of lateral root primordia development in the pericycle by VLCFAs  
134 production in the endodermis partly relies on the nuclear protein *ARABIDOPSIS ABERRANT*  
135 *LATERAL ROOT FORMATION (ALF4)*, a regulator of cullin-RING E3 ligases, whose expression is  
136 restricted to the pericycle (Bagchi et al., 2018; Shang et al., 2016).

137 However, the production of VLCFAs has to be precisely controlled both in time and space to fit  
138 the lateral root primordia pattern observed along the main root and thus avoid anarchical  
139 lateral root development. The AP2/EREBP transcription factor *PUCHI* controls cell proliferation  
140 during the early stages of lateral root formation (Hirota et al., 2007). Interestingly, *PUCHI*  
141 targets the spatial expression of genes involved in VLCFA synthesis such as *KCS1*, *KCS20*, *KCR1*,  
142 *PAS2* and *ECR/CER10* during lateral root primordia development (Figure 1) (Trinh et al., 2019).  
143 VLCFA mutants display lateral root formation defects and *puchi* mutant displays altered VLCFA

144 content with a reduction of C22 fatty alcohols and C24 sphingolipids (Trinh et al., 2019). At later  
145 stages of lateral root development, *PUCHI* expression disappears from the center of lateral root  
146 primordia and becomes confined to their base and flanks (Figure 1) (Trinh et al., 2019).  
147 Consistently with a role of *PUCHI* in restricting cell proliferation in boundaries, the distance  
148 between two lateral root primordia is reduced in the *puchi* mutant compared to the wild-type  
149 (Trinh et al., 2019). Lateral inhibition of lateral root primordia development by *PUCHI* is further  
150 supported by the finding that *PUCHI* is a downstream element of the peptide hormone TARGET  
151 OF LBD SIXTEEN 2 (TOLS2) which is induced by auxin, through the transcription factor LOB  
152 DOMAIN-CONTAINING PROTEIN 16 (LBD16), and which is perceived by the RECEPTOR-LIKE  
153 KINASE7 (RLK7) (Toyokura et al., 2019). TOLS2 is induced by auxin in lateral root founder cells  
154 and has the ability to move nearby to repress the initiation of other lateral roots in close  
155 proximity of the developing primordia (Figure 1) (Toyokura et al., 2019).

156

### 157 **VLCFA-containing membrane lipids as executive producer of the hormonal transcriptional** 158 **script**

159 Auxin induces cell proliferation and pluripotency in new lateral root primordia and at the same  
160 time separately activates the production of a mobile peptide that will trigger the  
161 TOLS2/RLK7/*PUCHI* pathway in neighboring cells to restrict cell proliferation through the  
162 induction of VLCFA synthesis (Figure 1). Hence, the precise induction of VLCFA synthesis  
163 delineates a zone of the root where cell proliferation induced by auxin is controlled and this will  
164 define a periodic pattern along the main root. However, an important question to address is  
165 how do VLCFAs execute the hormone-induced developmental program? VLCFAs are found  
166 mainly in the sphingolipids where they constitute more than 80% of sphingolipids in plants  
167 (Wattelet-Boyer et al., 2016). VLCFAs are additionally found in phosphatidylcholine in  
168 mammals, phosphatidylinositol in yeast and in phosphatidylserine in plants (Murata et al.,  
169 1984; Poulos, 1995; Schneider et al., 2004)

170 Beside membrane lipids, extracellular lipids such as the cuticle and suberin contain VLCFAs. The  
171 cuticle is a hydrophobic barrier at the surface of epidermal cells and is composed from cutin, a  
172 polymer of C16 and C18 fatty acids, and cuticular waxes, a polymer of VLCFA-derived  
173 compounds (Bernard and Joubes, 2013; Delude et al., 2016b; Kunst and Samuels, 2003).

174 Suberin is a polymer made of VLC  $\omega$ -hydroxyacids, dicarboxylic acid and fatty alcohols (Delude  
175 et al., 2016b; Franke et al., 2005). VLCFAs presence in extracellular lipids could be of particular  
176 importance for targeted development programs since unusual cutin-like compounds were  
177 found to be enriched in a cell layer overlaying the lateral root primordia and play a role in  
178 lateral root emergence (Berhin et al., 2019). Moreover, the LONG-CHAIN FATTY ACID  
179 SYNTHETASE 2 (*LACS2*) enzyme is required for cutin synthesis and lateral root development

180 (Macgregor et al., 2008). Hence, modulation of cuticle lipids plays a role in lateral root  
181 formation most probably by regulating the permeability of the cuticle to ions and osmotic  
182 solutes (Berhin et al., 2019; Macgregor et al., 2008). However, targeted auxin/TOLS2/PUCHI-  
183 mediated lateral root primordia formation is unlikely to occur through cuticle as unusual cutin-  
184 like compounds detected in developing lateral root primordia are not VLCFAs and the level of  
185 typical suberin and cutin compounds was found to be similar between wild-type and *puchi*  
186 mutant (Trinh et al., 2019).

187 Aside from extracellular lipids and membrane sphingolipids, the acyl-chain length of  
188 phosphatidylserine and the quantity of phosphatidylserine at the plasma membrane vary  
189 during plant development (Li et al., 2014; Platre et al., 2019). Phosphatidylserine is required for  
190 auxin-mediated meristem maintenance of the shoot apex (Liu et al., 2013). Moreover,  
191 phosphatidylserine is a crucial lipid which stabilizes auxin-induced nano-clustering of Rho of  
192 Plants 6 (ROP6), a small guanosine triphosphatase (GTPase) involved in auxin signaling (Platre et  
193 al., 2019). Interestingly, in animal cells, phosphatidylserine is located in the cytoplasmic leaflet  
194 of the plasma membrane (Yeung et al., 2008) and has the ability to induce the nanoclustering of  
195 GPI-anchored proteins located at the outer leaflet, through trans-bilayer coupling (Raghupathy  
196 et al., 2015). This coupling requires long acyl-chains of 18 carbons for both GPI and  
197 phosphatidylserine (e.g. PS 18:0/18:1) (Raghupathy et al., 2015; Skotland and Sandvig, 2019).  
198 Hence, phosphatidylserine does not necessarily require VLCFA to induce nano-clustering of  
199 proteins in animal cells. However, whether VLCFA of phosphatidylserine is required to target  
200 specific developmental programs is currently unknown in plant cells.

201 Contrastingly, VLCFAs of sphingolipids are known to be instrumental in executing hormone-  
202 induced developmental program.  $\alpha$ -hydroxylated VLCFAs, specific of sphingolipids (Box 1), were  
203 found to be a target of the auxin/PUCHI-mediated lateral root primordia formation and this is  
204 consistent with the role of the ceramide synthases LONGEVITY ASSURANCE GENE 1  
205 HOMOLOGUE (LOH) in lateral root development (Bach et al., 2011). In *loh* mutant, the auxin  
206 influx carrier AUX1 and the auxin efflux carrier PIN1, both required for lateral root formation,  
207 are mis-localized in endosomal compartments, which indicates an involvement of sphingolipids  
208 in endomembrane trafficking of auxin carriers (Bach et al., 2011). Interestingly, pharmacological  
209 reduction of acyl-chain length of sphingolipids, which does not alter the total quantity of  
210 sphingolipids, inhibits secretory sorting of the auxin efflux carrier PIN2 at a specific sub-domain  
211 of the TGN, a major post-Golgi sorting station (Wattelet-Boyer et al., 2016). Immuno-isolation  
212 of intact TGN compartments revealed that the TGN sub-domain involved in PIN2 secretory  
213 sorting is enriched in  $\alpha$ -hydroxylated VLCFAs, the specific hallmark of sphingolipids, similarly to  
214 what was found in TGN-derived vesicles in yeast (Klemm et al., 2009; Wattelet-Boyer et al.,  
215 2016). Mis-sorting of PIN2 at the TGN upon the reduction of the acyl-chain length of  
216 sphingolipids resulted in the alteration of the capacity of plants to orient their root growth

217 towards the gravity vector (Wattelet-Boyer et al., 2016). Hence, the acyl-chain length of  
218 sphingolipids is not only involved in developmental programs but also in the perception and  
219 response of plants to the environment. Identifying the mechanisms through which  
220 sphingolipids act in protein sorting at the TGN is a future challenge shared in all communities  
221 working on sphingolipids.

222 In summary, auxin triggers cell proliferation and pluripotency, which create a niche of stem  
223 cells. In parallel, auxin activates signaling pathways that will restrict this zone in space, as well  
224 as activation of VLCFAs biosynthesis, which will feedback on auxin transport to inhibit cell  
225 proliferation and organize coordinated growth of the new organ.

