

Root Apical Meristem Pattern: Hormone Circuitry and Transcriptional Networks

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Abstract In higher plants, growth and development relies on the spatiotemporal regulation of gene expression, which is under the control of both endogenous signals and external stimuli. In this chapter, recent advances in defining signaling machinery and genetic frameworks that underlie RAM patterning and maintenance are reviewed, with a focus on the interplay between different hormone classes. The evidence for an epigenetic control of the root developmental program is also briefly considered. Conceivably, many other aspects are still to be elucidated. Future challenges deal with, on the one hand, understanding how signaling and genetic programs are modulated to achieve adaptative traits under environmental pressure and, on the other, how cell fate is reprogrammed *in vivo* and *in vitro*. We conclude that knowledge from the plant model, *Arabidopsis thaliana*, could enhance our understanding of more complex species encountered in crop

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systems and could provide relevant perspectives for both crop improvement and plant biotechnology.

1 Introduction

In higher plants, root growth is achieved by pronounced elongation of cells that are descendants of the root apical meristem (RAM), a specialized structure present at the root tip, which produces cells for virtually all of the root tissues formed during postembryonic development. In this localized microenvironment, meristematic cells can reside for potentially an indeterminate period of time and produce progeny cells while self-renewing, thus exhibiting key features of a stem-cell niche (Ohlstein et al. 2004; Li and Xie 2005; Dinneny and Benfey 2008; Morrison and Spradling 2008). This behavior relies on the capacity of RAM cells to undergo asymmetric cell division, which is another defining feature of stem-cells dictating that one daughter cell retains the meristematic fate and another daughter cell is programmed to differentiate into a specialized cell (McCulloch and Till 2005; Scheres 2007; Morrison and Spradling 2008). The acquisition of differential potential of the two daughter cells can result from an unequal partitioning of cell fate determinants through the asymmetric positioning of the cell division plate as well as from a different signaling from their surroundings.

The maintenance of the RAM is assured by a balance between the production of new meristematic cells and their displacement toward the differentiation process. However, in some cases, the RAM is genetically determined to become exhausted, and root growth shifts from an indeterminate to a determinate developmental pattern. Recently, an extensive review on the determinacy/indeterminacy of root growth has been provided by Shishkova et al. (2008), but it is outside the scope of this chapter.

Here, we review the recent advances in understanding transcriptional networks and hormone cross-talk underlying the establishment and regulation of the RAM. In particular, signals between the organizing region and the stem-cell population in the RAM as well as signals released by neighboring cells will be emphasized. Mainly, we focus on studies on the model plant, *Arabidopsis thaliana*.

2 An Overview of RAM Organization in Plants

Precociously determined during embryogenesis, and indeed before the establishment of the shoot apical meristem (SAM), the RAM exhibits a proliferative multicellular dome that includes apical initials and their derivative cells (Fig. 1) (Clowes 1976; Barlow 1997). The number of initials varies according to species (Webster and McLeod 1996), but they are almost permanent in position and divide continuously, producing at each division one cell that continues to act as an initial

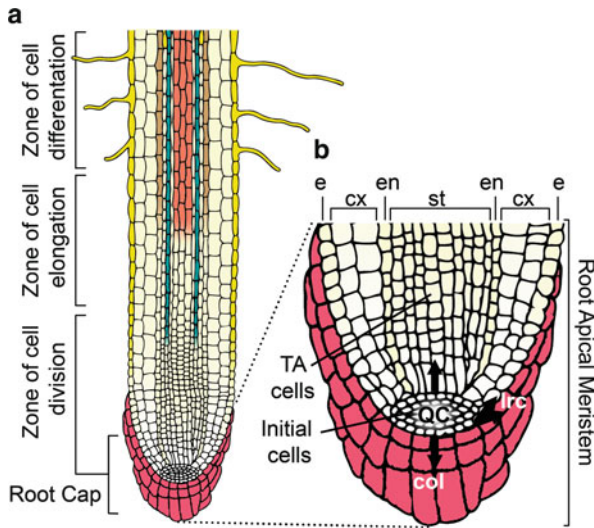


Fig. 1 Schematic of a higher plant primary root tip in longitudinal section (**a**) featuring zones of cell division, elongation, and differentiation. In (**b**), arrows indicate the direction of cell division; *col* columella; *cx* cortex; *e* epidermis; *en* endodermis; *lrc* lateral root cap; *QC* quiescent center; *st* stele; *TA* transit amplifying cells; adapted from Taiz and Zeiger (1998)

and one derivative cell. Derivative cells undergo numerous additional cell divisions producing a transit amplifying (TA) cell population, which is progressively displaced from initials and then allowed to differentiate (Fig. 1a). In the RAM, the initials divide proximally, laterally, or distally, thus giving rise to derivative cells for root cortex/endodermis/stele, epidermis/lateral rootcap, and columella, respectively (Fig. 1b). As a consequence, the RAM dome is located subterminally and is covered by the root cap, which protects the apical meristem, produces mucilage to facilitate a passage for the growing root, and serves as a gravity perceiving tissue. Behind the RAM is the root elongation zone where cells continue to elongate, followed by the differentiation zone, where cells acquire the characteristics of the mature differentiated primary tissues (Fig. 1a). Hence, the blue print for root function is laid down remarkably early during embryogenesis.

In angiosperms, the apical dome exhibits either an open or closed configuration (Clowes 1981; Heimsch and Seago 2008; and references cited therein). In the closed configuration, cell boundaries between cortical, epidermis, and root-cap regions are clearly distinguishable, while they are lacking in the open meristem (Fig. 2). Within both configurations, a large range of specific features exist in the Angiospermae (i.e., Basal angiosperms, Monocots, Eudicots) and in root ranks (i.e., primary vs. adventitious, primary vs. lateral, ephemeral vs. permanent), and have been extensively reviewed by Heimsch and Seago (2008). The specific organization of plant RAM is established early (i.e., the first day) after germination (Guttenberg 1968; Clowes 2000). However, some species exhibit RAMs that can change from

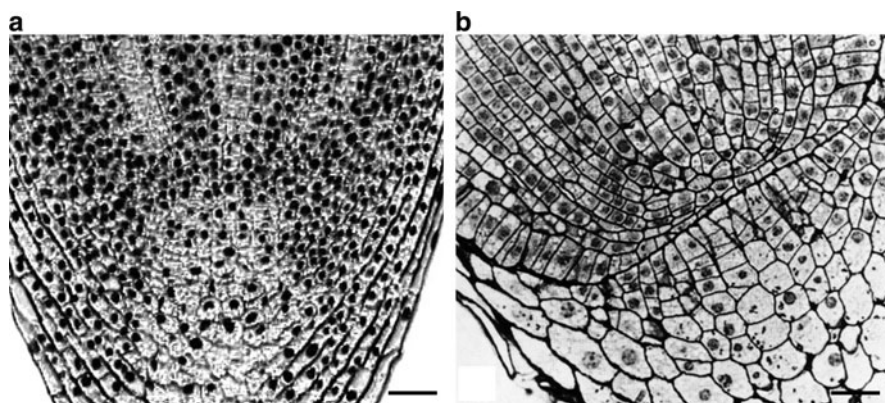


Fig. 2 Longitudinal sections through the root tip of (a) *Allium cepa* and (b) *Zea mays* [adapted from Webster and McLeod (1996)] exhibiting an open and closed organization, respectively. Bar scale (a) 70 μm ; (b) 100 μm

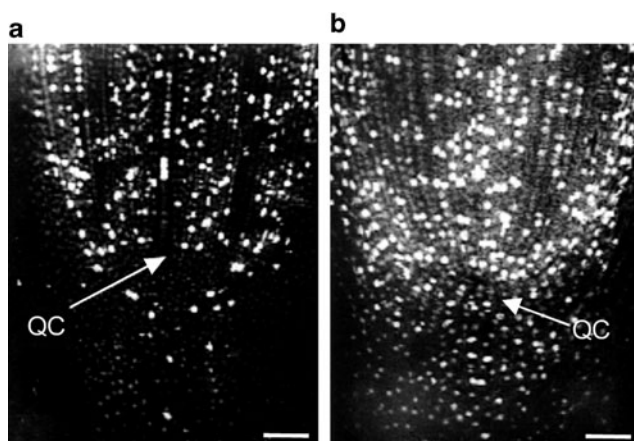


Fig. 3 Autoradiographs of longitudinal sections through the *Allium cepa* root apical meristem (RAM) of seedlings exposed to $[\text{Me-}^3\text{H}]$ thymidine for 24 h: (a) root grown in water and (b) root supplied with ascorbic acid. Note the different size of the QC in (a) and (b), represented by area within the RAM exhibiting unlabeled nuclei. Bar scale = 120 μm (adapted from Liso et al. 1988)

closed to open configuration and vice-versa as the root grows (Seago and Heimsch 1969; Armstrong and Heimsch 1976).

