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AP2/ERF transcription factors orchestrate Very Long Chain Fatty Acids biosynthesis during Arabidopsis lateral root development

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One key factor defining root system architecture is root branching i.e. the process of the formation of new root axes, called lateral roots (LR), from an existing root. In the model plant *Arabidopsis thaliana,* LR originate from pre-selected pericycle cells that divide to create a LR primordium (LRP) that grows and eventually forms a new root apical meristem (Banda et al., 2019). During this developmental process, cell fate acquisition is very plastic and depends on positional information rather than on cell lineage (Banda et al., 2019). Importantly, LR formation occurs inside the parent root and the LRP must grow through overlying tissues before emerging into the soil. This lateral root emergence process is controlled by dynamic intercellular signaling and involves a tight coordination of cellular responses in the LRP and surrounding tissues (Banda et al., 2019). In particular, the growing LRP rapidly pushes against the overlying endodermis, a layer of cells mechanically reinforced by the Casparian strip. The crossing of the endodermis by the LRP between stage IV and V has been proposed as an important developmental checkpoint during lateral root development (Banda et al., 2019).

The AP2/ERF transcription factors PUCHI and ERF13 regulate LR development

The APETALA2/ETHYLENE-RESPONSIVE FACTOR (AP2/ERF) transcription factors (TFs) form a large group of TFs (147 genes in Arabidopsis) that are mainly found in plants (Feng et al., 2020). One AP2/ERF TF, named PUCHI, was shown to be an important regulator of LR development in Arabidopsis (Hirota et al., 2007). The *puchi-1* loss-of-function mutant exhibits higher LR initiation rate and forms LRPs with an abnormal shape that are delayed in development and emergence (Hirota et al., 2007; Trinh et al., 2019). Interestingly, an increased proportion of LRPs are blocked before the endodermis crossing checkpoint in *puchi* compared to wild type (Trinh et al., 2019). Altogether, all published data indicate that the AP2/ERF TF PUCHI coordinates cell proliferation during LR development, while the downstream targets of this TF were not known.

Recently, Lv et al. (Lv et al., 2020) reported that another Arabidopsis AP2/ERF TF, ERF13, played an important role in LR development. Auxin, a key regulator of different steps of LR development (Banda et al., 2019),

activates the MITOGEN-ACTIVATED PROTEIN KINASE 14 (MPK14; (Lv et al., 2020)). A loss-of-function mutation in *MPK14* or treatment with a map kinase kinase inhibitor (U0126) disturbed the progression of LRP development and in particular the transition between stage IV and V (Lv et al., 2020). Using different *in vitro* and *in vivo* assays, they demonstrated that MPK14 phosphorylates ERF13 leading to its degradation (Lv et al., 2020). While an *erf13* loss-of-function mutant did not show any LR phenotype, *ERF13* overexpression led to reduced LR development and an increased proportion of arrested LRP at the stage IV to V transition, reminiscent of the *mpk14* phenotype (Lv et al., 2020). This indicates that ERF13 is a negative regulator of LR development that is degraded upon auxin perception through a MPK14-dependent pathway to allow LRP development to proceed through the stage IV to V developmental checkpoint (Figure 1A).

AP2/ERF as master regulators of VLCFA biosynthesis during LR development

Recent studies revealed that both AP2/ERF TFs PUCHI and ERF13 control LR development, at least in part, through regulation of Very Long Chain Fatty Acid (VLCFA) biosynthesis. VLCFAs are fatty acids of at least 20 carbon atoms (C20) that can either be present as free CoA-VLCFAs or incorporated into lipid pools, mainly into sphingolipids (SLs) and phosphatidylserine (PS) for membrane lipids, and into suberin and cuticle/cutin for extracellular lipids (Li-Beisson et al., 2013). VLCFAs are synthesized by the elongase complex through a 4-step mechanism: a condensation by a β-ketoacyl-CoA synthase (KCS, 21 genes in *Arabidopsis*), a reduction by a β-ketoacyl-CoA reductase (2 genes *KCR1* and *KCR2* in *Arabidopsis*), a dehydration catalyzed by the β-hydroxyacyl-CoA dehydratase HCD/PAS2 and a reduction catalyzed by the enoyl-CoA reductase ECR/CER10. While KCR, PAS2 and CER10 have a broad range of substrates, KCS enzymes display specificities towards the acyl-chain length of their substrate (Haslam and Kunst, 2013).

