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► **To cite this version:**

Duy Chi Trinh, Laurent Laplaze, Soazig Guyomarc'H. Lateral Root Formation: Building a Meristem de novo. Wiley Online Library, 1, 2018, Annual Plant Reviews online, 10.1002/9781119312994.apr0650 . hal-03193294

HAL Id: hal-03193294

<https://hal.science/hal-03193294>

Submitted on 5 Dec 2022

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1 **Title: Lateral root formation: building a meristem *de novo***

2 Author names: Duy Chi Trinh^{1,2}, Laurent Laplaze², and Soazig Guyomarc'h³

3 Author affiliations:

4 ¹Department of Pharmacological, Medical and Agronomical Biotechnology, University of
5 Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Hanoi,
6 Vietnam

7 ²Institut de Recherche pour le Développement, Unité Mixte de Recherche (UMR) Diversité
8 Adaptation et Développement des plantes (DIADE), Montpellier 34394 Cedex 5, France.

9 ³Université de Montpellier, Unité Mixte de Recherche (UMR) Diversité Adaptation et
10 Développement des plantes (DIADE), Montpellier 34394 Cedex 5, France.

11

12 **Abstract**

13 The complex and adaptable architecture of the plant root system in soil is of paramount
14 importance for crop growth and performance. Root growth depends on the activity of the root
15 apical meristem, an organized population of proliferating progenitor cells continuously
16 replenished from a stem cell niche. Root branching, which greatly contributes to root system
17 architecture in most dicot species, consists in *de novo* formation of new root meristems in
18 existing root tissues. This phenomenon illustrates the ability of plants to repeatedly generate new
19 tissues specialized in post-embryonic continuous growth and greatly impacts the elaboration of
20 the root system architecture and its adaptation to environmental constraints. Here, we review the
21 recent findings and models related to lateral root organogenesis in the dicot species *Arabidopsis*

22 *thaliana*, with emphasis on the mechanisms controlling *de novo* root meristem formation.

23 Experimental evidence suggests that critical regulatory modules are common between embryonic

24 and post-embryonic root meristem organogenesis, and that the lateral root formation molecular

25 pathway is in part common with organ regeneration from callus.

26 **Keywords:** lateral root, root branching, root meristem formation, *Arabidopsis*, auxin,

27 organogenesis, stem cell niche

28

29 **1. Introduction**

30 The root system fulfils multiple essential roles for the plant, including soil exploration and water
31 and nutrient uptake, interactions with surrounding biotic and abiotic environments, plant
32 anchorage to the substrate, and in some instances, vegetative reproduction or storage of
33 photosynthates (Beeckman, 2009). In doing so, it greatly influences crop performance and yield
34 (Rogers & Benfey, 2015). For example, changes in root system and water uptake explain a large
35 part of the continuous increase in maize yield in the U.S. over the past 70 years (Hammer *et al.*,
36 2009). Root system architecture (RSA), which refers to the spatial configuration of the whole
37 root system of a plant in soil, is a potent parameter influencing root system function and crop
38 growth (Lynch, 1995, 2007; de Dorlodot *et al.*, 2007; Rogers & Benfey, 2015). RSA traits have
39 been frequently overlooked in past breeding programs due to the difficulty to access and quantify
40 them. However, recent advances in phenotyping technologies and physiological modelling open
41 the way for smart crop breeding programs targeting root traits and especially RSA (Smith & De
42 Smet, 2012; Kuijken *et al.*, 2015). These new breeding strategies offer a valuable approach to
43 meet the demand in crop production in the current challenging context of increasing global
44 human population and intensifying adversary soil and climatic conditions (Godfray *et al.*, 2010;
45 Smith & De Smet, 2012; Tai *et al.*, 2014).

46 Plant RSA is modulated by 1) root growth, *i.e.* increase in root length, 2) root angle and 3) root
47 branching, *i.e.* the formation of new roots such as lateral roots (LRs, originating from existing
48 roots including lateral roots) and adventitious roots (emerging from shoot tissues, such as stem
49 bases; Osmont *et al.*, 2007; Bellini *et al.*, 2014). Root growth relies on the activity of specialized
50 tissues called root apical meristems that are organized populations of dividing cells including a
51 self-maintained stem cell niche (Aichinger *et al.*, 2012; Choe & Lee, 2017; Figure 1). Secondary

52 root formation consists of *de novo* organogenesis of new root meristems from seemingly
53 differentiated tissues. Contribution of post-embryonic root organogenesis to the elaboration of
54 the plant RSA varies greatly depending on the species. While the primary root and LRs emerging
55 from it contribute to a significant extent to RSA development in dicot species, adventitious roots
56 are predominant in RSA of most monocot species (Bellini *et al.*, 2014). Root growth and
57 branching are influenced by endogenous physiological cues as well as by environmental factors,
58 such as soil texture, nutrient and water availability, and microbial interactions (Malamy, 2005;
59 Bao *et al.*, 2014; Tian *et al.*, 2014b; Morris *et al.*, 2017). This plasticity in RSA is of paramount
60 importance for plant adaptation to environmental constraints.

61 <Figure 1 near here>

62 Meristems are complex tissues that consist of cells with little differentiation and that retain
63 mitotic activity (Aichinger *et al.*, 2012). Division and differentiation of these cells are tightly
64 regulated through a network of genetic factors and cell-to-cell communications providing plants
65 with the ability to continuously generate new tissues and organs after germination. As a result,
66 meristematic cells express repertoires of cell cycle-related genes but also specific factors
67 influencing cell fate, such as chromatin modifiers and hormone and peptide signalling pathways
68 (reviewed in Lee *et al.*, 2013; Chiatante *et al.*, 2018). Regulators of the structural properties of
69 the tissue such as cytoskeleton organization, cell membrane dynamics, as well as primary cell
70 wall formation are also of great importance for meristematic activity (Sassi & Traas, 2015).
71 Importantly, emergent properties in this intricate regulation network generate long-lasting
72 dynamic organization at the tissue scale, and especially, the maintenance of a central stem cell
73 niche. Stem cells are undifferentiated cells able to divide with no apparent limit, renewing the
74 stem cell pool as well as producing progenitor cells that will participate in the production of one

75 or more differentiated cell types (Spradling *et al.*, 2001; Laux, 2003). In plant meristems, stem
76 cells are prevented from differentiating through signalling from another group of cells, the
77 meristem organizing centre (Doerner, 1998). Specifically in root apical meristems, the stem cells
78 are called “initials” and the organizing centre is termed “quiescent centre” (QC; Choe & Lee,
79 2017). As in animal stem cell niches (Fortunel, 2003; Zipori, 2004), the transcriptomic signature
80 of plant stem cells remains elusive, although association of some specific transcription factors
81 has been shown to be important (Galinha *et al.*, 2007; Sarkar *et al.*, 2007; Scheres, 2007; de Luis
82 Balaguer *et al.*, 2017). However, hormonal, epigenetic, and transcriptional regulators have been
83 identified that play a critical role in stem cell niche establishment, organization, and maintenance
84 (Choe & Lee, 2017). Not surprisingly, some factors, such as cell cycle effectors, are important
85 for both shoot and root meristems. In addition, common schemes involving related molecules
86 participate in both shoot and root meristem organization. For example, the ratio between auxin
87 and cytokinin hormonal signalling greatly influences the balance between cell division and
88 differentiation, although with seemingly different outputs in shoot and root contexts (Galinha *et*
89 *al.*, 2009; Vanstraelen & Benková, 2012). Additionally, in both shoot and root meristems
90 transcription factors specifically expressed in the organizing centre inhibit the differentiation of
91 neighbouring stem cells and are targets of a negative feedback mediated by non-cell autonomous
92 peptides and membrane-located receptors (Stahl *et al.*, 2013). Nevertheless, other aspects of
93 meristem regulation, and especially hormonal and peptide signal transduction and its impact on
94 cell differentiation, are specific to root or shoot development (Galinha *et al.*, 2007).

95 The root apical meristem activity continuously generates new cells that participate to root growth
96 as well as to renewal of the root cap, an important interface of the root meristem with the
97 environment (Perilli *et al.*, 2012; Petricka *et al.*, 2012; Sozzani & Iyer-Pascuzzi, 2014). Still,

98 only one or few root apical meristems are generated during plant embryogenesis. A significant
99 proportion of the root system of a growing plant thus originates from post-embryonic root
100 formation through a tightly regulated sequence of cell division and differentiation. This
101 organogenesis process implies precise changes in cell cycle activities as well as modifications in
102 cell gene expression programs (Birnbaum, 2016).

103 This review focuses on lateral root (LR) development, *i.e.* root organogenesis from existing root
104 tissues, with emphasis on the processes generating a new functional root meristem. To that
105 purpose, we will predominantly consider the model plant *Arabidopsis thaliana*, in which LR
106 formation has been extensively studied. *Arabidopsis* is a dicot plant whose RSA consists of a
107 primary root, LRs of multiple orders, *e.g.* tertiary roots, and few adventitious roots (Gutierrez *et al.*,
108 *al.*, 2012; Smith & De Smet, 2012). *Arabidopsis* primary roots and LRs have a relatively simple
109 anatomy, making them a valuable model for developmental biology studies (Lavenus *et al.*,
110 2013). The young *Arabidopsis* root is made of one layer of each tissue namely from the outside
111 to the inside, the epidermis, the cortex, the endodermis and the pericycle enclosing the vascular
112 tissues, which includes two xylem and two phloem poles (Dolan *et al.*, 1993; Figure 1). In
113 addition, *Arabidopsis* is amenable to LR induction protocols, live imaging techniques, and
114 genetic reporter and mutant strategies (Koornneef & Meinke, 2010; Jansen *et al.*, 2013). A
115 wealth of information has been gathered on the processes regulating embryonic root meristem
116 establishment, primary root meristem maintenance and plasticity, as well as on the cellular and
117 molecular events underlying post-embryogenesis LR formation. Here, we synthesize recent
118 published data highlighting observed properties of post-embryonic root meristem formation and
119 aim to identify remaining gaps in our understanding of this biological process that greatly
120 contributes to plant development and crop production.

121 2. Tissue context and early events of lateral root primordium organogenesis

122 2.1. Competence of pericycle cells for root organogenesis

123 LR formation in *Arabidopsis* originates exclusively from pericycle cells (Figure 2). The
124 pericycle is a single layer of cells representing the outermost cells of the vascular cylinder
125 (Beeckman & De Smet, 2014). Pericycle cells are produced by inner initials of the primary root
126 meristem. Due to this anatomical position, some pericycle cells neighbour either xylem pole or
127 phloem pole cells. Interestingly this relative positioning is of functional importance, since lateral
128 root primordia (LRPs) originate exclusively from xylem-pole pericycle (XPP) cells in many
129 plants such as *Arabidopsis* (Dubrovsky *et al.*, 2000; Parizot *et al.*, 2008), and only from phloem
130 pole pericycle cells in others such as maize (Jansen *et al.*, 2012).

131 <Figure 2 near here>

132 What makes those pericycle cells competent for LRP initiation is not clearly understood.
133 However, a transcriptomic analysis in maize showed that compared to non-pericycle cells,
134 pericycle cells preferentially express a subset of genes related to protein synthesis, transcription,
135 and signal transduction, which could explain their competence for cell division (Dembinsky *et*
136 *al.*, 2007). In addition, analyses of the expression pattern of cell cycle regulators in roots
137 suggested that stele tissues, including pericycle, retained S-phase related gene expression longer
138 than other root tissues (Beeckman *et al.*, 2001). Consistent with this hypothesis, a recent analysis
139 of mitosis distribution in *Arabidopsis* root tips showed that, together with endodermis and
140 vascular cells, pericycle cells retained mitotic activity longer than cortex cells or epidermis cells
141 (Lavrekha *et al.*, 2017). The pericycle actually consists of a heterogeneous population of cells:
142 *Arabidopsis* XPP cells in the mature root retain some meristematic cell-specific features such as
143 fragmented vacuoles, large nuclei and dense cytoplasm, and expression of S-phase related cell

144 cycle regulators in contrast to “differentiated” pericycle cells (Beeckman *et al.*, 2001; Casimiro
145 *et al.*, 2003; Parizot *et al.*, 2008). De Almeida Engler *et al.* (2009) identified 16 cell cycle genes
146 displaying preferential expression in the Arabidopsis XPP cell file. The D-type Cyclin *CYCD4;1*
147 gene is expressed in XPP cells in the root meristem, and its loss of function causes a premature
148 elongation of these cells associated with a reduced LR density (Nieuwland *et al.*, 2009).
149 Conversely, phloem pole pericycle cells were shown to differentiate and become quiescent
150 significantly earlier than other pericycle cells, including XPP cells (Lavrekha *et al.*, 2017).
151 Longer cell cycle activity in XPP cells exiting the root meristem is dependent on the activity of
152 *ABERRANT LATERAL ROOT FORMATION 4 (ALF4)* as in the *alf4* mutant XPP cells only
153 weakly express the G2-to-M transition transgene *CYCB1;1::GUS* (Celenza *et al.*, 1995;
154 DiDonato *et al.*, 2004). *ALF4* is widely expressed in plant tissues and encodes a plant-specific
155 regulator modulating the activity of SCF complexes required for the signalling of the plant
156 hormones auxin and gibberellin (Bagchi *et al.*, 2018). Consistent with this longer cell cycle
157 activity of pericycle cells being instrumental for LR formation, the *alf4* mutant plants display a
158 strong reduction in root branching, even in presence of the root-formation promoting hormone
159 auxin (DiDonato *et al.*, 2004; Bagchi *et al.*, 2018).