Within the RAM, meristematic cells undergo division at differential rates. One major demarcation of differential rates of division can be observed in a group of cells located centrally (Fig. 3a), which rarely divide and therefore was named the quiescent center (QC) and bears some analogies to the central zone in the SAM (Clowes 1954, 1956). Since its discovery in roots of *Zea mays* (Clowes 1954), QC

cells have long been considered a geometric necessity for root patterning as well as a reservoir of potentially meristematic cells that can be activated into division upon damage inflicted on other parts of the RAM (Clowes 1975; Barlow 1978). In line with this latter role, it has been demonstrated that QC cells are not permanently quiescent. Certainly, they can be induced to divide rapidly after irradiation (Clowes 1959, 1961), mechanical damage (Barlow 1974), carbohydrate starvation (Webster and Langenauer 1973), exposure to low temperature (Clowes and Stewart 1967), or exposure to ascorbic acid (Liso et al. 1988; Innocenti et al. 1990). However, and notably, in ascorbate-treated roots, a threshold number of QC cells were not induced to divide consistent with the existence of a minimal QC (Fig. 3b). Unlike the other treatments, the functions of meristem were not affected in these roots, thus suggesting a role for a minimal QC, maintaining and controlling RAM activity (Innocenti et al. 1990). As will be discussed later on, more recent evidence indicates that the QC represses cell differentiation of surrounding cells and therefore acts as the organizing center (OC) of the RAM stem niche (Van den berg et al. 1997).

3 Environmental Cues and RAM Patterning

In plants, both endogenous developmental factors and environmental cues can modulate cell differentiation and morphogenesis. Regarding root systems, an extensive literature exists about structural and functional modifications in a range of unrelated species, in response to water and nutrient availability, mechanical and gravitational stimuli, and responses to magnetic fields, as well as in relation to symbiotic or pathogenic interactions with heterologous organisms (Audus 1960; Behrens et al. 1982; Goodman and Ennos 1996; Fusconi et al. 1999; Stange et al. 2002; Waisel et al. 1997; Swarup et al. 2005; Potters et al. 2009).

However, with the exception of the effects on QC homeostasis, little attention has been paid to the effects induced by abiotic factors on RAM pattern as a whole (Feldman and Torrey 1975; Doncheva et al. 2005; Sanchez-Calderon et al. 2005; Sharma and Dubey 2007; De Tullio et al. 2009). Nevertheless, some data exist showing that exposure of *Zea mays* seedlings to a continuous electromagnetic field induced a stimulation in the rate of root elongation and resulted in a significant increase in cell expansion, in both the acropetal (proximal metaxylem cell lineage) (Fig. 4a, b) and basipetal (distal root cap cells) (Fig. 4e–h) directions. Concomitantly, a significant reduction in the size of the QC occurred (Fig. 4c, d) in roots exposed to this magnetic field together with an advanced differentiation of initials previously in surface contact with the QC (Bitonti et al. 2006). These results from a rather more complex system than *Arabidopsis* again underlie the importance of signals generated by cell-to-cell surface contact influencing differentiation and controlling root pattern.

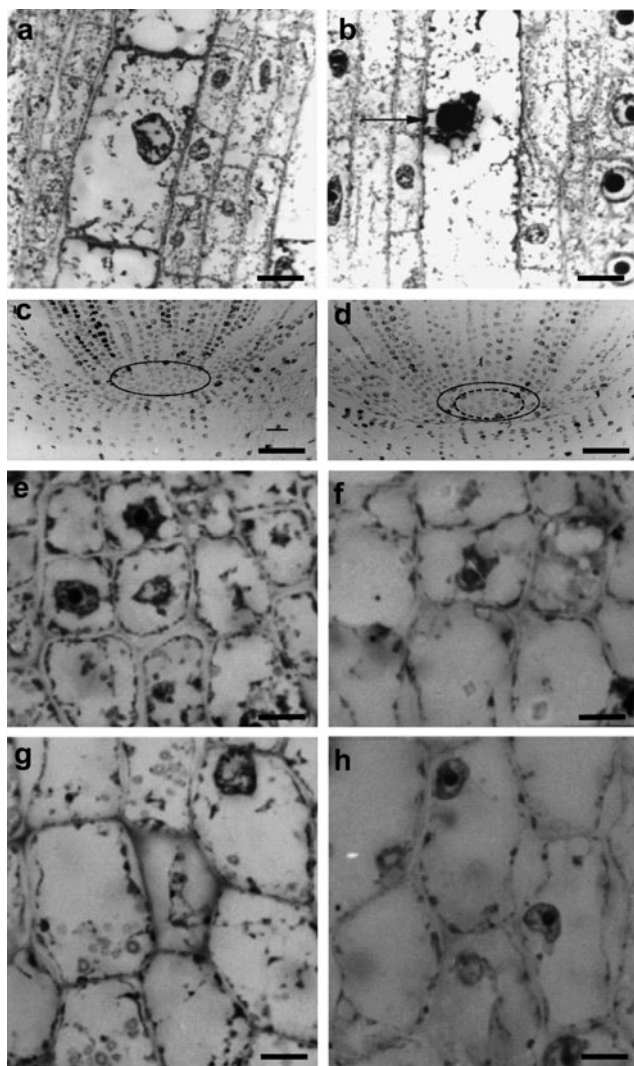


Fig. 4 Longitudinal sections through maize root tips in (a, c, e, g) control roots and (b, d, f, h) roots after 30-h exposure to a magnetic field. (a, b) Metaxylem cells at 800 μm from the cap-junction, scale bar = 40 μm ; note differences in length between control (a) and exposed root (b). (c, d) Autoradiograph of root apical meristem of seedlings exposed to $[\text{Me-}^3\text{H}]\text{thymidine}$ for 24 h; ring indicates quiescent centre (QC) characterized by absence of labeling, scale bar = 150 μm ; note differences in QC size between control (c) and exposed root (d). (e, f) root cap cells at the junction with the RAM; (g, h) root cap cells in the distal zone of columella, scale bar = 15 μm ; note differences in cell size and structure between control (e, g) and exposed root (f, h). Adapted from Bitonti et al. (2006)

4 *Arabidopsis thaliana*

4.1 *Morphogenetic Establishment of the RAM During Embryogenesis*

During embryogenesis, the morphogenetic organization of the future plant is defined through the establishment of two overlapping developmental patterns along the longitudinal (apical–basal) and radial axes. Along the longitudinal axis, the mature embryo exhibits the SAM, located between two cotyledons, the embryo axis or hypocotyl, and spatially separate from the RAM (Fig. 5a). The establishment of the radial pattern relies on the determination of three different tissues: protoderm, ground meristem, and procambium, which will generate cells that are fated to differentiate into epidermis, endodermis, and other cortical tissues and vascular tissues, respectively (Fig. 5b). In order to highlight the composite origin of the RAM, the morphogenetic events leading to its development are summarized in Fig. 6. Briefly, the stele, endodermis, cortex, epidermis, and lateral cap derive from the apical cell established at the bicellular embryo stage, while the QC and columella derive from the basal cell. It is evident that in plants very early during development (i.e., from the first zygotic division), cell fate determination relies on asymmetric cell division. Indeed, apical cell will give rise to most of the embryo proper while the basal cell will form the suspensor, a structure that physically positions the embryo within the seed and plays an active role in nutrient acquisition during early embryo development. In such a way, embryo polarity is established early through a mechanism that is common to zygotic embryo development in *Fucus* (Kropf et al. 1999). The importance of asymmetric cell division can be seen

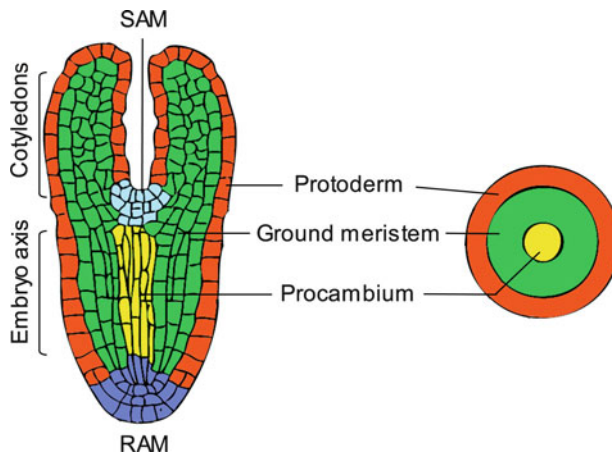


Fig. 5 Schematic of (a) longitudinal and (b) cross section of *Arabidopsis thaliana* mature embryo featuring the establishment of apical-basal and radial pattern, respectively. Adapted from Altamura et al. (2007)

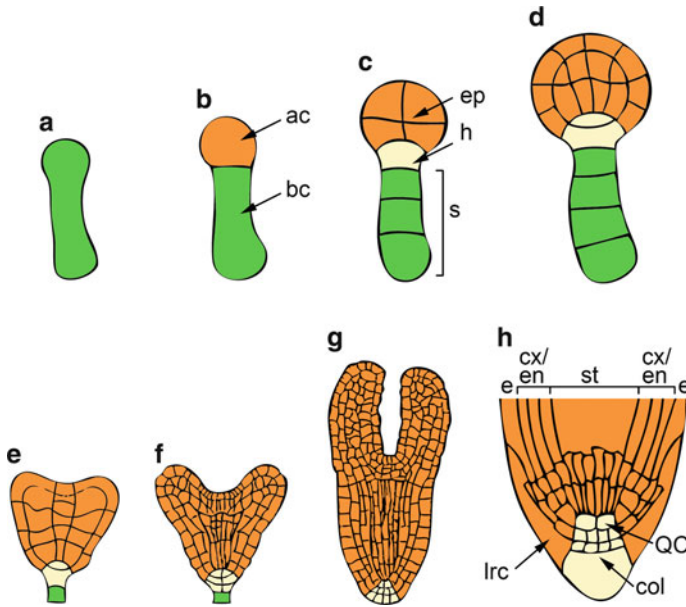


Fig. 6 Cartoon illustrating the main stages of *Arabidopsis thaliana* embryo development and the composite origin of the root pole. (a) zygotic cell, (b) bicellular stage, (c) octant stage, (d) globular stage, (e) triangular stage, (f) late-heart stage, (g) torpedo stage, (h) adult root. *ac* apical cell; *bc* basal cell; *col* columella; *e* epidermis; *en/cx* endodermis/cortex; *ep* embryo proper; *h* hypophysis; *lrc* lateral root cap; *QC* quiescent centre; *s* suspensor; *st* stele. Adapted from Altamura et al. (2007)

through phenotypic analysis of mutants that have lost this feature of cell partitioning. For example, the *gnome* (*gn*) mutant that does not exhibit asymmetric zygotic division is unable to organize an embryo with the normal polarity featuring spatially separated RAM and SAM (Mayer et al. 1993). However, it must be underlined that asymmetric division does not inherently block cell fate as demonstrated by *twin* mutants, which are able to produce viable additional embryos from the basal cell of the bicellular stage, suggesting that cell-to-cell communication is a relevant player in developmental processes (Vernon and Meinke 1994). Hence, as we will discuss later, asymmetric cell division, although a notable feature of cell fate determination, is by no means the sole determinant of tissue specification.