Using a systems biology approach, Trinh et al. (Trinh et al., 2019) identified a subset of genes, including *KCS1*, *KCS2*, *KCS20*, whose expression was induced in a PUCHI-dependent manner in developing LRP (Figure 1). Accordingly, lipidomic profiling revealed that *puchi-1* display reduced content in VLCFA compared to WT. Moreover, mutants perturbed in VLCFA biosynthesis showed similar lateral root developmental defects as *puchi-1*. Hence, PUCHI activates a subset of VLCFA biosynthetic genes to regulate cell proliferation and allows correct organogenesis during lateral root development.

Similarly, using transcriptomics to compare gene expression in WT and *ERF13* loss and gain-of-function lines, Lv et al. (Lv et al., 2020) found that expression of 3 *KCS* genes (*KCS8*, *KCS16*, and *KCS18*) was negatively regulated downstream of ERF13 (Figure 1). Accordingly, VLCFA content was reduced in *35S:ERF13* lines compared to WT. Furthermore, a *kcs16* loss-of-function mutant showed similar LR development defects to *35S:ERF13* lines and overexpression of *KCS16* or treatment with VLCFA could rescue the LR development phenotype of *35S:ERF13* lines (Lv et al., 2020).

Thus, the AP2/ERF TFs ERF13 and PUCHI have opposite effects on LR development but both act, at least in

66 part, through regulation of VLCFA biosynthesis via the transcriptional control of multiple *KCS* genes (Figure
67 1A). In both cases, VLCFA biosynthetic genes were indirect targets of PUCHI and ERF13.

68

69 **Distinct pools of VLCFAs might have different roles in LR development**

70 This raises the question of the role of VLCFAs during LR development. VLCFAs can be incorporated into
71 extracellular lipids, including suberin and cuticle, and integrated into the cell wall. In *Arabidopsis*, an atypical
72 cutin layer covers the root apical meristem and developing LRP (Berhin et al., 2019). Perturbing the deposition
73 of the cuticle-like layer over the LRP leads to delay in LRP emergence and deformation of LRP, possibly due
74 to mechanical interactions between the LRP and cortical cells (Berhin et al., 2019). Thus, the cuticle-like layer
75 covering LRP may act as a "lubricant" to facilitate emergence through cortical and epidermal cell layers.
76 However, this process may not primarily involve VLCFAs, given that the main components are C18 fatty acids
77 (Berhin et al., 2019).

78 VLCFAs are also included into suberin, which is mainly present in the root endodermis. Suberin biosynthesis
79 and degradation play a role in LR formation (Li et al., 2017). However, *ERF13* loss and gain-of-function lines
80 or *puchi* loss-of-function do not seem to alter suberin formation (Trinh et al., 2019; Lv et al., 2020) suggesting
81 that VLCFA-suberin biosynthesis is not the main target of *ERF13* and *PUCHI* during LR formation.

82 Membrane lipids are other potential targets especially given that SLs, which contain VLCFA, are known to act
83 in LR formation (Roudier et al., 2010). Importantly, different profiles of VLCFAs incorporated into
84 endomembranes and plasma membranes could impact their structural and functional properties, e.g. fluidity or
85 integration of transmembrane proteins (Wattelet-Boyer et al., 2016).

86

87 **Future directions**

88 Published results suggest that PUCHI and ERF13 do not regulate the same KCS gene sets during LR
89 development (Trinh et al., 2019; Lv et al., 2020) thus hinting that different pools of VLCFA-containing lipids
90 are produced in a very dynamic and localized manner during LR development. Indeed, membrane or
91 extracellular VLCFA-containing lipids differ in fatty acid composition, for instance for chain length or
92 modification of the chain by hydroxylation. Thus, a metabolic channeling might direct specific VLCFA
93 signature to different pools of lipids at precise domains in LRP. The identification of the direct regulators of
94 VLCFA biosynthetic genes could reveal if specific VLCFA signatures are differentially triggered during LR
95 development. Moreover, the characterization of the distinct lipid pools containing VLCFA signatures, as well
96 as their localization in LRP and their impact in cell membrane composition and properties constitute another
97 important target.

98

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140

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145

146

147 **Figure legend**

148 **Figure 1.** Expression of sets of VLCFA biosynthetic genes is regulated by AP2/ERF transcription factors during
149 lateral root development.

