Formation and Function of the Quiescent Center – The Major Regulators

Master Thesis – Cancer Genomics and Developmental Biology

Miriam Tange

Supervised by Dr. R. Heidstra

February 25, 2011

Index **Page**

Abstract

Plants can grow indeterminately. The ongoing growth of roots is made possible by the capability of the plant to maintain a pool of stem cells. These stem cells keep proliferating and do not differentiate. The daughters of the stem cells differentiate and form the different structures of the root.

In the plant root, stem cells are located in the root tip. The stem cells are located in an organized structure, the stem cell niche. The stem cell niche functions as a microenvironment where, by short range cell signaling, the stem cells are maintained. The Quiescent Center (QC) lies in the centre of the root stem cell niche and provides the signals for maintenance of the surrounding stem cells.

The organization of the niche is set up early in embryogenesis and stays unchanged in the mature plant. Essential for root patterning in embryogenesis is the phytohormone auxin. Several transcription factors are involved in both root patterning in the embryo and stem cell maintenance in the mature plant. The processes of organization and maintenance of root stem cells are very well studied in the A*rabidopsis thaliana* and new insights from this model organism will be discussed here.

1. Introduction

1.1. Embryogenesis

After fertilization the *Arabidopsis* zygote passes through several developmental stages to form a seedling (Figure 1). During these stages the embryo has to establish the apical-basal axis, a radial orientation and bilateral symmetry and form root and shoot meristems.[1, 2]

To form the apical-basal axis, the zygote first elongates and divides asymmetrically into an apical and a basal cell. The apical cell undergoes highly coordinated divisions and forms the spherical proembryo. The basal cell divides horizontally and forms the suspensor, which connects the embryo to maternal tissue. At the octant stage the apical-basal axis in the proembryo is established by the specification of a lower and an upper tier of cells.[4-6]

Patterning of the radial axis takes place during the transition from the octant stage to the dermatogen stage. The cells of the embryo proper divide tangentially, forming an inside and outside cell layer. The outside cell layer is the protoderm, which is a precursor of the epidermis. The protoderm cells continue to divide anticlinally and therefore stay the outer cell layer of the embryo. The inside cell layer of the embryo will later form the ground tissue and the vasculature.[4-5]

The formation of the root apical meristem (RAM) starts in the globular stage. Important for the patterning of the QC is the formation of the hypophysis. The hypophisis is derived from the uppermost suspensor cell. In the transition stage the hypophysis divides asymmetrically into an apical small lens-shaped cell and a large basal cell. The lens-shaped cell forms the QC after two vertical divisions in the heart stage. The basal cell will form the columella stem cells and root cap tissue. Stem cells for the proximal meristem are derived from cells apical of the QC.[4-5]

Patterning of the shoot apical meristem (SAM) and cotyledons starts at the globular stage and proceeds during the heart stage. In the upper tier of the embryo the shoot meristem is formed between the two cotyledon primordia. The cotyledons serve as the first leaves of the embryo and their formation initiates bilateral symmetry.[4-5]

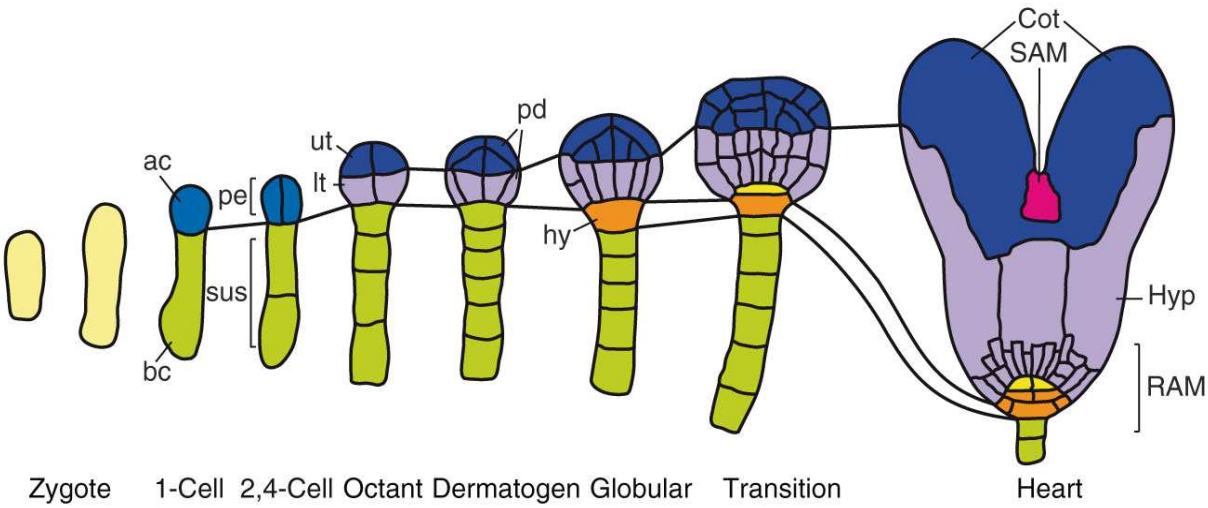

Figure 1. Schematic overview of stages of *arabidopsis* embryogenesis. Apical cell (ac), basal cell (bc), proembryo (pe), suspensor (sus), upper tier (ut), lower tier (lt), protoderm (pd), hypophysis (hy), cotyledon (Cot), Shoot Apical Meristem (SAM), Root Apical Meristem (RAM), hypocotyls (future stem) (Hyp). Adapted from Peris et.al[5].

1.2. Root anatomy

The root consists of a number of tissues that are radially organized (Figure 2.A). The epidermis forms the outside layer. The ground tissue follows to the inside; consisting of the cortex and endodermis layers. In the centre of the root lies the stele, containing the pericycle and the xylem and phloem tissues that make up the vasculature of the root. The distal tip of the root is covered by the lateral root cap and central columella.[7, 8]

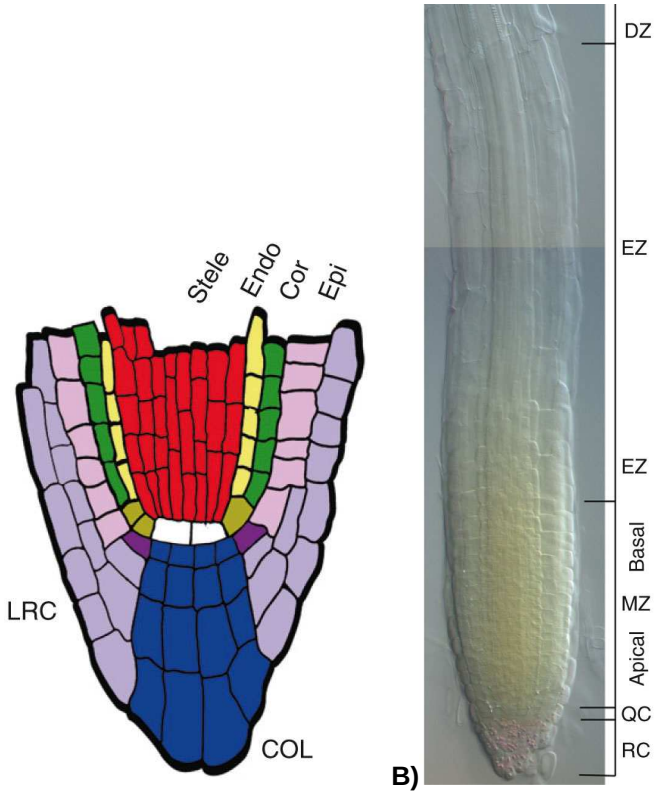

Figure 2. A). Schematic view of the distal root tip. Lateral root cap (LRC), columella (COL), epidermis (Epi), cortex (Cor), endodermis (Endo). The white cells depict the QC. Next to the QC lie the progenitor cells of the endodermis, cortex and epidermis, depicted in a different color.
B). A longitudinal view of the Arabidopsis root. Root cap (RC), quiescent centre (QC), meristem zone (MZ), elongation zone (EZ), differentiation zone (DZ) Adapted from Bennet et.al.[7]