160 Importantly, XPP cells express specific repertoires of genes as exemplified by the J0121
161 enhancer trap line that displays robust *GFP* expression in XPP cell files from the elongation zone
162 of the meristem onwards, as well as, interestingly, in related shoot tissues competent for root
163 formation (Casimiro *et al.*, 2001; Laplaze *et al.*, 2005; Sugimoto *et al.*, 2010). Another enhancer
164 trap line, Rm1007, showed *GFP* expression specifically in XPP cells and in the corresponding
165 initials, adjacent to the root meristem QC, including in young embryos, indicating that the

166 genomic region highlighted by the Rm1007 insertion drives very early XPP cell fate-specific
167 expression (Parizot *et al.*, 2008).

168 The close relationship between vascular pole differentiation and pericycle cell competence for
169 LR formation suggests a robust crosstalk between these two cell populations in the primary root
170 meristem. For example, the *Arabidopsis lonesome highway (lhw)* mutant, that has only one
171 xylem and one phloem pole, specifically produces LRP along that single xylem strand (Parizot *et*
172 *al.*, 2008). Conversely, roots of the *wooden leg (wol)* mutant lack phloem specification and the
173 J0121 marker is present throughout the pericycle. Interestingly, despite this characteristic, LR
174 formation is severely affected in the *wol* mutant background, suggesting that both xylem and
175 phloem specification in the primary root is required for functional LR formation (Parizot *et al.*,
176 2008). Patterning of xylem and phloem vascular identities in the central domain of the root
177 meristem is regulated through a complex network of transcriptional signalling and hormonal
178 crosstalk (reviewed in Vaughan-Hirsch *et al.*, 2018). A combination of experimental and
179 modelling approaches suggested that mutual inhibition between auxin and cytokinin signalling
180 patterned the alternating of xylem and phloem poles in the *Arabidopsis* root meristem (Bishopp
181 *et al.*, 2011a). Signalling between vasculature identity and competence of pericycle cells for LR
182 formation could involve auxin maxima in the xylem axis and phloem axis, in *Arabidopsis* and in
183 maize, respectively (Jansen *et al.*, 2012; el-Showk *et al.*, 2015). Indeed, treatments by exogenous
184 auxins or auxin transport inhibition alter the anatomical distinction between XPP and non XPP
185 cells in *Arabidopsis* (Parizot *et al.*, 2008). However, neither auxin nor cytokinin short term
186 treatments modify the expression pattern of J0121 and Rm1007 transgenes, indicating a robust
187 patterning mechanism of XPP cell identity in the root meristem (Parizot *et al.*, 2008).

188 2.2. Oscillatory LR priming by endogenous cues

189 In *Arabidopsis* LRs originate only from XPP cells, but not every XPP cell develops into a LRP.
190 Indeed in the basal meristem of the parental root tip, oscillating mechanisms periodically select
191 subsets of XPP cells and prepare them to enter LR formation, a phenomenon called “priming”
192 (Möller *et al.*, 2017). This selection process has a major impact on RSA by defining the potential
193 sites for root branching. The molecular processes underlying this priming event are still not fully
194 understood, but are associated with changes in auxin signalling (De Smet *et al.*, 2007).
195 Monitoring expression of the synthetic auxin-responsive *DR5* (*DIRECT REPEAT5*) promoter
196 revealed regular pulses of auxin signalling activity in the two protoxylem strands in the basal
197 meristem. Transcriptomic analyses showed that the expression levels of hundreds of genes were
198 also oscillating in that segment of the root, according to the same period as auxin signal
199 oscillations (Moreno-Risueno *et al.*, 2010). While cells exit the basal meristem, those temporal
200 fluctuations progressively cease and high signal stabilizes into local auxin maxima correlating
201 with future sites of LR formation (De Smet *et al.*, 2007; Xuan *et al.*, 2015). The specification of
202 these sites with high and stable *DR5* signal, termed prebranch sites, is dependent on auxin signal
203 transduction and probably on a complex network of transcription factors (Moreno-Risueno *et al.*,
204 2010). The root cap plays a crucial role in creating these auxin pulses. First, a defect in auxin
205 synthesis in the root cap impairs the amplitude of auxin signalling oscillations in the basal
206 meristem, suggesting that auxin molecules that signal XPP priming may originate from the root
207 cap (Xuan *et al.*, 2015). Second, periodic programmed cell death occurs in lateral root cap cells
208 creating rhythmic influxes of auxin into inner cells of the elongation zone of the meristem, which
209 tightly correlate with prebranch site formation (Xuan *et al.*, 2016). Pulsating fluxes of auxin from

210 the root cap to the meristem might participate in generating peaks in auxin signalling activity in
211 the protoxylem cell files, contributing to prebranch site specification.

212 Basipetal (from the root tip shootward) auxin fluxes from the columella through the lateral root
213 cap and the root epidermis have been extensively studied in Arabidopsis in particular in relation
214 to gravitropism (Rashotte *et al.*, 2000; Wisniewska *et al.*, 2006; Band *et al.*, 2012). Consistent
215 with the model of root cap-derived auxin priming XPP cells, parental root waving, mechanical
216 bending, or gravistimulation modulate LR formation, possibly because of gravity-modulated
217 auxin fluxes or changes in auxin routes due to tissue bending that affects shootward auxin
218 distribution (Ditengou *et al.*, 2008; Laskowski *et al.*, 2008; Lucas *et al.*, 2008; Scheres &
219 Laskowski, 2016). However, even agar-constrained roots, growing with only little deviation
220 from gravity, display oscillation in auxin signalling and regularly spaced prebranched sites
221 (Moreno-Risueno *et al.*, 2010). This suggests that an endogenous clock mechanism produces
222 uniformly spaced pre-branch sites to recruit pericycle cells and make them competent for *de*
223 *novo* organogenesis.

224 Recently, Laskowski and ten Tusscher (2017) analysed the properties of the priming process in
225 order to elucidate the molecular steps controlling auxin signalling oscillations and subsequent
226 stabilization of auxin signal maximum. Both could rely on emergent properties (i.e. properties
227 that arise from the collaborative functioning of a system, but do not belong to any one part of
228 that system) of the global genetic system controlling auxin distribution, auxin signal
229 transduction, and cell fate specification in the meristem (Alon, 2007). Feedback mechanisms can
230 generate oscillating gene expression patterns that result in locally fluctuating transcriptomes (De
231 Caluwé *et al.*, 2016) or, when combined with cell-to-cell communication, create spatial
232 patterning of distinct cell identities (reviewed in Green and Sharpe, 2015). Other network motifs,

233 *i.e.* specific association of gene regulatory interactions, can instead buffer variations in gene
234 expression (*e.g.* Vernoux *et al.*, 2011). Particularly interesting in a developmental perspective are
235 regulatory network properties that make the system “choose” between two potential dynamic
236 trends, causing bifurcation in system state trajectories, distinct cell fates and potentially
237 symmetry breaking at a tissue scale (Bishopp *et al.*, 2011a). Such critical properties are described
238 in various aspects of plant developmental regulation by auxin, including in root tissues.
239 Feedback of cell parameters on auxin transporter expression and polarization can create and
240 enhance non-uniform auxin distribution across tissues in a robust manner. As a result,
241 asymmetric auxin distribution, locally transduced by cell specific signalling mechanisms, can
242 create shifts in cell identity among neighbours. Van Norman *et al.* (2013) propose that the
243 priming process, caused by auxin signal pulses, could enhance cell sensitivity to later auxin
244 accumulation and its competence to translate it into the onset of a new organogenesis program.
245 Bistable properties of auxin signalling modules could explain the “memorization” of that auxin
246 signal in the prebranch sites only. Such a toggle-switch behaviour of an auxin-signalling motif
247 has been described (Lau *et al.*, 2011).

248 **2.3. Lateral root founder cell specification**

249 Within some prebranch sites, a subset of pericycle cells is later specified to become lateral root
250 founder cells. Founder cells (FCs) refer to pre-existing cells that initiate the formation of a new
251 organ through cell division (Laskowski *et al.*, 1995); thus FC specification corresponds to the
252 last apparent transition in cell identity before the onset of organogenesis. FC specification again
253 involves a local increase in auxin signalling (De Smet *et al.*, 2007; De Rybel *et al.*, 2010;
254 Moreno-Risueno *et al.*, 2010; Goh *et al.*, 2012). Selective stimulation of auxin production in one
255 or several pericycle cells is sufficient to transform them into FCs and trigger LR organogenesis

256 (Dubrovsky *et al.*, 2008). Conversely, in normal conditions, emergent properties of the auxin
257 transport and signalling network may explain why, while XPP cell specification, priming and
258 prebranch site definition may occur symmetrically on both xylem poles of the Arabidopsis
259 vascular cylinder (De Smet *et al.*, 2007; Goh *et al.*, 2012), effective initiation of LR development
260 only occurs on one of those two sides (Goh *et al.*, 2012; el-Showk *et al.*, 2015).

261 Auxin transcriptomic signalling is known to involve degradation of Aux/IAA proteins and
262 activation of associated AUXIN RESPONSE FACTOR (ARF)-family transcription factors
263 (reviewed in Weijers and Wagner, 2016). Interestingly, an auxin signalling pathway involving
264 IAA28 and several ARFs it interacts with is required for FC specification (De Rybel *et al.*,
265 2010). This auxin signalling module induces the expression of the transcription factor *GATA23*
266 in pericycle cells that will develop into a LRP before any sign of LR initiation (Figure 2).
267 Artificially inducing *GATA23* expression in the pericycle greatly increases LRP density,
268 indicating that it positively regulates LR formation (De Rybel *et al.*, 2010). *GATA23* is therefore
269 considered a marker for LR FCs, but its targets relevant for LR formation remain unknown.
270 Interestingly, *GATA23* expression is also positively controlled by RAPID ALKALINIZATION
271 FACTOR 34 (RALFL34), a small signalling peptide that could participate in auxin-mediated
272 regulation of FC specification (Murphy *et al.*, 2016).

273 Chromatin remodelling factors, for example Polycomb group (PcG) proteins, are known to
274 regulate developmental transitions in both plants and animals by modulating the expression of
275 key developmental genes (Schuettengruber *et al.*, 2017). PcG proteins can form two multiprotein
276 complexes: Polycomb repressive complex 1 (PRC1) and PRC2 which modify histone marks on
277 chromatin to repress gene expression (Derkacheva & Hennig, 2014). Arabidopsis mutants
278 impaired in subunits of the PRC2 complex CURLY LEAF (CLF) and EMBRYONIC FLOWER

279 2 (EMF2) displayed increased density of LR FCs and emerged LRs, suggesting that the PRC2
280 complex might inhibit LR FC specification (Gu *et al.*, 2014). *CURLY LEAF* is expressed in the
281 basal meristem and in presumptive FCs, and directly represses the expression of the auxin efflux
282 carrier-encoding gene *PIN-FORMED 1 (PIN1)* via histone modification (Gu *et al.*, 2014). *PIN-*
283 *FORMED 1* participates to auxin signal concentration in the root tip and in LRPs (Benková *et*
284 *al.*, 2003; Blilou *et al.*, 2005). Accordingly, an increase in the auxin-sensitive *DR5* promoter
285 activity was reported in root apical meristems and in FCs of a loss-of-function *clf* mutant (Gu *et*
286 *al.*, 2014). Other chromatin remodelling processes were shown to participate in the regulation of
287 cell fate transition during LR founder cell specification, such as histone deacetylation (Singh *et*
288 *al.*, 2012), ATP-dependent chromatin remodelling (Fukaki *et al.*, 2006; Ho *et al.*, 2013), or
289 histone variant deposition (Manzano *et al.*, 2012). The precise hierarchy of these regulatory
290 levels in combination with the auxin signalling network remains to be elucidated.