226

### 227 **VLCFAs are regulating cell-to-cell transport during defined developmental programs**

228 Cell-to-cell transport is known to partly rely on plasmodesmata which are membrane-lined  
229 channels connecting the cytoplasm between adjacent cells. Lipid characterization of isolated  
230 plasmodesmata revealed that they are significantly enriched in sterols and sphingolipids  
231 containing  $\alpha$ -hydroxylated VLCFAs and phytosphinganine (tri-hydroxylated long chain base  
232 t18:0) (Grison et al., 2015; Liu et al., 2020). Phytosphinganine binds plasmodesmata-located  
233 protein 5 (PDLP5) which regulates plasmodesmata cell-to-cell connectivity (Liu et al., 2020;  
234 Sager et al., 2020). Furthermore, the sphingolipid biosynthesis *phloem unloading modulator*  
235 (*plm*) mutant has reduced level of VLCFA-ceramides and displays enhanced cell-to-cell  
236 trafficking between the pericycle and the endodermis (Yan et al., 2019). VLCFA-mediated  
237 restriction of cell-to-cell trafficking between the pericycle and the endodermis is particularly  
238 relevant when considering the restriction of lateral root primordium development at the  
239 pericycle by VLCFAs production in the endodermis (see discussion in previous section; (Shang et  
240 al., 2016; Trinh et al., 2019)). Indeed, during lateral root initiation, the plasmodesmata-  
241 mediated connectivity between the initial auxin-primed pericycle cell and the neighboring cells  
242 is reduced as soon as the first pericycle cell division occurs (Benitez-Alfonso et al., 2013). Auxin  
243 is a small molecule that can likely diffuse through plasmodesmata, as shown previously during  
244 the tropic growth of *Arabidopsis* hypocotyl in response to unilateral light (Han et al., 2014), in  
245 the root meristem (Mellor et al., 2020) or in leaves (Gao et al., 2020). An advantage of reducing  
246 cell-to-cell connectivity in the early stages of lateral root primordia development could be to  
247 concentrate auxin at a restricted area of the pericycle to trigger cell proliferation and regulate  
248 the spatial patterning of lateral root primordia along the main root. Sphingolipids-mediated  
249 regulation of cell-to-cell connectivity at plasmodesmata occurs through both the external  
250 deposition of callose (a  $\beta$ -(1,3)-glucan polymer), induced by the recruitment of PDLP5, and the  
251 internal modulation of plasmodesmata membrane structure (Liu et al., 2020; Yan et al., 2019).  
252 Additionally, a non-negligible number of receptor-like kinases have been identified at

253 plasmodesmata such as CLAVATA1 (CLV1) acting in stem cell specification, and its receptor  
254 kinase interactor CRINKLY4 (Stahl et al., 2013). Another plasmodesmata-located receptor, the  
255 Qiān Shǒu kinase (QSK1), associates more strongly to plasmodesmata when the acyl-chain  
256 length of fatty acids was reduced by pharmacological treatment (Grison et al., 2019).  
257 Interestingly, QSK1 is necessary for callose deposition and lateral root development (Grison et  
258 al., 2019). Hence, VLCFA-modulation of plasmodesmata occurs through several mechanisms to  
259 regulate cell-to-cell connectivity during defined developmental programs.

260

### 261 **Membrane sterols participate to non-cell autonomous auxin-mediated tissue patterning**

262 It is known from a long time that membrane sterols are crucial for plant development as the  
263 first genetic screens performed in *Arabidopsis* identified from the start several sterol-  
264 biosynthesis genes as master regulator of cell division, embryogenesis, body patterning and  
265 growth axes polarity (Mayer et al., 1991). More precisely, the ratio between 24-ethyl sterols ( $\beta$ -  
266 sitosterol and stigmasterol, see Box 1) and 24-methyl sterols (campesterol, see Box 1) has been  
267 found to be critical during general plant development (Schaeffer et al., 2001). This is important  
268 since this ratio is controlled by the last steps of sterol-biosynthesis and could be a way for  
269 plants to finely tune morphogenesis in a given tissue. The 24-ethyl/24-methyl sterols ratio is  
270 balanced by the C24-methylation of 24-methylene lophenol which is synthesized by the  
271 HYDRA1 enzyme. Interestingly, while *hydra1* mutant displays root patterning defects in the  
272 epidermis, columella and vascular tissues, tissue-specific expression of *HYDRA1* in the epidermis  
273 significantly rescued the cell patterning defects in all tissues (Figure 2) (Short et al., 2018;  
274 Souter et al., 2002). Contrastingly, expression of *HYDRA1* in the pericycle, endodermis or  
275 vascular tissues had limited effects on the rescue of root cell patterning defects of the *hydra1*  
276 mutant (Short et al., 2018). Thus, sterols are involved in the transit of a non-cell autonomous  
277 signal from the epidermis to the other tissues of the root. Campesterol is the precursor of the  
278 plant hormone brassinosteroids and could be a mobile signal; nonetheless, exogenous  
279 application of brassinosteroids does not rescue any of the cell patterning defects of the *hydra1*  
280 mutant (Short et al., 2018). Interestingly, *Arabidopsis* mutants in sterol-biosynthesis steps  
281 upstream the 24-ethyl/24-methyl sterols checkpoint all display defects in the localization of PIN  
282 auxin efflux carriers and altered tissue distribution of auxin (Men et al., 2008; Pan et al., 2009;  
283 Souter et al., 2002; Willemsen et al., 2003). Moreover, specific expression of *HYDRA1* in  
284 epidermis rescues PIN localization defects in the *hydra1* mutant (Figure 2) (Short et al., 2018).  
285 Hence, it is very tempting to hypothesize that auxin would be a non-cell autonomous signal  
286 controlled by membrane sterols.

287 Using *Arabidopsis* root hairs, which develop as a projection from trichoblast epidermal cell,  
288 auxin was found to act as a non-cell autonomous signal controlling the positioning of the root

289 hair in the plane of epidermal cells (Figure 2) (Boutte and Grebe, 2009; Fischer et al., 2004;  
290 Ikeda et al., 2009). Interestingly, at the root hair initiation site, sterols accumulate with ROP  
291 GTPases, ROP2 and ROP6 and are necessary for correct ROP positioning. Moreover, sterols are  
292 required for the polar localization at early hair bulging sites of the AGC kinases of the D6PK  
293 family, which are involved in auxin transport (Figure 2) (Armengot et al., 2016; Barbosa et al.,  
294 2014; Stanislas et al., 2015; Stanislas and Jaillais, 2019; Zourelidou et al., 2014). Hence, sterols  
295 feed back on auxin transport during auxin-regulated cell polarization.

296

### 297 **Phosphatidylserine gradients regulate Rho-mediated auxin signaling at the tissue level.**

298 Phosphatidylserine is produced in the ER by the PHOSPHATIDYLSERINE SYNTHASE1 (PSS1)  
299 enzyme (Yamaoka et al., 2011). However, in the ER, phosphatidylserine is oriented toward the  
300 lumen and is not accessible to cytosolic proteins (Platre and Jaillais, 2016, 2017). Analysis of  
301 phosphatidylserine biosensors revealed that instead, phosphatidylserine is present on the  
302 cytosolic leaflets of the plasma membrane and endosomal compartments (Platre et al., 2018;  
303 Simon et al., 2016). Quantitatively, phosphatidylserine appears to form a concentration  
304 gradient from the cell surface to the late endocytic pathway, including the vacuolar membrane  
305 (i.e. the tonoplast) (Figure 3A) (Platre et al., 2018). Interestingly, this gradient is not  
306 homogeneous but appears to be developmentally controlled (Platre et al., 2019). Indeed, in  
307 Arabidopsis root epidermis, meristematic cells have a very sharp concentration gradient of  
308 phosphatidylserine from the plasma membrane to endosomal compartments (Figure 3B). By  
309 contrast, elongating epidermal cells have a more balanced phosphatidylserine cellular gradients  
310 with less phosphatidylserine sensor localized at the plasma membrane and proportionally more  
311 at the surface of endosomes/TGN (Figure 3B) (Platre et al., 2019). Hence, there is not only a  
312 cellular phosphatidylserine gradient within the endomembrane system but also a tissue-level  
313 phosphatidylserine gradient (Figure 3A-B) (Colin and Jaillais, 2019). The variations of  
314 phosphatidylserine accumulation at the plasma membrane at the tissue scale appears to be  
315 functionally relevant as it impacts the signaling activity of the Rho GTPase ROP6 (Platre et al.,  
316 2019). ROP6 coordinates cytoskeleton dynamics and intracellular trafficking during the root  
317 response to gravity and is activated in response to auxin (Armengot et al., 2016; Chen et al.,  
318 2012; Lin et al., 2012). Phosphatidylserine is required to stabilize ROP6 into plasma membrane  
319 nanoclusters, which is itself needed for downstream signaling activity (Platre et al., 2019).  
320 Importantly, phosphatidylserine is not only required but is rate limiting for this process. Indeed,  
321 a decreased phosphatidylserine synthesis leads to attenuated ROP6 nanoclustering and  
322 response, while an increased phosphatidylserine production leads to constitutive ROP6  
323 nanoclustering and downstream signaling (Platre et al., 2019). Therefore, variations of  
324 phosphatidylserine accumulation at the plasma membrane during cell differentiation impact  
325 the cellular sensitivity of root cells to auxin (Figure 3C) (Colin and Jaillais, 2019). Accordingly,

326 elongating epidermal cells, which have less PS at the plasma membrane, have a weaker  
327 response to ROP6-mediated auxin signaling than root meristematic cells. Interestingly, auxin  
328 exogenous application promotes phosphatidylserine accumulation at the plasma membrane  
329 (Platre et al., 2019). Therefore, phosphatidylserine appears to be at the heart of a feedback  
330 regulatory loop, by which auxin impacts on the subcellular accumulation of phosphatidylserine  
331 to regulate ROP6 signaling and hence a subset of the auxin responses (Figure 3C) (Jaillais and  
332 Ott, 2020; Platre et al., 2019). There are many unknown components in this regulatory loop,  
333 including which of the many auxin pathways (e.g. ROP6 signaling, TIR/AFB-mediated nuclear  
334 auxin signaling, non-canonical TIR/AFB signaling) feedbacks on phosphatidylserine subcellular  
335 accumulation and what are the phosphatidylserine metabolic pathways that are targeted by  
336 the auxin signal (e.g. biosynthesis, flipping from luminal to cytosolic leaflet, transport). Although  
337 these metabolic intermediates are currently unknown, this example nicely illustrates how a  
338 membrane lipid, such as phosphatidylserine, may regulate a given signaling output, while at the  
339 same time being targeted for regulation by that signaling pathway.

340

341 **Opposites attract: membrane targeting by electrostatics in the regulation of hormone**  
342 **signaling.**

343 Phosphatidylserine, together with phosphoinositides (e.g. PI4P, PI(4,5)P<sub>2</sub>) and phosphatidic acid  
344 are anionic lipids (Colin and Jaillais, 2019). As such, they impact a key physicochemical property  
345 of biological membranes: their electrostatic field (Noack and Jaillais, 2017; Platre and Jaillais,  
346 2017). The extensive use of lipid and electrostatic biosensors revealed that the endomembrane  
347 system of plant cells may be roughly divided in two cellular territories (Platre et al., 2018; Simon  
348 et al., 2016). The first one includes membrane that are not electrostatics (i.e. no accumulation  
349 of anionic lipids on their cytosolic leaflet) and that corresponds to ER-derived membranes. The  
350 second is an electrostatic territory and is composed of post-Golgi compartments, including the  
351 TGN, the plasma membrane, endosomes and the tonoplast (Platre et al., 2018). The separation  
352 of the endomembrane system into two distinct membrane territories has a strong impact on  
353 protein localization, including both peripheral proteins that may be recruited to their target  
354 membrane through interactions with anionic lipids, but also for integral transmembrane  
355 proteins, which can be stabilized or destabilized by membrane electrostatics.