Longitudinally the root can be divided in different zones (Figure 2.B). These zones comprise different stages of root development. Above the root cap lies the QC, surrounded by one layer of stem cells. Directly above the stem cells lies the meristem zone. The meristem zone itself can be divided into two regions. Closest to the QC is the apical meristem, where cells divide rapidly and are called transit-amplifying cells (TA), in anology with animal systems[9]. In the basal meristem cells divide less and start differentiating.[7]

When mitosis of TA cells stops, they reach the elongation zone, where the cells elongate[10]. Finally the cells reach the differentiation zone, where the cells fully differentiate and all radial structures of the root are formed. This zone is distinctive by formation of root hairs and a mature vasculature.[7]

1.3. Root stem cell niche

The mature stem cell niche of the root has a specific arrangement (Figure 3). The four QC cells are located in the centre and are surrounded by the stem cells. The surrounding stem cells are also called initials[4]. The QC cells divide rarely to renew both the QC and the stem cells[7, 11].

The stem cells are under the influence of growth signals from the meristem. In addition, the stem cells need to keep their properties of pluripotency and endless division. The QC is responsible for the maintenance of the stem cells identity (chapter 5).

The position of the stem cells in the meristem correlates to their fate. After an asymmetric division the cell next to the QC remains a stem cell. The daughter cell will become a TA cell and differentiate according to the adjacent structures. However, the stem cells of the cortex and endodermis first need to undergo an extra anticlinal division to produce their specified daughters. Also the lateral root cap and epidermis tissues originate from the same stem cell. For clarity the columella stem cells are called the distal stem cells. The stele and ground tissue stem cells are called the proximal stem cells. Two days after germination (DAG) there is one tier of columella stem cells and four tiers of differentiated columella cells in the root tip. The differentiated columella cells are characterized by the presence of starch granules.[7, 12]

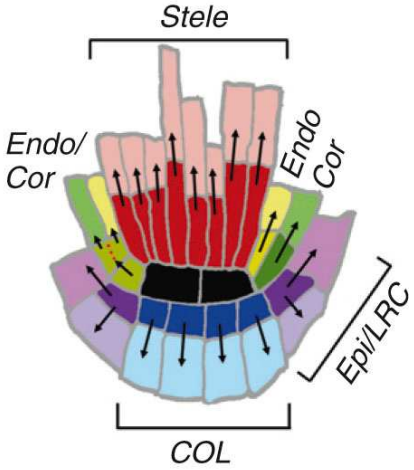

Figure 3. Schematic view of the root niche. Arrows indicate growth direction. Columella (COL), epidermis (Epi), lateral root cap (LRC), endodermis (endo), cortex (cor). The left side shows the anticlinal division that the endo/cor stem cell undergoes. The right side shows two separate stem cells for endodermis and cortex, as occurs in ongoing development of the plant. Adapted from Bennet et.al.[7]

2. Auxin

2.1. Auxin synthesis and signaling

Auxin is essential for embryonic root development. Auxin is synthesized directly from indole or by different enzymes from tryptophan (Figure 4). Genetic interference with auxin synthesis enzymes, such as YUCCA and TAA1[13, 14] or auxin receptors, such as TIR1[15] inhibit root growth[16], however, auxin is not synthesized in all of the tissues where it is involved in signaling[5, 17]. When auxin enters a cell it is recruited by its receptor, the SCF-TIR1/AFB ubiquitin ligase. Auxin itself recruits transcriptional repressors of the Aux/IAA family. Subsequently Aux/IAA is ubiquitinated and degraded in the proteasome. Aux/IAA proteins are inhibitors of the AUXIN RESPONSE FACTORS (ARFs) family of transcription factors. Under low levels of Auxin, Aux/IAA inhibits ARF function through binding. Under high levels of Auxin, Aux/IAAs are degraded and ARFs can perform their function on their target genes.[18]

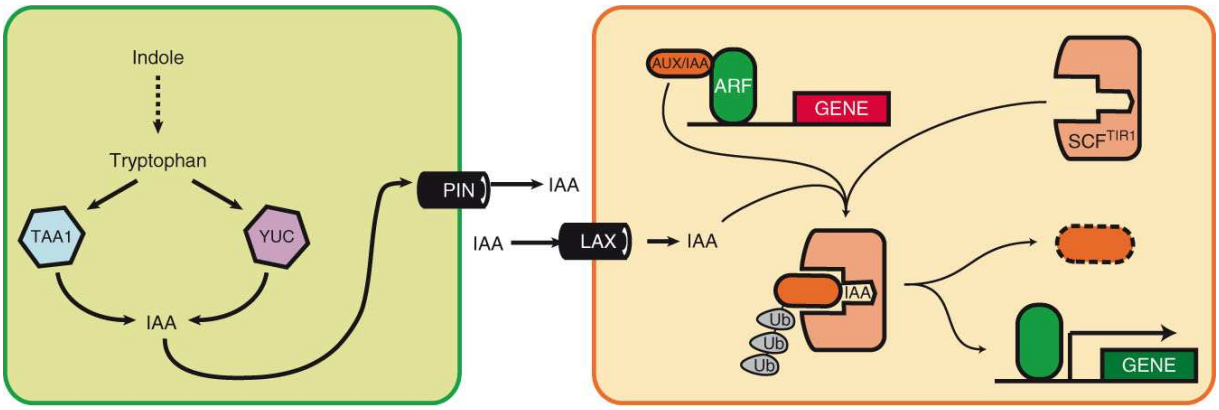

Figure 4. Schematic view of Auxin synthesis, transport and function.
On the left side auxin is synthesized by the enzymes TAA1 and YUCCA (YUC). Indole acetic acid (IAA) is the most abundant form of auxin. Auxin is exported from the cell by PINs. Auxin import occurs freely or by the LAX importers.
On the right side auxin binds to its receptor and recruits the Aux/IAA protein to the ubiquitination machinery. This prevents the inhibition of the ARF by Aux/IAA. Adapted from Peris et.al.[5]

2.2. Auxin distribution

The efflux carriers PIN-FORMED (PIN) mediated distribution of auxin is essential for auxin signaling. E.g. *pin 1,3,4,7* mutants are not able to form roots[19]. PINs export auxin from the cell, as there is no free auxin efflux in *Arabidopsis*. The location of the PINs in the cells determines the direction and gradient of auxin flow[5, 20].

Auxin activity can be measured by the expression reporter DR5[19]. In the globular stage an auxin response maximum is observed in the apical proembryo (Fig 5). In later stages DR5

expression is redirected to the uppermost suspensor cells by changes in cellular PIN localization[19]. In the globular stage PIN7 is present in the suspensor cells, there it relocates from the apical to the basal cell side. Simultaneously PIN1, located in the proembryo inner cells, moves to the basal cell side[16, 19].