4.2 RAM Pattern

The cellular organization of the RAM in *Arabidopsis* seedlings was well defined by Dolan et al. (1993). The *Arabidopsis* RAM exhibits a dome typically organized as a three-tiered closed meristem, where distinct cell layers of initials give rise to the stele (S), endodermis/cortex (EC), and root-cap/epidermis (RCE) tissues (Fig. 7).

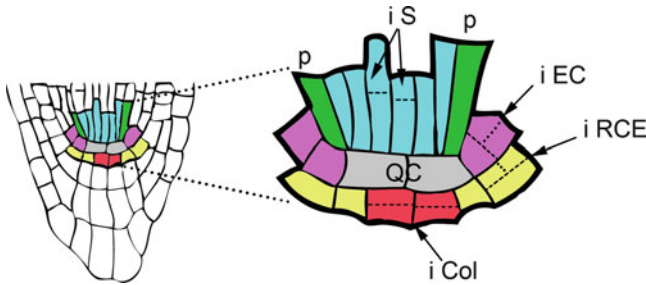


Fig. 7 Cartoon illustrating the organization of root apical meristem of *Arabidopsis thaliana* and the division planes of initial cells. *iCol* columella cell initial; *iEC* endodermis/cortex cell initial; *iRCE* lateral root cap/epidermis cell initial; *iS* stele cell initial

The QC is constituted by four founder cells surrounded by initials. Based on asymmetric division, the RCE initial firstly divides periclinally to give rise to one lateral root cap cell (the outer cell) and one inner that retains the role of an initial that divides anticlinally producing one epidermal cell (the upper cell) and one initial cell (the lower cell). In turn, the cortical and endodermal cells derive from two consecutive asymmetric cell divisions: the EC initial first undergoes an anticlinal division and the daughter cell in contact with the QC maintains the stem-cell fate, while the other derivate cell divides periclinally producing an inner and outer cell, which will differentiate into the endodermis, the innermost layer of the cortex and the next outer cortical layer, respectively. Finally, the S initial divides periclinally giving origin to xylem along only one central axis (i.e., 4/5 cell tiers in contact with QC) and phloem tissues. The most external S initials divide transversely to form the pericycle. Distally, columella initials below the QC divide periclinally producing central cells of the root cap (Fig. 7).

Cell ablation experiments have largely demonstrated that positional information more than clonal lineage is relevant in defining cell fate. Indeed, if an initial cell is ablated, an adjacent cell in contact with this cell divides, producing a new initial. Moreover, when a differentiated cell is ablated along a differentiating cell line, only an adjacent derivate or TA cell, which maintains the contact with a differentiated overlapping cell, does undergo asymmetric division to replace the ablated cell. Thus, a positional effect and a short-range cell-to-cell signal have a role in inducing asymmetric cell division (Van den Berg et al. 1995; Scheres 2001).

4.3 Positional Signaling and Genetic Network Operating in Root Patterning

Positional information established through both long-distance signals and cell-to-cell interactions as well as specific transcription factor activity are known to be

important throughout plant development (Meyerowitz 2002; Vernoux and Benfey 2005; Qu and Zhu 2006; Veit 2006; Sablowski 2007; Scheres 2007; Benjamins and Scheres 2008; Kiefer 2009; Robert and Friml 2009; Stahl and Simon 2010).

Cell-to-cell interactions involve plasmodesmata and the cell wall. It is known that plasmodesmata dynamically guarantee or interrupt symplastic connection between cells, thus controlling the trafficking of molecules, which play key roles in developmental events. In particular, specific transcription factors at the protein or mRNA level but also micro (mi)RNAs can be translocated through plasmodesmata (Lucas and Lee 2004; Jackson 2005; Lee and Cui 2009). For example, the transcription factor, *SHORTROOT* (*SHR*), is specifically translocated into the QC and endodermis (Nakajima et al. 2001). More of this later (see Sect. 4.3.2).

The cell wall is also a relevant component of both long- and short-distance intercellular signaling. Apoplastic continuity along xylem vascular elements represents a route for long-distance signaling played by several types of molecules such as different hormones and sugars (Davies 1995; Francis and Halford 2006). Moreover, cell wall components can act as polarization markers. For example, *LIPID TRANSFER PROTEIN* (*AtLTP1*) is required at the protein level for cuticle formation. It is expressed in the globular stage of embryogenesis, at the level of the protoderm, but thereafter, its expression is more polar being confined to the developing hypocotyl and cotyledons but is excluded from the pole of the root where cuticle is neither formed nor required (Fig. 8) (Vroemen et al. 1996). Importantly, *ROOT-SHOOT-HYPOCOTYL-DEFECTIVE* (*RSH*), which encodes a cell wall hydroxyproline-rich glycoprotein, and *KNOLLE*, which encodes syntaxin, a specific cytokinesis protein, are necessary for the correct positioning of the cell plate during cytokinesis (Lauber et al. 1997; Hall and Cannon 2002). This is essential for defining the first asymmetric division of the zygote and subsequent normal embryo development. Indeed, *knolle* mutant embryos show highly irregular shapes comprising binucleate cells along with unusually shaped cells (Waizenegger et al. 2000).

An important role in both early embryogenesis and postembryonal root development that involves positional signaling is played by hormones. Polar auxin transport through specific alignment of plasma membrane-based influx and efflux carriers plays a major role in providing positional cues for developmental processes; localized auxin biosynthesis also contributes to create hormone gradients

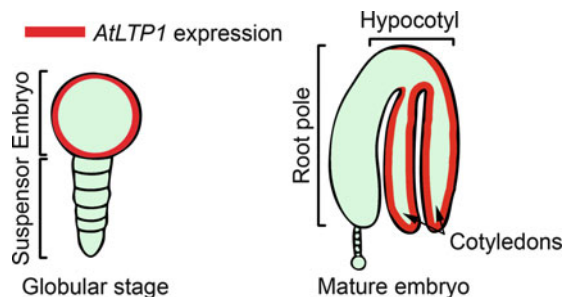


Fig. 8 Schematic of polarized accumulation of *AtLTP1* transcripts in the *Arabidopsis thaliana* embryo at globular and bent-cotyledon stages. Adapted from Altamura et al. (2007)

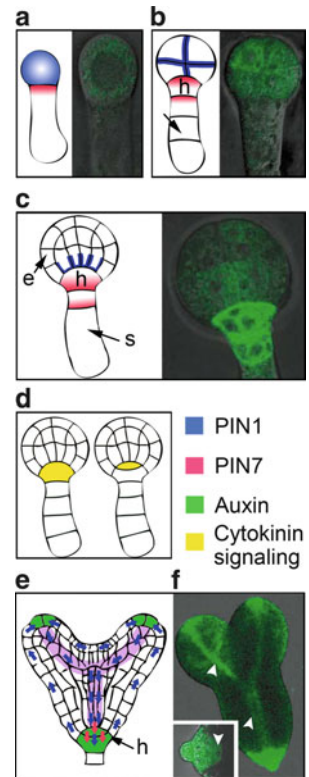
(Friml 2003; Ljung et al. 2005; Grieneisen et al. 2007; Benjamins and Scheres 2008; Stepanova et al. 2008; Ikeda et al. 2009; Petràšek and Friml 2009). Moreover, recent work has highlighted an antagonistic and, at times, transient interaction between auxin and cytokinins in determining positional information for specifying cell fate and organ pattern (Dello Ioio et al. 2007, 2008; Muller and Sheen 2008; Perilli et al. 2009).