356 Phosphatidylserine is the only anionic lipid that accumulates in all post-Golgi compartments  
357 and thus can be seen as a hallmark of the electrostatic territory in plants (Platre et al., 2018). By  
358 contrast, PI4P and phosphatidic acid strongly accumulate at the plasma membrane and  
359 together they power a very strong electrostatic field which is a key determinant of the identity  
360 of this membrane (Figure 3D) (Platre and Jaillais, 2017; Platre et al., 2018; Simon et al., 2016).  
361 Consequently, proteins that contains a polybasic region engage electrostatic interactions with

362 anionic lipids and may be targeted specifically to the plasma membrane. This is for example the  
363 case of several key regulators of hormonal pathways, including the AGC kinases PINOID and  
364 D6PKs, which are master regulators of PIN-mediated auxin transport (Barbosa et al., 2016; Lee  
365 et al., 2018; Marhava et al., 2020; Simon et al., 2016; Stanislas et al., 2015). Mutants versions of  
366 PINOID or D6PKs that are not able to interact with anionic lipids, fail to interact with the plasma  
367 membrane and are not functional (Barbosa et al., 2016; Simon et al., 2016). Another example of  
368 proteins regulated by plasma membrane electrostatics is the BRI1 KINASE INHIBITOR1 (BKI1)  
369 (Simon et al., 2016). BKI1 is an inhibitor of the BRASSINOSTEROID INSENSITIVE1 (BRI1) receptor  
370 kinase (Jaillais and Vert, 2016; Wang and Chory, 2006). It is an intrinsically disordered protein,  
371 which binds to the plasma membrane via a polybasic membrane hook (Jaillais et al., 2011).  
372 Interestingly, the localization and thereby function of BKI1 is not only regulated by anionic lipids  
373 but also by brassinosteroid signaling itself (Figure 3D) (Jaillais et al., 2011; Wang and Chory,  
374 2006). Here, brassinosteroid does not target the metabolic production of anionic lipids, but  
375 directly the BKI1 proteins (Jaillais et al., 2011; Wang and Chory, 2006). Indeed, upon  
376 brassinosteroid perception, BRI1 phosphorylates BKI1 on a conserved tyrosine within its  
377 polycationic membrane hook (Jaillais et al., 2011). This tyrosine phosphorylation triggers the  
378 rapid dissociation of BKI1 from the plasma membrane and allows further downstream BRI1  
379 signaling. A likely mechanistic explanation for this membrane release is that the  
380 phosphorylation, which is negatively charged, creates a local electrostatic clash with the highly  
381 anionic plasma membrane, thereby releasing BKI1 into the cytosol (Jaillais and Vert, 2016). In  
382 favor of this hypothesis, a BKI1 mutant in which the tyrosine residue within the membrane  
383 hook is substituted for a negatively charged aspartic acid, is unable to bind to the plasma  
384 membrane (Jaillais et al., 2011). This example shows that hormonal pathways may not only  
385 target lipid metabolism to modulate their signaling activity, but may also directly manipulate  
386 protein-lipid interactions, for example via protein phosphorylation.

387

388 **Reinforcing cell polarity: a regulatory feedback loop between auxin and phosphoinositide**  
389 **metabolism.**

390 PROTEIN KINASE ASSOCIATED WITH BRX (PAX) is an AGC kinase involved in root protophloem  
391 differentiation (Marhava et al., 2018). PAX is specifically expressed in root protophloem and  
392 localized on the rootward pole of these cells (Marhava et al., 2018). PAX directly  
393 phosphorylates PIN proteins in protophloem to enhance polar auxin efflux, which decreases the  
394 intracellular auxin level (Marhava et al., 2018) (Figure 4A). PAX activity is counterbalanced by its  
395 protein partner BREVIS RADIX (BRX), which negatively regulates PAX, thereby decreasing auxin  
396 efflux and increasing intracellular auxin concentration (Marhava et al., 2018; Rodriguez-Villalon  
397 et al., 2015). Heightened intracellular auxin dissociates BRX from the membrane into the

398 cytosol and as such promotes PAX activity and auxin efflux, creating a regulatory feedback loop  
399 akin to a molecular rheostat (Marhava et al., 2018; Scacchi et al., 2009) (Figure 4A-B).

400 Interestingly, like PINOID or D6PKs, PAX is targeted to the plasma membrane via electrostatic  
401 interactions with anionic phospholipids (Barbosa et al., 2016) and its localization is dependent  
402 on two PI4P kinases PIP5K1/PIP5K2, which produce PI(4,5)P<sub>2</sub> (Figure 4C) (Marhava et al., 2020).  
403 PIP5Ks are coimmunoprecipitated by both BRX and PAX and the *pip5k1k2* double mutant shows  
404 a protophloem differentiation phenotype similar to *pax* and *brx* mutants. PIP5K1/2, PAX and  
405 BRX are all localized on the rootward pole of protophloem cells in a plasma membrane domain  
406 at the center of the cell and referred to as a muffin-like pattern. This muffin-like membrane  
407 patch is complementary to PIN1 localization, which accumulates in a donut-like membrane  
408 domain surrounding PIP5Ks/PAX and BRX (Figure 4D-F) (Marhava et al., 2020). The PIN1 donut-  
409 like localization is altered in *pip5k1k2*, *pax* and *brx* mutants suggesting that the activity of the  
410 complex is involved in PIN1 localization. Thus, PIN1 localization in donut appears to be  
411 important for protophloem differentiation. However, the exact function of this annular pattern,  
412 by opposition to a localization on the entire face of the cell's rootward pole, is unclear.

413 Because PIP5K1/2 are localized in the muffin-like patch, it is tempting to speculate that their  
414 lipid product, PI(4,5)P<sub>2</sub>, is also enriched in this domain. A local increase in PI(4,5)P<sub>2</sub> may create a  
415 high local electrostatic field, which could contribute to the polar recruitment of PAX specifically  
416 in the muffin-like patch (Figure 4D). In animals, PI(4,5)P<sub>2</sub> is essential for endocytosis as it  
417 mediates the recruitment of many key regulators of clathrin-mediated endocytosis (Posor et al.,  
418 2015). A similar scenario is likely in plants, since most of these core regulators of endocytosis  
419 are conserved and PI(4,5)P<sub>2</sub> is known to positively contribute to endocytosis (Ischebeck et al.,  
420 2013). Interestingly, DYNAMIN-RELATED PROTEIN 1A (DRP1A), a dynamin-like protein involved  
421 in clathrin-mediated endocytosis in plants, localizes in the muffin-like domain (Marhava et al.,  
422 2020). This suggests that DRP1A may participate in an endocytic process, which locally removes  
423 PIN1, thereby promoting its donut-like localization (Figure 4D-E). Thus, PI(4,5)P<sub>2</sub> and their  
424 biosynthetic enzymes, PIP5Ks, are part of a self-organizing polarity module (Marhava et al.,  
425 2020; Weijers, 2020). On the one hand, they contribute to localizing PAX/BRX to the muffin-like  
426 polar patch and on the other hand, they are critical to establish the PIN1 donut domain (Figure  
427 4F). Because it is a rate limiting component in cellular auxin efflux, PIN1 determines  
428 intracellular auxin levels, which itself impacts on BRX localization (Marhava et al., 2018; Scacchi  
429 et al., 2009), but also *PIP5K* and *BRX* expression (Mei et al., 2012; Mouchel et al., 2006; Tejos et  
430 al., 2014). This example highlights how lipids participate in self-organizing systems to obtain  
431 robust polarity maintenance, which in this case is required for the differentiation of the root  
432 vasculature. Interestingly, auxin regulates this polarity module in several ways, including the  
433 control of BRX localization, by a so far uncharacterized mechanism, and gene expression. The  
434 fact that auxin acts through multiple pathways on lipid metabolism is a concept that will likely

435 be extended to many other developmental outputs, starting with the ROP6/phosphatidylserine  
436 module (Figure 3C). Indeed, each auxin pathways will likely function at different time scales  
437 and/or at different concentrations, thereby expanding the regulatory repertoire that  
438 membrane lipids have to offer to control plant development.

439

## 440 **Conclusion**

441 In the examples described above, it is clear that most membrane lipid classes have key roles in  
442 the hormonal control of plant development. Their metabolism is directly regulated by these  
443 pathways, notably in response to auxin. The modification of membrane lipid homeostasis is  
444 therefore part of the downstream cellular toolkit, which executes given developmental  
445 responses. In addition, developmentally-controlled modifications of lipid metabolism often  
446 feedback on those signaling pathways to either downregulate or activate them, providing  
447 negative feedback regulations or self-reinforcing loops. In the case of auxin, which have several  
448 paralleled signaling pathways, membrane lipids appear as key factors involved in the crosstalk  
449 between the different arms of the auxin responses. However, the importance of lipid  
450 metabolism in plant development is only beginning to be uncovered as we still do not fully  
451 appreciate to what extent phytohormones impact membrane lipids, notably at the single cell  
452 level. In addition, there are still few cases in which we understand the downstream impacts of  
453 targeted membrane lipid alterations on the development of growing tissues and organs. The  
454 cell wall is now recognized as a major target of developmental regulators to control plant  
455 morphogenesis (Echevin et al., 2019; Eng and Sampathkumar, 2018; Kierzkowski and Routier-  
456 Kierzkowska, 2019; Landrein and Ingram, 2019; Zhao et al., 2018). We propose that membrane  
457 lipids, which build the very interface between the cytoplasm and the cell wall (Ackermann and  
458 Stanislas, 2020), should also be considered on an equal footing with cell wall modifying  
459 processes when considering plant morphogenesis.