In later embryogenesis stages this directed auxin distribution remains and is essential for QC specification. In the hypophysis and its uppermost daughter cell *PIN4* is expressed. From the heart stage the columella precursor cells express *PIN3*. *PIN2* is expressed in cortex and epidermis layers. [16, 19]

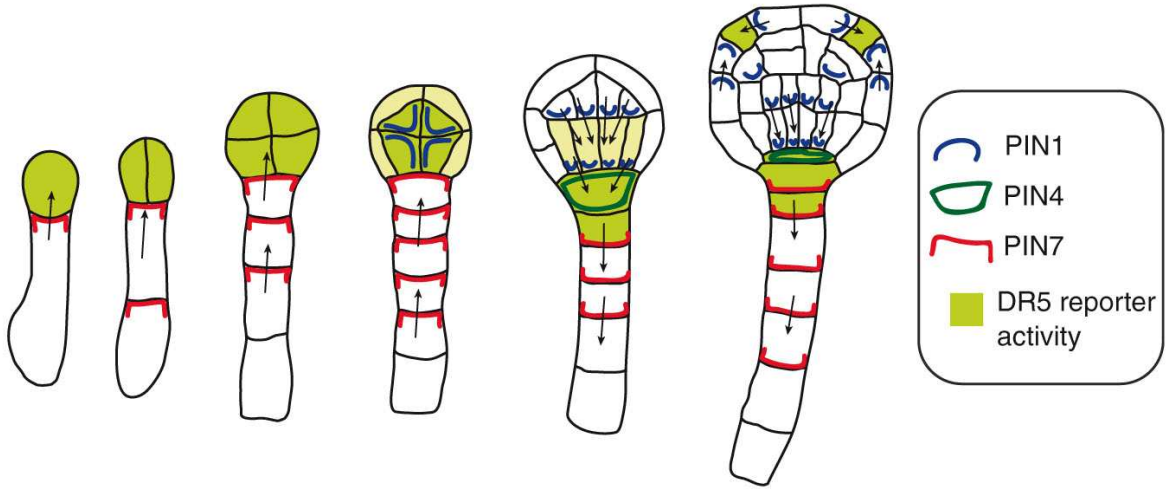

Figure 5. Schematic view of changed PIN polarization and auxin activity in the dermatogen, globular and transition stages of embryogenesis. Arrows indicate the direction of auxin distribution. Auxin activity is visualized by DR5-GFP (light green). Colored lines indicate cellular PIN localization of PIN1 (blue), PIN4 (dark green) and PIN7 (red). Adapted from Peris et.al.[5]

During embryogenesis auxin is not only distributed to the basal side in the embryo. In the transition stage auxin distribution at the apical side is necessary for cotyledon formation (Figure 5). The Aux/IAA IAA18-1 protein is expressed in the apical domain of the embryo from the globular stage on. The *iaa18-1* gain of function mutant is a stabilized Aux/IAA protein. The *iaa18-1* mutant embryo shows disrupted PIN1 localization at the globular stage in the apical side of the embryo and disrupted cotyledon outgrowth.[21] Interestingly, *iaa18-1* mutants also show reduced root growth and differentiated cells more closely to the meristem; auxin distribution in the root is shifted apical compared to wild type as well. Moreover, in a *bdl* (an AUX/IAA protein) or *tir1* mutant background the *iaa18-1* mutation increases the rootless phenotype frequency, suggesting that the auxin signaling pathway is more disrupted. This suggests that apical regulation of auxin distribution by PIN1 in the embryo also

influences auxin signaling in root development. So PIN mediated auxin transport from the apical to the basal domain of the embryo is necessary for root initiation.[21]

In contrast with auxin efflux, auxin influx is free[5, 22], therefore the role of the AUX1/LAX family of auxin influx carriers (Figure 4) on auxin distribution was not expected to be of regulatory influence. The quadruple *aux1 lax1 lax2 lax3* mutant embryo however, shows an increased size of the root cap, ectopic divisions of QC cells and disrupted columella organization. This phenotype could be caused by increased levels of free auxin that disrupt the auxin gradient, therefore also regulation of auxin influx is important for QC patterning.[23]

2.3. Intracellular PIN transport

PIN proteins are continually transported in the cell by endo- and exocytosis. Basal directed PIN transportation through endosomal recycling requires the ARF GTPase exchange factor *GNOM* [24, 25]. Targeting of the PIN proteins is also dependent on their phosphorylation status. Dephosphorylated PIN proteins are targeted to the basal plasma membrane, in contrast phosphorylated PIN proteins are targeted to the apical cell side[26-30].

Basal auxin efflux is inhibited by 1-naphthylphthalamic acid (NPA). When NPA is added to wild type *Arabidopsis*, the intracellular localization of PINs is altered. This eventually results in cell fate changes in epidermal and ground tissue cells in the mature root. When NPA is added during embryogenesis the apical/basal polarity and embryogenesis is disrupted.[31-36]

A novel factor important for intracellular PIN localization is the nuclear factor NO VEIN (NOV). *NOV* is expressed in the embryo and RAM and SAM. *nov* mutants show embryonic phenotypes similar to those of auxin transport or auxin signaling mutants, which are characterized by fused cotyledons and size reduction. The *nov* mutant seedlings have shorter roots and a disorganized RAM also columella stem cells are not maintained. *nov* mutants show disrupted PIN2 polarization and decreased PIN3 and PIN7 expression, specifically in the mature root cap. There is an auxin maximum detected in mature *nov* mutants at the QC, however distribution of auxin into the root tip is reduced. In the *nov* mutant embryo auxin is distributed over the whole suspensor, in the wild type however, auxin distribution is confined to the uppermost suspensor cell and the hypophysis. Remarkably in the root the *nov* mutant represses the effect of NPA. In the shoot, however, the *nov* mutant shows an increased NPA phenotype.[37] Like other PIN polarization factors, NOV is necessary for correct intracellular PIN localization and subsequently essential for correct auxin distribution. The different NOV dependent responses to NPA of the root and the shoot suggest that regulated PIN localization is involved in the specification of these structures.[37]

3. MONOPTEROS and hypophysis specification

3.1. MP encodes an ARF

Once the auxin gradient is established at the basal side of the proembryo the hypophysis precursor is recruited (Figure 1, Figure 5). Important for the recruitment of the hypophysis to the proembryo is the auxin response factor *ARF5/MONOPTEROS (MP)*. *mp* mutants show abnormal hypophysis specification and do not produce roots[38]. *MP* is expressed in the provascular cells apical of the hypophysis precursor. *MP* is inhibited by the IAA/AUX BODENLOS (BDL/IAA12). The increased auxin distribution around the hypophysis precursor in the proembryo induces degradation of BDL, thus stimulates *MP* expression. In *mp* mutants *PIN1* expression is diminished, thus MP regulates PIN1 expression. Thereby MP directs auxin flow to the hypophysis in a feedback loop, increasing the auxin maximum at the hypophysis.[39, 16] The expression of *MP* apical of the hypophysis precursor contrasts with the auxin maxima in hypophysis precursor itself and the later uppermost hypophysis daughter. Therefore there is probably another ARF active in the hypophysis precursor itself that responds to the auxin maximum in this process (Figure 6).

3.2. MP recruits the hypophysis non-cell-autonomously

In the *bdl* mutant *PIN1* expression is only lost in the *BDL*-expressing domain, also auxin is not distributed to the hypophysis, however it is still distributed to the adjacent suspensor cells. In both *bdl* and *mp* mutants 2,4-D auxin analog treatment could not rescue hypophysis specification. Thus the directed auxin transport by MP is not restricted to the hypophysis and not sufficient for hypophysis formation.[39] Therefore it is possible that MP recruits the hypophysis by cell-cell signaling independent of auxin signaling. This suggests that other targets of MP must be involved in hypophysis recruitment. Schlereth et. al. discovered with mRNA microarray under BDL inhibition the basic helix-loop-helix (bHLH) transcription factor *TARGET OF MONOPTEROS 7 (TMO7*) as a target of MP. *TMO7* is expressed in *MP* expressing cells in the globular stage, in later stages *TMO7* is expressed in root stem cell precursrs. In *mp* mutants *TMO7* is not expressed and with a ChIP assay the direct binding of MP to the *TMO7* promoter was shown. Thus *TMO7* expression is dependent on MP and it is a direct target of MP.[40]

Suppression of *TMO7* results in aberrant hypophysis divisions and rootless seedlings[40]. In the embryo the TMO7-GFP fusion protein is not only detected in the *MP* expressing cells, but also in the hypophysis nucleus. The rootless phenotype of the weak *mp* mutant is rescued by

misexpression of TMO7 in the uppermost suspensor cell. Thus TMO7 regulates hypophysis recruitment and division in the hypophysis under regulation of MP.[40] This shows that MP recruits the hypophysis to the proembryo non-cell-autonomously, using both directed auxin flow and cell-cell signaling (Figure 6). [40,39]