150 (A) The AP2/ERF transcription factor PUCHI induces *KCS1*, *KCS2*, *KCS20* expression upon auxin-dependent
151 stimulation by LBD16 (Goh et al., 2019; Trinh et al., 2019). Auxin-dependent MPK-mediated phosphorylation
152 and subsequent degradation of the AP2/ERF transcription factor ERF13 results in induction of *KCS16*, *KCS8*
153 and *KCS18* expression in developing LRP (Lv et al., 2020). Expression of the different KCSs leads to the
154 production of VLCFAs.

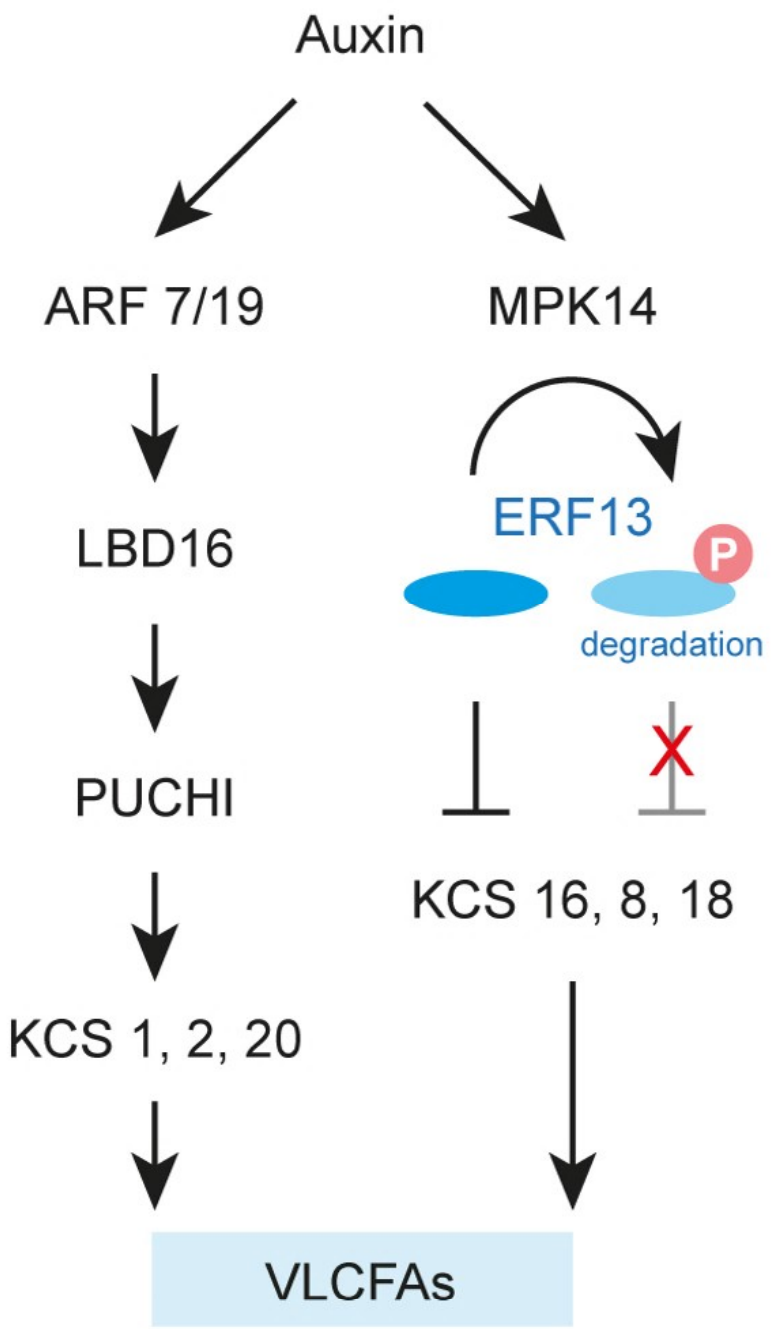
155 (B) PUCHI and ERF13 regulate the expression of *KCS* genes in developing LRP. In both cases the controlled
156 induction of VLCFA biosynthesis is necessary for correct LRP development and especially breaking through
157 the endodermis layer and proceeding to LRP emergence. Synthesized VLCFAs may incorporate into
158 hydrophobic cell wall materials (cuticle, suberin) and/or participate to endomembrane and plasma membrane

159 structures and functional dynamics in the LRP.