460

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467

## 468 **Declaration of interest:**

469 The authors declare no competing interests.

470

## 471 **References**

- 472 Ackermann, F., and Stanislas, T. (2020). The Plasma Membrane-An Integrating Compartment for  
473 Mechano-Signaling. *Plants (Basel, Switzerland)* 9.
- 474 Armengot, L., Marques-Bueno, M.M., and Jaillais, Y. (2016). Regulation of polar auxin transport by  
475 protein and lipid kinases. *Journal of experimental botany* 67, 4015-4037.
- 476 Bach, L., Gissot, L., Marion, J., Tellier, F., Moreau, P., Satiat-Jeunemaitre, B., Palauqui, J.C., Napier, J.A.,  
477 and Faure, J.D. (2011). Very-long-chain fatty acids are required for cell plate formation during  
478 cytokinesis in *Arabidopsis thaliana*. *Journal of cell science* 124, 3223-3234.
- 479 Bagchi, R., Melnyk, C.W., Christ, G., Winkler, M., Kirchsteiner, K., Salehin, M., Mergner, J., Niemeyer, M.,  
480 Schwechheimer, C., Calderon Villalobos, L.I.A., *et al.* (2018). The *Arabidopsis* ALF4 protein is a regulator  
481 of SCF E3 ligases. *The EMBO journal* 37, 255-268.
- 482 Barbosa, I.C., Shikata, H., Zourelidou, M., Heilmann, M., Heilmann, I., and Schwechheimer, C. (2016).  
483 Phospholipid composition and a polybasic motif determine D6 PROTEIN KINASE polar association with  
484 the plasma membrane and tropic responses. *Development* 143, 4687-4700.
- 485 Barbosa, I.C., Zourelidou, M., Willige, B.C., Weller, B., and Schwechheimer, C. (2014). D6 PROTEIN  
486 KINASE activates auxin transport-dependent growth and PIN-FORMED phosphorylation at the plasma  
487 membrane. *Developmental cell* 29, 674-685.
- 488 Belkhadir, Y., and Jaillais, Y. (2015). The molecular circuitry of brassinosteroid signaling. *The New*  
489 *phytologist* 206, 522-540.
- 490 Benitez-Alfonso, Y., Faulkner, C., Pendle, A., Miyashima, S., Helariutta, Y., and Maule, A. (2013).  
491 Symplastic intercellular connectivity regulates lateral root patterning. *Developmental cell* 26, 136-147.
- 492 Berhin, A., de Bellis, D., Franke, R.B., Buono, R.A., Nowack, M.K., and Nawrath, C. (2019). The Root Cap  
493 Cuticle: A Cell Wall Structure for Seedling Establishment and Lateral Root Formation. *Cell* 176, 1367-  
494 1378.e1368.
- 495 Bernard, A., and Joubes, J. (2013). *Arabidopsis* cuticular waxes: advances in synthesis, export and  
496 regulation. *Progress in lipid research* 52, 110-129.
- 497 Bigay, J., and Antonny, B. (2012). Curvature, lipid packing, and electrostatics of membrane organelles:  
498 defining cellular territories in determining specificity. *Developmental cell* 23, 886-895.
- 499 Boutte, Y., and Grebe, M. (2009). Cellular processes relying on sterol function in plants. *Current opinion*  
500 *in plant biology* 12, 705-713.
- 501 Boutte, Y., and Moreau, P. (2014). Modulation of endomembranes morphodynamics in the  
502 secretory/retrograde pathways depends on lipid diversity. *Current opinion in plant biology* 22, 22-29.
- 503 Chen, X., Naramoto, S., Robert, S., Tejos, R., Lofke, C., Lin, D., Yang, Z., and Friml, J. (2012). ABP1 and  
504 ROP6 GTPase signaling regulate clathrin-mediated endocytosis in *Arabidopsis* roots. *Current biology : CB*  
505 22, 1326-1332.
- 506 Colin, L.A., and Jaillais, Y. (2019). Phospholipids across scales: lipid patterns and plant development.  
507 *Current opinion in plant biology* 53, 1-9.
- 508 Delude, C., Fouillen, L., Bhar, P., Cardinal, M.J., Pascal, S., Santos, P., Kosma, D.K., Joubes, J., Rowland, O.,  
509 and Domergue, F. (2016a). Primary Fatty Alcohols Are Major Components of Suberized Root Tissues of  
510 *Arabidopsis* in the Form of Alkyl Hydroxycinnamates. *Plant physiology* 171, 1934-1950.
- 511 Delude, C., Moussu, S., Joubes, J., Ingram, G., and Domergue, F. (2016b). Plant Surface Lipids and  
512 Epidermis Development. *Sub-cellular biochemistry* 86, 287-313.
- 513 Echevin, E., Le Gloanec, C., Skowronska, N., Routier-Kierzkowska, A.L., Burian, A., and Kierzkowski, D.  
514 (2019). Growth and biomechanics of shoot organs. *Journal of experimental botany* 70, 3573-3585.

515 Eng, R.C., and Sampathkumar, A. (2018). Getting into shape: the mechanics behind plant  
516 morphogenesis. *Current opinion in plant biology* 46, 25-31.

517 Faure, J.D., Vittorioso, P., Santoni, V., Fraiser, V., Prinsen, E., Barlier, I., Van Onckelen, H., Caboche, M.,  
518 and Bellini, C. (1998). The PASTICCINO genes of *Arabidopsis thaliana* are involved in the control of cell  
519 division and differentiation. *Development* 125, 909-918.

520 Fischer, U., Men, S., and Grebe, M. (2004). Lipid function in plant cell polarity. *Current opinion in plant*  
521 *biology* 7, 670-676.

522 Franke, R., Briesen, I., Wojciechowski, T., Faust, A., Yephremov, A., Nawrath, C., and Schreiber, L. (2005).  
523 Apoplastic polyesters in *Arabidopsis* surface tissues--a typical suberin and a particular cutin.  
524 *Phytochemistry* 66, 2643-2658.

525 Gao, C., Liu, X., De Storme, N., Jensen, K.H., Xu, Q., Yang, J., Liu, X., Chen, S., Martens, H.J., Schulz, A., *et*  
526 *al.* (2020). Directionality of Plasmodesmata-Mediated Transport in *Arabidopsis* Leaves Supports Auxin  
527 Channeling. *Current biology : CB*.

528 Grison, M.S., Brocard, L., Fouillen, L., Nicolas, W., Wewer, V., Dormann, P., Nacir, H., Benitez-Alfonso, Y.,  
529 Claverol, S., Germain, V., *et al.* (2015). Specific membrane lipid composition is important for  
530 plasmodesmata function in *Arabidopsis*. *The Plant cell* 27, 1228-1250.

531 Grison, M.S., Kirk, P., Brault, M.L., Wu, X.N., Schulze, W.X., Benitez-Alfonso, Y., Immel, F., and Bayer,  
532 E.M. (2019). Plasma Membrane-Associated Receptor-like Kinases Relocalize to Plasmodesmata in  
533 Response to Osmotic Stress. *Plant physiology* 181, 142-160.

534 Han, X., Hyun, T.K., Zhang, M., Kumar, R., Koh, E.J., Kang, B.H., Lucas, W.J., and Kim, J.Y. (2014). Auxin-  
535 callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling.  
536 *Developmental cell* 28, 132-146.

537 Haslam, T.M., and Kunst, L. (2013). Extending the story of very-long-chain fatty acid elongation. *Plant*  
538 *science : an international journal of experimental plant biology* 210, 93-107.

539 Hirota, A., Kato, T., Fukaki, H., Aida, M., and Tasaka, M. (2007). The auxin-regulated AP2/EREBP gene  
540 PUCHI is required for morphogenesis in the early lateral root primordium of *Arabidopsis*. *The Plant cell*  
541 19, 2156-2168.

542 Holthuis, J.C., and Levine, T.P. (2005). Lipid traffic: floppy drives and a superhighway. *Nature reviews*  
543 *Molecular cell biology* 6, 209-220.

544 Holthuis, J.C., and Menon, A.K. (2014). Lipid landscapes and pipelines in membrane homeostasis. *Nature*  
545 510, 48-57.

546 Ikeda, Y., Men, S., Fischer, U., Stepanova, A.N., Alonso, J.M., Ljung, K., and Grebe, M. (2009). Local auxin  
547 biosynthesis modulates gradient-directed planar polarity in *Arabidopsis*. *Nature cell biology* 11, 731-738.

548 Ischebeck, T., Werner, S., Krishnamoorthy, P., Lerche, J., Meijon, M., Stenzel, I., Lofke, C., Wiessner, T.,  
549 Im, Y.J., Perera, I.Y., *et al.* (2013). Phosphatidylinositol 4,5-bisphosphate influences PIN polarization by  
550 controlling clathrin-mediated membrane trafficking in *Arabidopsis*. *The Plant cell* 25, 4894-4911.

551 Jaillais, Y., Hothorn, M., Belkhadir, Y., Dabi, T., Nimchuk, Z.L., Meyerowitz, E.M., and Chory, J. (2011).  
552 Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release  
553 of its kinase inhibitor. *Genes & development* 25, 232-237.

554 Jaillais, Y., and Ott, T. (2020). The nanoscale organization of the plasma membrane and its importance in  
555 signaling - a proteolipid perspective. *Plant physiology*, pp.01349.02019.

556 Jaillais, Y., and Vert, G. (2016). Brassinosteroid signaling and BRI1 dynamics went underground. *Current*  
557 *opinion in plant biology* 33, 92-100.

558 Kierzkowski, D., and Routier-Kierzkowska, A.L. (2019). Cellular basis of growth in plants: geometry  
559 matters. *Current opinion in plant biology* 47, 56-63.

560 Klemm, R.W., Ejsing, C.S., Surma, M.A., Kaiser, H.J., Gerl, M.J., Sampaio, J.L., de Robillard, Q., Ferguson,  
561 C., Proszynski, T.J., Shevchenko, A., *et al.* (2009). Segregation of sphingolipids and sterols during  
562 formation of secretory vesicles at the trans-Golgi network. *The Journal of cell biology* 185, 601-612.

563 Kunst, L., and Samuels, A.L. (2003). Biosynthesis and secretion of plant cuticular wax. *Progress in lipid*  
564 *research* 42, 51-80.

565 Landrein, B., and Ingram, G. (2019). Connected through the force: mechanical signals in plant  
566 development. *Journal of experimental botany* 70, 3507-3519.