4.3.1 RAM Establishment

Early during embryogenesis, the polarization of different auxin carriers determines the formation of auxin gradients and their directional flow (Benková et al. 2003; Friml et al. 2003; Petràšek and Friml 2009; Zazimalová et al. 2010). In particular, auxin transport is under the control of influx AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) symporters, efflux PINFORMED (PIN) transporters, and ABCB/P-GLYPROTEIN (PGP) efflux/conditional transporters (Vieten et al. 2007; Petràšek and Friml 2009). Notably, the transcription of all these carriers is influenced by auxin itself, an action that is only a component of the fine-tuning mechanism by which auxin acts as one of the key regulators of its own transport (Vieten et al. 2007; Petràšek and Friml 2009). A coordinate action of different efflux carriers is essential in embryo patterning with a major role for PIN-dependent auxin transport. At the proembryo bicellular stage, *PIN* genes are already expressed and a non-polarized distribution of PIN1 protein is detected in the apical cell, while PIN7 localizes along the upper plasmalemma of the basal cell upper side, thus driving auxin flux toward the proembryo (Fig. 9a). At the eight cell stage, PIN7 is localized along the plasmalemma of the upper side of the hypophysis, the founder cell of the root stem-cell niche, and suspensor contacting cell (Fig. 9b). Thus, the auxin flux continues to be driven toward the developing embryo. At the globular stage, PIN protein localization changes dramatically. PIN1 relocates to the membrane of the lower side of the basal cells, while PIN7 shifts to the membrane of the lower side of the hypophysis and suspensor contacting cell (Fig. 9c). In such a way, auxin accumulates strongly at the base of the embryo (hypophysis) (Friml et al. 2003). As mentioned above, positional information is also related to a polarized repression of cytokinin signaling. In particular, early cytokinin activity is detectable in the hypophysis and maintained in its apical, lens-shaped derivative cell, which will form the QC. Conversely, cytokinin output signal is suppressed in the basal cell lineage through an auxin-mediated transcriptional activation of A-type *ARABIDOPSIS RESPONSE REGULATOR 7* and *15* (type-A *ARR7* and *ARR15*), which encode two negative regulators of cytokinin signaling (Fig. 9d) (Muller and Scheen 2008; To and Kieber 2007). Consistent with this, *arr7* and *arr5* mutants are unable to specify a stem-cell niche required for the establishment of a normal embryogenic root (Muller and Sheen 2008).

As embryogenesis proceeds, auxin preferentially accumulates in the developing root and at the torpedo stage, when the QC is already established; an auxin maximum is achieved in columella initial cells below it (Fig. 9e, f). *pin1* and

Fig. 9 (a, b, c, e) Schematic of the localization of PIN1 and PIN7 auxin carriers and whole-mount visualization of auxin accumulation through DR5rev::GFP expression during embryo development in *Arabidopsis thaliana*. (d) Schematic of cytokinin signaling in *Arabidopsis* globular embryo. (a) bicellular stage; (b) octant stage; (c, d) globular stage; (e) heart stage; (f) torpedo stage; *h* hypophysis; *e* embryo; *s* suspensor. Adapted from Altamura et al. (2007), Friml et al. (2003), and Petrášek and Friml (2009)



pin7 mutants fail to develop a polar longitudinal axis, thus supporting the key role for PIN-dependent auxin transport in the morphogenetic process (Friml et al. 2003). In this context, we may recall that subcellular trafficking and targeting of auxin transporters to direct auxin flow, as well as transport activity, relies on several cellular and molecular mechanisms, which involve posttranslational modifications of transporters themselves, membrane composition, endocytosis, and endosomal sorting/recycling (reviewed by Petrášek and Friml 2009, Zazimalova et al. 2010). Consistent with this is the similarity between *pin* and *gn* mutants, which are unable to organize a longitudinal axis, a deleterious phenotype that begins with the first division of the zygote (Mayer et al. 1993). *GNOME* (*GN*) encodes an ARF-GEF (ADP Ribosylation Factor-Guanilic Exchange Factor) protein involved in vesicular trafficking from the trans-Golgi (TGN Trans Golgi Network) to the plasmalemma. Moreover, the *gn* phenotype is linked to nonpolar accumulation of high auxin concentrations due to a wrong localization of PIN1 protein in embryo cells during development (Steinmann et al. 1999; Richter et al. 2010). Confirming that vesicular trafficking is integral to the polar distribution of auxin carriers, these observations indicate how *PIN* and *GN* genes operate on the same genetic pathway.

Notably, three relevant genes for embryo pattern such as *MONOPTEROS* (*MP*), *BODENLOS* (*BDL*), and *AUXINRESISTANCE6* (*AXR6*) are either under the control of, or are responsive to, high auxin levels (Hardtke and Berleth 1998; Hamann et al. 2002; Hellmann et al. 2003; Weijers et al. 2005). Indeed, *MP* encodes a transcription factor belonging to the AUXIN RESPONSIVE FACTORS (ARF) family, which interacts with specific DNA sequences, named Auxin Responsive Elements (AuxRE) (Fig. 10). *BDL* encodes an AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) protein, which is able to bind ARF transcription factors, such as MP, forming an inactive complex. *AXR6* protein is a component of a ubiquitin-ligase complex involved in the degradation of BDL protein. Moreover, high auxin levels on the one hand promote the interaction of MP-AuxRE but on the other inhibits BDL-MP binding (Fig. 10) According to this network, in both loss-of-function *mp* mutants, gain-of-function *bdl* mutants and loss-of-function *axr6* mutants, target AuxRE sequences are not transcribed despite the presence of auxin. This is consistent with the similar phenotypes of single mutants defective in the above genes; they all fail to exhibit an embryonic root and all have a reduced vascular system (Hardtke and Berleth 1998; Hamman et al. 1999; Hobbie et al. 2000).

Possible gene targets of MP and BDL activity belong to the *WUSCHEL-RELATED HOMEODOMAIN* (*WOX*) gene family, which includes 14 members. Among these, four are differentially expressed during embryogenesis both spatially and temporally (Fig. 11) (Haecker et al. 2004). On the basis of their expression pattern, and as well as by the *wox* phenotypes, specific roles have been postulated for each member. In particular, at the early globular stage, a role in the specification of stem-cells, which will form the QC and embryo root, has been defined for *WOX*

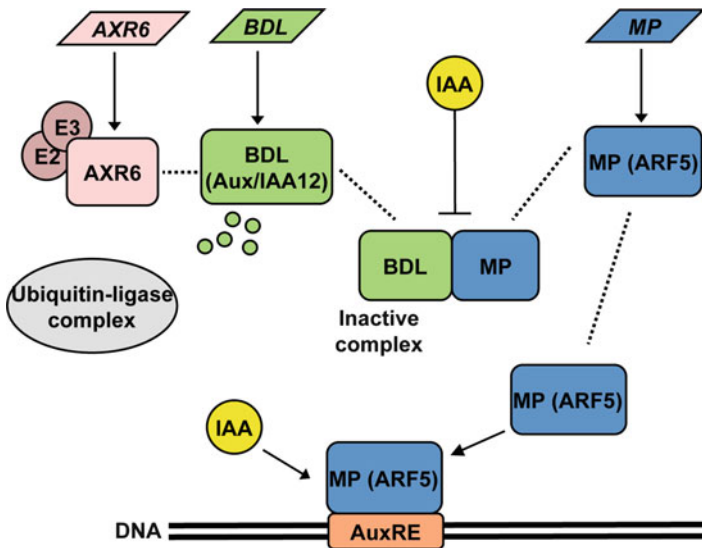


Fig. 10 Scheme depicting the interaction between *MP*, *BDL*, and *AXR6* gene activities in the context of auxin signaling (see text for further details). Adapted from Altamura et al. (2007)

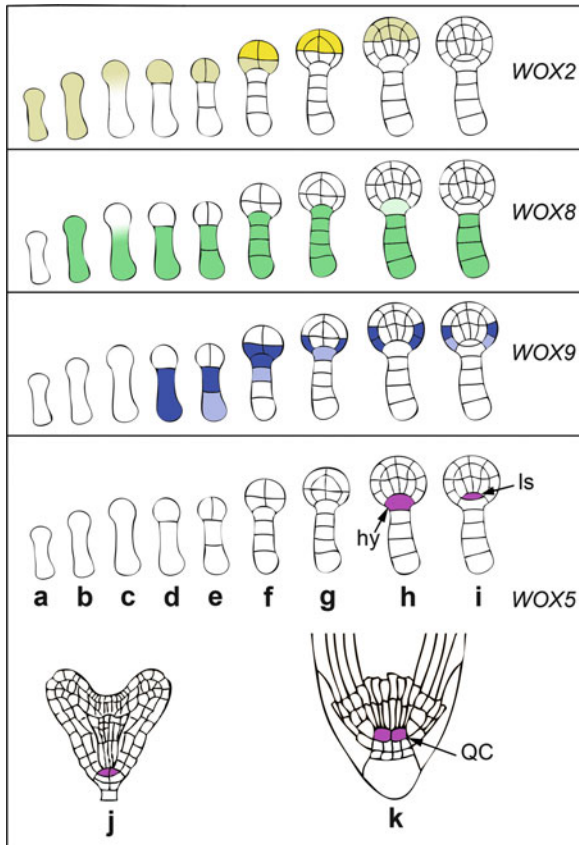


Fig. 11 Schematic of spatial and temporal expression pattern of (a–j) *WOX* genes during early embryogenesis and (j–k) of *WOX5* gene at embryo heart stage and adult root of *Arabidopsis thaliana*. (a–c) zygotic cell; (d–i) from bicellular to advanced globular stage; (j) heart stage; (k) adult roots; *hy* hypophysis; *ls* lens-shaped cell; *QC* quiescent centre. Adapted from Altamura et al. (2007)

5 and *WOX 9*, respectively. In particular, at the early globular stage, *WOX 5* expression first occurs in the embryonic cell lineage that will give rise to the QC. After the division of the hypophysis, *WOX5* transcripts are present in the upper lens-shaped cell that gives rise to the QC, while they are absent in the lower cell that gives rise to the central root cap (Fig. 11h, i). Subsequently, at the heart and bent cotyledon stages, *WOX5* expression is detectable in the direct descendants of the lens-shaped cell represented by the four cells of the QC (Fig. 11j, k). As discussed in further detail later, in the adult root, *WOX5* is involved in maintaining cell-stem state in a noncell-autonomous manner (Haecker et al. 2004; Sarkar et al. 2007; Breuninger et al. 2008). Evidence is available that the BDL/MP-mediated auxin signaling is required for *WOX5* and *WOX9* expression. For example, *WOX5*

expression was rarely detected in either *mp* or *bdl* mutants and *WOX9* expression was affected in *mp bdl* double mutants (Haecker et al. 2004; Sarkar et al. 2007).