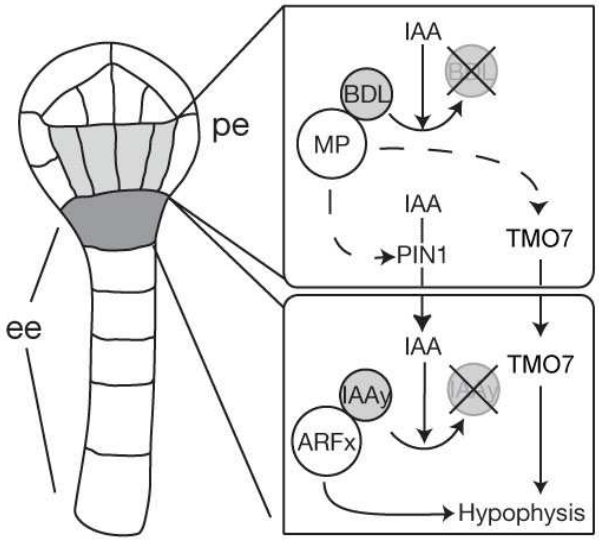

Figure 6. Schematic view of hypopysis recruitment by MP and auxin. In the pro-embryo (pe) cells (light-grey) auxin (IAA)-induced BDL degradation allows MP activity. This includes transport of auxin (through PIN1) and TMO7 to the adjacent extra-embryonic (ee) cell (dark-grey). Here, an auxin-activated ARFx-IAAy pair and TMO7 specify hypophysis fate. Adapted from Schlereth et.al.[40]

4. Specification of the niche

4.1. PLT and SHR/SCR pattern the QC

Once the hypophysis is recruited to the embryo it undergoes a regulated asymmetrical division to form the lens-shaped cell and the basal cell. The daughters of the lens-shaped cell will form the QC during the heart stage[41]. The *PLETHORA* (*PLT*) family of AP2-type transcription factors exists of several homologue genes[12,42]. *PLT1* and *PLT2* are expressed at the basal half of the proembryo and in the provascular and lens-shaped cell in the globular stage and in the root meristem at later stages[12]. In the *plt1plt2* mutant mature embryo the hypophysis is not correctly divided and the divisions of the lens-shaped cell and its daughters are wrongly orientated. The QC are cells not formed during the heart stage and the *plt1plt2* mature embryo does not express QC markers. This indicates that *PLT* is necessary for correct hypophysis divisions and a proper patterning of the QC[12, 42].

Members of the GRAS family of putative transcription factors *SHORT-ROOT (SHR*) and *SCARECROW* (*SCR*) are known for their role in radial organization and stem cell maintenance in the mature root. In the mature root *SHR* is expressed in the vasculature, from there it moves to the ground tissue and QC, where it activates *SCR* transcription, thus SHR acts non-cell-autonomously in the QC. SCR in turn restricts SHR movement by confining it to the nucleus.[43-46] Interestingly, *SHR* and *SCR* are also expressed in the mature embryo and proposed to play a role in QC patterning. s*cr* and *shr* mutants show a distorted root development phenotype, like the *plt1plt2* mutants, with reduced RAM size and differentiation of RAM cells.[7, 47,43-46] In *scr* and *shr* mutants however, *PLT* expression is only mildly reduced. In *plt* mutants the expression of *SCR* and *SHR* in the embryo and post-embryonic is not changed and the phenotype of the *plt-shr* and *plt-scr* mutants is more severe than in the single mutants. This indicates that PLT does not influence the transcription of SHR and SCR and vice versa, and that they do not function in the same pathway. However they are all necessary for QC specification and probably function in parallel pathways to organize and maintain the root stem cell niche.[12]

4.2. PLT acts antagonistically with apical patterning factors

The presence of PLT in the auxin maximum region in the root suggests that the expression of *PLT* is regulated by auxin and ARFs. When auxin is added to *Arabidopsis* seedlings *PLT* expression increases, but only in a late response. This indicates that PLT is not directly regulated by auxin, but likely by ARFs. In ARF mutants, such as *mp* the expression of *PLT* is

reduced in the embryo from the globular stage, but the reduction is not complete. This suggests that other ARFs might regulate *PLT* expression.[12, 40]

A possible regulator of *PLT* expression is TOPLESS (TPL), a transcriptional co-repressor that is required by BDL and inhibits *MP* transcription[48]. *tpl-1* mutants do not form shoots instead they form extra apical roots in the place of the shoot.[50] Ectopic *PLT2* expression also turns the shoot into a root, similar to the *tpl* phenotype[3,12]. Therefore it is possible that PLT is responsible for the *tpl* phenotype. In *tpl* mutants *PLT* is apically misexpressed in embryos starting at the heart stage. With ChIP it was shown that TPL binds the promoters of the *PLT1* and *PLT2* genes, this indicates that TPL apically represses *PLT* and that *PLT* is a direct target of TPL.[50]

The *tpl-plt1-plt2* triple mutant does not form an apical root, indicating that PLT is necessary for its apical root phenotype. This suggests that PLT activity also inhibits the development of an apical shoot meristem. The apically expressed transcription factors CLASS III HOMEODOMAIN leucine zippers (HD-ZIPIII) *PHABULOSA (PHB*) and REVOLUTA (*REV*) play an important role in early shoot development[49,50]. The PHB gain of function mutation *phb-14d* is able to rescue the *tpl* phenotype. In the wild type plant PHB is restricted to the apical region of the embryo by a microRNA-dependent pathway[51, 52]. When PHB is not restricted to the apical side, embryos do not form a QC and QC markers are not expressed, due to aberrant division of the hypophysis. PHB is also sufficient to induce a basal shoot meristem when basal misexpressed in the embryo.[50] This shows that both PHB and PLT can influence ectopic meristem formation.

Interestingly, there is no change in the expression of basal PLT when PHB is overexpressed, lower PLT levels would be expected because of the inability of these embryos to form a QC. In addition, the *tpl phb-14d* mutant shows no misexpressed PLT in the developing shoot meristem cells, but there is misexpressed PLT in other apical structures present. This suggests that PHB locally represses PLT in the developing shoot meristem. In contrast the triple mutant of a REV gain of function mutation *rev* combined with the *plt1plt2* mutation shows increased basal misexpression of REV, indicating that PLT in turn represses REV expression. Thus *PLT* and *HD-ZIPIII* genes act antagonistically in the developing embryo, thereby restricting their expression to the basal or apical side of the embryo. This restriction is necessary for embryonic patterning, as misexpression of both genes initiates ectopic meristem formation.[49,50]

5. Maintaining the niche

5.1. PLTs regulate RAM growth dose dependent

The *plt1plt2* mutant seedling displays an increased number of columella and QC cells and shows a reduced RAM growth rate and less meristem cells, also the cell division rate in the meristem is decreased, measured by cyclin expression, resulting in an extreme reduced root growth phenotype. In addition the QC cells do not express their markers and the columella stem cells contain starch granula, a marker for differentiation, thus the identity and organization of the QC and surrounding stem cells in the seedling is lost. Therefore PLT is also needed for the maintenance of the QCs and stem cell zone and meristematic growth in *Arabidopsis.*[12]