291 Plasma membrane-located signalling processes during FC specification are suggested by the
292 impaired conversion of prebranch sites into LRP in a mutant defective in the *MEMBRANE-*
293 *ASSOCIATED KINASE REGULATOR4 (MAKR4)* gene (Xuan *et al.*, 2015). The *MAKR4* protein
294 belongs to the same protein family as *MAKR5* (Kang & Hardtke, 2016) and *BRI1 KINASE*
295 *INHIBITOR 1 (BKI1)* (Wang & Chory, 2006; Jiang *et al.*, 2015), that are regulators of receptor
296 kinases-associated signalling pathways. Expression of *MAKR4*, which is induced by auxin, is
297 strongly enhanced in pericycle cells before cell division occurs and displays oscillations similar
298 to that of *DR5::LUC* reporter expression. Importantly, the *makr4* mutant has a normal number of
299 prebranch sites but produces fewer LRP, and overexpression of *MAKR4* promotes LRP
300 formation. The data indicates that *MAKR4* participates in the transition of a prebranch site into a
301 LRP (Xuan *et al.*, 2015).

302 Proper cell-to-cell communication via plasmodesmata is also necessary for correct LR FC
303 specification (Benitez-Alfonso *et al.*, 2013). In *plasmodesmal-localized b-1,3-glucanase 1*
304 (*pdbg1*), *pdbg2* single and double mutants whose increased callose deposition in the stele and
305 LRPs block plasmodesmata, LRs are formed at higher density, sometimes even fused to each
306 other (Benitez-Alfonso *et al.*, 2013). Closer inspection revealed expanded domains of *GATA23*
307 expression and auxin signalling activity (as reported by *DR5* promoter-based transgene
308 expression) around FCs, indicating that the correct function of these plasmodesmata-located
309 proteins is necessary to restrict FC specification to a subset of selected cells. How exactly
310 symplastic communication keeps LR formation in check is currently not understood, but possibly
311 through the movement of non-cell autonomous factors, such as transcription factors, peptides,
312 metabolites or hormones (Bishopp *et al.*, 2011b; Van Norman *et al.*, 2011; Benitez-Alfonso *et*
313 *al.*, 2013; Benitez-Alfonso, 2014; Yue & Beeckman, 2014).

314 **2.4. Lateral root primordia initiation**

315 In Arabidopsis, LRP initiation is first visually recognized by the swell of LR FCs, together with
316 the shrink in volume of overlaying endodermal cells (Vermeer *et al.*, 2014). Subsequently, nuclei
317 of two longitudinally adjacent FCs, in each row, migrate to the common cell wall (De Rybel *et*
318 *al.*, 2010). Then these two cells undergo an anticlinal and asymmetric division yielding two
319 shorter daughter cells next to each other (Casimiro *et al.*, 2001; De Rybel *et al.*, 2010). This
320 typical cell division occurs in 4 to 6 abutting pericycle cell files concomitantly (Goh *et al.*, 2016;
321 von Wangenheim *et al.*, 2016). Because this asymmetric cell division produces two daughter
322 cells with distinct shape and fate, it is a formative rather than a proliferative cell division
323 (Gunning *et al.*, 1978; Smolarkiewicz & Dhonukshe, 2013). Occurring independently of an
324 established stem cell niche context and in a seemingly differentiated tissue, it is a landmark of

325 the initiation of a new developmental program (De Smet & Beeckman, 2011). This first round of
326 pericycle cell division produces the so-called stage I LRP (Malamy & Benfey, 1997). LRP
327 initiation can also occur in a less common way from files of single pericycle cells (Dubrovsky *et*
328 *al.*, 2001; De Smet *et al.*, 2006).

329 The swelling of FCs prior the first asymmetric cell division is assisted by volume loss of
330 endodermal cells induced by auxin perception (Vermeer *et al.*, 2014). Indeed, when endodermal
331 cells expressed the stabilized form of SHY2, an Aux/IAA auxin signalling inhibitor, they were
332 unable to lose their volume, hence maintained their turgidity. In those conditions underlying
333 pericycle cells could not swell nor initiate asymmetric cell division, indicating that this space
334 accommodation interaction between pericycle and endodermis is necessary for LR initiation
335 (Vermeer *et al.*, 2014). Moreover, ablation of an overlying endodermal cell triggers pericycle
336 cell division, although in a periclinal manner, unless auxin was added (Marhavý *et al.*, 2016).

337 An early characteristic feature of LR initiation is the change of cell polarity revealed by nuclear
338 migration in pericycle cells preceding the asymmetric cell division. This is controlled by auxin-
339 triggered expression of the *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)* family
340 transcription factors *LBD16*, *LBD18* and *LBD33* in pericycle cells (Goh *et al.*, 2012). The
341 expression of *LBD16* in XPP and FCs is dependent on the SLR/IAA14-ARF7-ARF19 auxin
342 signalling pathway (Goh *et al.*, 2012). Interestingly, artificially converting *LBD16* into a
343 transcriptional repressor does not affect auxin signal in LR FCs but blocks the polar nuclear
344 migration and subsequent anticlinal division of these cells, suggesting that the SLR/IAA14-
345 ARF7-ARF19-LBD16 signalling cascade transduces auxin signalling into changes in pericycle
346 cell polarity (Goh *et al.*, 2012). The transcription factor bZIP59 physically interacts with LBDs,
347 including *LBD16*, *LBD17* and *LBD29* to control LRP initiation (Xu *et al.*, 2018).

348 Transcriptomic analysis of auxin-induced LR initiation shed light on the early molecular events
349 leading to pericycle cell division (Vanneste *et al.*, 2005; De Smet *et al.*, 2008; De Almeida
350 Engler *et al.*, 2009). Cell cycle progression is tightly regulated by an intricate and dynamic
351 network involving transcription regulators (such as E2F and RBR) and cyclin-dependent kinases
352 whose activity is post-transcriptionally regulated by phosphorylation, protein-protein interaction,
353 as well as APC/C-mediated ubiquitination and degradation (Komaki & Sugimoto, 2012; Breuer
354 *et al.*, 2014; Genschik *et al.*, 2014). One G1-to-S phase specific gene (*CYCD3;2*), one S-specific
355 gene (*CYCA2;4*) and two G2-to-M related genes (*CYCB2;5* and *CDKB2;1*) are upregulated in
356 roots submitted to auxin-induced LR formation (Vanneste *et al.*, 2005). Conversely, some genes
357 encoding cell cycle inhibitors such as *KRP2*, *KRP4*, *KRP7* and *WEE1* are downregulated in the
358 pericycle upon auxin treatment (De Almeida Engler *et al.*, 2009). In normal conditions, the
359 *CYCB1;1* gene, encoding a G2-to-M transition specific cyclin, is strongly expressed in XPP cells
360 undergoing the first division (Beeckman *et al.*, 2001; Himanen, 2002).

361 In addition to the change in LR FC cell polarity, SLR/IAA14-dependent auxin signalling is
362 essential for their first divisions. Either *slr* gain of function mutation or loss of function mutation
363 of its two ARF partners ARF7 and ARF19 prevents LR formation because the first anticlinal and
364 subsequent periclinal divisions of pericycle cells are blocked (Fukaki *et al.*, 2002). The
365 implication of SLR/IAA14 in priming and FC specification can be excluded because it is not
366 expressed in basal meristem (Fukaki *et al.*, 2002) and competence for LR FC specification of *slr*
367 pericycle cells is still maintained (De Smet *et al.*, 2007; De Rybel *et al.*, 2010). The
368 transcriptomic analysis by Vanneste *et al.* (2005), demonstrated that specific G1-to-S, S, and G2-
369 to-M cell cycle regulators act downstream of IAA14-ARF7-ARF19 mediated auxin signalling. In
370 addition, active ARF7 and ARF19 transcription factors stimulate the expression of *LBD18* and

371 *LBD33* which in turn stimulate the expression of *E2Fa*, a potent activator of S-phase promoting
372 gene expression (Berckmans *et al.*, 2011). Another pathway for auxin-mediated cell cycle
373 reactivation in XPP cells could consist in the downregulation of *KRP2*, an inhibitor of
374 CYCD2;1-CDKA complex activity which could stimulate G1-to-S transition (Sanz *et al.*, 2011).

375 The first asymmetric cell division in LRP formation is also regulated by a peptide belonging to
376 the GOLVEN/root growth factor/CLE-like (GLV/RGF/CLEL) family, *GLV6* (Fernandez *et al.*,
377 2015). *GLV6* is expressed in FCs preceding the migration of nuclei, and *GLV6* silencing or
378 overexpressing lines display decreased or increased FC division, respectively. In addition,
379 overexpression of *GLV6* or treatment of roots with *GLV6* peptides disrupts the migration of FC
380 nuclei to the common cell wall without disturbing cell cycle progression, converting the
381 asymmetric cell division into a symmetric division (Fernandez *et al.*, 2015). Thus cell-to-cell
382 communication mediated by *GLV6* and possibly other GLV/RGF/CLEL family peptides is
383 probably involved in the correct coordination of the asymmetric LR FC division (Fernandez *et*
384 *al.*, 2015).

385 The double mutant impaired in AURORA kinases (*aur1 aur2*) shows a strong defect in division
386 plane orientation during LR formation, as early as the first asymmetric cell division. Although
387 LR initiation density and polar localization of FC nuclei are unaffected, the positioning of the
388 new cell wall is uncontrolled (Van Damme *et al.*, 2011). Interestingly, while this results in a
389 reduced proportion of emerged LRPs, this does not totally preclude LR formation, indicating the
390 robustness of this organogenesis program (Van Damme *et al.*, 2011).

391 **2.5. Control of lateral root spacing/density**

392 Mechanisms controlling LR FC specification and LRP initiation along the primary root deeply
393 impact the overall root branch production, *i.e.* LRP density (number of LRPs divided by the

394 length of the parent root). In addition, other factors are required for efficient LRP spacing. The
395 gene encoding the membrane localized receptor-like kinase *ARABIDOPSIS CRINKLY4 (ACR4)*
396 is expressed specifically in the short daughter cells after the first asymmetric cell division. The
397 *acr4* mutant produces LRPs at higher densities and these LRPs usually stretch, sometimes even
398 fuse together, indicating that ACR4, and possibly related kinases, control cell proliferation and
399 spacing of newly initiated LRPs (De Smet *et al.*, 2008). ACR4 was proposed to be the receptor
400 of GLV6 and other related peptides (Fernandez *et al.*, 2013), and possibly to transduce perceived
401 signals inside the cell through a phosphorylation cascade involving the PROTEIN
402 PHOSPHATASE 2A (PP2A; Yue *et al.*, 2016). Cytokinins synthesized at the flanks of existing
403 LRP was found to act as an inhibitor of LRP initiation, thereby preventing closely formed LRPs
404 (Chang *et al.*, 2015). Interestingly, expression of *ACR4* and *GLVs* were significantly reduced in
405 mutants defective in cytokinin biosynthesis, but ACR4 and cytokinins seems to act in partially
406 separate pathways (Chang *et al.*, 2015). Another peptide, C-TERMINALLY ENCODED
407 PEPTIDE 5 (CEP5), is also involved in the regulation of LRP initiation and positioning (Roberts
408 *et al.*, 2016). Overexpression of the *CEP5* gene and exogenous treatment by the CEP5 peptide
409 reduce LRP initiation events and induce the formation of closely positioned LR, while *CEP5*
410 *RNAi* lines display an increase in LRP stage I and II density. Remarkably, *CEP5* is expressed
411 specifically in phloem pole pericycle cells and adjacent phloem cells, indicating an unknown
412 crosstalk between different pericycle populations (Roberts *et al.*, 2016). Similarly, the
413 PLETHORA (PLT) transcription factors PLT3, PLT5, PLT7 act redundantly downstream of the
414 ARF7- and ARF19-mediated auxin signalling pathway to regulate LRP spacing (Hofhuis *et al.*,
415 2013).