567 Lee, B.H., Weber, Z.T., Zourelidou, M., Hofmeister, B.T., Schmitz, R.J., Schwechheimer, C., and Dobritsa,  
568 A.A. (2018). Arabidopsis Protein Kinase D6PKL3 Is Involved in the Formation of Distinct Plasma  
569 Membrane Aperture Domains on the Pollen Surface. *The Plant cell* 30, 2038-2056.

570 Lee, S.B., Jung, S.J., Go, Y.S., Kim, H.U., Kim, J.K., Cho, H.J., Park, O.K., and Suh, M.C. (2009). Two  
571 Arabidopsis 3-ketoacyl CoA synthase genes, KCS20 and KCS2/DAISY, are functionally redundant in  
572 cuticular wax and root suberin biosynthesis, but differentially controlled by osmotic stress. *The Plant*  
573 *journal : for cell and molecular biology* 60, 462-475.

574 Li, W., Song, T., Wallrad, L., Kudla, J., Wang, X., and Zhang, W. (2019). Tissue-specific accumulation of  
575 pH-sensing phosphatidic acid determines plant stress tolerance. *Nat Plants* 5, 1012-1021.

576 Li, Y., Zheng, G., Jia, Y., Yu, X., Zhang, X., Yu, B., Wang, D., Zheng, Y., Tian, X., and Li, W. (2014). Acyl chain  
577 length of phosphatidylserine is correlated with plant lifespan. *PloS one* 9, e103227.

578 Li-Beisson, Y., Shorrosh, B., Beisson, F., Andersson, M.X., Arondel, V., Bates, P.D., Baud, S., Bird, D.,  
579 Debono, A., Durrett, T.P., *et al.* (2013). Acyl-lipid metabolism. *The Arabidopsis book* 11, e0161.

580 Lin, D., Nagawa, S., Chen, J., Cao, L., Chen, X., Xu, T., Li, H., Dhonukshe, P., Yamamuro, C., Friml, J., *et al.*  
581 (2012). A ROP GTPase-dependent auxin signaling pathway regulates the subcellular distribution of PIN2  
582 in Arabidopsis roots. *Current biology : CB* 22, 1319-1325.

583 Liu, C., Yin, H., Gao, P., Hu, X., Yang, J., Liu, Z., Fu, X., and Luo, D. (2013). Phosphatidylserine synthase 1 is  
584 required for inflorescence meristem and organ development in Arabidopsis. *Journal of integrative plant*  
585 *biology* 55, 682-695.

586 Liu, N.J., Zhang, T., Liu, Z.H., Chen, X., Guo, H.S., Ju, B.H., Zhang, Y.Y., Li, G.Z., Zhou, Q.H., Qin, Y.M., *et al.*  
587 (2020). Phytosphinganine Affects Plasmodesmata Permeability via Facilitating PDLP5-Stimulated Callose  
588 Accumulation in Arabidopsis. *Molecular plant* 13, 128-143.

589 Macgregor, D.R., Deak, K.I., Ingram, P.A., and Malamy, J.E. (2008). Root system architecture in  
590 Arabidopsis grown in culture is regulated by sucrose uptake in the aerial tissues. *The Plant cell* 20, 2643-  
591 2660.

592 Mamode Cassim, A., Gouguet, P., Gronnier, J., Laurent, N., Germain, V., Grison, M., Boutte, Y., Gerbeau-  
593 Pissot, P., Simon-Plas, F., and Mongrand, S. (2019). Plant lipids: Key players of plasma membrane  
594 organization and function. *Progress in lipid research* 73, 1-27.

595 Marhava, P., Aliaga Fandino, A.C., Koh, S.W.H., Jelinkova, A., Kolb, M., Janacek, D.P., Breda, A.S.,  
596 Cattaneo, P., Hammes, U.Z., Petrasek, J., *et al.* (2020). Plasma Membrane Domain Patterning and Self-  
597 Reinforcing Polarity in Arabidopsis. *Developmental cell* 52, 223-235.e225.

598 Marhava, P., Bassukas, A.E.L., Zourelidou, M., Kolb, M., Moret, B., Fastner, A., Schulze, W.X., Cattaneo,  
599 P., Hammes, U.Z., Schwechheimer, C., *et al.* (2018). A molecular rheostat adjusts auxin flux to promote  
600 root protophloem differentiation. *Nature* 558, 297-300.

601 Mayer, U., Ruiz, R.A.T., Berleth, T., Miséra, S., and Jürgens, G. (1991). Mutations affecting body  
602 organization in the Arabidopsis embryo. *Nature* 353, 402-407.

603 Mei, Y., Jia, W.J., Chu, Y.J., and Xue, H.W. (2012). Arabidopsis phosphatidylinositol monophosphate 5-  
604 kinase 2 is involved in root gravitropism through regulation of polar auxin transport by affecting the  
605 cycling of PIN proteins. *Cell research* 22, 581-597.

606 Mellor, N.L., Voss, U., Janes, G., Bennett, M.J., Wells, D.M., and Band, L.R. (2020). Auxin fluxes through  
607 plasmodesmata modify root-tip auxin distribution. *Development* 147.

608 Men, S., Boutte, Y., Ikeda, Y., Li, X., Palme, K., Stierhof, Y.D., Hartmann, M.A., Moritz, T., and Grebe, M.  
609 (2008). Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier  
610 polarity. *Nature cell biology* 10, 237-244.

611 Morineau, C., Gissot, L., Bellec, Y., Hematy, K., Tellier, F., Renne, C., Haslam, R., Beaudoin, F., Napier, J.,  
612 and Faure, J.D. (2016). Dual Fatty Acid Elongase Complex Interactions in Arabidopsis. *PLoS one* *11*,  
613 e0160631.

614 Mouchel, C.F., Osmont, K.S., and Hardtke, C.S. (2006). BRX mediates feedback between brassinosteroid  
615 levels and auxin signalling in root growth. *Nature* *443*, 458-461.

616 Murata, N., Sato, N., and Takahashi, N. (1984). Very-long-chain saturated fatty acids in  
617 phosphatidylserine from higher plant tissues. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid*  
618 *Metabolism* *795*, 147-150.

619 Noack, L.C., and Jaillais, Y. (2017). Precision targeting by phosphoinositides: how PIs direct  
620 endomembrane trafficking in plants. *Current opinion in plant biology* *40*.

621 Nobusawa, T., Okushima, Y., Nagata, N., Kojima, M., Sakakibara, H., and Umeda, M. (2013). Synthesis of  
622 very-long-chain fatty acids in the epidermis controls plant organ growth by restricting cell proliferation.  
623 *PLoS biology* *11*, e1001531.

624 Pan, J., Fujioka, S., Peng, J., Chen, J., Li, G., and Chen, R. (2009). The E3 ubiquitin ligase SCFTIR1/AFB and  
625 membrane sterols play key roles in auxin regulation of endocytosis, recycling, and plasma membrane  
626 accumulation of the auxin efflux transporter PIN2 in Arabidopsis thaliana. *The Plant cell* *21*, 568-580.

627 Platre, M.P., Bayle, V., Armengot, L., Bareille, J., Marques-Bueno, M.D.M., Creff, A., Maneta-Peyret, L.,  
628 Fiche, J.B., Nollmann, M., Miege, C., *et al.* (2019). Developmental control of plant Rho GTPase nano-  
629 organization by the lipid phosphatidylserine. *Science* *364*, 57-62.

630 Platre, M.P., and Jaillais, Y. (2016). Guidelines for the Use of Protein Domains in Acidic Phospholipid  
631 Imaging. *Methods Mol Biol* *1376*, 175-194.

632 Platre, M.P., and Jaillais, Y. (2017). Anionic lipids and the maintenance of membrane electrostatics in  
633 eukaryotes. *Plant signaling & behavior* *12*, e1282022.

634 Platre, M.P., Noack, L.C., Doumane, M., Bayle, V., Simon, M.L.A., Maneta-Peyret, L., Fouillen, L.,  
635 Stanislas, T., Armengot, L., Pejchar, P., *et al.* (2018). A Combinatorial Lipid Code Shapes the Electrostatic  
636 Landscape of Plant Endomembranes. *Developmental cell* *45*, 465-480 e411.

637 Posor, Y., Eichhorn-Grunig, M., and Haucke, V. (2015). Phosphoinositides in endocytosis. *Biochimica et*  
638 *biophysica acta* *1851*, 794-804.

639 Poulos, A. (1995). Very long chain fatty acids in higher animals--a review. *Lipids* *30*, 1-14.

640 Raghupathy, R., Anilkumar, A.A., Polley, A., Singh, P.P., Yadav, M., Johnson, C., Suryawanshi, S., Saikam,  
641 V., Sawant, S.D., Panda, A., *et al.* (2015). Transbilayer lipid interactions mediate nanoclustering of lipid-  
642 anchored proteins. *Cell* *161*, 581-594.

643 Rodriguez-Villalon, A., Gujas, B., van Wijk, R., Munnik, T., and Hardtke, C.S. (2015). Primary root  
644 protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and  
645 systemically affects root branching. *Development* *142*, 1437-1446.

646 Roudier, F., Gissot, L., Beaudoin, F., Haslam, R., Michaelson, L., Marion, J., Molino, D., Lima, A., Bach, L.,  
647 Morin, H., *et al.* (2010). Very-long-chain fatty acids are involved in polar auxin transport and  
648 developmental patterning in Arabidopsis. *The Plant cell* *22*, 364-375.

649 Sager, R., Wang, X., Hill, K., Yoo, B.C., Caplan, J., Nedo, A., Tran, T., Bennett, M.J., and Lee, J.Y. (2020).  
650 Auxin-dependent control of a plasmodesmal regulator creates a negative feedback loop modulating  
651 lateral root emergence. *Nature communications* *11*, 364.

652 Scacchi, E., Osmont, K.S., Beuchat, J., Salinas, P., Navarrete-Gomez, M., Trigueros, M., Ferrandiz, C., and  
653 Hardtke, C.S. (2009). Dynamic, auxin-responsive plasma membrane-to-nucleus movement of  
654 Arabidopsis BRX. *Development* *136*, 2059-2067.