The *pl1t-plt2-plt3* triple mutant seedling shows a rootless phenotype. When a weak *plt1*$^{+/-}$-*plt2-plt3* mutant, or any other combination, is grown this phenotype is less severe. The mature double *plt1-plt2* mutant has an even less severe root phenotype, this indicates that the regulating effect of PLT in the RAM is dose-dependent.[42] Accordingly, *PLT* promoter activity in the root tip shows a gradient. The highest expression is in the stem cell niche, including the QC, subsequently the PLT levels decrease to low levels at the elongation zone. All PLT family members show a different radial expression pattern in the root tip, but with the same longitudinal gradient.[42] When *PLT* is expressed under a different promoter that is less active in the stem cell zone, but normally active in the transition and elongation zone, there is no stem cell activity from 7 DAG. The TA cells are lost at 12 DAG. So intermediate levels of PLT induce TA cells. When *PLT* is expressed under a promoter that shows a longitudinally steeper gradient the root and RAM are shorter, but the stem cells are correctly maintained. This suggests that there are three zones of *PLT* expression that cause different effects in target cells. High *PLT* expression in the QC is needed for stem cell maintenance, lower expression induces mitotic activity of the stem cell daughters in the meristem and even lower *PLT* levels allow differentiation.[42]

5.2. TPST regulates post-embryonic stem cell niche organization

Recently the gene *ACTIVE QUIESCENT CENTRE1 (AQC1)* was identified to encode a tyrosylprotein sulfotransferase (TPST) protein. *TPST* is first expressed in the hypophysis and surrounding cells in the heart stage, in the mature plant *TPST* is expressed in the stem cell niche and the differentiated columella cells. Tyrosin sulfation by TPST is needed for the

activation of Tyr-sulfated peptides, these peptides can perform regulatory functions in *Arabidopsis* and have also been shown to be involved in RAM growth.[53-56] Therefore it is possible that activation of these peptides by TPST is important for RAM growth. The *tpst* mutant shows reduced root growth from 2 DAG and reduced RAM growth, this is probably a result of loss of QC function. The QC cells in the *tpst* mutant are increased in number and the morphology is disrupted from 1 DAG. *WOX5* is not strictly expressed in the QC cells, but expanded to the surrounding cells and the QC cells show more mitotic divisions than wild type. In addition the layer of starch granule containing columella cells starts directly after the aberrant QC cells, thus stem cells are not maintained. This indicates that *TPST* is required for correct QC organization and function post-embryonically.[57]

The expression pattern of *TPST* overlaps with that of the auxin maximum in the root tip, suggesting that, as many other genes, it is regulated by auxin. Indeed, after IAA treatment the TPST expression increases. In the *tpst* mutant the directed distribution of auxin is slightly expanded, but the maximum is at the QC cells, as it is in wild type also, free auxin levels are increased in the root tip, but not in the whole embryo. The expression of PIN4, PIN3 and PIN7 and auxin biosynthesis enzymes in the root meristem is reduced in the *tpst* mutant, this indicates that TPST can locally affect auxin distribution, by regulating the expression of transport and biosynthesis genes.[57]

5.3. PLT is post-transcriptional regulated by RGFs

The phenotype of the *tpst* mutant is similar to the *plt1plt2* mutant phenotype, where also PIN4, -3 and -7 expression is decreased[32, 42, 50] and RAM growth is disrupted (chapter 5.1), this suggests that TPST is involved in PLT regulation. *PLT2* overexpression can rescue the *tpst* phenotype and the triple mutant *tpst-plt1-plt2* shows a more severe disrupted root stem cell niche than the *tpst* or *plt1plt2* mutants individually, however the *tpst-plt* mutant still shows a RAM at 4 DAG indicating that TPST and PLT act in the same pathway[57]. In the *tpst* mutant PLT levels are reduced, however the transcription levels of *PLT* are only slightly decreased in the *tpst* mutant. PLT levels in the *tpst* mutant can also still be upregulated by auxin, however not to the wild type levels. In addition, the *plt1plt2* phenotype cannot be rescued by TPST, therefore it is likely that TPST regulates PLT protein levels post-transcriptional.[56]

Recently, a family of peptides containing a tyrosine sulfation residu was found to be present in the root stem cells and innermost layer of collumella cells, named root meristem growth factors (RGFs). These RGFs are activated by TPST mediated tyrosine sulfation. The *rgf* triple

mutant shows a short-root phenotype with a smaller RAM, similar to the *tpst* phenotype, this phenotype can be rescued in both *rgf* and *tpst* mutants by adding RGF peptides. Therefore RGFs are involved in postembryonic RAM organization and function in the TPST pathway.[56] PLT levels are reduced in the *rgf* and *tpst* mutants and the levels of PLT in the *rgf* and *tpst* mutants can be rescued by RGF peptide treatment, thus the RGFs positively regulate PLT expression. In wild type seedlings RGF treatment induces PLT expression expanding into the basal meristem region. The transcription levels of *PLT* accumulate only in the stem cell region after RGF treatment. When RGF treatment is ended in the seedling the PLT expression levels are restored, thus RGFs positively regulate PLT levels post-transcriptionally. The root RGFs possibly function in the stabilization of PLT after transcription and therefore allow a wider PLT distribution.[56]

5.4. WOX5 maintains stem cells

There is similarity in the signaling mechanisms of the maintenance of the root and shoot stem cell niches. For example the transcription factors WUSCHEL (WUS) in the shoot and WUS-RELATED HOMEOBOX5 (WOX5) in the root are interchangeable.[58, 59]

The QC cells in the *Arabidopsis* seedling express *WOX5*. This suggests that *WOX5* is involved in stem cell maintenance in the mature root. In *wox5* mutants the QC cells are abnormally shaped and enlarged and expression of some QC markers is reduced. In addition, the adjacent columella stem cells are also enlarged and contain starch-granules, indicating that they are differentiated. In both the *plt-wox5* or *scr/shr/wox5* mutants also the proximal stem cells differentiate.[60] In *WOX5* overexpressing embryos there are small cells present that do not contain starch-granules, in the place of differentiated columella cells. The QC itself is not affected by *WOX5* overexpression. In the wild type plant, ablation of the QC causes collapse of the stem cell niche, however, QC ablation does not increase differentiation of columella stem cells when *WOX5* is ectopically expressed.[60] Thus WOX5 is essential for QC function of stem cell maintenance and plays a minor role in QC specification and *WOX5* expression is sufficient for columella stem cell maintenance and possibly acts redundantly with *PLT* and *SHR*/*SCR* in stem cell maintenance of the other root tissues.[60]

The auxin maximum that is established during embryogenesis is maintained in the mature root tip, indicating that auxin signaling is involved in organization of the stem cell niche. Accordingly, in *mp* or *bdl* mutants *WOX5* expression in the QC is lost, which is expected because of the inability to form the QC in these mutants. In the *plt* mutant *WOX5* expression is slightly expanded. In the *scr-shr* mutant however, *WOX5* expression is heavily reduced,

thus *WOX5* expression is dependent on *SHR*/*SCR* expression and indirectly on auxin expression.[61] Exogenous auxin or NPA treatment, or endogenous overexpression of the auxin biosynthesis gene IAAM in the root tip in wild type roots promotes distal stem cell differentiation and reduces *WOX5* expression. In contrast, in *yucca* or *pin3* mutants auxin distribution is decreased and distal stem cell differentiation is delayed. The effect of auxin on stem cell differentiation suggests that downstream factors of auxin play a role in inducing meristem growth. The IAA17/AXR3 protein is well characterized in auxin signaling and the *axr3* mutant shows a delay of distal stem cell differentiation and expansion of *WOX5* expression. Two ARFs that are repressed by AXR3 are the transcription factors *ARF10* and *ARF16*. When ARF10 and ARF16 are repressed there is a delay in distal stem cell differentiation and *WOX5* expression is not restricted to the QC. In addition, *ARF16* overexpression represses WOX5 expression and causes increased distal stem cell differentiation.[61] Thus auxin stimulates columella stem cell differentiation, by indirectly restricting WOX5 expression to the QC through an ARF10 and ARF16 signaling pathway.[61]

5.5. CLE40 regulates stem cell niche size

QC signaling only affects its adjacent cells; this suggests that QC signals are repressed in further away localized cells. The class A group of the CLV3/ENDOSPERM SURROUNDING REGION (CLE) peptide family is involved in the inhibition of meristem size. This class includes CLV3, CLE19, and CLE40, which are involved in meristem growth inhibition[9] CLV3 is known to inhibit stem cell maintenance activity of *WUS* in the shoot meristem[58, 59]. Because *WOX5* is a homolog of *WUS* it is possible that such a feedback system is also present in the root meristem.