416 In summary, cells competent for LR formation are pericycle cells that have been progressively
417 selected through a series of regulatory events: competence acquisition (or conservation) in the
418 root meristem along with vascular strands patterning; priming of subsets of these cells through an
419 endogenous clock mechanism followed by memorization of the prebranch site status, and
420 eventually initiation of the LR organogenesis program revealed by anticlinal asymmetric cell
421 division of so-called LR FCs. At each of these steps, local auxin signalling is involved in cell
422 selection and state transition. Regular LR patterning along the root results from a robust
423 emergent behaviour of the endogenous regulatory system (Figure 2). Still, this system allows the
424 integration of environmental inputs, which translate into changes in auxin distribution, thus
425 allowing adaptive responses of LR initiation to environmental constraints (Yu *et al.*, 2015,
426 2016).

427 **3. Building a new organ: a coherent program of coordinated cell divisions, with newly set** 428 **axis of growth and boundaries**

429 After initiation, LRPs evolve in terms of tissue organization and morphology, developing in a
430 direction perpendicular to the main root axis. LRP development is categorized into stages
431 characterized by structural and positional features (Malamy & Benfey, 1997; Figure 3). Briefly,
432 LRPs of stage I, II, III and IV are one, two, three and four cell layers thick, respectively, thanks
433 to successive periclinal divisions. Stage V LRPs penetrate the endodermis and are characterized
434 by two cuboidal cells of the second outermost layer. At stage VI, LRPs have the typical dome
435 shape reminiscent of the primary root tip, and its outermost layer has around 12 cells. LRPs of
436 stage VII are more complex structurally and touching the epidermis, ready for emergence
437 (Malamy & Benfey, 1997). From founder cells to an emerged LR, LRP organogenesis relies on a

438 series of cell divisions in different planes that need to be well coordinated to ensure proper
439 organization and development of the new organ.

440 <Figure 3 near here>

441 **3.1. Control and sequence of cell division and division planes**

442 As mentioned above, cell division is instrumental in LRP organogenesis, and in Arabidopsis
443 conserved landmarks define important steps in the process of new LR formation. However, in
444 contrast to embryo organogenesis which follows a highly regular cell lineage (Palovaara *et al.*,
445 2016), cell division in developing LRPs do not follow a fully stereotypic sequence, leading to
446 significant differences in cell numbers between LRPs of the same stage (Lucas *et al.*, 2013; von
447 Wangenheim *et al.*, 2016). Nevertheless, modelling and experimental observation revealed that
448 division of LRP cells may follow a small set of rules: (i) cells tend to follow the “shortest wall”
449 principle, meaning that the positioning and geometry of newly formed cell wall frequently
450 minimize the surface contact between the two daughter cells, (ii) cells often alternate their
451 division plane (*i.e.* periclinal and anticlinal) between two consecutive divisions, and (iii) the
452 outer layer predominantly divides periclinally prior to the inner layer (von Wangenheim *et al.*,
453 2016). Remarkably, despite variation in cell number, LRP shapes at a particular stage appear to
454 be consistent (Lucas *et al.*, 2013), indicating some higher levels of regulation contributing to the
455 dome shape formation. Differential contribution of each pericycle founder cell to the LRP
456 organogenesis participates in this dome shape development. In live time course analysis, ~60%
457 of the LRP cell population derived from two or five founder cells (von Wangenheim *et al.*,
458 2016). Importantly, mutants of AURORA kinases, that are impaired in the control of cell
459 division planes, can still form LRPs having typical domed-shape but lacking layered

460 organization (Van Damme *et al.*, 2011; Lucas *et al.*, 2013). All the data points to a great
461 conservation in shape formation of developing LRPs.

462 Changes in cell division planes guide major transitions during the LRP morphogenesis, namely,
463 the establishment of a new axis of growth, perpendicular to the main axis of the parental root,
464 and the transition from bilateral to radial development. The first transition corresponds to the
465 first periclinal divisions of stage I primordium cells, creating two cell layers characteristic of the
466 stage II primordium. What controls this perpendicular shift in cell division plane orientation is
467 currently not clearly understood. A long-standing model for cell division plane orientation in
468 plants, which can be formulated as the “shortest wall” rule, proposes that the new cell wall
469 follows one of the possible configurations that minimize the interface area between the two
470 daughter cells. This model fits observed cell division planes in many cases, especially in
471 embryos, shoot meristems, and LR development (Yoshida *et al.*, 2014; Louveaux *et al.*, 2016;
472 von Wangenheim *et al.*, 2016). However even in those contexts in some instances, this model
473 could not predict division plane orientation. Monitoring of the first periclinal division in the
474 developing LRP using light sheet microscopy showed that only 50% of the dividing cells
475 followed the “shortest wall” rule (von Wangenheim *et al.*, 2016). More recently, a mechanical,
476 tension-based rule for cell division plane orientation was proposed (Louveaux *et al.*, 2016). This
477 rule could explain cell division plane orientation better than the “shortest wall” principle in
478 tissues submitted to anisotropic forces, which could be the case for the swelling pericycle cells
479 constrained between the underlying XPP cells, the overlaying endodermis, and the neighbouring
480 pericycle cells. Interestingly, endodermis cell ablation, which modifies mechanical constraints in
481 the surrounding tissues, provokes the periclinal cell division of the underlying pericycle cell
482 (Marhavý *et al.*, 2016). Alternatively, high auxin concentration was proposed to instruct dividing

483 cells to escape the default “shortest wall” rule during embryo development (Yoshida *et al.*,
484 2014). Dividing pericycle cells are known to be sites of high auxin signalling, dependent from
485 convergent auxin transport from neighbouring pericycle cells as well as overlaying endodermal
486 cells (Benková *et al.*, 2003; Marhavý *et al.*, 2013). Strikingly, auxin supplementation after
487 endodermis cell ablation causes ectopic pericycle cell division plane to shift from periclinal to
488 anticlinal (Marhavý *et al.*, 2016). Altogether, this data supports the hypothesis that auxin
489 distribution across the tissue and/or mechanical constraints influence formative periclinal cell
490 plane orientation and the onset of the LRP growth axis.

491 Interestingly, by meticulously documenting the orientation of cell division in 3D of hundreds
492 LRPs from stage I to VI, Lucas *et al.* (2013) found that cells on the flanks of LRPs undergo
493 either tangential or radial division. As a consequence, these cells form a ring surrounding the
494 mass of daughter cells originated from the central cell files, contributing to the transition from
495 the bilateral structure of the newly initiated LRP to its radial structure at later stages (Lucas *et al.*,
496 2013). Because of the particular position of these cells at the flanks of the developing
497 primordium, their specific division geometry could also be influenced by local mechanical or
498 hormonal cues.

499 **3.2. LRP cellular organization and anatomical patterning**

500 During regular Arabidopsis LR development, the sequence of cell divisions, although not fully
501 stereotyped, produce conserved anatomical landmarks in the elaboration of the LRP cellular
502 patterning (Malamy & Benfey, 1997). Notably, important transitions are (i) the first round of
503 periclinal cell divisions at the stage I to II transition, (ii) the organisation of a small group of
504 subapical cells reminiscent of a meristem-organizing centre at the stage IV to V transition,
505 concomitant with LRP breaking through the endodermis, acquiring dome shape and radial

506 symmetry (Goh *et al.*, 2016), and (iii) the elongation of basal cells during LRP emergence
507 (Malamy & Benfey, 1997; Figure 3). These transitions highlight changes in the identity and
508 behaviour of some LRP cells and contribute to the LRP functional patterning. For instance,
509 specific gene expression profiles distinguish the outer layer from the inner layer cells in stage II
510 LRPs. The expression of *SCARECROW (SCR)* is first detected specifically in the outer layer of
511 stage II LRP, while *SHORT-ROOT (SHR)* expression is detected weakly from stage I and in both
512 inner and outer layers of stage II LRP (Malamy & Benfey, 1997; Tian *et al.*, 2014a; Goh *et al.*,
513 2016). Gene expression patterns become more and more complex as the LRP grows and
514 organizes into a new root apical meristem. The molecular events controlling *de novo* meristem
515 patterning will be addressed in the next section. First, we will consider major regulators of
516 overall LRP morphogenesis and patterning.

517 Analysis of auxin signalling (as reported by *DR5*-based reporter expression) in developing
518 primordia shows that while being strongly and homogenously enhanced in stage I LRP as
519 compared to surrounding tissues, auxin activity is gradually confined to the central cells in outer
520 cell layers of developing LRPs, and to the tip of emerged LRs (Benková *et al.*, 2003). In various
521 tissues, gradients of auxin distribution are known to be predominantly controlled by the polar
522 localization of PIN-FORMED family auxin carriers, although other auxin transporters, or auxin
523 biosynthesis or degradation, can participate (Petrasek & Friml, 2009; Zhao, 2010; Tang *et al.*,
524 2017). Several members of the *PIN* family are expressed in developing primordia, including
525 *PIN1* and *PIN3*, although for some genes experimental results differ depending on the reporter
526 constructs used (Benková *et al.*, 2003; Guyomarc'h *et al.*, 2012). Notably the *pPIN1::PIN1:GFP*
527 reporter construct (rescuing the *pin1* mutant phenotype) is expressed early during LRP
528 development and the recombinant protein displays a polar distribution in the cell membrane

529 pointed to the primordium tip, consistent with *DR5*-reported auxin signalling gradients. LRPs
530 produced in a context of impaired auxin transport, either through genetic mutations or chemical
531 treatments, display dramatic alterations in shape and organization and eventually often fail to
532 emerge (Benková *et al.*, 2003; Geldner *et al.*, 2003; Moriwaki *et al.*, 2011). Conversely,
533 artificially inducing pericycle cell division, either by cyclin transgenic expression or by
534 endodermal cell ablation, provokes the accumulation of cells without any sign of primordium
535 patterning unless auxin is added (Vanneste *et al.*, 2005; Marhavý *et al.*, 2016). This data
536 collectively demonstrates that non-uniform auxin distribution in the primordium is instructive for
537 its cellular organization.

538 The distribution pattern of PIN proteins is regulated by the *PLT* genes, a family of
539 AINTEGUMENTA-like transcription factors which have multiple roles in auxin-regulated root
540 development (Blilou *et al.*, 2005; Galinha *et al.*, 2007; Du & Scheres, 2017; Scheres & Krizek,
541 2018). While *PLT3*, *PLT5* and *PLT7* are expressed in some pericycle cells prior to the first
542 asymmetric cell division of LRP, *PLT1*, *PLT2* and *PLT4* are expressed in LRP of later stages
543 (Hofhuis *et al.*, 2013). The expression of *PLT3*, *PLT5* and *PLT7* is required for the expression of
544 *PLT1*, *PLT2* and *PLT4*, so that the triple mutant *plt3 plt5 plt7* is actually a PLT null mutant in the
545 context of LRP development (Du & Scheres, 2017). A majority of LRP in the triple mutant *plt3*
546 *plt5 plt7* lack periclinal cell division in early stages and show abnormal cell division planes at
547 later stages, resulting in abnormally shaped LRPs. These LRPs have very low expression of *PINs*
548 including *PIN1* and *PIN3*. Very few LRPs of this mutant are able to emerge (Du & Scheres,
549 2017), again pointing to the role of polar auxin distribution in LRP development.

550 Some transcription factors were shown to play a critical role during the first step of LRP cellular
551 organization. The GRAS family transcription factor *SHORTROOT* (*SHR*) gene is expressed in

552 primary meristem stele, including the pericycle and stage I developing LRP, and the SHR protein
553 is also detected in the overlying endodermis layer (Goh *et al.*, 2016). As early as stage II, the
554 cytoplasmic SHR protein is confined to the inner layer or the developing LRP, while nuclear
555 SHR proteins are detected in the outer cell layer (Goh *et al.*, 2016). Concomitantly expression of
556 an SHR target, the related transcription factor *SCR* gene, is initiated in the outer layer of stage II
557 primordia, and later is restricted to the second outermost layer, contacting the *SHR*-expression
558 domain (Goh *et al.*, 2016). Importantly, analysis of loss-of-function mutants showed that SHR
559 function is required for proper lateral LRP organization (Lucas *et al.*, 2011a; Goh *et al.*, 2016).
560 In addition, SCR controls specifically the cell division in the outer layer of stage II primordia. In
561 *scr-3* mutant primordia, this division is blocked whereas cell division in the inner layer continues
562 with little modifications compared to the wild type. As a consequence, the *scr-3* LRP patterning
563 is severely affected from stage II onwards (Goh *et al.*, 2016).