4.3.2 RAM Maintenance

During postembryonic development, root distal pattern is related to auxin distribution and its antagonistic interplay with cytokinins. In growing roots, auxin polar transport and local biosynthesis act together to produce auxin gradients and maxima at the root tip, with its highest concentration in the QC and likely columella initials (Benkova et al. 2003; Blilou et al. 2005; Grieneisen 2007; Ikeda et al. 2009; Petersson et al. 2009). Several members of PIN gene family are differentially expressed in the root, and each transporter exhibits both a tissue-specific and cell-polarized localization, which, in effect, configures the root pole as a sink for auxin (Benková et al. 2003; Friml et al. 2003; Blilou et al. 2005; Petrášek and Friml 2009).

Just like during embryogenesis, the auxin gradient modulates the activity of the auxin-inducible *PLETHORA 1* and *2* (*PLT1* and *2*) genes (Fig. 12a, b), which encode AP2 class transcription factors that have an essential role in the specification of stem-cells. In particular, stem-cell fate is promoted by high *PLT* expression,

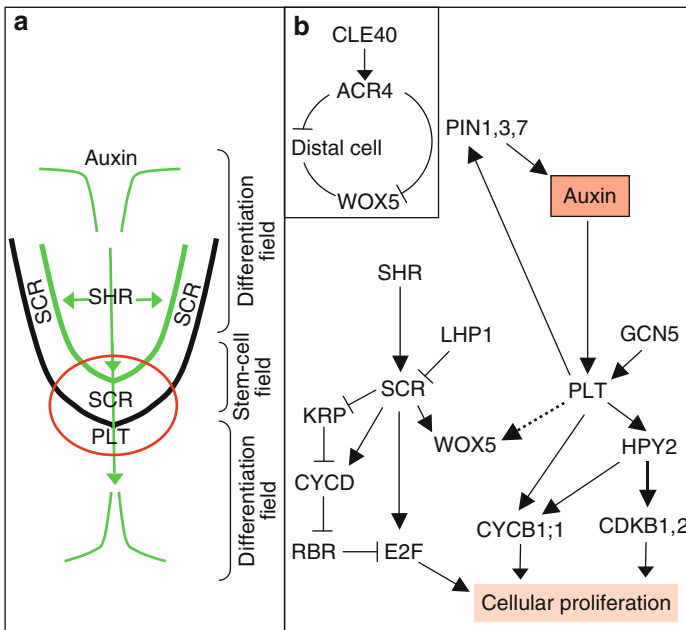


Fig. 12 Scheme depicting (a) auxin polar flux (adapted from Vernoux and Benfey 2005) and (b) its interaction with a transcriptional network in root patterning (see text for further details). Arrows indicate positive regulation; barred lines indicate negative regulation

whereas lower and lowest *PLT* expression promotes daughter cell proliferation and differentiation, respectively (Aida et al. 2004; Blilou et al. 2005; Galinha et al. 2007). Through a feedback mechanism, *PLT* proteins promote *PIN* expression, assuring a steady-state high level of auxin and therefore maintaining the stem-cell niche. A histone acetyltransferase activity, General Control Nonrepressed protein5 (*GCN5*), is also necessary to promote *PLT2* expression (Fig. 12b) (Kornet and Scheres 2009). Accordingly, the *gcn5* mutants exhibit a short root meristem and fail to maintain a QC, but their phenotype can be reversed by *PLT2* overexpression. Plant specific Cyclin-dependent kinases *CDKB1* and 2 and *CYCLIN B1;1* (*CYCB1*), which act at the G2-to-M phase transition of cell cycle, have been suggested as putative target genes of *PLT2* (Fig. 12b) (Aida et al. 2004; Ishida et al. 2009; Stahl and Simon 2010). It is known that CYCs and CDKs are the major drivers of the mitotic cell cycle, and a reduction in CDK activity is a primary feature of cells that enter the endocycle, in which cells replicate their DNA without undergoing cell division (Larkins et al. 2001). The transition from the mitotic cell cycle into the endocycle is often coupled with the switch from cell proliferation to cell differentiation, and therefore, it is tightly integrated into plant developmental programs (Francis 2007). Interestingly, *PLT1* and 2 induce the expression and/or accumulation of HIGH PLOIDY2 (*HPY2*), a nuclear-localized SMALL UBIQUITIN-RELATED MODIFIER E3-ligase (SUMO E3-ligase), which is expressed in proliferating cells of the RAM and is a negative regulator of endoreduplication (Ishida et al. 2009). *CDKB1* and 2 and *CYCB1* levels were reduced in *hpy2-1* mutants suggesting that they are among the targets of *HPY2* for sumoylation in order to modulate their activities (Ishida et al. 2009). Consequently, a picture emerges in which auxin maintains root meristem homeostasis through *PLT1/2* expression and high *HPY2* expression, which prevents endoreduplication and promotes cell proliferation (Ishida et al. 2009).

The activity of plant-specific GRAS family of transcription factors such as SCARECROW (*SCR*) and SHORTROOT (*SHR*) is also essential for root meristem patterning (Pysh et al. 1999; Nakajima et al. 2001; Sabatini et al. 2003). GRAS factors are required for correct function of the stem niche: *SHR* is expressed in the provascular cells of the stele and its encoded protein moves to adjacent QC cells and the endodermal initial cell layer (Fig. 12a) where it interacts with *SCR* through its central binding domain and is sequestered in the nucleus thereby preventing further *SHR* movement (Nakajima et al. 2001; Cui et al. 2007). The interaction results in the activation of target genes, including *SCR* itself (Fig. 12b), which is required for the specification of QC identity and stem-cell homeostasis as well as for the promotion of asymmetric cell division, which underlies tissue specification in the E/C initials surrounding the QC (Di Laurenzio et al. 1996; Sabatini et al. 2003).

In this context, we must underline a trait that is distinctive of the *Arabidopsis* root compared to other plant species, that is, the absence of further asymmetric cell division after endodermis specification and the consequent maintenance of a two-layer ground tissue (i.e., endodermis and cortex) for a long period after germination (2 weeks) in spite of the presence of the *SHR* and *SCR* transcription factors

(Helariutta et al. 2000; Cui et al. 2007). However, more than 2 weeks after germination, additional divisions occur and a second or third layer of cortical cells form the middle cortex (Paquette and Benfey, 2005). Note that *scr* mutants are characterized by a premature middle cortex elevating the role of SCR in promoting the first but repressing additional asymmetric division in the endodermal cell layer. Posttranslational modification as well as interactions with other proteins could account for SCR's dual activities. Recently, a number of putative SCR-interacting proteins have been identified, which specifically interact with its N-terminal domain. Unlike the C-terminal domain, which interacts only with SHR thus activating asymmetric cell division, the N-terminal region was found to be a versatile interaction domain necessary to repress further cell divisions. The transcriptional repressor LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), highly expressed in the root elongation zone, was included among the SCR interacting proteins (Cui and Benfey 2009). In such a way, SCR could either activate or inhibit gene expression depending on the interacting partner (Fig. 13). Notably, both LHP1 and SCR bind to the promoter of MAGPIE (MGP), a zinc finger protein previously confirmed as an SCR target, which has a role in ground tissue patterning by restricting SHR action (Welch et al. 2007). Moreover, *MGP* and *SCR* expression are enhanced in the *lhp1* mutant, which exhibits a premature middle cortex. This led to the conclusion that LHP1 plays a role in cortex formation by acting together with SCR in preventing further asymmetric cell divisions (Fig. 12b). (Cui and Benfey 2009; Welch et al. 2007). On the basis of the above-described genetic network, the stem-cell niche can be identified as the domain where the highest expression levels of *PLT1*, *SHR*, and *SCR* overlap (Fig. 12a) (Sabatini et al. 2003; Aida et al. 2004; Nawy et al. 2005; Galinha et al. 2007). Moreover, *SCR* acts cell autonomously to

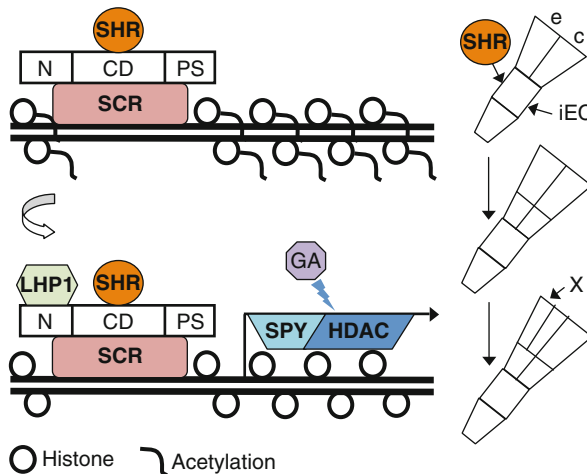


Fig. 13 Scheme depicting the interaction of LHP1, SPY, and GA signaling in a common epigenetic regulation of SCR activity in cortex formation (see text for further details). *c* cortex; *iEC* endodermis/cortex initial; *e* endodermis. Adapted from Cui and Benfey (2009)

maintain QC cell identity, whereas a nonautonomous signal from the QC maintains adjacent stem-cell division as demonstrated through cell ablation experiments. Indeed, the ablation of one QC cell induces a precocious differentiation of columella initials in surface contact with the ablated cell (Van den Berg et al. 1997). Besides featuring the QC as the root OC, this genetic network is consistent with the reestablishment of a new functional QC from proximal initials after cell ablation: initials undergo division and auxin accumulates in these new cells. This in turn induces the expression of *PLT* genes, which activate *SCR*, thus respecifying QC identity.