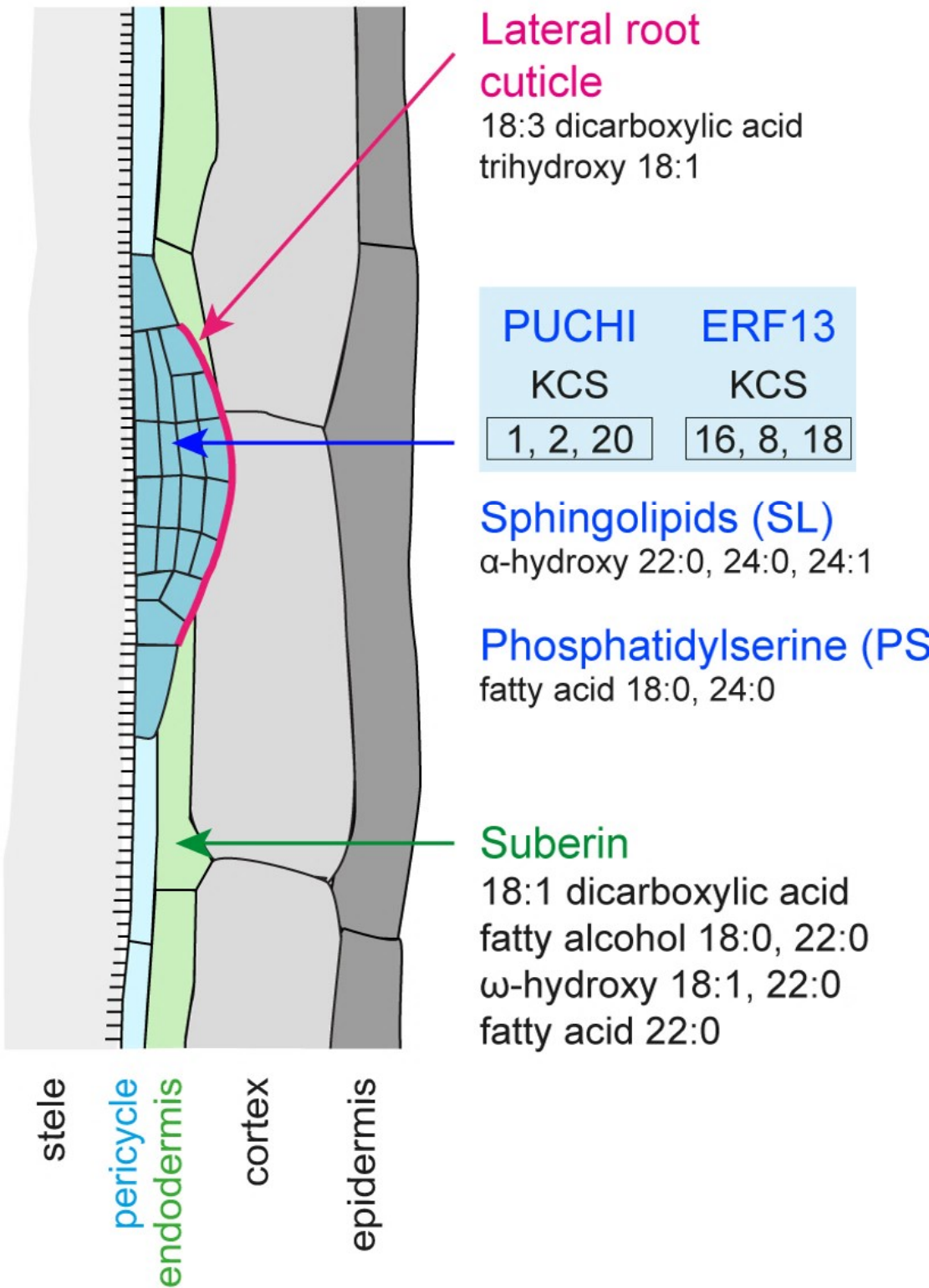
160

161

A


Auxin
ARF 7/19
MPK14
LBD16
ERF13
P
degradation
PUCHI
KCS 16, 8, 18
KCS 1, 2, 20
VLCFAs

B


Lateral root cuticle
18:3 dicarboxylic acid
trihydroxy 18:1
PUCHI
KCS
1, 2, 20
ERF13
KCS
16, 8, 18
Sphingolipids (SL)
α-hydroxy 22:0, 24:0, 24:1
Phosphatidylserine (PS
fatty acid 18:0, 24:0
Suberin
18:1 dicarboxylic acid
fatty alcohol 18:0, 22:0
ω-hydroxy 18:1, 22:0
fatty acid 22:0
stele
pericycle
endodermis
cortex
epidermis