564 **3.3. Lateral root primordia boundary**

565 To describe the organization of a LRP, the concepts of boundary (or flank/periphery) and centre
566 domains are frequently used. LRP boundary and centre domains can be delimited by the ring of
567 cells originated from tangential and radial cell division described above. These cells seem to act
568 as a structure to support the rapidly developing LRP apex and are termed “buttress cells” (Lucas
569 *et al.*, 2013; Figure 1). Their cell division pattern could be distinctively controlled since several
570 cell cycle genes are preferentially expressed in the boundary (De Almeida Engler *et al.*, 2009).

571 A few genes involved in the regulation of LRP boundary have been reported. The auxin
572 transporter *PIN6* gene is expressed specifically in the boundary of developing LRPs and is
573 involved in auxin homeostasis during plant development. LRPs of seedlings overexpressing
574 *PIN6* undergo more rounds of anticlinal cell division, resulting in misshaped LRPs (Cazzonelli *et*

575 *al.*, 2013). *PUCHI* encodes a transcription factor of the AP2/EREBP family involved in the
576 control of LRP morphogenesis. Its expression is detected in the whole LRP of early stages and
577 gradually confines to the flank domain of LRPs of later stages. Periphery cells of LRPs of *puchi-*
578 *1* mutant have extra anticlinal cell divisions, and some of those cells may be enlarged, suggesting
579 that *PUCHI* regulates cell division pattern and cell differentiation during LRP development
580 (Hirota *et al.*, 2007). *PUCHI* functions downstream of auxin signalling and is proposed to co-act
581 with LBD16/18 to control LRP development (Hirota *et al.*, 2007; Kang *et al.*, 2013). Its targets
582 are currently unknown.

583 Loss of function of *ARABIDOPSIS HOMOLOG of TRITHORAX1 (ATX1/SDG27)*, a chromatin
584 remodelling factor, impacts the coordination of cell division and cell differentiation in both
585 primary root meristems and developing LRPs. Developing LRPs lacking *ATX1* function are
586 delayed, disorganized, and frequently exhibit enlarged flanking cells (Napsucialy-Mendivil *et al.*,
587 2014). Still, the auxin signalling pattern in *atx1* (as reported by *DR5::GFP*) looked similar to
588 normal LRPs suggesting that *ATX1* controls LRP patterning and cell proliferation independently
589 of auxin response (Napsucialy-Mendivil *et al.*, 2014).

590 Similarly, loss of function of the transcription factor *MYB36* causes misspecification of the LRP
591 boundary (Fernández-Marcos *et al.*, 2017). In the primary root meristem *MYB36* is a target of
592 *SCR* and a master regulator of endodermis cell differentiation (Lieberman *et al.*, 2015). In LRP,
593 *MYB36* is expressed specifically in boundary cells and regulates genes involved in reactive
594 oxygen species homeostasis. It is hence proposed that *MYB36* regulates cell proliferation of LRP
595 boundary domain by controlling their redox status (Fernández-Marcos *et al.*, 2017).

596 3.4. LRP shape progression through surrounding tissues and LRP emergence

597 As LRP cells divide, the LRP grows in contact with overlaying tissues, the first one being the
598 endodermis. While the LRP develop from stage I to stage IV, the overlying endodermis, which
599 contains lignified and suberized cell walls (Naseer *et al.*, 2012; Vishwanath *et al.*, 2015), is
600 subjected to important cell wall modifications (Vermeer *et al.*, 2014). LRPs of mutants having
601 increased deposition of suberin or lignin in the endodermis (*i.e.* more physical constraints) or
602 plants in which the auxin-mediated response of endodermis has been impaired, are flatter
603 compared to those of WT, leading to a delay in LRP emergence (Lucas *et al.*, 2013). By tracking
604 the height of LRP during their development, Goh *et al.* (2016) showed that LRP heights increase
605 gradually from stage I to IV, but display a dramatic increase in LRP height during stage V when
606 LRP crosses the endodermis. That sudden change can be explained by cell expansion rather than
607 cell division (Goh *et al.*, 2016). The shape of LRP also changed from flat-topped to dome-shaped
608 after breaking through the endodermis (Lucas *et al.*, 2013; Goh *et al.*, 2016).

609 The Casparian strips surrounding endodermis cells and linking them together form a
610 supracellular network that is sturdy and resistant to pectinases (Geldner, 2013), meaning that it is
611 difficult for endodermal cells to separate to accommodate LRP penetration. Overlaying
612 endodermal cells lose their cell volumes instead and become flatter. Plasma membranes of these
613 cells are sometimes even fused together, but their integrity largely remains intact (Vermeer *et al.*,
614 2014). Consequently, endodermal cells are separated at small breaking points just enough for the
615 penetration of an LRP (Vermeer *et al.*, 2014).

616 Although the volume loss mechanism of endodermal cells minimizes the impact of LRP on
617 Casparian strip integrity, it still can be compromised. A layer of suberin is deposited at the site of
618 LRP penetration, covering both the endodermal cells and LRP surface (Li *et al.*, 2017). This

619 layer of suberin is thought to compensate to the damage of the Casparian strip, ensuring the
620 function as an apoplastic barrier of the endodermis (von Wangenheim *et al.*, 2017).

621 Although the cortex and epidermis pose less physical constraints to a developing LRP, they are
622 also important for LRP development and participate actively to the process (reviewed in Stoeckle
623 *et al.*, 2018). Epidermal cells of two adjacent cell files are separated from one another to
624 facilitate the progression of a LRP (Swarup *et al.*, 2008). This separation is regulated by activity
625 of cell-wall remodelling enzymes in cells directly overlaying a developing primordium.
626 Expression of these enzymes is regulated by an ARF7-LBD29-LAX3-LBD18 cascade (Swarup
627 *et al.*, 2008; Lee & Kim, 2013; Porco *et al.*, 2016) and other players such as *BRS1* (Deng *et al.*,
628 2017).

629 Water movement between cells is partially controlled by water channels called aquaporins
630 integrated in cell membranes (Li *et al.*, 2014). A combination of experimental analysis of root
631 tissue permeability to water and plasma membrane intrinsic aquaporins (PIPs) gene expression
632 suggested that a complex spatio-temporal regulation of water fluxes across the root tissues, at
633 least partially mediated by auxin, was critical for LRP emergence (Péret *et al.*, 2012). A
634 modelling approach proposed that for optimal LRP emergence, auxin-mediated fine tuning of
635 water transport could participate in increasing cell turgor in the LRP while decreasing it in the
636 overlaying cortical and epidermal cells (Péret *et al.*, 2012). Moreover, during LRP development,
637 tonoplast intrinsic aquaporin (TIP) protein levels are also regulated spatio-temporally, and
638 defects in TIPs led to defects in LRP emergence. It was proposed that TIPs finely control the
639 movement of water from surrounding tissues into cells at the base of LRP, allowing these cells to
640 expand rapidly, thereby facilitating the emergence of LRPs (Reinhardt *et al.*, 2016). This model
641 is consistent with the observation that the emergence of LRPs through overlying tissues is largely

642 due to the rapid expansion of these basal cells (Malamy & Benfey, 1997; Goh *et al.*, 2016). A
643 recent study showed that during LRP formation, the circadian clock is re-phased, and this
644 modulation regulates auxin-related genes and is required for LR emergence (Voß *et al.*, 2015).
645 One possible explanation is that the re-phased circadian clock helps differentially control
646 hydraulic properties of cells within LRP and in surrounding layers, thereby facilitating LRP
647 emergence (Voß *et al.*, 2015).

648 **3.5. Connectivity between developing lateral root primordia and the primary root**

649 The LR vasculature needs to connect to that of the primary root for proper functioning. Stele
650 connection happens only when the LR already emerges as revealed by phloem unloading and
651 stele marker expression studies (Oparka *et al.*, 1995; Malamy & Benfey, 1997). In LRPs, the
652 connection with the primary root stele relies on symplastic connectivity, *i.e.* plasmodesmata.
653 Using the symplastic reporter *pSUC2-GFP*, it was shown that symplastic connection is present at
654 early stages of LRP development, but is completely blocked at later stages until a functional stele
655 is formed in emerged LRs (Guyomarc'h *et al.*, 2012; Benitez-Alfonso *et al.*, 2013). This change
656 is correlated with the progressive deposition of callose at plasmodesmata, a process regulated by
657 callose-degrading enzymes encoded by *PdBG* genes (Benitez-Alfonso *et al.*, 2013).

658 **4. Meristem formation and activation during lateral root formation**

659 The root apical meristem is a specialized tissue ensuring root elongation and root cap
660 regeneration through tightly regulated cell division and differentiation. In Arabidopsis, primary
661 root apical meristem formation during embryogenesis and its post-embryonic maintenance have
662 been thoroughly studied (reviewed in ten Hove *et al.*, 2015; Figure 3B). Arabidopsis root apical
663 meristem displays a simple and stereotyped cellular organization with a central stem cell niche
664 surrounding the organizing centre, called QC, and providing new progenitors for all the cell

665 types of the root and the root cap (Figure 1). Stem cells, called initials, can be recognized from
666 their location, close to the QC, and from their ability to produce progenitor cells through
667 asymmetric cell division. Progenitor cells, or transit amplifying cells, can be recognized by the
668 expression of cell fate specific marker genes as well as by proliferative cell divisions tending to
669 conserve clonally related cell file structures. Key players during the embryonic root apical
670 meristem formation and maintenance include auxin and its regulators, and transcription factors
671 such as WUSCHEL RELATED HOMEBOXs (WOXs), PLTs and SHR-SCR (Figure 4B).

672 <Figure 4 near here>

673 **4.1. Common molecular mechanisms controlling embryonic root apical meristem and LRP** 674 **meristem formation**

675 Recent studies have pointed to a parallel in the regulation of meristem formation and
676 maintenance during embryogenesis and LRP development.

677 *WUSCHEL RELATED HOMEBOX 5 (WOX5)*, a marker for the primary root QC (Haecker *et*
678 *al.*, 2004), is also expressed during lateral root formation. The *WOX5* function in columella stem
679 cell maintenance appears to be conserved between primary root, lateral roots, and adventitious
680 roots (Sarkar *et al.*, 2007; Tian *et al.*, 2014a; Hu & Xu, 2016). *WOX5* is expressed at least from
681 the stage IV-stage V transition onwards, in the 2 or 4 central cells presumptive of the QC
682 anatomical organisation (Goh *et al.*, 2016; Rosspopoff *et al.*, 2017), although its expression may
683 start even earlier, as suggested by other reporter constructs (Ditengou *et al.*, 2008; Tian *et al.*,
684 2014a; Du & Scheres, 2017). The importance of the stage IV-V transition for presumptive QC
685 establishment is further supported by the onset of the QC-specific enhancer trap QC25
686 expression in the subapical cells of the developing primordium (Goh *et al.*, 2016).

687 Expression of *WOX5* during embryonic root formation is dependent on the SHR-SCR module
688 (Sarkar *et al.*, 2007). In the embryo, *SHR* is transcribed in the root stele (vascular tissues and
689 pericycle), and SHR controls *SCR* expression in the endodermis and QC in a non-cell
690 autonomous fashion (Nakajima *et al.*, 2001; Cui *et al.*, 2007; Fisher & Sozzani, 2016). Similarly,
691 during LRP development, SHR is found in presumptive stele tissues, whereas *SCR* expression is
692 confined to the endodermis precursor layer (Lucas *et al.*, 2011b; Goh *et al.*, 2016). Expression of
693 *SHR* in the inner layer but not in the outer layer is an early patterning event in stage II LRP.
694 Moreover, *SCR* expression seems to be activated by SHR, and is required for proper QC
695 specification in the developing LRP (Sabatini *et al.*, 2003; Goh *et al.*, 2016). SHR is also
696 required for LRP initiation and development and for asymmetric divisions of cortex/endodermis
697 initials in LRs (Lucas *et al.*, 2011b).