655 Schaeffer, A., Bronner, R., Benveniste, P., and Schaller, H. (2001). The ratio of campesterol to sitosterol  
656 that modulates growth in Arabidopsis is controlled by STEROL METHYLTRANSFERASE 2;1. *The Plant*  
657 *journal : for cell and molecular biology* *25*, 605-615.

658 Schneider, R., Brugger, B., Amann, C.M., Prestwich, G.D., Epand, R.F., Zellnig, G., Wieland, F.T., and  
659 Epand, R.M. (2004). Identification and biophysical characterization of a very-long-chain-fatty-acid-  
660 substituted phosphatidylinositol in yeast subcellular membranes. *The Biochemical journal* *381*, 941-949.  
661 Shang, B., Xu, C., Zhang, X., Cao, H., Xin, W., and Hu, Y. (2016). Very-long-chain fatty acids restrict  
662 regeneration capacity by confining pericycle competence for callus formation in *Arabidopsis*.  
663 *Proceedings of the National Academy of Sciences of the United States of America* *113*, 5101-5106.  
664 Short, E., Leighton, M., Imriz, G., Liu, D., Cope-Selby, N., Hetherington, F., Smertenko, A., Hussey, P.J.,  
665 Topping, J.F., and Lindsey, K. (2018). Epidermal expression of a sterol biosynthesis gene regulates root  
666 growth by a non-cell-autonomous mechanism in *Arabidopsis*. *Development* *145*.  
667 Simon, M.L., Platre, M.P., Marques-Bueno, M.M., Armengot, L., Stanislas, T., Bayle, V., Caillaud, M.C.,  
668 and Jaillais, Y. (2016). A PtdIns(4)P-driven electrostatic field controls cell membrane identity and  
669 signalling in plants. *Nat Plants* *2*, 16089.  
670 Skotland, T., and Sandvig, K. (2019). The role of PS 18:0/18:1 in membrane function. *Nature*  
671 *communications* *10*, 2752.  
672 Souter, M., Topping, J., Pullen, M., Friml, J., Palme, K., Hackett, R., Grierson, D., and Lindsey, K. (2002).  
673 hydra Mutants of *Arabidopsis* are defective in sterol profiles and auxin and ethylene signaling. *The Plant*  
674 *cell* *14*, 1017-1031.  
675 Stahl, Y., Grabowski, S., Bleckmann, A., Kuhnemuth, R., Weidtkamp-Peters, S., Pinto, K.G., Kirschner,  
676 G.K., Schmid, J.B., Wink, R.H., Hulsewede, A., *et al.* (2013). Moderation of *Arabidopsis* root stemness by  
677 CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. *Current biology : CB* *23*, 362-371.  
678 Stanislas, T., Huser, A., Barbosa, I.C., Kiefer, C.S., Brackmann, K., Pietra, S., Gustavsson, A., Zourelidou,  
679 M., Schwechheimer, C., and Grebe, M. (2015). *Arabidopsis* D6PK is a lipid domain-dependent mediator  
680 of root epidermal planar polarity. *Nat Plants* *1*, 15162.  
681 Stanislas, T., and Jaillais, Y. (2019). *Plant Cell Biology: How to Give Root Hairs Enough ROPs?* *Current*  
682 *biology : CB* *29*, R405-R407.  
683 Stanislas, T., Platre, M.P., Liu, M., Rambaud-Lavigne, L.E.S., Jaillais, Y., and Hamant, O. (2018). A  
684 phosphoinositide map at the shoot apical meristem in *Arabidopsis thaliana*. *BMC Biol* *16*, 20.  
685 Tejos, R., Sauer, M., Vanneste, S., Palacios-Gomez, M., Li, H., Heilmann, M., van Wijk, R., Vermeer, J.E.,  
686 Heilmann, I., Munnik, T., *et al.* (2014). Bipolar Plasma Membrane Distribution of Phosphoinositides and  
687 Their Requirement for Auxin-Mediated Cell Polarity and Patterning in *Arabidopsis*. *The Plant cell* *26*,  
688 2114-2128.  
689 Toyokura, K., Goh, T., Shinohara, H., Shinoda, A., Kondo, Y., Okamoto, Y., Uehara, T., Fujimoto, K.,  
690 Okushima, Y., Ikeyama, Y., *et al.* (2019). Lateral Inhibition by a Peptide Hormone-Receptor Cascade  
691 during *Arabidopsis* Lateral Root Founder Cell Formation. *Developmental cell* *48*, 64-75.e65.  
692 Trinh, D.C., Lavenus, J., Goh, T., Boutte, Y., Drogue, Q., Vaissayre, V., Tellier, F., Lucas, M., Voss, U.,  
693 Gantet, P., *et al.* (2019). PUCHI regulates very long chain fatty acid biosynthesis during lateral root and  
694 callus formation. *Proceedings of the National Academy of Sciences of the United States of America* *116*,  
695 14325-14330.  
696 Vittorioso, P., Cowling, R., Faure, J.D., Caboche, M., and Bellini, C. (1998). Mutation in the *Arabidopsis*  
697 PASTICCINO1 gene, which encodes a new FK506-binding protein-like protein, has a dramatic effect on  
698 plant development. *Molecular and cellular biology* *18*, 3034-3043.  
699 Wang, X., and Chory, J. (2006). Brassinosteroids regulate dissociation of BKI1, a negative regulator of  
700 BRI1 signaling, from the plasma membrane. *Science* *313*, 1118-1122.  
701 Wattelet-Boyer, V., Brocard, L., Jonsson, K., Esnay, N., Joubes, J., Domergue, F., Mongrand, S., Raikhel,  
702 N., Bhalerao, R.P., Moreau, P., *et al.* (2016). Enrichment of hydroxylated C24- and C26-acyl-chain  
703 sphingolipids mediates PIN2 apical sorting at trans-Golgi network subdomains. *Nature communications*  
704 *7*, 12788.  
705 Weijers, D. (2020). Assorted Pastries at the South Pole. *Developmental cell* *52*, 137-138.

706 Willemsen, V., Friml, J., Grebe, M., van den Toorn, A., Palme, K., and Scheres, B. (2003). Cell polarity and  
707 PIN protein positioning in Arabidopsis require STEROL METHYLTRANSFERASE1 function. *The Plant cell*  
708 *15*, 612-625.

709 Yamaoka, Y., Yu, Y., Mizoi, J., Fujiki, Y., Saito, K., Nishijima, M., Lee, Y., and Nishida, I. (2011).  
710 PHOSPHATIDYLSERINE SYNTHASE1 is required for microspore development in Arabidopsis thaliana. *The*  
711 *Plant journal : for cell and molecular biology* *67*, 648-661.

712 Yan, D., Yadav, S.R., Paterlini, A., Nicolas, W.J., Petit, J.D., Brocard, L., Belevich, I., Grison, M.S., Vaten, A.,  
713 Karami, L., *et al.* (2019). Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem  
714 unloading. *Nat Plants* *5*, 604-615.

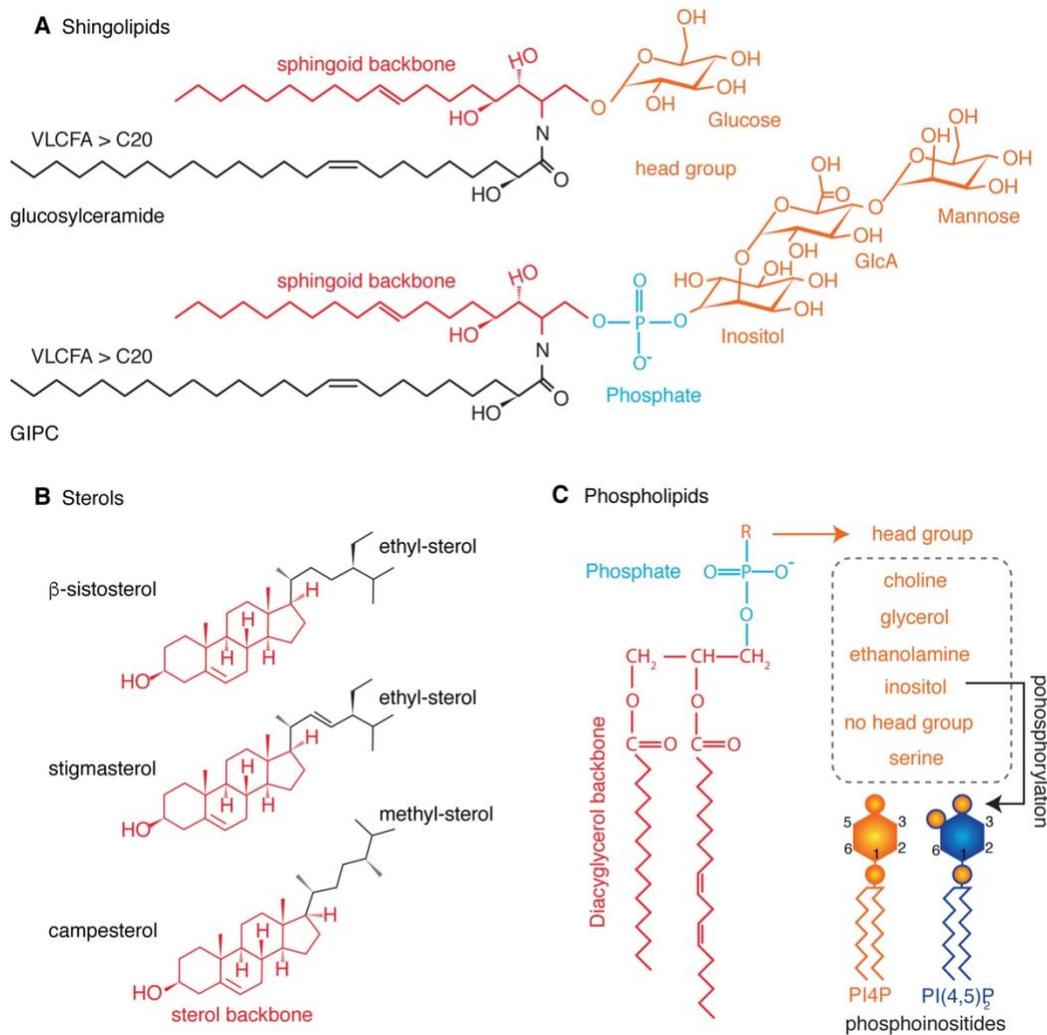
715 Yeung, T., Gilbert, G.E., Shi, J., Silvius, J., Kapus, A., and Grinstein, S. (2008). Membrane  
716 phosphatidylserine regulates surface charge and protein localization. *Science* *319*, 210-213.