Although *PLT* and *SHR-SCR* pathways are essential for the maintenance of the stem-cell population, loss-of-function of each these genes differentially affects it. Presumably, all these transcription factors do not control the same target genes with the possible exception of *WOX5*. Expression of *WOX* is confined to the QC (Fig. 11k) and plays a role in maintaining the stem-cell state and, most likely, controls QC-specific gene expression (Haecker et al. 2004; Sarkar et al. 2007.) Consistent with such a key role, *wox5-1* mutants exhibit precocious differentiation of columella cells, whereas the proximal meristem remains unaffected; ubiquitous *WOX5* activation maintains an undifferentiated state (Sarkar et al. 2007). However, *WOX5* expression is differentially modulated by the above-mentioned pathways. In particular, *WOX5* transcripts are undetectable or reduced in *shr* and *scr* mutants. Conversely, *PLT1* and *PLT2* play only a minor role since in *plt1*, *plt2* double mutant, while *WOX5* expression can be occasionally expanded, but *PLT1* expression is normal in *wox5-1*.

Note the unidirectionality of *WOX5* signaling, which is only required for columella stem-cell maintenance that resembles the unidirectional control played by *WUS* on the stem-cell niche in the SAM (Sarkar et al. 2007). Moreover, *WUS* and *WOX* genes exhibit comparable patterns of spatial expression being confined to the organizing centers of the SAM and RAM, respectively (Mayer et al. 1998; Sarkar et al. 2007). All these features, together with the high degree of sequence similarity between the two genes, have been interpreted as a homology in the homeostasis mechanisms operating in the SAM and RAM (Sablowski 2007). That both genes can functionally replace each other offers further support of such an interpretation (Gallois et al. 2004; Sarkar et al. 2007). This is consistent with the hypothesis that the RAM has evolved from the SAM as an adaptive strategy for water and nutrient uptake and soil anchorage, following water emersion (Jiang and Feldman 2005). Based on recent evidence showing that gymnosperms exhibit only one *WUS/WOX5* proortholog, separation of *WUS* and *WOX* genes has occurred relatively recently during the evolution of angiosperms (Nardmann et al. 2009).

As in the SAM, RAM totipotent cells progressively undergo a determinate fate. As a consequence, SAM/RAM homeostasis relies on a balance between the number of cells that are maintained in the stem-cell state and the number of TA cells, progressively displaced toward the differentiation zone. In the SAM, a feedback loop is established between *WUS* and members of the *CLAVATA* gene family that dynamically confines the organizing center into a defined number of cells (Brand et al. 2000). In particular, *WUS* is expressed in the organizing center keeping

stem-cells undifferentiated and inducing the expression of *CLV3* that encodes a small ligand polypeptide for the *CLV1* encoded receptor-like kinase that, in turn, assists in maintaining the size of the *WUS*-expressing region (Mayer et al. 1998; Brand et al. 2000; Schoof et al. 2000). The closest homologs of *CLV3* that are expressed in root systems are members of *CLV3/ENDOSPERM SURROUNDING REGION (CLE)* family. Some of the *CLE* genes act to reduce root meristem size (e.g., *CLV3*, *CLE19*, *CLE40*) whilst others promote cell proliferation in the stele (*CLE41*) (Hobe et al. 2003; Fiers et al. 2006). More recently, a clearer role has been assigned to *CLE40* in controlling cell proliferation in the distal root meristem. It was proposed that *CLE40* protein is secreted from columella cells into the QC and represses *WOX5* expression therein (Fig. 12b). Seemingly, this effect is brought about through *CLE40*'s putative receptor, *ARABIDOPSIS CRINKLY4 (ACR4)*, which is mainly expressed in the distal meristem and locally restricts cell division activity interfering with columella stem-cell maintenance (De Smet et al. 2008). Accordingly, *cle40* and *acr4* mutants exhibit opposite phenotypes (De Smet et al. 2008; Stahl and Simon 2009; Stahl et al. 2009). Hence, in the RAM, a *CLE40/WOX5* pathway parallels the *CLV3/WUS* pathway in the SAM. However, a clear difference exists in that the feedback signal from differentiated descendant cells control stem-cell proliferation in the root, while in the shoot, signaling is derived within the stem-cell area (Brand et al. 2000; Schoof et al. 2000). Moreover, in the RAM but not in the SAM, this role is exerted through a cell-to-cell signal analogous, perhaps to cell to cell signaling in stem cell niches in animals (e.g., Spradling et al. 2001).

5 Hormonal Circuitry in Determining RAM Size and Pattern

A distinctive feature of plant hormones is to act synergically or antagonistically in several morphogenetic events during plant development. Recent advances in resolving the cross-talk between different hormone classes in controlling root patterning are assessed here.

5.1 Auxin/Cytokinin Interplay

Root meristem size is determined by the rate of cell division of stem and TA cells in the division zone and by the rate of cell elongation prior to differentiation of cells in the differentiation zone. Clearly, there is widespread evidence for auxin in promoting and controlling cell division in the RAM. Auxin action is mediated by auxin receptors, such as *TRANSPORT INHIBITOR RESPONSE1 (TIR1)*. This protein is a component of the E3 ubiquitin ligase complex, which targets the transcriptional repressors of the *Aux/IAA (AUXIN/INDOLE-3-ACETIC ACID)* family for proteasome-mediated degradation (Fig. 14) (Kepinski and Leyser 2005). In such

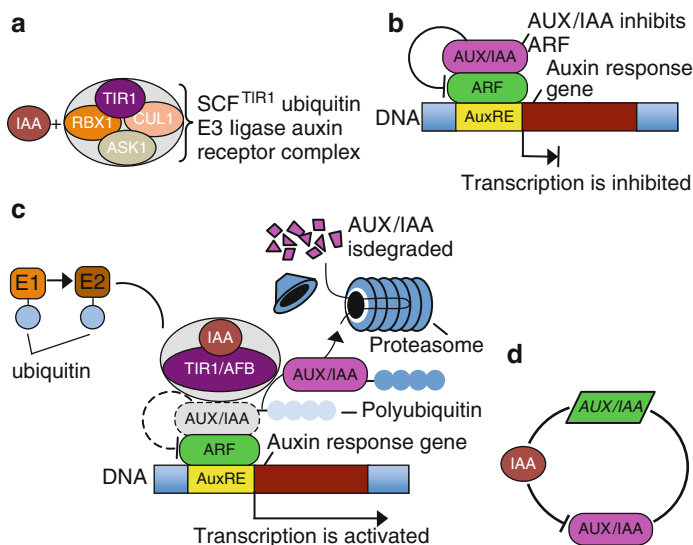


Fig. 14 Scheme depicting mechanisms of auxin on the coregulator-transcription factor duo AUX/IAA-ARF. Adapted from Taiz and Zeiger (2002)

a way, auxin response factors (ARFs), which are inhibited by Aux/IAA, are released to activate the transcription of auxin-responsive genes. A negative-feedback loop is active since auxin negatively regulates Aux/IAA abundance while their respective genes are themselves auxin-induced (Fig. 14) (Benjamins and Scheres 2008). Auxin action has also been linked to changes in cellular redox status (De Tullio et al. 2009; Eckardt 2010; Jiang et al. 2010), as well as alternative auxin-responsive pathways, and an even more complex signaling has been described (Benjamins and Scheres 2008; Lau et al. 2008; Strader et al. 2008). However, detailed reviewing of this mechanism is beyond the scope of the current chapter.

As recently demonstrated, from the earliest phases of embryogenesis, cytokinins act antagonistically to auxin in controlling root pattern through to postembryonic development (Muller and Sheen 2008; Moubayidin et al. 2009; Perilli et al. 2009; Werner and Schmülling 2009). In particular, during embryogenesis, cytokinin/auxin interplay has an essential role for the specification of the first root stem-cell niche, which is marked by an auxin concentration maximum occurring in a single cell (i.e., hypophysis) (Fig. 9). As mentioned above (see Sect. 4.3.1), a high level of auxin at the level of the hypophysis activates the expression of two negative regulators of cytokinin signaling, thus suppressing cytokinin output (Muller and Sheen 2008).