In the globular stage *CLE40* is expressed in the whole embryo, in later stages *CLE40* expression is restricted to the basal side of the embryo. In the seedling *CLE40* is expressed in the differentiation zone of the stele and columella, therefore CLE40 could be involved in *WOX5* regulation. The *cle40* mutant shows shorter roots, a disorganized root tip, an increase of columella stem cells at a more distal location and expanded *WOX5* expression. This indicates that CLE40 is involved in the regulation of *WOX5* expression and that is confines *WOX5* expression to the QC. In this way only the adjacent cells of the QC are influenced by *WOX5* expression and maintained as stem cells.[62]

When CLE40 peptide is added to wild type seedlings increased differentiation, ectopic starch granule accumulation and loss of stem cells is seen. Also the expression of *WOX5* and a QC marker moves proximal in these mutants. This suggests that CLE40 regulates the location of

WOX5 expression and so regulates the location and size of the root stem cell niche, however, when CLE40 peptide is added to the *wox5* mutant the expansion of differentiated columella cells is even more increased. This suggests that CLE40 also functions in a pathway parallel of *WOX5* to maintain the root stem cell niche size.[62]

Interestingly the *ARABIDOPSIS CRINKLY4 (ACR4)* receptor-like kinase mutant shows the same phenotype as the *cle40* mutant. When CLE40 peptide is added to the *acr4* mutant there is only a minor change in WOX5 expression, indicating that ACR4 is involved in the CLE40 pathway to repress *WOX5* expression. *ACR4* is expressed in the columella stem cells and differentiated cells, but not in the QC. When CLE40 peptide is added to the wild type seedling also the expression of *ACR4* moves proximal, to the previous QC position. This shows that CLE40 regulates *WOX5* expression through regulation of *ACR4* expression and activity. In this way CLE40 acts in a feedback mechanism with *WOX5* from differentiated cells to the QC to maintain the root stem cell niche size and location (Figure 7).[9, 62]

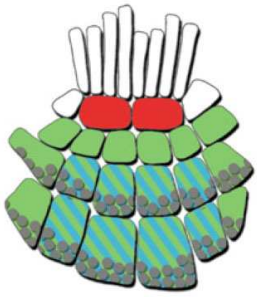

Figure 7. Schematic view of the expression of *WOX5* (red), CLE40 (blue) and *ACR4* (green) in the mature distal root tip. *WOX5* is only expressed in the QC. *CLE40* is only expressed in differentiated columella cells, marked by starch granules (grey). *ACR4* is expressed in both columella stem cells and differentiated cells, but not in the QC. Adapted from Stahl et.al.[62]

5.6. SCZ specifies ground tissue stem cells

Another factor that is important for radial organization is *SCHIZORIZA (SCZ)*[3, 63, 64]. SCZ encodes for the HEAT SHOCK TRANSCRIPTION FACTOR B4. SCZ is first expressed in the QC progenitors in the embryo and from the heart stage on it is expressed in the QC and ground and vascular tissue stem cells and their immediate daughters[64]. In the mature root *scz* mutants show a disrupted organization of the QC and columella cells and reduced root and RAM growth, however, in the embryo the QC markers and WOX5 are correctly expressed. The earliest defect observed in the *scz* mutant is in the first ground tissue stem cell, which divides periclincal, instead of first anticlinal in the wild type (Figure 3). This results in the formation of an ectopic ground tissue layer at the torpedo stage, which is maintained in the mature plant.[3, 63, 64] Overexpression of *WOX5* in the mature *scz* mutant rescues QC identity

and columella stem cell maintenance, however the radial organization and root growth are not rescued. Overexpression of SCZ results in extra stem cells in the root, this indicates that SCZ is not essential for QC function, but is involved in early radial organization and formation of stem cells[3, 64].

Radial organization of the root is also dependent on SCR. The mature *scr-scz* double mutant shows reduced root growth and a severely disrupted radial organization where the cortex and endodermis layers are not observed. In the embryo *scr-scz* mutant the epidermis/lateral root cap stem cell formation is delayed and the formation of ground tissue stem cells is not visible [3]. This shows that SCZ is necessary for the early establishment of the ground tissue stem cells and their maintenance in the mature root. So together with SCR, SCZ regulates the development of specific stem cells and radial patterning[3, 63, 64].

6. Conclusion and discussion

Discussed here are the major regulators of QC patterning and function. The actual mechanism of hypophysis recruitment, QC specification and stem cell maintenance is much more detailed, including many factors involved in cell division and specification, which have not yet been identified.

6.1. Three levels of regulation

The regulation of *Arabidopsis* patterning can be roughly divided into three levels. First global regulation in the whole embryo by auxin, second local regulation by ARFs and third cellular regulation by ARF targets. These levels of regulation are maintained in the mature plant.

Auxin is distributed throughout the mature plant and in the embryo, with local varying maxima. Auxin can be seen as a meta regulator of *Arabidopsis* as it is involved in many processes, such as development and responses to external factors, throughout the whole plant. The distribution of auxin by the PIN efflux carriers is therefore essential for normal growth. This distribution is regulated over long distances in the whole plant, from the shoot to the root. As shown by the lack of RAM formation when apical PIN function is lost in the embryo[21].

As auxin is present in the whole plant it cannot be directly involved in the specification of different structures. The specification is regulated by ARFs, these are expressed where auxin is accumulated, but they also have to be under the regulation of another specific factor. Not much is known, however, about why certain ARFs are expressed in certain areas. In QC specification, for example, it could be that the hypophysis provides the specific signals for PLT expression, to promote root patterning. It was shown that ectopic PLT expression can induce ectopic RAM formation[12, 50]. Therefore the hypophysis does not appear to be essential for QC formation itself, but only for the correct localization of the QC in the root. PLT repressors, however, also play an important role in confining PLT expression to the basal embryo and mature root tip[42, 50].

The regulation of major processes by transcription factors is mediated by the local actions of their downstream factors. Downstream factors of ARFs are mainly involved in intracellular processes, such as cell division, histone modification and intracellular signaling pathways [18, 65-68]. The QC induces stem cell identity by a WOX5 mediated signaling pathway. To know the exact mechanism of WOX5 function its targets need to be found. ARFs can also influence

signaling of auxin by regulating the expression of PINs and auxin synthesis enzymes. Thus the three levels of regulation in *Arabidopsis* can interact and influence expression levels in an feedback manner.

6.2. Non-autonomous cell-cell signaling

Interestingly the major regulating processes in root development and stem cell maintenance are regulated by non-autonomous cell-cell signaling. This is visible at hypophysis recruitment by MP and in the mature root in QC function. MP is essential for the recruitment of the hypophysis to the embryo, however MP is only expressed apical of the hypophysis precursor. It could be that this is needed because the hypophysis precursor is at this stage not yet so far specified to be able to act cell-autonomously. Also there is probably another ARF active in the hypophysis itself. So the recruitment of the hypophysis is not solely non-cell-autonomously regulated[39, 40].

The activity of the QC in the mature root however, is dependent on cell-cell signaling. SHR needs to move from the vasculature to the QC to activate SCR and WOX5[60]. Also the maintenance of the stem cells is regulated non-cell-autonomously. Stem cell identity is stimulated by WOX5, which is expressed by the adjacent QC[60]. WOX5 itself is confined to the QC by auxin and CLE40, to restrict the stem cell niche size[61,62]. In this way an equilibrium of signals is established at the stem cells that allows for this organization non-cell-autonomously.