717 Zhao, F., Chen, W., and Traas, J. (2018). Mechanical signaling in plant morphogenesis. *Current opinion in*  
718 *genetics & development* *51*, 26-30.

719 Zourelidou, M., Absmanner, B., Weller, B., Barbosa, I.C., Willige, B.C., Fastner, A., Streit, V., Port, S.A.,  
720 Colcombet, J., de la Fuente van Bentem, S., *et al.* (2014). Auxin efflux by PIN-FORMED proteins is  
721 activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *eLife* *3*.

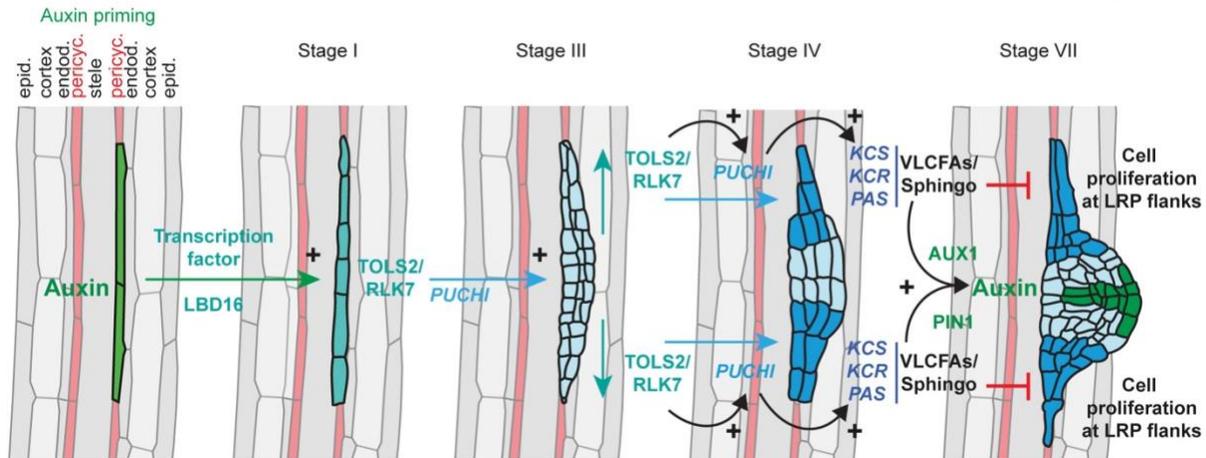
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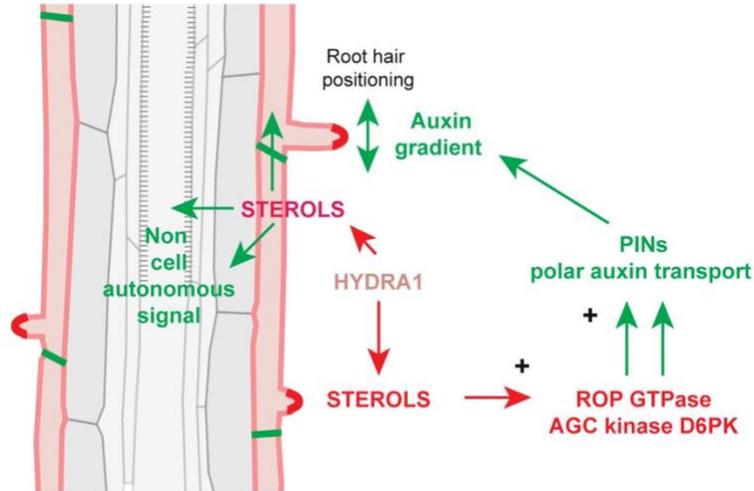
725 **Box 1: Typical structures of the three-main lipids of the Arabidopsis endomembrane system.** Membrane lipids in  
 726 the plant endomembrane system are made of three main lipid classes, sphingolipids, sterols and phospholipids. **A)**  
 727 Sphingolipids are lipids with a sphingoid backbone grafted with a Very-Long Chain Fatty Acid (VLCFA) and are  
 728 abundant in plants (up to 10% of total lipids) (Mamode Cassim et al., 2019). The main sphingolipids in plants are  
 729 glucosylceramide (~35%) and glycosyl inositol phosphoryl ceramide (GIPC, ~65%) (Mamode Cassim et al., 2019). They  
 730 are mostly located in the outer leaflets of the plasma membrane but also in the lumen of the *trans*-Golgi Network  
 731 (TGN), endosomes and the vacuolar membrane, where they can represent about 10 to 20% of total lipids (Jaillais and  
 732 Ott, 2020; Mamode Cassim et al., 2019). **B)** Sterols are membrane lipids constituted from four rings and a lateral chain  
 733 (Boutte and Grebe, 2009). This basic structure of free sterols can be further modified by addition of a fatty acid,  
 734 producing steryl esters, a sugar, producing steryl glucoside, or both, resulting in acyl steryl glycosides. Plants produce  
 735 three major sterols amongst which β-sitosterol and stigmasterol are both 24-ethyl sterols, and campesterol, a 24-methyl  
 736 sterol acting also as a precursor of brassinosteroids, a class of phytohormones involved in multiple plant developmental  
 737 processes (Belkhadir and Jaillais, 2015; Mamode Cassim et al., 2019). **C)** Phospholipids are glycerolipids made of a  
 738 glycerol backbone linking two long chain fatty acids to a hydrophilic head group with a phosphate group (Colin and  
 739 Jaillais, 2019). They can have diverse head groups, which determine the phospholipid class. Some phospholipids are  
 740 relatively abundant, such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (Colin and Jaillais,  
 741 2019). Low abundant phospholipids are also important functionally and often acts as lipid signal in membranes. These  
 742 low abundant phospholipids are anionic and include phosphatidic acid, phosphatidylserine and the phosphorylated  
 743 derivative of phosphatidylinositol, the phosphoinositides, such as phosphatidylinositol 4-phosphate (PI4P) and  
 744 phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>) (Colin and Jaillais, 2019).



745

746 **Figure 1: Feedbacks between auxin and VLCFAs during development of lateral root primordium.** Schematic  
 747 representation of auxin-triggered VLCFAs feedbacks on cell proliferation and auxin transport. In pericycle (light red), an  
 748 auxin maximum (green) triggers the synthesis of several transcription factor, including LBD16 (LOB DOMAIN-  
 749 CONTAINING PROTEIN 16) which will activate the synthesis of the peptide TOLS2 (TARGET OF LBD SIXTEEN  
 750 2, sea green). TOLS2 induces cell signaling through the RECEPTOR-LIKE KINASE 7 RLK7, and triggers the  
 751 synthesis of the transcription factor PUCHI (light blue). The TOLS2 peptide is able to move to neighboring cells and  
 752 reinforce the synthesis of PUCHI (blue) which targets transcription of genes involved in the synthesis of Very Long  
 753 Chain Fatty Acids (VLCFAs) such as KCS (3-KETOACYL-COA SYNTHASE), KCR (3-KETOACYL-COA  
 754 REDUCTASE) and PAS (PASTICCINO). Within membrane lipids, VLCFAs are mainly contain in sphingolipids  
 755 (Sphingo) that repress cell proliferation at lateral root primordium flanks and regulate polar auxin transport, thereby it  
 756 will positively feedback on the establishment of an auxin maximum in the new developing root. AUX1: AUXIN  
 757 TRANSPORTER1 (auxin-influx carrier), PIN1: PIN-FORMED1 (auxin-efflux carrier), VLCFA: very-long-chain fatty  
 758 acids, epid.: epidermis, endod.: endodermis, pericyc.: pericycle. Figure based on  
 759 <https://doi.org/10.6084/m9.figshare.5143987.v4> and <https://doi.org/10.6084/m9.figshare.4786357.v1>

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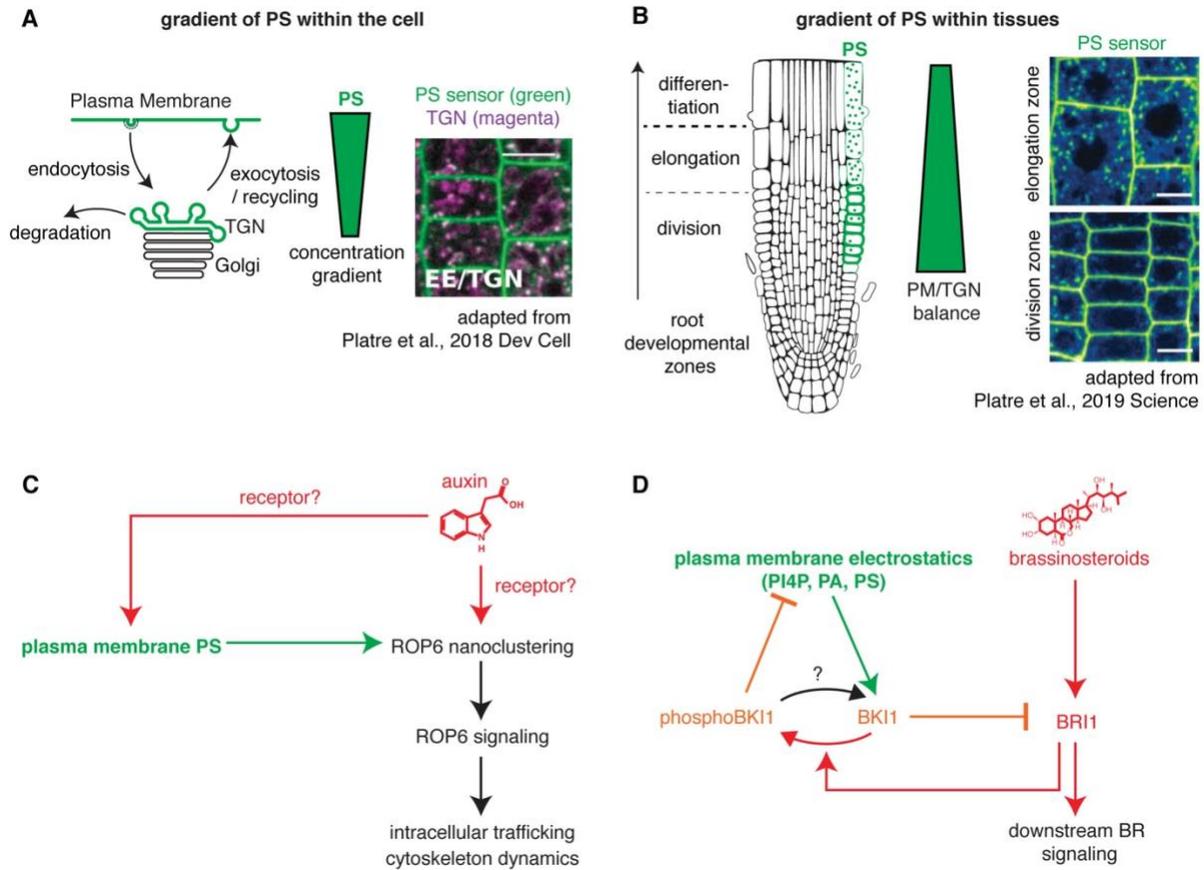