698 PLTs, key players in embryonic root development, are also important for LRP formation. PLT1,
699 PLT2 and PLT3 redundantly control embryonic root meristem establishment, since the triple
700 mutant *plt1plt2plt3* produces rootless seedlings and the *plt1plt2* double mutant embryos only
701 produce abnormal root meristems with no expression of QC marker genes (Aida *et al.*, 2004).
702 *PLT3*, *PLT5* and *PLT7* are expressed as early as in stage I primordia where they control the
703 asymmetric periclinal divisions giving rise to the stage II primordium. The activity of PLT3,
704 PLT5 and PLT7 is necessary to switch on the differential expression of *SHR* and *SCR* in the two
705 layers of the stage II primordium, as well as to induce expression of other *PLTs* including *PLT1*,
706 *PLT2* and *PLT4* (Du & Scheres, 2017). Interestingly, as the LRP progresses *PLT5* expression is
707 progressively confined to the base of the LRP, while the others are predominantly expressed in
708 the central region comparable to the situation in the primary root meristem (Du & Scheres,
709 2017). In addition, the expression of *PLTs* is necessary to restrict auxin signalling maximum at

710 the tip of the developing primordia by controlling expression of auxin transporter *PIN* genes,
711 again similar to the situation in the primary root apical meristem (Galinha *et al.*, 2007; Du &
712 Scheres, 2017). As a result, in the *plt3 plt5 plt7* mutant background LRPs are usually severely
713 disorganized and contain enlarged cells. Very few of these LRPs emerge, indicating that LRPs of
714 the *plt3 plt5 plt7* mutant could not form a functional meristem (Hofhuis *et al.*, 2013; Du &
715 Scheres, 2017). Instead the double mutant *plt1 plt2* can produce lateral roots but the meristem of
716 these roots are disorganized and differentiate shortly after LR emergence, similar to the
717 phenotype of the primary root apical meristem (Aida *et al.*, 2004). Together the data reveal a
718 master role of *PLT3*, *PLT5*, and *PLT7* genes in initiating the regulatory cascade that controls the
719 functional patterning of the LRP centre.

720 ARF5/MONOPTEROS (MP) is critical for embryonic root formation, since loss-of-function *mp*
721 alleles, or dominant gain-of-function alleles of its inhibitor IAA12/BODENLOS, result in
722 rootless seedlings (ten Hove *et al.*, 2015). MP exerts its functions by positively controlling
723 expression of multiple auxin transporter genes, including *PIN1*, *PIN4*, *PIN7*, *AUX1* and *LAX2*, to
724 trigger polar auxin accumulation necessary for embryonic root specification (Weijers *et al.*,
725 2006; ten Hove *et al.*, 2015). Recently, using a mutant background which allows studying the
726 roles of MP post-embryonically, Krogan *et al.* (2016) demonstrated that MP directly binds to
727 promoters of *PIN1*, *PIN3*, and *PIN7* to regulate their expression levels and auxin distribution in
728 developing LRP. Polar distribution of PIN1:GFP proteins in LRPs of the mutant also diminishes,
729 coincident with the defects in LRP development. These mutant LRPs are disorganized and
730 unable to develop after emergence, suggesting that a functional meristem is not established or
731 maintained (Krogan *et al.*, 2016).

732 Experimental evidences described above point to a similarity in molecular mechanisms
733 governing primary and lateral root development, although differences possibly linked to the
734 distinct tissue context, can be noted. Especially, it is interesting to stress the contrasting roles of
735 PLT3,5,7 and PLT1,2,4 in lateral root development, the former being required for the onset of
736 expression of the latter, by the stage when *WOX5* expression in the presumptive QC starts. In
737 addition, specific regulatory network properties might be involved to organise the developing
738 lateral root primordia and define its boundaries as well as its central domain, in which the root
739 meristem can be patterned (Lavenus *et al.*, 2015).

740 **4.2. Lateral root meristem activation**

741 A first approach to determine the formation of a new root apical meristem in the developing LRP
742 relies on the identification of a cellular pattern reminiscent of the primary root meristem cellular
743 anatomy. Cytological analyses of developing LRP by Malamy and Benfey (1997) highlighted
744 conserved features in longitudinal sections of developing primordia. Especially, while a first
745 sequence of anticlinal and periclinal cell divisions generated a regular four-layered primordium,
746 subsequent cell division created a group of 2 square-shaped cells in the subapical position, which
747 corresponds to the position of the future QC. This landmark cell division corresponds to stage V
748 primordia. Subsequent cell divisions maintain this cellular organisation, and position of
749 presumptive initials can soon be recognized, while the developing LRP grows through the cortex
750 and epidermis cell layers (Malamy & Benfey, 1997).

751 However, analysing cellular organization is not enough to determine if a meristem-like structure
752 indeed functions like a meristem. In general, an active root meristem is recognized as a group of
753 dividing and differentiating cells that is continuously replenished thanks to the maintenance of a
754 stem cell niche. Therefore, applying this criterion to developing LRPs with the aim to identify a

755 functional meristem requires either to analyse the expression of meristem cell fate markers
756 and/or to precisely monitor dynamics in cell division and differentiation in developing LRPs.

757 Investigating cell division in developing primordia has long been difficult, but recent progresses
758 in live imaging are now contributing to overcome this technical challenge (Goh *et al.*, 2016; von
759 Wangenheim *et al.*, 2016; Du & Scheres, 2017). In their detailed analysis of Arabidopsis LRP
760 development, Malamy and Benfey (1997) described conserved cell divisions creating the cellular
761 organization similar to that of the primary root meristem around stage VI, but subsequent cell
762 divisions were difficult to track. During LRP emergence, primordium apical cell number remains
763 almost constant while basal cells quickly expand, thus supporting the model of cell elongation-
764 driven LR emergence and post-emergence lateral root meristem activation (Malamy & Benfey,
765 1997).

766 As reported earlier, analyses of marker gene expression in developing primordia support the
767 hypothesis of QC being set as early as from stage V onwards, although *WOX5* might already be
768 expressed at earlier stages (Goh *et al.*, 2016; Du & Scheres, 2017). Whether these cells do
769 actually behave like a stem cell niche organizing centre remains to be elucidated, since *WOX5*
770 stem-cell promoting activity was only shown in the primary and lateral root columella stem cells
771 (Tian *et al.*, 2014a; Pi *et al.*, 2015). Conversely, tracking initial stem cell fate remains difficult
772 because no cell marker is available to clearly identify root “stem cell-ness” quality. However,
773 *SHR* is expressed in inner cells abutting the *WOX5*-expressing cells in a comparable manner as in
774 root meristem vascular initials (Figure 4A; Goh *et al.*, 2016). Comparably, *SCR* is expressed in
775 cells flanking the presumptive QC, the characteristic position of cortex/endodermis initials (Goh
776 *et al.*, 2016; Du & Scheres, 2017). NAC domain transcription factors *FEZ* and *SOMBRERO*
777 (*SMB*) are expressed in the primary root cap where they control the division of root cap stem

778 cells (Willemsen *et al.*, 2008). *FEZ* is also expressed in as early as stage VI LRP, at the very tip
779 of the dome and in cells in contact with the presumptive QC, similar to the situation in mature
780 root meristems (Du & Scheres, 2017). Expression of *SMB* is detected in stage VI LRP, in the
781 outermost layer of the primordium tip, consistent with its described role in specifying root cap
782 progenitor cells (Du & Scheres, 2017). Last, expression of the MYB related transcription factor
783 *WEREWOLF* involved in epidermis specification (Lee & Schiefelbein, 1999) is also detected in
784 the presumptive epidermis layer of emerging LRPs (Du & Scheres, 2017). Thus, many markers
785 for root stem cell identity are already expressed in developing LRP before emergence, supporting
786 the hypothesis that a complete root meristem stem cell niche is organized before LRP
787 emergence.

788 As a functional test to identify meristematic activity, the ability of LRP of different stages to
789 develop independently of exogenous auxin supply was assayed using excision protocols
790 (Laskowski *et al.*, 1995). LRPs with no less than 3 to 5 cell layers (which corresponds
791 approximately to LRP of stages III to V) are able to develop after excision from the parental root
792 (Laskowski *et al.*, 1995; Malamy & Benfey, 1997), in accordance with the report that LRPs at
793 these stages are able to synthesize auxin IAA on their own (Ljung, 2005). Detailed analysis of
794 LRP development by Goh *et al.* (2016) supports the model that LRP development is a biphasic
795 process, comprising an early morphogenesis phase and a later meristem formation phase.
796 Importantly the transition happens at around stage V, coincident with the shift from bilateral
797 symmetry to radial symmetry of LRPs (Lucas *et al.*, 2013; Goh *et al.*, 2016), and with the onset
798 of expression of both *WOX5* and *QC25*, typical markers of root QC, in the central cells of the
799 second outermost layer (Goh *et al.*, 2016; Rosspopoff *et al.*, 2017). In the root apical meristem, a
800 QC is specified at the overlap between *SHR-SCR* and *PLTs*, which are activated by local auxin

801 signaling maximum (Aida *et al.*, 2004; Sablowski, 2007). If this criterion is applied to lateral
802 roots, then the *WOX5*- and *QC25*-positive cells in stage IV-V LRPs can be considered a QC
803 (Goh *et al.*, 2016; Du & Scheres, 2017). Rosspopoff *et al.* (2017) suggested that these QC
804 marker positive-cells constitute a functional stem cell niche that is required for the direct
805 conversion of a LRP into a shoot meristem during the artificial cytokinin-induced shoot
806 regeneration process.

807 Taken together, the data suggests the progressive establishment of a stem cell niche organization
808 from the stage II, with SCR/SHR-dependent outer layer specification, to lateral root emergence,
809 with the induction of epidermis and root cap identities. A critical step in this *de novo* root
810 meristem organogenesis process occurs at the stage IV-V transition, when the primordium breaks
811 through the overlying endodermis layer, develops a radial symmetry centred on a QC-specific
812 marker expressing niche, and acquires autonomy relative to the auxin fluxes from the parent root.
813 Interestingly, this newly established root stem cell niche may only be active after LR emergence
814 (Malamy & Benfey, 1997). The characterization of the *aberrant lateral root formation 3 (alf3)*
815 mutant, that is unable to sustain LR growth after emergence unless exogenous auxin is supplied
816 (Celenza *et al.*, 1995), also supports the hypothesis that a major transition in LRP meristem
817 activity takes place at LR emergence.

818 **5. LRP organogenesis as a common basis for organ regeneration from callus**

819 Organ regeneration is a process of *de novo* organogenesis of shoots or roots from wounded or
820 detached plant tissues. It is widely used in plant tissue culture to multiply plants quickly. Tissue
821 culture usually goes through two steps: first callus formation, and then root or shoot
822 organogenesis induction using different combinations of phytohormones. More and more

823 evidence indicate that the process of organ regeneration employs similar regulatory mechanisms
824 to that of LRP formation, especially at the first step of callus formation (Figure 4).

825 First of all, callus and LR formation share a similar cellular origin. When cultivated in auxin-rich
826 medium, callus develops from pericycle cells in root explants and from cells surrounding
827 vasculature bundles in other aerial organs (Sugimoto *et al.*, 2010). The *alf4-1* mutant is defective
828 in pericycle cell divisions, consequently unable to produce any LRs. Consistent with this, callus
829 formation from *alf4-1* root explants is blocked when cultivated in multiple combinations of auxin
830 and cytokinin concentrations (Sugimoto *et al.*, 2010). Interestingly, callus formation from aerial
831 organs, including cotyledons and petals, is also inhibited in the *alf4-1* mutant. Close inspection
832 of the pericycle marker line J0121 showed *GFP* expression in cells surrounding the midvein of
833 the cotyledons and the petals. This expression gradually diminishes when callus formation
834 progresses, similar to what happens in root pericycle cells during LRP and callus formation in
835 roots (Laplaze *et al.*, 2005; Che *et al.*, 2007; Sugimoto *et al.*, 2010). These pericycle-like cells
836 are also present in leaves and are involved in hormone-free root regeneration (Liu *et al.*, 2014;
837 Bustillo-Avendaño *et al.*, 2018). This data indicates that pericycle and pericycle-like cells are
838 essential for both LRP formation and callus formation from explants of various origins.

839 Contrary to the previous perception that callus is an unorganized mass of undifferentiated cells,
840 callus is actually an organized structure resembling a LRP. Both callus and LRP originate from
841 pericycle or pericycle-like cells, and then develop into structures of multiple cell layers (Atta *et*
842 *al.*, 2009; Sugimoto *et al.*, 2010). Cell identity markers in the root apical meristem and LRP are
843 also expressed in callus in comparable domains (Figure 4). For example, *pSCR::GFP*, a marker
844 for endodermal and QC cells in the primary root meristem, is expressed in a single cell layer
845 under the epidermis, while *pSHR::SHR:GFP*, the marker for stele, endodermal and QC cells, is

846 expressed in multiple subepidermal cell layers comparable to the stele (Sugimoto *et al.*, 2010).
847 The outer layer of the callus expresses the epidermis-specific markers *MERISTEM LAYER 1* and
848 *GLABRA2*, and even displays root cap functional characteristics, such as the presence of
849 statoliths (Atta *et al.*, 2009; Sugimoto *et al.*, 2010). Moreover, the QC marker *WOX5* whose
850 expression is activated during LRP development (Tian *et al.*, 2014a; Goh *et al.*, 2016;
851 Rosspopoff *et al.*, 2017) is also expressed in a single subepidermal cell layer in the callus,
852 although its expression range is expanded probably because of the disturbed auxin gradient
853 provided by the callus induction medium (Sugimoto *et al.*, 2010). These analyses indicate that
854 callus shares important characteristics with the root apical meristem and the LRP. Indeed,
855 transcriptomic analyses showed that callus forming from various organs is enriched in root tip-
856 expressed genes. In addition, there is a large overlap in genes upregulated in root, cotyledon and
857 petal explants during callus formation, indicating that this process shares common mechanisms
858 in these different contexts. However, the number of genes upregulated in aerial explants are
859 much higher than those in root explants, suggesting that callus formation from aerial organs
860 requires intermediate steps (Sugimoto *et al.*, 2010).