6.3. Proximal and distal meristem regulation

The essential function of WOX5 for columella stem cell maintenance is clear[60]. The function of WOX5 in the maintenance of proximal stem cells however, is less clear. Effects of *wox5* mutants on the proximal meristem are not often detected. Also, other factors, such as SCR and SCZ, have been shown to be essential for specific proximal stem cell development and maintenance[3]. This suggests that WOX5 is not essential for the maintenance of these stem cells. However, in *plt1plt2-wox5* and *scr wox5* and *shr wox5* mutants the proximal stem cells are earlier differentiated. Thus WOX5 function is also necessary for proximal stem cell maintenance , where it probably functions in redundant pathways[60].

6.4. Perspectives

Several steps in QC patterning and function are not yet clear. Some are already described above. Other unanswered questions are for example the onset of changed PIN polarization in

the globular stage. Many processes are regulated by auxin, however when PIN polarization is changed there is not yet an auxin gradient established. So there must be another factor involved to regulate this process, which is not yet found. Once the auxin gradient is established by PIN polarization it is maintained in the mature plant. Involved in the maintenance of the auxin flow are besides PINs also auxin biosynthesis enzymes. Many auxin downstream signaling factors play a role in the regulation of PINs and these enzymes. The exact mechanism, however of auxin distribution and feedback regulation by downstream signaling factors is not known. It is possible that the same pathways are involved during embryogenesis and in the mature root. The auxin distribution, however, could also be maintained by different mechanisms during different growth stages, as for example auxin is first directed to the hypophysis by MP, which is not active in later stages.

To date not all factors involved in hypophysis recruitment have been found. It is shown that MP is necessary for hypophysis recruitment and that it induces TMO7 expression that moves to the hypophysis precursor[40], however additional targets of MP are expected to be involved in this process. As described, there is an auxin maximum in the hypophysis precursor, indicating that an unknown ARF is active in this cell[39]. Moreover in the globular stage distribution of auxin is not exclusively directed to the hyphophysis precursor and its apical cells, but it is also distributed to the apical side of the embryo and involved in cotyledon formation, thus the auxin signal is not specific in this region. This suggests that the multiple signals in and around the hypophysis precursor contribute to the specificity of this process. It is possible that a specific combination of signals is needed for the correct localization and recruitment of the hypohysis.

Once the hypophysis is recruited PLT is necessary for QC formation[12,42], it regulates the specific cell divisions of the hypophysis and the lens-shaped cell. Moreover, PLT is sufficient to initiate ectopic root meristems. Therefore PLT expression is required for the establishment of a QC identity, this includes a slow division rate and its function in stem cell maintenance. Not much is known about the regulation of QC division, therefore more PLT targets need to be found.

The function of PLT in root stem cell maintenance is also not clear. A lack of PLT induces stem cell differentiation, suggesting that PLT stimulates stem cell fate. The dose-dependent regulation of RAM growth by PLT shows that it can also induce differentiation of cells at lower levels. This indicates that high levels of PLT inhibit differentiation and that lower levels allow differentiation. To conclude this however, more PLT targets need to be found.

Stem cell maintenance by the QC is also dependent on SHR/SCR[43-46], indicating that non-cell-autonomous signaling plays an important role in QC function. Therefore it is expected that there are more feedback signaling systems present in the root stem cell niche, besides SHR/SCR (from the vasculature) and CLE/WOX (from the columella)[43-46,62]. These feedback mechanisms, from stem cells and differentiated cells, contribute to the regulation of the stem cell niche size. Furthermore, not much is known about the regulation of the expression of SHR and CLE40.

The regulation of the formation of the different types of stem cells in the root is not clear. It is not known how the stem cells are recruited in the embryo. The stem cells start to specify after the QC is formed, indicating that its function is important for this process. Furthermore transcription factors SHR/SCR and SCZ are involved in the specification of certain types of stem cells[3], suggesting that more transcription factors are involved in the specification of the other stem cell types.

In general, there are still transcription factors and downstream targets that are involved in QC patterning and function that are not yet found. In addition, of many involved transcription factors the direct regulation is not clear. For example, the ARFs are known to be induced by auxin, but the complete activation pathway of many of these transcription factors is not known. Overall, the roles of the major regulators of root patterning are becoming more clear. In the coming years the knowledge of their roles will become more detailed. Also the identity of the QC and its ability to maintain stem cells provide an interesting research field to be further explored.

7. References

1. De Smet, I. & Jurgens, G. Patterning the axis in plants--auxin in control. *Curr. Opin. Genet. Dev.* **17**, 337-343 (2007).

2. Scheres, B. Stem-cell niches: nursery rhymes across kingdoms. *Nat. Rev. Mol. Cell Biol.* **8**, 345-354 (2007).

3. Pernas, M., Ryan, E. & Dolan, L. SCHIZORIZA Controls Tissue System Complexity in Plants. **20**, 818-823 (2010).

4. De Smet, I., Lau, S., Mayer, U. & Jurgens, G. Embryogenesis - the humble beginnings of plant life. *Plant J.* **61**, 959-970 (2010).

5. Peris, C. I., Rademacher, E. H. & Weijers, D. Green beginnings - pattern formation in the early plant embryo. *Curr. Top. Dev. Biol.* **91**, 1-27 (2010).

6. Mansfield, S. G. & Briarty, L. G. Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* **69**, 461-476 (1991).

7. Bennett, T. & Scheres, B. in *Current Topics in Developmental Biology* (ed Marja C.P. Timmermans) 67-102 (Academic Press, 2010).

8. Dolan, L. *et al*. Cellular organisation of the Arabidopsis thaliana root. *Development* **119**, 71-84 (1993).

9. Stahl, Y. & Simon, R. Plant primary meristems: shared functions and regulatory mechanisms. *Curr. Opin. Plant Biol.* **13**, 53-58 (2010).

10. Baluaka, F., Mancuso, S., Volkmann, D. & Barlow, P. W. Root apex transition zone: a signallingresponse nexus in the root. **15**, 402-408 (2010).

11. Kidner, C., Sundaresan, V., Roberts, K. & Dolan, L. Clonal analysis of the Arabidopsis root confirms that position, not lineage, determines cell fate. *Planta* **211**, 191-199 (2000).

12. Aida, M. *et al*. The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. *Cell* **119**, 109-120 (2004).

13. Cheng, Y., Dai, X. & Zhao, Y. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in Arabidopsis. *Plant Cell* **19**, 2430-2439 (2007).

14. Stepanova, A. N. *et al*. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **133**, 177-191 (2008).

15. Dharmasiri, N., Dharmasiri, S. & Estelle, M. The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441-445 (2005).

16. Moller, B. & Weijers, D. Auxin control of embryo patterning. *Cold Spring Harb Perspect. Biol.* **1**, a001545 (2009).

17. Woodward, A. W. & Bartel, B. Auxin: regulation, action, and interaction. *Ann. Bot.* **95**, 707-735 (2005).

18. Thomas, C. L., Schmidt, D., Bayer, E. M., Dreos, R. & Maule, A. J. Arabidopsis plant homeodomain finger proteins operate downstream of auxin accumulation in specifying the vasculature and primary root meristem. *Plant J.* **59**, 426-436 (2009).

19. Friml, J. *et al*. Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature* **426**, 147-153 (2003).

20. Wisniewska, J. *et al*. Polar PIN localization directs auxin flow in plants. *Science* **312**, 883 (2006).

21. Ploense, S. E., Wu, M., Nagpal, P. & Reed, J. W. A gain-of-function mutation in IAA18 alters Arabidopsis embryonic apical patterning. *Development* **136**, 1509-1517 (2009).

22. Petrasek, J. & Friml, J. Auxin transport routes in plant development. *Development* **136**, 2675-2688 (2009).

23. Ugartechea-Chirino, Y. *et al*. The AUX1 LAX family of auxin influx carriers is required for the establishment of embryonic root cell organization in Arabidopsis thaliana. *Ann. Bot.* **105**, 277-289 (2010).