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762 **Figure 2: Non-cell autonomous sterol function in root hair positioning through ROP GTPase, AGC kinase and**  
 763 **polar auxin transport.** In epidermis (red), HYDRA1 controls the ratio between 24-ethyl sterols ( $\beta$ -sitosterol and  
 764 stigmasterol) and 24-methyl sterols (campesterol) in a non-cell autonomous manner. Sterols concentrate at the root hair  
 765 initiation site and are involved in polar positioning of RHO-OF-PLANTS (ROP) GTPase and the D6P-Kinase, which  
 766 activate polar auxin transport, create an auxin gradient and define the position of the root hair initiation site at epidermal  
 767 cells. Sterols mediate the patterning of tissues adjacent to epidermis through a non-cell autonomous signal which could  
 768 be auxin. Figure based on <https://doi.org/10.6084/m9.figshare.5143987.v4>

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771

772 **Figure 3: Feedback regulations between anionic phospholipids and auxin/brassinosteroid signaling.** A)

773 Schematic representation of the phosphatidylserine accumulation gradient at the cell level (left), and example of

774 colocalization between a phosphatidylserine sensor and a *trans*-Golgi Network (TGN) marker (right, image adapted from

775 Platre et al., 2018). B) Schematic representation of the phosphatidylserine accumulation gradient at the tissue level (left),

776 and example of confocal images showing the localization of a phosphatidylserine sensor in the Arabidopsis root

777 epidermis elongation zone (top right) and meristem (bottom right, images adapted from Platre et al., 2019). C) Auxin

778 triggers RHO-OF-PLANTS6 (ROP6) nanoclustering, which is phosphatidylserine-dependent, and subsequent

779 downstream signaling, while at the same time auxin also controls phosphatidylserine subcellular accumulation. Note that

780 the receptor that perceives auxin upstream of ROP6 is unknown. Similarly, the auxin signaling pathway involved in

781 regulating the phosphatidylserine gradients at the cell and tissue scales are unknown, but could involve the ROP6

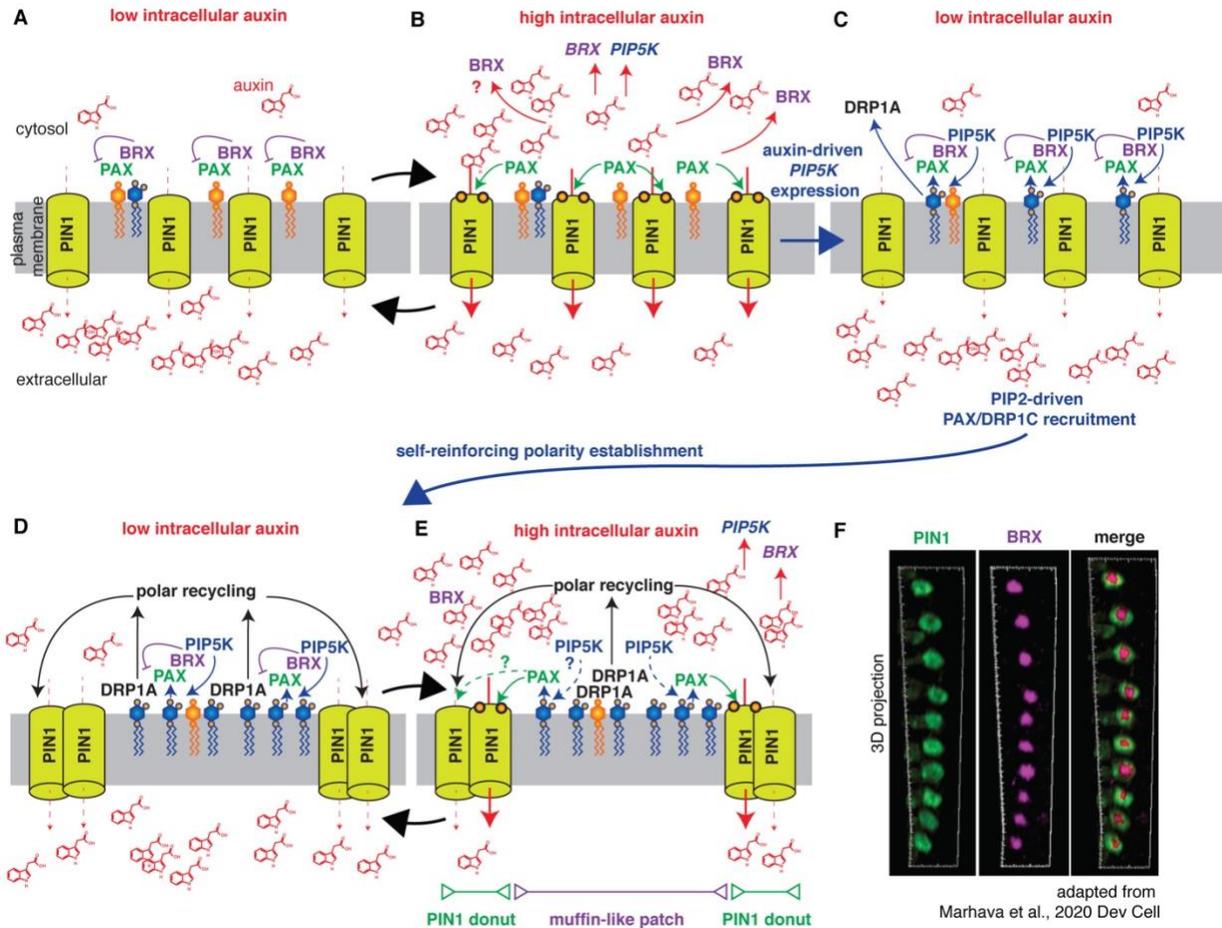
782 pathway itself. C) Anionic lipids antagonize brassinosteroid signaling by recruiting BRI1 KINASE INHIBITOR1

783 (BKI1) to the plasma membrane, which inhibits BRASSINOSTEROID INSENSITIVE1 (BRI1). Brassinosteroid

784 signaling, via BRI1 kinase activity, releases this inhibition by phosphorylating BKI1, thereby blocking the interaction

785 between BKI1 and anionic lipids and removing BKI1 from the plasma membrane. BR: brassinosteroid, PS:

786 phosphatidylserine, PA: phosphatidic acid.



787

788 **Figure 4: Feedback mechanisms between auxin and PI(4,5)P<sub>2</sub> in the regulation of polar auxin transport in**  
 789 **protophloem.** A) PROTEIN KINASE ASSOCIATED WITH BRX (PAX) is recruited to the plasma membrane via  
 790 electrostatic interactions with anionic phospholipids. When the intracellular auxin concentration is low, BREVIS  
 791 RADIX (BRX) interacts with PAX and negatively regulates its activity. PIN-FORMED1 (PIN1) is not phosphorylated  
 792 by PAX thus has a low auxin efflux activity. B) As a result of low PIN1 activity, the intracellular auxin concentration  
 793 rises, which triggers the release of BRX from the plasma membrane (by an unknown mechanism). In the absence of  
 794 BRX, PAX phosphorylates PIN1, which activates polar auxin efflux. The original molecular rheostat hypothesis  
 795 proposed that PIN1 activation allows the system to come back to the situation depicted in A (Marhava et al., 2018).  
 796 However, high intracellular auxin triggers the expression of BRX and PI4P-5-Kinases (PIP5Ks), which can further lead to  
 797 the situation depicted in C. C) Once intracellular auxin levels are lowered, BRX and PIP5Ks are recruited to the plasma  
 798 membrane by PAX. PIP5K phosphorylates PI4P into PI(4,5)P<sub>2</sub> (PIP2), which in turn enhances PAX plasma membrane  
 799 interaction. PIP2 also recruits components of the clathrin-mediated endocytosis machinery, including DYNAMIN-  
 800 RELATED PROTEIN 1A (DRP1A). D) The local increase in PIP2 increases PAX targeting to the plasma membrane,  
 801 which further lead to enhanced recruitment of PIP5Ks to the plasma membrane, leading to a self-amplifying loop, which  
 802 fixes PAX/BRX/PIP5K polarity at the center of the cell (muffin-like patch). Increased PIN1 endocytosis in this domain  
 803 could exclude it from the muffin-like patch and participate in the donut-like localization of PIN1. E) Revised version of  
 804 the molecular rheostat model, with two distinct polar domains for PIN1 and PAX/BRX/PIP5K/DRP1A (Marhava et  
 805 al., 2020). F) 3D reconstruction of confocal pictures illustrating the complementary localization of PIN1 (green) and  
 806 BRX (purple) in root protophloem cells (adapted from Marhava et al., 2020). From panel A to E, only the rootward pole  
 807 of root protophloem cell is depicted.

808