861 Callus formation also depends on the auxin signalling module comprising ARF7, ARF19 and
862 their *LBD* targets (*LBD16*, *LBD17*, *LBD19*, *LBD29*) which plays critical roles in controlling LRP
863 initiation, development and emergence (Okushima *et al.*, 2007; Lee *et al.*, 2009; Goh *et al.*,
864 2012). *LBD* genes (*LBD16*, *LBD17*, *LBD18*, *LBD29*) are also strongly induced in both shoot and
865 root explants cultivated in callus induction medium (Fan *et al.*, 2012). These LBDs physically
866 interact with the transcription factor AtbZIP59 to control callus formation (Xu *et al.*, 2018).
867 Overexpression of these genes is sufficient to induce spontaneous callus formation at various
868 degrees and positions in the seedlings (Fan *et al.*, 2012; Xu *et al.*, 2018). Conversely,

869 suppression of LBD function reduces LR formation from primary roots and almost abolishes
870 callus formation capability of root explants (Fan *et al.*, 2012). Like in LRP development, *LBD*
871 genes also act downstream of auxin via *ARF9* and *ARF19* in the context of callus formation (Fan
872 *et al.*, 2012; Lee *et al.*, 2017).

873 Interestingly, the formation of callus-like structures in leaf vascular tissues induced by root-knot
874 nematodes also depends on LBD16 (Olmo *et al.*, 2017). Another *LBD* gene, *LBD29*, which
875 regulates LR initiation through maintaining cell division competence of pericycle cells (Feng *et*
876 *al.*, 2012), also controls callus formation on callus induction medium (Xu *et al.*, 2017). During
877 callus formation, LBD29 activates genes of different functional categories such as transcriptional
878 regulation, ROS and lipid metabolism, and cell wall hydrolysis. Overexpression of some putative
879 targets induces spontaneous callus formation at various degrees, similar to the phenotype of
880 *LBD29*-overexpressing plants (Xu *et al.*, 2017).

881 Callus formation is an important intermediate step for *de novo* organ regeneration by hormonal
882 treatment. In this procedure, explants are first incubated in auxin-rich callus induction medium to
883 induce the formation of calli that acquire pluripotency for subsequent shoot regeneration. When
884 transferred to a shoot induction medium that has high cytokinin-to-auxin ratio, some callus cells
885 transform into shoot progenitors that will eventually develop into shoots (Kareem *et al.*, 2015).
886 PLTs, key regulators in LRP and root apical meristem formation (Galinha *et al.*, 2007; Du &
887 Scheres, 2017), also play pivotal roles in hormone-induced organ-regeneration. Like in a
888 developing LRP, expression of *PLT3*, *PLT5* and *PLT7* genes is up-regulated during callus
889 formation and shoot regeneration (Kareem *et al.*, 2015; Du & Scheres, 2017). Single mutants in
890 these genes are able to regenerate shoots in shoot induction medium, but the regeneration
891 capability is completely lost in the triple mutant. Moreover, overexpression of *PLT5* or *PLT7* is

892 enough to induce shoot regeneration in the *plt3 plt5 plt7* mutant background in hormone-free
893 medium. This data indicates that PLT5 and PLT7 transcription factors are necessary and
894 sufficient for shoot regeneration from callus. Expression of key root stem cell maintenance
895 regulators, including *SCR*, *PLT1* and *PLT2* is upregulated in callus originated from wild type
896 shoot and root explants, but not detected in *plt3 plt5 plt7* calli (Kareem *et al.*, 2015). PLT3, PLT5
897 and PLT7 transcription factors also positively regulate the expression of the auxin efflux carrier
898 *PIN1* gene during callus formation. Altogether, the data points to a common regulatory pathway
899 involving *PLT* genes in LRP formation and *de novo* organ regeneration: in both processes (i)
900 *PLT* genes are required, and (ii) PLT3, PLT5 and PLT7 regulate *PLT1* and *PLT2* and also *PIN1*
901 expression in a similar manner (Kareem *et al.*, 2015; Du & Scheres, 2017).

902 As described for LR FC specification and LRP formation, chromatin-remodelling factors also
903 play critical roles in callus formation. ARABIDOPSIS TRITHORAX-RELATED 2 (*ATXR2*), a
904 trithorax group protein as *ATX1* mentioned above, modifies target gene expression by deposition
905 of positive histone marks. *ATXR2* binds to the promoter of *LBD16* and *LBD29* with the help of
906 *ARF7* and *ARF19* transcription factors to induce permissive chromatin conformation on these
907 loci. Consistent with the critical roles of LBDs in callus formation, the ability of leaf explants to
908 form calli is significantly reduced in the *atxr2* mutant background and enhanced in an *ATXR2*
909 overexpressing line (Lee *et al.*, 2017). Although it is not clear if this regulation of *ATXR2* on
910 *LBDs* is also true in LRP formation, lateral root production in the *atxr2* mutant is significantly
911 reduced compared to the wild type (Lee *et al.*, 2018). Finally, subunits of the Polycomb
912 repressive complex 2 (*PRC2*), which negatively regulates LR FC specification, were shown to
913 inhibit callus formation given that single or combined mutants of these subunits produce callus
914 spontaneously in tissue culture (Chanvivattana, 2004; Bouyer *et al.*, 2011).

915 Interestingly, although not exactly in the context of LRP development, callus formation and
916 adventitious root formation from leaf explants are demonstrated to share a common genetic
917 pathway. In Callus Induction Medium (CIM), leaf explants produce callus through two steps:
918 founder cell specification and callus formation (Yu *et al.*, 2017). In the first step, pericycle-like
919 (procambium) cells highly express *WOX11*, a *WOX5*-related transcription factor that acts
920 redundantly with *WOX12* to convert competent cells into callus founder cells. *WOX11* and
921 *WOX12* then activate the expression of *LBD16* and *WOX5* to induce founder cell division to
922 form a callus (Liu *et al.*, 2014; Hu & Xu, 2016; Sheng *et al.*, 2017). This two-step mechanism is
923 conserved in both monocot and dicot plants (Hu *et al.*, 2017). The genetic pathway comprising of
924 *WOX11*, *WOX12* and *WOX5*, along with other important genetic factors such as *PLT1*, *PLT2* and
925 *SHR*, is also involved in adventitious root founder cell specification and hormone-free root
926 regeneration from leaf explants (Liu *et al.*, 2014; Hu & Xu, 2016; Bustillo-Avendaño *et al.*,
927 2018; Sheng *et al.*, 2017). This newly unravelled molecular mechanisms for callus formation can
928 help bridge the gaps between LR and adventitious root formation.

929 **6. General conclusions**

930 Root system architecture greatly impacts plant growth and crop yield, and root branching
931 contributes to a major extent to root system development. Root branching consists in a strikingly
932 robust event of post-embryonic organogenesis that progressively generates, thanks to
933 coordinated proliferation of pericycle-derived founder cells, a new functional root meristem
934 including a stem cell niche. Major regulators of cell fate and cell behaviour, especially cell cycle,
935 are involved in triggering and then controlling this organogenesis process. Specific transcription
936 factors, chromatin modifiers, and signalling pathways impact gene expression in a precise time
937 and space dependent manner, although we are still far from having elucidated all their

938 interactions and understanding the properties of the entire network. Importantly non-cell
939 autonomous signals, such as mobile peptides, directional fluxes of the plant hormone auxin, and
940 transcription factors able to move through plasmodesmata, mediate cell-to-cell coordination
941 across tissues and the emergence of developmental patterns. Moreover, *de novo* root meristem
942 formation associated to root branching shares common regulatory mechanisms together with root
943 meristem specification in the embryo and plant regeneration from calli, raising interesting evo-
944 devo as well as biotechnological perspectives. However, many questions are still open regarding
945 key developmental events during LRP organogenesis. How pericycle cell competency for root
946 organogenesis is precisely regulated remains unclear. In addition, the precise molecular
947 mechanisms underlying pericycle cell priming, prebranch site formation and founder cell
948 specification are unknown. Last, the regulatory network properties controlling the organisation
949 of a new root apical meristem are not understood. Hopefully, the comparison of root formation
950 from embryonic, post-embryonic, and calli tissues will help to unravel the fundamental
951 properties of this organogenesis program.

952 **Acknowledgements**

953 Work by D. C. T., L. L. and S. G. is supported by the Institute of Research for Development, the
954 University of Montpellier, the University of Science and Technology of Hanoi and the French
955 National Research Agency (ANR-17-CE13-0004-01). We thank the anonymous reviewer for
956 useful comments on the manuscript and apologize to authors whose work could not be cited due
957 to space limitation.

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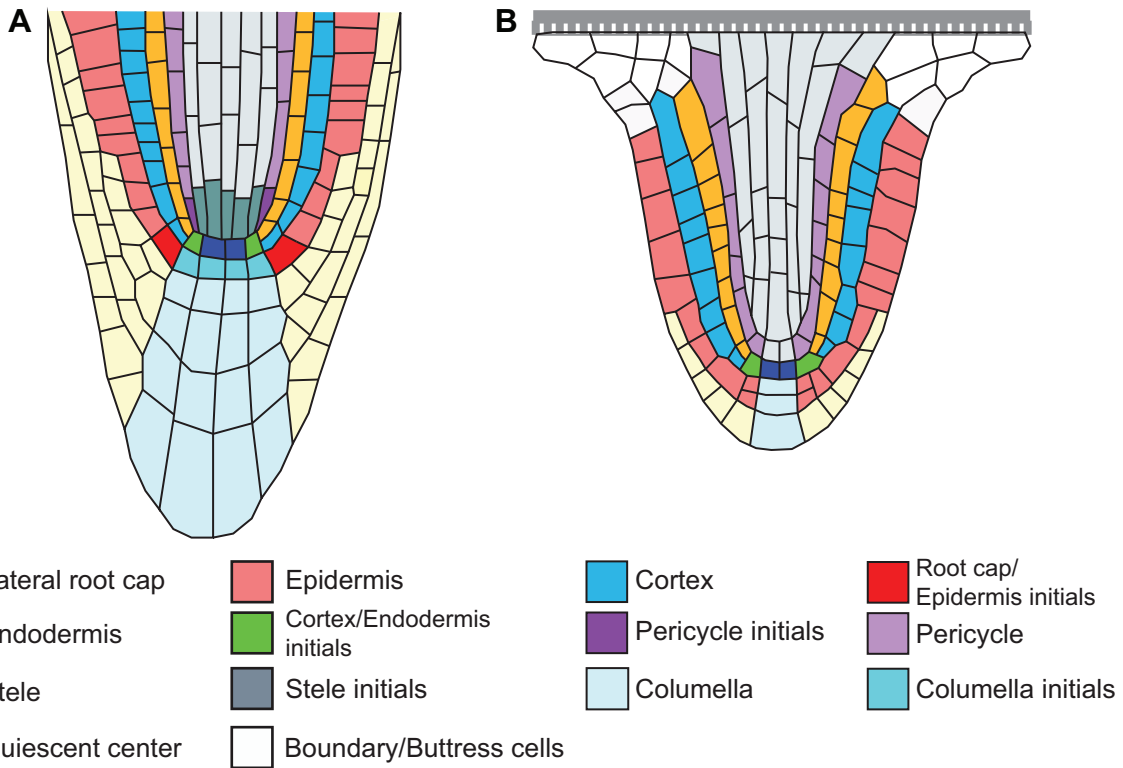
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1444 Captions for figures



1445

1. Quiescent center Boundary/Buttress cells

1446

Figure 1. **Arabidopsis primary root and lateral root meristems.** Schematic organization of the

1447

apical meristem of Arabidopsis primary root (A) and emerged lateral root (B). Cell types of

1448

primary root apical meristem and presumptive cell types of lateral root apical meristems are