24. Mayer, U., Buttner, G. & Jurgens, G. Apical-basal pattern formation in the Arabidopsis embryo: studies on the role of the gnom gene. *Development* **117**, 149-162 (1993).

25. Geldner, N. *et al*. The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* **112**, 219-230 (2003).

26. Huang, F. *et al*. Phosphorylation of conserved PIN motifs directs Arabidopsis PIN1 polarity and auxin transport. *Plant Cell* **22**, 1129-1142 (2010).

27. Zhang, J., Nodzynski, T., Pencik, A., Rolcik, J. & Friml, J. PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 918-922 (2010).

28. Kleine-Vehn, J. *et al*. PIN auxin efflux carrier polarity is regulated by PINOID kinase-mediated recruitment into GNOM-independent trafficking in Arabidopsis. *Plant Cell* **21**, 3839-3849 (2009).

29. Michniewicz, M. *et al*. Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* **130**, 1044-1056 (2007).

30. Friml, J. *et al*. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862-865 (2004).

31. Sabatini, S. *et al*. An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell* **99**, 463-472 (1999).

32. Blilou, I. *et al*. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* **433**, 39-44 (2005).

33. Weijers, D. *et al*. Maintenance of embryonic auxin distribution for apical-basal patterning by PIN-FORMED-dependent auxin transport in Arabidopsis. *Plant Cell* **17**, 2517-2526 (2005).

34. Mravec, J. *et al*. Interaction of PIN and PGP transport mechanisms in auxin distribution-dependent development. *Development* **135**, 3345-3354 (2008).

35. Hakman, I., Hallberg, H. & Palovaara, J. The polar auxin transport inhibitor NPA impairs embryo morphology and increases the expression of an auxin efflux facilitator protein PIN during Picea abies somatic embryo development. *Tree Physiol.* **29**, 483-496 (2009).

36. Peer, W. A. *et al*. Mutation of the Membrane-Associated M1 Protease APM1 Results in Distinct Embryonic and Seedling Developmental Defects in Arabidopsis. *Plant Cell* **21**, 1693-1721 (2009).

37. Tsugeki, R. *et al*. NO VEIN Mediates Auxin-Dependent Specification and Patterning in the Arabidopsis Embryo, Shoot, and Root. *Plant Cell* **21**, 3133-3151 (2009).

38. Berleth, T. & Jurgens, G. The role of the monopteros gene in organising the basal body region of the Arabidopsis embryo. *Development* **118**, 575-587 (1993).

39. Weijers, D. *et al*. Auxin triggers transient local signaling for cell specification in Arabidopsis embryogenesis. *Dev. Cell.* **10**, 265-270 (2006).

40. Schlereth, A. *et al*. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**, 913-916 (2010).

41. Scheres, B. *et al*. Embryonic origin of the Arabidopsis primary root and root meristem initials. *Development* **120**, 2475-2487 (1994).

42. Galinha, C. *et al*. PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. *Nature* **449**, 1053-1057 (2007).

43. Di Laurenzio, L. *et al*. The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. *Cell* **86**, 423-433 (1996).

44. Helariutta, Y. *et al*. The SHORT-ROOT gene controls radial patterning of the Arabidopsis root through radial signaling. *Cell* **101**, 555-567 (2000).

45. Nakajima, K., Sena, G., Nawy, T. & Benfey, P. N. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* **413**, 307-311 (2001).

46. Sabatini, S., Heidstra, R., Wildwater, M. & Scheres, B. SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. *Genes Dev.* **17**, 354-358 (2003).

47. Sena, G., Wang, X., Liu, H. Y., Hofhuis, H. & Birnbaum, K. D. Organ regeneration does not require a functional stem cell niche in plants. *Nature* **457**, 1150-1153 (2009).

48. Szemenyei, H., Hannon, M. & Long, J. A. TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science* **319**, 1384-1386 (2008).

49. Grigg, S. P. *et al*. Repression of Apical Homeobox Genes Is Required for Embryonic Root Development in Arabidopsis. **19**, 1485-1490 (2009).

50. Smith, Z. R. & Long, J. A. Control of Arabidopsis apical-basal embryo polarity by antagonistic transcription factors. *Nature* **464**, 423-426 (2010).

51. Grigg, S. P., Canales, C., Hay, A. & Tsiantis, M. SERRATE coordinates shoot meristem function and leaf axial patterning in Arabidopsis. *Nature* **437**, 1022-1026 (2005).

52. Prigge, M. J. & Wagner, D. R. The arabidopsis serrate gene encodes a zinc-finger protein required for normal shoot development. *Plant Cell* **13**, 1263-1279 (2001).

53. Matsubayashi, Y. & Sakagami, Y. Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of Asparagus officinalis L. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 7623-7627 (1996).

54. Matsubayashi, Y., Ogawa, M., Morita, A. & Sakagami, Y. An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science* **296**, 1470-1472 (2002).

55. Amano, Y., Tsubouchi, H., Shinohara, H., Ogawa, M. & Matsubayashi, Y. Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 18333-18338 (2007).

56. Matsuzaki, Y., Ogawa-Ohnishi, M., Mori, A. & Matsubayashi, Y. Secreted Peptide Signals Required for Maintenance of Root Stem Cell Niche in Arabidopsis. *Science* **329**, 1065-1067 (2010).

57. Zhou, W. *et al*. Arabidopsis Tyrosylprotein Sulfotransferase Acts in the Auxin/PLETHORA Pathway in Regulating Postembryonic Maintenance of the Root Stem Cell Niche. *Plant Cell* (2010).

58. Schoof, H. *et al*. The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* **100**, 635-644 (2000).

59. Dodsworth, S. A diverse and intricate signalling network regulates stem cell fate in the shoot apical meristem. *Dev. Biol.* **336**, 1-9 (2009).

60. Sarkar, A. K. *et al*. Conserved factors regulate signalling in Arabidopsis thaliana shoot and root stem cell organizers. *Nature* **446**, 811-814 (2007).

61. Ding, Z. & Friml, J. Auxin regulates distal stem cell differentiation in Arabidopsis roots. *Proceedings of the National Academy of Sciences* **107**, 12046-12051 (2010).

62. Stahl, Y., Wink, R. H., Ingram, G. C. & Simon, R. A Signaling Module Controlling the Stem Cell Niche in Arabidopsis Root Meristems. **19**, 909-914 (2009).

63. Ten Hove, C. A. & Heidstra, R. Who begets whom? Plant cell fate determination by asymmetric cell division. *Curr. Opin. Plant Biol.* **11**, 34-41 (2008).

64. ten Hove, C. A. *et al*. SCHIZORIZA Encodes a Nuclear Factor Regulating Asymmetry of Stem Cell Divisions in the Arabidopsis Root. **20**, 452-457 (2010).

65. Gagne, J. M. & Clark, S. E. The Arabidopsis Stem Cell Factor POLTERGEIST Is Membrane Localized and Phospholipid Stimulated. *Plant Cell* **22**, 729-743 (2010).

66. Anzola, J. M. *et al*. Putative Arabidopsis Transcriptional Adaptor Protein (PROPORZ1) is required to modulate histone acetylation in response to auxin. *Proceedings of the National Academy of Sciences* **107**, 10308-10313 (2010).

67. Kornet, N. & Scheres, B. Members of the GCN5 Histone Acetyltransferase Complex Regulate PLETHORA-Mediated Root Stem Cell Niche Maintenance and Transit Amplifying Cell Proliferation in Arabidopsis. *Plant Cell* **21**, 1070-1079 (2009).

68. Vanstraelen, M. *et al*. APC/CCCS52A complexes control meristem maintenance in the Arabidopsis root. *Proceedings of the National Academy of Sciences* **106**, 11806-11811 (2009).