As far as postembryonic root development is concerned, cytokinins promote cell differentiation at the boundaries between the division and elongation zones (i.e., the transition zone) by suppressing auxin signaling and transport, while auxin promotes cell division by suppressing cytokinin signaling (Blilou et al. 2005; Dello Ioio et al. 2007, 2008; Perilli et al. 2009; Ruzicka et al. 2009). In particular, increased

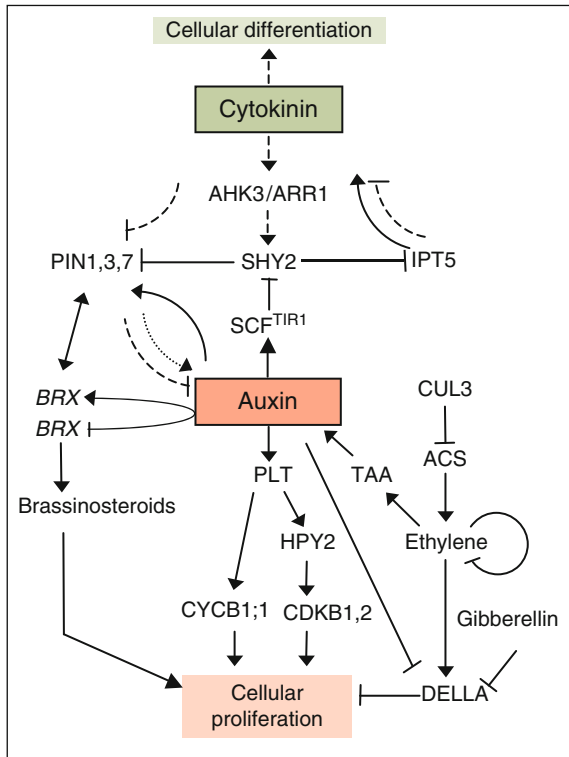


Fig. 15 Scheme depicting hormone interaction in transcriptional network underlying root patterning (see text for further details). Arrows indicate positive regulation; barred lines indicate negative regulation. Adapted from Stahl and Simon (2009)

cytokinin levels reduce root meristem size and inhibit root growth, by modulating PIN expression and therefore auxin distribution. This interplay relies on the convergence of both hormone classes on the same target gene, *SHORTHYPOCOTYL* (*SHY2*), which encodes an IAA-class repressor protein of the auxin signaling pathway (Fig. 15) (Benjamins and Scheres 2008). In particular, SHY2 prevents the activation of auxin-responsive genes by negatively regulating *PIN 1, 3, and 7* and therefore repressing auxin export at the vascular transition zone (Dello Ioio et al. 2008). Note that auxin and cytokinins control SHY2 in opposite ways; auxin drives SHY2 protein degradation, through a ubiquitin-ligase complex (SCF^{TIR1}), thus stabilizing PIN expression level and auxin distribution. Conversely, cytokinins promote SHY2 expression, via a two-component signaling pathway (AHK3/AA1), which activates ARR1, a primary type-B cytokinin response factor that binds to the SHY2 promoter specifically at the vascular tissue transition zone (Fig. 15) (Benjamin and Scheres 2008; Dello Ioio et al. 2008; Moubayidin et al. 2009). Thus, in the presence of cytokinins, auxin transport is limited, auxin-dependent cell division is antagonized, and cell elongation occurs. Recently, it has been shown in

maize that an ARR-mediated cytokinin signal is inactive in the QC and such repression could be related to the maintenance of the QC (Jiang et al. 2010). Finally, it must be underlined that a negative feedback control is active, since SHY2 protein downregulates *ISOPENTENYLTRANSFERASE* (*AtIPT*), which encodes an enzyme involved in a rate-limiting step of cytokinin biosynthesis (Fig. 15) (Sakakibara 2006; Dello Ioio et al. 2008). Note also that cytokinins antagonize auxin signaling and distribution during lateral root formation (Fukaki and Tasaka 2009). However, cytokinin-induced inhibition of root primordia development remains unaltered in auxin mutants, and auxin cannot reverse the cytokinin-induced inhibitory effect, suggesting that auxin and cytokinins likely control lateral root initiation through discrete pathways (Werner and Schmülling 2009)

5.2 Ethylene, Gibberellin, Absciscic Acid, and Brassinosteroids

In addition to auxin/cytokinins, an even more complex cross-talk between other hormone classes and transcription factors is active in the control of root pattern.

Ethylene and auxin interplay in maintaining RAM size. In particular, ethylene cooperates to inhibit cell elongation by stimulating localized auxin synthesis through TRYPTOPHAN AMINONOTRANFERASE (*TAA1*) activity; *TAA1* is expressed in the QC and is involved in the indol-3-pyruvic acid (IPA) branch of the auxin biosynthetic pathway (Stepanova et al. 2008). This relationship between local auxin synthesis and ethylene response is supported by phenotypic analyses of *taal* and *tar2* mutants, which exhibit root-specific ethylene insensitivity and a reduction in auxin concentration; *TRYPTOPHAN AMINONOTRANFERASE RELATED2* (*TAR2*) shows very close sequence homology to *TAA1*. Notably, both mutants exhibit an arrest of root growth due to precocious differentiation of meristematic cells leading to the complete loss of the stem-cell niche (Stepanova et al. 2008; Ortega-Martinez et al. 2007). On the other hand, ethylene production is modulated by the activity of CULLIN 3 (*CUL3*) proteins, members of a ubiquitin-ligase degrading complex, which targets 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (*ACS*) for degradation. Normally, synthase (*ACS*) proteins are rate-limiting for ethylene biosynthesis. A negative feedback mechanism between ethylene signaling and response is also active (Fig. 15) (Chae and Kieber 2005; Thomann et al. 2009). In line with the role of *CUL3* protein, the *cul3* mutant shows a reduced root meristem size and cell number, an effect due to a premature transition of cells to the elongation zone rather than to any alterations in cell division activity (Thomann et al. 2009). Notably, *CUL3* proteins also control distal root patterning by modulating the division of the stem-cell niche and columella cap cells. Although this role should be consistent with the involvement of *CUL3* proteins in ethylene synthesis and consequent increase of auxin level, an ethylene-independent role and a broader connection with auxin action has also been suggested for *CUL3* proteins (Ortega-Martinez et al. 2007; Thomman et al. 2009).

The interplay between auxin and ethylene in root patterning is strengthened by their convergence on the activity of DELLA proteins, a subgroup of the GRAS protein family, which stimulate the production of cell cycle inhibitors thus limiting cell proliferation rate, although without affecting the stem-cell niche (Achard et al. 2003). In particular, ethylene delays the degradation of DELLA proteins, whereas auxin negatively modulates their stability (Achard et al. 2003; Fu and Harberd 2003). In the context of hormone circuitry, it is also worth noting that the degradation of DELLA proteins is controlled by another important hormone class, the Gibberellins (GAs) (Achard et al. 2009). This is consistent with a promotive effect of auxin and a repressive effect of ethylene on GA regulation of root elongation (Fig. 15).

The role of GA signaling in *Arabidopsis* root development has been largely documented in relation to cortex cell proliferation and therefore radial pattern. We described above (see Sect. 4.3.2) the role of SCR in promoting the first but repressing additional asymmetric division in the endodermal cell layer, thus assuring the maintenance of a two-layer ground tissue (Cui et al. 2007). This occurs through the activity of LHP1, a transcriptional repressor of *SCR* expression (see Sect. 4.3.2), which physically interacts with SCR itself (Fig. 13) (Welch et al. 2007; Cui and Benfey 2009). Note that the *scr* phenotype, which exhibits a premature middle cortex, is suppressed by GA treatment but enhanced through the inhibition of GA biosynthesis (Paquette and Benfey 2005). Notably, the *lhp1* phenotype is also rescued by GA treatment, suggesting that LHP1 and SCR share a common mechanism based on a major role of GA in repressing premature cortex proliferation (Sung et al. 2006; Cui and Benfey 2009). Moreover, a premature middle cortex root phenotype is exhibited by mutants deficient in key components of GA signaling (Cui and Benfey 2009). Surprisingly, a normal ground tissue phenotype has been detected in the root of both *sleepy* (*sly*) 1-10 and *sneezy* (*sne*) mutants in which DELLA proteins are not degraded, and, therefore, a high steady-state level of GAs is believed to be present (McGinnis et al. 2003; Strader et al. 2004; Cui and Benfey 2009). Conversely, radial patterning defects were observed in *spindly* (*spy*) mutants in which GA signaling is not compromised (Swain et al. 2002; Cui and Benfey 2009). The possibility that *SPINDLY* (*SPY*), which encodes an O-GLNAc transferase, could act by postranscriptionally modulating SHR and SCR activities has been excluded based on experimental grounds (Cui and Benfey 2009). Note that in animal systems, a SPY homolog interacts with a histone deacetylases (HADC) (Yang et al. 2002). Since in wild-type roots a premature middle cortex phenotype has been also induced by inhibiting HADC activity, it was suggested that SPY could play a role in middle cortex formation through an epigenetic mechanism, perhaps negatively modulating cell division genes in the ground tissue (Cui and Benfey 2009). In the context of a more complex hormone circuitry, note also that a cross talk between GA and cytokinins has been suggested, which partially converge on SPY function (Greenboim-Wainberg et al. 2005). Indeed cytokinin signaling is affected in the *spy* mutant and GAs also repress the effects of cytokinins, by inhibiting induction of the cytokinin primary-response gene, type-A *Arabidopsis* response regulator 5 (ARF5). Thus, SPY acts as both

a repressor of GA responses and a positive regulator of cytokinin signaling (Greenboim-Wainberg et al. 2005).