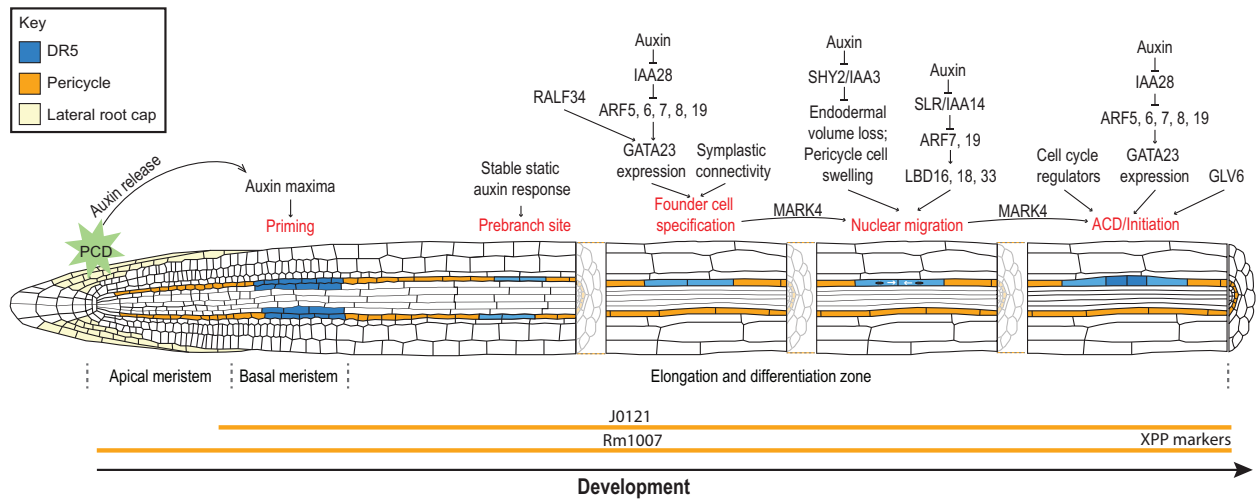
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indicated in the colour legend. In root apical meristems, the stem cells are called “initials” and

1450

the organizing centre is termed “quiescent centre” (QC).

1451 2.



1452

1453 **Figure 2. Key events during lateral root primordium initiation and their regulators**

1454 (modified from Lavenus *et al.*, 2013). Pericycle cells are primed partly through auxin released

1455 from programmed cell death (PCD) in the lateral root cap. Subsets of primed cells at opposite

1456 xylem poles that retain stable static auxin response are called prebranch sites, but usually only

1457 one will develop into founder cells. Founder cells then swell and their nuclei migrate to the

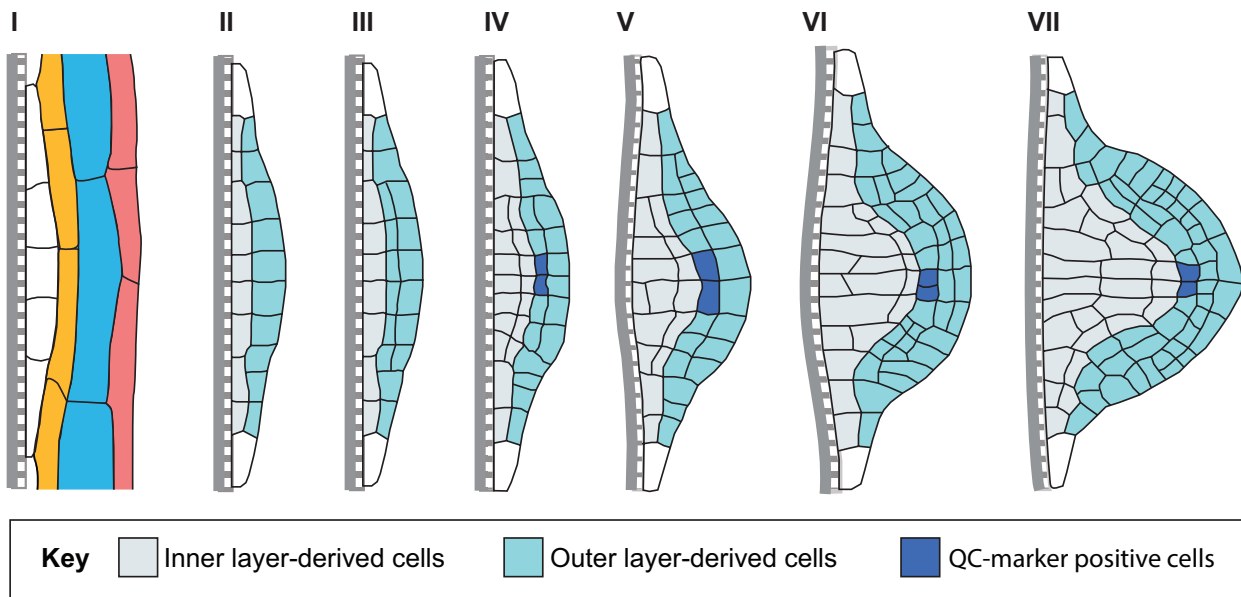
1458 common cell wall (white arrows). An asymmetric cell division (ACD) then occurs marking the

1459 initiation of the lateral root organogenesis program. Expression patterns of xylem-pole pericycle

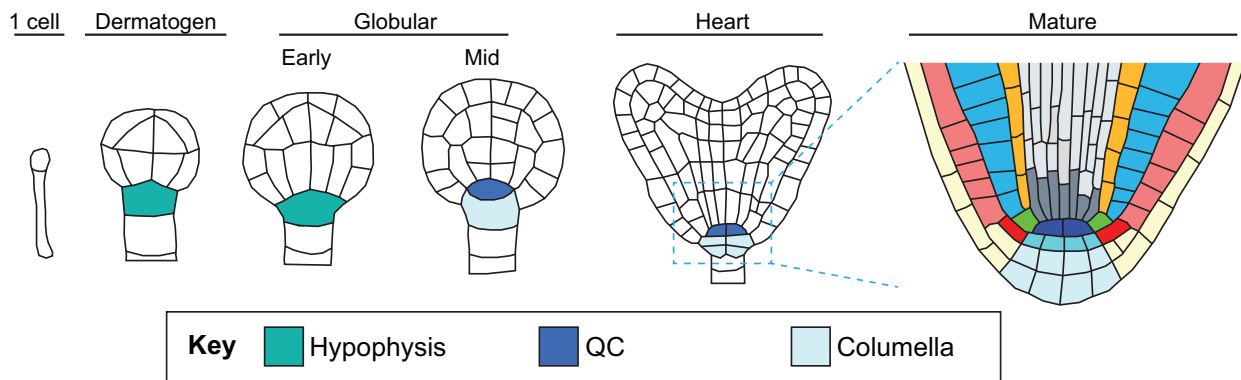
1460 (XPP) cell markers (J1021 and Rm1007) and developmental zones along the primary root are

1461 indicated.

A - Lateral root organogenesis



B - Embryonic root organogenesis



1463

1464 Figure 3. **Schematic presentation of LRP organogenesis and embryonic root organogenesis.**

1465 (A) LRP organogenesis is usually described as different stages (I-VII and finally emergence).

1466 The stage I LRP (4 green cells) divides periclinally to form stage II LRP having two cell layers.

1467 The inner layer of stage II LRP will eventually develop into ground tissues and pericycle (grey),

1468 while the outer layer develops into the endodermis, cortex, epidermis and root cap (light blue).

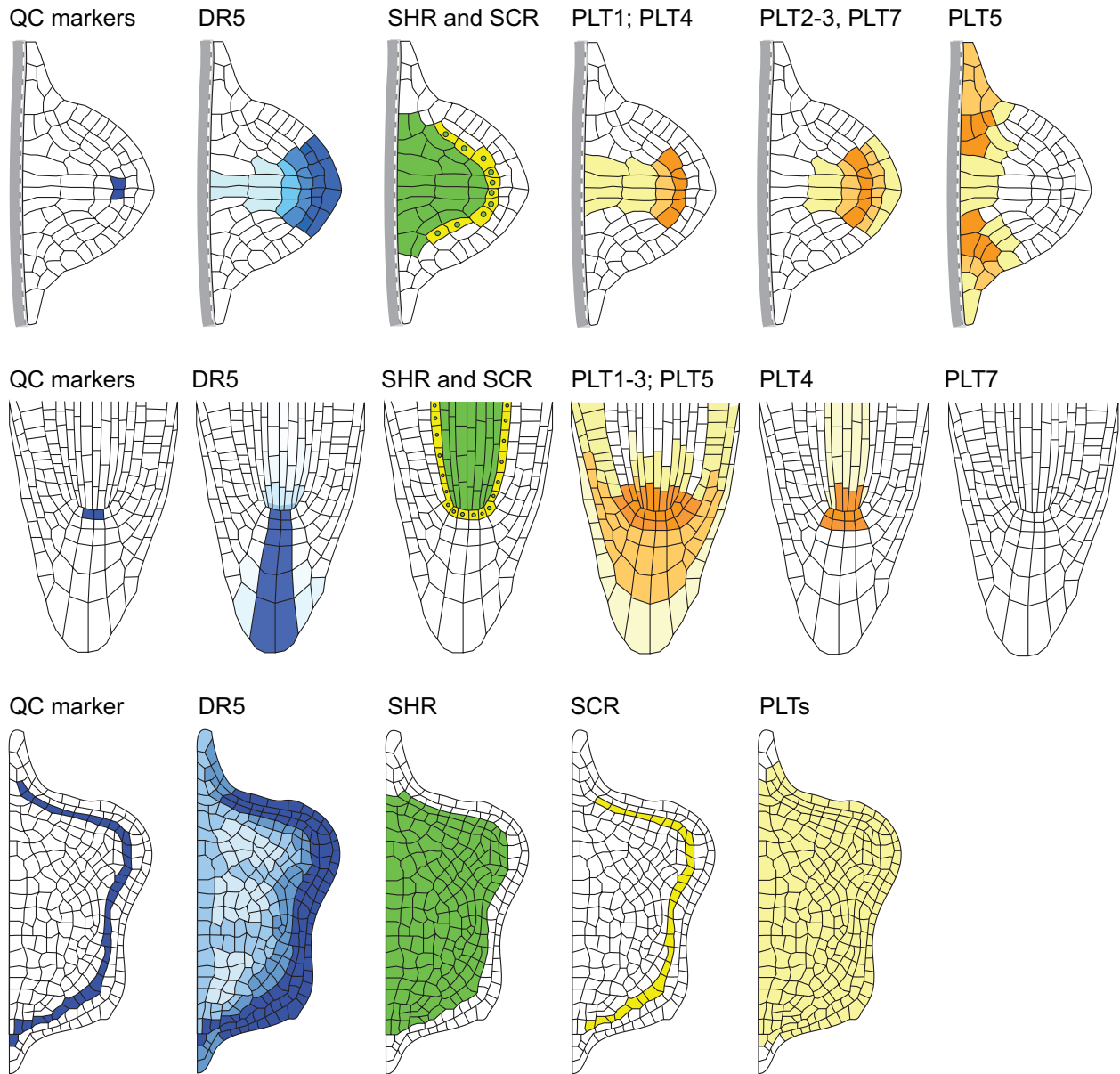
1469 From stage IV onwards, some central cells start expressing QC markers. For simplicity, the

1470 pericycle, endodermis, cortex and epidermis tissues of the parent root that are shown at stage I
1471 LRP are not shown in stage II-VII LRPs. White cells correspond to cell that do not divide
1472 periclinally at stage II.

1473 (B) After fertilization, the embryo develops from the zygote through different stages. At
1474 dermatogen stage, a hypophysis is established which will divide asymmetrically to form a lens-
1475 shape cell and a basal cell (mid globular stage), and eventually develop into the root apical
1476 meristem (from heart stage onwards). Colour legend for figure A-I (except the stage I LRP) and
1477 B-Mature is as of Figure 1.

1478

1479 4.



1480

1481 **Figure 4. Distribution patterns of some key regulators in different developmental contexts.**

1482 Auxin gradient revealed by *DR5* promoter, and expression patterns of QC markers, *SHR*, *SCR*

1483 and *PLTs* in stage VII lateral root primordia (A), primary root apical meristem (B) and callus in

1484 callus induction medium (C). *SHR* protein (green) is produced in stele and moves into the

1485 adjacent cell layer to activate the expression of *SCR* (yellow). Note the similarity in expression

1486 patterns of these regulators in these different contexts.