Cross-talk between auxin and brassinosteroids (BR) is also active in root patterning (Fig. 15). There is evidence that auxin strongly promotes the expression of *BREVIS RADIX* (*BRX*), which encodes a transcription factor that is rate-limiting for BR biosynthesis and auxin responsive gene expression. *brx* mutants are characterized by impaired root growth although they maintain a normal capacity to initiate lateral roots and respond normally to gravitropic stimulus. Moreover, in *brx* mutants, the expression of auxin-responsive genes is impaired, although they maintain an intact auxin signaling pathway (Mouchel et al. 2006; Scacchi et al. 2009). This is consistent with the BRX pathway lowering the level of constitutive repression of auxin-induced genes by impinging on the DNA-binding capacity of the repressive ARF2 (Vert et al. 2008). An autoregulatory feedback loop of *BRX* expression is also active in that BRX protein abundance is negatively regulated by auxin, likely being a target for auxin-induced proteasome degradation while BRX is itself an auxin-induced gene (Fig. 15). Recently, it has been also demonstrated that BRX protein colocalizes with PIN1 at the plasma membrane and moves to the nucleus in an auxin concentration- or flux-dependent manner (Mouchel et al. 2006; Scacchi et al. 2009). In spite of its reduced level in the nucleus, BRX plays a transcriptional modulatory role in cooperation with the B3 domain-type transcription factor NGATHA (NGA1), which in turn is related to ARFs. BRX protein and NGA 1 factors might represent a novel coregulator-transcription factor duo, analogous to AUX/IAA-ARF, which might act in conveying auxin efflux modulation into a different pattern of gene expression (Scacchi et al. 2009). The auxin response necessary for cytokinin-induced inhibition of lateral root formation was hypothesized to be lost in the *brx* mutant, which if correct, would establish a link between BRX and cytokinin action (Li et al. 2009). Moreover, an interaction occurs between brassinosteroids, auxin, and abscisic acid (ABA) in root development. It is well known that ABA-related glucose signaling is involved in the control of RAM maintenance and development through pleiotropic effects (Brocard-Gifford et al. 2004; Shishkova et al. 2008). Recently, mutants affected in BR signaling also showed enhanced ABA sensitivity while transcriptomic analysis revealed that ABA negatively affects auxin signaling (Rodrigues et al. 2009).

6 Stem-Cell State and Chromatin Remodelers

Chromatin remodeling, accomplished through posttranslational histone modifications and changes in DNA methylation level, is an important mechanism for regulating gene activity in both animals and plants (Huck-Hui and Bird 1999; Riechman 2002; Suzuki and Bird 2008; Law and Jacobsen 2009, 2010). In animals, there is evidence that a stem-cell-specific state of chromatin is related to the repression of genes, which promote differentiation while simultaneously promoting

stem-cells and proliferation factors (Boyer et al. 2006). Moreover, in murine embryonic stem-cells, repressive and promotive chromatin motifs have been identified within key development genes (Bernstein et al. 2006; Mikkelsen et al. 2007). This could be a mechanism for silencing differentiation genes while keeping them primed for activation. In mammalian stem-cells, a role has been demonstrated for the Polycomb group (PCg) proteins complexes PRC2 and PRC1 in gene silencing. Notably, PRC-complex-binding sites harbor trimethylated histone (H3K27met) (Azuara et al. 2006; Boyer et al. 2006; Schwaz et al. 2006; Schwarz and Pirrotta 2007).

Whether reversibility in chromatin accessibility is also related to stem-cell function in plants is of current debate. Despite the presence in plants of several PRC2 complexes with specific functions and the interaction of a PRC2 complex in repressing *SHOOTMERISTEMLESS (STM)* in the differentiated cells of the SAM, evidence for their putative role in a plant stem-cell niche is not yet available (Hsieh et al. 2003; Katz et al. 2004). However, there is evidence of a role for chromatin organization in plant stem-cell specification. For example, in the SAM, there is an involvement of plant-CHROMATIN-ASSEMBLY FACTOR-1 (CAF1), which is necessary for maintaining chromatin integrity, and SPLAYED (SYD), a chromatin remodeling ATPase belonging to a class of SFN2 transcriptional regulators (Kaya et al. 2001; Kwon et al. 2005). Further observations in RAMs deal with the activity of PICKLE (PKL), a CHD3 chromatin remodeling factor, and TOPLESS (TPL), a nuclear protein that bears some similarity to a transcriptional corepressor. PKL represses embryonic genes in germinating seedlings, while TPL seemingly participates in a chromatin-based stabilization of the embryo axis through the repression of root-promoting genes (Ogas et al. 1999; Long et al. 2006). Consistent with this role, *tpl* mutants were rescued by mutation of *HAG1*, which encodes a histone acetyltransferase (HAT), and this was enhanced by a mutation in *HAD19*, which encodes a histone deacetylase, acting in promoting and repressing target genes, respectively (Long et al. 2006). RNA-directed DNA methylation, which leads to chromatin modification and represents an additional mechanism of epigenetic regulation, has also been related to a correct development of the SAM (Kidner and Martienssen 2005).

Concerning the RAM, LHP1, which as mentioned above (see Sects. 4.3.2 and 4.2) has a role in ground tissue patterning, seemingly acts as a transcriptional repressor by modifying chromatin conformation (Sung et al. 2006; Turck et al. 2007; Welch et al. 2007). However, LHP1 seems not to change histone modification, and unlike the animal homolog, HP1, LHP1 targets both heterochromatin and euchromatin and binds to H3 histones that are trimethylated at lysine 27 (Turck et al. 2007). Moreover, the weakness of *lhp1* phenotypes compared to that of both *spy-3* and roots treated with HDAC inhibitor, suggested that LHP1 enhances the silencing effect of other mechanisms rather than directly causing gene silencing itself (Cui and Benfey 2009). So, it has been proposed that LHP1, SPY, and GA signaling act together in a common epigenetic mechanism involving histone deacetylation, which allows SCR to acts as a repressor of cell division gene activity (Fig. 13) (Cui and Benfey 2009). Notably and as described above (see Sect. 4.3.2),

a histone acetyltransferase activity (GCN5) has also been found to act in the PLT pathway by modulating a PLT gradient (Kornet and Scheres 2009).

Additional evidence of epigenetic control of the stem-cell condition in plants is through the understanding of the RETINOBLASTOMA-related (RBR) protein, a master negative regulator of cell-cycle progression, which in animal systems interacts with differentiation promoting factors and chromatin remodeling proteins (Macaluso et al. 2006). In plants, reduced RBR activity induced additional division of columella daughter cells, which acquired stem-cell potential, thus expanding the stem-cell pool, while increased RBR expression promoted premature differentiation (Wildwater et al. 2005). Notably, RBR, which can be locally modulated by a root-specific RNAi, primarily affected cell differentiation rather than the cell cycle as seen by unaffected cell cycle progression in the expanding columella stem-cell population under reduced RBR activity (Wildwater et al. 2005). A model that provides robustness to the involvement of epigenetic regulation mechanisms has been proposed, in which KRYPTONYTE2 (KPR2), a histone H3 methyltransferase (Jackson et al. 2002), inhibits cyclinD/kinase, which inhibits RBR, which in turn inhibits cell cycle-promoting transcription factors (E2F), thus modulating the progression of a stem-cell to differentiation (Fig. 12b) (Wildwater et al. 2005). Recently, increased *RBR2;1* expression in the QC, together with *ARGONAUTE* (*AGO*)4-mediated siRNA events have been postulated to play a role in maize root repatterning (Jiang et al. 2010).

Finally, an overexpression of genes that play a role in chromosome organization and biogenesis, including genes involved in histones and DNA modifications, has been detected in the SAM stem-cell niche of *Arabidopsis*, through a high-resolution gene expression map. On this basis, it has been proposed that in plants, stem-cell chromatin is maintained in a flexible state in order to dynamically balance gene expression (Yadav et al. 2009).

7 Conclusions and Perspectives

Despite the huge quantity of data on transcriptional networks that underlie root developmental pattern and its interplay with hormone signaling, the spatiotemporal complexity and the dynamic framework of root transcriptional program are still far from being resolved. In the future, major understanding could be achieved by extending recent approaches, which have been refined to address and define expression profiles at the level of individual cell types (Birnbaum et al. 2003; Schmid et al. 2005; Levesque et al. 2006; Brady et al. 2007; Yadav et al. 2009), as well as by combining different tools. For example, genome-wide expression analysis has very high resolution and is efficient in identifying rare transcripts. Multiplex *in situ* hybridization allows one to simultaneously localize transcripts at the cellular level. Live-imaging has been developed to follow intracellular and spatial distribution of signaling molecules and regulatory factors, and therefore the dynamics of cell identity. ChIP/chip and ChIP/seq techniques enable identification of large numbers

of transcription factors and target genes. Genome-wide insertional mutagenesis allows large gene functional characterization. Informatic elaboration enables the management of huge data sets and the elaboration of predictive computational models (Heisler et al. 2005; Brady et al. 2007; Kiefer 2009; Yadav et al. 2009).

With the development of such techniques, we appear to be on the cusp of major breakthroughs in understanding plant development. However, in the context of current knowledge, two major unanswered questions are, on the one hand, how transcriptional networks are temporally regulated during the progression of cells toward a particular developmental fate and, on the other, how such networks are modulated in relation to environmental stimuli and selection pressures. In particular, besides defining transcriptional changes, it will become increasingly important to identify how different genes interact in determining adaptive traits. Moreover, a related and poorly explored field deals with genetic networks and signaling that underlie stem-cell reprogramming within the context of differentiated tissues, which in plants frequently occurs both during intrinsic developmental programs (i.e., lateral root formation, activity of lateral meristems, axillary buds), as well as in relation to adventitious morphogenesis and in vitro regeneration processes (Chiappetta et al. 2006, 2009; Costa and Shaw 2006; Xu et al. 2006; Sena et al. 2009). Such knowledge should also be relevant for the development of refined plant biotechnology.

Finally, since root patterning is largely diversified within the plant kingdom, it will be interesting to extend our knowledge to different species and use natural variation to understand regulatory networks that control developmental processes. In particular, the transfer of this knowledge to crop plants should be of benefit in improving crop yield and quality and making them adapted to survive in our ever-changing global environment.

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